Structure of an integral membrane sterol reductase from *Methylococcus alcaliphilum*

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Sterols are essential biological molecules in the majority of life forms. Sterol reductases including Δ⁷ sterol reductase (C14SR, also known as TM7SF2), 7-dehydrocholesterol reductase (DHCR7) and 24-dehydrocholesterol reductase (DHCR24) reduce specific carbon–carbon double bonds of the sterol moiety using a reducing cofactor during sterol biosynthesis. Lamin B receptor² (LBR), an integral inner nuclear membrane protein, also contains a functional C14SR domain. Here we report the crystal structure of a inner nuclear membrane protein, also contains a functional C14SR.

Sterols, amphipathic molecules, are widespread in animals, plants, fungi and some prokaryotes and a large variety exist, including ergosterol, hopanoids, phytosterol and cholesterol. The most abundant sterol from *Methylomicrobium alcaliphilum*²⁰Z is an aerobic methanotroph, the cell membrane of *Methylomicrobium alcaliphilum*²⁰Z is an aerobic methanotroph, the cell membrane of *Methylomicrobium alcaliphilum*²⁰Z, MaSR1, which shares 38–45% sequence identity and 51–62% similarity to human C14SR, DHCR7 and the C-terminal portion of LBR (Extended Data Fig. 2). *Methylococcus alcaliphilum* 20Z is an aerobic methanotroph, the cell membrane of which contains significant levels of sterols and hopanoids⁴⁴.

Expression of MaSR1 complements the deletion of the Δ⁷ sterol reductase gene (*ERG24*) in yeast, indicating that MaSR1 is a bona fide sterol reductase (Extended Data Fig. 3, lanes 1–3). To test whether MaSR1 can function in human cholesterol biosynthesis, we performed the sterol reductase activity assay of MaSR1 after expression in human HEK293 cells and employed 5α-cholesta-8,14-dien-3β-ol (C27Δ⁸,1⁴), a human cholesterol biosynthetic intermediate analogue¹⁵–¹⁷ of 4,4-dimethylcholesta-8,14-dien-3β-ol (C29Δ⁸,1⁴, Extended Data Fig. 1), as the substrate (Fig. 1a). This assay has been used for initial identification¹⁵ and further investigation¹⁶,¹⁷ of mammalian sterol reductases. The catalytic efficiency of MaSR1 is about 75% of that of human C14SR (Fig. 1b, c). We conclude that MaSR1 can function like human C14SR and specifically reduce the double bond of the approximate cholesterol biosynthetic intermediate.

We crystallized MaSR1 in space group P1 with NADPH. The diffraction of the crystal is anisotropic (Methods and Extended Data Fig. 4). The structure was determined by selenium-based single-wavelength anomalous dispersion and refined at 2.74 Å resolution (Extended Data Tables 1 and 2). Introduction of additional selenium anomalous scatterers by selective mutation and preparation of platinum derivatives confirmed that the atomic model was correct (Extended Data Fig. 5 and Extended Data Table 3).

Two MaSR1 molecules (rotated by 180°) pack into a crystallographic dimer that forms the asymmetric unit. The dimensions of the MaSR1 monomer are 50 × 45 × 58 Å. The enzyme contains ten transmembrane helices (TM1–10). Its catalytic domain comprises the carboxy-terminal half (containing TM6–10) and envelopes two interconnected pockets, one of which faces the cytoplasm and houses NADPH, while the other one is accessible from the lipid bilayer. Comparison with a soluble steroid 5β-reductase structure⁶ suggests that the reducing end of NADPH meets the sterol substrate at the junction of the two pockets. A sterol reductase activity assay proves that MaSR1 can reduce the double bond of a cholesterol biosynthetic intermediate, demonstrating functional conservation to human C14SR.

Therefore, our structure as a prototype of integral membrane sterol reductases provides molecular insight into mutations in DHCR7 and LBR for inborn human diseases.

