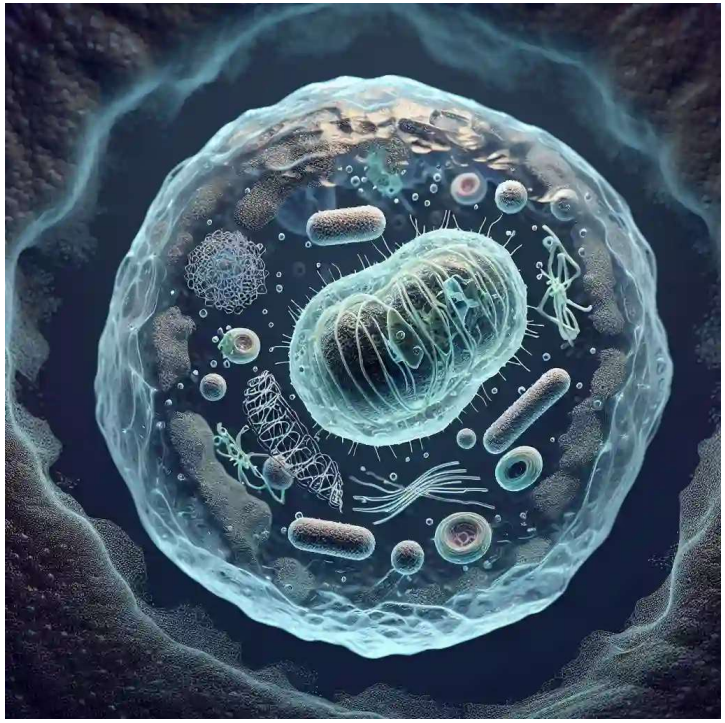
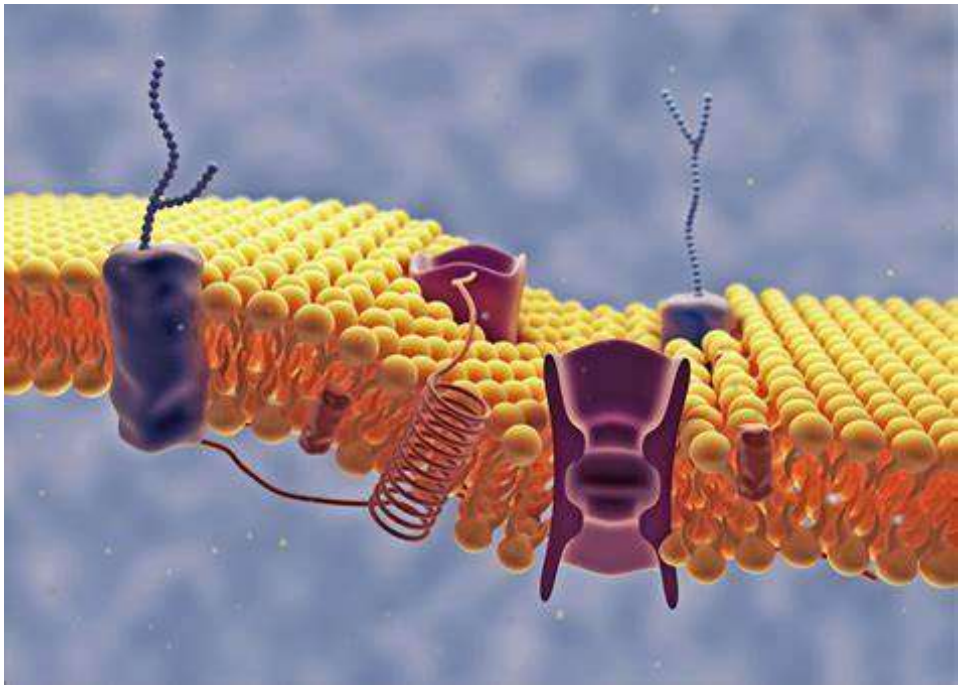
使用**Charmm-Gui**进行膜蛋白模拟

