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## Supplementary Information

### Structures of a P4-ATPase lipid flippase in lipid bilayers

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## 25 **Supplemental results and discussion**

26

### 27 **Results**

28           In both E1-ATP and E2P structures, ctCdc50p has extensive interactions with ctDnf1p. On the  
29 cytoplasmic side, the N-terminal peptide (residues 23-46) of ctCdc50p runs along one face of ctDnf1p,  
30 interacting with the cytosolic loops of ctDnf1p, including the segment connecting TM4 and the P domain,  
31 the loop between TMs 6 and 7 (L6/7), L8/9, and a C-terminal amphipathic helix that was suggested to  
32 undergo conformational changes upon PI4P activation in scDrs2p<sup>1,2</sup> (Fig. S10). The C-terminal tail of  
33 ctCdc50p (residues 384-398), which is invisible in the scCdc50p and hCDC50a structures, could be  
34 traced to run towards ctDnf1p. Thus, the C-terminal tail and the N-terminal peptide of ctCdc50p are in  
35 such a conformation that sandwiches the C-terminal amphipathic helix of ctDnf1p (Fig. S10). The two  
36 TMs of ctCdc50p form hydrophobic interactions with TM7 and TM10 of ctDnf1p. The ectodomain of  
37 ctCdc50p has the largest interfaces with ctDnf1p, interacting with all the exoplasmic loops except L1/2  
38 (Fig. 1a). Thus, ctCdc50p acts as a 3-way clamp to hold TMs 3-10 of ctDnf1p in a relatively fixed  
39 conformation in both E1-ATP and E2P structures.

40           The E1-ATP and E2P structures have a lipid-binding site in common in a cavity formed by TMs  
41 7, 8, and 10, on the opposite side of TMs 2, 4, and 6 (Fig. S11). Phospholipid density is observed at the  
42 cytoplasmic border of the membranes. The cavity was suggested to be a PI4P binding site for scDrs2p  
43 activation<sup>1</sup>. However, in our structures, the head group does not insert as deep as PI4P in scDrs2p E2P<sup>inter</sup>  
44 or E2P<sup>active</sup>. Instead, it is likely to be the head group of PS as modeled in scDrs2p E2P<sup>inhib</sup><sup>1</sup>.

45

### 46 **Discussion**

#### 47 **Phospholipid flipping coupled with the E1-E2 transition**

48           Four lipid-binding sites are identified in the groove formed by TMs 2, 4, and 6, two from E1-ATP  
49 and two from E2P. The four sites arranged in such a way that they could relay the phospholipid substrates  
50 through the groove during the E1-E2 transition (Fig. S12). Conformational changes of TM1 and TM2  
51 guide the phospholipids to move from the exoplasmic leaflet to the cytoplasmic leaflet. ATP binding to  
52 the lipid flippase in the E1-ATP state detaches the A domain from the N and P domains. The large motion  
53 of the A domain increases the flexibility of TMs 1 and 2 and exposes a negatively charged patch formed  
54 by the residues from TMs 2 and 4. The local lipid bilayers are distorted and the membranes are thinned by  
55 almost a half. In the distorted membranes, the phospholipid molecules are more likely to tilt parallel to the

56 membrane plane. The lipid head groups are in an inward-facing orientation, ready to enter the groove via  
57 E1-site2 (Fig. S12a). In the E2P state, the A domain is associated with the P and N domains tightly,  
58 leading to a relatively rigid conformation of TMs 1 and 2. The local lipid bilayer structures are restored.  
59 TMs 2, 4, and 6 create a cavity (E2-site1) in the exoplasmic leaflet for shielding the polar head group of  
60 the phospholipid that has been picked up from E1-site2 (Fig. S12b). E2-site1 is disrupted in the E1-ATP  
61 state as TM2 moves towards TMs 4 and 6 and two polar residues of TM4 (Q549 and N550) lean towards  
62 the membranes. The lipid head group is likely to be squeezed out and to move forward to the cytoplasmic  
63 leaflet via E1-site1, a shallow hydrophilic cleft (Fig. S12c). Finally, the phospholipid reaches E2-site2 and  
64 is held by a clamp between TMs 2 and 4 in a flipped conformation (Fig. S12d). The phospholipid  
65 substrate is ready to be laterally released into the cytoplasmic leaflet when the clamp is disrupted in the  
66 E1-ATP state. In the scenario proposed above, it takes two E1-E2 cycles to flip one phospholipid  
67 substrate, but costs one ATP molecule per phospholipid on average because two phospholipids are  
68 present at the same time in each state. However, as we are missing several intermediate states, e.g. E2 and  
69 E1P, the exact cycle number and ATP cost per substrate need further investigation. During lipid transport,  
70 the hydrophilic head group of the phospholipid substrate is constantly protected from the hydrophobic  
71 environment by sliding through the binding sites in the positively charged groove (Fig. 2b, d). The groove  
72 provides the only continuous hydrophilic pathway in the M domain during the E1-E2 transition (Compare  
73 Fig. 2b, d and Fig. S11c). The positive charges are mainly contributed by K174, R181, and K1121 on the  
74 TMs. The highly conserved K1121 has been shown to be important for substrate binding and ATPase  
75 activity<sup>3</sup>. Consistent with biochemical data<sup>3</sup>, the groove has high affinity to the lipid substrate in the E2P  
76 state as evident by the strong phospholipid density, whereas the groove shows weak lipid binding to  
77 facilitate lipid entry and exit in the E1-ATP state as indicated by the fragmented lipid density in the  
78 structure. Similarly, a hydrophilic membrane-traversing groove is also present in the TMEM16F  
79 scramblase<sup>4</sup>.

80 The “hydrophobic gate” model suggests that TMs 1 and 2 move away from TMs 3 and 4 during  
81 lipid transport<sup>5</sup>. Indeed, our structures show that TM1 and TM2 becomes flexible in E1-ATP. The key  
82 residue, I364 of the “hydrophobic gate” (I554 in ctDnf1p), is at the interface between TMs 1, 2, and 4  
83 (Fig. S13). Thus the mutations of the residue may disrupt the E1-E2 equilibrium and hamper lipid  
84 flipping as observed in the mutagenesis studies<sup>5</sup>. The “two-gate” model suggests that the flippases  
85 recognize the phospholipid substrates by interacting with the head groups. Residues other than the  
86 classical ion binding residues in ion-pumping P-type ATPases are involved in recognition<sup>6-9</sup>. Consistent  
87 with the model, the four distinct binding sites in our structures mainly interact with the lipid head groups.  
88 However, the sites do not seem to provide a discrimination mechanism for different phospholipid  
89 substrates. As shown in the phospholipid-dependent ATPase activity assays, ctDnf1p may have different

90 substrate specificity from scDrs2p and hATP8A1. The amino acids that interact with the polar head group  
91 of the phospholipid substrate at E2-site1 are similar to those in the structures of scDrs2p and hATP8A1.  
92 The corresponding residues are Q549 and N550 of ctDnf1p, S503 and N504 of scDrs2p, and N352 and  
93 N353 of hATP8A1 (Fig. S1). The clamp residues of E2-site2 consist of both hydrophilic and hydrophobic  
94 residues, but are not conserved among P4-ATPases (Fig. S1). The serine residues at E1-site1 are highly  
95 conserved among P4-ATPases, and E1-site2 only provides a steric opening. Further studies on other  
96 intermediate states may provide clues on the substrate specificity.

97

## 98 **Materials and methods**

### 99 Protein expression and purification

100 Protein BLAST search identified three P4-ATPases in *C. thermophilum*, including one *S.*  
101 *cerevisiae* Drs2p homolog (ctDrs2p), one Dnf1p and Dnf2p homolog (ctDnf1p), and one Dnf3p homolog  
102 (ctDnf3p) (Fig. S1). Only one CDC50 protein was found in the *C. thermophilum* genome (ctCdc50p).  
103 After initial screening, ctDnf1p was chosen to co-express with ctCdc50p in yeast. The genes of ctDnf1p  
104 and ctCdc50p were cloned from the cDNA library of *Chaetomium thermophilum* (*var. thermophilum*  
105 strain: DSM1495, a gift from Dr. Stefan Schoebel). Superfolder green fluorescence protein (sfGFP)<sup>10</sup>, a  
106 Twin-Strep tag and a 3C protease cleavage site were fused to the N-terminus of ctDnf1p. sfGFP, a His<sub>9</sub>  
107 tag, and a 3C protease cleavage site were fused to the N-terminus of ctCdc50p. The expression plasmids  
108 pRS426-sfGFP-twinStrep-3C-ctDNF1 and pRS424-sfGFP-His<sub>9</sub>-3C-ctCDC50 were co-transformed into *S.*  
109 *cerevisiae* strain BJ5465 using the LiAc/SS carrier DNA/PEG method<sup>11</sup>. Yeast cells were cultured in  
110 synthetic drop-out medium supplemented with 2% raffinose at 30 °C for about 24h to reach an optical  
111 density (OD<sub>600</sub>) of about 5. The culture was induced by the addition of 2% galactose and continued for 20  
112 h at 25°C. The cells were harvested and stored at -80 °C until use.

113 The cells were suspended in the membrane extraction buffer (20 mM Tris-HCl pH 7.4, 150 mM  
114 NaCl, 5mM MgCl<sub>2</sub>, 1mM DTT, and protease inhibitor cocktails) and lysed by high pressure  
115 homogenization. The crude lysate was clarified by centrifugation (20,000×g, 25 min, 4°C). The  
116 membrane fraction was pelleted by ultracentrifugation (200,000×g, 1 h, 4°C) and washed once with the  
117 membrane extraction buffer. The membrane pellets were solubilized in 2% lauryl maltose neopentyl  
118 glycol (LMNG, Anatrace) in the membrane solubilization buffer (20 mM Tris-HCl pH 7.4, 150 mM  
119 NaCl, 5mM MgCl<sub>2</sub>, 1mM DTT, 10% glycerol, and protease inhibitor cocktails). After incubation at 4 °C  
120 for 1 h, the solution was clarified by ultracentrifugation (200,000×g, 1 h, 4°C). The supernatant was  
121 mixed with avidin (Sigma) and loaded onto a column pre-packed with StrepTactin resin (IBA

122 Lifesciences). The eluents were concentrated and incubated with 3C protease at 4°C overnight. The  
123 protein solution was then loaded onto a Superdex 200 10/300 column (GE Healthcare). The peak fractions  
124 were pooled and concentrated (Fig. S2). The purified protein was either reconstituted into nanodiscs or  
125 flash-frozen in liquid nitrogen and stored at -80 °C.

126 The purified protein was mixed with MSP1D1<sup>12</sup> and yeast polar lipids (Avanti Lipids, 40 mg/ml  
127 dissolved in 1% DDM) at a molar ratio of 1:2:25. Bio-beads SM2 (Bio-Rad) were then added to the  
128 mixture and incubated at 4 °C overnight to remove detergents. The complex was further purified by size-  
129 exclusion chromatography on a Superdex 200 10/300 column. The peak fraction had a protein  
130 concentration of 1.0 mg/ml (Fig. S2). It was immediately used for cryo-EM sample preparation without  
131 concentrating.

