X-ray structure of a mammalian stearoyl-CoA desaturase

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Stearoyl-CoA desaturase (SCD) is conserved in all eukaryotes and introduces the first double bond into saturated fatty acyl-CoAs1–4. Because the monounsaturated products of SCD are key precursors of membrane phospholipids, cholesterol esters and triglycerides, SCD is pivotal in fatty acid metabolism. Humans have two SCD homologues (SCD1 and SCD5), while mice have four (SCD1–SCD4). SCD1-deficient mice do not become obese or diabetic when fed a high-fat diet because of improved lipid metabolic profiles and insulin sensitivity5–6. Thus, SCD1 is a pharmacological target in the treatment of obesity, diabetes and other metabolic diseases. SCD1 is an integral membrane protein located in the endoplasmic reticulum, and catalyses the formation of a cis-double bond between the ninth and tenth carbons of stearoyl- or palmitoyl-CoA7–9. The reaction requires molecular oxygen, which is activated by a di-iron centre, and cytochrome b₅, which regenerates the di-iron centre10. To understand better the structural basis of these characteristics of SCD function, here we crystallize and solve the structure of mouse SCD1 bound to stearoyl-CoA at 2.6 Å resolution. The structure shows a novel fold comprising four transmembrane helices capped by a cytosolic domain, and a plausible pathway for lateral substrate access and product egress. The acyl chain of the bound stearoyl-CoA is enclosed in a tunnel buried in the cytosolic domain, and the geometry of the tunnel and the conformation of the bound acyl chain provide a structural basis for the regioselectivity and stereospecificity of the desaturation reaction. The dimetal active site is coordinated by a unique spatial arrangement of nine conserved histidine residues that implies a potentially novel mechanism for oxygen activation. The structure also illustrates a possible route for electron transfer from cytochrome b₅ to the di-iron centre.

A Δ2–23 amino-terminal truncation of mouse SCD1 was crystallized in lipidic cubic phase8 and the structure was solved by single-wavelength anomalous dispersion (Extended Data Table 1). SCD1 has four transmembrane helices (TM1–TM4) arranged in a cone-like shape with TM4 sandwiched between TM1 and TM2 (Fig. 1a). Residues in the membrane-spanning region are largely hydrophobic, with the notable exception of a conserved arginine (Arg249; Extended Data with the notable exception of a conserved arginine (Arg249; Extended Data with the notable exception of a conserved arginine (Arg249; Extended Data with the notable exception of a conserved arginine (Arg249; Extended Data)}

Residues that probably have a role in determining substrate length, the double bond is exclusively placed between C₉ and C₁₀, as agreed well with the observed preference of the enzyme for 18:0 (ref. 9). Tyr104 is highly conserved in animal SCD1. However, an atypical acyl-CoA desaturase (ChDes1) from the marine copepod Calanus hyperboreus has a threonine at the position corresponding to Tyr104 in mouse SCD1 (ref. 14). ChDes1 preferentially acts on very long-chain fatty acyl-CoAs (22:0–26:0), but when this threonine was mutated to tyrosine, desaturation of 26:0 was lost while desaturation of 18:0 was retained. Another conserved residue, Ala108, is located one helical turn above Tyr104 facing the substrate tunnel (Fig. 2c). We note that the narrow and kinked tunnel precisely positions the acyl chain for Δ9-regiospecific desaturation, an idea that was envisioned by Bloch over four decades ago9.
observations suggest that the tunnel-facing residues 104 and 108 on TM2 are critical determinants of the substrate chain length.

