Model Colloboration

This year, we are making colloborations with HBUT about modeling work. Knowing that they are doing the job about the NiCoT transporter, we are going to make a molecular dynamic simulation of the process that Nickle ion entering the cell through the NixA transporter protein.

NixA is the high-affinity cytoplasmic membrane nickel transport protein of Helicobacter pylori which is capable of importing Ni^{2+}into the cell for insertion into the active site of the urease metalloenzyme.[1] But how could the nickle ion go through the cytomembrane with the help of NixA? We want to use the molecular dynamic method to explain and simulate the process, and finally visualize it using videos.

At the very beginning, we need to find the protein NixA coordinate file (knowns as PDB files). We search the biggest protein tertiary structure base: RCSB PDB (Protein Data Bank). Unfortunately, we can't find anything about NixA, even any protein from the NiCoT family. We continue to search the Modbase and obtain the Model-predict structure by the sail lab (Primary Database Link is Q48262.2). However, there is a big hole in the center, which is different from common transporter protein, indicating that this structure is probably not so precise about its true structure. What's more, the model coverage sketch is from 23 to 275, which is much shorter than the 350 residues we need.

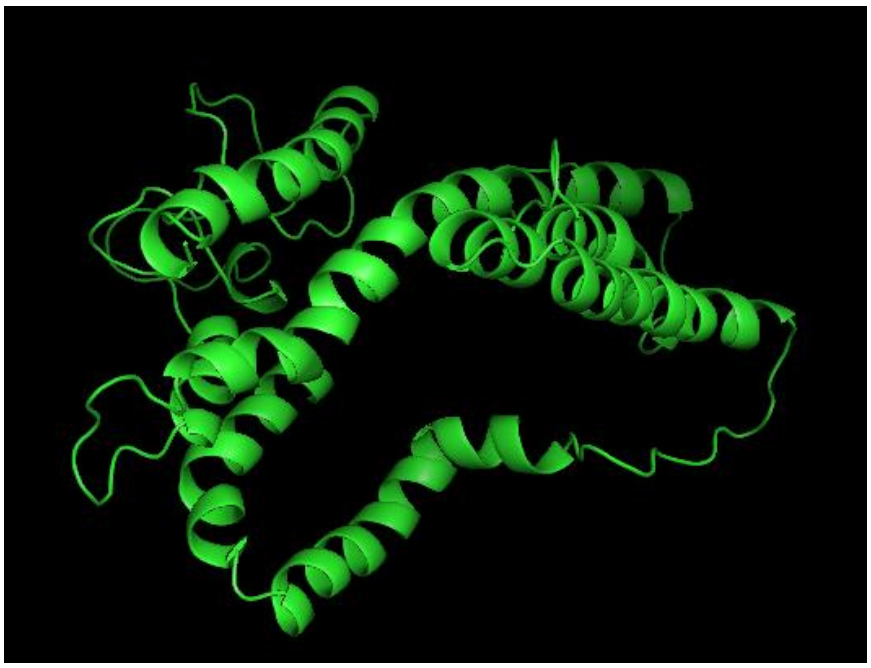

Fig1: NixA model-predict structure from Modbase.

Under that circumstance, we try another way to get the PDB file. That is Swiss Model. The protein sequence we use (provided by HBUT) are as follows:

MIKYHVMKEGYILTVFKNERLSWLPYIAIVILLHVIGFSFLWIAGKDHHILFGM
GILAYTLGLRHAFDADHIAAIDNTVRKLLQQRKDPSGVGFYFSIGHSSVVFLM
AVFLGVSVKWAKDELPHFQDIGGTIGTLVSGFFLVLIGVLNLIILISLINLFAKLR
REHIEEAEVDALLESRGLVSRFVGPYFKLITRSWHVLPLGFLFGLGFDTASEIA
LLALSSGASQQAISFIGILSLPILFASGMSLLDTLDGVVMKYAYNWAFFNPIRKI
YYNITITAISVMAALVIGMIELLQILADKLDLHGAFWAFIGSIEFDYLGYILVALF
LITWLISSLIWKFGRIEHKWSR.

