Simulation with Amber

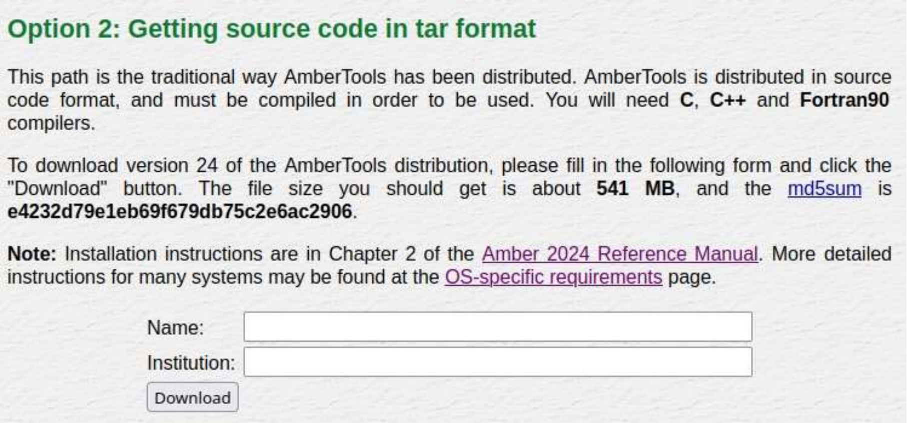
Naf Guo
2025

Download Amber24

https://ambermd.org/GetAmber.php

https://ambermd.org/GetAmber.php

从2024年的某一天开始，大善人amber学术免费了！我们需要下载amber24和ambertools24



Option 2: Getting source code in tar format

This path is the traditional way AmberTools has been distributed. AmberTools is distributed in source code format, and must be compiled in order to be used. You will need **C**, **C++** and **Fortran90** compilers.

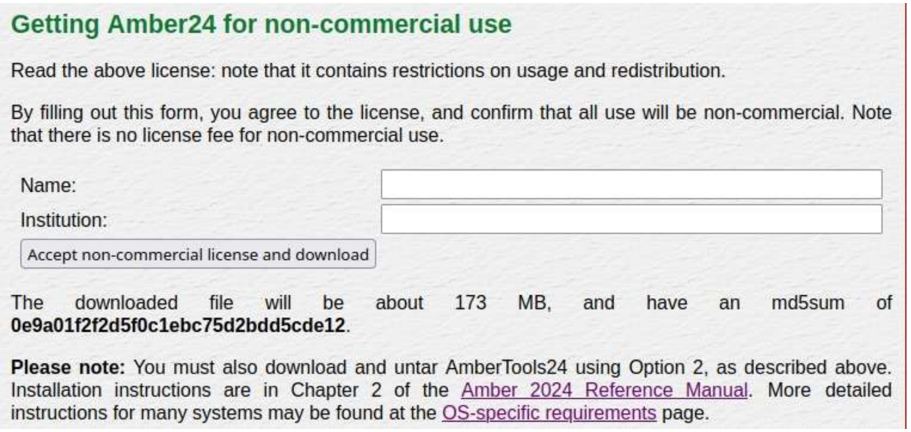
To download version 24 of the AmberTools distribution, please fill in the following form and click the "Download" button. The file size you should get is about **541 MB**, and the md5sum is **e4232d79e1eb69f679db75c2e6ac2906**.

Note: Installation instructions are in Chapter 2 of the Amber 2024 Reference Manual. More detailed instructions for many systems may be found at the OS-specific requirements page.

Name:

Institution:

Ambertools24的安装包



Getting Amber24 for non-commercial use

Read the above license: note that it contains restrictions on usage and redistribution.

By filling out this form, you agree to the license, and confirm that all use will be non-commercial. Note that there is no license fee for non-commercial use.

Name:

Institution:

The downloaded file will be about 173 MB, and have an md5sum of **0e9a01f2f2d5f0c1ebc75d2bdd5cde12**.

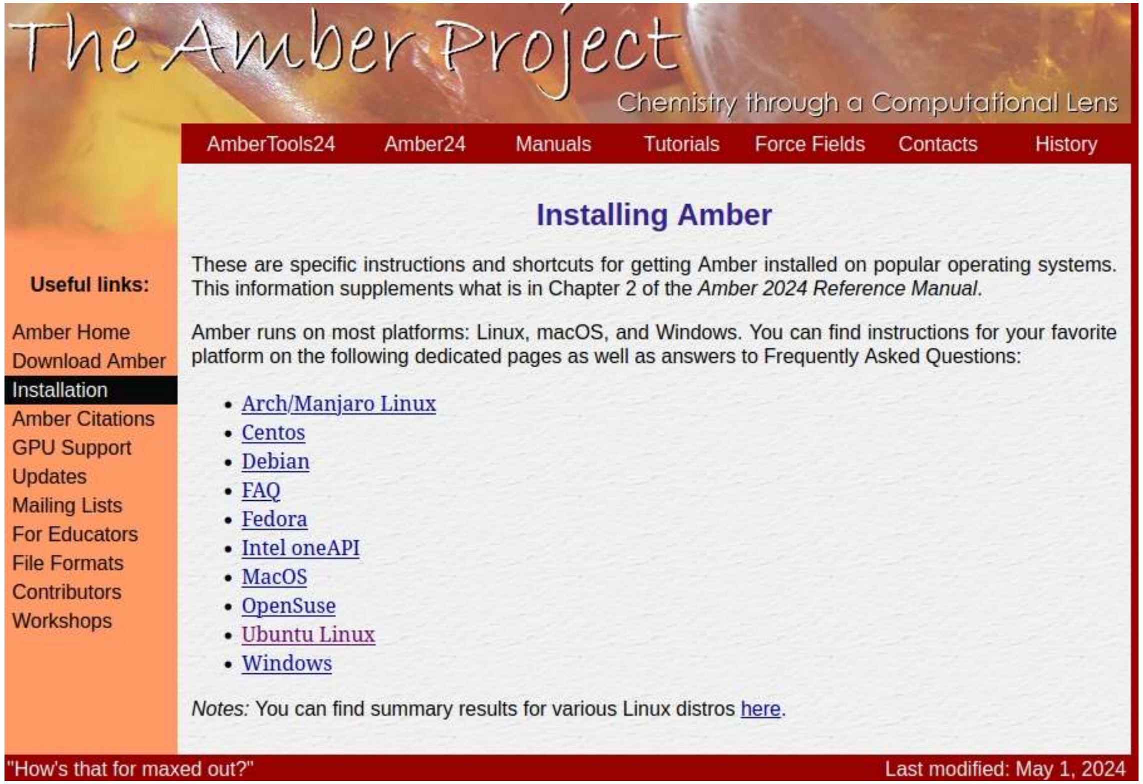
Please note: You must also download and untar AmberTools24 using Option 2, as described above. Installation instructions are in Chapter 2 of the Amber 2024 Reference Manual. More detailed instructions for many systems may be found at the OS-specific requirements page.