To explore the relationship between the structure of the substrate tunnel in mouse SCD1 and acyl chain selectivity further, we transformed yeast monounsaturated fatty acid auxotroph L8–14C with either mouse SCD1 or SCD3, which allowed growth in media lacking unsaturated fatty acids. Although SCD1 and SCD3 share 89% primary sequence identity, they yield remarkably different total fatty acid profiles in the yeast host cells, probably reflecting differences in their preferences for reaction with 16:0 and 18:0 (Fig. 2e and ref. 16). In

Figure 2 | Architecture of the acyl-CoA binding site. a, Two views of the SCD1 structure with the C1 domain coloured teal and the C2 domain coloured magenta. The bound stearoyl-CoA is shown as yellow, red and blue sticks, and the two zinc ions as grey spheres. b, A close-up view of the CoA binding site shown as a surface representation (left), or with residues interacting with the CoA moiety shown as sticks (right). c, A close-up view of the substrate tunnel housing the acyl chain shown as a surface cross-section (top), or with residues forming the kink (Thr257, Gln143 and Trp149), hydrogen bonding to the acyl oxygen (Trp258), and capping the end of the substrate tunnel (Tyr104 and Ala108) shown as sticks (bottom). d, The locations of SCD3 mutations studied by yeast complementation experiments are mapped onto the SCD1 structure as green spheres. Residue positions are labelled according to the SCD3 sequence. e, Monounsaturated fatty acids in the total lipid of yeast L8–14C transformed with mouse SCD1, SCD3 or mutated SCD3 enzyme. SCD1 produces a mixture of 16:1 and 18:1 fatty acids, while SCD3 produces 16:1 nearly exclusively. The combination of Ile112Ala and Glu113Leu mutations converts SCD3 into an enzyme that yields a proportion of monounsaturated fatty acids indistinguishable from SCD1. The corresponding residues in SCD1, Ala108 and Leu109, lie at the end of the substrate binding tunnel. Error bars are s.d. of 3 technical replicates. f, Expression levels of the SCD3 mutants were similar as detected by western blotting. Uncropped gel is shown in Extended Data Fig. 6.
SCD1, Ala108, Leu109, Ala288 and Val289 line the distal end of the substrate binding channel, Ala115 is near the position of double bond formation, and Gln277 and Ser278 are on the cytoplasmic surface opposite to the CoA binding site. The corresponding residues in SCD3 are Ile112, Glu113, Ser292 and Met293, Val119, and Asp281 and Pro282, respectively (Fig. 2d). The stacked mutations Ile112Ala/ Glu113Leu were able to convert SCD3 from exclusively a 16δ desaturase into a predominantly 18δ desaturase (Fig. 2e, f and Extended Data Fig. 6). The stacked mutations Val119Ala/Asp281Gln/ Pro282Ser, which are located away from the end of the substrate tunnel, caused no change in the reaction specificity.

In addition to the bound stearoyl-CoA molecule, SCD1 also contains two metal ions. The metal ions in the current structure were identified as zinc by X-ray fluorescence, and by diffraction data collected at a wavelength near the zinc absorption edge that yielded two prominent anomalous difference peaks in each protein (Extended Data Fig. 5b–e). Incorporation of zinc instead of iron into the protein was probably an artefact of protein overexpression, and zinc remained the predominant metal species even when the growth media and purification solutions were supplemented with iron.

The dimetal cluster sits at the kink in the substrate tunnel adjacent to C9 and C10 on the substrate, where the double bond is introduced. Zinc 1 (M1) is positioned 5.2 Å from C9, while zinc 2 (M2) is 4.7 Å from C10 (Fig. 3a). M1 and M2 are coordinated by four and five histidine residues, respectively, provided by the helices TM2, TM4, H2 and H8 (Fig. 3b and Extended Data Fig. 7a). The coordination of both zinc ions is consistent with an octahedral geometry with one missing ligand. The nine histidines are highly conserved (Extended Data Fig. 6), and eight of them belong to three histidine-containing motifs (two HXXHH motifs and one HX 4H motif in SCD1) that are characteristic of integral membrane desaturases, alkane hydroxylases and xylene monoxygenases.1,3,7 The predominance of histidine ligands in SCD1 is consistent with the assignment of nitrogen-rich ligation of the di-iron centre in alkane hydroxylase from Mössbauer isomer shifts.8,9 Mutation of any of these eight histidines into an alanine in rat SCD1 led to a nonfunctional enzyme.17 The ninth histidine (His265) is conserved in other SCDs but had not been previously identified. The water molecule coordinating M1 is hydrogen bonded to an asparagine residue, Asn261 on TM4. Interestingly, this asparagine and His265 belong to a NXXH motif that is symmetrically equivalent to the HX 4H motif interacting with M2 (Fig. 3c). Likewise, the two HXXHH motifs have symmetrical interactions with M1 and M2 (Fig. 3c).