In order to make our simulation more accurate, we use a transporter protein as a template (PDB ID: 2XUT, whch demonstrate the crystal structure of a proton dependent oligopeptide (POT) family transporter.) and obtain a well-predict model structure of NixA.

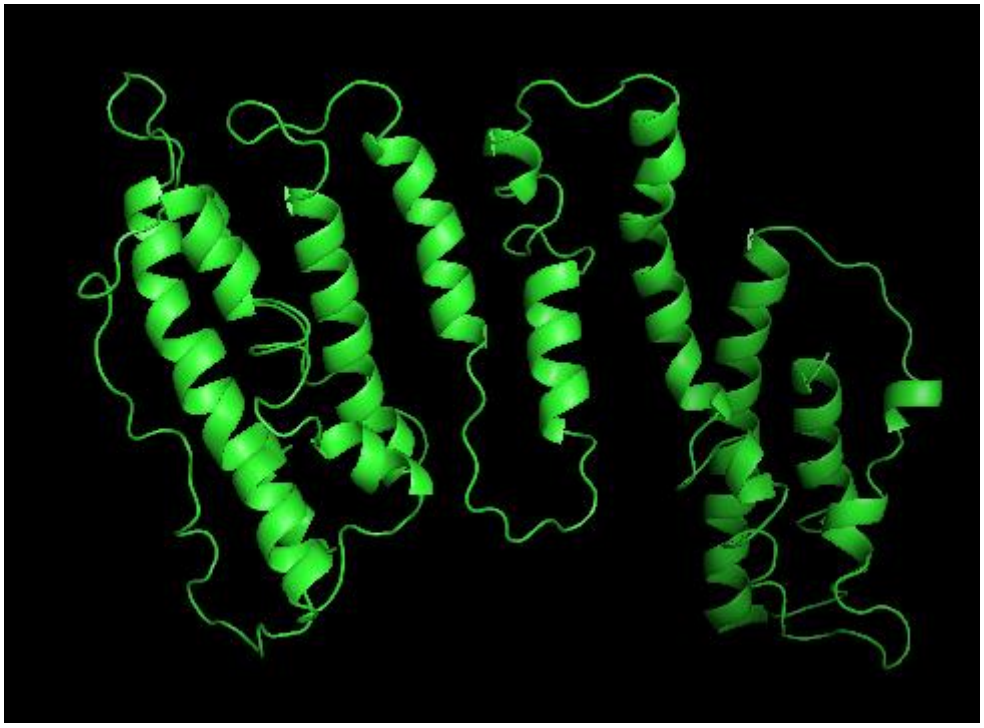

Fig2 SWISS model-predict structure of NIxA.

This model has a eight-transmembrane-domain, which is well-qualified by the characteristics of Nickel/cobalt transporters (NiCoTs)(Fig3). So we take it as a our NixA model.

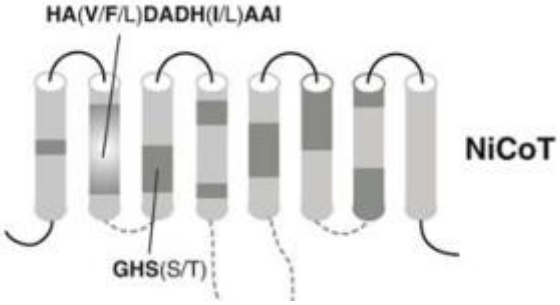

Fig3: Topology of nickel/cobalt transporters (NiCoT).[2]

However, there is no paper demonstrating where Ni^{2+} connects to the NixA and how to go through it, we have to do it with another simulaion in silico. We making docking between Ni^{2+} and NixA protein using autodock. Due to the fact that autodock couldn't accept single ion docks with the protein (we

also try the FoldX, neither can accompolish satisfying result), so we use the $NiCl_2$ which is very common in the acidic waste water to dock. We get the sdf files of $NiCl_2$ from PubChem and transfer it into pdb format using Openbabel.

