DGAT1 (EC 2.3.1.20) is an integral membrane protein that synthesizes triacylglycerides from two substrates: diacylglycerol (DAG) and fatty acyl-CoA (Extended Data Fig. 1). In humans, DGAT1 is highly expressed in epithelial cells of the small intestine and its activity is essential for the absorption of dietary fats. DGAT1 is also found in the liver, in which it synthesizes fat for storage and in female mammary glands, in which it produces fat in the milk. "Dgat1" mice are viable, and show substantially reduced levels of triacylglycerides in all tissues and resistance to obesity when kept on a high-fat diet. These results have generated considerable interest in DGAT1 as a potential target for the treatment of hypertriglyceridaemia and fatty liver disease.

DGAT1 belongs to the large MBOAT superfamily (http://pfam.xfam.org/family/MBOAT), members of which are found in all kingdoms of life. In mammals, the MBOAT family includes enzymes that modify lipids or proteins, such as acyl-CoA:cholesterol acyltransferase (ACAT) and protein-serine O-palmitoleoyltransferase (PORCUPINE). Members of the MBOAT family have a highly conserved histidine residue that is required for their transferase activity, and are predicted to have eight to eleven transmembrane segments. The crystal structure of a bacterial MBOAT, DltB, has previously been described (Extended Data Fig. 1). However, the structure of DltB may not be a suitable model for human DGAT1 because the sequence identity of the two proteins is low (around 20%).

In vitro activity of purified human DGAT1

Full-length human DGAT1 was overexpressed and purified. DGAT1 purified in the detergent lauryl maltose neopentyl glycol (LMNG) exists mainly as a stable dimer that is partially resistant to the denaturing conditions of SDS–PAGE (Extended Data Fig. 2a, Methods). When DGAT1 was purified with a milder detergent, glyco-diosgenin (GDN), a substantially higher fraction of DGAT1 was in the tetrameric form. Both the dimeric and tetrameric forms of human DGAT1 seem stable in terms of their oligomeric state (Extended Data Fig. 2b). DGAT1 from plants and mammals was previously shown to form either a dimer or a tetramer; however, it is not clear whether the oligomeric state has an effect on enzymatic functions.

We established an in vitro functional assay to measure the activity of human DGAT1 (Extended Data Fig. 2d–f, Methods). The initial rate of the enzymatic reaction in different concentrations of oleoyl-CoA can be fitted with a Michaelis–Menten equation for both the dimeric and the tetrameric DGAT1 (Extended Data Fig. 2g, Extended Data Table 2). The tetrameric DGAT1 has a slightly higher velocity of enzyme-catalysed reaction at infinite concentration of substrate ($V_{\text{max}}$) and the $V_{\text{max}}$ values are equivalent to a turnover rate of around one molecule per second for each DGAT1 protomer. Both forms have a similar Michaelis constant ($K_m$). We also measured the activity of DGAT1 in cell membranes and found that the $V_{\text{max}}$ is about 50% higher than that of DGAT1 in detergent.
The structure of human DGAT1 was solved by single-particle cryo-electron microscopy (cryo-EM) (Extended Data Fig. 3a–e, Methods). A density map was reconstructed to an overall resolution of 3.1 Å with an imposed C2 symmetry. The resolution for helices that are density defined is 2.7 Å, whereas regions close to the periphery of the dimer have lower resolution, probably owing to their relatively higher mobility (Extended Data Fig. 3f).

The density map is of sufficient quality to allow de novo building of residues 64 to 224 and 239 to 481—which include all the transmembrane helices, one oleoyl-CoA molecule and five partially resolved lipid or detergent molecules—and the structure was refined to proper geometry (Extended Data Fig. 4a–c, Extended Data Table 1). The first 63 residues, last 5 residues and residues 225–238 (which are part of a cytosolic loop) were not resolved. Residues 112 to 120 (part of a luminal loop) were partially resolved and built as poly-alanines.

The DGAT1 dimer is around 105 Å by 55 Å by 48 Å, and is similar in shape to a canoe (Fig. 1a–d). On the basis of the positive-inside rule16, the N terminus of DGAT1 resides at the cytosolic side (Extended Data Fig. 4d). The approximate position of the ER membrane is marked with grey shading. The ER membrane is marked with grey shading. The approximate position of the ER membrane is marked with grey shading.

Whereas the $K_m$ is similar (Extended Data Fig. 2h, Extended Data Table 2). Both the $V_{max}$ and the $K_m$ values reported here are comparable to those previously reported for human DGAT1 in microsomes16. The enzymatic activity was also measured in different concentrations of DAG for both dimeric and tetrameric DGAT1 and the two forms show similar activity (Extended Data Fig. 2i, Extended Data Table 2). Notably, both datasets were better fit with an allosteric sigmoidal equation (Methods), suggesting that DAG has a regulatory role on DGAT1.

