The summary of comparison between homology models between the models built by Alpha Fold 2 and the model built by a traditional method

1. The available crystal structure on Protein Data Bank.

There are not many crystal structures of ATPase available on the Protein Data Bank. Via blast, I found that the crystal structure of the Ca^{2+}-ATPase with BeF is the most similar with *PfATP4*. The PDB ID is 6ZHF. The organism is *Listeria monocytogenes.* The length of 6ZHF is 1035aa. The identity between 6ZHF and *PfATP*4 is 30.0483, and the similarity is 46.57. Figure 1 shows the 6ZHF structure. In figure 1A, the blue and red dots show the lipid bilayer which is also from the crystal structure. In the figure 1A, the blue dots show the lipid layer between the cytosol and SR membrane, the red dots show the lipid layer between the SR membrane and lumen. However, there is no ligand bound to this structure. We cannot tell where the binding pocket is. In 2020, there was released a crystal structure of the Ca^{2+}-ATPase bound to an inhibitor. The resolution of this crystal structure is 3.40 Å. The organism is *Oryctolagus cuniculus* (Rabbit). The length of 6YAA is 1043aa. The identity between 6YAA and *PfATP4* is 16.7785, and the similarity is 31.4477. Table 1 shows the comparison information between 6ZHF, 6YAA, and *PfATP4.* The crystal structure 6YAA is crystalized with an inhibitor, which shows the binding pocket. Figure 2A shows the crystal structure of 6YAA. Same as figure 1A, the blue and red dots show the lipid bilayer. The blue dots show the membrane between the cytosol and SR membrane, the red dots show the membrane between the SR membrane and lumen (figure 2A). Figure 2B is the zoomed-in view of the binding pocket, which is around the lipid layer between the cytosol and SR membrane. The pocket entrance is above the lipid layer, and part of the pocket is below the lipid layer (figure 2B). There is a helix (labeled in figure 2B) around the pocket entrance, located around the lipid layer. Comparing these two crystal structures, the binding pockets' entrances are above the lipid layer between the cytosol and SR membrane. Figure 3A shows the two crystal structures superimposed. Figure 3B shows the binding pocket surface zoomed-in view. In the labeled helix of 6YAA (figure 3B), the binding mode is lower than the labeled helix of 6ZHF (figure 2B). Without a ligand however, both helices are around the lipid layer and the binding pocket entrances are above the layer (Figure 1, 2 and 3). The lipid layers in figure 3 are from crystal structure 6ZHF.

2. Alpha Fold 2 homology models

Alpha Fold 2 was used to build homology models. There are three relaxed models, and three unrelaxed models. The differences of RMSD between each are within 1.255 Å (Table 3). The RMSD results are shown in Table 1. Figure 4 shows the superimposed structures of the 6 homology models. All 6 homology model structures are quite similar. In figure 4B the labeled helices are located in a similar location. Here we are using relaxed model 1 to keep discussing the binding pocket of the homology model.

2.1 Alpha Fold 2 homology model and crystal structure 6ZHF

6ZHF has the highest identity and similarity in the available crystal structures. The identity between the Ca^{2+}-ATPase and *PfATP4* is 30.0483. The similarity between the Ca^{2+}-ATPase and *PfATP4* is 46.57 (Table 1). Figure 5A shows the superimposed view between 6ZHF and Alpha Fold 2 model. The RMSD between these two structures is 6.239 Å. The structure details (in figure 5B) show the two helices below the lipid layer. However, the Alpha Fold 2 model's

labeled helix is deeper than 6ZHF's. This means the Alpha Fold 2 model's binding pocket entrance is buried in lipid. This is not the only big difference between 6ZHF and Alpha Fold 2 homology model. Figure 5C is the view from the right side angle of figure 5A. In figure 5C the labeled helices show more difference between the crystal structure and the homology model. The distance between these two labeled helices is around 7 Å (shown in SI figure 3). This shows the Alpha Fold 2 homology model is quite different, especially in the binding pocket region. However, 6ZHF is a crystal structure without ligand.

2.2 Alpha Fold 2 homology model and crystal structure 6YAA

6YAA crystal structure is the Ca^{2+}-ATPase bound to an inhibitor, and the organism is *Oryctolagus cuniculus* (Rabbit). This structure is a very good reference to compare with. The identity and similarity between these two proteins is 16.7785 and 31.4477, respectively (shown in Table 1). These two structures' RMSD is 3.723 Å. Based on RMSD with 6YAA and 6ZHF, this homology model is a quite good result. From the crystal structures' comparison, it shows more details. In figure 6, 6YAA is in light pink, Alpha Fold 2 model is in light gray, and the ligand from 6YAA is in magenta. This ligand shows where the binding pocket is, which is labeled with a black circle in figure 6A. Figure 6B shows the zoomed-in view of the binding pocket. The labeled helix is still the same as the comparison with 6ZHF. The 6YAA helix is around the lipid layer and the Alpha Fold 2 helix is inside of the lipid layer. This homology model's helix makes the pocket be buried by lipid molecules. Figure 6C is the surface of the homology model, it is clearly shown that the pocket entrance is totally buried by the lipid layer. It also shows the difference of the two helices from the figure 6B (SI figure 4).

3. HLN homology model

In figure 7 it shows the HLN homology model with a docked ligand. Figure 7B shows the binding pocket of the HLN homology model. The ligand looks to fit well in the binding pocket.

3.1 HLN homology model and crystal structure 6ZHF

Comparing to the crystal structure of 6ZHF, the RMSD between these two structures is 4.182 Å (Table 2). Figure 8 shows the HLN homology model and crystal structure 6ZHF. Figure 8A shows the superimposed view of the two structures. The transmembrane part of the proteins is similar, especially the region around ligand (figure 8B).

3.2 HLN homology model and crystal structure 6YAA

6YAA was released in 2020-05 and has not been used to build this homology model. Between these two structures the RMSD is 2.060 Å. Based on the RMSD result, these two structures are quite similar. Without taking advantage of 6YAA, the HLN model is already quite a good homology model. The HLN model is more similar with 6YAA than 6ZHF. It is not only the binding pocket that is similar with 6YAA's transmembrane domain, but also the entire protein (figure 9A). In figure 9A the structure 6YAA is in light pink, its ligand is in magenta, the HLN model is in cyan, and its ligand is in turquoise. The binding pocket is labeled by a black circle. The zoomed-in view of the binding pocket is shown in figure 9B. The two labeled helices are both around the lipid layer and around a similar position. The two inhibitors are also around a similar position (figure 9B and 9C). In comparison to 6YAA, the docked HLN model is a quite reasonable result.

4. The Alpha Fold 2 homology model and HLN homology model

Here, I am going to compare two models in this session and use the crystal structure 6YAA as the reference to determine the difference between the homology models. Figure 10 shows the comparison results. Figure 10A shows the superimposed view of the Alpha Fold 2 homology model and the HLN homology model. The lipid bilayer is from the crystal structure of 6YAA. The Alpha Fold 2 homology model is in light gray. The HLN homology model is in cyan. The RMSD value between the two homology models is 4.471 Å. Both are more similar in the transmembrane domain than other regions. Figure 10B shows the superimposed view of the two homology models and 6YAA. 6YAA is in light pink and its inhibitor is in magenta. The binding pocket is labeled with a black circle. As a reference, 6YAA's helix around the binding pocket is around the lipid layer. The helix from the Alpha Fold 2 model is buried by lipid molecules same as was seen before. This causes the binding pocket's entrance to be buried by lipid molecules as well, which is not consistent with the crystal structure. The HLN homology model makes much more sense than the Alpha Fold 2 homology model for this reason. The binding pocket entrance of the HLN homology model is above the lipid layer and part of the pocket is inside of the lipid layer, which is consistent with the crystal structure (figure 9, 10B, 10C, and 10D).

These comparisons are only examining the main backbone of the two models with the crystal structures, 6ZHF and 6YAA. For the Alpha Fold 2 model, there is already a huge difference in the binding pocket even though the RMSD is only 3.723 Å with 6YAA. Since there is no crystal structure of *PfATP4*, consistency with the available crystal structure is important, not just comparing the RMSD values.