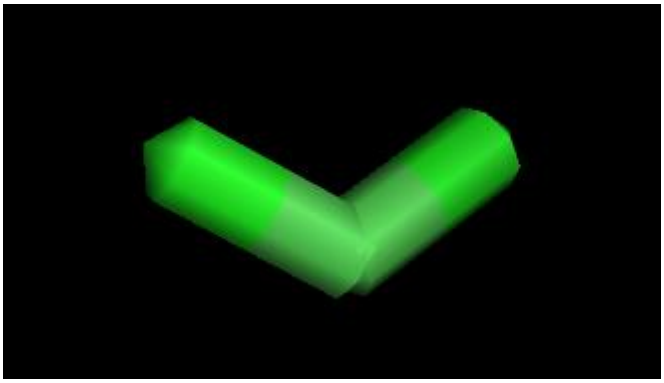

Fig4 $NiCl_2$ 3Dstructure.

Docking proves to be very difficult because Ni^{2+} is not so common as other metal ion like Cu^{2+}, Ca^{2+}, so autodock couldn't recognize its atom type. Finally, we get the parameters from the links below and solve the problem. (http://mgldev.scripps.edu/pipermail/autodock/2009-March/005439.html). Fully unware of the connecting location, we set the grid box large enough to contain the whole protein, hoping to get a propriate docking conformation. The certain parameter when setting the box are shown in Fig 5. We get 50 docking results of the $NiCl_2$ and NixA and choose the one which $NiCl_2$ is above the NixA protein and close to the transmembrane domains II (Fig6), since requisite motifs for Ni^{2+} transport locates entirely within transmembrane domains II and III[1].

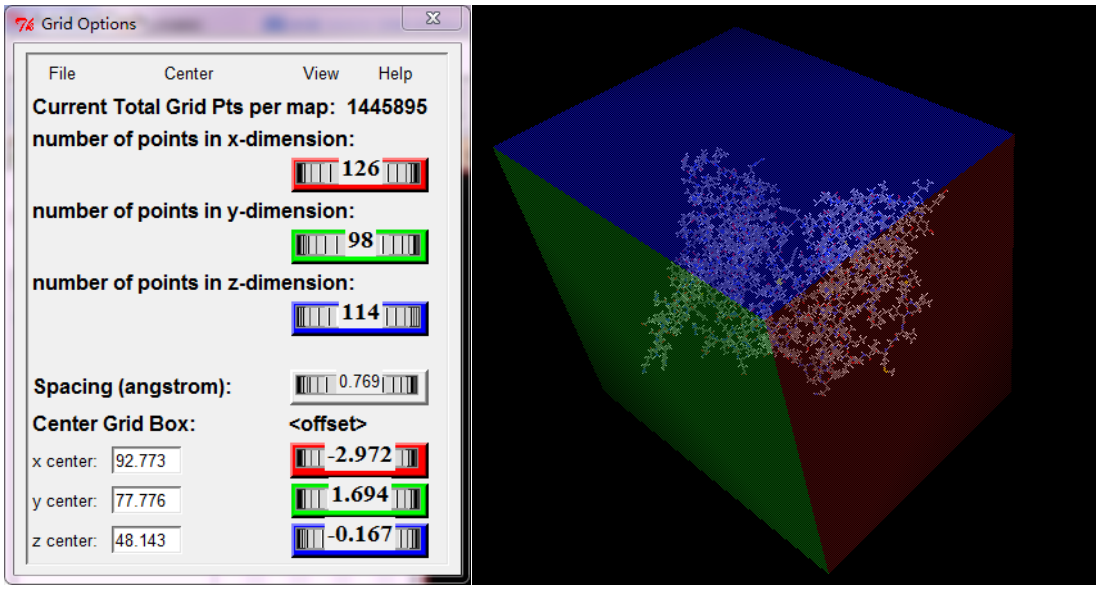

Fig5: Parameter we ues when setting the docking box.