**Overall structure of human DGAT1**

The structure of human DGAT1 was solved by single-particle cryo-electron microscopy (cryo-EM) (Extended Data Fig. 3a–e, Methods). A density map was reconstructed to an overall resolution of 3.1 Å with an imposed C2 symmetry. The resolution for helices that are density defined is 2.7 Å, whereas regions close to the periphery of the dimer have lower resolution, probably owing to their relatively higher mobility (Extended Data Fig. 3f).

The density map is of sufficient quality to allow de novo building of residues 64 to 224 and 239 to 481—which include all the transmembrane helices, one oleoyl-CoA molecule and five partially resolved lipid or detergent molecules—and the structure was refined to proper geometry (Extended Data Fig. 4a–c, Extended Data Table 1). The first 63 residues, last 5 residues and residues 225–238 (which are part of a cytosolic loop) were not resolved. Residues 112 to 120 (part of a luminal loop) were partially resolved and built as poly-alanines.

The DGAT1 dimer is around 105 Å by 55 Å by 48 Å, and is similar in shape to a canoe (Fig. 1a–d). On the basis of the positive-inside rule16, the N terminus of DGAT1 resides at the cytosolic side (Extended Data Fig. 4d). This assignment is also consistent with the previous consensus from biochemical studies11,18 and allows for unambiguous placement of the C terminus to the luminal side of the endoplasmic reticulum (ER) (Fig. 1e, f). Each DGAT1 protomer has nine transmembrane helices, TM1–TM9, and three long loops: an ER luminal (extracellular) loop EL1 between TM1 and TM2; an intracellular loop IL1 between TM4 and TM5; and a second intracellular loop IL2 between TM6 and TM7. In each protomer, TM2–TM9 and the two intracellular loops IL1 and IL2 form a distinctive structural fold that we name the MBOAT fold (Figs. 1e, f, 2a–d). TM1, which is not part of the MBOAT fold, is isolated and linked to the MBOAT fold by the long ER luminal loop EL1 (residues 110 to 125). EL1 is partially structured and extends around 35 Å along the luminal side of the protein (Fig. 1e, f).

**The dimer interface**

Although TM1 seems to be suspended in the membrane when a protomer is viewed in isolation, the space between the TM1 and the rest of the protomer (the MBOAT fold) is partially filled by the TM1 from the neighbouring protomer so that the two form a domain-swapped homodimer (Fig. 1b, g–i). The C terminus of TM1 makes contact at residues Ser83 and Asn84, which are located close to the intracellular side of the membrane. The rest of the space between the two protomers is filled with two detergent molecules and four partially resolved lipid molecules (Extended Data Figs. 4c, 5a–e). Because these bound detergent and lipid molecules have extensive interactions with DGAT1, they may have important roles in both the structure and the function of DGAT1.

Previous studies on a plant DGAT1 identified part of the N terminus as intrinsically disordered protein, and showed that deletion of the entire
The reaction chamber and oleoyl-CoA-binding site. a–d, The reaction chamber (grey surface) is shown in four orientations with the surrounding helices in cartoon representation. e, An oleoyl-CoA molecule is shown as spheres with carbon atoms coloured in yellow. The side chains of the conserved active site residues (SXXHEY) are shown as magenta spheres. Inset, oleoyl-CoA is shown as sticks and its density as green mesh. f, Residues at the oleoyl-CoA-binding site are shown as sticks with carbon atoms coloured in magenta. AH, amphipathic helix. g, Interaction between the FYXDWWN motif (magenta) and the N terminus of the neighbouring protomer (cyan).

The reaction chamber
The MBOAT fold of DGAT1 (TM2–TM9, IL1 and IL2) carves out a large hollow chamber in the hydrophobic core of the membrane (Fig. 2a–d). His415, which is almost universally conserved in the MBOAT family of enzymes, is found inside the reaction chamber and on TM7. TM2–TM9 segregate into three groups that form three sidewalls of the chamber. TM2, TM3 and TM4 pack into a bundle that forms the first sidewall; TM5 and TM6 are both very long (almost 40 amino acids each) and the two helices coil into a unit that tilts roughly 56° to the membrane norm to form the second sidewall; and TM7, TM8 and TM9 form a panel and the third sidewall (Fig. 2a–d). The cytosolic ends of TM7 and TM8 are around 19 Å apart, creating a cytosolic entrance to the reaction chamber (Fig. 2a, b). IL1 and IL2 form the floor of the chamber at the cytosolic side. IL1 (residues 222 to 261) is composed of a helix flanked by two long strands, whereas IL2 (residues 352 to 396) has a long amphipathic helix (residues 380 to 394) preceded by a short helix and a loop.