5. Alpha Fold 2 homology model's docking

Even though the binding pocket comparison with 6YAA does not perform well, I still performed docking to seek additional information. Here, I used Glide software to dock the inhibitor into the receptor. Figure 11 shows the docking results of the Alpha Fold 2 model. Figure 11A shows the overall results of the Alpha Fold 2 model. The Alpha Fold 2 is in light gray, the inhibitor is in dark gray. Figure 11B shows the zoomed-in view of the binding region. It clearly shows the inhibitor interacts with the receptor, however, the binding region does not seem like a pocket. Half of the ligand surface is exposed to the solvent (lipid layer). Figure 11C is the view from right side looking at the binding region. Figure 11D is the HLN model docked mode. The inhibitor from the HLN model interacts with the receptor very well. Figure 11E is the crystal structure 6YAA. The inhibitor from 6YAA interacts with the receptor very well and is also tightly packed inside the receptor. Compared to the 6YAA and HLN structure, the inhibitor cannot be docked into the Alpha Fold 2 model well. The docking score also shows the Alpha Fold 2 model is not a good model to use with docking. The Glide score of Alpha Fold 2 model is only -4.877. This is the only docking output. I also tried to use other docking software to dock the inhibitor into the Alpha Fold 2 model. The inhibitor cannot even dock into the model. Overall, the HLN homology model performs better than the Alpha Fold2.

Table 1 The available crystal structure comparison with PfATP4

Organisms	PDB ID	Length	Identity	Similarity
Listeria monocytogenes	6ZHF	1035	30.0483	46.57
Oryctolagus cuniculus (Rabbit)	6YAA	1043	16.7785	31.4477

*Table 2 The RMSD between 6YAA, 6ZHF, HLN_model, and AF2_model **

RMSD	6YAA	6ZHF	HLN_model	AF2_model
6YAA	/	3.906 Å	2.060 Å	3.723 Å
6ZHF	3.906 Å	/	4.182 Å	6.239 Å
HLN_model	2.060 Å	4.182 Å	/	4.471 Å
AF2_model	3.723 Å	6.239 Å	4.471 Å	/

* The RMSD values were calculate by PyMOL.

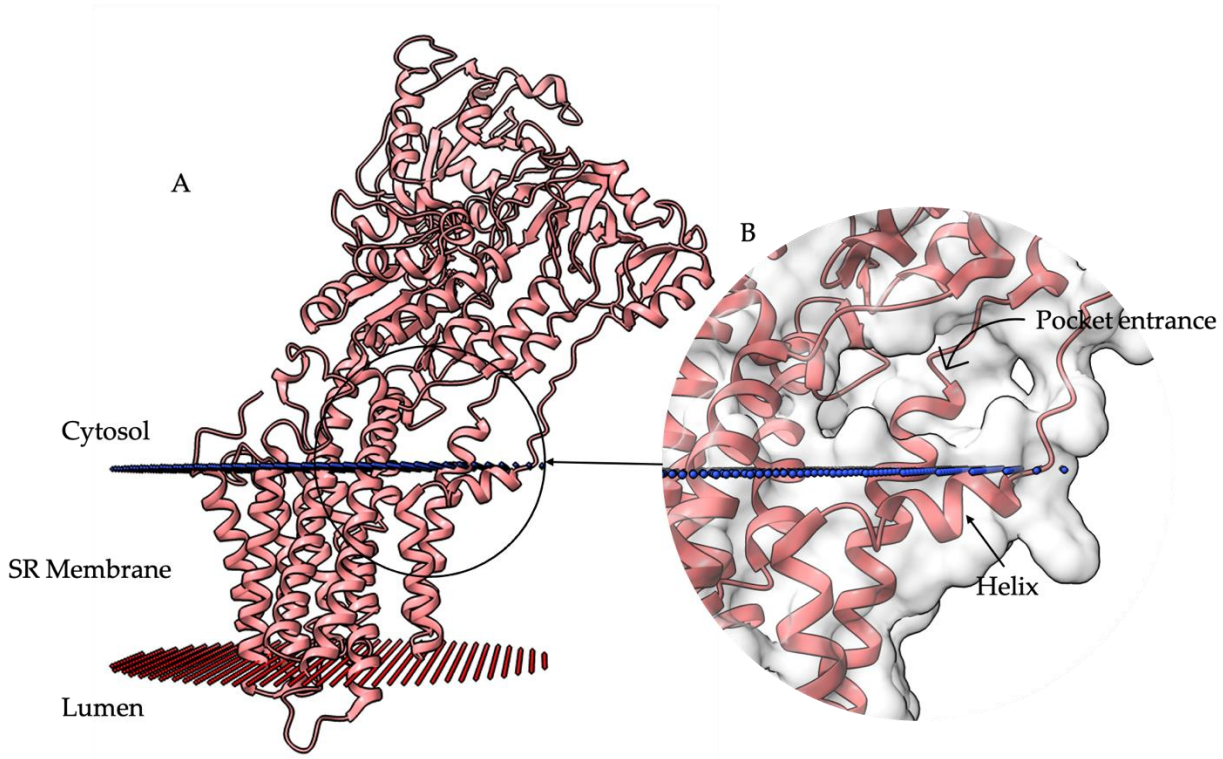


Figure 1 This is the crystal structure of the Ca^{2+}-ATPase with BeF. The organism is Listeria monocytogenes. (PDB ID: 6ZHF) A) shows the ATPase structure. The blue and red dots show the lipid bilayer. B) is the zoomed-in view of the binding pocket surface. The binding pocket's entrance is above the lipid layer and faces the cytosol. The labeled helix is a little bit below the lipid layer.

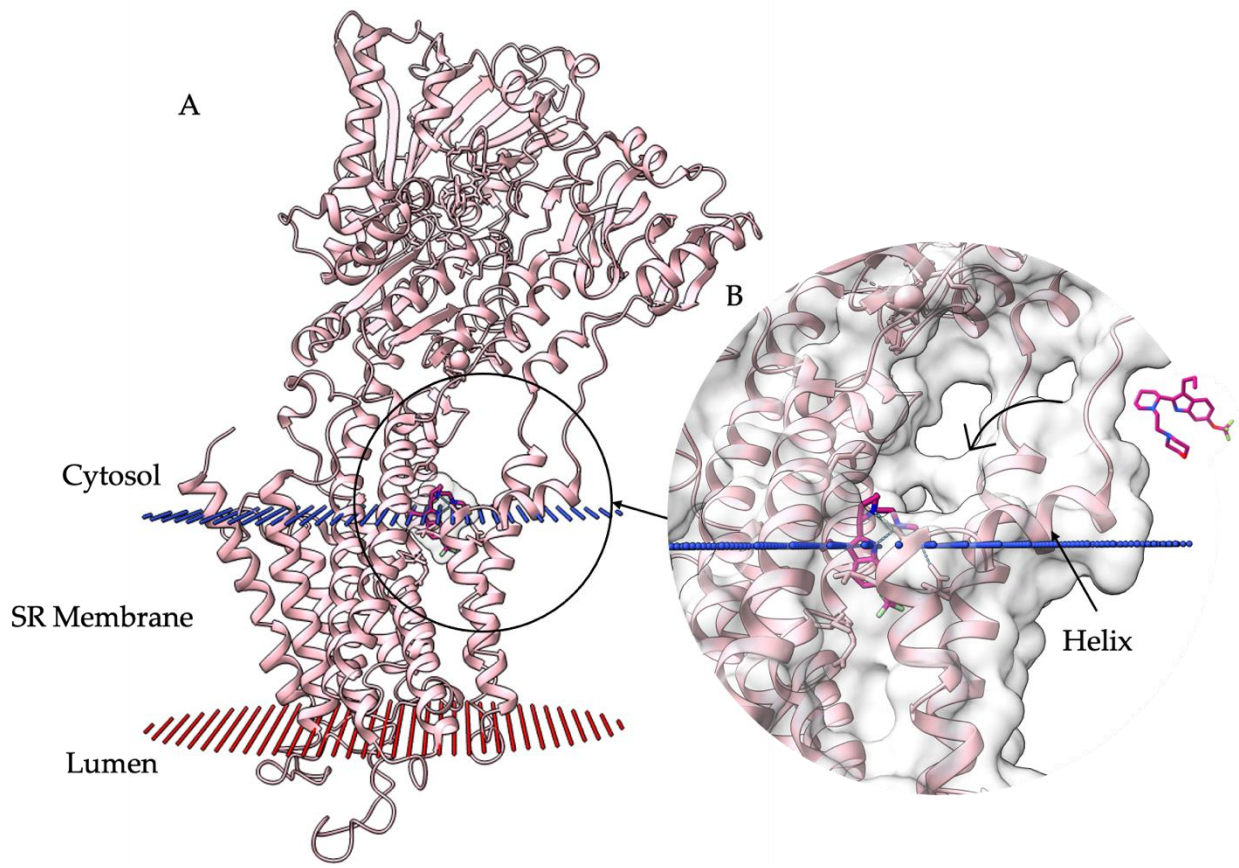


Figure 2 This is the crystal structure of the Ca^{2+}-ATPase bound to the inhibitor compound CAD204520. The organism is Oryctolagus cuniculus (rabbit), PDB ID is 6YAA. A) shows the ATPase crystal structure. The blue and red dots show the lipid bilayer. The inhibitor CAD204520 is in magenta, its surface is in light gray. B) is the zoomed-in view of the receptor binding pocket surface. The binding pocket is around the cytosol side of the lipid layer. The pocket entrance is facing to the cytosol and above the lipid layer.