Amber24的安装包

Install Amber24

https://ambermd.org/Installation.php

https://ambermd.org/Installation.php



Installing Amber

These are specific instructions and shortcuts for getting Amber installed on popular operating systems. This information supplements what is in Chapter 2 of the *Amber 2024 Reference Manual*.

Amber runs on most platforms: Linux, macOS, and Windows. You can find instructions for your favorite platform on the following dedicated pages as well as answers to Frequently Asked Questions:

- Arch/Manjaro Linux
- Centos
- Debian
- FAQ
- Fedora
- Intel oneAPI
- MacOS
- OpenSuse
- Ubuntu Linux
- Windows

Notes: You can find summary results for various Linux distros here.

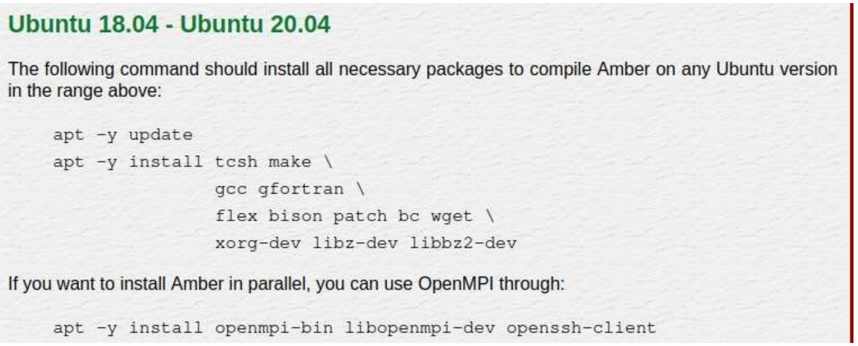
"How's that for maxed out?" Last modified: May 1, 2024

经过gromacs的洗礼以后，我们对怎么安装软件应该有一定的熟练度了，amber官方提供了不同操作系统下的安装教程。

Install Amber24 on Ubuntu

https://ambermd.org/InstUbuntu.php

首先使用apt安装依赖



Ubuntu 18.04 - Ubuntu 20.04

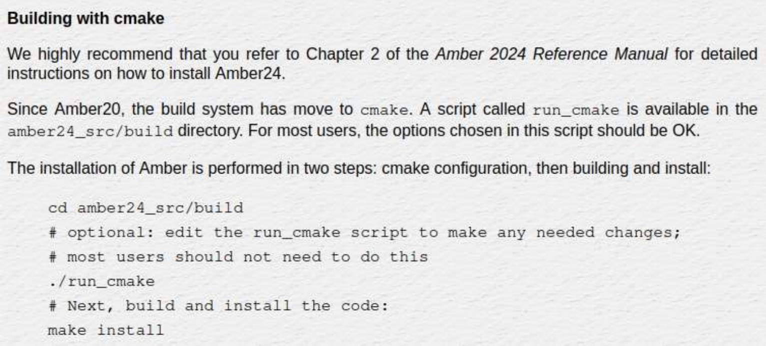
The following command should install all necessary packages to compile Amber on any Ubuntu version in the range above:

```
apt -y update
apt -y install tcsh make \
               gcc gfortran \
               flex bison patch bc wget \
               xorg-dev libz-dev libbz2-dev
```

If you want to install Amber in parallel, you can use OpenMPI through:

```
apt -y install openmpi-bin libopenmpi-dev openssh-client
```

之后使用amber提供的run_cnake脚本安装amber



Building with cmake

We highly recommend that you refer to Chapter 2 of the *Amber 2024 Reference Manual* for detailed instructions on how to install Amber24.

Since Amber20, the build system has move to `cmake`. A script called `run_cmake` is available in the `amber24_src/build` directory. For most users, the options chosen in this script should be OK.

The installation of Amber is performed in two steps: cmake configuration, then building and install:

```
cd amber24_src/build
# optional: edit the run_cmake script to make any needed changes;
# most users should not need to do this
./run_cmake
# Next, build and install the code:
make install
```

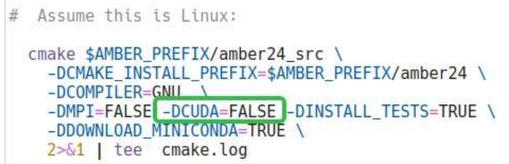
对ubuntu24.04

```
sudo apt install tcsh make gcc gfortran flex bison patch bc wget xorg-dev libz-dev libbz2-dev
```

```
tar xvfj AmberTools24.tar.bz2
```

```
tar xvfj Amber24.tar.bz2
```

解压缩



```
#  Assume this is Linux:

  cmake $AMBER_PREFIX/amber24_src \
    -DCMAKE_INSTALL_PREFIX=$AMBER_PREFIX/amber24 \
    -DCOMPILER=GNU  \
    -DMPI=FALSE -DCUDA=FALSE -DINSTALL_TESTS=TRUE \
    -DDOWNLOAD_MINICONDA=TRUE \
    2>&1 | tee  cmake.log
```

之后极其重要的一步：修改build文件夹内的run_cmake脚本，修改DCUDA后为TRUE以编译支持GPU加速的pmemd

```
./run_cmake
```

```
make install -j 4
```

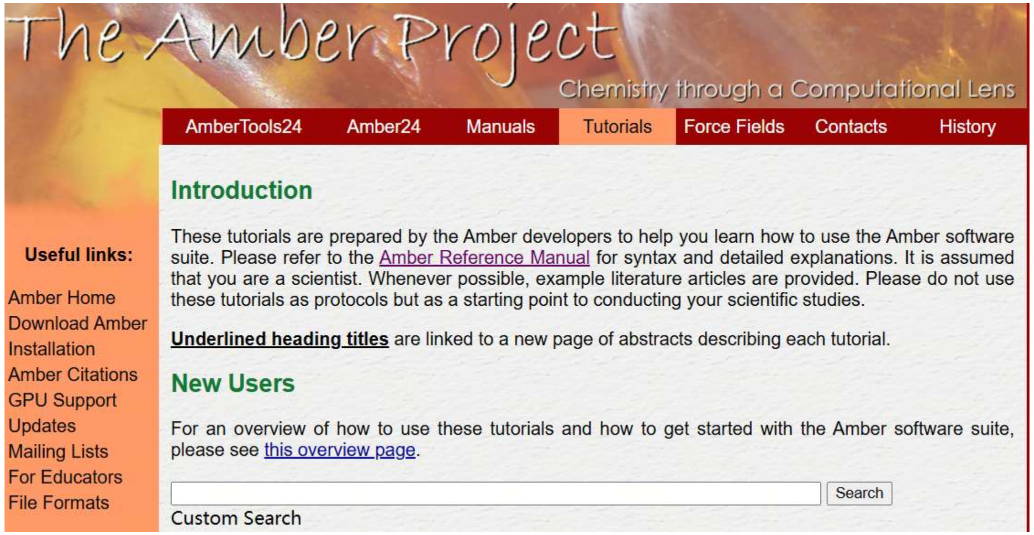
```
source /home/path-to-amber-dir/amber24/amber.sh
```

Amber Tutorials

https://ambermd.org/tutorials/

Amber具有丰富的官方教程

https://ambermd.org/tutorials/



Introduction

These tutorials are prepared by the Amber developers to help you learn how to use the Amber software suite. Please refer to the Amber Reference Manual for syntax and detailed explanations. It is assumed that you are a scientist. Whenever possible, example literature articles are provided. Please do not use these tutorials as protocols but as a starting point to conducting your scientific studies.