One notable aspect of the structure is that the two metal ions are separated by 6.4 Å (Fig. 3a). This is longer than in any previously solved structures of soluble di-iron enzymes18–20, including the soluble plant acyl-ACP desaturases that catalyse the same reaction as SCD1. In these soluble enzymes, the two iron ions are bridged by a glutamate residue with bidentate coordination (Extended Data Fig. 7b) that constrains the inter-iron distance to roughly 3–4 Å (refs 19–21), and permits formation of reaction intermediates such as cis-μ-1,2 peroxo22,23 and dipheryl24. In SCD1, there is no coordinate coordination between the metal ions. This is probably not an artefact caused by Zn2+, as the closest glutamate or aspartate residues are over 6 Å away from the metal centres and provide hydrogen-bonding interactions to the metal-bound His residues (Extended Data Fig. 7a). Since Zn2+ has an ionic radius of 0.88 Å, it may have served as a reasonable substitute for Fe2+ (ionic radius 0.92 Å) in terms of size and charge during heterologous expression. Notably, the bound Zn2+15, which often have tetrahedral coordination, have octahedral coordination in the SCD1 structure as typical for iron ions. Given the similar B-values for residues around the observed metal sites, the presence of stearoyl-CoA bound in an appropriately kinked configuration relative to the metals, and the absence of reasonably positioned carboxylate residues that might serve as bridging ligands, we propose that the unfortunate presence of Zn2+ has not significantly altered the structure and that the SCD1 structure indicates a new avenue for activation of O2 in biological oxidation reactions.

Two known aspects of the desaturase reaction are compatible with this active site. Removal of the pro-R hydrogen atom from C9 proceeds with kD/kE of ~6–7 (refs 9, 25), indicating that this step is rate-limiting. The distance from the pro-R C9 hydrogen atom to the water bound to M1 is 3.5 Å, possibly corresponding to the direction through which the desaturation reaction will initiate. Furthermore, no oxygen atom transfer to carbon is anticipated during the desaturation reaction26,27. The enforcement of a long distance between the acyl chain and metals would be consistent with promotion of an electron transfer mechanism in which O atoms are retained on an oxidizing metal centre as electrons and protons are extracted from the bound and sterically configured acyl chain.

Release of a desaturated acyl chain from the active site merits additional consideration. Given the kink and the narrow aperture of the substrate tunnel, it seems unlikely that substrate entrance or product release can occur by simple linear diffusion in and out of the tunnel. However, a break in the hydrogen bond between Gln143 and Thr257 below the kink in the substrate tunnel would create a fenestration into the hydrophobic core of the membrane (Fig. 2c), allowing lateral transfer of substrates and products into and out of the well-formed substrate tunnel. A separation between TM4 and the loop between helices H1 and H2 could break this bond (Extended Data Fig. 8).

In SCD1, the electrons needed for the desaturase reaction are obtained from cytochrome b5 (cytb5), which in turn obtains electrons from NAD(P)H via cytochrome b5 reductase. Although it is known that cytb5 consists of an N-terminal haem-binding domain and a C-terminal membrane anchor domain,7 and must be membrane-anchored to