The structure of human DGAT1 was solved in the presence of 2 mM oleoyl-CoA. A large non-protein density is found at the cytosolic side of the reaction chamber close to IL2 and it extends deep into the reaction chamber (Extended Data Fig. 4b). An oleoyl-CoA molecule is modelled into this density, with the adenosine 3′,5′-diphosphate of the CoA moiety at the cytosolic entrance, the 4-phosphate pantothenic acid, β-alanine and β-mercapto-ethylamine extending progressively into the reaction chamber and the acyl chain residing in a hydrophobic pocket inside the reaction chamber (Fig. 2e, f, Extended Data Fig. 6a–h). The activated thioester is located in the vicinity of His415, poised for an attack from the hydroxyl group of DAG. The position of the thioester could be stabilized by interactions between the carboxyl oxygen of the fatty acid and the side chain of Gln465 on TM9 (Fig. 2f, Extended Data Fig. 6h).

IL2 has a crucial role in acyl-CoA binding. The V-shaped helix-turn-helix motif of IL2 forms the binding site for the adenosine 3′,5′-diphosphate moiety of acyl-CoA (Fig. 2g). The loop preceding the helix-turn-helix motif contains a highly conserved FYXDWWN sequence that is found in both DGAT1 and the related ACAT, and mutational studies suggest that these residues may coordinate acyl-CoA.23 (Extended Data Fig. 7a, c). However, only Trp364, the first tryptophan in the FYXDWWN sequence, forms part of the hydrophobic pocket for the acyl chain and the rest of the sequence does not have direct contact with the acyl-CoA. FYXDWWN packs tightly against the helix-turn-helix motif of IL2 and also interacts extensively with the N terminus from the neighbouring protomer (Fig. 2g). We speculate that mutations to this sequence and deletion of the N terminus could affect the enzymatic activity by perturbing these interactions.

To assess the functional effect of residues at the active site and those that line the acyl-CoA-binding site, we introduced point mutations and measured the enzymatic activities of these different DGAT1 mutants. Point mutations to residues that line the entrance of the acyl-CoA-binding site (Thr371, Tyr390, Lys400 and Arg404) reduce the enzymatic activity by 30–70%, whereas point mutations to residues of the rest of the binding pocket (Trp377, Asn378, His382 and Ser411) have a larger effect, resulting in a loss of enzymatic activity of more than 80%. Mutations to residues of the active site (His415 and Glu416) abolish enzymatic activity (Extended Data Fig. 6i, j).

N terminus before TM1 led to loss of the enzymatic activity18–20. The current structure shows that although the N terminus is not structured, it interacts with the highly conserved elements in the MBOAT fold. We next examined the functional effects before TM1 led to loss of the enzymatic activity implications of the domain-swapped N terminus of DGAT1. We progressively shortened the N terminus by constructing the dimer with the longest N-terminal deletion (ΔN84) and were able to purify 2–75 (ΔN75), 2–80 (ΔN80) and 2–84 (ΔN84), and were able to purify all of these as stable dimers (Extended Data Fig. 5f). We found that the enzymatic activity is progressively lower as more N-terminal residues are deleted, and the dimer with the longest N-terminal deletion (ΔN84) has no activity (Fig. 1j, Extended Data Table 2). These results indicate that the N terminus could regulate enzymatic function by its interaction with the MBOAT fold.

Acyl-CoA-binding site
The MBOAT fold of DGAT1 (TM2–TM9, IL1 and IL2) carves out a large hollow chamber in the hydrophobic core of the membrane (Fig. 2a–d). His415, which is almost universally conserved in the MBOAT family of enzymes, is found inside the reaction chamber and on TM7. TM2–TM9 segregate into three groups that form three sidewalls of the chamber. TM2, TM3 and TM4 pack into a bundle that forms the first sidewall; TM5 and TM6 are both very long (almost 40 amino acids each) and the two helices coil into a unit that tilts roughly 56° to the membrane norm to form the second sidewall; and TM7, TM8 and TM9 form a panel and the third sidewall (Fig. 2a–d). The cytosolic ends of TM7 and TM8 are around 19 Å apart, creating a cytosolic entrance to the reaction chamber (Fig. 2a, b). IL1 and IL2 form the floor of the chamber at the cytosolic side. IL1 (residues 222 to 261) is composed of a helix flanked by two long strands, whereas IL2 (residues 352 to 396) has a long amphipathic helix (residues 380 to 394) preceded by a short helix and a loop.
We propose that when the ber also has a large opening to the hydrophobic core of the membrane, the lateral opening would allow the entrance of DAG and exit of triacylglyceride. The product, triacylglyceride, could return into the hydrophobic core of the membrane while coenzyme A dissociates into the cytosol (Fig. 3c).