Underlined heading titles are linked to a new page of abstracts describing each tutorial.

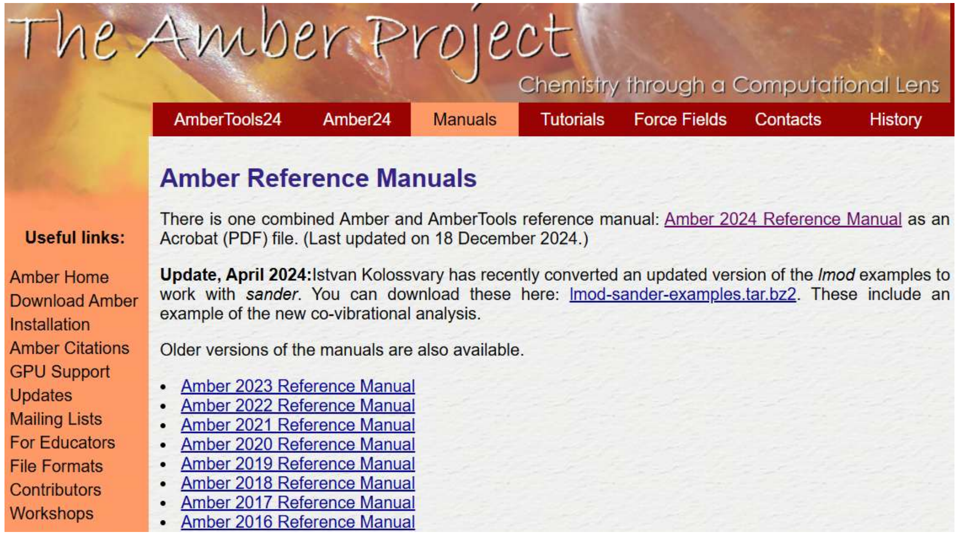
New Users

For an overview of how to use these tutorials and how to get started with the Amber software suite, please see this overview page.

Custom Search

Amber具有详细的文档

https://ambermd.org/Manuals.php



Amber Reference Manuals

There is one combined Amber and AmberTools reference manual: Amber 2024 Reference Manual as an Acrobat (PDF) file. (Last updated on 18 December 2024.)

Update, April 2024: Istvan Kolossvary has recently converted an updated version of the *lmod* examples to work with *sander*. You can download these here: lmod-sander-examples.tar.bz2. These include an example of the new co-vibrational analysis.

Older versions of the manuals are also available.

- Amber 2023 Reference Manual
- Amber 2022 Reference Manual
- Amber 2021 Reference Manual
- Amber 2020 Reference Manual
- Amber 2019 Reference Manual
- Amber 2018 Reference Manual
- Amber 2017 Reference Manual
- Amber 2016 Reference Manual

几个与模拟强相关的教程

https://ambermd.org/tutorials/basic/tutorial7/index.php

https://ambermd.org/tutorials/basic/tutorial8/index.php

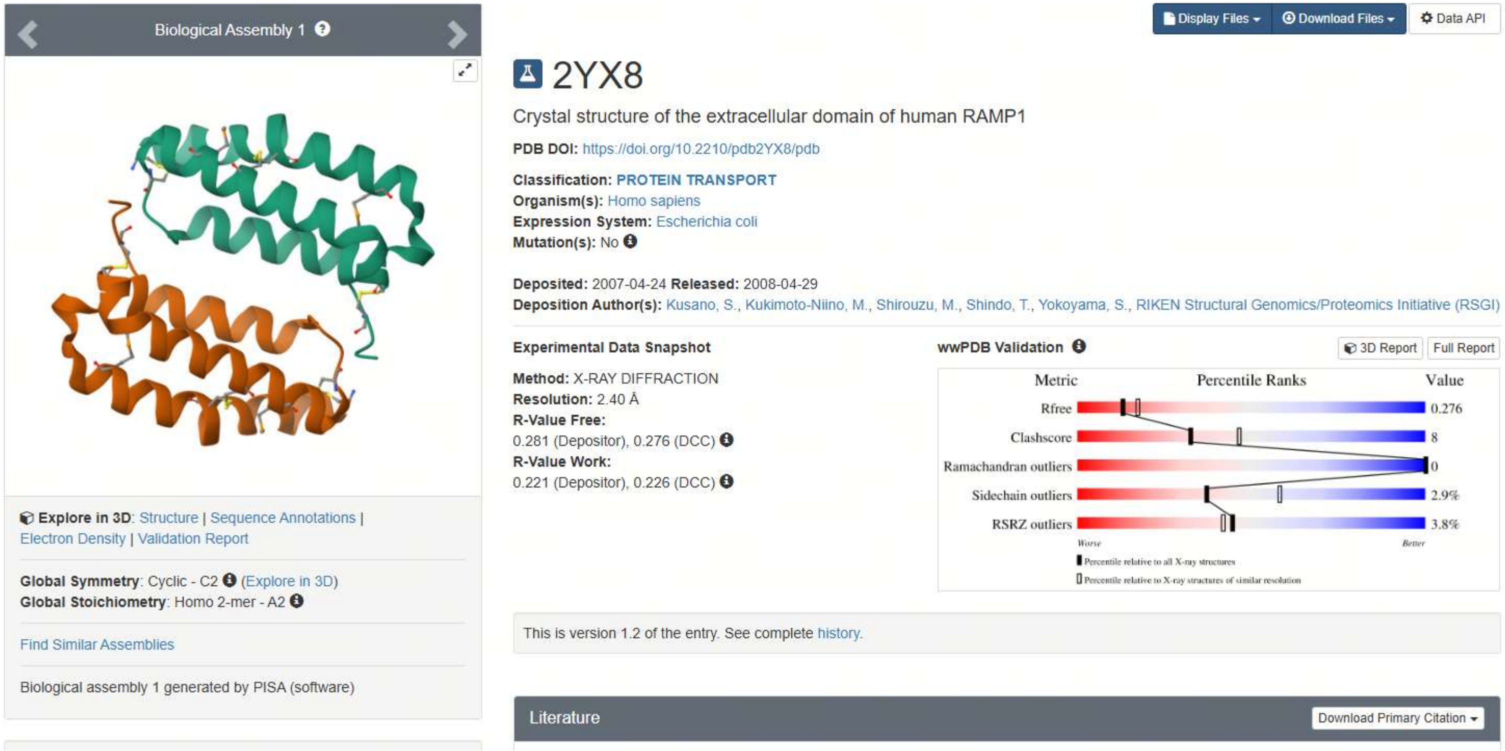
https://ambermd.org/tutorials/basic/tutorial13/index.php

