Structure of the human frataxin-bound iron-sulfur cluster assembly complex provides insight into its activation mechanism

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The core machinery for de novo biosynthesis of iron-sulfur clusters (ISC), located in the mitochondria matrix, is a five-protein complex containing the cysteine desulfurase NFS1 that is activated by frataxin (FXN), scaffold protein ISCU, accessory protein ISD11, and acyl-carrier protein ACP. Deficiency in FXN leads to the loss-of-function neurodegenerative disorder Friedreich’s ataxia (FRDA). Here the 3.2 Å resolution cryo-electron microscopy structure of the FXN-bound active human complex, containing two copies of the NFS1-ISD11-ACP-ISCU-FXN hetero-pentamer, delineates the interactions of FXN with other component proteins of the complex. FXN binds at the interface of two NFS1 and one ISCU subunits, modifying the local environment of a bound zinc ion that would otherwise inhibit NFS1 activity in complexes without FXN. Our structure reveals how FXN facilitates ISC production through stabilizing key loop conformations of NFS1 and ISCU at the protein–protein interfaces, and suggests how FRDA clinical mutations affect complex formation and FXN activation.
Iron-sulfur clusters (ISC) are inorganic cofactors essential in all life forms with common roles in electron transfer, radical generation, and structural support. In eukaryotes, the de novo ISC assembly machinery is located in the mitochondrial matrix and requires a core complex comprising the proteins NFS1, ISD11, ACP, and ISCU (SDAU)\(^{-1,2}\). The NFS1 cysteine desulfurase facilitates a pyridoxal 5’-phosphate (PLP) cofactor to generate the sulfane sulfur from L-cysteine, and deliver it to the ISC scaffold protein\(^{-3,4}\). The accessory protein ISD11/LYRM4 is unique in eukaryotes, and was shown to stabilize NFS1 and interact directly with the acyl carrier protein ACP/NDUFAB1\(^{-3,4}\). ISCU utilizes three of its conserved cysteine residues (Cys69, Cys95, Cys138) to combine the sulfane sulfur from NFS1 with an iron source, resulting in ISC formation. ISCU then exploits the highly conserved LLPVK motif for interaction with the chaperones, such as GRP75/HSCB\(^{-7}\), for the downstream delivery to apoproteins. Whereas electrons required for ISC assembly most likely involve mitochondrial ferredoxin/ferredoxin reductase contribution\(^{-8}\), the iron source remains unclear.

An intronic GAA repeat of FXN gene, resulting in deficiency of the frataxin (FXN) protein, causes autosomal recessive Friedreich’s ataxia (FRDA)\(^{-9}\). The in vivo loss of FXN results in ISC deficiency and iron accumulation in the mitochondria, rendering FRDA a fatal and debilitating condition. Several roles of FXN with respect to ISC biosynthesis, including that of an iron source\(^{-10}\), have been speculated. The emerging role is that FXN acts as an allosteric regulator of ISC assembly, and stimulates NFS1 activity by binding the SDAU complex to form the five-way active SDAUF complex\(^{11-13}\). ISC biosynthesis must be heavily regulated to avoid iron and sulfur toxicity in the cell, rendering FXN essential in eukaryotes. Zn\(^{2+}\) ion has been found to bind ISCU and completely inhibit the SDAU complex in vitro although its activity is restored by addition of FXN\(^{14}\), and the in vivo relevance of the Zn\(^{2+}\) effect remains to be determined.

Recently, crystal structures of SDA/SDAU/SDAU-Zn\(^{2+}\) complexes without the key component FXN have been published\(^{15,16}\), which attributed the zinc inhibition to the sequestration of key NFS1 catalytic residue Cys381, but could not serve as template to understand the molecular roles of FXN activator. To this end, we pursued structure determination of the SDAUF complex, coupled with FXN binding studies, to decipher the FXN-mediated activation mechanism.