Naf Guo
2025

Membrane Protein



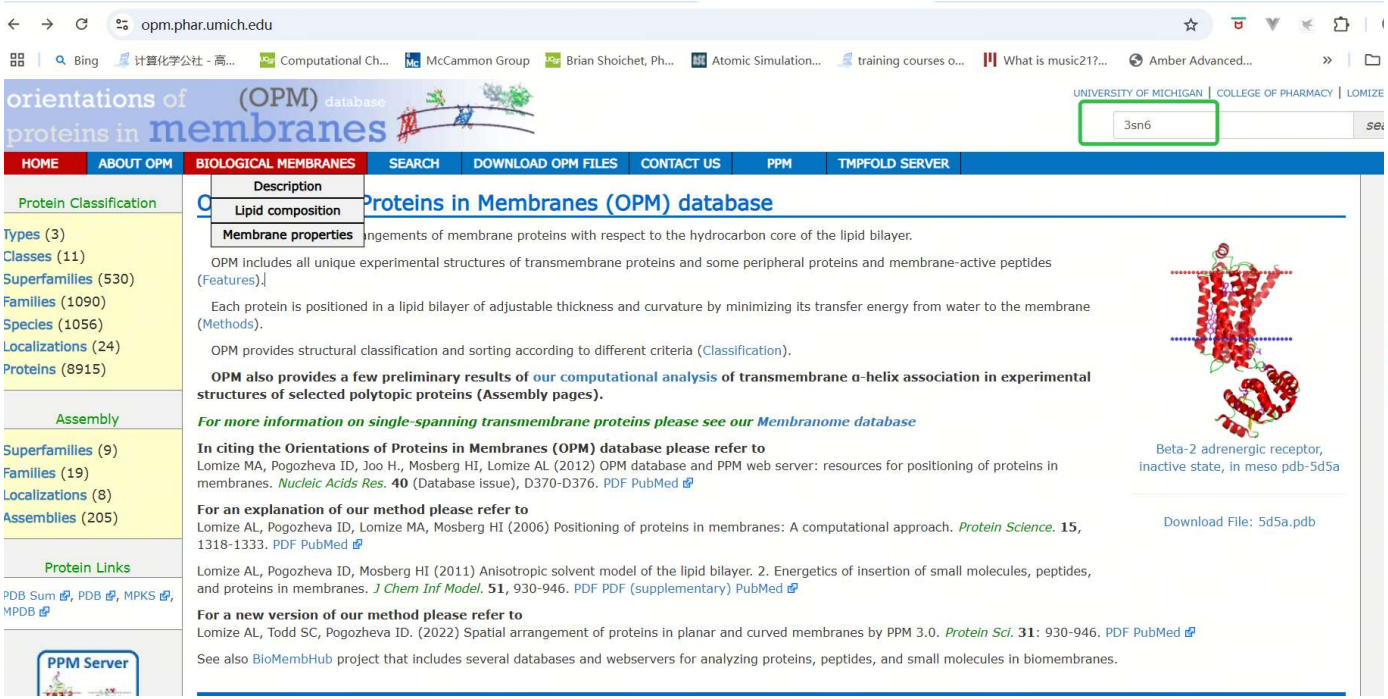


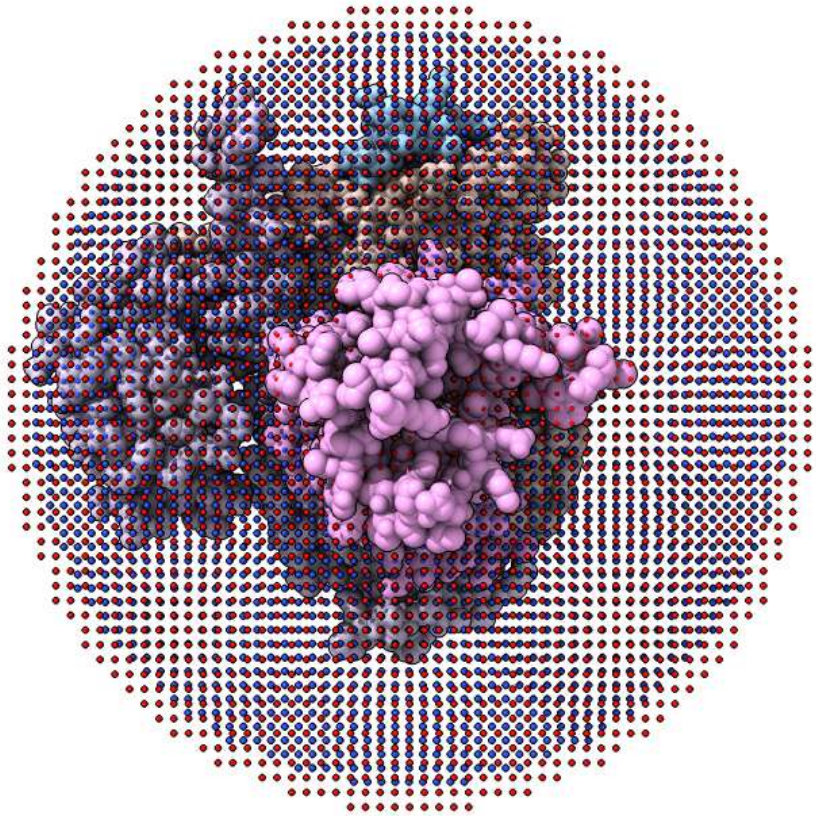
- 我们都知道细胞表面有双层磷脂组成的细胞膜，细胞膜上分布着许多蛋白；细胞膜作为细胞内外的分界，其上的许多蛋白具有沟通胞内外信息的作用；
- 对膜蛋白而言，它和水溶性蛋白最大的区别就是它被磷脂膜包裹在中间；
- 在进行膜蛋白的模拟时，我们必须考虑膜环境。

Get Membrane Position from OPM Database

https://opm.phar.umich.edu/

- 为了设置适当的膜环境，我们需要获得膜相对的位置
- 如我们要对PDB 3SN6进行模拟，在OPM数据库搜索该结构，可以获得标注了膜位置的PDB文件
- 如果没有3SN6，也可以找同一蛋白或者同源蛋白的结构代替