https://ambermd.org/tutorials/basic/tutorial14/index.php

Download PDB

https://ambermd.org/tutorials/basic/tutorial7/index.php

我们就按照官方教程，下载2YX8作为测试体系



2YX8

Crystal structure of the extracellular domain of human RAMP1

PDB DOI: https://doi.org/10.2210/pdb2YX8/pdb

Classification: PROTEIN TRANSPORT
Organism(s): Homo sapiens
Expression System: Escherichia coli
Mutation(s): No

Deposited: 2007-04-24 **Released:** 2008-04-29
Deposition Author(s): Kusano, S., Kukimoto-Niino, M., Shirouzu, M., Shindo, T., Yokoyama, S., RIKEN Structural Genomics/Proteomics Initiative (RSGI)

Experimental Data Snapshot

Method: X-RAY DIFFRACTION
Resolution: 2.40 Å
R-Value Free:
0.281 (Depositor), 0.276 (DCC)
R-Value Work:
0.221 (Depositor), 0.226 (DCC)

wwPDB Validation

This is version 1.2 of the entry. See complete history.

Literature

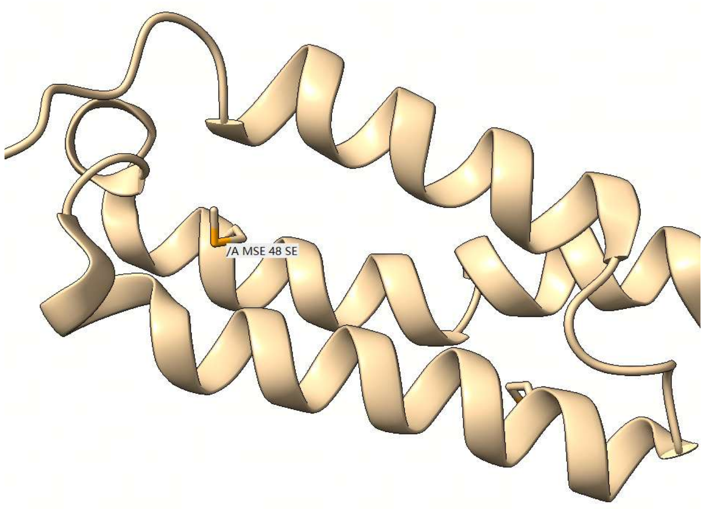
Explore in 3D: Structure | Sequence Annotations | Electron Density | Validation Report

Global Symmetry: Cyclic - C2 (Explore in 3D)
Global Stoichiometry: Homo 2-mer - A2

Find Similar Assemblies

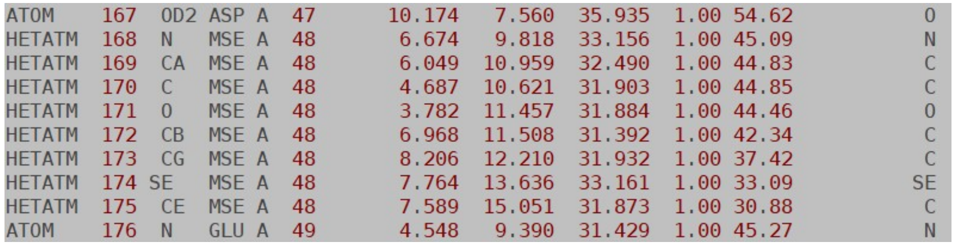
Biological assembly 1 generated by PISA (software)

Protein Preparation

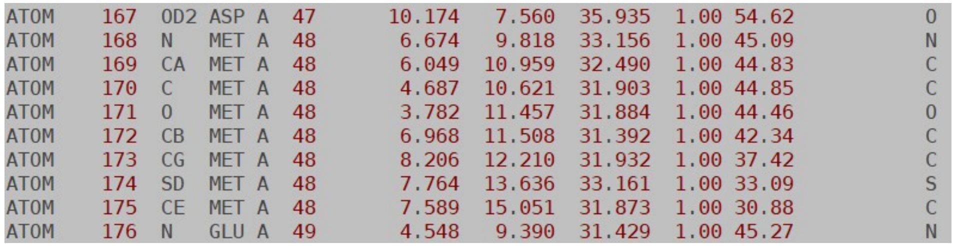


此结构有一个有趣的地方，就是它有二个残基是MSE，所谓MSE，就是把甲硫氨酸MET里的硫（S）换成了硒（Se）

需要手动修改回MET



```
ATOM    167  OD2 ASP A  47      10.174   7.560  35.935  1.00 54.62           O
HETATM  168  N   MSE A  48       6.674   9.818  33.156  1.00 45.09           N
HETATM  169  CA  MSE A  48       6.049  10.959  32.490  1.00 44.83           C
HETATM  170  C   MSE A  48       4.687  10.621  31.903  1.00 44.85           C
HETATM  171  O   MSE A  48       3.782  11.457  31.884  1.00 44.46           O
HETATM  172  CB  MSE A  48       6.968  11.508  31.392  1.00 42.34           C
HETATM  173  CG  MSE A  48       8.206  12.210  31.932  1.00 37.42           C
HETATM  174 SE   MSE A  48       7.764  13.636  33.161  1.00 33.09          SE
HETATM  175  CE  MSE A  48       7.589  15.051  31.873  1.00 30.88           C
ATOM    176  N   GLU A  49       4.548   9.390  31.429  1.00 45.27           N
```