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-020-2280-2.

**Discussion**

The structure of human DGAT1 defines a conserved MBOAT structural fold, which forms a reaction chamber in the ER membrane to shield the acyl transfer reaction from the hydrophobic core of the membrane. The reaction chamber has a tunnel to the cytosolic side and its entrance recognizes the hydrophobic coenzyme A motif of an acyl-CoA molecule. The tunnel has a slit between TM7 and TM8 that could allow the entry of the acyl chain of an acyl-CoA molecule into the chamber, reaching the hydrophobic pocket inside the chamber (Fig. 3a, b). The reaction chamber also has a large opening to the hydrophobic core of the membrane, which could allow entry of a DAG molecule. We propose that when the glycerol backbone of a DAG molecule approaches the catalytic centre at His415, the two hydrophobic aliphatic acyl chains of DAG could remain partially outside of the protein, accommodated in the hydrophobic core of the membrane (Fig. 3b). The conserved His415 would facilitate the acyl transfer reaction by activating the free hydroxyl group on DAG, and Glu416 could enhance the activation. The activated hydroxyl oxygen then attacks the thioester on the fatty acyl-CoA to form a new ester bond (Fig. 3d). The product, triacylglyceride, could return into the hydrophobic core of the membrane while coenzyme A dissociates into the cytosol (Fig. 3c).

**Fig. 3** | Proposed catalytic mechanism of human DGAT1. a, b A DGAT1 monomer is shown as a trapezoid in light blue and the reaction chamber is shown in the shape of an inverted flask coloured in grey. TM7–TM9, acyl-CoA and DAG are shown schematically. The catalytic His415 residue is marked in red on TM7. The CoA moiety of an acyl-CoA molecule binds to DGAT1 at the cytosolic entrance of the tunnel and the hydrophobic acyl chain slides into the reaction chamber through a slit between TM7 and TM8. The glycerol backbone of a DAG molecule approaches the catalytic centre at His415, the two hydrophobic aliphatic acyl chains of DAG could remain partially outside of the protein, accommodated in the hydrophobic core of the membrane (Fig. 3b). The conserved His415 would facilitate the acyl transfer reaction by activating the free hydroxyl group on DAG, and Glu416 could enhance the activation. The activated hydroxyl oxygen then attacks the thioester on the fatty acyl-CoA to form a new ester bond (Fig. 3d). The product, triacylglyceride, could return into the hydrophobic core of the membrane while coenzyme A dissociates into the cytosol (Fig. 3c).
Methods

Data reporting
No statistical methods were used to predetermine sample size. The experiments were not randomized and the investigators were not blinded to allocation during experiments and outcome assessment.

Cloning, expression and purification of human DGAT1
The human DGAT1 gene (accession number NP_036211) was codon-optimized and cloned into a modified pFastBac Dual vector22 for production of baculovirus by the Bac-to-Bac method (Thermo Fisher Scientific). High Five cells (Thermo Fisher Scientific) at a density of around 3 x 10⁶ cells ml⁻¹ were infected with baculovirus and grown at 27 °C for 48–60 h before collection. Cell membranes were isolated following a previous protocol22 and flash-frozen in liquid nitrogen.

Isolated cell membranes were thawed and homogenized in 20 mM HEPES, pH 7.5, 150 mM NaCl and 2 mM β-mercaptoethanol, and then solubilized with 1% (w/v) LMNG (Anatrace) at 4 °C for 2 h. After centrifugation (55,000g, 45 min, 4 °C), DGAT1 was purified from the supernatant using a cobalt-based affinity resin (Talon, Clontech) and the His₆-tag was cleaved by incubation with tobacco etch virus (TEV) protease for 1 h at room temperature. Oleoyl-CoA (20 μM) was added to reduce aggregation, and DGAT1 was then concentrated to around 5 mg ml⁻¹ (Amicon 100 kDa cut-off, Millipore) and loaded onto a size-exclusion column (SRT-3C SEC-300, Sepax Technologies) equilibrated with 20 mM HEPES, pH 7.5, 150 mM NaCl and 0.02% GDN (Anatrace). Purified DGAT1 was mixed with 2 mM oleoyl-CoA and concentrated to around 20 mg ml⁻¹ for cryo-EM grid preparation.

When LMNG is used in the extraction step, most of the DGAT1 is homodimer and only a small fraction is homotetramer. To obtain tetrameric DGAT1, 1% GDN was used for extraction and 0.02% GDN for purification. The dimeric DGAT1 produces substantially better cryo-EM grids and was given priority for structure determination.

DGAT1 mutants were generated using the QuikChange method and the entire cDNA was sequenced to verify the mutation. Mutants were expressed and purified following the same protocol as wild type.