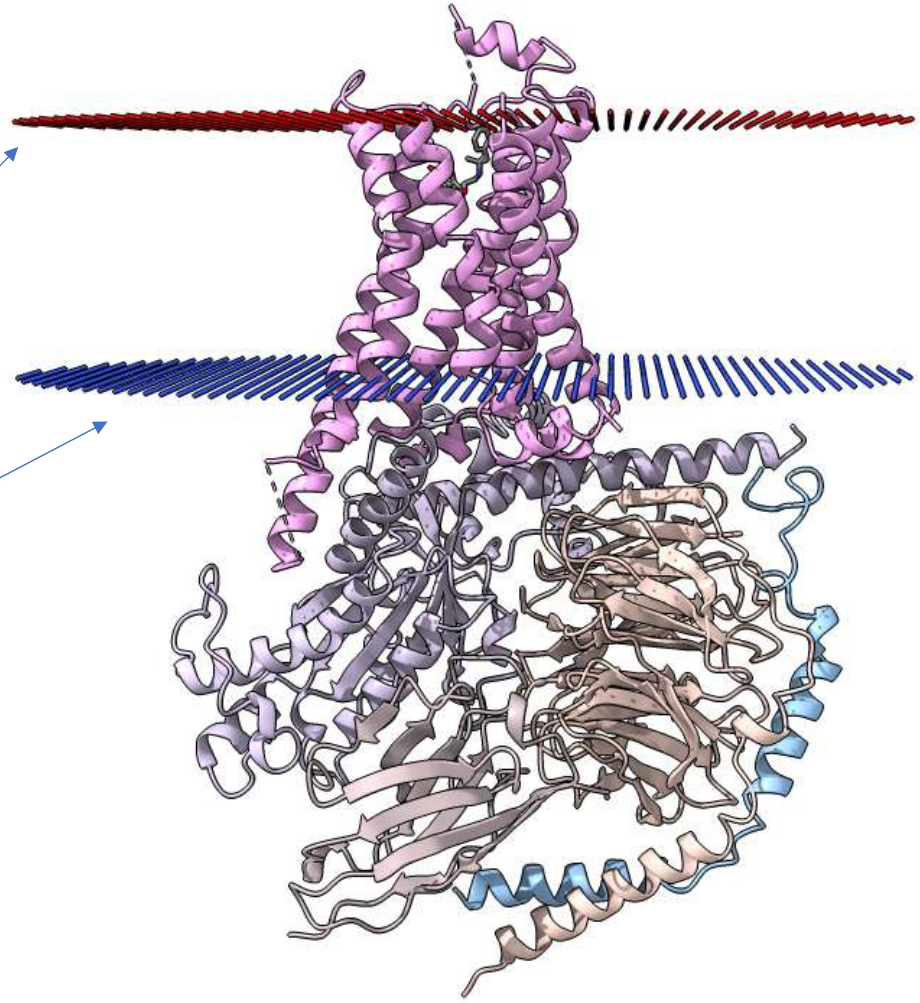




Get Membrane Position from OPM Database

https://opm.phar.umich.edu/

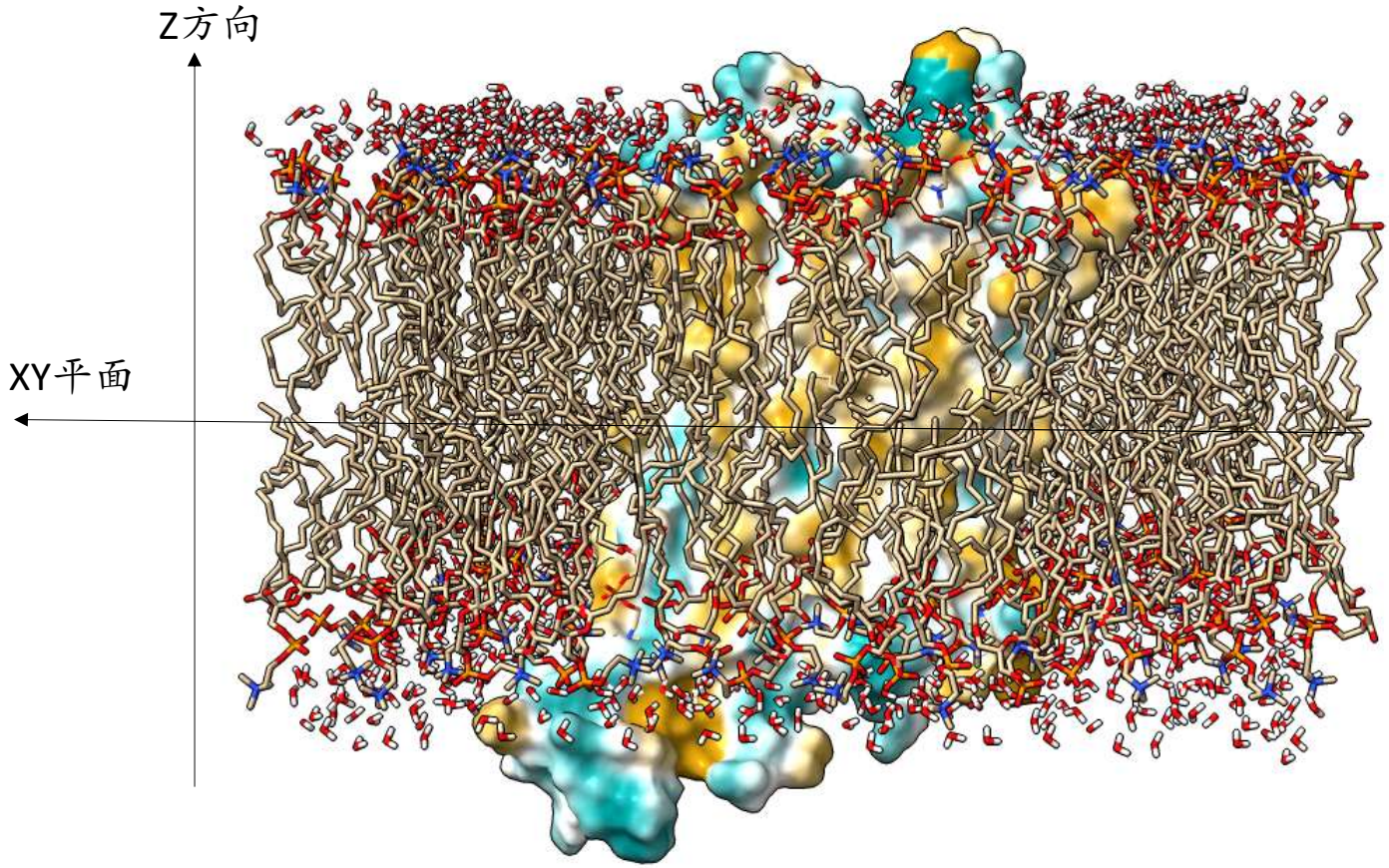
- 从OPM获得的结构中，通过**DUM**原子标记了双层膜的位置
- 文件的第一行**REMARK**标记了膜的厚度



```
REMARK    1/2 of bilayer thickness:   15.5
ATOM      1  N   THR A   9     -22.316  41.823 -24.680  1.00171.15      A    N
ATOM      2  CA  THR A   9     -23.632  41.941 -24.066  1.00171.98      A    C
ATOM      3  C   THR A   9     -24.727  41.980 -25.130  1.00166.96      A    C
ATOM      4  O   THR A   9     -24.655  41.278 -26.139  1.00166.22      A    O
ATOM      5  CB  THR A   9     -23.725  43.210 -23.190  1.00174.37      A    C
ATOM      6  OG1 THR A   9     -23.688  44.371 -24.029  1.00172.10      A    O
ATOM      7  CG2 THR A   9     -22.568  43.264 -22.208  1.00175.31      A    C

HETATM11872  O   DUM 11872       8.000   4.000  15.500
HETATM11873  N   DUM 11873       8.000   6.000 -15.500
HETATM11874  O   DUM 11874       8.000   6.000  15.500
HETATM11875  N   DUM 11875       8.000   8.000 -15.500
HETATM11876  O   DUM 11876       8.000   8.000  15.500
HETATM11877  N   DUM 11877       8.000  10.000 -15.500
HETATM11878  O   DUM 11878       8.000  10.000  15.500
HETATM11879  N   DUM 11879       8.000  12.000 -15.500
HETATM11880  O   DUM 11880       8.000  12.000  15.500
```