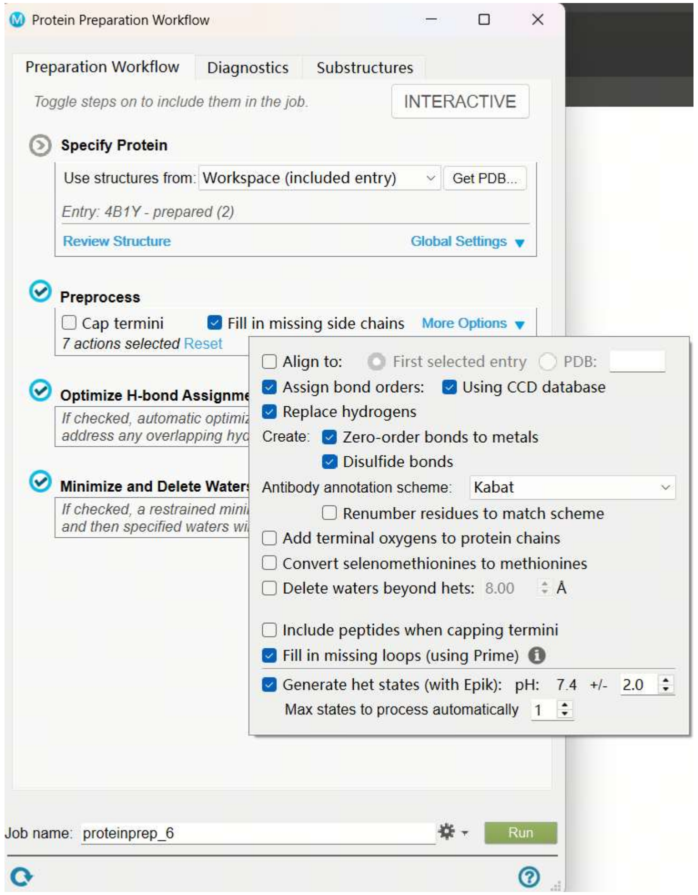


```
ATOM    167  OD2 ASP A  47      10.174   7.560  35.935  1.00 54.62           O
ATOM    168  N   MET A  48       6.674   9.818  33.156  1.00 45.09           N
ATOM    169  CA  MET A  48       6.049  10.959  32.490  1.00 44.83           C
ATOM    170  C   MET A  48       4.687  10.621  31.903  1.00 44.85           C
ATOM    171  O   MET A  48       3.782  11.457  31.884  1.00 44.46           O
ATOM    172  CB  MET A  48       6.968  11.508  31.392  1.00 42.34           C
ATOM    173  CG  MET A  48       8.206  12.210  31.932  1.00 37.42           C
ATOM    174  SD  MET A  48       7.764  13.636  33.161  1.00 33.09           S
ATOM    175  CE  MET A  48       7.589  15.051  31.873  1.00 30.88           C
ATOM    176  N   GLU A  49       4.548   9.390  31.429  1.00 45.27           N
```

https://ambermd.org/tutorials/basic/tutorial7/index.php

Protein Preparation

之后就是熟悉的流程，使用maestro或者pdbfixer进行蛋白准备



Protein Preparation Workflow

Preparation Workflow | Diagnostics | Substructures

Toggle steps on to include them in the job. INTERACTIVE

Specify Protein

Use structures from: Workspace (included entry) Get PDB...

Entry: 4B1Y - prepared (2)

Review Structure Global Settings

Preprocess

☐ Cap termini ☑ Fill in missing side chains More Options

7 actions selected Reset

☐ Align to: ○ First selected entry ○ PDB:

☑ Assign bond orders: ☑ Using CCD database

☑ Replace hydrogens

Create: ☑ Zero-order bonds to metals

☑ Disulfide bonds

Antibody annotation scheme: Kabat

☐ Renumber residues to match scheme

☐ Add terminal oxygens to protein chains

☐ Convert selenomethionines to methionines

☐ Delete waters beyond hets: 8.00 Å

☐ Include peptides when capping termini

☑ Fill in missing loops (using Prime)

☑ Generate het states (with Epik): pH: 7.4 +/- 2.0

Max states to process automatically 1

Optimize H-bond Assignme

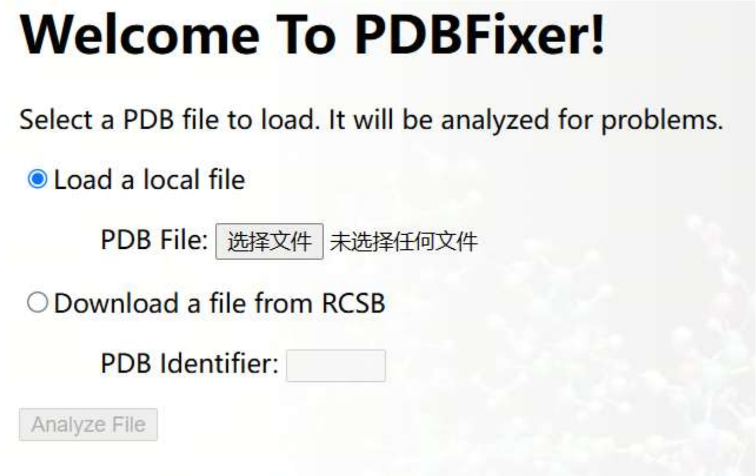
If checked, automatic optimiz address any overlapping hyd

Minimize and Delete Waters

If checked, a restrained mini and then specified waters wi

Job name: proteinprep_6 Run

https://htmlpreview.github.io/?https://github.com/openmm/pdbfixer/blob/master/Manual.html



Welcome To PDBFixer!

Select a PDB file to load. It will be analyzed for problems.

● Load a local file

PDB File: 选择文件 未选择任何文件

○ Download a file from RCSB

PDB Identifier:

Analyze File

```
pdbfixer 4b1y.pdb --output protein.pdb --keep-heterogens=none --add-residues --verbose
```

此处需要记录一下准备好的蛋白带电量，比如-3

Protein Preparation

之后记得处理质子化状态

使用maestro分配的质子化状态

https://github.com/Sept-naf/gromacs-tutorials/blob/main/maestro2amber.py

```
python maestro2amber.py maestro_format.pdb amber_readable.pdb
```

使用pdb2pqr分配质子化状态

https://pdb2pqr.readthedocs.io/en/latest/index.html

```
pip install pdb2pqr -i https://pypi.tuna.tsinghua.edu.cn/simple
```

对一个已经使用maestro或者pdbfixer处理好了的pdb文件

```
pdb2pqr input.pdb output.pdb --ffout AMBER --with-ph 7.4
```