**Results and discussion**

**Cryo-electron microscopy of recombinant SDAUF complex.** FXN binding to the SDAU complex is dynamic, yielding low-\(\mu\)M dissociation constants (\(K_d\)) by bio-layer interferometry (BLI) (Supplementary Fig. 1a-c), hence presenting challenges to isolate the SDAUF complex intact with all 5 components in proper stoichiometry. Our several attempts to generate the SDAUF complex by reconstitution of individually expressed components (Supplementary Fig. 2a, b) did not fully incorporate FXN. To remedy this, we co-expressed in *E. coli* a plasmid containing His\(_{12}\)-ISD11-NFS1-ISCU, with a plasmid containing His\(_{6}\)-FXN-FXN (Supplementary Fig. 2c). This produced excess FXN, shifting equilibrium towards formation of a stable and active SDAUF complex comprising human SDUF co-purified with *E. coli* ACP (ACP\(_{ec}\)). We attempted to generate the 5-way all-human complex, without ACP\(_{ec}\), by inserting human ACP (NDUFAB1) into the second site of the plasmid containing His\(_{6}\)-ISD11-NFS1-ISCU, we observed a heterogeneous complex containing an approximately equimolar mixture of the desired human ACP and contaminating ACP\(_{ec}\) (Supplementary Fig. 2d). Based on previous reports\(^5\), and the functional conservation of human and *E. coli* ACP, we continued our experiments with a homogenous complex containing ACP\(_{ec}\) and human SDUF (hereafter SDAUF). The as-isolated five-way complex could still be inhibited by Zn\(^{2+}\) (Supplementary Fig. 3, SDAUF), as shown previously with the four-way complex\(^{14}\) (Supplementary Fig. 3, SDAU), due to the dissociation equilibrium of FXN. Addition of more purified ISCU to the five-way complex further exacerbated the Zn\(^{2+}\) inhibition (Supplementary Fig. 3, SDAUF + U), while Zn\(^{2+}\) inhibition was fully reversed upon further FXN supplementation to the five-way complex (Supplementary Fig. 3, SDAUF + F and SDAUF + U + F), explaining how excess FXN is needed to maintain its bound state within the five-way complex for the activation.

We determined the single-particle cryo-electron microscopy (cryo-EM) structure of the 186-kDa SDAUF complex to 3.2 \(\AA\) resolution (Supplementary Fig. 4 and Supplementary Table 1), allowing model building of entire complex components, with unambiguous placement of cofactor pyridoxal 5’-phosphate covalently-linked to NFS1 Lys258, a Zn\(^{2+}\) ion in each ISCU, and a 4’-phosphopantetheine (4’-PP) acyl-chain attached to ACP\(_{ec}\) Ser37 (Supplementary Fig. 5). LC-MS revealed a mixture of ACP components with different acyl-chains\(^{16}\), and further top-down MS\(^3\) enabled the detailed structure elucidation on these acyl-chains through accurate mass measurements of both parent and fragment ions of the 4’-PP acyl chains which were readily ejected from ACP in the gas phase and interrogated further by tandem MS (Supplementary Fig. 6 and Supplementary Table 2). The relative abundances of the ACP components were estimated on the basis of extracted ion chromatograms in the LC-MS measurements, showing that ACP with longer acyl-chains are clearly the dominant species (Supplementary Table 2).

**Overall architecture of the SDAUF complex.** Our human SDAUF-Zn\(^{2+}\) structure, the only FXN-bound complex structure to date from any organism, is a symmetric heterodecamer comprising 2 copies of each of the five proteins i.e. (NFS1)\(_{2}\)(ISD11)\(_{2}\)(ACP\(_{ec}\))\(_{2}\)(ISCU-Zn\(^{2+}\))\(_{2}\)(FXN)\(_{2}\). Structurally it constitutes a (NFS1-ISD11-ACP\(_{ec}\))\(_{2}\) homodimeric core, with one ISCU appended to each long end of the core, and one FXN fitted into the cavity next to each ISCU (Fig. 1a, b). This architecture agrees with small-angle x-ray scattering (SAXS) analysis (Fig. 1c, d and Supplementary Fig. 7a, b), whereby a theoretical SAXS profile back-calculated from our SDAUF-Zn\(^{2+}\) cryo-EM structure shows a good fit to experimental scattering data (\(\chi^2 = 1.98\)). While our five-way complex superimposes well with four-way SDAU/SDAU-Zn\(^{2+}\) structures from Boniecki et al.\(^3\) within the (NFS1-ISD11-ACP\(_{ec}\))\(_{2}\) core (rmsd 0.6 Å, over 756 C\(^{\alpha}\) atoms), there is significant displacement of ISCU, up to 2.0 Å away from the core, in our FXN-bound complex (Supplementary Fig. 7c).