#### 132 Cryo-EM sample preparation and data collection

133 The freshly prepared samples were incubated with 1mM BeF<sub>3</sub> or 1mM AMPPCP on ice for  
134 30min before vitrification. The cryo-grid preparation was performed at 4 °C and 100% humidity in an FEI  
135 Vitrobot Mark IV. 4 µl sample was applied to each freshly glow-discharged grid (Quantifoil, R1.2/1.3).  
136 The grids were then plunge-frozen in liquid ethane. The cryo-grids were screened with a 200 kV FEI  
137 Talos Arctica microscope equipped with a FEI Ceta camera. The data were collected on a 300 kV FEI  
138 Titan Krios TEM with a K2 summit camera and GIF Quantum energy filter (Gatan). The images were  
139 collected at a magnification of 130,000× with a calibrated pixel size of 1.055 Å. The dose rate was set at  
140 8 e<sup>-</sup>/s/pixel and the exposure time was 8 s, corresponding to a total dose of 57.5 e<sup>-</sup>/Å<sup>2</sup>. Movie stacks (32  
141 frames each) were recorded with the software SerialEM<sup>13</sup> under low-dose conditions with defocuses  
142 ranging from -1 to -2 µm.

#### 143 Image processing

144 The movie stacks were subject to motion correction and electron-dose weighting by using  
145 MotionCor2<sup>14</sup> (Fig. S3a, S4a). The program Gctf<sup>15</sup> was used to estimate the contrast transfer function  
146 (CTF) parameters. Images of high quality were selected for further image processing on the basis of the  
147 CTF power spectra of the corrected images. The following calculations are performed with RELION3.0<sup>16</sup>.  
148 Particles of high quality were selected according to 2D classification (Fig. S3b, S4b) and 3D classification  
149 results. The selected particles were subject to several rounds of CTF refinement and polishing. After  
150 mask-based post-processing, the final maps had resolutions of 3.40 Å and 3.48 Å for the AMPPCP and  
151 BeF<sub>3</sub><sup>-</sup> samples, respectively (Fig. S3, S4). All the resolution estimations were based on gold-standard  
152 Fourier Shell Correlation (FSC) 0.143 criteria.

153 The model for the E2P (BeF<sub>3</sub><sup>-</sup>) structure was built manually in Coot<sup>17</sup>, with the guidance of the scDrs2p  
154 structures. The model was refined in real space using Phenix<sup>18</sup>. For the model building of E1-ATP  
155 (AMPPCP), the E2P model was fit in the E1-ATP density map. Each domain is subject to rigid body  
156 refinement. Due to the local resolution limits, the A and N domains were not refined further. The rest  
157 parts of the E1-ATP model were refined in real space with Phenix. Model validation was done with  
158 MolProbity<sup>19</sup>. The cryo-EM maps have been deposited in the Electron Microscopy Data Bank under  
159 accession numbers 0872 (BeF<sub>3</sub><sup>-</sup>) and 0873 (AMPPCP). The atomic structure coordinates have been  
160 deposited in the Protein Data Bank under the accession number 6LCP (BeF<sub>3</sub><sup>-</sup>) and 6LCR (AMPPCP). All  
161 other data can be obtained from the corresponding author upon reasonable request.

162

### 163 ATPase activity assay

164 The ATPase activity assays were carried out by using BIOMOL® Green (Enzo) to measure the  
165 free phosphate concentrations. The reaction solutions consisted of 0.05mg/ml protein, 0.01% LMNG,  
166 0.02% C<sub>12</sub>E<sub>9</sub> (Anatrace), 150 mM NaCl, 20 mM HEPES-NaOH pH 7.5, 5mM MgCl<sub>2</sub>, 1mM DTT, 2.5mM  
167 ATP, and lipids at the indicated concentrations. The reactions were carried out at 30 °C for 20 min, and  
168 then immediately diluted 10 times for color development. 100 µl reagent was added to 50 µl sample and  
169 the mixture was incubated at room temperature for 20 min. The absorbance at 650 nm was measured in a  
170 microplate reader (BioTek Cytation5). The phosphate concentration was determined by calibration with  
171 the phosphate standard (BML-KI102).

172

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219

## 220 **Supplementary Figures**

### 221 **Fig. S1 Sequence alignment of selected P4-ATPases.**

222  
223 Sequence alignment of ctDnf1p, ctDrs2p and other P4-ATPases in yeast, bovine, human, and *A. thaliana*,  
224 aligned by T-coffee<sup>48</sup>. The conserved domains and transmembrane helices of ctDnf1p are indicated above  
225 the sequences. The conserved residues are indicated in red letters. The amphipathic helix of TM1 is  
226 highlighted with a green bar above the alignment. The phosphorylation site of the P domain is highlighted  
227 with a green dot. The residues involved in the negatively charged patch are highlighted with red dots. The  
228 residues contribute to the positive charge of the groove are highlighted with blue dots. The residues  
229 involved in E1-site1 are highlighted with orange dots. The residues involved in E2-site1 are highlighted  
230 with magenta dots. The residues involved in E1-site2 are highlighted with purple dots. The key isoleucine  
231 residue in the hydrophobic gate model is highlighted with a grey dot. Ct, *Chaetomium thermophilum*; Sc,  
232 *Saccharomyces cerevisiae*; Bt, *Bos Taurus*; Hs, *Homo sapiens*; At, *Arabidopsis thaliana*. Uniprot  
233 accession numbers: ScDRS2, P39524; ScDNF1, P32660; ScDNF2, Q12675; BtATP8A2, C7EXK4;  
234 HsATP8A1, Q9Y2Q0; HsATP8A2, Q9NTI2; HsATP11A, P98196; HsATP11C, Q8NB49; HsATP8B1,  
235 O43520; HsATP8B2, P98198; HsATP10A, O60312; AtALA2, P98205; AtALA10, Q9LI83.

236  
237

### 238 **Fig. S2 Purification and characterization of the ctDnf1p-Cdc50p complex.**

239 **a**, Flow chart of ctDnf1p-Cdc50p purification. **b**, Size-exclusion chromatography profile of the protein  
240 complex reconstituted into nanodiscs. The gray-shaded area (Fraction 27) was used for cryo-EM analysis.  
241 **c**, SDS-PAGE analysis of fractions from the SEC purification in **b**. **d**, ATPase activity of ctDnf1p-  
242 Cdc50p complex stimulated by phospholipids. Data points represent the mean  $\pm$  SEM of at least three  
243 experiments. POPC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine. POPS, 1-palmitoyl-2-oleoyl-sn-  
244 glycero-3-phospho-L-serine.

245

### 246 **Fig. S3 Cryo-EM single particle analysis of ctDnf1p-Cdc50p with AMPPCP**

247 **a**, Representative image after motion correction. **b**, Representative results of 2D classification. **c**,  
248 Workflow of the single particle analysis. **d**, Local resolution map of the final sharpened map, shown with  
249 (left) and without (right) the nanodisc. **e**, Fourier shell correlation (FSC) curve with estimated resolution  
250 according to the gold standard.

251

### 252 **Fig. S4 Cryo-EM single particle analysis of ctDnf1p-Cdc50p with BeF<sub>3</sub>.**

253 **a**, Representative image after motion correction. **b**, Representative results of 2D classification. **c**,  
254 Workflow of the single particle analysis. **d**, Local resolution map of the final sharpened map shown with  
255 (left) and without (right) the nanodisc. **e**, Fourier shell correlation (FSC) curve with estimated resolution  
256 according to the gold standard.

257

### 258 **Fig. S5 Examples of the fit of models into the density map.**

259 **a-g**, Density map and model in selected regions of each domain and TMs. Residues at the beginning and  
260 end of each polypeptide segment are indicated. **h**, Density map and model of  $\text{BeF}_3^-$ ,  $\text{Mg}^{2+}$ , and D606 in  
261 the E1-ATP structure. **i**, Density map and model of AMPPCP,  $\text{Mg}^{2+}$ , and D606 in the E1-ATP structure.

262

### 263 **Fig. S6 Comparison of ctDnf1p-Cdc50p structures in the E1-ATP and E2P states.**

264 **a**, Overlay of the E1-ATP and E2P structures by superimposing TMs 3-10 of ctDnf1p and ctCdc50p  
265 (grey). The A domain is yellow in E1-ATP and blue in E2P. The N domain is red in E1-ATP and cyan in  
266 E2P. TM2 is purple in E1-ATP and TMs 1 and 2 are green in E2P. **b**, same as **a**, except the N domains are  
267 omitted for clarity. The motion distance of the A domain between the E1-ATP and E2P states is labeled. **c**,  
268 same as **a**, except the A domains are omitted. The movement of the N domain between the E1 and E2  
269 states is indicated. **d**, same as **a**, except rotating by 90 degrees and the A and N domains are omitted to  
270 show the movements of TMs 1 and 2 between the E1-ATP and E2P states.

271

### 272 **Fig. S7 Comparison of AMPPCP in different E1-ATP structures**

273 **a-d**, AMPPCP conformations from different P-type ATPases are shown as sticks. The protein structures  
274 from which AMPPCP are extracted are labeled.

275

### 276 **Fig. S8 Comparison of phospholipid binding at E2-site1 in ctDnf1p and hATP8A1**

277 **a**, Lipid binding at E2-site1 of ctDnf1p. The protein is shown as tan ribbon representation. The density of  
278 the lipid is shown as a grey mesh. The lipid (green) and its interacting residues (tan) are shown as sticks.  
279 **b**, Lipid binding in the E2Pi-PL structure of hATP8A1 (PDB ID: 6K7M, EMDB number: 9941). The  
280 protein is colored purple. The lipid is yellow. **c**, Superimposition of the two lipid binding sites. The  
281 yellow arrow and green arrow indicate the extension directions of the lipid acyl chains in hATP8A1 and  
282 ctDnf1p, respectively.

283

### 284 **Fig. S9 Comparison of E1-site1 among P4-ATPase structures**

285 **a**, E1-site1 in ctDnf1p. The density of the possible phospholipid substrate is shown as a grey mesh. The  
286 conserved serine residue is labeled. **b-c**, same as in **a**, except showing scDrs2p and hATP8A1,  
287 respectively.

288

### 289 **Fig. S10 Interaction of the two terminal segments of ctCdc50p with ctDnf1p on the cytoplasmic side.**

291 The N-terminal and C-terminal segments of ctCdc50p are colored yellow. The rest of ctCdc50p is pink.  
292 The ctDnf1p fragments that interact with ctCdc50p are colored red. The rest of ctDnf1p is tan. Interacting  
293 segments and TMs are labeled.

294

### 295 **Fig. S11 A common lipid binding site in E1-ATP and E2P**

296 **a-b**, Lipid binding site in E1-ATP (**a**) and E2P (**b**). The density of the lipid is shown as grey meshes. The  
297 lipid molecules are shown as sticks. **c**, Electrostatic potential surfaces of E1-ATP (left) and E2P (right),  
298 showing the lipid binding environment. The lipid molecules are shown as sticks. The surfaces showing  
299 here are on the opposite side of the surfaces showing in Fig. 2**b** and **d**.

300

### 301 **Fig. S12 Model of phospholipid flipping by P4-ATPases**

302 **a**, Cartoon drawing of E1-ATP. Domains are labeled and colored as in Fig 1**a**. The membrane is colored  
303 grey. The lipid molecules with light green heads are arranged to show the distortion of bilayers. The lipid  
304 molecule with the dark green head represents a substrate that is entering the transport pathway via E1-  
305 site2. **b**, Cartoon drawing of E2P. The lipid substrate is trapped in E2-site1. **c**, As in **a**, with the lipid  
306 bound at E1-site1. **d**, As in **b**, but the lipid substrate has been flipped to the cytosolic leaflet and waits at  
307 E2-site2 to be released.