Cryo-EM sample preparation and data collection
Cryo grids were prepared using the Thermo Fisher Vitrobot Mark IV. Quantifoil R1.2/1.3 Cu grids were glow-discharged in air for 40 s at medium level using the Plasma Cleaner (Harrick Plasma, PDC-32G-2). Concentrated DGAT1 (3.5 μl) was applied to each glow-discharged grid. After blotting with filter paper (Ted Pella, Prod. 47000-100) for 3.5 s, the grids were plunged into liquid ethane cooled with liquid nitrogen. Movie stacks were collected using SerialEM on a Titan Krios at 300 kV with a Quantum energy filter (Gatan) and a Cs corrector (Thermo Fisher Scientific), at a nominal magnification of ×105,000 and with defocus values of −2.0 μm to −1.2 μm. A K2 Summit direct electron detector (Gatan) was paired with the microscope. Each stack was collected in the super-resolution mode with an exposing time of 0.175 s per Å⁻² for 2 × 2 binned (2 x 2) so that the pixel size was 1.11 Å. Dose weighting was performed during motion correction, and the defocus values were estimated with Gctf.

Cryo-EM data processing
A total of 2,749,110 particles were automatically picked (RELION 2.129) from 3,510 images and imported into cryoSPARC20. Out of 200 two-dimensional (2D) classes, 101 (containing 1,000,063) particles were selected for ab initio three-dimensional (3D) reconstruction, which produced one good class with recognizable structural features and three bad classes that do not have structural features (Extended Data Fig. 3). Although human DGAT1 can form both dimers and tetramers, as only the dimer fraction was used in grid preparation we found no tetramer during 2D classification. Both the good and bad classes were used as references in the heterogeneous refinement (cryoSPARC) and yielded a good class at 4.1 Å from 408,945 particles. After handedness correction, non-uniform refinement (cryoSPARC) was performed with C2 symmetry and an adaptive solvent mask, which yielded a map with an overall resolution of 3.1 Å (Extended Data Fig. 3b).

Further heterogeneous refinement yielded a class with 275,945 particles and after non-uniform refinement, a map was yielded that had similar resolution but improved density of TM2, TM3, TMβ and lipids (Extended Data Fig. 3c). Resolutions were estimated using the gold-standard Fourier shell correlation with a 0.143 cut-off28 and high-resolution noise substitution25. Local resolution was estimated using ResMap.

Model building and refinement
Structure models were built de novo into the density map starting with poly-alanine, and side chains were then added onto the model based on the map. Model building was conducted in Coot27. Structure refinements were carried out in PHENIX in real space with secondary structure and geometry restraints. The EMRinger Score was calculated as described.

DGAT1 activity assay
DGAT1 activity was measured using a fluorescence-based coupled-enzyme assay29 in a quartz cuvette at 37 °C (Extended Data Fig. 2d). The reaction was monitored in a FluoroMax-4 spectrofluorometer (HORIBA) with 340-nm excitation and 465-nm emission at 15-s intervals. All assays were done in a buffer with 20 mM HEPES, pH 7.5, 150 mM NaCl, 2 mM β-mercaptoethanol, 0.5 mM DDM and 1% TritonX-100. Final concentrations of NAD⁺, thiamine pyrophosphate and α-ketoglutarate were 0.25 mM, 0.2 mM and 2 mM, respectively. α-Ketoglutarate dehydrogenase (αKDH) was prepared from bovine heart purchased from a meat market, following a published protocol38. An appropriate amount of αKDH was used to ensure that the DGAT1 reaction is the rate-limiting step. When oleoyl-CoA concentrations were varied, the DAG concentration was fixed at 200 μM. When DAG concentrations were varied, the oleoyl-CoA concentration was fixed at 100 μM. All reactions were initiated with the addition of oleoyl-CoA. The initial rate versus different concentrations of oleoyl-CoA can be fit with a Michaelis–Menten equation. The initial rates in various concentrations of DAG were not well fit with the traditional Michaelis–Menten equation, but could be fit with an allosteric sigmoidal equation:

\[
V = V_{\max} \times \frac{X_h}{(K_m + X)^n}
\]

In which \(X\) is the DAG concentration and \(h\) is the Hill coefficient.

When assaying the activity of DGAT1 dimer or tetramer, the protein concentration was kept at 2.4 μg ml⁻¹ (around 40 nM). When measuring DGAT1 in the cell membrane, crude membrane containing DGAT1 was used and the amount of DGAT1 in the membrane was estimated on the basis of the yield of DGAT1 from the same batch of cells. We did not observe substrate inhibition up to 200 μM of oleoyl-CoA.