FXN occupies a cavity at the interface of both NFS1 and one ISCU subunits (Fig. 1e and Supplementary Fig. 8a), burying ~1345 Å\(^{2}\) of FXN accessible surface. No direct FXN-ISD11 interaction is observed, contrasting previous predictions with oligomeric FXN\(^{17,18}\). A key feature of FXN binding is its simultaneous interactions with both NFS1 protomers of the complex (Fig. 2a), which definitively supports previous predictions from crosslinking, SAXS and NMR studies\(^3,19,20\). Importantly, this requires a homodimeric arrangement of NFS1 within the complex, consistent with the SDAU conformation observed by Boniecki et al.\(^3\), while incompatible with the SDAF\(_{ec}\) crystal structure from Cory et al.\(^4\) whereby an extensive NFS1 homodimer interface was not observed\(^{15}\). The SDAF conformation from Cory et al., not observed in all cysteine desulfurase structures published to date, would therefore not be conducive to the FXN activation mechanism, and could possibly instead depict a FXN-independent function.
FXN interacts with one NFS1 protomer via potential salt-bridges, and with the other NFS1 protomer through van der Waals contacts (Fig. 2a). The salt-bridge interface, highly conserved across orthologues (Supplementary Fig. 9), involves an acidic ridge of FXN (end of \(\alpha_1\), loop \(\alpha_1-\beta_1\), start of \(\beta_1\)) and a positively-charged Arg-rich patch (Arg272-Arg277) on one NFS1 protomer (Figs. 1d, 2a and Supplementary Fig. 8a, b). For example, FXN Asp124 can potentially form salt-bridges with NFS1 Arg289, while carbonyl backbones of FXN residues Glu121 and Tyr123 form potential H-bonds with NFS1 Arg119 and Arg272 (Fig. 2a). The FXN(D124A) variant, aimed at abolishing these salt-bridges, binds SDAU with 4-fold weakened \(K_d\) compared to wild-type (WT) (Table 1 and Supplementary Fig. 1d). FXN contacts the other NFS1 protomer, and ISCU, using the \(\beta\)-sheet (\(\beta_1-\beta_5\)) surface (Fig. 2a–d). In this interface, FXN directly contacts the NFS1 loop containing the catalytic residue Cys381 (Cys-loop) (Fig. 2a), via hydrophobic interaction between FXN Trp155 and NFS1 Leu386, and potential H-bond between FXN Asn146 and NFS1 Ala384 carbonyl backbone. The clinical variant FXN(N146K)
FXN binds to two key regions on ISCU. One ISCU-FXN interface is through the conserved ISCU Ala-loop (Ala66-Asp71), containing the conserved Cys69 that is required for ISC biosynthesis and interacts with FXN Asn151, as well as the Zn$^{2+}$ coordinating ligand Asp71. This interaction (Fig. 2c), which may account for the weaker binding caused by the FXN(N151A) variant (Table 1), is mediated by significant changes of the ISCU Ala-loop conformation in SDAUF as compared with SDAU-Zn$^{2+}$ complex.

![Fig. 2 FXN-ISCU interactions and FXN mutagenesis. a. Interactions of FXN (orange) with both NFS1 subunits (NFS1, light slate; NFS1', blue). Residues studied by site-directed mutagenesis are asterisked. Dashed lines denote potential hydrogen bonds. b. Interface of FXN β-sheet with ISCU LPPVK-region and NFS1 Cys-loop. c. Interface of FXN with ISCU Ala-loop, LPPVK-region, and Zn$^{2+}$ ion (sphere). Inset shows viewpoints of panels b and c within SDAUF-Zn$^{2+}$ complex]

**Table 1 Dissociation constant ($K_d$) and melting temperature ($T_m$) of FXN variants**

| FXN Variant | BLI ($K_d$ (μM)) | Error | DSF $T_m$ (°C) | Error |
|-------------|-----------------|-------|----------------|-------|
| WT          | 3.32            | 0.94  | 66.45          | 0.23  |
| D124A       | 14.36           | 1.53  | 65.33          | 0.70  |
| N146K       | 177.2           | 43.8  | 72.51          | 0.86  |
| W155R       | 9.45            | 2.45  | 66.05          | 2.33  |
| P163G       | 253.3           | 67.3  | 60.85          | 0.60  |
| R165C       | 38.45           | 7.16  | 66.34          | 1.19  |
| R165C       | 134.2           | 8.7   | 68.96          | 1.16  |