Stoichiometrically, the multifunctional lamin B receptor² (LBR), located in the inner nuclear envelope membrane, also contains a domain in its C-terminal portion that is highly homologous to human sterol reductase (Extended Data Fig. 2). Indeed, complementary DNA of human LBR complements reductase gene (*ERG24*) deletion in yeast, supporting the idea that LBR can substitute for sterol reductase activity. Mutations in LBR and DHCR7 lead to various human genetic diseases (Pelger–Hüet anomaly¹⁰ (PHA) and Greenberg skeletal dysplasia¹¹ (also known as hydrops-ectopic calcification-moth-eaten skeletal dysplasia, HEM) related to LBR; and Smith–Lemli–Opitz syndrome²²,²³ (SLOS) related to DHCR7). However, structural knowledge of these important membrane-embedded enzymes is lacking, and therefore a mechanistic understanding cannot be developed.

To gain more insight into sterol reductases, we set out to determine the crystal structure of one of its family members. In screening for crystal-forming representatives of integral membrane sterol reductases, we found a homologue from the methanotrophic bacterium *Methylococcus alcaliphilum* 20Z, MaSR1, which shares 38–45% sequence identity and 51–62% similarity to human C14SR, DHCR7 and the C-terminal portion of LBR (Extended Data Fig. 2). *Methylococcus alcaliphilum* 20Z is an aerobic methanotroph, the cell membrane of which contains significant levels of sterols and hopanoids⁴⁴.

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with the substrate. In our structure, the absence of a sterol substrate probably fails to coordinate the nicotinamide ring and hence causes it to be disordered. By contrast, the other half of the NADPH molecule is well defined in the electron density and stabilized by a hydrogen bond facing cavity of MaSR1 is the likely candidate for the sterol binding pocket (Fig. 3c). Notably, the sterol binding pockets of both enzymes contain a ‘signature’ motif forming triangular hydrogen bonds that coordinate the β3 hydroxyl of either sterol or steroid; for MaSR1, this signature motif includes Tyr 241 bonded to Asp 363 (Fig. 3c). The distance between Tyr 58 and Glu 120 (4.1 Å) in steroid 5β-reductase, Tyr 58 bonded to Glu 120 (4.1 Å). The reduced products (peak 4) were detected in MaSR1, Flag–MaSR1 and C14SR but little in untransfected cells. d, The catalytic efficiency of Flag–MaSR1 mutants. All mutants expressed by anti-Flag western blot (WB) detection. The plotted values in the grey bars are the average of two different experiments. The thin black lines show the spread between the two individual measurements c, GC–MS chromatograms of Δ14-sterol reductase assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay. Peak 1, mass to charge ratio (m/z) 372, 5α-cholestan-8,14-sterol reductase activity assay.
and Tyr 387 residues enforce the interaction of TM7 and TM10 (Extended Data Fig. 6d). Notably, in each monomer of the crystallographic dimer there is the extra electron density of an unidentified molecule in front of the cavity (Figs 2b and 3c). Although the molecular identity of this density could not be unambiguously determined (maybe an endogenous molecule from *Escherichia coli* or the detergent used for purification), it may represent a substrate for the putative sterol binding pocket. We modelled that two binding pockets bring the reducing end of NADPH into close proximity to the sterol (steroid) carbon–carbon double bond to be reduced (Fig. 3d), similar to the aldo-keto reductase (AKR) family of enzymes involved in human steroidogenesis[20] (for example, AKR1C3 and AKR1C2).

Owing to the high sequence homology with human LBR and human DHCR7, we generated structural models based on MaSR1 to highlight disease-related mutations (Fig. 4). The PHA/HEM-related mutations of LBR and the SLOS-related mutations of DHCR7 could be almost entirely mapped to the sterol reductase catalytic domain affecting the cofactor binding or sterol entry/binding sites. The similarities in pathogenesis between PHA/HEM and SLOS could therefore arise from a defect in sterol reduction.

Intriguingly, substrate recognition for sterol reductases is not very specific. MaSR1 could reduce the double bond of both C27Δ8,14 (Fig. 1b) and of the yeast sterol substrate ergosta-8,14-dien-ol (Extended Data Fig. 3). This is consistent with previous observations for LBR: it can reduce C27Δ8,14 [ref. 16]; complement C14SR function in C14SR−/− (also known as Tm7sf2−/−) mice[21]; and also reduce different yeast sterol substrates[9]. Finally, our structure also provides insight into the function of LBR. A DALI search for structural homologues of MaSR1 shows}