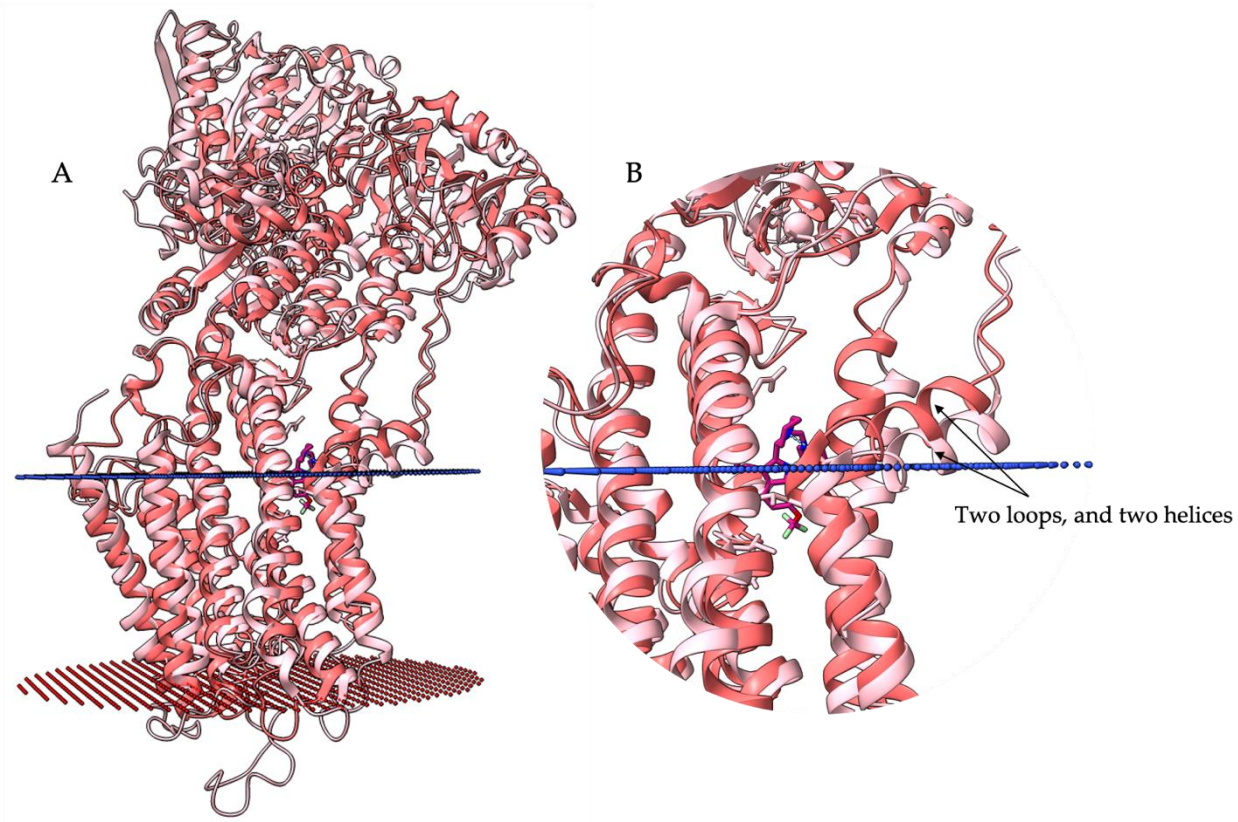


Figure 3 The two crystal structures of the Ca^{2+}-ATPase. 6YAA is in pink. 6ZHF is in salmon. A) shows the two crystal structures superimposed. The blue and red dots show the lipid bilayer. The lipid bilayer location is from the crystal structure 6YAA. B) is the binding pocket zoomed-in view. The loops and helices labeled in B) are both above the lipid layer.

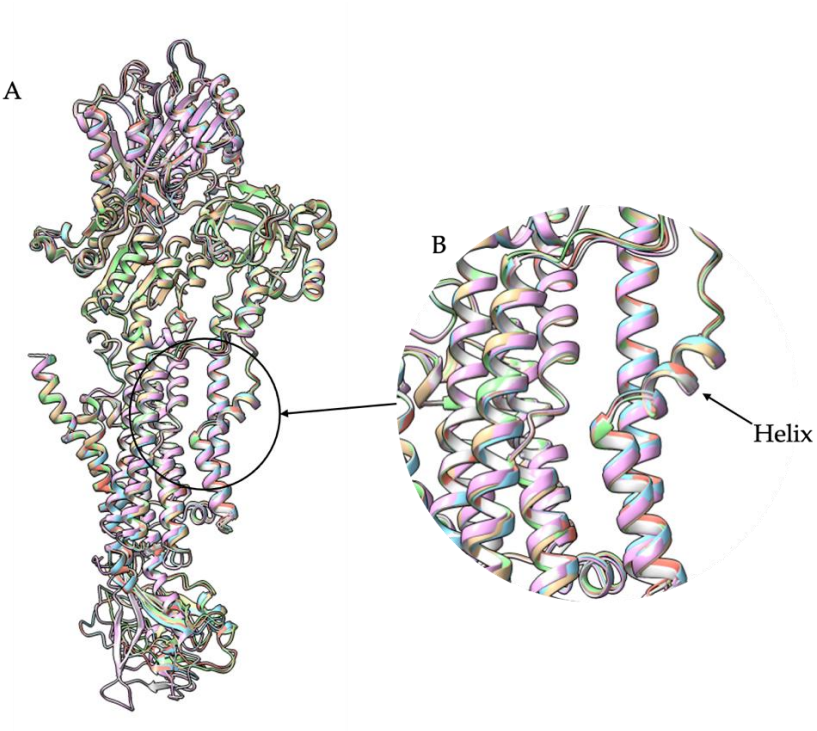


Figure 4 The homology models were built by Alpha Fold 2. The helices are almost at the same location.

Table 3 The RMSD between each other of homology models built by Alpha Fold2

Model Name	Model Name	RMSD
Model 1-relaxed	Model 2-relaxed	0.750 Å
Model 1-relaxed	Model 3-relaxed	1.255 Å
Model 2-relaxed	Model 3-relaxed	0.890 Å
Model 1-relaxed	Model 1-unrelaxed	0.116 Å
Model 1-relaxed	Model 2-unrelaxed	0.646 Å
Model 1-relaxed	Model 3-unrelaxed	0.937 Å
Model 2-relaxed	Model 1-unrelaxed	0.645 Å
Model 2-relaxed	Model 2-unrelaxed	0.118 Å
Model 2-relaxed	Model 3-unrelaxed	0.692 Å
Model 3-relaxed	Model 1-unrelaxed	0.937 Å
Model 3-relaxed	Model 2-unrelaxed	0.693 Å
Model 3-relaxed	Model 3-unrelaxed	0.122 Å
Model 1-unrelaxed	Model 2-unrelaxed	0.622 Å
Model 1-unrelaxed	Model 3-unrelaxed	0.911 Å
Model 2-unrelaxed	Model 3-unrelaxed	0.667 Å