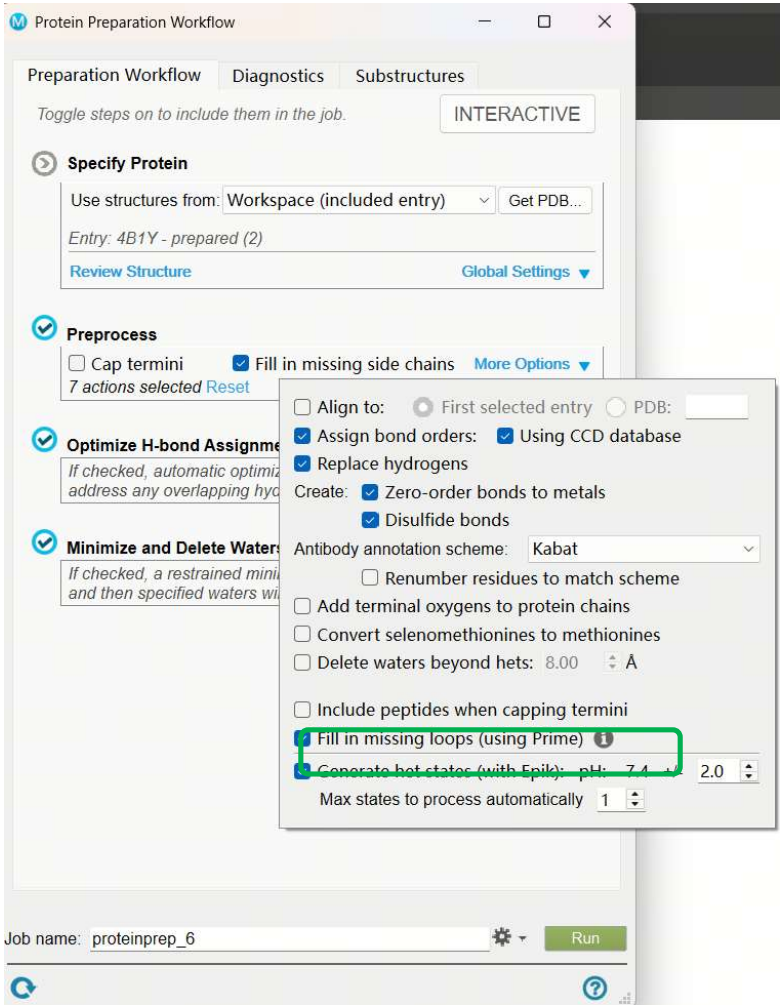
Membrane Protein



- 从OPM下载下来的结构，膜平面一定是与XY平面平行的，水只会出现在膜蛋白的Z方向上
- 与普通模拟的区别，首先要加膜
- 其次由于XY平面是膜，Z方向是水，控制压力的时候会有区别

Protein Preparation

无论如何，进行模拟之前一定要做蛋白质准备

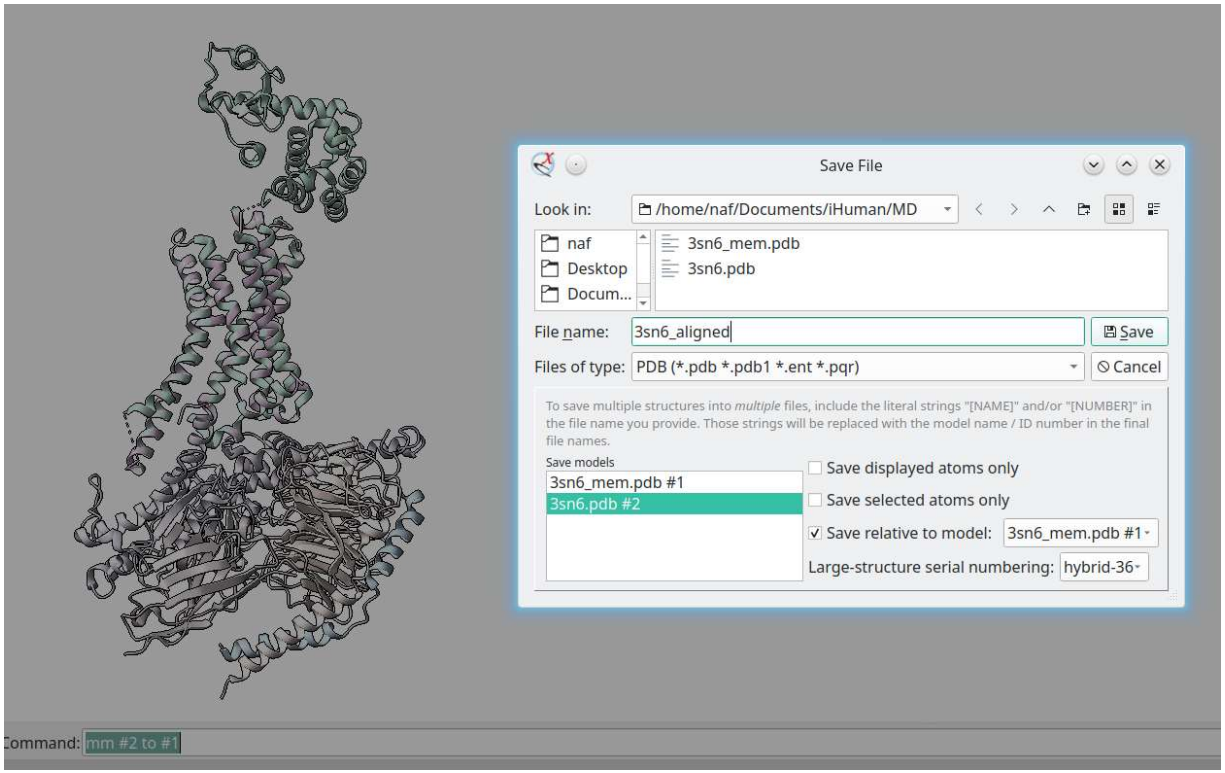


- 补充缺失的残基
- 补充缺失的侧链
- 复原结构解析时引入的氨基酸突变
- 删除不需要的组分
- 修正配体的质子化状态，键级等

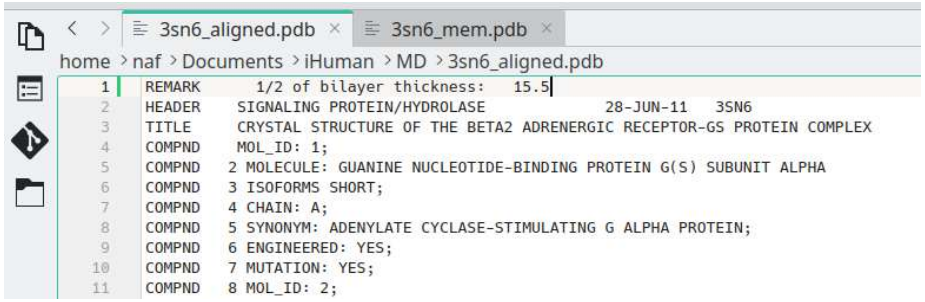
- 保存一个同时具有蛋白和配体的PDB文件
- 以及一个只有配体的sdf文件，同时记录一下配体带电量