此时得到的文件就带有amber力场格式的pH7.4下的质子化状态

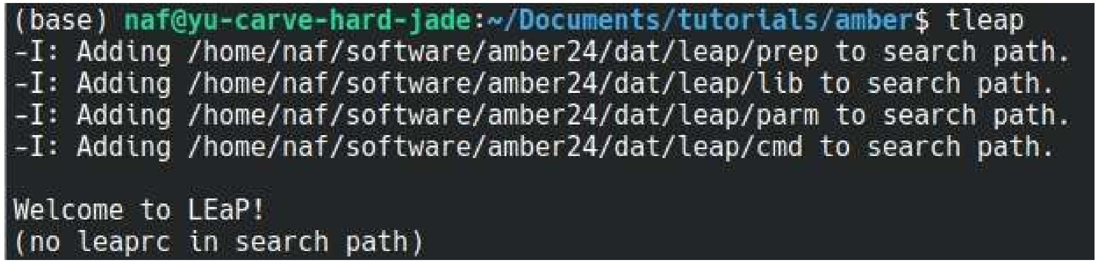
Build Simulation System

https://ambermd.org/tutorials/basic/tutorial7/index.php

到这里蛋白处理好了，可以搭建模拟体系了，amber搭建体系需要用tleap

```
tleap
```

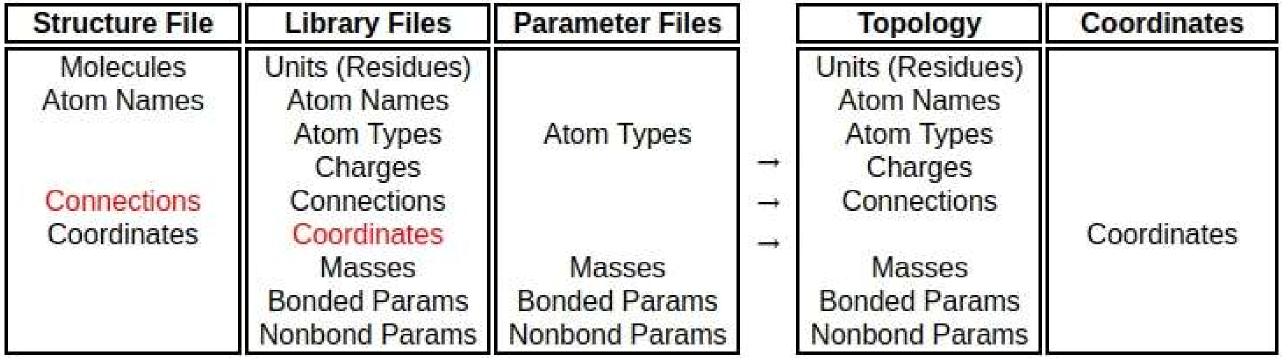
使用tleap之后会进入tleap的界面



```
(base) naf@yu-carve-hard-jade:~/Documents/tutorials/amber$ tleap
-I: Adding /home/naf/software/amber24/dat/leap/prep to search path.
-I: Adding /home/naf/software/amber24/dat/leap/lib to search path.
-I: Adding /home/naf/software/amber24/dat/leap/parm to search path.
-I: Adding /home/naf/software/amber24/dat/leap/cmd to search path.

Welcome to LEaP!
(no leaprc in search path)
```

通过左边进入tleap的提示是不是已经可以猜测amber力场参数文件都在哪找了

https://ambermd.org/tutorials/pengfei/index.php



Structure File	Library Files	Parameter Files		Topology	Coordinates
Molecules	Units (Residues)			Units (Residues)	
Atom Names	Atom Names			Atom Names	
	Atom Types	Atom Types		Atom Types	
	Charges		→	Charges	
Connections	Connections		→	Connections	
Coordinates	Coordinates		→		Coordinates
	Masses	Masses		Masses	
	Bonded Params	Bonded Params		Bonded Params	
	Nonbond Params	Nonbond Params		Nonbond Params	

Amber官方提供了leap的教学，它将PDB与力场参数文件组织起来，变成能进行模拟的拓扑文件和坐标文件

Build Simulation System

https://ambermd.org/tutorials/basic/tutorial7/index.php

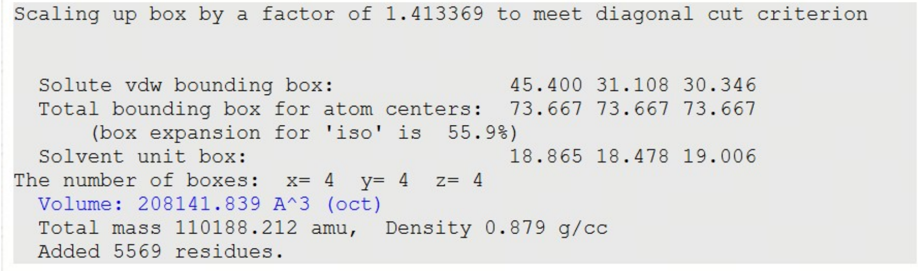
tleap

在tleap内

source leaprc.protein.ff19SB source leaprc.water.opc	导入蛋白，水和离子的力场参数
ramp=loadpdb 2YX8_prepared.pdb	导入做好了蛋白质准备的，处理好了质子化状态的蛋白，且将其命名为ramp
bond ramp.27.SG ramp.82.SG bond ramp.40.SG ramp.72.SG bond ramp.57.SG ramp.104.SG	手动连接所有的二硫键
addIons2 ramp Na+ 0	由于前面知道蛋白带负电，这里用Na+中和电荷；对正电体系则用Cl-
solvateOct ramp OPCBOX 10.0	给ramp添加水盒子
addIonsRand ramp Na+ 19 Cl- 19	添加离子，使体系盐浓度为0.15M，计算方法在下一页
SaveAmberParm ramp RAMP.prmtop RAMP.inpcrd	存储拓扑文件prmtop和坐标inpcrd

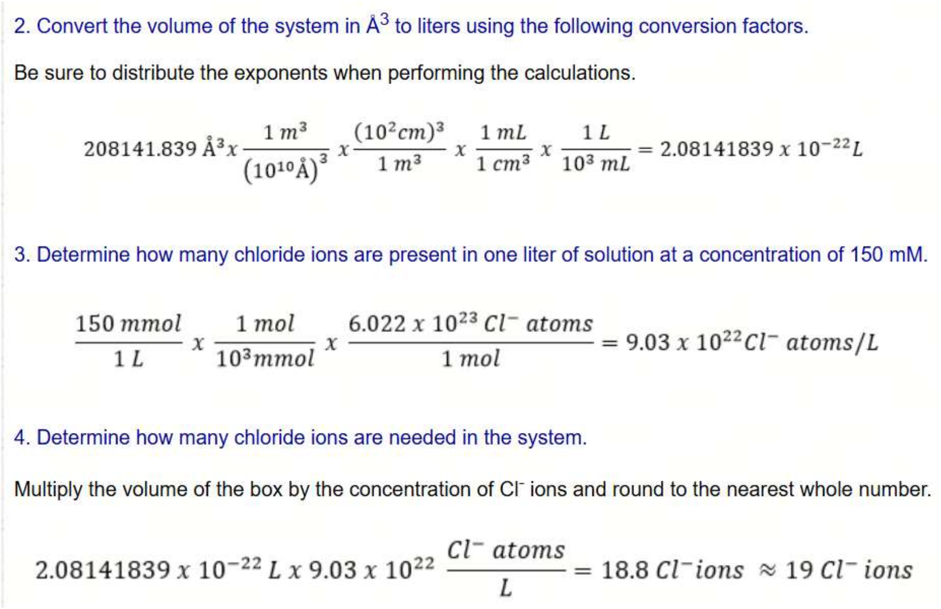
Calculating Salt in an Explicit Water System

https://ambermd.org/tutorials/basic/tutorial8/index.php



```
Scaling up box by a factor of 1.413369 to meet diagonal cut criterion


  Solute vdw bounding box:              45.400 31.108 30.346
  Total bounding box for atom centers:  73.667 73.667 73.667
      (box expansion for 'iso' is  55.9%)
  Solvent unit box:                     18.865 18.478 19.006
The number of boxes:  x= 4  y= 4  z= 4
  Volume: 208141.839 A^3 (oct)
  Total mass 110188.212 amu,  Density 0.879 g/cc
  Added 5569 residues.
```