Detection of triacylglycerides by thin-layer chromatography
To validate the functional assay described in the previous section, we confirmed triacylglyceride production directly. After initiating an enzymatic reaction, 100 μl of the sample was taken at each indicated time point and extracted with 400 μl chloroform. The organic phase containing triacylglyceride was dried under argon and then resuspended in 40 μl chloroform out of which 4 μl was spotted onto a KC18 reversed-phase thin-layer chromatography plate (Whatman Chemical Separation). The mobile phase is 100:1 (chloroform: acetic acid, v/v) and triacylglyceride was visualized in an I₅ chamber.

Reporting summary
Further information on research design is available in the Nature Research Reporting Summary linked to this paper.
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| MBOAT | DGAT | DltB |
|-------|------|------|
| **Acyl-donor** | ![Acyl-CoA](image) | ![DltC-Ser35-Pi.png](image) |
| **Acyl-acceptor** | ![DAG](image) | ![Glc-Glc-Diacylglycerol](image) | ![LTA](image) |
| **Product** | ![Product](image) | ![Product](image) | ![Product](image) |

**Substrates distribution**

**MBOAT fold**

**Tunnel**

*Extended Data Fig. 1* | See next page for caption.
**Extended Data Fig. 1 | Side-by-side comparison of human DGAT1 and DltB.**

Both human DGAT1 and DltB have an acyl donor and an acyl acceptor. In the acyl-donor row, the red dashed lines indicate the bonds that are broken during acyl-transfer reactions. In the acyl-acceptor row, the hydroxyl groups are highlighted in red. In the substrates distribution row, DGAT1 and the DltB–DltC complex are shown as cartoon and the membrane as dashed lines. The position of the catalytic histidine in each protein is marked with a yellow star. In human DGAT1, the acyl-CoA comes from the intracellular side whereas the DAG comes from the hydrophobic core of the membrane. In DltB, the 4′-phosphopantetheine–DltC is intracellular whereas the lipoteichoic acid (LTA) is extracellular. In the MBOAT fold row, the MBOAT folds of DGAT1 and DltB are shown in cartoon representation and viewed from the same orientation. Equivalent helices have the same colour. The tunnel row shows the cut-away surface illustrations of DGAT1 and DltB, showing their cytosolic tunnels. The position of the conserved histidine residue in each protein is marked with a yellow star. In DltB, the intracellular loops are placed more towards the centre of the membrane and as a result, the MBOAT fold in DltB does not carve out a reaction chamber in the membrane. Overall, DltB is shaped similarly to an hourglass that allows the two substrates to approach the reaction centre from either side of the membrane, and the transfer of an acyl group across the membrane. These observations highlight the versatility of the MBOAT fold.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | Purification and functional characterization of human DGAT1. 

**a**, Size-exclusion chromatograph profile of human DGAT1 extracted with LMNG. Elution volumes of membrane proteins of known molecular weight—bcMalT (about 100 kDa, green)\(^3\) and mouse (m)SCD1 (about 40 kDa, blue)\(^2\)—are marked with arrows. Inset, SDS–PAGE of the purified DGAT1. DGAT1 has a main peak with an elution volume of around 11.7 ml that corresponds to a dimer and a minor peak at around 10.4 ml that corresponds to a tetramer. 

**b**, Size-exclusion chromatography profiles of DGAT1 extracted with LMNG (left) or GDN (right). The main peak from the first run (red trace) was collected and reinjected onto the same column after 1 h. In both **a** and **b**, the detergent in the mobile phase is GDN. 

**c**, A white layer of fat appeared after membrane solubilization and centrifugation, indicating that the heterologously expressed DGAT1 is active in cells. 

**d**, The DGAT1 reaction is coupled to that of αKDH to monitor production of coenzyme A (CoA-SH) in real time. 

**e**, Fluorescence of NADH plotted versus time. Rapid production of coenzyme A occurs in the presence of oleoyl-CoA, 1,2-dioleoyl-sn-glycerol (1,2-DAG) and the purified dimeric DGAT1. By contrast, production of coenzyme A was not observed when either 1,2-DAG or DGAT1 was omitted from the reaction mixture, indicating that hydrolysis of oleoyl-CoA is tightly coupled to the enzymatic reaction. In addition, coenzyme A production was almost completely suppressed in the presence of 5 μM T863, a known DGAT1 inhibitor\(^1\). 

**f**, Production of triacylglyceride over time, detected by thin-layer chromatography. The first lane from the left is a triacylglyceride standard. 

**g–i**, Initial rate of reaction versus oleoyl-CoA concentration measured using the purified dimeric DGAT1 (**g**), tetrameric DGAT1 (**h**) or DGAT1 in cell membrane (**i**). 