Figure 3 | The dimetal centre. a, b. Two views of the dimetal centre and coordinating residues, marked with distances between the zinc ions and C9 and C10 on the substrate (a), and coordination distances (b). The zinc ions and an ordered water molecule are shown as grey and red spheres, respectively. See Extended Data Fig. 7 for a stereo view. c. Schematic showing the locations of the coordinating His and Asn residues in four conserved motifs on TM2, TM4, H2 and H8.
function in the desaturation reaction, the cytb5 binding site on SCD is unknown. Examination of the mouse SCD1 structure shows that the dimetal centre could be accessible from the cytoplasm via a groove formed between the soluble domains C1 and C2. In the crystal structure, the N terminus of the protein lies along this groove (Extended Data Fig. 9); however, it forms few interactions with the cytoplasmic domain and potentially could be displaced by cytb5. Electrostatic calculations on the two proteins demonstrate that the predominantly positive surface of SCD1 is well complemented by the mostly negative surface of cytb5 (Fig. 4a). Placement of the cytb5 functional domain along this groove would place the electron donor groups within 14 Å of the dimetal centre, an acceptable distance for electron transfer between biological redox centres (Fig. 4b). His157 (H2) and His298 (H8) sit directly above the two metal ions and are potential candidates to form an electron transfer interface. This proposed complex also places negatively charged residues on cytb5 demonstrated to be necessary for complex formation close to positively charged residues on H4 of SCD. The structure of mouse SCD1 enables us to address questions related to basic mechanisms of the desaturation reaction more precisely.

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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1. Goren, M. A. & Fox, B. G. Wheat germ cell-free translation, purification, and assembly of a functional human stearoyl-CoA desaturase complex. Protein Expr. Purif. 62, 171–178 (2008).
2. Paton, C. M. & Ntambi, J. M. Biochemical and physiological function of stearoyl-CoA desaturase. Am. J. Physiol. Endocrinol. Metab. 297, E28–E37 (2009).
METHODS

No statistical methods were used to predetermine sample size.

Desaturase expression and purification. To identify an optimal SCD candidate for crystallization, we tested 24 eukaryotic homologs for overexpression in High Five (Trichoplusia ni) insect cells using the Bac-to-Bac system (Invitrogen) to generate virus. During the process of identifying suitable homologs, we found that the InterPro fatty acid desaturase family 1 motif30, G-E-X-[FYN]-H-N-[FY]-H-X-F-P-X-D-Y, is found in more than 7,000 eukaryotic sequences (fatty acid desaturase type 1, InterPro accession IPR005804) from animals, plants and fungi. The three His residues from this motif are now known to provide ligands to both metal ions, and so provide an excellent indicator for the presence of a mouse SCD1-like protein fold. Inclusion of bacterial genes having a related three-His motif expands the database to more than 22,000 sequences with 90 distinct domain architectures where a mouse SCD1 construct was fused to domains with a redox centre such as cyt b5, plant-type and Rieske-type ferredoxins, rubredoxins and thioredoxins, as well as domains for peptidases, hydrolases, kinases, phosphatases, and many others. This diversity of domain architectures suggests a considerably greater versatility for the SCD1 fold than the originally anticipated desaturase and alkane hydroxylase activities31.

The pFastBac vector was modified to include a tobacco etch virus (TEV) protease cleavage site before the C-terminal polyhistidine tag. The majority of the 24 homologues expressed, but the mouse SCD1 clone (GI:13398635, Life Technologies Open Biosystems) gave the highest yield and stability. Since full-length mouse SCD1 was prone to aggregation and precipitation, we designed five more mouse SCD1 constructs of various N-terminal truncations based on secondary structure prediction and sequence conservation. The construct containing residues 24–355 was stable and yielded diffracting crystals.