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**Figure 2** | The molecular architecture of MaSR1. **a**, Overall structure of MaSR1 viewed parallel to the membrane. The ten transmembrane segments are divided into an N-terminal half (TM1–5 and α1, grey) and a C-terminal half (TM6–10 and α2, yellow). The two black lines show the approximate location of the lipid bilayer. **b**, MaSR1 structure cartoon with NADPH shown by stick representation. 2Fo–Fc map for an unidentified molecule (blue mesh) and simulated annealing (SA)-omit map (Fo–Fc densities) for NADPH (magenta mesh) both contoured at 2σ. **c**, The cavity is indicated by an orange circle in an electrostatic surface representation. The right and left panels represent two perpendicular views.

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**Figure 3** | NADPH, putative sterol binding pockets and homology modelling with steroid 5β-reductase. **a**, Close-up view of the NADPH binding pocket. NADPH is shown in stick representation with the phosphates in red. The interactions between NADPH and MaSR1 are indicated by dashed lines. **b**, Close-up view of the active pockets of steroid 5β-reductase (PDB accession number 3COT). Tyr 58 and Glu 120 clamp the 3 carbonyl oxygen of progesterone. **c**, The putative sterol binding site is accessible from the lipid bilayer. An unidentified ligand density is shown with 2Fo–Fc map (blue mesh) at 2.5σ. **d**, Modelling of MaSR1 putative sterol binding pocket. On the basis of the active sites of steroid 5β-reductase, the missing nicotinamide-ribose moiety (purple) of NADPH was docked into the NADPH pocket and C29Δ8,14 (light green) modelled into the pocket of the MaSR1 structure.
no similar entry for the entire MaSR1 structure. However, it identified the membrane-embedded isoprenylcysteine carboxyl methyltransferase (ICMT, PDB accession number 4A2N) as the closest entry for the TM6–10 segments of MaSR1 (Extended Data Fig. 7). The function of ICMT, which recognizes and then carboxymethylates the farnesylated cysteine of its substrate, points towards a similar role of the C14SR domain of LBR, which may recognize the farnesylated cysteine of either prelamin A or lamin B as the ligand11.

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. Porter, F. D. & Herman, G. E. Malformation syndromes caused by disorders of cholesterol synthesis. J. Lipid Res. 52, 6–34 (2011).
2. Worman, H. J., Yuan, J., Blobel, G. & Georgatos, S. D. A lamin B receptor in the nuclear envelope. Proc. Natl Acad. Sci. USA 85, 8531–8534 (1988).
3. Di Costanzo, L., Drury, J. E., Penning, T. M. & Christianson, D. W. Crystal structure of 4-3-ketosteroid 5,7-20Z-reductase. Biochemistry 40, 13413–13423 (1984).
4. Roberti, R. et al. Cloning and expression of sterol Δ14-reductase from bovine liver. Eur. J. Biochem. 269, 283–290 (2002).
5. Bennati, A. M. et al. Sterol dependent regulation of human TMSF2 gene expression: role of the encoded 3β-hydroxysterol Δ14-reductase in human cholesterol biosynthesis. Biochim. Biophys. Acta 1761, 677–685 (2006).
6. von Heijne, G. Control of topology and mode of assembly of a polytopic membrane protein by positively charged residues. Nature 341, 456–458 (1989).
7. Kim, H., Melen, K., Osterberg, M. & von Heijne, G. A global topology map of the Saccharomyces cerevisiae membrane proteome. Proc. Natl Acad. Sci. USA 103, 11142–11147 (2006).
8. Miller, W. L. & Auchus, R. J. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. Endocr. Rev. 32, 81–151 (2011).
9. Bennati, A. M. et al. Disruption of the gene encoding 3β-hydroxysterol Δ14-reductase (Tm7sd2) in mice does not impair cholesterol biosynthesis. FEBs J. 275, 5034–5047 (2008).
10. Yang, J. et al. Mechanism of isoprenylcysteine carboxyl methylation from the crystal structure of the integral membrane methyltransferase ICMT. Mol. Cell 44, 997–1004 (2011).
11. Ennekens, H. & Nigg, E. A. The role of isoprenylation in membrane attachment of nuclear lamins. A single point mutation prevents proteolytic cleavage of the lamin A precursor and confers membrane binding properties. J. Cell Sci. 107, 1019–1029 (1994).
**METHODS**