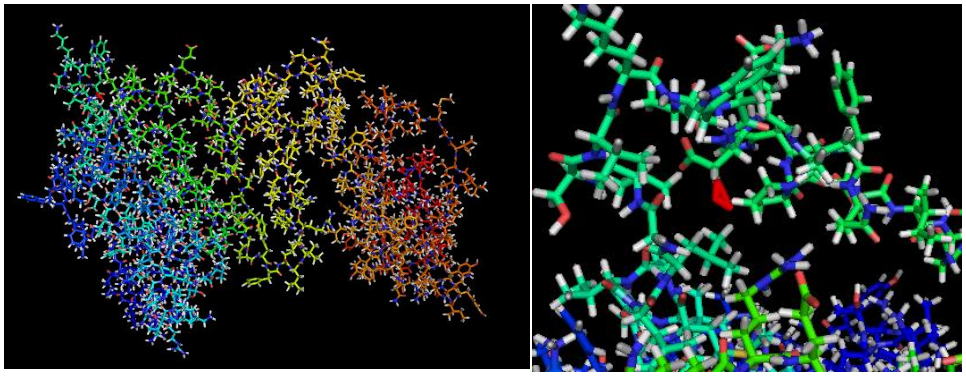

Fig6: Docking result we choose. The red triangle represents NiCl2 while the green section represents transmembrane domains II of NixA.

For some PDB files, there may be some missing heavy atoms in flexible regions that could not be clearly resolved from the electron density. This may include anything from a few atoms at the end of a sidechain to entire loops or there may be multiple locations listed for some atoms[3]. So it's necessary to fix the PDB files before next move. We use PDBfixer to fix the docking result file.

After fixing, we use Membrane Builder tools of CHARMM-GUI to build a protein/membrane complex for

molecular dynamics simulations. We use insertion method to insert NixA protein into DPPC membrane since it's ubiquitous and a classic membrane model. The result are shown as Fig7(a). We compare it to the topoiogical model ol NixA in H. pylori Fig7(b) and find some similarities between each other, further proving the accuracy of the model prediction.

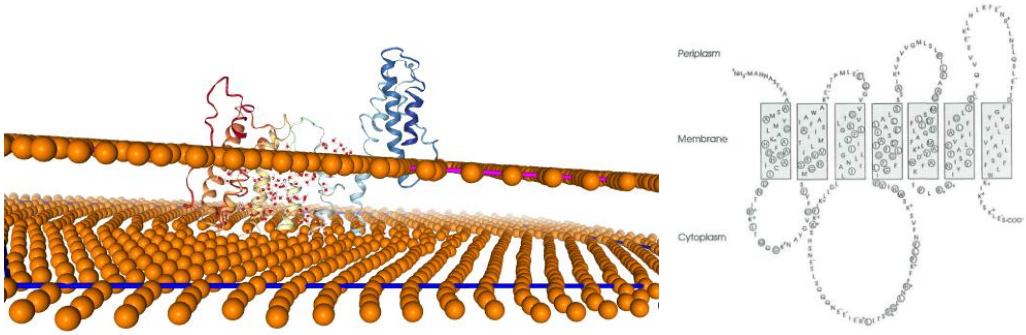

Fig 7. Comparison between model and previous paper work. (a)The result of CHARMM-GUI, the membrane is DPPC. (b)Topoiogical model ol NixA in H. pylori[4].

Then we put the protein\membrane complex into a water box and add 0.15M K^+ and Cl^- to make charge balance and systems stable. The specific parameters of the box are as follows.

Determined System Size:

Box Type	Rectangle		
Crystal Type	TETRAGONAL		
System Size	A	210.100985	Dimension along the A (X) axis
	B	210.100985	Dimension along the B (Y) axis
	C	118.550127	Dimension along the C (Z) axis
Crystal Angle	Alpha	90.0	Angle between the axis B and C
	Beta	90.0	Angle between the axis A and C
	Gamma	90.0	Angle between the axis A and B
# of Lipids	on Top	677	
	on Bottom	671	
Z Center	-12.9852237		Center of the system along the Z axis

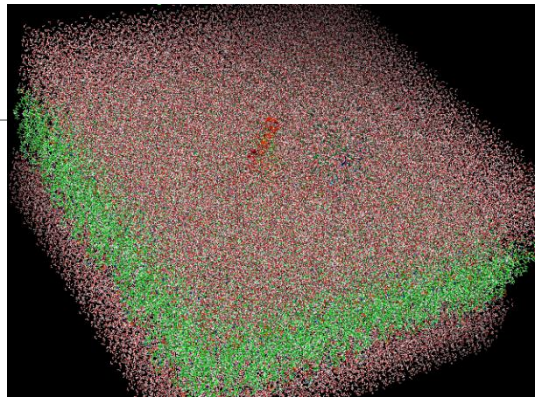

Fig 8. The final result of CHARMM-GUI. (a)The certain parameters of the water box we built in tthis simulation. (b)Visual result of the whole system. Pink dots represents solvent (that is, TIP3), green dots represnts DPPC while red dots in the center of the box represents NixA protein.