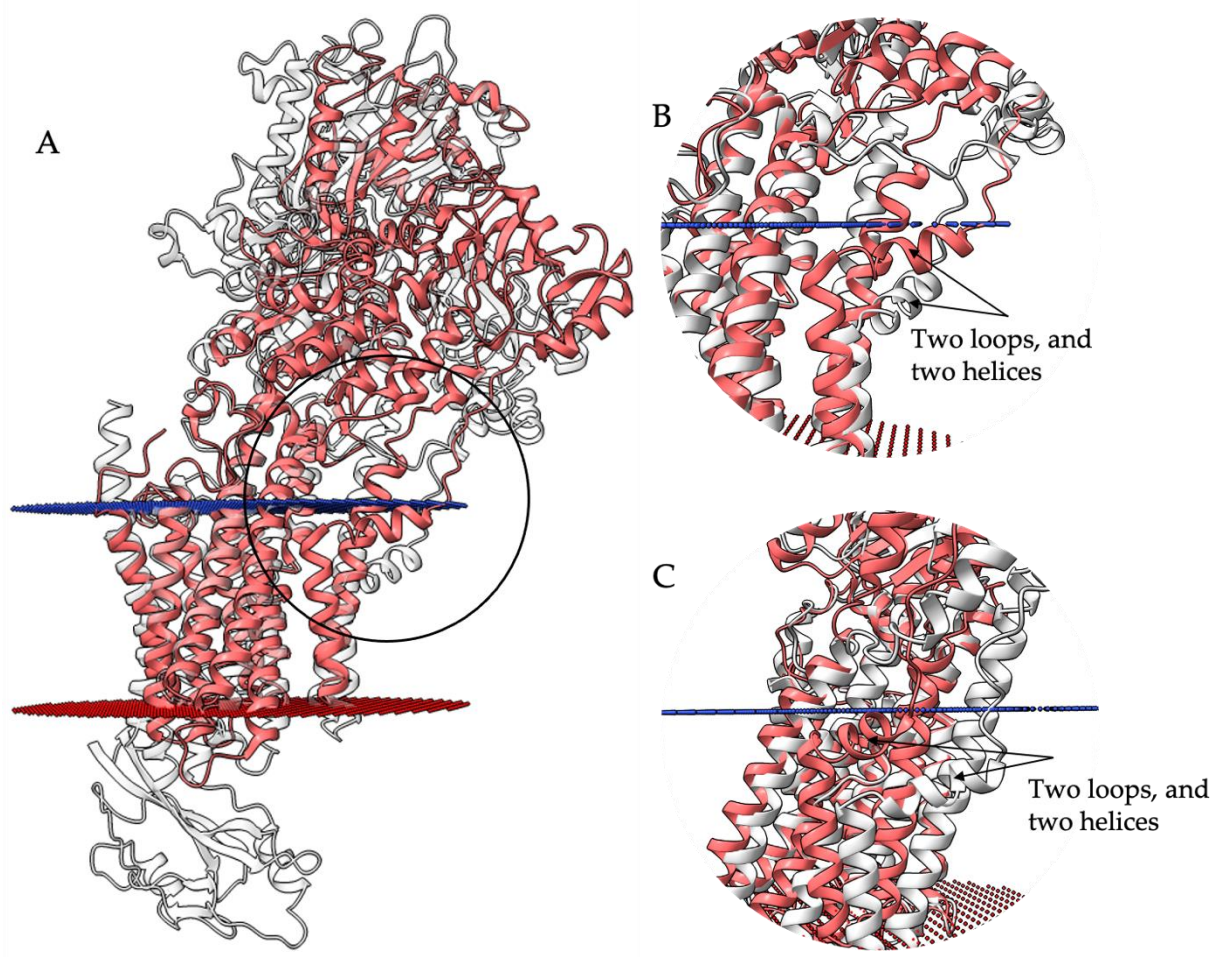


Figure 5 is the structures of the Alpha Fold 2 homology model (light gray) and the crystal structure of 6ZHF (salmon). A) is the superimposed view of the two structures. B) and C) are the zoomed-in view of the binding pocket. B is from the same angle as A to look at the structures. C) is from the right side of A) to look at the structures. There are two labeled helices in figure 3B. The Alpha Fold 2 homology model's helix is lower than 6ZHF's helix.

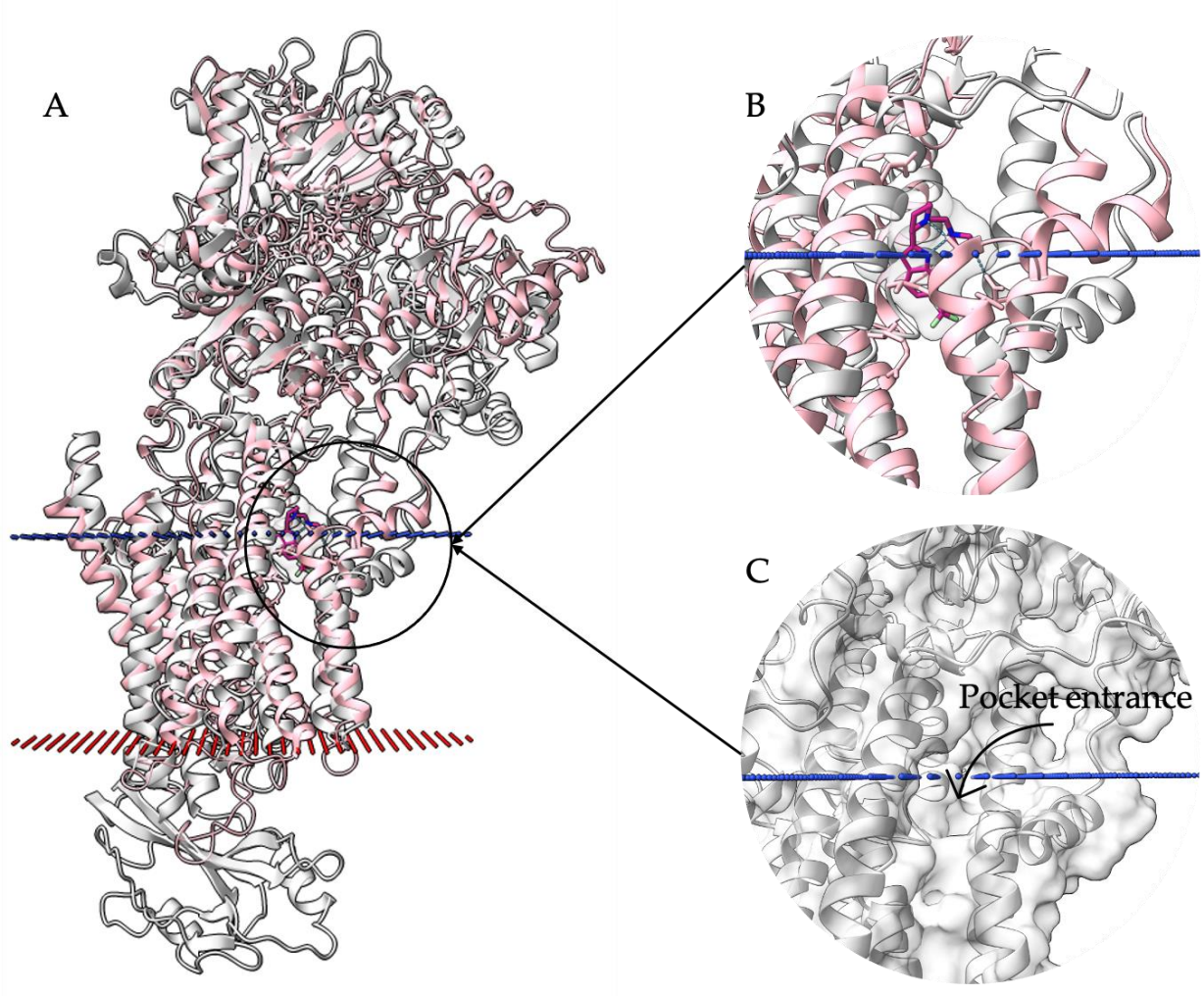


Figure 6 This is the comparison between the crystal structure of the Ca^{2+}-ATPase and PfATP4. A) shows the superimposed crystal structure of the Ca^{2+}-ATPase (PDB ID: 6YAA) and the homology model built by Alpha Fold 2. 6YAA is in pink. Alpha Fold 2 homology model (AF2 model) is in light gray. The blue dots and red dots are to show the lipid bilayer. The inhibitor is in magenta. The inhibitor location is around the lipid level. B) It is the binding pocket zoomed in view. The AF2 model's binding pocket is lower than the crystal structure 6YAA, and it is also lower than lipid. C) shows the surface of the Alpha Fold 2 homology models' binding pocket surface. The binding pocket is buried inside of the lipid layer.