Protein expression and purification. We expressed a homologue of eukaryotic sterol reductases from the methanotrophic bacterium *Methylomicrobium alcaliphilum* 20Z (MaSR1, NCBI GI number: 503913803). Its cDNA was cloned into pET-21b (Novagen) with an N-terminal 6-His tag and expressed in *E. coli* C43(DE3) (Lucigen). The transformed cells were grown to an optical density of 1.0 at OD600 and induced with 0.2 mM isopropyl-β-D-thiogalactopyranoside (IPTG). Cells were disrupted using a French press with two passes at 15,000 p.s.i., in a buffer containing 25 mM Tris-Cl, pH 8.0, and 150 mM NaCl (buffer A). After low-speed centrifugation, the resulting supernatant was centrifuged at high speed to sediment a membrane fraction, which then was incubated in buffer A with 2% (v/v) N-dodecyl-β-D-maltopyranoside (DDM, Anatrace) for 1 h at 4 °C. The lysate was centrifuged again and the supernatant was loaded onto Ni²⁺-NTA affinity column (Qiagen). After washing twice, the protein was eluted with 25 mM Tris-Cl, pH 8.0, 150 mM NaCl, 300 mM imidazole, and 0.1% DDM, and concentrated by centrifugon for subsequent gel filtration (Superdex-200, GE Healthcare) in buffer A with 0.4% (v/v) N-octyl-β-D-glucopyranoside (β-NG, Anatrace). The peak fraction was collected for crystallization. All mutations were generated using two-step PCR. Selenomethionine (SeMet)-labelled protein was purified similarly with the exception that 1 mM Tris (2-carboxyethyl) phosphine (TCEP) was included during the purification process.

**Crystallization.** Before crystallization, the protein solution was incubated with 2 mM NADPH (Sigma-Aldrich). Crystals were grown at 20 °C by the hanging-drop vapour-diffusion method. The crystals appeared after 5 days in the well buffer containing 0.1 M Tris-Cl pH 7.0, 0.2 M NaHAc, 30% (v/v) pentaerythritol ethoxylate (15/4 EO/0H). DDM was added into crystallization buffer at 1% (v/v) final concentration to improve diffraction. SeMet-labelled protein was crystallized in the same buffer supplemented with 6 mM dithiothreitol (DTT). Platinum derivatives were obtained by soaking native crystals for 12 h in mother liquor plus 10 mg ml⁻¹ K₃Pt(NO₃)₂. All crystals were directly flash-frozen in a cold nitrogen stream at 100 K.

**Data collection and structure determination.** The data were collected at National Synchrotron Light Source (NSSL) beamline X29. All data sets were processed using HKL2000 (ref. 24). Owing to the anisotropic diffraction properties, the outlier reflections were rejected based on extreme-value Wilson statistics using the program XTRIAGE(25) in the PHENIX package(26). The anomalous signal in the SeMet-derivative data was further magnified with the local-scaling algorithm using the program SOLVE(27). Then, the selenium sites were determined using the program SHELXD(28). The identified sites were refined and the initial phases were generated in the program XTRIAGE25 in the PHENIX package26. The anomalous signal in the SeMet-derivative reflections were rejected based on extreme-value Wilson statistics using the program DAMMIF(19). The electron density map allowed placement of the amino acid residues in the active site cavity of the protein. The structure was refined using the program REFMAC(29). The final model of the protein includes 271 residues of catalytic subunit, 15 residues of linker, and 106 residues of SeMet labels (42%). The model was validated using the program MolProbity(30). Introduction of additional selenium anomalous scatterers by selective mutation and preparation of platinum derivatives confirmed the correctness of the atomic model.

The homology models of human LBR and human DHCR7 were generated by the program MODELLER(31) on the basis of the structure of MaSR1 in which the N-terminal regions (1–200 of LBR and 1–58 of DHCR7) were excluded because of poor convergence. Protein structure determination was performed by Bradford method, using bovine serum albumin as a standard. Proteins of the membrane fraction were separated by SDS–PAGE, blotted on PVDF and probed with mouse monoclonal anti-Flag M2 (Sigma–Aldrich) and peroxidase-conjugated goat anti-mouse (Santa Cruz). The protein was detected using Super Signal West Pico Chemiluminescent Substrate (Pierce).