We use output of CHARMM-GUI as the input of GROMACS. Further molecule dynamic simulations are all carried out in GROMACS, and we refer to GROMACS Tutorial--Membrane Protein: KALP15 in DPPC[5] to guide us for the rest work. We run the energy minimization, nvt equilibration, npt equilibration and production MD in GROMACS. We use a temperature that is higher than the phase transition temperature of the lipid molecule, which is 323 K. As for tc-grps, we use four groups: protein, DPPC, SOL_CL and $NiCl_2$ to make simulation more accurate.

We use server to run the GROMACS simulations. Due to lake of time, the simulation only lasts for 1.34ns. The group consists of DPPC membrane, protein NixA and $NiCl_2$. The simulation generates about 6G pdb files. Constrained by computing power of PC, such file is far too large for PyMOL to accept. We have to extract first 1000 frames to generate pdb file and use PyMOL to visualize it. The result is shown in Fig 9.

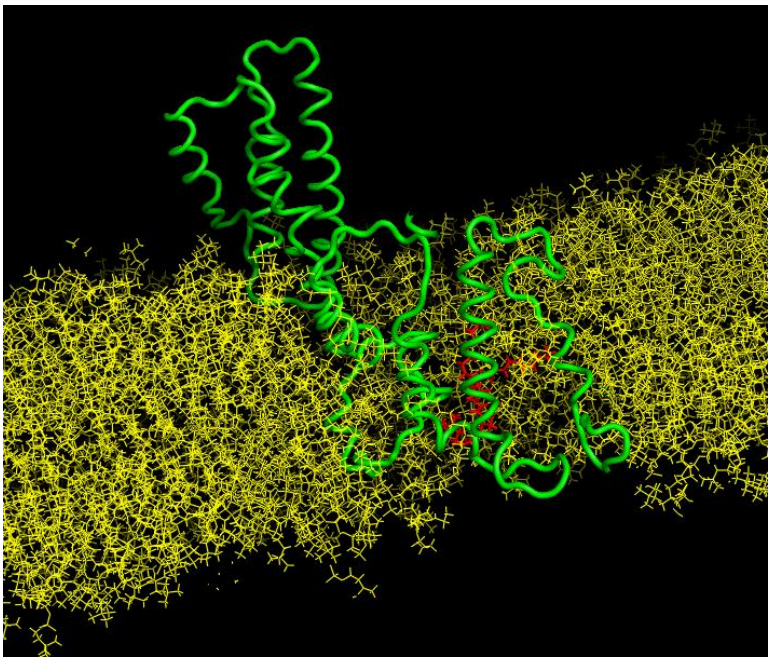

Fig 9. Visualization of the simulation using PyMOL. The green part represents NixA protein, red part represents NiCl2, and yellow part represents DPPC membrane.

We make a 8s video to display the process of Ni^{2+} going through the membrane. As we extract more frames, the video becomes longer, so it can show more parts of the process. We believe we can see finally the Ni^{2+} goes from periplasm side to the cytoplasm side in this way.

Reference:

1. Fulkerson J F , Mobley H L T . Membrane Topology of the NixA Nickel Transporter of Helicobacter pylori: Two Nickel Transport-Specific Motifs within Transmembrane Helices II and III[J]. Journal of Bacteriology, 2000, 182(6):1722-1730.

2. Eitinger T , Suhr J , Moore L , et al. Secondary Transporters for Nickel and Cobalt Ions: Theme and Variations[J]. Biometals, 2005, 18(4):399-405.

3. http://htmlpreview.github.io/?https://raw.github.com/pandegroup/pdbfixer/master/Manual.html PDBfixer manual

4. Mobley H L T , Garner R M , Bauerfeind P . Helicobacter pylori nickel-transport gene nixA: synthesis of catalytically active urease in Escherichia coli independent of growth conditions[J]. Molecular Microbiology, 1995, 16(1).

5.http://www.mdtutorials.com/gmx/membrane_protein/index.html