Align Prepared Protein to OPM structure

在ChimeraX中将待模拟的蛋白align到从OPM下载的结构上，存储。



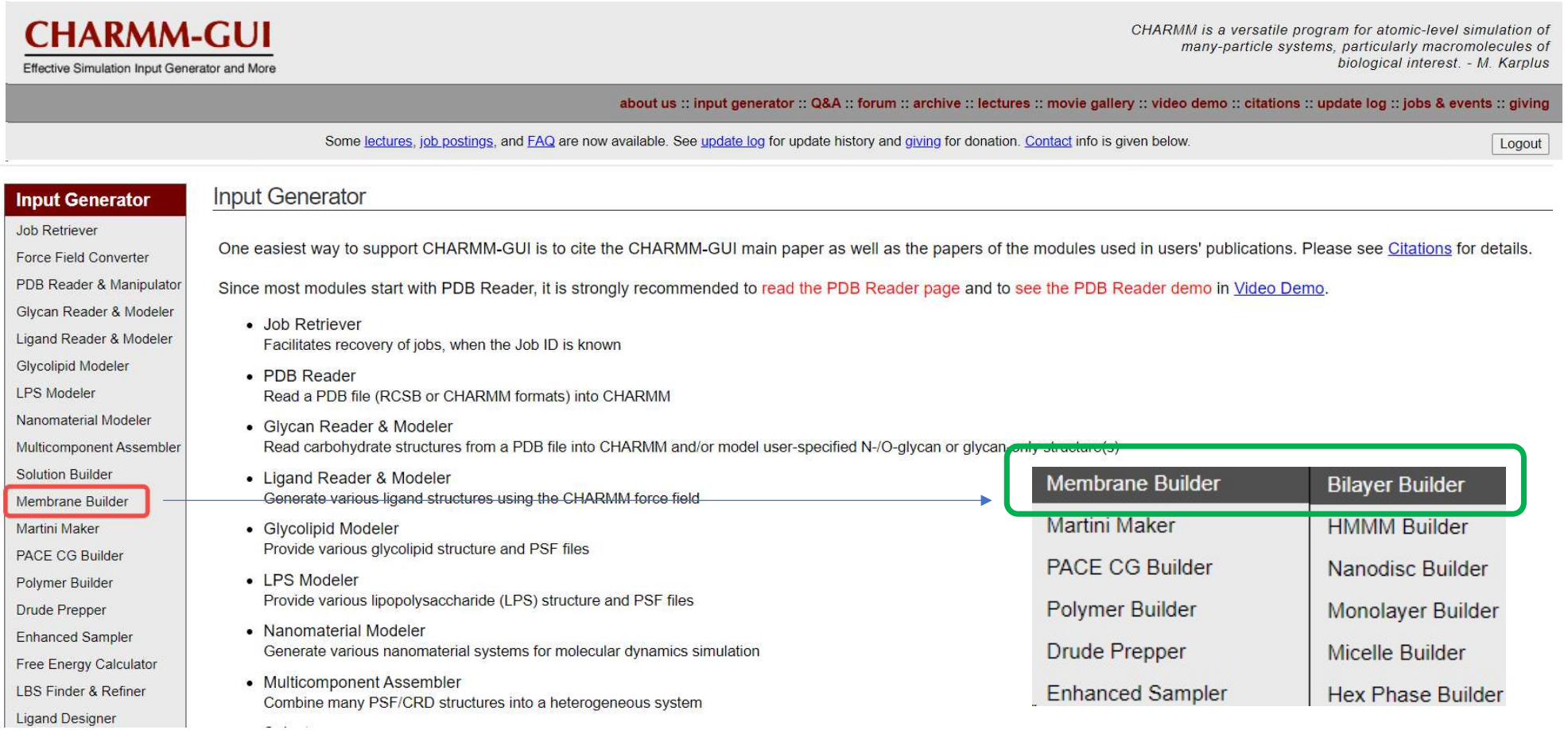
将第一行复制到align好的文件里。



ChimeraX命令 mm #2 to #1
会将第二个结构，我这里是3sn6.pdb，对齐到第一个结构，也就是OPM上的3sn6.pdb上

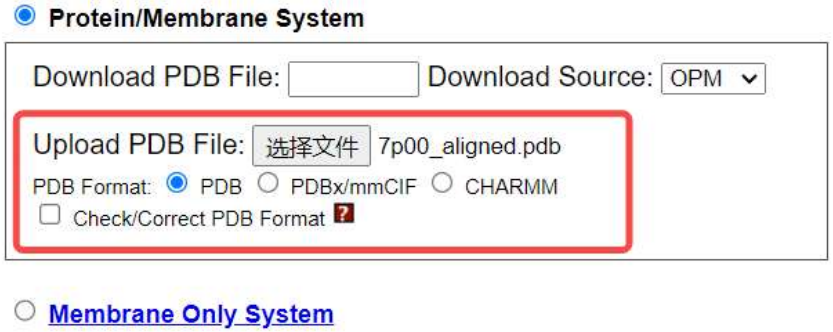
CHARMM-GUI

https://charmm-gui.org/?doc=input



CHARMM-GUI

https://charmm-gui.org/?doc=input

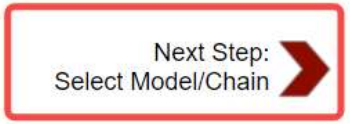


选择对齐好了的，准备好了的蛋白，在此上传；
如果要做蛋白-配体复合物模拟，记得上传的PDB里别漏了配体。

References for Lipid Force Fields:

J.B. Klauda, R.M. Venable, J.A. Freites, J.W. O'Connor, D.J. Tobias, C. Mondragon-Ramirez, I. Vorobyov, A.D. MacKerell, Jr., and R. W. Pastor (2010)
Update of the CHARMM all-atom additive force field for lipids: Validation on six lipid types *J Phys Chem B. 114(23): 7830–7843*

R.M. Venable, A.J. Sodt, B. Rogaski, H. Rui, E. Hatcher, A.D. MacKerell, Jr., R.W. Pastor, and J.B. Klauda. (2014)
CHARMM All-Atom Additive Force Field for Sphingomyelin: Elucidation of Hydrogen Bonding and of Positive Curvature. *Biophys J. 107(1):134-45*