**Δ¹⁴-reductase activity was assayed in the membrane fractions obtained from transfected cells (0.25 mg protein per assay) using 5α-cholesta-8,14-dien-3β-ol (C27Δ¹⁴) as a substrate(15–17). After the addition of 5α-cholestan-5β (1 μg) as an internal standard, sterols were extracted with petroleum ether, desiccated under nitrogen stream and converted to trimethylsilyl derivatives using N,O-Bis (trimethylsilyl) trifluoroacetamide (BSTFA) and pyridine (1:1, v/v). Gas chromatography–mass spectrometry (GC–MS) analysis was performed in multiple ion detection mode using a Varian GC-MS 2000 apparatus with a Varian CP-Sil8 CB low bleed capillary column. The trimethylsilylation of steroid products yields a molecular mass increase of 72 Da. Sterol retention times were: 15.31 min, 5α-cholestanol (m/z 372); 19.90 min, cholesterol (m/z 485); 20.16 min, 5α-cholesta-8,14-dien-3β-ol (C27Δ¹⁴, m/z 456); 20.34 min, 5α-cholesta-8-ene-3β-ol (C27AΔ⁸, m/z 458). Δ¹⁴-reductase catalytic efficiency is calculated as the peak area ratio C27AΔ⁸/(C27AΔ¹⁴ + C27Δ¹⁴). The C27AΔ⁸ sterol was undetectable at zero incubation time.

24. Otwinowski, Z. & Minor, W. Processing of X-ray diffraction data collected in oscillation mode. *Methods Enzymol.* 276, 307–326 (1997).

25. Zwart, P. H., Grosse-Kunstleve, R. W. & Adams, P. D. Xtriage and Fest: automatic assessment of X-ray data and substructure structure factor estimation. CCP4 Newsletter 43, 27 (2005).

26. Adams, P. D. et al. PHENIX: a comprehensive Python-based system for macromolecular structure solution. *Acta Crystallogr.* D 66, 213–221 (2010).

27. Terwilliger, T. C. & Berendzen, J. Automated MAD and MR structure solution. *Acta Crystallogr.* D 55, 849–861 (1999).

28. Schneider, T. R. & Sheldrick, G. M. Substructure solution with SHELXD. *Acta Crystallogr.* D 58, 1772–1779 (2002).

29. McCoy, A. J. et al. Phaser crystallographic software. *J. Appl. Crystallogr.* 40, 658–674 (2007).

30. Cowtan, K. D.: An automated procedure for phase improvement by density modification. *Joint CCP4 and ESF-EACBM Newsletter on Protein Crystallogr.* 31, 34–38 (1994).

31. Emsley, P. & Cowtan, K. Coot: model-building tools for molecular graphics. *Acta Crystallogr.* D 60, 2126–2132 (2004).

32. Chen, V. B. et al. MolProbity: all-atom structure validation for macromolecular crystallography. *Acta Crystallogr.* D 66, 12–21 (2010).

33. Fiser, A. & Sali, A. Modeller: generation and refinement of homology-based protein structure models. *Methods Enzymol.* 374, 461–491 (2003).

34. Thompson, J. D., Higgins, D. G. & Gibson, T. J. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22, 4673–4680 (1994).

35. Clayton, P. et al. Mutations causing Greenberg dysplasia but not Pelger anomaly uncouple enzymatic from structural functions of a nuclear membrane protein. *Nucleus* 1, 354–366 (2010).