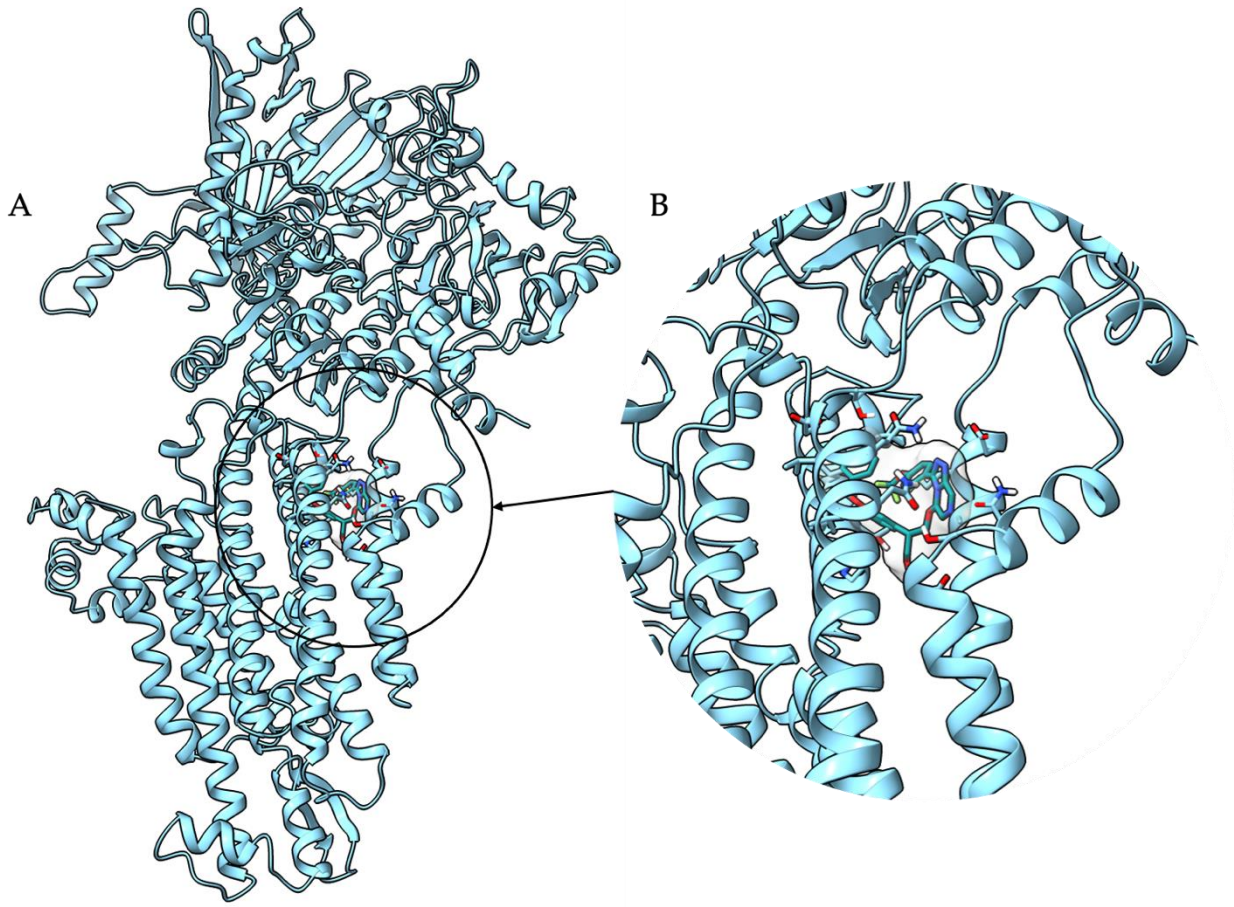


Figure 7 A) is the HLN homology model. B) shows the zoomed-in view of the ligand binding pocket. The ligand is shown in turquoise, the ligand surface is shown in gray. The ligand looks to be fitting in the binding pocket quite well.

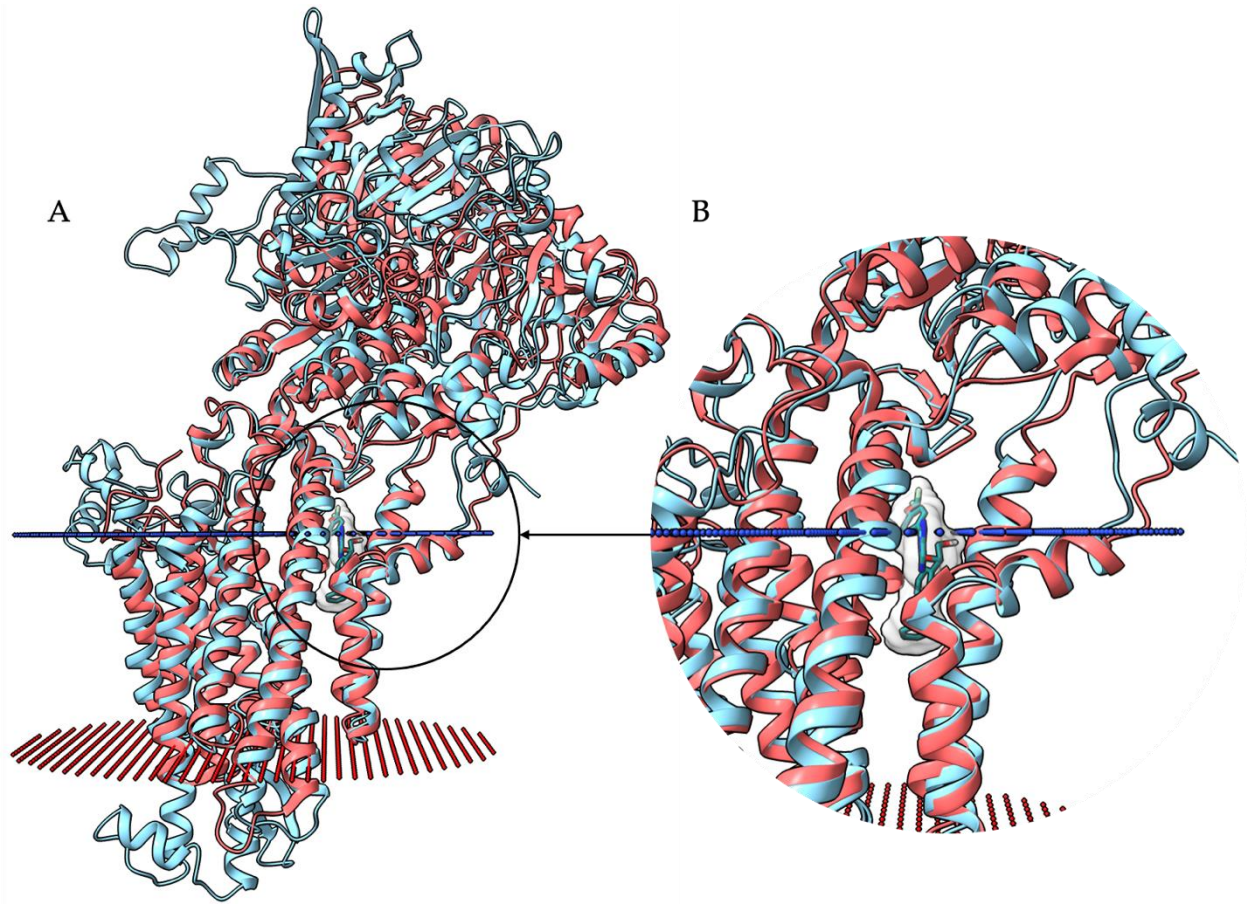


Figure 8 A) is the superimposed view of 6ZHF and the HLN homology model. The blue and red dots show the lipid bilayer which is from the 6ZHF crystal structure. 6ZHF is in salmon. HLN homology model is in cyan. The docked ligand is in turquoise, and the ligand's surface in light gray. B) shows the zoomed-in view of the binding pocket.