Cells were infected with baculovirus at a density of $3 \times 10^5$ cells ml$^{-1}$ and were grown at 27°C for 48–56 h before being centrifuged at 2,000g for 20 min. Cell membranes were isolated from cell pellets following a published protocol31. In brief, cell pellet from 1 l of culture was lysed in 50 ml hypotonic buffer containing 0.1% (w/v) Nonidet P40, 5 mM MgCl$_2$, 0.25 M sucrose and 1 mM PMSF for 20 min at 4°C. After centrifugation, the supernatant was washed with 50 ml high-osmotic buffer containing 25 mM HEPES, pH 7.5 and 1 M NaCl. Purified membranes were re-suspended in a low-osmotic buffer containing 25 mM HEPES, pH 7.5 and 1 M NaCl. Purified membranes were thawed and dounced in (10 ml per gram membrane) 20 mM HEPES, pH 7.5, 150 mM NaCl and 2 mM Mg-mercaptoethanol, and solubilized with 2% (w/v) n-decyl-β-D-maltoside (Anatrace) at 4°C for 2 h. After centrifugation (55,000g, 45 min, 4°C), the desaturase was purified from the supernatant using a cobalt-based affinity resin (Talon, Clontec) and the His-tag was cleaved by TEV protease (leaving an extra ENLYFQ peptide at the C terminus).

Purified desaturase was collected and concentrated to 5 mg ml$^{-1}$ (Amicon 50 kDa cutoff, Millipore) and loaded onto a size-exclusion column (Superdex 200 10/300 GL, GE Health Sciences) equilibrated with 25 mM HEPES, pH 7.5, 150 mM NaCl, 0.18% (v/v) n-decyl-β-D-maltoside and 5 mM Mg-mercaptoethanol. The peak fractions containing desaturase were pooled and immediately used for crystallization.

Lipidic cubic phase crystallization. Crystallization trials with detergent-solubilized protein and the bicelle method32 failed to yield crystals, whereas the in meso method33 succeeded. For lipidic cubic phase crystallization, the purified desaturase was concentrated to 50 mg ml$^{-1}$ and two volumes of protein solution and three volumes of molten monolein (Sigma) were mixed with a coupled syringe device. Crystallization trials were performed with 96-well glass sandwich plates (Molecular Dimensions) and a Gryphon crystallization robot using 50 nL protein-lipid mixture overlaid with 800 nL precipitant solution in each well. The initial crystal hits were systematically optimized by screening against salt and PEG concentrations, pH values, and different lipids. The best crystals grew to a final size of $50 \times 50 \times 20$ μm within 5 days in 100 mM MES buffer, pH 6.7–7.1, containing 33–37% (v/v) PEG 400, 200 mM NaCl, 4% (v/v) ethylene glycol. Crystals were collected directly from the protein-lipid mixture using 50 μm MiTeGen MicroMounts and immediately flash frozen in liquid nitrogen.

X-ray data collection and processing. X-ray diffraction data were collected at beamline 24ID-C (NE-CAT) at the Advanced Photon Source at Argonne National Laboratory. A data set collected from a single crystal at a wavelength of 1.254 Å with a resolution of 2.6 Å was used for phasing (Extended Data Table 1). The phasing data were processed with XDS34 and scaled with AIMLESS35. The presence of zinc ions was confirmed with fluorescence excitation spectra using an Amptek SDD fluorescence detector, and analysis of the anomalous signal. A second data set with a resolution of 2.6 Å was collected on a second crystal at a wavelength of 0.9795 Å for use in molecular replacement. The higher resolution data were indexed, integrated, and scaled using HKL2000 (ref. 36). The crystals belonged to the space group P2$_1$2$_1$2$_1$, with unit cell dimensions of $a = 77.06$ Å and $c = 141.70$ Å.

Structure determination and refinement. SHELDX/SHELEX37 found four anomalous scatterers per asymmetric unit and the resulting density modified map was used to build an all-atomic partial model of one protein molecule. The phased translation function from MOLREP38 was then used to locate a second protein molecule. This partial model and the heavy atom sites were input into PHASER39, which was run in MR-SAD phasing mode. Further density modification was carried out with RESOLVE40. The structure model was then further built through successive rounds with COOT41 and refinement with phenix.refine42. This model was then used as the input model for molecular replacement using PHASER with the 2.6 Å diffraction data. The model was completed through successive rounds of model building with COOT and refinement with phenix.refine. The crystallographic map was easily interpretable. In the final stages of refinement, TLS groups were determined using TLSMD43 and protein geometry was validated with MolProbity44. Figures were produced with PyMOL (Schrödinger LLC.). Electrostatic surfaces were generated with Chimera45. For Fig. 4, coordinates were used from rat cytochrome $b_5$ (PDB accession 1BXX, ref. 46), which is known to cytochrome $b_5$, over the depicted residue range.