点击下一步。

CHARMM-GUI

https://charmm-gui.org/?doc=input

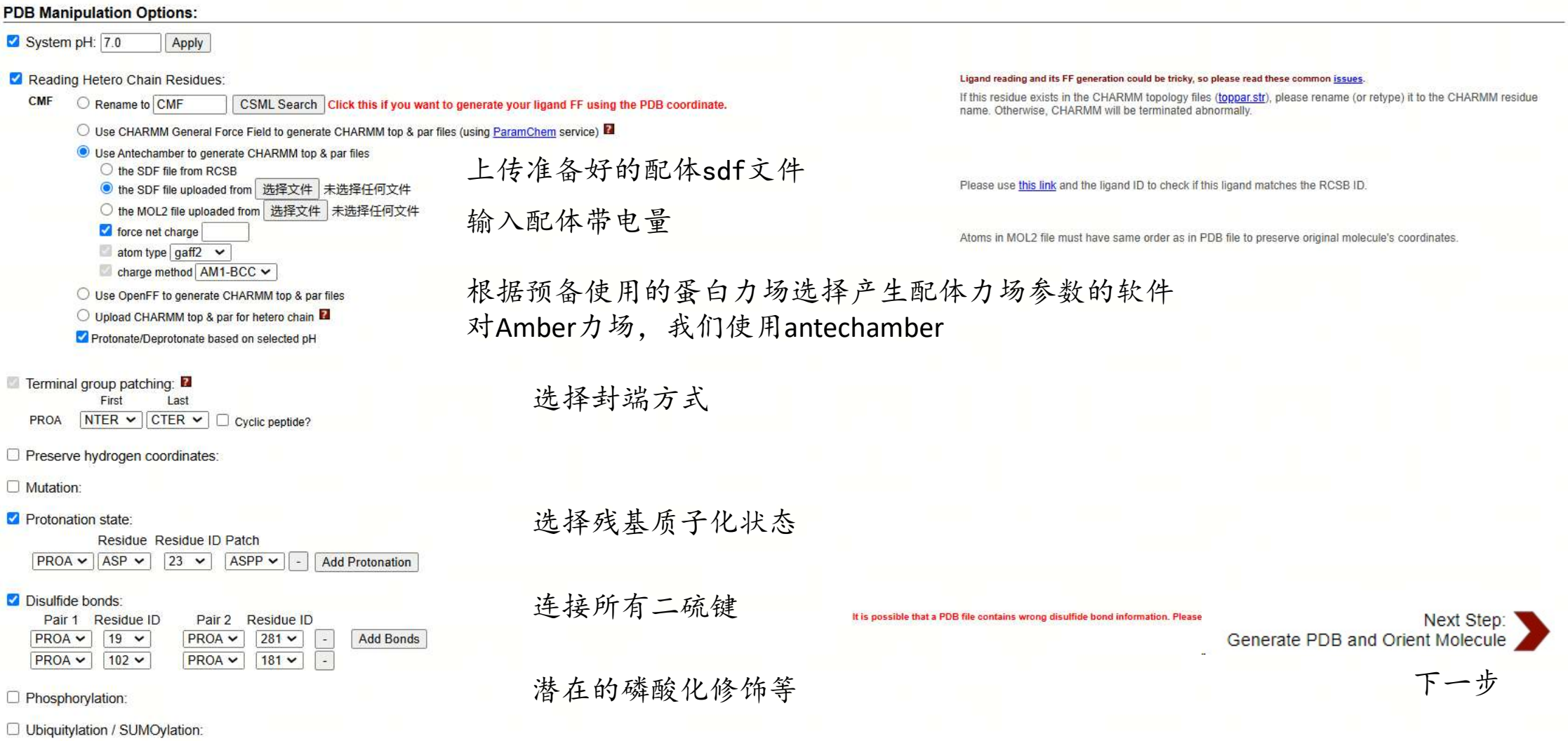
记住ID号，可以用于回到任务

选择需要进行模拟的蛋白和配体

下一步

CHARMM-GUI

https://charmm-gui.org/?doc=input



上传准备好的配体sdf文件

输入配体带电量

根据预备使用的蛋白力场选择产生配体力场参数的软件
对Amber力场，我们使用antechamber

选择封端方式

选择残基质子化状态

连接所有二硫键

潜在的磷酸化修饰等

下一步

CHARMM-GUI

https://charmm-gui.org/?doc=input

Computed Energy:

Please beware of that the computed energy is CHARMM single-point energy and is displayed to make sure all the coordinates are defined.

ENER ENR:	Eval#	ENERgy	Delta-E	GRMS		
ENER INTERN:		BONDs	ANGLes	UREY-b	DIHEdrals	IMPRopers
ENER CROSS:		CMAPs	PMF1D	PMF2D	PRIMO	
ENER EXTERN:		VDWaals	ELEC	HBONds	ASP	USER
ENER>	0	1089.17985	0.00000	8.12560		
ENER INTERN>		552.02091	1065.34547	53.94700	2607.69352	14.06989
ENER CROSS>		92.20264	0.00000	0.00000	0.00000	
ENER EXTERN>		-1551.08363	-1745.01596	0.00000	0.00000	0.00000

Orientation Options:

- (●) Use PDB Orientation — This option is suggested for an oriented structure from http://opm.phar.umich.edu
- ( ) Align the First Principal Axis Along Z — This option is suggested for small helical bundle or homo-oligomer.
- ( ) Align a Vector (Two Atoms) Along Z — This option is suggested for an irregular, hetero-oligomer.
- ( ) Run PPM 2.0 — This option run executable for given input structure at https://opm.phar.umich.edu/ppm_server2_cgopm It may take some minutes depending on protein size.

我们已经将结构按OPM对齐了

Positioning Options:

- [ ] Rotate Molecule respect to the X axis [ ] Degree
- [ ] Rotate Molecule respect to the Y axis [ ] Degree
- [ ] Translate Molecule along Z axis [ ] Angstrom
- [ ] Flip Molecule along the Z axis

Area Calculation Options:

- [ ] Generate Pore Water and Measure Pore Size

Next Step:
Calculate Cross-Sectional Area

下一步

System Size Determination Options:

Homogeneous Lipid "Homogeneous Lipid" option is no longer supported. You can use "Heterogeneous Lipid" option even for homogeneous lipid bilayer building.

Heterogeneous Lipid

1. Box Type: Rectangular (Currently, only CHARMM, NAMD, and GROMACS support the hexagonal box)

默认的膜体系搭建方式

2. Length of Z based on:
   - Water thickness 22.5 (Minimum water height on top and bottom of the system)
   - Hydration number 50 (Number of water molecules per one lipid molecule)

3. Length of XY based on:
   - Ratios of lipid components
   - Numbers of lipid components

选择膜脂数目

XY Dimension Ratio: 1
(The system size along the X and Y must be the same)

Show the system info click this once you fill the following table:

选择好膜脂数目后，点击，
如果没报错就可以了

Lipid Type	Charge [e]	Tail Info. [sn1/sn2]	Images	# of Lipid on Upperleaflet	# of Lipid on Lowerleaflet	Surface Area
▸ Sterols						
▸ PA (phosphatidic acid) Lipids						
▾ PC (phosphatidylcholine) Lipids						
DDPC	0	10:0 / 10:0	[Image]	0	0	64.1
DCPC	0	11:0 / 11:0	[Image]	0	0	64.1
DLPC	0	12:0 / 12:0	[Image]	0	0	64.1
DMPC	0	14:0 / 14:0	[Image]	0	0	64.1
DPPC	0	16:0 / 16:0	[Image]	0	0	63.0
DSPC	0	18:0 / 18:0	[Image]	0	0	65.6
PSPC	0	16:0 / 18:0	[Image]	0	0	68.3
PYPC	0	16:0 / 16:1	[Image]	0	0	64.1
POPC	0	16:0 / 18:1	[Image]	90	90	68.3
PLPC	0	16:0 / 18:2	[Image]	0	0	65.2