在加水盒子时能看到整个体系的体积，记录下来



2. Convert the volume of the system in Å^3 to liters using the following conversion factors.

Be sure to distribute the exponents when performing the calculations.

$$208141.839\ Å^3 x \frac{1\ m^3}{\left(10^{10}Å\right)^3} x \frac{(10^2 cm)^3}{1\ m^3} x \frac{1\ mL}{1\ cm^3} x \frac{1\ L}{10^3\ mL} = 2.08141839\ x\ 10^{-22} L$$

3. Determine how many chloride ions are present in one liter of solution at a concentration of 150 mM.

$$\frac{150\ mmol}{1\ L} x \frac{1\ mol}{10^3 mmol} x \frac{6.022\ x\ 10^{23}\ Cl^-\ atoms}{1\ mol} = 9.03\ x\ 10^{22} Cl^-\ atoms/L$$

4. Determine how many chloride ions are needed in the system.

Multiply the volume of the box by the concentration of Cl$^-$ ions and round to the nearest whole number.

$$2.08141839\ x\ 10^{-22}\ L\ x\ 9.03\ x\ 10^{22} \frac{Cl^-\ atoms}{L} = 18.8\ Cl^- ions\ \approx 19\ Cl^-\ ions$$

把体积单位从立方埃换算成升

计算对这么多升溶液，需要多少离子能达到0.15M

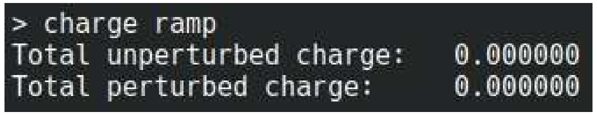
计算具体需要的离子数目

208141.839 * 9.033e-05

总结其实就是Volume乘以一个确定的数值，然后四舍五入（或者四舍六入五成双）

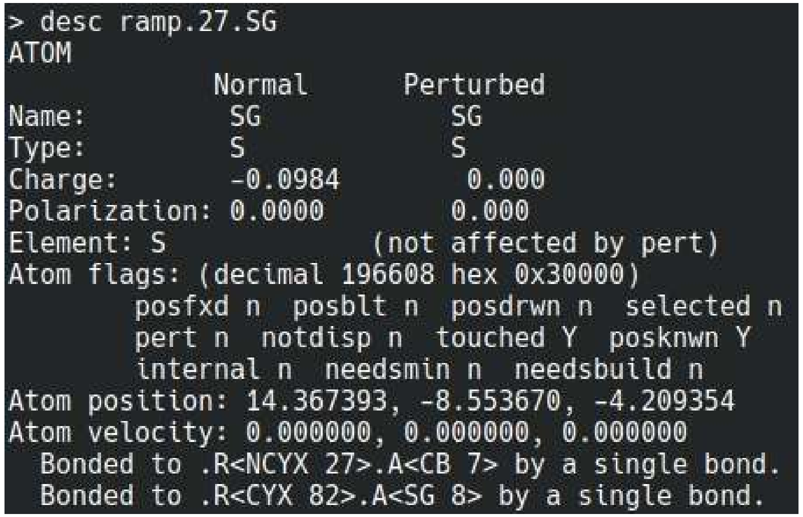
More detail

我们已经做好模拟前的准备了，但是，出于学习的考虑，再更进一步前，我们看几条leap命令



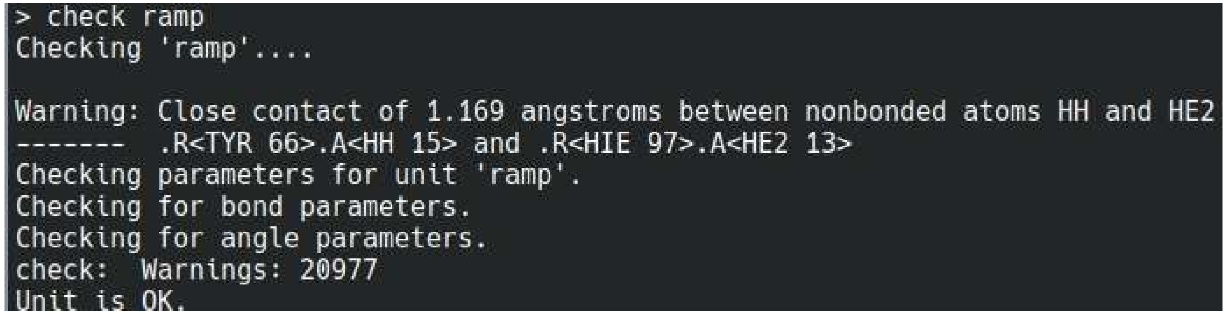
```
> charge ramp
Total unperturbed charge:   0.000000
Total perturbed charge:     0.000000
```

charge命令，检查体系电量



```
> desc ramp.27.SG
ATOM
             Normal      Perturbed
Name:         SG            SG
Type:         S             S
Charge:       -0.0984        0.000
Polarization: 0.0000        0.000
Element: S            (not affected by pert)
Atom flags: (decimal 196608 hex 0x30000)
        posfxd n  posblt n  posdrwn n  selected n
        pert n  notdisp n  touched Y  posknwn Y
        internal n  needsmin n  needsbuild n
Atom position: 14.367393, -8.553670, -4.209354
Atom velocity: 0.000000, 0.000000, 0.000000
  Bonded to .R<NCYX 27>.A<CB 7> by a single bond.
  Bonded to .R<CYX 82>.A<SG 8> by a single bond.
```

desc命令，检查体系部分的情况

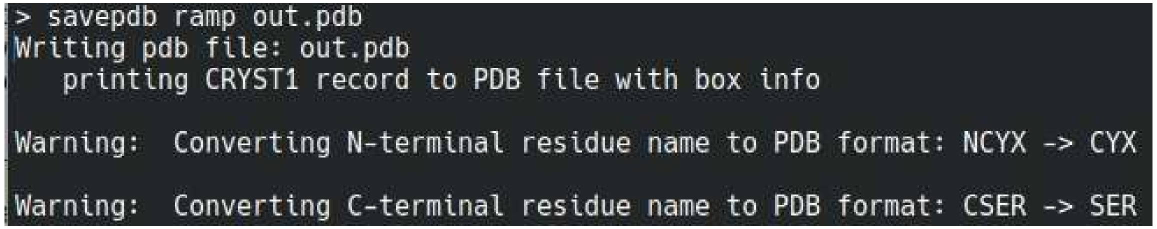


```
> check ramp
Checking 'ramp'....

Warning: Close contact of 1.169 angstroms between nonbonded atoms HH and HE2
-------  .R<TYR 66>.A<HH 15> and .R<HIE 97>.A<HE2 13>
Checking parameters for unit 'ramp'.
Checking for bond parameters.
Checking for angle parameters.
check:  Warnings: 20977
Unit is OK.
```