308

### 309 **Fig. S13 The “hydrophobic gate” residue I554 in cfDnf1p**

310 **a**, Top view of the I554 and its interacting residues in E2P. The interacting residues are shown as sticks.

311 **b**, Top view of I554 in E1-ATP. TM4 is in the same orientation as it is in **a**.

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**Table S1 Cryo-EM data collection, refinement and validation statistics**

	ctDnf1p-Cdc50p with AMPPCP (EMDB-0873) (PDB 6LCR)	ctDnf1p-Cdc50p with BeF <sub>3</sub> <sup>-</sup> (EMDB-0872) (PDB 6LCP)
<b>Data collection and processing</b>		
Magnification	130,000	130,000
Voltage (kV)	300	300
Electron exposure (e-/Å <sup>2</sup> )	57.5	57.5
Defocus range (µm)	-1.0 to -2.0	-1.0 to 2.0
Pixel size (Å)	1.055	1.055
Symmetry imposed	C1	C1
Initial particle images (no.)	2,397,258	2,820,251
Final particle images (no.)	272,912	249,694
Map resolution (Å)	3.40	3.48
FSC threshold 0.143		
Map resolution range (Å)	3.3-8.2	3.3-7.8
<b>Refinement</b>		
Model resolution (Å)	3.4	3.5
Map sharpening B factor (Å <sup>2</sup> )	-94	-84
<u>Model composition</u>		
Non-hydrogen atoms	11590	12406
Protein residues	1420	1514
Ligands	17	18
<u>B factors (Å<sup>2</sup>)</u>		
Protein	41.2	50.0
Ligand	45.6	51.5
<u>R.m.s. deviations</u>		
Bond lengths (Å)	0.012	0.010
Bond angles (°)	1.055	1.088
<u>Validation</u>		
MolProbity score	2.16	2.35
Clashscore	15.5	18.1
Poor rotamers (%)	0.31	0.25
<u>Ramachandran plot</u>		
Favored (%)	89.8%	88.1%
Allowed (%)	10.2%	11.7%
Disallowed (%)	0%	0.2%

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	TM2 cont.	A domain	
CtDNF1	YRRTLLDIELNNA	PVHRLQGWENNVNVEKDNVSLWRRFFKANSRFFGSIWHL-IERLWKEDAQSMR-----QR-FASADPRMSIETRTAPW	262
CtDRS2	YRKQADKALNMSKTRVLRG-		341
ScDRS2	IKRANSDEKELNNSAEIFSE-		289
ScDNF1	SRRTVLDLEVNNTKTHILEGVENENVSTDNISLWRRFFKANSRLLFKFIQYCKEHLTEEGKKRMRQRKHELVRVQKTVGTSGPRSSLDSID---		353
ScDNF2	SRRTVLDLEVNNTRTHILSGVKNENAVDENVSLWRRFFKANTRALIKIFEYFSENLAAGREKLLQKKREELRRKRNSRSFSGPRGSLDSIG---		391
BtATP8A2	FKRHKADNAVNNKKTIVLRN-		119
HsATP8A1	IKRHKADNAVNNKKTQVLRN-		140
HsATP8A2	FKRHKADNAVNNKKTIVLRN-		159
HsATP11A	WLRHKADNAMNQCVPVHFQIH-		135
HsATP11C	CLRHRADNEVNNKSTVYIIEEN-		133
HsATP8B1	VARHKMDKEINNRICEVIKD-		183
HsATP8B2	YFRHKSQDNQVNNRQSQVLIN-		139
HsATP10A	YSRHRSDHKINHLGCLVFSRE-		151
AtALA2	YHRYLSDKKANEKEVWIVKQ-		108
AtALA10	WRKQQDIEVNNRKKVKVHGD-		147

	A domain	
CtDNF1	DPSHRRSVASHTEEIQMTPVPSVPVPHDPDPTVSSAIENEATLLQ--NLKGLDINHEIPVSGKA--RFHKDANKNLVVGDFVRIYNDDELPA	351
CtDRS2		369
ScDRS2		319
ScDNF1	--SYR-----VSADYGRPSLDYDNLQEQAG-----EANIVDRSLPRTDC--KFAKNYKGVKVGDIVRIHNNDEIPAD	418
ScDNF2	--SYR-----MSADFGRPSLDYENLNQMTSQANRYNDGENLVDRTLQPNPEC--RFAKDYKNNVVGDIVRVHNNDEIPAD	463
BtATP8A2		147
HsATP8A1		168
HsATP8A2		187
HsATP11A		163
HsATP11C		161
HsATP8B1		211
HsATP8B2		167
HsATP10A		180
AtALA2		136
AtALA10		176

	A domain	
CtDNF1	IIILATSDPDGAGYVEETKNDLGETNLKVRQALRCGRITLK-HA-RDCERAQFVIESEEPQPNLYKYNGAIRWKQRPVWPDPHGEPREMSEPIGIDN	443
CtDRS2	LVLASSEPEGLCYIETANLDGETNLKIKQALPETASLV-SS-TELSRLGRLRSEQENSSLYTYEATLTLQTGG-----GEKELPLNPEQ	453
ScDRS2	TIILSSSEPEGLCYIETANLDGETNLKIKQSRVETAKFI-DV-KTLKNMNGKVVSEQENSSLYTYEGTMTLN-----DRQPLSPDQ	399
ScDNF1	IIILSTSDPDGAGYVEETKNDLGETNLKVRQSLKCTNTIR-TS-KDIARTKFWIESEGEHNSNLYTYQGNMKWRNLA---DG--EIRNEPITINN	504
ScDNF2	MILSTSDVDGAGYVEETKNDLGETNLKVRQSLKCSKIIK-SS-RDITRTKFWVIESEGEHANLYSYQGNFKWQDTQ---NG--NIRNEPVNINN	549
BtATP8A2	VVLLSSSEPEQAMCYIETANLDGETNLKIRQGLSHADMQ-TR-EVLMKLSGTIECEGENRHLYDFTGNNLNDG-----KSPVALGPDQ	228
HsATP8A1	LISLSSSEPEQAMCYIETANLDGETNLKIRQGLPATSDIK-DV-DSLMRISGRIECESENRLHYDFVGNIRLDG-----HGTVPLGADQ	249
HsATP8A2	VVLLSSSEPEQAMCYIETANLDGETNLKIRQGLSHADMQ-TR-EVLMKLSGTIECEGENRHLYDFTGNNLNDG-----KSLVALGPDQ	268
HsATP11A	LIFLSSNRDGTGHVTHASLDGESSHRTHYAVDQTKGFH-TE-EDIGGLHATIECEQEPDPLYKFGVRINYSYL---N---DPVVRPLGSEN	248
HsATP11C	LILSSCTDGTGYVTASLDGESSNCRTHYAVRDITIALC-TA-ESIDTLRAAIECEQEPDPLYKFGVRINYSNS---L---EAVARSLGPEEN	246
HsATP8B1	LILSSSEPEPNSLYVEETAEALDGETNLKFKMSLEITDQYLORE-DTLATFDGFIECEBENNRDLKFTGTFLWR-----NTSFPDLADK	292
HsATP8B2	LILSSSEPEGLCYIETAEALDGETNLKVRQAIPTSELG-DI-SKLAKFDGEVIECEPENNRDLKFTGTFLWR-----ENKFPPLSNQN	247
HsATP10A	LILSSSDPDGLGHITAEALDGETNLKRRQVVRGFSELV-SE-FNPLTFTSVEIECEBENNRDLKFTGTFLWR-----GKKAGLYKEN	261
AtALA2	LVLGTSDPDGAGYVEETAEALDGETNLKTRVIPSAC-VGI-DL-ELLHKMGVIECEPVEDKDIRFDANMRLFPFP---I---DNDVCSLTIKN	220
AtALA10	LLLSSSYEDSVYVEETAEALDGETNLKVRQGLEATSSLL-NQSDSDFDKFRGVVRCBENNVNLYVFGTLALEE-----ERFPLSIQQ	257

	A domain	TM3	
CtDNF1	LLRGRGHLRNTAEWALVVVFTGHDTRIMNAGITPSSRRARIARELNFNVICNFGILLIMCLIAAANGIAW--G--KTDASL-AWFEYSGIGG-		531
CtDRS2	LLRGTATLRNTAWIHVVVFTGHEHTKLMRNATAAPIKRTVEKQNLKLVMLVGMVLSVISTAGDLIMR--G--V-AGRS-FEYLDLDGIT-		540
ScDRS2	MLRGTATLRNTAWIFGLVIFVFTGHEHTKLLRNATATPIKRTAVEKIINRQIIALFTVLIVLILISSIGNVIMS--T--A-DAKH-LSYLYLEGTN-		486
ScDNF1	VLRRGTALRNTKAWMVFMTGHTKRLMNSGITPTKRSRISRELNFSVIVNFVLLFICLFCVSGIANGVYV--D--KGRSR-FSYFPGTIAG-		592
ScDNF2	LLRGTALRNTKAWMVFMTGHTKRLMNSGITPTKRSRISRELNFSVILNFFVLLFICLFTAGIVNGVYV--K--QKPRSR-DYFEGTIGG-		637
BtATP8A2	ILRGTALRNTQWGFIVVFTGHDTRKLMQNSTKAPLRRSNVEKVTNVQILVLFGLLVMALVSVSGALYWN--G--S-QGGK-NWYIKKMDAT-		315
HsATP8A1	ILRGAQLRNTQWVHGIIVVFTGHDTRKLMQNSTSPPLRLSNVERITNVQIILIFCILIAMSLVCSVSGSAIWN--R--R-HSGK-DWYLNLYGG-		336
HsATP8A2	ILRGTALRNTQWVHGIIVVFTGHDTRKLMQNSTKAPLRRSNVEKVTNVQILVLFGLLVMALVSVSAGALYWN--R--S-HGK-NWYIKKMDT-		355
HsATP11A	LLRGTALRNTKTEKIFGVAIYTGEMTKMALNYQSKSQRRSAVEKSMNAFLIVLYLCILISKALINTVLKYMWQ--S--EPPRDE-PWYNQKTESER		337
HsATP11C	LLRGTALRNTKTEKIFGVAIYTGEMTKMALNYQSKSQRRSAVEKSMNAFLIVLYLCILISKALINTVLKYMWQ--S--TPYND-PWYNQKTKER		335
HsATP8B1	ILRGCVIIRNTDFCHGLVIFACADTRKIMKNSGKTRFRRTKIDYLMNYMVTIIFVLLLSAGLAIGHAYWE--A--Q-VGNS-SWYLYDGE-D-		378
HsATP8B2	MLRGCVLNRTEWCFGLVIFAGPDTKLMQNSGRTKFRRTSIDRLMNTLVLWIFGLVCMGVILAIGNAWE--H--E-VGMRFOVYLPWDEAV-		335
HsATP10A	LLRGTALRNTDAVVGIVYACHETKALNNNSGPRYRRSKLERQMNCDVLCVLLVCMMSLFSAVGHGLWIRY--Q--EKKS-LFYVPKSGSS		351
AtALA2	TLRQSCYLNRTEWACVSVYTGNTKLGMSRGAEPRLTAMDAMDKLTGAIFFVQIVVVLVLAGIAGNVK--D--T-EARK-QWYVQYPEEA-		307
AtALA10	ILLRDSKLRNTEVYVCAVVFTGHDTRKVIQNSTDPPSSRRSRIERTMDKIYYLMFGLVFLMSFVGSIIFGVETREDKVKNGRTE-RWYLPKDDADI		350