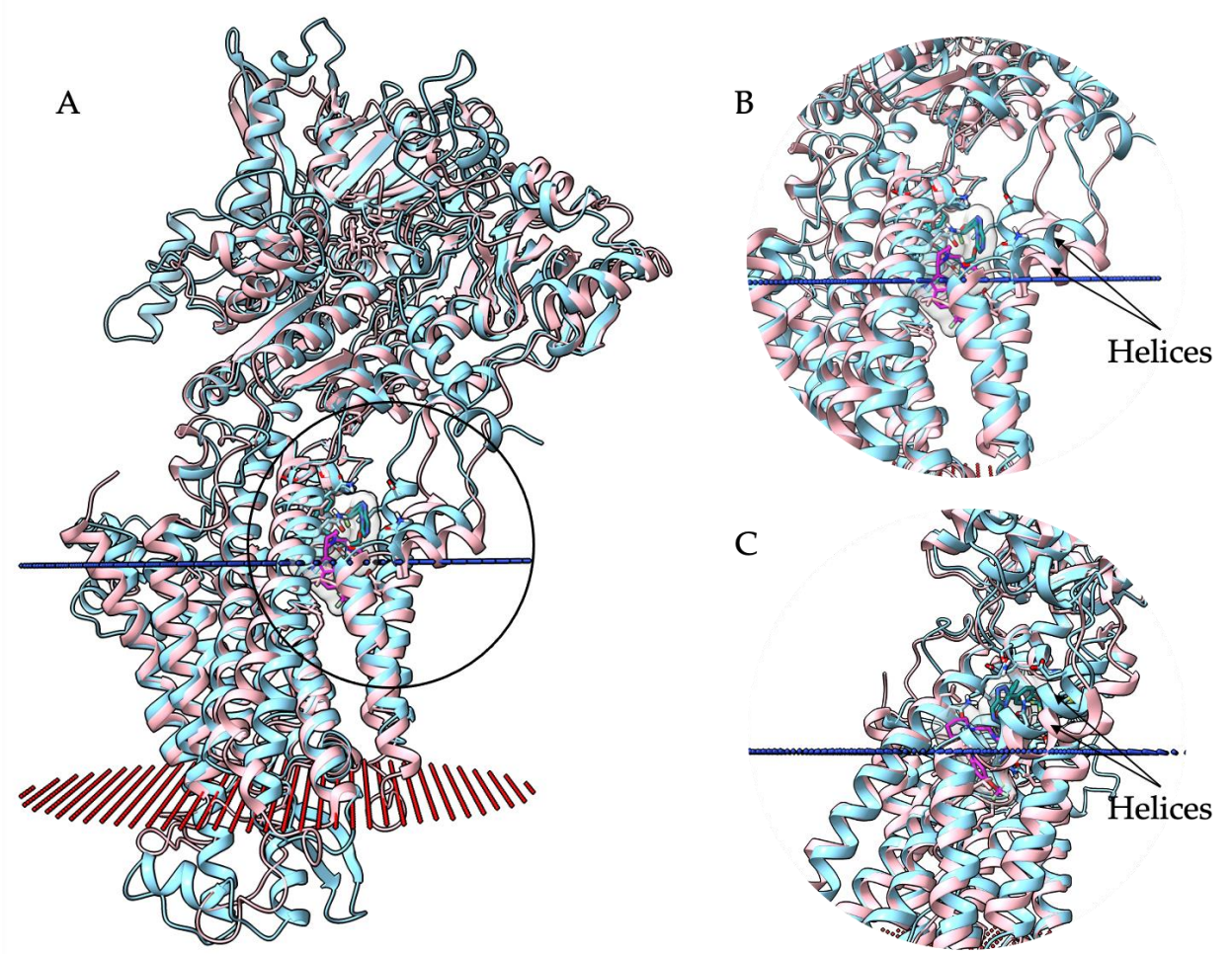


Figure 9 This is the crystal structure 6YAA and HLN model. The crystal structure 6YAA is in light pink, and the inhibitor is in magenta. The blue and red dots show the lipid bilayer. The HLN model is in cyan, and the inhibitor is in turquoise. A) shows the superimposed view of 6YAA and the HLN model. The black circle labels the binding pocket of these two structures. B) shows the zoomed-in view of the binding pocket. The two labeled helices are around similar locations. Both of the inhibitors are located around the lipid layer and the labeled helices. C) shows the zoomed-in view from right side looking at A. the labeled helices are located similarly.

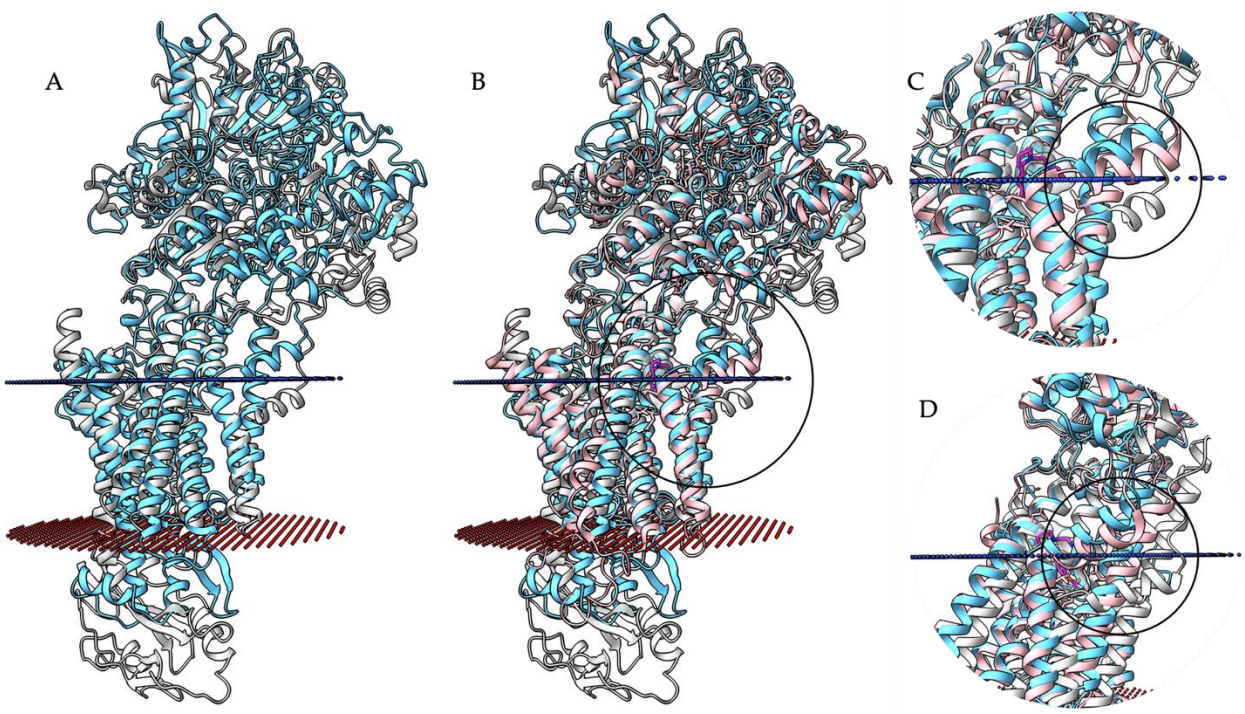


Figure 10 This is the crystal structure 6YAA, Alpha Fold 2 homology model, and HLN homology model. A) is the superimposed view of the Alpha Fold 2 homology model and the HLN homology model. B) is the superimposed view of the Alpha Fold 2 homology model, the HLN homology model, and 6YAA. 6YAA's inhibitor is in magenta. The binding pocket is labeled by a black circle. The most notable difference between the three structures is the helices labeled in the black circle. 6YAA and HLN model's helices are above or around the lipid layer. However, the alpha fold 2 model's helix is inside of SR membrane. C) is the zoomed-in view of the binding pocket. The three helices from different structures are labeled by a black circle. D) is the view from the right side to look at C.