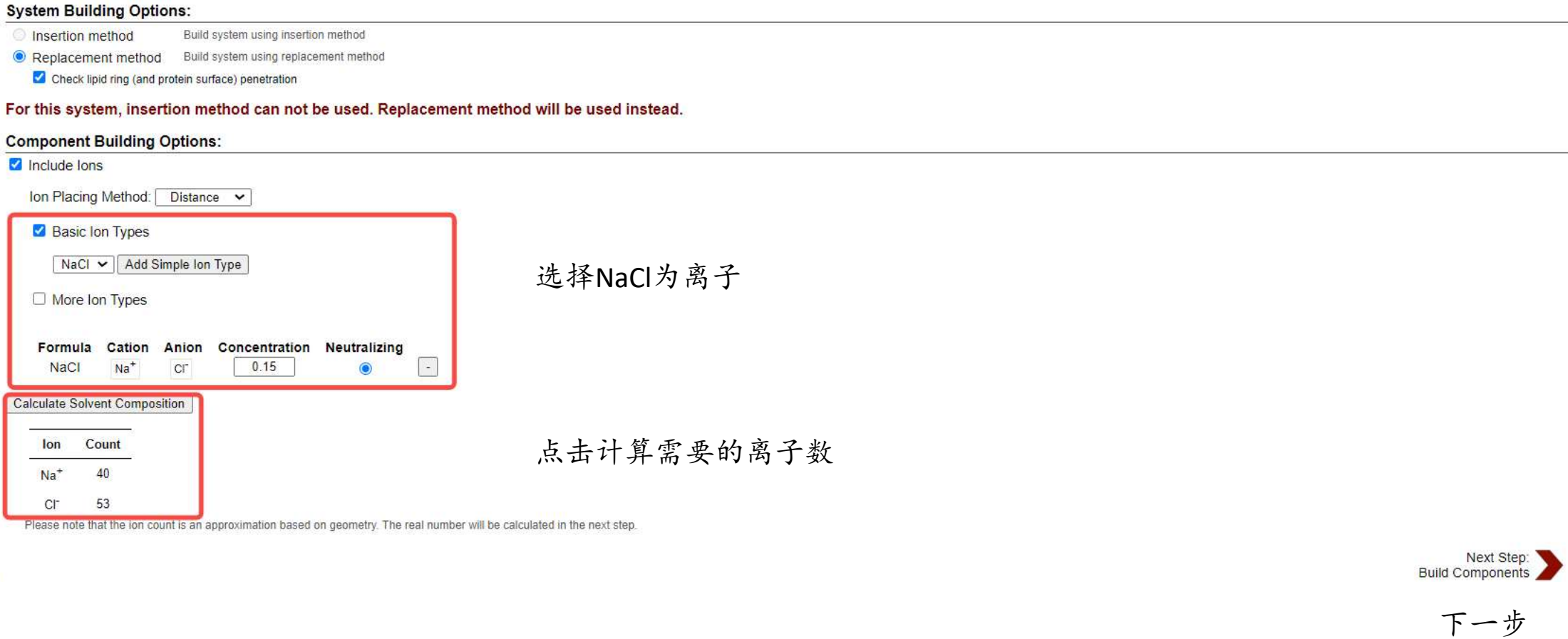
Calculated XY System Size:

	Upperleaflet	Lowerleaflet
Protein Area	1156.13503	1186.81708
Lipid Area	6147	6147
# of Lipids	90	90
Total Area	7303.13503	7333.81708
Protein X Extent	27.44	
Protein Y Extent	21.79	
Average Area	7318.48	
A	85.55	
B	85.55	

对GPCR，初始设置上下各90个，然后微调直到不报错

Next Step:
Determine the System Size

下一步



选择NaCl为离子

点击计算需要的离子数

下一步

CHARMM-GUI

https://charmm-gui.org/?doc=input

Membrane Builder

User Profile

Bookmark this link, if you want to comeback to this page

PDB Info | STEP 1 | STEP 2 | STEP 3 | STEP 4 | STEP 5 | STEP 6

JOB ID: 0805166713

download.tgz

Oriented PDB: step2_orient.pdb (view structure)
Component Input: step4_lipid.inp
Component Output: step4_lipid.out
Component Number: step4_components.str
Component PDB: step4_lipid.pdb (view structure)

Check lipid pentration

The protein surface penetration check finds the lipid tails that go beyond the protein surface, and the lipid ring penetration check detects the lipid tails that pass through the cyclic groups (e.g., cholesterol ring) in the simulation systems. Energy minimization can resolve many of these bad contacts, but one might need to visually check the following lipid molecules to ensure the following contacts are resolved. The user can regenerate the lipid bilayer if necessary.

Protein surface penetration:

```
No protein surface penetration is found.
```

Lipid ring penetration:

```
No lipid ring penetration is found.
```

Building Ion and Waterbox

Membrane components are generated. Due to time constrains, we first generate the lipid bilayer then generate ions and the water box. Click "Next Step" to generate ions and the water box.

没报错就可以下一步了

Next Step:
Assemble Components

CHARMM-GUI

https://charmm-gui.org/?doc=input

没报错就可以继续下一步了

CHARMM-GUI

https://charmm-gui.org/?doc=input

Force Field Options:

AMBER
CHARMM36m
CHARMM36
AMBER

elds

DNA	RNA	Glycan	Lipid	Water	Ligand
OL15	OL3	GLYCAM_06j	Lipid21	TIP3P	GAFF2

FF14SB

☐ Hydrogen mass repartitioning
☐ 12-6-4 ion

Glycolipids and lipoglycans are not supported in current CHARMM-GUI Amber FF implementation.

选择力场，charmm36m及amber14SB力场均可

对amber19SB力场，只有软件amber能识别；对gromacs，最多使用到14SB力场。

Input Generation Options:

☑ AMBER
☑ GROMACS
☑ OpenMM
☐ NAMD
☐ GENESIS
☐ Tinker

选择进行MD的软件，会生成勾选的软件的需要模拟的全套文件

Equilibration Options:

☐ P21 image transformation (only available for CHARMM)
☐ CHARMM DOMDEC (only available for CHARMM)
◉ Generate grid information for PME FFT automatically
○ Explicit grid information for PME FFT

X Y Z

◉ NPT ensemble
○ NPAT ensemble
○ NPgT ensemble

Surface Tension 0 (dyne/cm)