	TM4	P domain	N	
CtDNF1	-----T-PAL-T-GFTFWAAAVIVFQNLVPIISLYISLEIVRTLQAFETIYSVGVMYEIKIQPCIPKSNWISDDVQIIEYIFSDKTGTTLTQNVME			617
CtDRS2	-----GA-IAVFKIFIKDMVYIWFSSLVPIISLFTVLEMVYWHGILINDLDIYDVDTDPANCRSSSLVEELGMVEYVFSDKTGTTLTQNVME			629
ScDRS2	-----KA-GLF---FKDFTLFWLFSNLVPIISLFTVLELVKYYQAFMINDSLLDYLYEKTDTPTVVRTSSLVEELQIIEYIFSDKTGTTLTQNVME			571
ScDNF1	-----S-AAT-N-GFVSFWVAVILYQSLVPIISLYISVEIKTAQAATFYGVLLYNAKLDYPCPTPKSNWISDDVQIIEYIFSDKTGTTLTQNVME			678
ScDNF2	-----S-AST-N-GFVSFWVAVILYQSLVPIISLYISVEIKTAQAATFYGVLLYNAKLDYPCPTPKSNWISDDVQIIEYIFSDKTGTTLTQNVME			723
BtATP8A2	-----S-DN---FGYNLLTFIILYNNLPIISLVTLEVVKYTQALFINWTDMMYIYGNTPAMARTSNLNEELQVYIFSDKTGTTLTQNVME			399
HsATP8A1	-----A-SN---FGLNFLTFFIILFNLLPIISLVTLEVVKYTQALFINWTDMMYIYGNTPAMARTSNLNEELQVYIFSDKTGTTLTQNVME			420
HsATP8A2	-----S-DN---FGYNLLTFIILYNNLPIISLVTLEVVKYTQALFINWTDMMYIYGNTPAMARTSNLNEELQVYIFSDKTGTTLTQNVME			439
HsATP11A	-----QRNLF-L-K-AFTDFLAFMVLFNFIIVFSVMVTVEMQKFLGSFFITWEDMDEETGEGPLVNTSDLNEELQVYIFSDKTGTTLTQNVME			425
HsATP11C	-----ETLKV-L-K-MFTDFLSFMVLFNFIIVFSVMVTVEMQKFLGSFFITWEDMDEETGEGPLVNTSDLNEELQVYIFSDKTGTTLTQNVME			423
HsATP8B1	-----DT-PSY-R-GFLIFWGYIIVLNTMVIISLYVSEVIRLQSHFINWDLQMYAEKDPAKARTTTLNEELQIIEYIFSDKTGTTLTQNVME			465
HsATP8B2	-----DS-AFF-S-GFLSFSYIILNTVVIISLYVSEVIRLQSHFINWDLQMYAEKDPAKARTTTLNEELQIIEYIFSDKTGTTLTQNVME			422
HsATP10A	-----LS-PVT-A-AVYSFLTMIIVLQVLIPIISLYVSEIVKACQVYFINWDMQYDEETDSQLQCRALNITEDIQIIEYIFSDKTGTTLTQNVME			438
AtALA2	-----PWY-E-LLVIPLRFELLCSTMIPIISIKVSLDLVGLYAKFITWEDMDEETGEGPLVNTSDLNEELQVYIFSDKTGTTLTQNVME			392
AtALA10	FFDPER-APM-A-AIYHFFATMLYSYFPIISLYVSEIVKVLQYKFINRDIHMYYEETDKPAQARTSNLNEELQVYIFSDKTGTTLTQNVME			441

	N domain	
CtDNF1	FKKATINGQFYGEAYTEAQAG--M--DRRRGINVEEEA-----KVIREEIAAAKVR-----AIRGL	669
CtDRS2	FKACSIAGVMYAESVPEDR-V--A--TIEDGV-----	656
ScDRS2	FKSCSIAGHCYIDKIPEDK-T--A--TVEDGI-----	598
ScDNF1	FKKCTINGVSYGRAYTEALAG--L--RKRQGIDVETEG-----RREKAEIAKDRDT-----MIDEL	730
ScDNF2	FKKCTINGVSYGRAYTEALAG--L--RKRQGVVVESEG-----RREKEEIAKDRDT-----MIDEL	775
BtATP8A2	FKKCSIAGVTYGHFPELTR-E--P--SSDDFS--R-----	427
HsATP8A1	FKKCTIAGVAYGHVPEPEDYD--C--SPDEWQ-----	448
HsATP8A2	FKKCSIAGVTYGHFPELAR-E--P--SSDDFC--R-----	467
HsATP11A	FKKCCIEGHVYVPHVICNGQV--L--PESSGID-----	454
HsATP11C	FIECCIDGHKYGKVTQ--EVDG--L--SQTDGTL-----	451
HsATP8B1	FKKCCINGQIYGDHRDASQ--H--NH-NKIEQVDFS-----	496
HsATP8B2	FNKCSIAGHSYGDVDFVLG--HKAELGERPEPVDFS-----	456
HsATP10A	FRRCTVSGVEYSHDANAQR--L--ARYQEADEEEVVRGGSVSRQSGISG----HQSVRVVHRTQSTKSHRRTGSRAEAKRASMLSKH	520
AtALA2	FRCCIGIFYGNENGD-----	410
AtALA10	FKKCSIAGKAYGRGITEVERA--M--AVRSGGS-----PLV-----NEDLDV-----VVD--	482

	N domain	
CtDNF1	RELHDNPFYLHDEDMTFTA--PDFVEDLAGK-----NPGPEQQQATEHFMALALALCHTVVAEKQ-----	724
CtDRS2	-----EVGIHDFKRLKDNLKN-----GHPTAQAIIDHFLTLLATCHTVIPEQK-----	698
ScDRS2	-----EVGYRKFDDLLKKLND-----PSDEDSPIINDFLTLLATCHTVIPEFQ-----	641
ScDNF1	RALSGNSQFYPEEVTFVS--KEFVRDLKGA-----SGEVQQRCEHFMALALALCHSVLVEAN-----	785
ScDNF2	RMSDNTQFCPEDLTFVS--KEIVEDLKGS-----SGDHQKQKCEHFMALALALCHSVLVEPN-----	830
BtATP8A2	-----IPPPSDSCDFDD--PRLLKNIED-----NHPTAPCIQEFLLTLAVCHTVVPERD-----	475
HsATP8A1	-----NS-QFGDEKTFDD--SLLLENLQN-----NHPTAPIICEFLTMMAVCHTAVPERE-----	495
HsATP8A2	-----MPPPCSDSCDFDD--PRLLKNIED-----RHPTAPCIQEFLLTLAVCHTVVPEKD-----	515
HsATP11A	-----MID-----SSPS-----V--NGREREELFRALCLCHTVQVKDDSDVDGP-----	492
HsATP11C	-----TYFD-----K-----VDKNREELFRALCLCHTVEIKTNDAVD-----	484
HsATP8B1	-----WNTYADGKLAFYD--HYLIEQIQS-----G--KEPEVRQEFLLAVCHTVMVDR-----	541
HsATP8B2	-----FNPLADKKFLFD--PSLLEAVKI-----G--DPHTHEFRLLSLCHTVMSEEK-----	501
HsATP10A	TA-----FSSPM--EKDITPD--PKLLEKVSCEDKSLAVARHQEHLHLAHLSPESLSDVDF--FIALTICNTVVVTSVDQ---PRTKVRVRFELKSPVKTI	606
AtALA2	-----LKD--AQLLNLAITS-----GSTDVIRFLTVMAICNTVLPVQ-----	444
AtALA10	-----QSGPKVKGFNFD--ERVMMGNWV-----RQPEAAVLQKFRLLAVCHTAVPETD-----	530

	N domain	
CtDNF1	-----	738
CtDRS2	-----DSGEIKYQASSP	710
ScDRS2	-----SDGSIKYQASSP	653
ScDNF1	-----PDNPKKLDLKAQSP	799
ScDNF2	-----KDDPKKLDIKAQSP	844
BtATP8A2	-----GDSIVYQASSP	486
HsATP8A1	-----GDKIYQASSP	506
HsATP8A2	-----GDNIYQASSP	526
HsATP11A	-----RKSPDGGKSCVYISSP	509
HsATP11C	-----G--ATESAELTYISSP	499
HsATP8B1	-----TDGQLNYQASSP	553
HsATP8B2	-----NEGELYKAQSP	513
HsATP10A	EDFLRRFTPSCLTSGCISIGSLAANKSSHKLGSFSPSTPSSDGMLLRLEERLQOPTSAIASNGYSSQADNWAELAQE--QESERELRYEASSP	698
AtALA2	-----SKAGDIVYKAQSQ	457
AtALA10	-----EESGNVSYEASSP	543

	N domain	
CtDNF1	DEAALVATARDMGFTVLGMSDGGIN--VNV-----MGKDMHFVLSIIEFNSSRKRMSSTIVRM--P-----DGRILLFCCKGADSVIYSLRKKG	817
CtDRS2	DEGALVEGAVQLGYRFLARKPRAVI--ITV-----NGQLEYELLAVCFENSTRKRMSSTIYRC--P-----DGKIRIYCKGADTVILERLNDQ	789
ScDRS2	DEGALVQGGADLGKFIIRKPNVSVTLLEE-----TGEEKEYQLNICEFNSTRKRMSAIFRF--P-----DGSIKLFCCKGADTVILERLDD	734
ScDNF1	DEAALVATARDVGFSGVFKTKKGLI--IEM-----QGIQKEFEILNLEFNSSRKRMSSTIVKI--PGLNPGDEPRALLICKGADSVIYSLRSRQ	884
ScDNF2	DESALVSTARQLGYSFVGSSKGLI--VEI-----QGVQKEFOVLNVLEFNSSRKRMSSTIYKI--PGSTPKDEPKALLICKGADSVIYSLRLDRT	929
BtATP8A2	DEAALVKGARKLGFVFTARTPPYSVI--IEA-----MGQEQTFGLNVLEFNSSDRKRMSSTIVRT--P-----SGQLRLYCKGADNVI FERLSKD	565
HsATP8A1	DEGALVRAAKQLNFVFTGRTPDSVI--IDS-----LGQEERYELLNLEFNSTRKRMSSTIVRT--P-----SGKRLRYCKGADTVIYDRLAET	585
HsATP8A2	DEAALVKGAKKLGFVFTARTPPFSVI--IEA-----MGQEQTFGLNVLEFNSSDRKRMSSTIVRT--P-----SGRLRLYCKGADNVI FERLSKD	605
HsATP11A	DEVALVEGQRLGFTYLRKLDNYME--ILN-----RENHIERFELLEILSDSVRRRMSSTIVKS--A-----TGEIYLFCKGADSVIFPRVIEG	589
HsATP11C	DEHALVKGAKRYGFTPLGNRNGYMR--VEN-----QRKEIEEYELNHTLNDAVRRRMSSTIVKT--Q-----EGDILLFCCKGADSAVFPVRQNH	579
HsATP8B1	DEGALVNAARNFGFAFLARTQNTIT--ISE-----LGTERTYVLAALDENS DRKRMSSTIVRT--P-----EGNIKLYCKGADTVIYERLHRM	632
HsATP8B2	DEGALVTAARNFGFVFRSRTPKTIT--VHE-----MGTAITYQLAALDNNIRKRMSSTIVRN--P-----EGKIRLYCKGADTILLDRLHHS	592
HsATP10A	DEAALVYAARAYNCVLERLHDQVS--VEL-----PHLG--RLTFELHTLCLDSVRRRMSSTIVRH--PL-----TDEINVTYKGDSSVMDLQPC	779
AtALA2	DEDALVIAASKLHMVFGKNANLLE--IRF-----NGSVIRYEVLEILFETS DRKRMSSTIVKDCQ-----NGKILLCKGADSAI LPYARA	536
AtALA10	DEAALVVAAREFGFEFNRTQNGIS--FRELDLVSGEKVERVYRLNVLEFNSTRKRMSSTIVRD--D-----DGKLLLSCKGADNVMFERLAKN	628