36. Holm, L. & Rosenstrom, P. Dalii server: conservation mapping in 3D. *Nucleic Acids Res.* 38, W545–W549 (2010).
Extended Data Figure 1 | Cholesterol biosynthesis pathway* and sterol reductase family. Acetyl-CoA is the precursor for cholesterol biosynthesis. After several reactions, the intermediate lanosterol is synthesized. Conversion of lanosterol to cholesterol (Bloch pathway) involves many reactions, some of which are catalysed by C14SR, LBR and DHCR7 (MaSR1 homologues, in red). C14SR, LBR and DHCR7 are homologues of NADPH-dependent reductases that catalyse the reduction of the sterol double bonds indicated in the green circles.
Extended Data Figure 2 | Sequence alignment of MaSR1 with human C14SR, DHCR7 and C-terminal domain of LBR. Secondary structural elements of MaSR1 are indicated above the sequences. Disordered regions in the MaSR1 structure are shown by a dashed line. Invariant amino acids are highlighted in blue (invariant in 3 of 4 proteins) and purple (invariant in all proteins). Putative cholesterol hydroxyl group binding sites are highlighted in red, NADPH binding sites are highlighted in cyan. Human disease mutations are also highlighted by different symbols. Sequence alignment was carried out using ClustalW**.
Extended Data Figure 3 | Yeast complementation assay. MaSR1 can rescue the growth of a Saccharomyces cerevisiae Δ14-sterol reductase Erg24 (yeast MaSR1 homologue) deletion strain (ΔErg24). ΔErg24 yeast expressing wild-type MaSR1, ScErg24 and mutated MaSR1 from a URA3 shuttle vector can grow under URA selection (upper panel). Growth of yeast expressing MaSR1, ScErg24 and various mutated MaSR1 versions in the presence of sub-inhibitory concentrations of cycloheximide (20 ng ml⁻¹) for 24 to 48 h (lower panel). The yeast expressing MaSR1 or ScErg24 is able to grow in the presence of cycloheximide. R395A (lane 8) corresponds to R583Q in LBR which has been reported to lead to loss of activity in yeast³⁵. Results are representative of three independent experiments.
Extended Data Figure 4 | MaSR1 crystal and X-ray diffraction image. a, Photograph of MaSR1 crystal. b, A representative X-ray diffraction image of MaSR1 crystals with various resolution rings indicated by the circles.
Extended Data Figure 5 | Anomalous difference Fourier electron density.

a, Overview of the anomalous difference Fourier map for selenium atoms in an asymmetric unit. The electron density is contoured at 4.5σ (purple mesh). Two molecules (MolA, molecule A; MolB, molecule B) were observed in each asymmetric unit. b, Examination of the atomic model in TM4 by selenium anomalous difference signals. Left panel shows wild-type SeMet anomalous difference signals; right panel shows mutated SeMet anomalous difference signals at 3σ (purple mesh). c, Examination of the atomic model in TM8 by selenium anomalous difference signals. Left panel shows wild-type SeMet anomalous difference signals, right panel shows mutated SeMet anomalous difference signals at 3σ (blue mesh). d, A view of the anomalous difference Fourier map for platinum atoms in an asymmetric unit. The electron density is contoured at 3σ (purple mesh). There are four platinum atoms binding to histidine residues in molecule A (yellow), but there are eight platinum atoms binding to six histidine and two methionine residues in molecule B (red). e, An overall view of the 2Fo – Fc electron density, contoured at 2σ, in one asymmetric unit.
Extended Data Figure 6 | NADPH binding pocket and interaction between Trp 274 and Tyr 387 of MaSR1. a, The structure of NADPH with the missing moiety in the MaSR1 structure indicated in the black circles. b, Overview of the NADPH-bound MaSR1. SA-omit map ($F_o - F_c$ densities, magenta mesh) for NADPH contoured at 2σ. The right panel is an enlargement of the left panel (same orientation as Fig. 2a), rotated by 180°. c, The rebuilt missing moiety (purple) of NADPH in MaSR1. d, The surface representation shows Trp 274 (orange) and Tyr 387 (blue) located in the back of the sterol binding pocket. $2F_o - F_c$ map for an unidentified ligand (blue mesh) contoured at 2σ.
Extended Data Figure 7 | Comparison of MaSR1 structure with ICMT structure. a, A comparison of MaSR1 (grey and yellow) and ICMT (cyan) structure with S-adenosyl-l-homocysteine (SAH) bound (PDB accession number 4A2N). DALI search shows the closest entry (Z-score of 7.5) to MaSR1 is the structure of ICMT, consisting of 5 transmembrane helices, which had 193 Cα atoms aligned to MaSR1 (TM6–10 and α2) with r.m.s.d. of 2.8 Å.