Functional studies of mouse SCDs. For expression using the galactose-inducible yeast expression vector pYES-DEST52 (Invitrogen), mouse SCD1 and SCD3 genes were cloned with an 81 nucleotide leader sequence encoding the N terminus of Saccharomyces cerevisiae desaturase (ole1) appendaged to the S end. Mutations of mouse SCD3 were made using QuickChange (Stratagene). Expression plasmids were transformed into L-8-14C (ref. 48), an ole1 yeast strain, and cultured on agar plates containing 0.5 mM each of oleic acid and palmitic acid49. SCD expression was induced on agar containing 2% galactose. SCD expression was detected by western blotting with a His-Tag monoclonal antibody (Novagen, 70976-3), goat anti-mouse IgG AP conjugate secondary antibody (Novagen, 69266-3), and alkaline phosphatase reagent (Novagen). Total fatty acids were determined as methyl esters using gas chromatography and mass spectroscopy49.
Extended Data Figure 1 | Sequence alignment of mouse SCD1 with other integral membrane desaturases. The N terminus of mouse SCD1 is not shown. For all the other sequences, only the region aligning to mouse SCD1 is included. Secondary structure elements from the mouse SCD1 crystal structure are labelled. Residues discussed in the text are highlighted in red (histidines in the primary coordination sphere of the dimetal unit), purple (carboxylates in the secondary coordination sphere of the dimetal unit), blue (acyl-chain binding site), yellow (CoA-binding site), green (residues that may determine the length of bound acyl chains), black (mutations that change the substrate specificity in mouse SCD3) and grey (Arg249 in the transmembrane region). The accession numbers for sequences included in the alignment are: mouse SCD1 (GI: 31543675), mouse SCD3 (GI: 13277368), human SCD1 (GI: 53759151), zebrafish SCD1 (GI: 28394115), D. melanogaster desat2 (GI: 24646295), C. hyperboreus ChDes9-1 (GI: 58983495), C. elegans FAT5 (GI: 544604099), delta-9 desaturase from Synechocystis sp. PCC 6803 (GI: 339274799), delta-9 desaturase from A. thaliana (GI: 18402641), and yeast OLE1 (GI: 1322552).
Extended Data Figure 2 | Structural role of Arg249. The conserved arginine residue Arg249, located on TM4 within the transmembrane region of the protein, forms a hydrogen bond with the carbonyl oxygen of Cys222 on TM3. This interaction may help stabilize the kink in TM3 caused by Pro226 on the following turn.
Extended Data Figure 3 | Structure of the SCD1 cytoplasmic domain. Four views of the cytoplasmic domain. The proposed amphipathic helices are coloured blue, while the other helices forming the cytoplasmic domain are green.
Extended Data Figure 4 | The mouse SCD1 crystal lattice. Cross-sections of the crystal lattice for the $P2_12_12_1$ mouse SCD1 lipidic cubic phase crystals, viewed from two perpendicular directions. One asymmetric unit is coloured blue. Within an individual asymmetric unit, interactions between the two chains are mediated by residues from a C-terminal cloning artefact. All interactions with chains in neighbouring asymmetric units involve antiparallel orientations of the interacting monomers and have small interface areas.
Extended Data Figure 5 | Electron density maps for acyl-CoA and the dimetal centre. a, Stearoyl-CoA bound to SCD1 is superposed with the weighted $2F_o - F_c$ electron density contoured at 1.5 $\sigma$ (left) or $F_o - F_c$ electron density calculated with the substrate molecule omitted and contoured at 2.3 $\sigma$ (right). b, Stereoview of the dimetal centre and coordinating histidines, shown with the weighted $2F_o - F_c$ density contoured at 2 $\sigma$. c-e, The dimetal centre superposed with the anomalous difference map, contoured at 5 $\sigma$ (c), the $F_o - F_c$ density calculated with the zinc atoms omitted, contoured at 3 $\sigma$ (d), and the $F_o - F_c$ density calculated with the ordered water molecule between M1 and Asn261 omitted, contoured at 3 $\sigma$ (e).
Extended Data Figure 6 | Western blot analysis of SCD expression. 