exhibited 50-fold weakened $K_d$ towards SDAU (Table 1 and Supplementary Fig. 1d). Beyond the two extensive interfaces with FXN, NFS1 further contributes its C-terminal 20 aa (Ser437-His457) to wrap around the ISCU surface, with terminal residues Gln153-His457 anchored by FXN Asn151, Tyr175 and His177 via potential H-bonds (Supplementary Fig. 10). FXN(N151A) variant further held in place by FXN Arg165 (pi-stacking interaction) and His457) to wrap around the ISCU surface, with terminal residues Gln153-His457 anchored by FXN Asn151, Tyr175 and His177 via potential H-bonds (Supplementary Fig. 10). FXN(N151A) variant further held in place by FXN Arg165 (pi-stacking interaction) and His457) to wrap around the ISCU surface, with terminal residues Gln153-His457 anchored by FXN Asn151, Tyr175 and His177 via potential H-bonds (Supplementary Fig. 10). FXN(N151A) variant further held in place by FXN Arg165 (pi-stacking interaction) and His457) to wrap around the ISCU surface, with terminal residues Gln153-His457 anchored by FXN Asn151, Tyr175 and His177 via potential H-bonds (Supplementary Fig. 10). FXN(N151A) variant further held in place by FXN Arg165 (pi-stacking interaction) and His457) to wrap around the ISCU surface, with terminal residues Gln153-His457 anchored by FXN Asn151, Tyr175 and His177 via potential H-bonds (Supplementary Fig. 10).
prokaryotic position (Fig. 3b), obviated the need for Yfh1 (FXN equivalent) and reversed ΔYfh1 phenotype. Our structure shows that ISCU Met140 packs against FXN Pro163 (Fig. 3c), and a M140I substitution (present in the SDAU/SDAU-Zn$^{2+}$ structures) sterically clashes with Pro163, unless the 163 position adopts the bacterial equivalent amino acid, Gly (E. coli Gly68; Fig. 3b). Our structure hence illustrates the evolutionarily distinct Met:Pro pairing in eukaryotes and Ile:Gly in prokaryotes. FXN could mimic the ISC in aerobic in vitro experiments, it remains to be seen how FXN unlocks the zinc inhibition of SDAU complex, to make way for binding ferredoxin (FDX) or the GRP75/HSCB chaperones. If so, the complex would cycle between the electron donation (mediated by FDX) and sulfur transfer (mediated by FXN) steps in ISC biosynthesis. Since only reduced FDX is known to activate NFS1 into a conformation that is now poised for its chaperone targets that remain intractable for X-ray crystallography.

**Methods**

**Protein expression and purification.** For bi-cistronic co-expression of NFS1-ISD11, a DNA fragment encoding His-tagged ISD11 and non-targeted NFS1 (Δ1-55), interspersed by a non-ribosomal binding site, was sub-cloned into the pNICE28-6a4 vector (GenBank ID: EF198196). For tri-cistronic co-expression of NFS1-ISD11-ISCU, a DNA fragment encoding His-tagged ISD11, non-targeted NFS1 (Δ1-55) and non-targeted ISCU (Δ1-34), interspersed by two in-frame ribosomal binding sites, was sub-cloned into the pNICE28-6a4 vector. Construct encoding human ISCU (Δ1-34) isoform 1 was sub-cloned into the pNICE28-6a4 vector. Primers used in this study are listed in Supplementary Table 3.