b, Comparison of NADPH and SAH binding pockets of MaSR1 (grey) and ICMT (cyan). The orientation of adenine–ribose moiety of SAH and NAPDH is similar with respect to the coordinating tyrosine residues in the cofactor pockets of these two enzymes.
Extended Data Table 1 | Data collection and refinement statistics

|                                | Native       | Se-SAD      |
|--------------------------------|--------------|-------------|
| Wavelength (Å)                 | 1.0750       | 0.9791      |
| Space group                    | P1           | P1          |
| Unit cell (Å) (a, b, c; α, β, γ) | 74.66, 74.62, 79.55; 66.00, 90.37, 86.86 | 74.81, 75.88, 80.27; 64.96, 89.95, 86.30 |
| Resolution (Å)                 | 50–2.74 (2.84–2.74) | 50–3.3 (3.42–3.3) |
| Rmerge (%)                     | 4.1 (41.5)   | 12.0 (64.6) |
| I/σ(I)                         | 21.1 (1.4)   | 20.4 (1.7)  |
| Completeness* (%)              | 74.8 (28.5)  | 83.0 (53.0) |
| Number of measured reflections | 63,092       | 136,260     |
| Number of unique reflections   | 30,595       | 19,876      |
| Redundancy                     | 2.1 (1.9)    | 6.9 (5.4)   |
| Wilson B factor (Å²)           | 76.7         | 85.1        |
| Rwork / Rfree (%)              | 23.29 / 28.37|             |
| Molecules in ASU               | 2            |             |
| Number of atoms / B-factor:    |              |             |
| All atoms                      | 6487 / 89.89 |             |
| Main chain                     | 3128 / 89.13 |             |
| Side chain                     | 3297 / 90.60 |             |
| Other entities                 | 62 / 90.56   |             |
| Ramachandran plot (%):         |              |             |
| Favored/Allowed/Disallowed     | 90.8/92.0/0  |             |
| RMS-deviation in:              |              |             |
| Bond distances (Å)             | 0.013        |             |
| Bond angles (°)                | 1.646        |             |

Values in parentheses are for the highest resolution shell. Rfree was calculated with 5% of the reflections selected in the thin shell. ASU, asymmetric unit.

* See Extended Data Table 2 of the native data completeness of each shell.
### Extended Data Table 2 | Native data completeness of each shell

| Resolution (Å) | Completeness (%) |
|----------------|------------------|
| 50.00 – 6.09   | 95               |
| 6.09 – 4.83    | 98               |
| 4.83 – 4.23    | 97               |
| 4.23 – 3.84    | 98               |
| 3.84 – 3.56    | 97               |
| 3.56 – 3.35    | 89               |
| 3.35 – 3.19    | 73               |
| 3.19 – 3.05    | 60               |
| 3.05 – 2.93    | 49               |
| 2.93 – 2.83    | 37               |
| 2.83 – 2.74    | 28               |
| Overall        | 74.8             |
Extended Data Table 3 | Data collection statistics for the MaSR1 mutants I151M, L304M and Pt-derivatives

| Data Set                  | I151M       | L304M       | Pt-derivatives |
|---------------------------|-------------|-------------|---------------|
| Wavelength (Å)            | 0.9791      | 0.9791      | 1.0717        |
| Space group               | P1          | P1          | P1            |
| Unit cell (Å) (a,b,c; α,β,γ) | 74.77, 75.38, 79.75; 65.39, 89.86, 86.19 | 74.80, 76.81, 81.37; 63.69, 90.171, 86.67 | 78.63, 75.00, 74.48; 94.20, 65.88, 90.80 |
| Resolution (Å) (outer shell) | 50–3.2 (3.31–3.2) | 50–4.3 (4.45–4.3) | 50–3.51 (3.64–3.51) |
| Rmerge (%)                | 8.4 (53.8)  | 17.3 (81.5) | 13.1 (79.3)   |
| I/σ (outer shell)         | 24.0 (2.2)  | 7.3 (1.2)   | 12.8 (1.1)    |
| Completeness (%)          | 86.9 (59.0) | 81.2 (68.1) | 82.2 (49.7)   |
| Number of measured reflections | 197,021     | 56,642      | 102,898       |
| Number of unique reflections | 22,484      | 8,813       | 15,845        |
| Redundancy                | 8.9 (7.5)   | 6.4 (5.1)   | 6.5 (4.7)     |

Values in parentheses are for the highest resolution shell.