	N domain	P	
CtDNF1	E-----QADMRRETAQHLEMFAVEGLRRLCIAERELSEEEYERWREHDLAATAL--ENREKLEEVADKIRDLTLLEGTAIEDRLQDQVVP		902
CtDRS2	N-----PHVDQTLRHLEEYASEGLRRLCLAFREVPQEFQEWYQVYDKAOTTVGGTRAQELDKAAEIEPKDFYLLGATAIEDRLQDQVVP		873
ScDRS2	A-----NQVVEATMRHLEDYASEGLRRLCLAMRDISGEYEWNSIYNEAATTL--DNRAEKLDEAANLEPKNLLIIGATAIEDRLQDQVVP		818
ScDNF1	SG-----SNSEAI LEKTALHLEQYATEGLRRLCIAQRELSWSEYKWKNEYDIAAASL--ANRDELEVVADSIRELILLEGTAIEDRLQDQVVP		972
ScDNF2	QN-----DATALLEKTALHLEEYATEGLRRLCIAQRELTWSYERWVVKYDVAASV--TNRREELDKVTDVIRELILLEGTAIEDRLQDQVVP		1015
BtATP8A2	S-----KYMEETLCHLEYFATEGLRRLCVAYADLSEYEWLKVYQEAATIL--KDRARQLLEECYIEIPKNLLLLGATAIEDRLQDQVVP		648
HsATP8A1	S-----KYKEITLKHLEQFATEGLRRLCFAVAEISESDFQEWRAVYQRASTSV--QNRLLKLEESYELIPKNLQLLGGATAIEDRLQDQVVP		668
HsATP8A2	S-----KYMEETLCHLEYFATEGLRRLCVAYADLSEYEWLKVYQEAATIL--KDRARQLLEECYIEIPKNLLLLGATAIEDRLQDQVVP		688
HsATP11A	-----KVDQIRARVERNAVEGLRRLCVAYADLSEYEWLKVYQEAATIL--KDRARQLLEECYIEIPKNLLLLGATAIEDRLQDQVVP		670
HsATP11C	-----EIELTKHVVERNAMDGYRRLCFAVKEIAPDDYERINRQLIEAKMAL--QDREKMEKVFDDIETNMNLIGATAIEDRLQDQVVP		660
HsATP8B1	N-----PTKQETQDALDIFANETRLCLCYKEIEKEFEWNNKFFMAASVAS--TNRDEALDKVYEEIPKDLILLGATAIEDRLQDQVVP		715
HsATP8B2	T-----QELLNFTMDHLNEYAGEGLRRLVLAAYKDLDEEYEWAEARLQASLAQ--DSREDRLASIYEEVNNMMLLGGATAIEDRLQDQVVP		676
HsATP10A	SSVDARGRHQKIRSKTQNYLNVYAAEGLRRLCIAKRVLSKEYEACWLQSHLEAESSL--ENSEELLFQSAIRLETNLHLLGATGIEDRLQDQVVP		872
AtALA2	-----GQQ--TRTIGDAVEHYSQLGLRRLCLAWRELEENEYLEWVSKFKEASSL--VDREWRIRAEVQQRLEHDLYILGATAIEDRLQDQVVP		619
AtALA10	-----GRQFEAKTQEHVNQYADAGLRLVLAAYREVDENEYIEFNKSFNEAKASVSEDRREALIDETDKMIRDLILLGATAIEDRLQDQVVP		713



TM10

CtDNF1 FYQA-APQVYQELTFWMCLIVTPALCLLPRLLVVKCIQKQRFPYDVDIIREQAN--R--GDFAAAD-----AAAV-----AALGGPE 1362  
 CtDRS2 FFEV-IPRLFSNPSFWLQMPTLAILCLARDFAWKFSKRLWKPEAYHHVQEIQK--Y--NI----- 1300  
 ScDRS2 YYGV-VKHTYGSVFWLTLVLPPIFALVDRFLWKYKRMYPETVHYVQEMQK--Y--NI----- 1246  
 ScDNF1 FFKA-AARIYGAPSFVAVFFVAVLFCLLPRFTYDSQKFFYPPTDVEIVREMWO--H--GHFDHYP-----PGYDPTDPNRPKVKAGQHGEK 1446  
 ScDNF2 FYKG-AARVFAQPAYWAVLFGVGLFCLLPRFTIDCIRKIFYPKDIEIVREMWL--R--GDFDLYP-----QGYDPTDPSRPRINEIRPLT-D 1488  
 BtATP8A2 MKGQ-ATMVLSSAHFWLGLFLVPTACLIEDVAWRAAKHTCKKTLLEEVQEM--K--SR----- 1072  
 HsATP8A1 MSGE-AAMLFSGGVFWMGLLFIPVASSLLLDVVYKVIKRTAFKTLVDEVQLEA--K--SQ----- 1092  
 HsATP8A2 MRGQ-ATMVLSSAHFWLGLFLVPTACLIEDVAWRAAKHTCKKTLLEEVQELT--K--SR----- 1112  
 HsATP11A MYVY-FIQMLSSGPAWLAIVLLVTISLLPDVLLKVLQRQLWPTATERVQTKSQ--C--LSVE----- 1118  
 HsATP11C MYFV-FAQMLSSVSTWLAIIILLIFISLFFPELLIVLKNVRRRSARRNLSRRASDS--LSAR----- 1114  
 HsATP8B1 FTGT-ASNALRQPYIWLTIILAVAVCLLPPVAIRFLSMTIWPESDKIQKHKR--R--LK----- 1186  
 HsATP8B2 FVGN-AQNTLAQPTVWLTIVLTTVVCIMPVAVFRFLRLNLKPDLSDTVRYTQL--VRKKQK----- 1129  
 HsATP10A PYWT-MQALLGDPVFYLTCLMTPVAALLPRLFFRSLOGRVFPPTQLQLARQLTR--K--SPRCSAPKETFAQGRLPKDS----- 1334  
 AtALA2 MYTI-MFRLCSPSYWITMFLIVGAGMGPIFALKYFRYTYRPSKINILQQAER--M--GGPIL-----TLG----- 1036  
 AtALA10 AYMVFLEALAPAPSYWLTTLFVMIFFALIPYFVYKSVQMRFFPKYHQMIQWIRY--E--GHSN----- 1157

CtDNF1 RVEGESLG-SLSSSGKSGRSKSKKHQQYASVDEDRRPIYPPSIATHNTRAQNGSD-----GTT----- 1420  
 CtDRS2 ----- 1420  
 ScDRS2 ----- 1420  
 ScDNF1 IIEGIALSDNL---GGSNYSR-----DSVVTIEIPMTF-MH-----GED-----GSPSGYQKQETW-----M 1494  
 ScDNF2 FKEPISLDTHF---DGVSHSQ-----ETIVTIEIPMSI-LN-----GEQ-----GSRKGYRVSTTLERRDQLSPVTTTN 1548  
 BtATP8A2 ----- 1420  
 HsATP8A1 ----- 1420  
 HsATP8A2 ----- 1420  
 HsATP11A ----- 1420  
 HsATP11C ----- 1420  
 HsATP8B1 ----- 1420  
 HsATP8B2 ----- 1420  
 HsATP10A ---GT---EHSSGR-----TV-KTSVPLSQPS-----WHTQQPVCSEASGEPSS----- 1371  
 AtALA2 ---N---IETQPR-----TIEKDLSPISITQ-----PKNRSPVY----- 1064  
 AtALA10 -----DPE----- 1160

CtDNF1 -----YIMQSRTS-----TELQQEMPFDREETPAVR-PSIERTRPSYDRIRR--SIDRVRPSEASNDFT 1480  
 CtDRS2 -----QDYRP-----RME---QFQK---AIRKVR----- 1318  
 ScDRS2 -----SDSRP-----HVQ---QFQN---AIRKVR----- 1264  
 ScDNF1 TSPKETQDLLQSPQFQQAQTFG-----R-----GPSTNVRS--SLDRTREQMIATNQLD 1541  
 ScDNF2 NLPRRSMASAR-----G--NKLRT--SLDRTREEMLANHQLD 1581  
 BtATP8A2 -----VMGRAMLR-DSNGKRMNERDRLK--RLSRKTP----- 1102  
 HsATP8A1 -----DPGAVVL-----GKSLTERAQLLK--NVFKKNH----- 1118  
 HsATP8A2 -----VLGKAVLR-DSNGKRLNERDRLIK--RLGRKTP----- 1142  
 HsATP11A -----QSTI----- 1122  
 HsATP11C -----PSVR----- 1118  
 HsATP8B1 -----AE-----EQWQRROQVFR----- 1199  
 HsATP8B2 -----AQ-----HRCMR--RVGR----- 1140  
 HsATP10A -----TVDMSPVREHTLLEGLSAP-APMSSAPGEAVLR-SPGGCPEESKVR-A--STGRVTP----- 1425  
 AtALA2 -----EP-----LLSDSPN-ATR-----R--SFGPGTP----- 1084  
 AtALA10 -----FV-----EMVRQR-SIRPTTVGYTARR-AA----- 1183

CtDNF1 SAARL-----SRIESTHSSL--GHTYSHQRESYAG--ESSGAQQGQEP--QORRFNLATVRKRGLSAFSSKKSIDTT--EGE----- 1548  
 CtDRS2 -----QVQRM--RKQRGYAFSMA--DE-----SQTR--VLQAY-----DTT--RHR----- 1351  
 ScDRS2 -----QVQRM--KKQRGYAFSQA--EEG-----GQEK--IVRMY-----DTT--QKR----- 1298  
 ScDNF1 NRYSV-----ERARTSL-----DL-----PGVTN-----A-----ASL-----I--GTQ----- 1568  
 ScDNF2 TRYSV-----ERARASL-----DL-----PGINH-----A-----ETL-----L--SQR----- 1608  
 BtATP8A2 --PT-L-----FRGSSLQQSMPHGYAFSQE--EHGA--V-----TQEE--IVRAY-----DTT--KQK----- 1144  
 HsATP8A1 --VN-L-----YRSESLQQNLLHGAYAFSQD--ENGI--V-----SQSE--VIRAY-----DTT--KQR----- 1160  
 HsATP8A2 --PT-L-----FRGSSLQQGVPHGYAFSQE--EHGA--V-----SQEE--VIRAY-----DTT--KKK----- 1184  
 HsATP11A --FML-S-----QTSSS-----LSF----- 1134  
 HsATP11C --PLL-L-----RTFSD-----ESN-----VL----- 1132  
 HsATP8B1 -----RGVST--RRSAYAFSHQ--RGYADLI-----SSGRSIRKKRS-----PLDAIVADGTAE-- 1244  
 HsATP8B2 -----T-GSRRSGYAFSHQ--EGFGELI-----MSGKNMRLSSL-----ALSSFTTRSSSSWI 1185  
 HsATP10A --LSSLFSLPTFSLNWISSWLVSRRLGSLVQLQFSRT--EQLADGQ--AGRG-----L-----PVQ--PHS----- 1477  
 AtALA2 --FE-F-----FQSQSR--LSSSSGYTRNCK--D----- 1106  
 AtALA10 -----SV-----RRS----- 1188