For recombinant expression, E. coli BL21(DE3)-R3-pRARE2 cells transformed with the above plasmids were cultured in Terrific Broth, induced with 0.1 mM isopropyl β-D-thiogalactopyranoside (IPTG), and incubated for 16 h at 18°C. Harvested cell pellets were resuspended in binding buffer containing 50 mM Hepes pH 7.5, 500 mM NaCl, 20 mM imidazole, 5% glycerol, 2 mM TCEP and EDTA-free protease inhibitor (Merck). Resuspended cells were lysed by sonication.
and clarified by centrifugation. To purify samples containing NFS1, binding buffer was removed by 300 kDa ultrafiltration with 150 μM pyridoxin-5′-phosphate (PPE) and dialysis. To purify proteins for enzymatic and biophysical studies (SDA, SDAO, SDAF, ISCU and FXN), centrifuged supernatants were incubated with 2.5 mM Ni Sepharose 6 fast flow resin (GE Healthcare), washed with binding buffer containing 40 mM Imidazole, and eluted with binding buffer containing 250 mM Imidazole. Fractions containing target proteins were pooled, added with 10 mM DTT (for samples containing ISCU or NFS1), and applied to size exclusion column (Superdex S75 for ISCU and FXN, Superdex S200 for NFS1-ISD11 complex; GE Healthcare) pre-equilibrated with gel filtration buffer (50 mM HEPES pH 7.5, 200 mM NaCl, 5% glycerol, and 2 mM TCEP). Peak fractions were pooled for His-tag removal by treatment with His-TEV protease, and re-purified by passing onto Ni Sepharose 6 fast flow resin to remove residual His-TEV protease and cleaved His-tag. Purified target proteins were then buffer-exchanged into gel filtration buffer. Recombinantly expressed NFS1-ISD11 (from bi-cistronic plasmid) and NFS1-ISD11-ACP (SDA) complexes were performed at 0.99 Å wavelength Diamond Light Source at beamline B21 coupled to the Shodox KW403-4F' size exclusion column (Harwell, UK) and equipped with Pilatus 2 M two-dimensional detector at 4.014 m distance from the sample, 0.005 < q < 0.4 Å⁻¹ (q = 4π sin θ/λ, 2θ is the scattering angle). The samples were in a buffer containing 300 mM NaCl, 25 mM HEPES 7.5, 1 mM TCEP 2% Glycerol, 1% Sucrose and the measurements were performed at 20°C. The data were processed and analyzed with Scatter and the ATSAS program package. Scatter was used to calculate the radius of gyration Rq and forward scattering I(0) via Guinier approximation and to derive the maximum particle size via Guinier approximation and to derive the maximum particle size. SAXS experiments for the SDAU and SDAUF complexes were performed at 0.9 Å wavelength Diamond Light Source at beamline B21 coupled to the Shodox KW403-4F’ size exclusion column (Harwell, UK) and equipped with Pilatus 2 M two-dimensional detector at 4.014 m distance from the sample, 0.005 < q < 0.4 Å⁻¹ (q = 4π sin θ/λ, 2θ is the scattering angle). The samples were in a buffer containing 300 mM NaCl, 25 mM HEPES 7.5, 1 mM TCEP 2% Glycerol, 1% Sucrose and the measurements were performed at 20°C. The data were processed and analyzed with Scatter and the ATSAS program package. Scatter was used to calculate the radius of gyration Rq and forward scattering I(0) via Guinier approximation and to derive the maximum particle dimension Dmax and P(r) function. The ab initio model was derived using DAMMIF. 20 individual models were created, then overlaid and averaged using DAMAVER. FoxS servers were used for comparison of theoretical and experimental data. SAXS data and parameters are summarized in Supplementary Table 4.

LC-MS® analysis. Mass measurement was carried out using in Synapt G2 HDMS (Waters, Milford, MA) instrument equipped with Lockspray system, quadrupole mass analyzer, trap collision cell, and time-of-flight mass analyzer in tandem. Liquid chromatography was performed using an ACQUITY UPLC system with an Agilent PLRP-S column (1000 Å, 5 μm, 50 × 2.1 mm) at a flow rate of 0.30 ml/min. MS/MS/MS measurement on the acyl-chain conjugated with 4'-PPT was accomplished by ejecting them from ACP in the ESi source region through in-source CID with elevated sample cone voltage – 60 V, and then fragmented by trap CID with trap potential – 30 V after selection in the quadrupole. The Synapt G2 mass spectrometer was externally calibrated using NaCl solution and further lock-SDA and separated on Superdex 200 increase size exclusion column for isolation of the SDAU complex. Complexes were at 0.1 mg/mL and loaded to the streptavidin-coated sensor. The concentration for FXN used ranged from 500 μM to 1 mM. Measurements were taken in a programme consisting of a 90-second association step simulation of a 60-second dissociation step, on a black 384-well tilted assay plate (ForteBio). Prior to association, the baseline was allowed to stabilize for 30 sec, and signal from the reference sensors was subtracted from measurements in the protein-loaded sensors. Dissociation constants (Kd) were determined through plots of response of FXN, using the one site-specific binding fit in GraphPad Prism (GraphPad Software).

