

*Tutorial for physiologists:*

*“Practical Approaches to Protein Structural Information”*

Flowchart for preparation:

How to see protein structures using PyMOL

Lecturers:

Takushi Shimomura (NIPS)

Katsumasa Irie (Nagoya Univ.)

Organizer:

Yuichiro Fujiwara (Kagawa Univ.)

March 31<sup>st</sup>, 2019

am 8:00- 9:10

Room2: International Conference Room

## Table of Contents:

### *Dr. Shimomura's section*

1-Displaying protein structures

2-Analysis of structural information

with  
a “turtorial\_shimot.zip” file

### *Dr. Irie's section*

1-Making homology model

2-Making ligand binding model

3-Analysis ligand binding mode

with  
a “tutorial\_kirie.zip” file

## Install softwares:

Pymol 2.2: <https://pymol.org/2/#page-top>

(registration: <https://pymol.org/2/buy.html?q=buy>)

Ligplot<sup>+</sup>: <https://www.ebi.ac.uk/thornton-srv/software/LigPlus/>

(requiring java plug-in; <https://www.java.com/download/>)

## 1-Displaying protein structures

### Step1. Set up protein structures

1. Access to protein data bank (<https://www.rcsb.org/>) and download a PDB file \*6C9A; mouse TPC1 bound with PI(3,5)P<sub>2</sub>
2. Load the PDB files in PyMOL
3. Build the multimers of hERG (cryo-EM; 5VA3) and plant TPC1 (X-ray; 5E1J)

### Step2. Look and Move structures

### Step3. Change the appearance

1. Show as different mode (stick, ribbon, cartoon, sphere and surface)
2. Select specific residues (N648 and A281) and show their side chains as stick
3. Select each monomer and color differently
4. Show only the pore regions as cartoon, with the others shown as ribbon
5. Show only half of the pore regions as surface
6. Draw/Ray the displayed structure and save as a PNG file
7. Save the current view as PSE file (1-1.pse)

## 2-Analysis of structural information

### Step1. Analyze the interaction with PI(3,5)P<sub>2</sub> molecule

1. Search for the interacting residues with PI(3,5)P<sub>2</sub> (1-2.pse)
2. Mutate R224, a residue interacting with PI(3,5)P<sub>2</sub> to A (1-3.pse)
3. Show as electrostatic potential map around PI(3,5)P<sub>2</sub> using APBS
4. Interpret the PI(3,5)P<sub>2</sub> recognition mechanism based on the structure

### Step2. Integrate the structural and functional data

1. Mutate D86 to R and K87 to N
2. Convert PI(3,5)P<sub>2</sub> to PI(3,4)P<sub>2</sub> using Builder (1-4.pse)
3. Compare with the functional data for PI(3,4)P<sub>2</sub> action

## 1-Making homology model

### Step1. Template Search

1. Access to swiss-model site (<https://swissmodel.expasy.org/>)



Start Modelling

2. Click “Start Modelling” button

3. Click “Upload Target Sequence File” and upload FASTA format sequence file

\*Example seq in 1-primary\_Seq folder



+ Upload Target Sequence File...

4. Click “Search For Templates”

5. Wait a moment and drink coffee or tea

### Step2. Choosing template

1. Chose suitable template from results

\*In this tutorial, 6agf.1.A and 6a95.1.A are used

2. Click “Build Models” button



Build Models 1

3. Wait a moment and take a lunch

### Step3. Download homology model

2. Click “Models” button and download homology models as zip file

\*Result of homology model in 4-result



Models 2

## 2-Making ligand binding model      Guide file: [bindingmodel.pse](#)

### Step1. Structural alignment

1. Open three files with pymol  
(Nav1.7 on NavPas in homology model results. 6a95.pdb and 6agf.pdb in model\_templates)
2. Roughly align Nav1.7 homology model and Nav1.4 structure(6agf) to NavPas-TTX complex structure(6a95).
3. Chose each selectivity filter as independent selection.
  4. Detail align Nav1.7 and Nav1.4 to NavPas TTX complex using filter selection.

### Step2. Generation TTX binding model of Nav1.7 and Nav1.4

1. Make selection including TTX and Nav1.4 whole channel.
2. Generate new pdb file including TTX and Nav1.4 whole channel using “Export molecules” command.
3. Making TTX and Nav1.7 channel pdb as similar way as case of Nav1.4.  
(Please exclude Arg1608 of Nav1.7 for binding analysis)

**\*Result of binding model in 2-Generation\_binding\_model**

### 3-Analysis ligand binding mode

1. Open Nav1.4-TTX complex file with ligplus
2. Generate 2D graphic picture of the interaction between TTX and channel
3. Making TTX and Nav1.7 picture as similar way as case of Nav1.4.

\*Pictogram and pymol session file of binding mode in 3-Binding\_analysis