CtDNF1 ----PPREP-----PM----- 1555  
 CtDRS2 ----GR-YGEMAS-----SRT-IGI-----AQ----- 1367  
 ScDRS2 ----GK-YGELQDASANPFNDNGLGNSDFESAEPFIENPFADGNQNSNRFFSSSRDDI-----SF-DI--- 1355  
 ScDNF1 ----Q-N-N----- 1571  
 ScDNF2 ----S-RDR----- 1612  
 BtATP8A2 ----SR-KK----- 1148  
 HsATP8A1 ----PD-EW----- 1164  
 HsATP8A2 ----SR-KK----- 1188  
 HsATP11A ----- 1118  
 HsATP11C ----- 1118  
 HsATP8B1 --YR-R-TGD-----S----- 1251  
 HsATP8B2 ESLRRK-KSDSAS-----SPS-GGA-----DK-PLKG- 1209  
 HsATP10A ----GR-SG-----L-QGPDHRLLIASSRRSQ 1499  
 AtALA2 -----N----- 1107  
 AtALA10 ----AR-FHDQIY-----KDL-VGV----- 1202

Fig S2

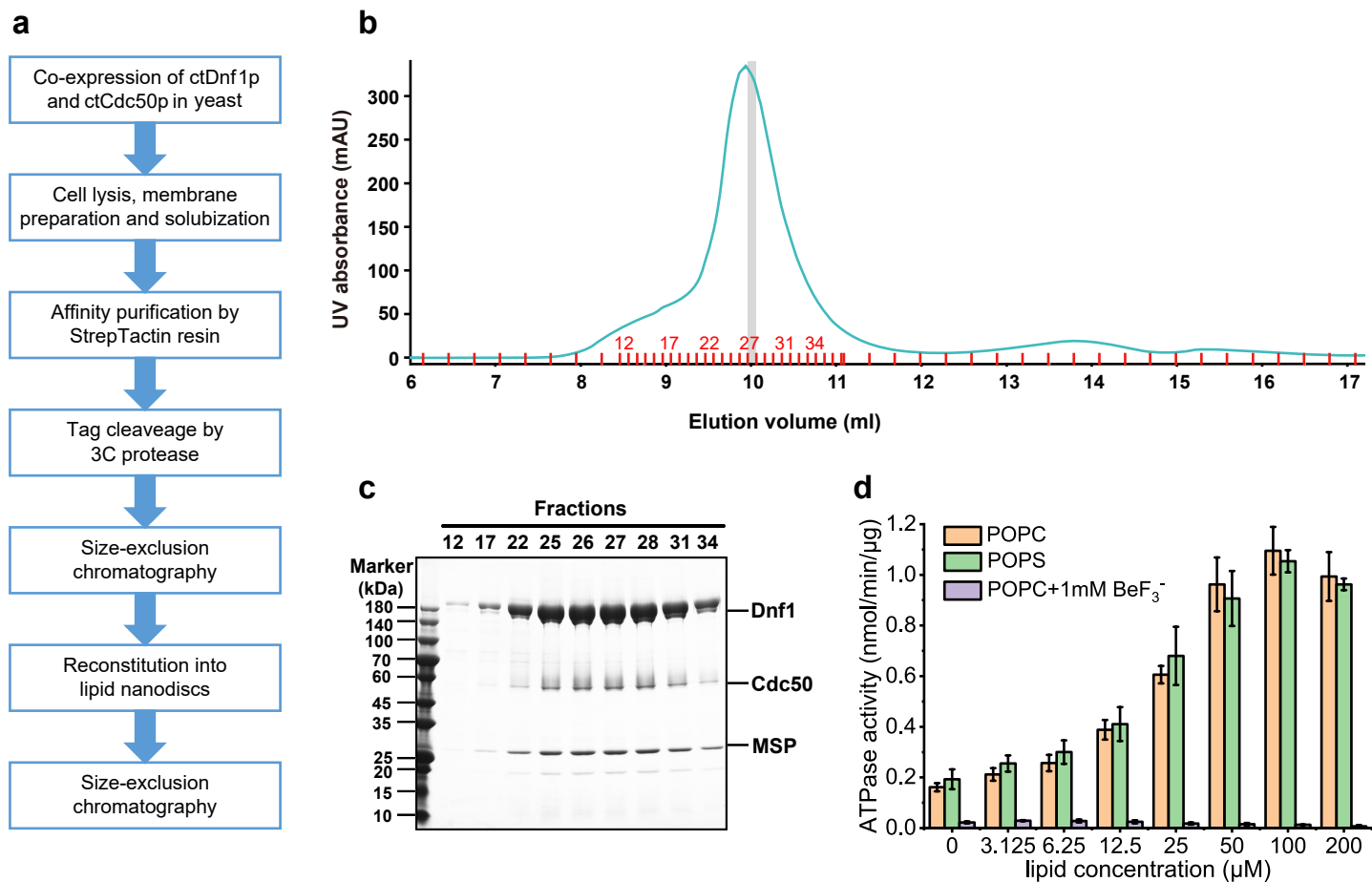
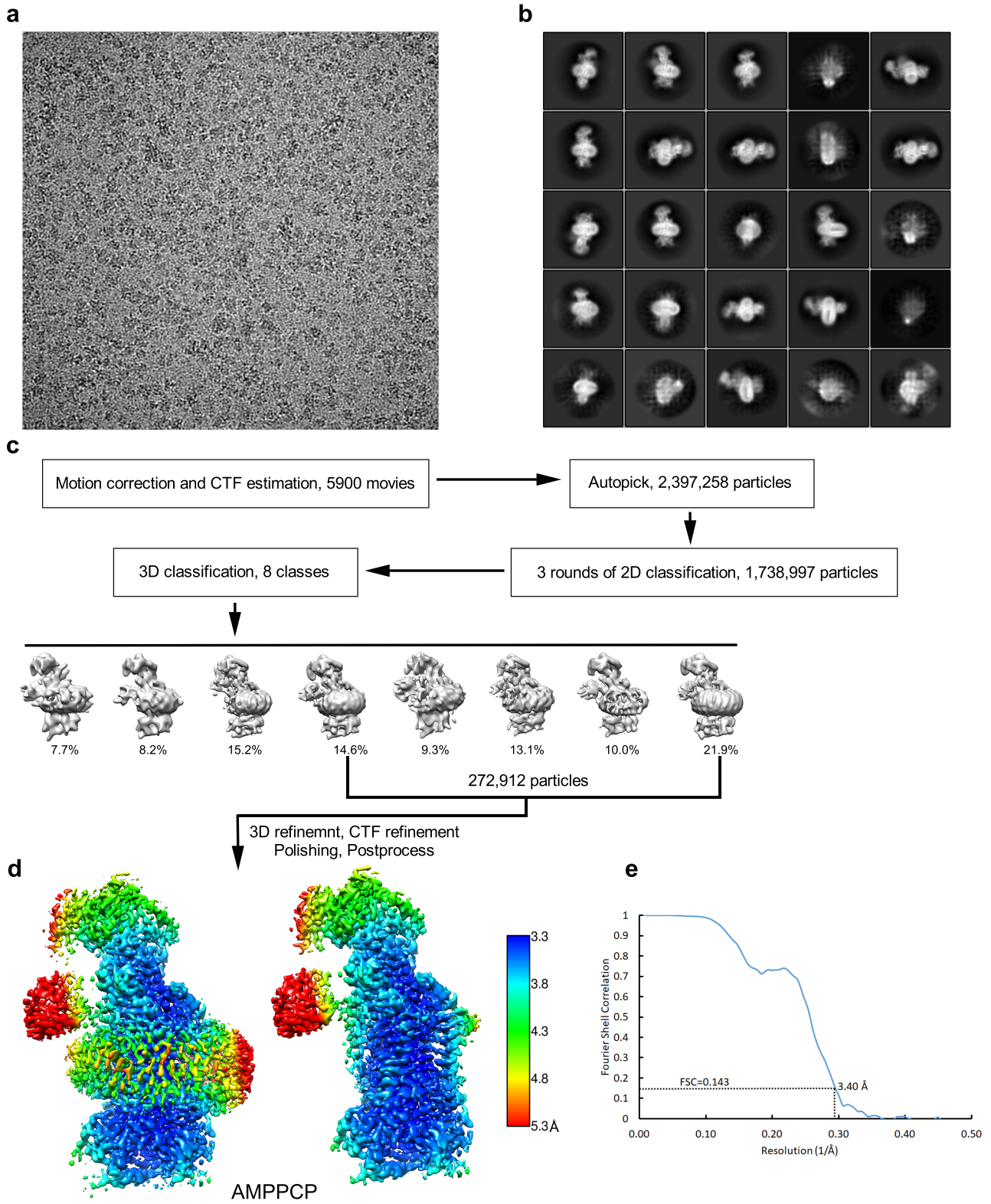
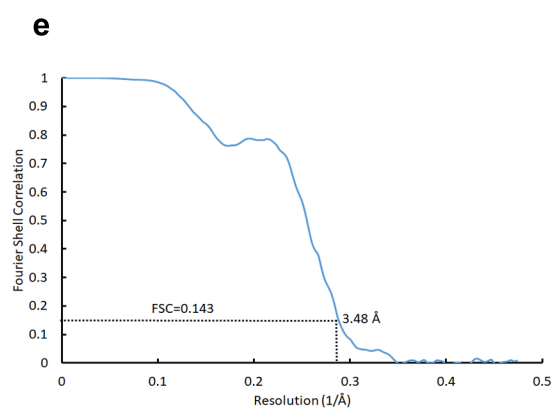
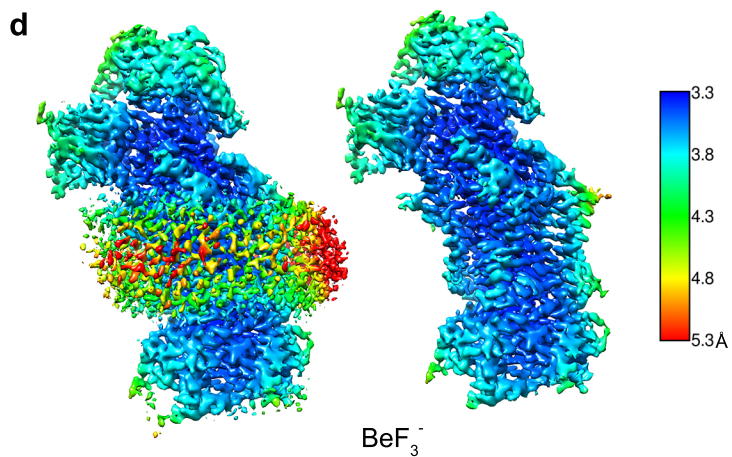
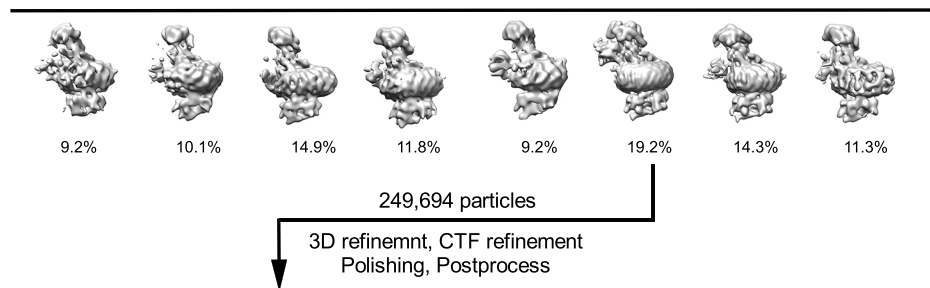
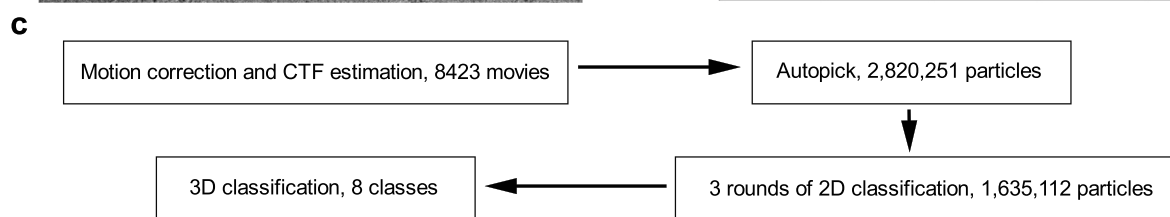
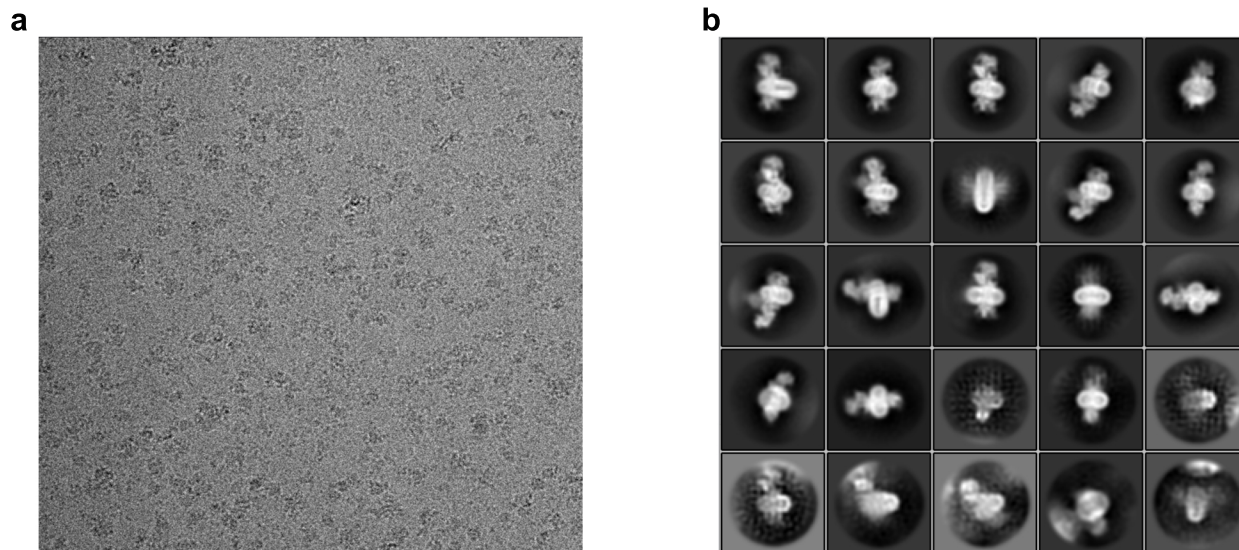
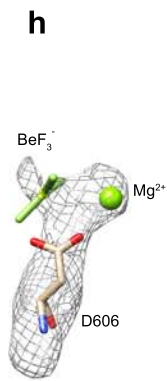
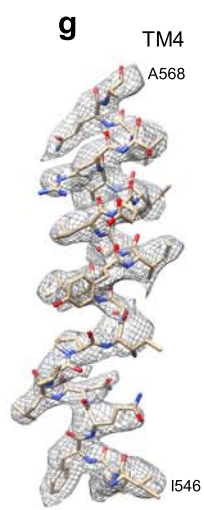
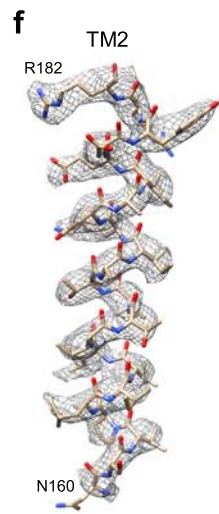
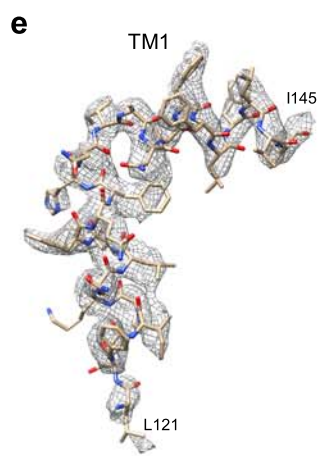
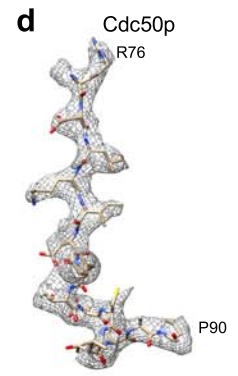
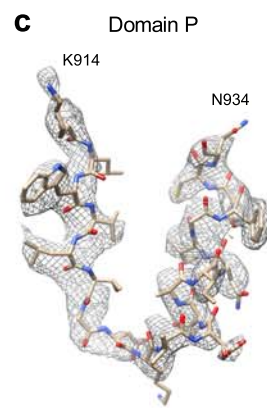
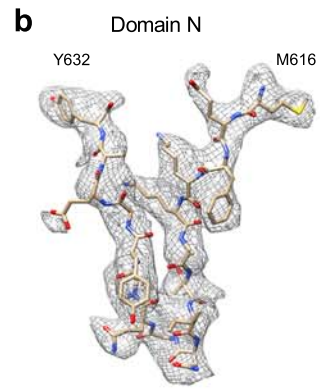
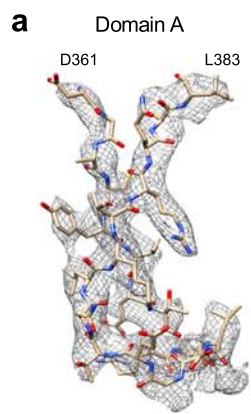
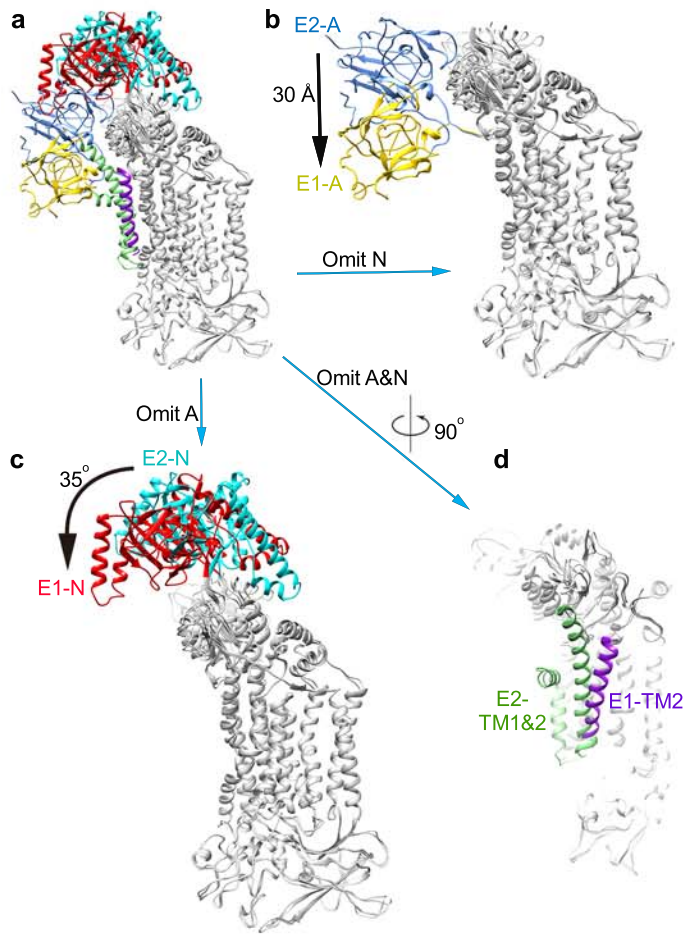