Small angle X-ray scattering. SAXS experiments for the SDAU and SDAUF complexes was performed at 0.9 Å wavelength Diamond Light Source at beamline B21 coupled to the Shodox KW403-4F’ size exclusion column (Harwell, UK) and equipped with Pilatus 2 M two-dimensional detector at 4.014 m distance from the sample, 0.005 < q < 0.4 Å⁻¹ (q = 4π sin θ/λ, 2θ is the scattering angle). The samples were in a buffer containing 300 mM NaCl, 25 mM HEPES 7.5, 1 mM TCEP 2% Glycerol, 1% Sucrose and the measurements were performed at 20°C. The data were processed and analyzed with Scatter and the ATSAS program package. Scatter was used to calculate the radius of gyration Rq and forward scattering I(0) via Guinier approximation and to derive the maximum particle dimension Dmax and P(r) function. The ab initio model was derived using DAMMIF. 20 individual models were created, then overlaid and averaged using DAMAVER. FoxS servers were used for comparison of theoretical and experimental data. SAXS data and parameters are summarized in Supplementary Table 4.
Grid preparation and data acquisition. 3.5 µL of 1.5 mg/ml purified SDAUF complex was applied to the glow-discharged Quantifoil Au R1/2/1.3 grid (Structure Probe), and was frequently vitrified using a Vitrobot Mark IV (FEI Company). In order to overcome an orientation bias, 0.067 % (w/v, final concentration) n-octyl-β-d-glucopyranoside (BOG, Anatrace) was added to the sample prior freezing. Cryo grids were loaded into a Titan Krios transmission electron microscope (Thermo Fisher Scientific) operating at 300 keV with a Gatan K2 Summit direct electron detector. Images were recorded with SerialEM in super-resolution mode with a super resolution pixel size of 0.543 Å and a defocus range of 1.2 to 2.5 µm. Data were collected with a dose rate of 5 electrons per physical pixel per second, and images were recorded with a 10 x exposure and 250 ms subframes (40 total frames) corresponding to a total dose of 42 electrons per Å². All details corresponding to individual datasets are summarized in Supplementary Table 1.

Electron microscopy data processing. A total of 4,260 dose-fractioned movies were gain-corrected, 2 x binned (resulting in a pixel size of 1.086 Å), and beam-induced motion correction using MotionCor236 with the dose-weighting option. The SDAUF particles were automatically picked from the dose-weighted, motion corrected average images using Gautomatch. CTF parameters were determined by Gctf37. A total of 1,316,416 particles were then extracted using Relion 2.038 with a box size of 200 pixels. The 2D, 3D classification and refinement were performed with Relion 2.0. Two rounds of 2D classification and one round of 3D classification were performed to select the homogenous particles. After selecting particle coordinates, per-particle CTF estimation was refined using the program Gctf37. One set of 267,153 particles was then submitted to 3D auto-refinement with C2 symmetry imposed and resulted in a 3.2 Å map (Supplementary Figs. 4, 5). All 3D classifications and 3D refinements were started from a 60 Å low-pass filtered version of an ab initio map generated with VIPER39. To evaluate the contribution of imposed symmetry in the result, 3D refinement was repeated using the same set of 267,153 particles without imposing symmetry and produced a 3.4 Å map (Supplementary Fig. 4). Since the overall structures with/without imposing symmetry are nearly identical, the C2 symmetry density map was used for model building. All resolutions were validated by applying a soft mask around the protein complex density and based on the gold-standard (two halves of data refined independently) FSC=0.143 criterion. Prior to visualization, all density maps were sharpened by applying different negative temperature factors using automated procedures40, along with the half maps, were used for model building. Local resolution was determined using ResMap51 (Supplementary Fig. 4).

Model building and refinement. The initial template of the SDAUF complex was derived from a homology-based model calculated by SWISS-MODEL based on the crystal structures of (NFS1-ISD11-ACP-ISCU)3 complex (PDB: 5WKP)5 and human frataxin (PDB: 354M)42 as the templates for NFS1, ISD11, ACP, ISCU, and FXN, respectively43. Each subunit was docked into the C2 symmetry full EM density map using Chimera44 and followed by manually adjustment using COOT45. To prevent overfitting and confidently refine the ligands, the complete model with the ligands was subjected to global refinement using the module phenix.real_space_refine with the ligands was subjected to global refinement using the module phenix.real_space_re

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Author contributions

C.B., S.H., W.W.Y. designed the experiments. N.G.F., X.Y., X.F., H.J.B., A.M., J.F.N. and C.S.-D. performed the experiments. N.G.F., S.H., and W.W.Y. wrote the manuscript.

Additional information

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