Temperature: 303.15 K

Next Step:
Generate Equilibration and Dynamics Inputs

下一步

CHARMM-GUI

https://charmm-gui.org/?doc=input

Membrane Builder

User Profile

Bookmark this link, if you want to comeback to this page

PDB Info | STEP 1 | STEP 2 | STEP 3 | STEP 4 | STEP 5 | STEP 6

JOB ID: 0805166713

download.tgz

Assembled PDB:	step5_assembly.pdb (view structure)	
Input Generator Input:	step5_input.inp	
Input Generator Output:	step5_input.out	
CHARMM Minimization:	step5_input_minimization.str	
Crystal Image:	crystal_image.str	
FFT Calculation:	checkfft.py	
Restraints:	membrane_restraint.str	Lipid Positional Restraint
	membrane_restraint2.str	Lipid Dihedral Restraint
Equilibration Inputs:	amber/step6.0_minimization.mdin	Minimization Input
	amber/step6.1_equilibration.mdin	Equilibration Step 1
	amber/step6.2_equilibration.mdin	Equilibration Step 2
	amber/step6.3_equilibration.mdin	Equilibration Step 3
	amber/step6.4_equilibration.mdin	Equilibration Step 4
	amber/step6.5_equilibration.mdin	Equilibration Step 5
	amber/step6.6_equilibration.mdin	Equilibration Step 6
Production Inputs:	amber/step7_production.mdin	Production Input

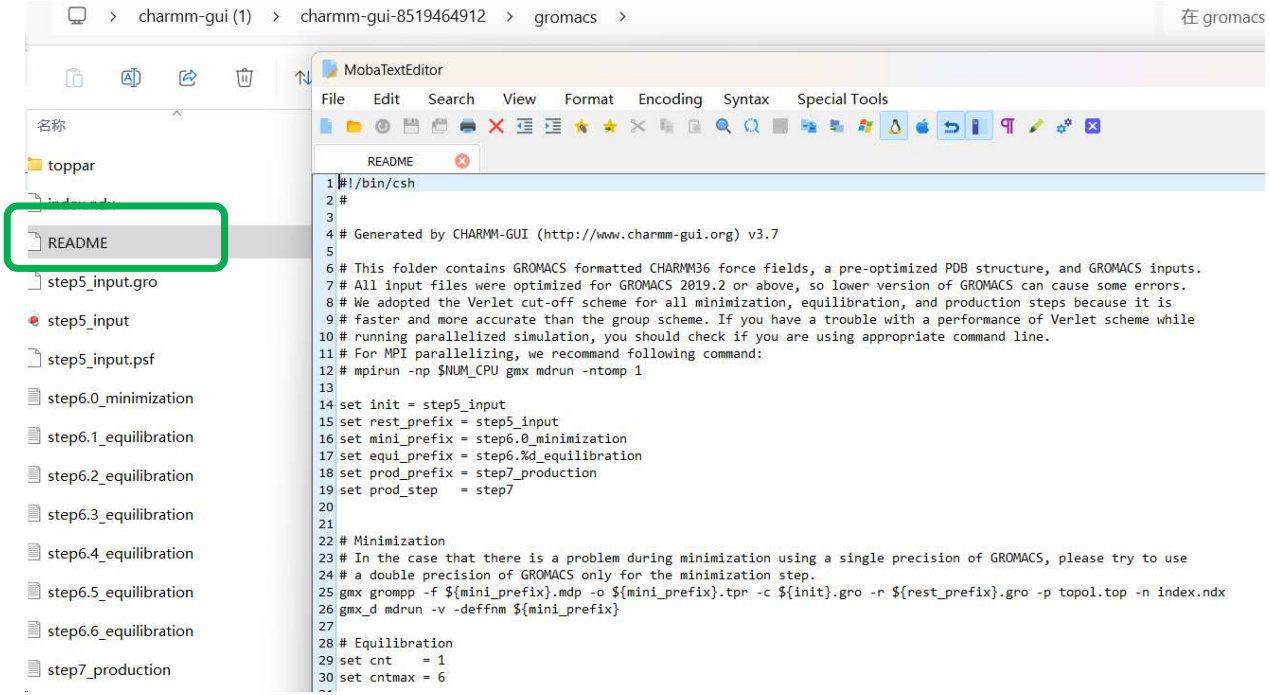
搞定，把结果下载下来即可

Equilibration Input Notes:

```
!
! Setup Restraints for Protein and Lipids (see @liptype_restraint.str)
!
! Suggested Equilibration Scheme [Reducing Force Constants]
! (5 Cycles, 1 cycle = 50 - 100 ps )
!
!          1 cycle    2 cycle    3 cycle    4 cycle    5 cycle    6 cycle
!
! BB         10.0        5.0        2.5        1.0        0.5        0.1
! SC          5.0        2.5        1.0        0.5        0.1        0.0
! wforce      2.5        2.5        1.0        0.5        0.1        0.0
! tforce      2.5        2.5        1.0        0.5        0.1        0.0
! mforce      2.5        2.5        1.0        0.5        0.1        0.0
! ion        10.0        0.0        0.0        0.0        0.0        0.0
!
!
```

CHARMM-GUI

https://charmm-gui.org/?doc=input



解压缩之后，readme内有运行MD需要的所有命令。

Charmm-gui产生的文件是宝藏，值得好好学习。

- README里有地方使用了双精度的gmx，我们可能没有安装，需要手动改成我们安装的gromacs名称，比如把gmx_d都改成gmx;
- 给README添加可执行权限，再直接执行它，就可以自动把模拟跑起来了
- 记得按需求修改一下step7_production.mdp里写的模拟时长

```
chmod +x README
./README
```

膜蛋白模拟与普通蛋白模拟重要区别

普通蛋白mdp文件：
- 各向同性控压 isotropic
- 指定1个compressibility，1个tau_p和ref_p即可

```
; 压力控制
pcoupl              = Parrinello-Rahman
pcoupltype          = isotropic   ; 各向同性压力
tau_p               = 5       ; 压力耦合时间常数
compressibility     = 4.5e-5      ; 压缩率
ref_p               = 1.0       ; 参考压力1.0 bar
```

普通蛋白mdp文件：
- 对xy方向和z方向分别进行压力控制，采用semiisotropic
- 指定2个compressibility，2个ref_p和2个tau_p

```
; 压力控制
pcoupl              = Parrinello-Rahman
pcoupltype          = semiisotropic   ;
tau_p               = 5 5       ; 压力耦合时间常数
compressibility     = 4.5e-5 4.5e-5     ; 压缩率
ref_p               = 1.0 1.0       ; 参考压力1.0 bar
```

半各向同性压力耦合是一种用于分子动力学模拟的方法，特别适用于涉及膜或双层系统的模拟。这种方法允许在x和y方向上进行各向同性压力耦合，但在z方向上进行不同的压力耦合。这对于膜模拟特别有用，因为脂质双层应该被允许在x-y平面上变形，但在z方向上则不应变形。