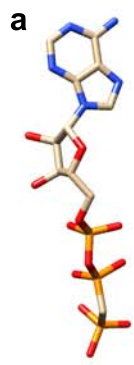
Fig S3



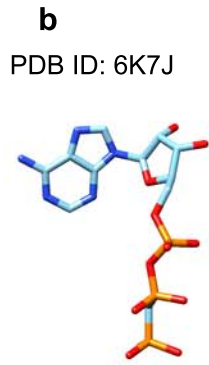




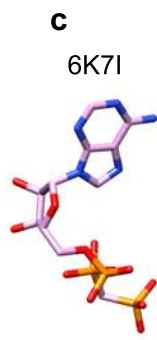




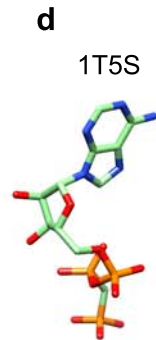
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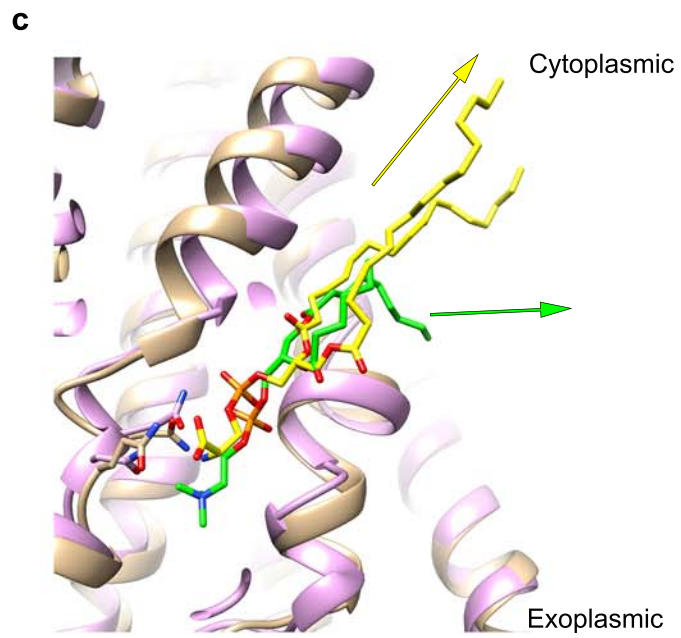
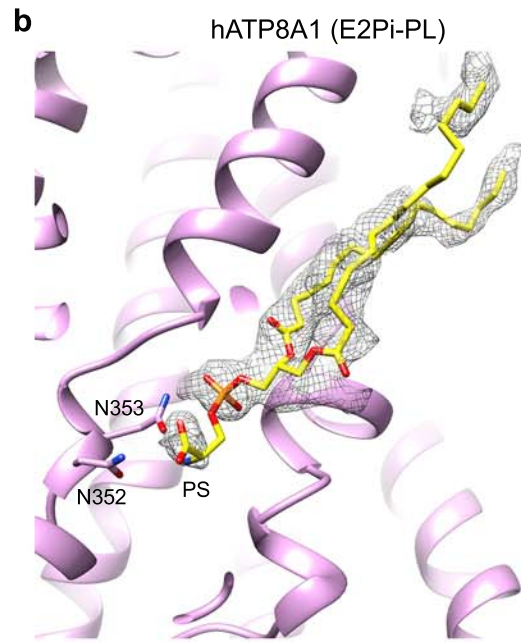
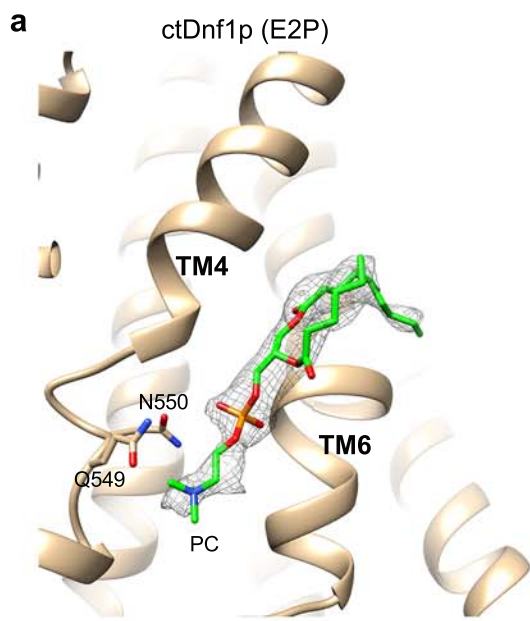
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E1-ATP  
(class1)