**j, k**, Initial rate of reaction versus DAG concentration measured using the dimeric (**j**) or tetrameric (**k**) DGAT1. Data are mean ± s.e.m. derived from three independent repeats. Experiments were repeated independently 10 times with similar results (**a, c**) or 3 times with similar results (**b, e, f**).
Extended Data Fig. 3 | Cryo-EM data and processing. a, A representative micrograph of DGAT1 (left), its Fourier transform (right) and representative 2D class averages (middle). Representative particles are highlighted with red circles. b, c, A flow chart for data processing and the final maps of DGAT1 (Methods). d, The gold-standard Fourier shell correlation (FSC) curve for the final map shown in c. e, Fourier shell correlation curves of the atomic model of DGAT1 versus the full map and individual half maps. f, Local-resolution map of DGAT1 shown in two orientations.
Extended Data Fig. 4 | Density maps and structural model of DGAT1. **a,** The overall map (left) and cartoon representation (right) of DGAT1. **b,** Individual secondary structures of DGAT1 shown as sticks, contoured in their density (green mesh). The density for oleoyl-CoA (green mesh) is shown at the same contour level as its neighbouring helix, TM4 (red mesh). **c,** Detailed view of each detergent or lipid molecule and its density. **d,** Electrostatic surface representations of the DGAT1 dimer in three orientations. The electrostatic potential was calculated using the APBS plug-in from PyMOL.
Extended Data Fig. 5 | Binding of the N terminus to the neighbouring protomer and its functional consequences. **a, b**, The DGAT1 dimer (cartoon) viewed in two orientations. **c–e**, Detailed view of the boxed regions shown in **a, b**. Residues involved in the interactions are shown as sticks. **f**, Size-exclusion profiles of N-terminal truncations. Experiments in **f** were repeated independently three times with similar results.
Extended Data Fig. 6 | Oleoyl-CoA-binding site. a–c, Oleoyl-CoA (spheres) bound to the DGAT1 protomer (cartoon) in three orientations. d–g, Detailed views of the boxed regions in a–c. Residues that coordinate oleoyl-CoA are shown as sticks with carbon atoms in magenta. h, LigPlus41,42 plot of the oleoyl-CoA-binding site. i, Normalized enzymatic activity of wild-type DGAT1 and various mutants. Data are mean ± s.e.m. derived from three independent repeats. j, Size-exclusion profiles of DGAT1 mutants. Experiments in I were repeated independently three times with similar results.
Extended Data Fig. 7 | DGAT1 sequence alignment. a, DGAT1 from human (Homo, Uniprot accession number O75907), mouse (Mus, Q922A7), frog (Xenopus, A0A1L8G0L4), fish (Danio, Q6P3J0) and thale cress (Arabidopsis, Q9SLD2) are aligned using the Clustal Omega server. Secondary structural elements of human DGAT1 are marked above the alignment. Residues are coloured on the basis of their conservation using the ESPript server. Residues at the acyl-CoA-binding site are marked with green triangles and those at the active site with red stars.

b, ConSurf mapped onto the human DGAT1 structure. DGAT1 is shown in different orientations in cartoon (top) and surface (bottom) representations. One DGAT1 monomer is coloured on the basis of the conservation score of each residue, calculated by the ConSurf server.

c, Sequence alignments of the FYXDWWN motif (red rectangle) from DGAT1 of different species and human ACAT1 (Uniprot P35610).
Extended Data Fig. 8 | Proposed gateway for DAG entry.  

(a–c), A large opening to the core of the membrane is framed by TM4 and TM6. A tubular density is observed extending into the reaction chamber.  

(d), Residues that line the opening are shown as magenta sticks.
## Extended Data Table 1 | Summary of cryo-EM data collection, processing and structure refinement

|                          | hDGAT1 (EMDB-21302) (PDB 6VP0) |
|--------------------------|--------------------------------|
| **Data collection and processing** |                               |
| Magnification            | 105,000                        |
| Voltage (kV)             | 300                            |
| Electron exposure (e-/Å²) | 50                             |
| Defocus range (μm)       | [-2.0, -1.2]                   |
| Pixel size (Å)           | 1.114                          |
| Symmetry imposed         | C2                             |
| Initial particle images (no.) | 2,749,110                   |
| Final particle images (no.) | 275,945                      |
| Map resolution (Å)       | 3.1                            |
| FSC threshold            | 0.143                          |
| Map resolution range (Å) | 2.7-4.3                        |
| **Refinement**           |                                |
| Initial model used (PDB code) | 6VP0                        |
| Model resolution (Å)     | 3.24                           |
| FSC threshold            | 0.5                            |
| Model resolution range (Å) | 3.24-3.33                    |
| Map sharpening B factor (Å²) | -100                          |
| Model composition        |                                |
| Non-hydrogen atoms       | 7212                           |
| Protein residues         | 808                            |
| Ligands                  | 12                             |
| B factors (Å²)           |                                |
| Protein                  | 70.5                           |
| Ligand                   | 78.1                           |
| R.m.s. deviations        |                                |
| Bond lengths (Å)         | 0.005                          |
| Bond angles (°)          | 1.129                          |
| Validation               |                                |
| MolProbity score         | 1.69                           |
| Clashscore               | 4.65                           |
| Poor rotamers (%)        | 1.96                           |
| Ramachandran plot        |                                |
| Favored (%)              | 96.5                           |
| Allowed (%)              | 3.5                            |
| Disallowed (%)           | 0                              |
Extended Data Table 2 | Functional parameters of wild-type human DGAT1 and various DGAT1 mutants