**a**, **b**, Analysis of two separate yeast expression trials after introduction of mutations to mouse SCD3 to impart catalytic specificity of mouse SCD1. Contents of lanes are as indicated in the gel. The position of SCD is indicated by a black star. Additional bands are other proteins detected by the polyclonal antibody. Dotted line in **a** shows the portion of the complete gel image included in Fig. 2f; dotted line in **b** shows the corresponding expression trials from the second experiment. **a**, Expression trial 1, with gel artefacts in lanes 2 and 3. **b**, Expression trial 2, with gel artefacts in lanes 4, 6 and 7.
Extended Data Figure 7 | Coordination in diiron-containing desaturases.

a, Stereoview of residues forming both the first and second coordination shell around the dimetal centre in mouse SCD1. 
b, Stereoview of the coordination of the dimetal centre in the stearoyl-acyl carrier protein desaturase from the castor bean (PDB accession 1AFR).
Extended Data Figure 8 | The Thr257–Gln143 hydrogen bond blocks product egress. The surface of the substrate tunnel housing the acyl chain is shown, with the structural elements AH1, H2 and TM4, and the hydrogen-bonded residues Thr257 and Gln143 highlighted in the inset. The proximity of these two residues creates the kinked shape of the substrate tunnel, and their separation would result in a larger opening capable of releasing the product into the bilayer.
Extended Data Figure 9 | The SCD1 N terminus. Two perpendicular views of mouse SCD1, from within the plane of the membrane and from the cytoplasmic side, showing the interaction between the N terminus (red ribbon) and the cytosolic domain (beige surface). The dashed yellow circles indicate the approximate location of the metal atoms.
Extended Data Table 1 | Crystallographic and structure refinement statistics

|                         | Phasing Data Set       | Refinement Data Set   |
|-------------------------|------------------------|-----------------------|
| Space group             | P2₁2₁2₁               | P2₁2₁2₁               |
| Unit Cell (Å)           | a=77.51, b=114.53, c=140.97 | a=77.06, b=113.77, c=141.70 |
| Wavelength (Å)          | 1.2541                 | 0.9795                |
| Resolution (Å)          | 38.8 - 2.8             | 47.4 - 2.6            |
| Rsym                    | 0.18 (1.17)            | 0.11 (0.53)           |
| lstd                    | 9.5 (1.3)              | 14.4 (3.1)            |
| Completeness (%)        | 99.1 (94.0)            | 98.0 (94.0)           |
| Redundancy              | 7.9 (7.5)              | 6.2 (5.0)             |
| Resolution (Å)          | 47.4 – 2.6             | 47.4 – 2.6            |
| No. reflections         | 38016                  | 38016                 |
| Rwork/Rfree             | 20.3 / 23.5            | 20.3 / 23.5           |
| No. atoms               | 5231                   | 5231                  |
| Protein                 | 5231                   | 5231                  |
| Ligand/ion              | 188                    | 188                   |
| Water                   | 86                     | 86                    |
| B-factors (Å²)          |                        |                       |
| Protein                 | 46.8                   |                       |
| Ligand/ion              | 51.5                   |                       |
| Water                   | 40.1                   |                       |
| R.m.s deviations        |                        |                       |
| Bond lengths (Å)        | 0.002                  |                       |
| Bond angles (°)         | 0.633                  |                       |
| Ramachandran Plot       |                        |                       |
| Favored (%)             | 93.8                   |                       |
| Allowed (%)             | 6.2                    |                       |
| Outliers (%)            | 0.0                    |                       |