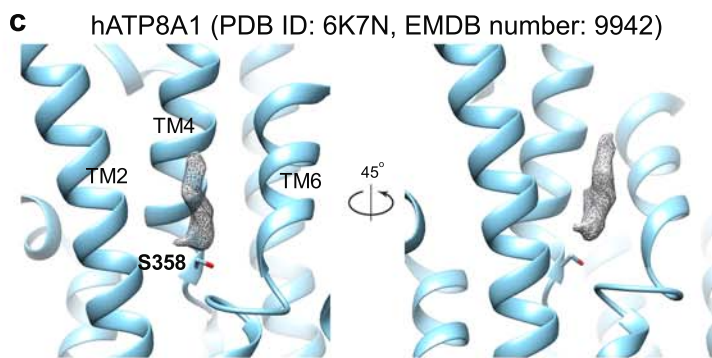
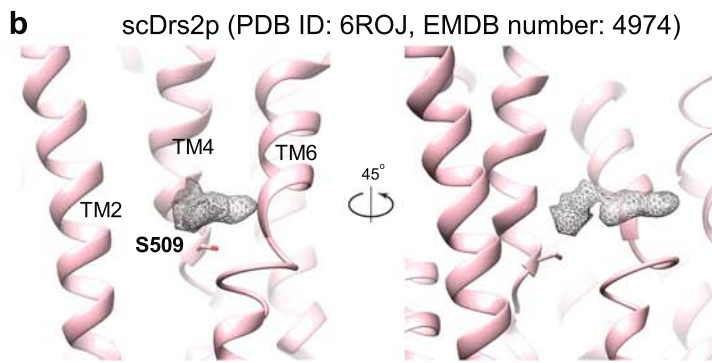
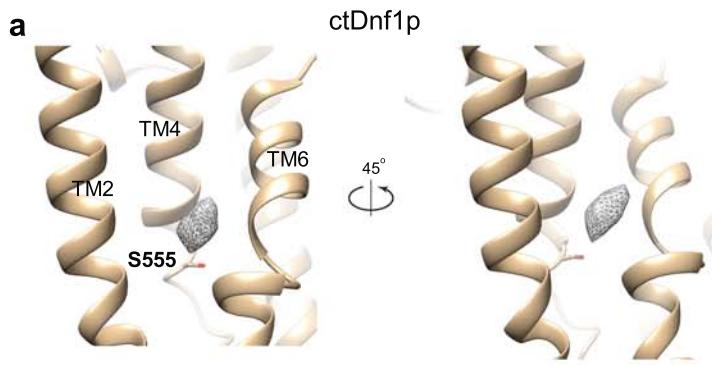


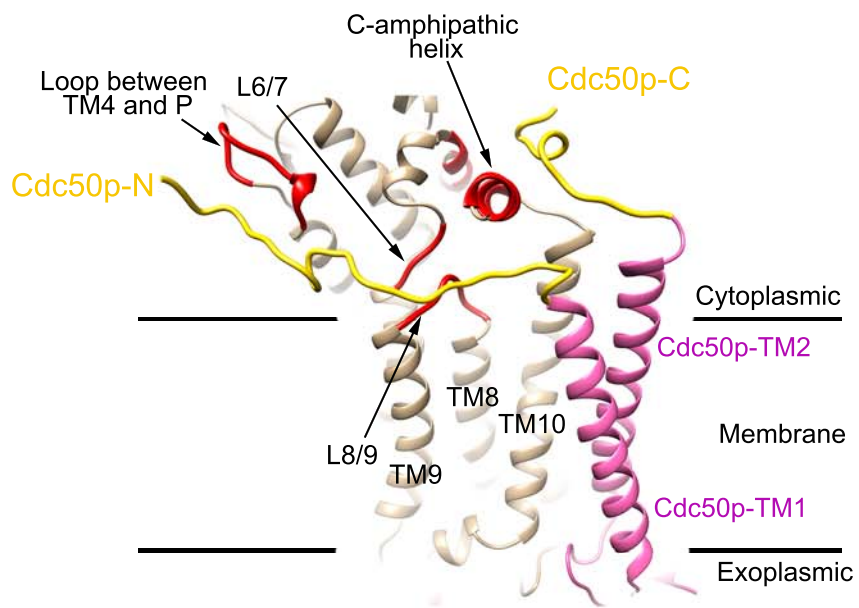
hATP8A1  
E1-ATP  
(class2)

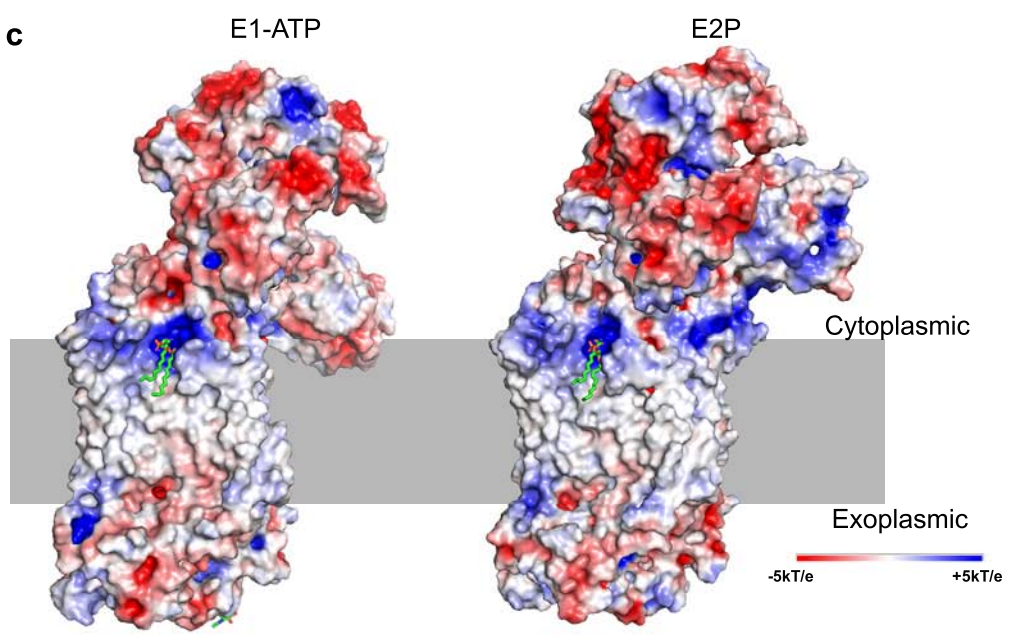
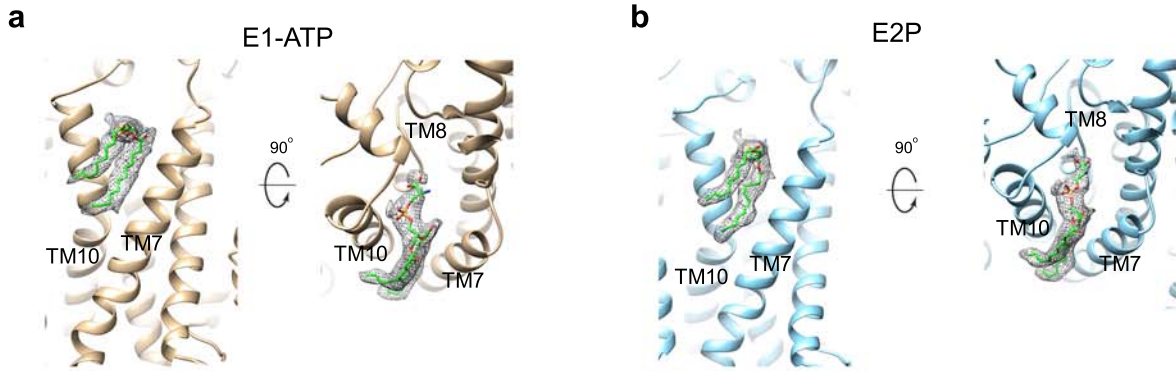


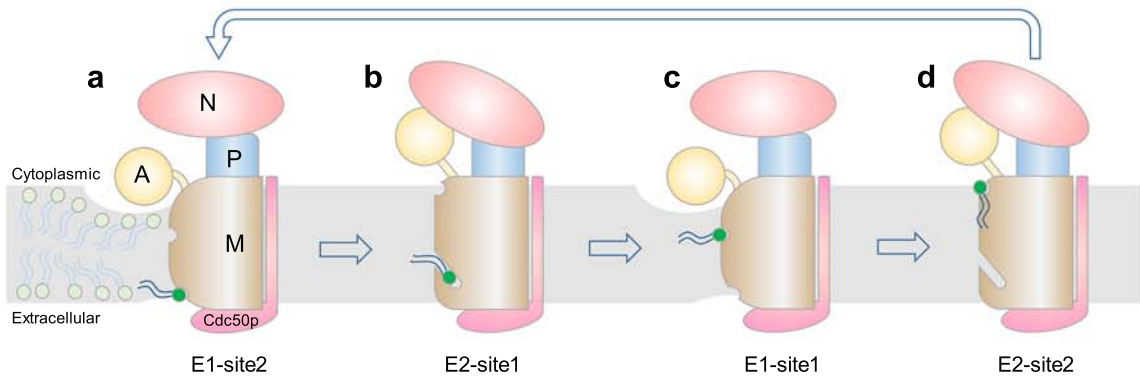
SERCA  
E1-ATP



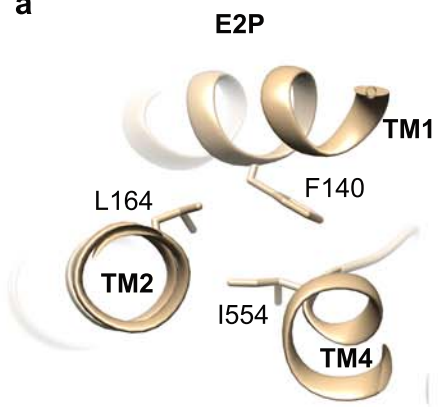








**a**



**b**