| substrates/mutants/truncations | $K_m$ (µM) | $V_{max}$ (pmol/min/µg) |
|-------------------------------|-----------|------------------------|
| oleoyl-CoA                    | 14.6 ± 1.3| 956.6 ± 36.1           |
| stearoyl-CoA                   | 8.6 ± 1.3 | 839.4 ± 49.9           |
| palmitoleoyl-CoA               | 6.2 ± 0.9 | 838.6 ± 31.6           |
| palmitoyl-CoA                  | 6.4 ± 1.1 | 767.8 ± 34.0           |
| octodecyl-CoA                  | 10.5 ± 1.4| 642.9 ± 25.0           |
| 1,2-dioleoyl-sn-glycerol       | 597.1 ± 94.5| 3310 ± 279.1 (h=1.50)  |
| 1,2-dioleoyl-sn-glycerol (tetramer) | 497.5 ± 57.6 | 3628 ± 187.4 (h=1.05)  |
| WT (dimer)                    | 14.6 ± 1.3| 956.6 ± 36.1           |
| WT (tetramer)                 | 16.6 ± 2.2| 1080.8 ± 45.3          |
| WT (membrane)                 | 15.9 ± 1.3| 1643.4 ± 36.4          |
| WT (membrane)                 | 15.9 ± 1.3| 1643.4 ± 36.4          |
| ΔN65                          | 13.9 ± 2.6| 563.9 ± 32.5           |
| ΔN70                          | 24.4 ± 1.5| 540.7 ± 25.5           |
| ΔN75                          | 28.2 ± 3.1| 497.8 ± 21.9           |
| ΔN80                          | 28.6 ± 4.2| 276.1 ± 16.4           |
| ΔN84                          | -         | -                      |

$K_m$ and $V_{max}$ values were obtained from fitting the initial rate versus concentration plots shown in Fig. 1, Extended Data Fig. 2 with the equations that are defined in Methods. The errors are 95% confidence intervals. For each concentration of substrate, the rate was measured three times independently.
Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- Confirmed
- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
- Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted. Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

- Data collection: Serial EM 3.7 is used to collect cryo-EM data.
- Data analysis: MotionCor2 1.1.0; GCTF 1.06; RELION 2.1; RELION-3.0-beta; Chimera 1.13; Coot 0.8.6.1; Phenix 1.13; Pymol 1.8.6.0; Prism 8.2.1; CryoSPARC 2.10.1.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

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Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

PDB coordinates has been deposited into the PDB database (6VP0). EM map has been deposited into the EMDB database (EMD-21302). All data will be available upon publication.
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☑ Life sciences       ☐ Behavioural & social sciences       ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | Sample sizes were chosen to give s.e.m. values of less than 10% of peak values based on prior experimental experience. |
|-------------|---------------------------------------------------------------------------------------------------------------|
| Data exclusions | No data were excluded from the analyses. |
| Replication | Each data point in enzymatic assay was measured in at least 3 independent experiments. |
| Randomization | No randomization is needed for enzymatic assay. |
| Blinding | All the mutants were tested and analyzed blindly to avoid bias. |

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|---------------------------------|---------|
| n/a                             | n/a     |
| ☑ Antibodies                    | ☐ ChiP-seq |
| ☐ Eukaryotic cell lines         | ☐ Flow cytometry |
| ☐ Palaeontology                 | ☐ MRI-based neuroimaging |
| ☐ Animals and other organisms   |         |
| ☐ Human research participants   |         |
| ☐ Clinical data                 |         |

Eukaryotic cell lines

Policy information about cell lines

| Cell line source(s) | Involved in the study |
|---------------------|-----------------------|
|                     | High Five Cells (invitrogen) |

Authentication

Authentication No further authentication was performed for commercially available cell lines.

Mycoplasma contamination

The cell lines were not tested for mycoplasma contamination.

Commonly misidentified lines (See ICLAC register)

No commonly misidentified lines were used.