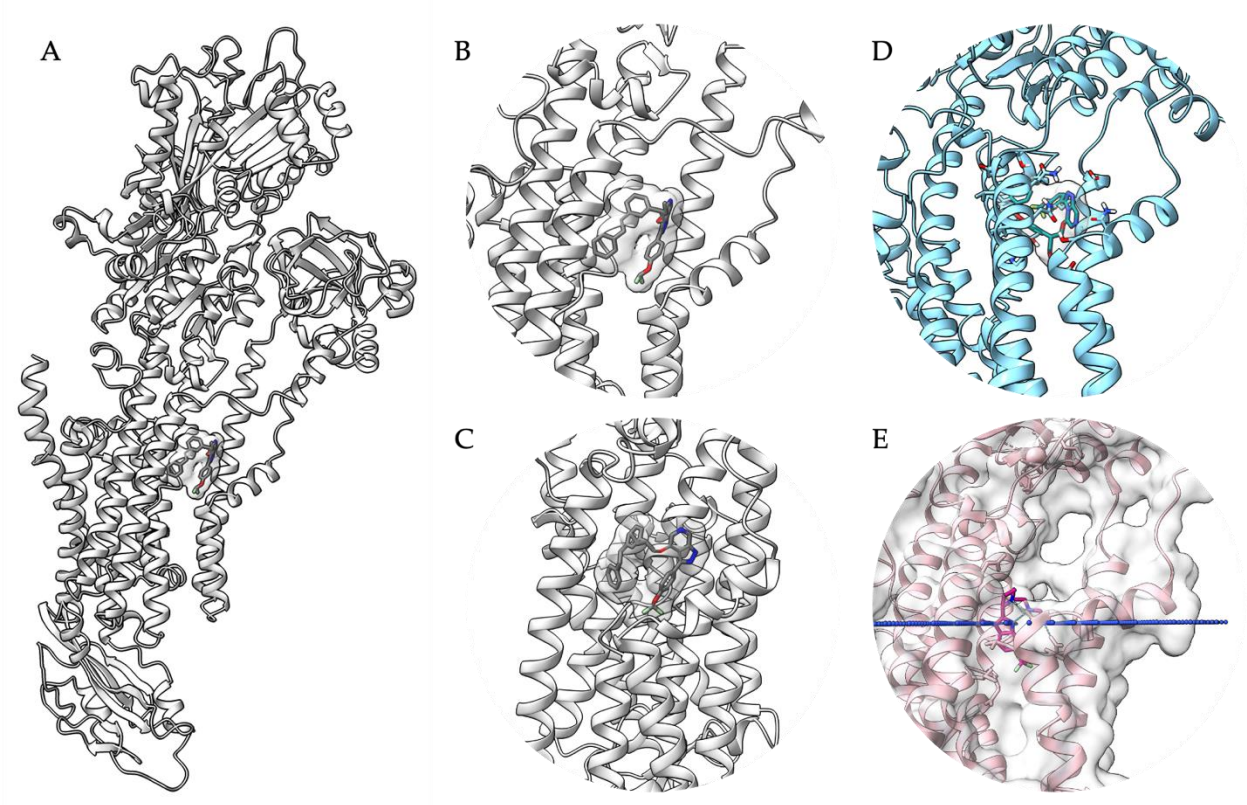
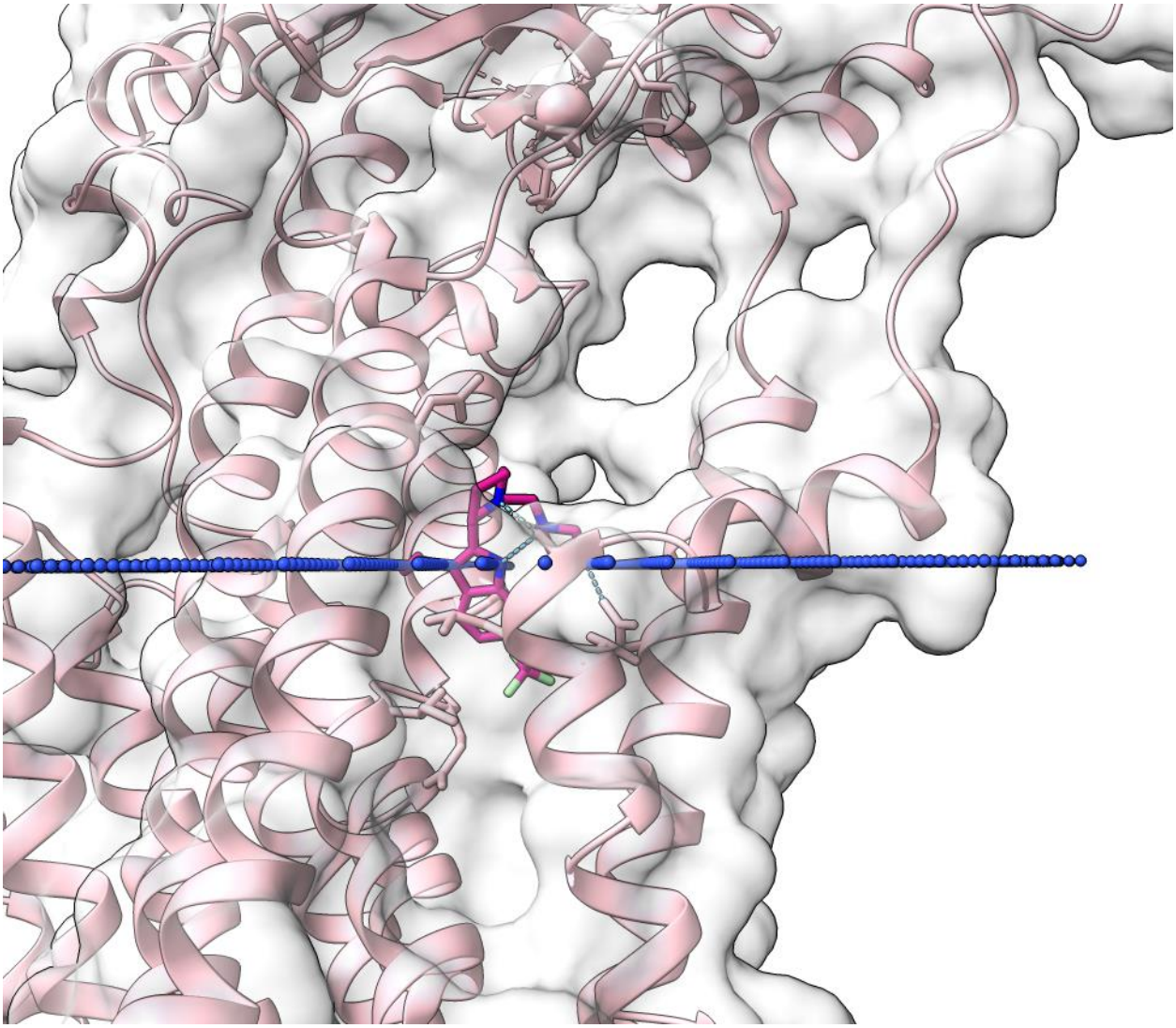
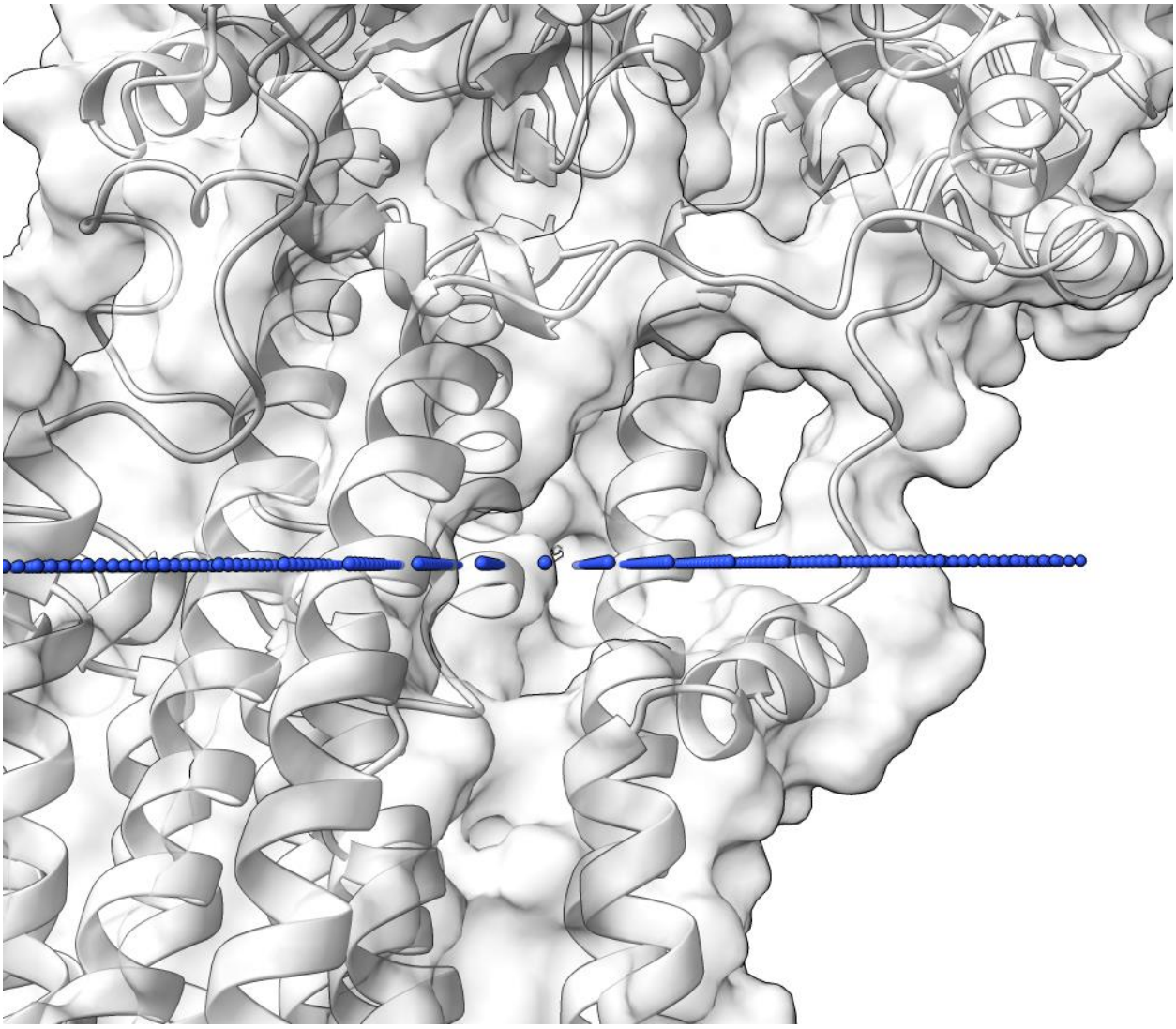


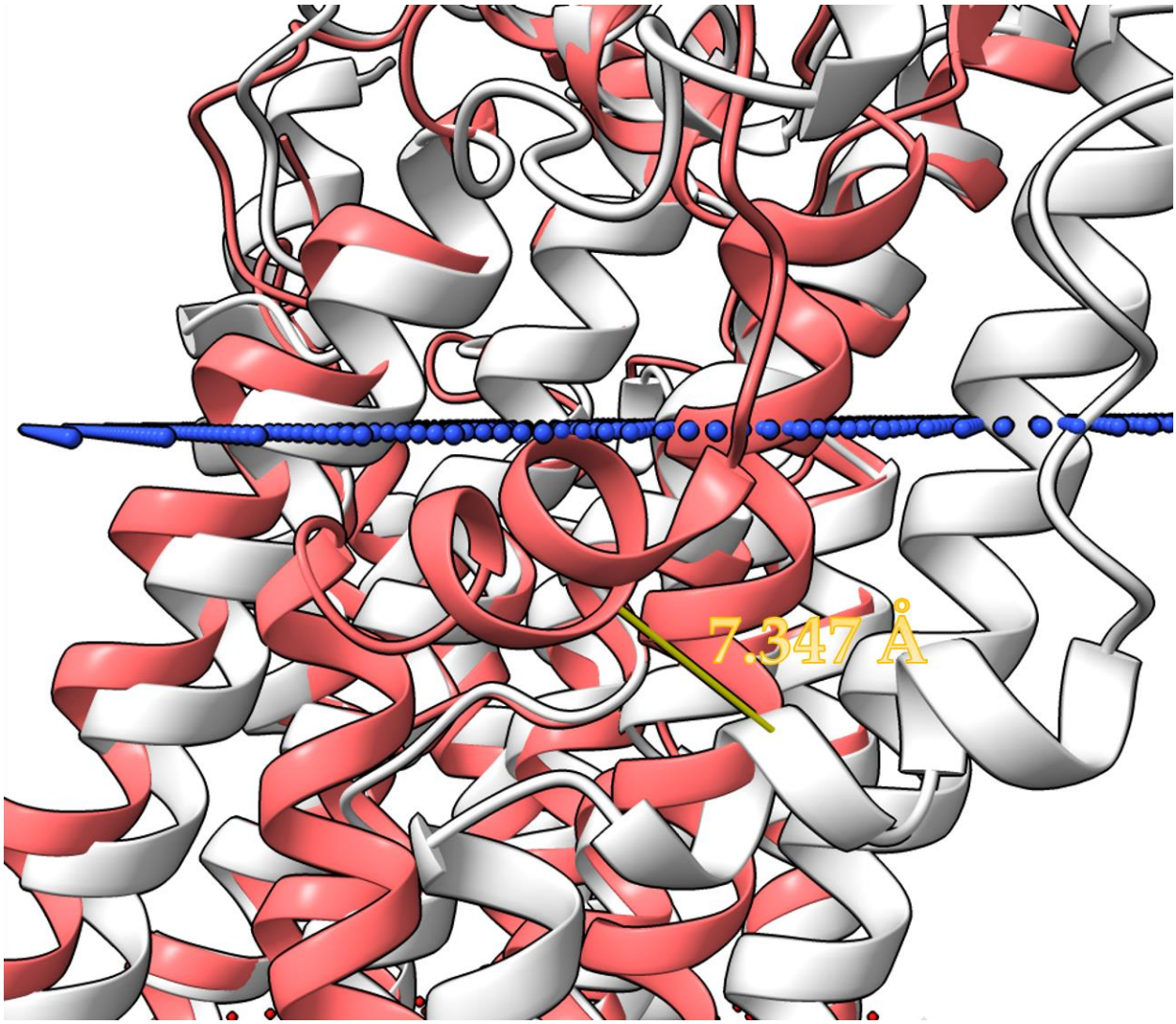
Figure 11 This is the inhibitor docked into the Alpha Fold 2 model. A) is the overall view of the inhibitor docked with the Alpha Fold 2 model. The ligand is in dark gray, and its surface is in light gray. B) is the zoomed-in view of binding region. The ligand does not interact with receptor very well. C) is from right side to look at B). D) and E) are the inhibitor docked with the HLN model and 6YAA, respectively.



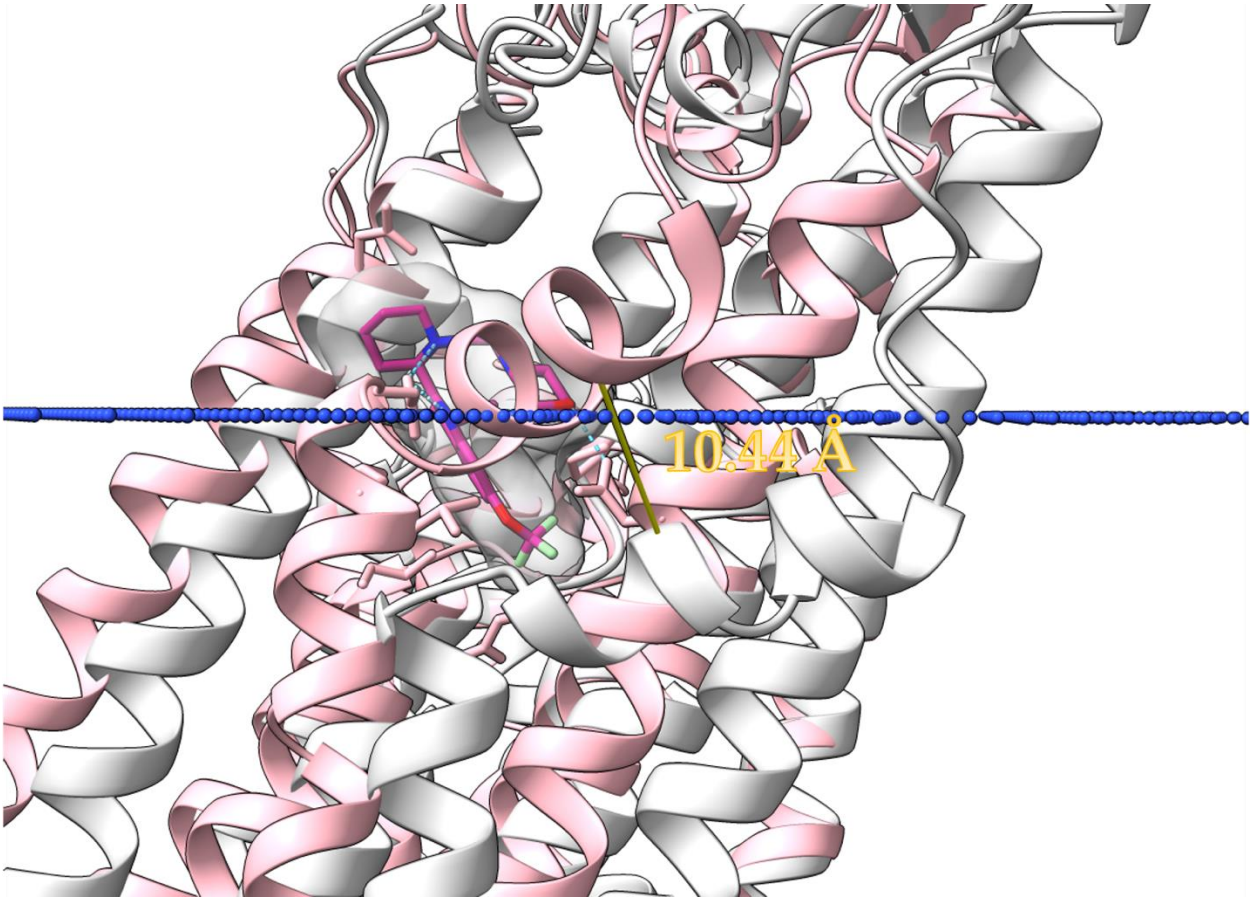
SI Figure 1 The binding pocket surface zoomed-in view. The crystal structure of the Ca^{2+}-ATPase bound to the inhibitor compound CAD204520. (PDB ID: 6YAA)



SI Figure 2 This is the binding pocket surface of homology model built by Alpha Fold 2. The blue dots show the lipid layer which is from the crystal structure 6YAA. The binding pocket entrance is buried inside of lipid layer.



SI Figure 3 This is Alpha Fold 2 homology model (light gray) and the crystal structure of 6ZHF (salmon). The two helices distance is around 7 Å (labeled in yellow).



SI Figure 4 From right side to look at figure 6A. 6YAA is in pink. Alpha Fold 2 model is in light gray. The ligand is in magenta. The ligand is around the lipid layer. The two labeled helices' distance is around 10 Å.