check命令，检查体系力场是否完整

Save PDB

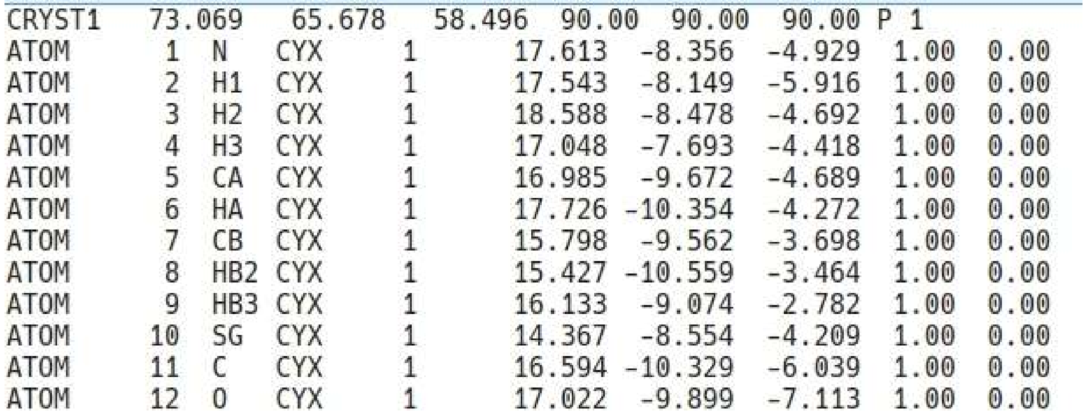
Amber有一个非常讨厌的地方，就是它会对你的体系残基重新编号，从1开始



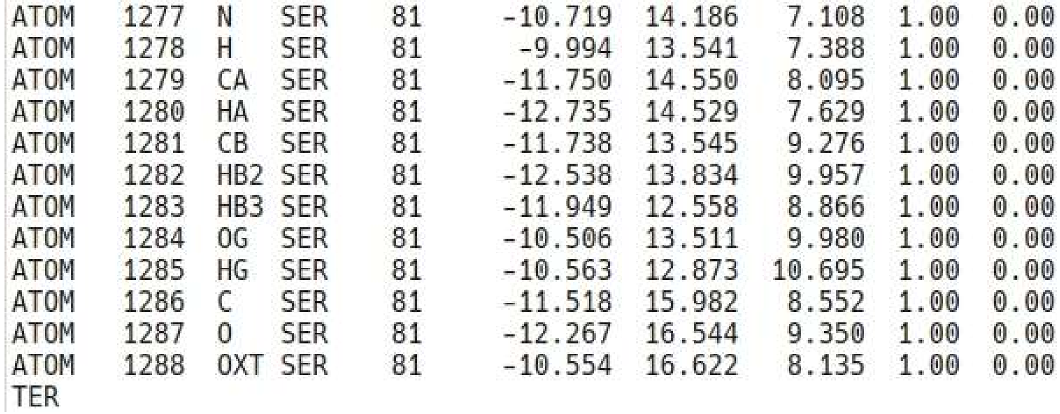
```
> savepdb ramp out.pdb
Writing pdb file: out.pdb
   printing CRYST1 record to PDB file with box info

Warning:  Converting N-terminal residue name to PDB format: NCYX -> CYX

Warning:  Converting C-terminal residue name to PDB format: CSER -> SER
```

将体系存一份PDB

打开你会发现，蛋白残基范围变成了1-81



```
CRYST1   73.069   65.678   58.496  90.00  90.00  90.00 P 1
ATOM      1  N   CYX     1      17.613  -8.356  -4.929  1.00  0.00
ATOM      2  H1  CYX     1      17.543  -8.149  -5.916  1.00  0.00
ATOM      3  H2  CYX     1      18.588  -8.478  -4.692  1.00  0.00
ATOM      4  H3  CYX     1      17.048  -7.693  -4.418  1.00  0.00
ATOM      5  CA  CYX     1      16.985  -9.672  -4.689  1.00  0.00
ATOM      6  HA  CYX     1      17.726 -10.354  -4.272  1.00  0.00
ATOM      7  CB  CYX     1      15.798  -9.562  -3.698  1.00  0.00
ATOM      8  HB2 CYX     1      15.427 -10.559  -3.464  1.00  0.00
ATOM      9  HB3 CYX     1      16.133  -9.074  -2.782  1.00  0.00
ATOM     10  SG  CYX     1      14.367  -8.554  -4.209  1.00  0.00
ATOM     11  C   CYX     1      16.594 -10.329  -6.039  1.00  0.00
ATOM     12  O   CYX     1      17.022  -9.899  -7.113  1.00  0.00
```

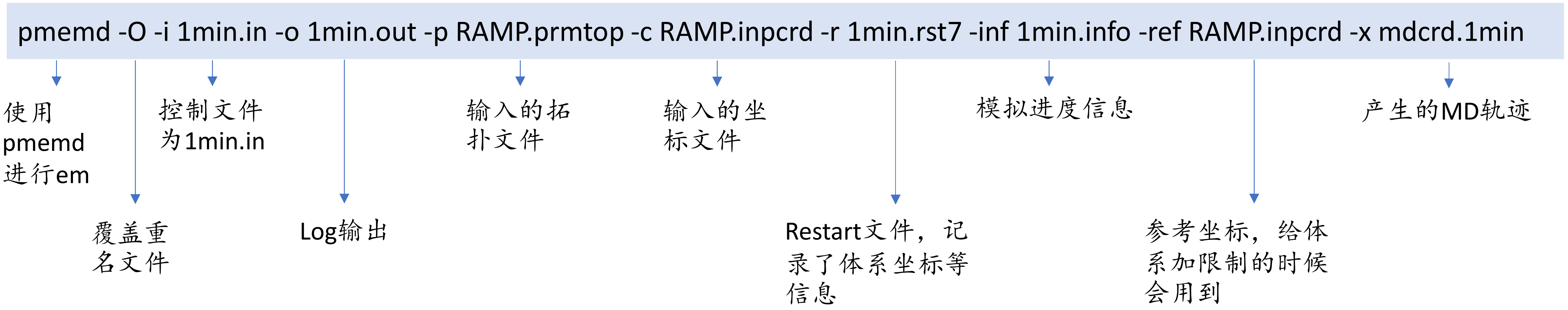


```
ATOM   1277  N   SER    81     -10.719  14.186   7.108  1.00  0.00
ATOM   1278  H   SER    81      -9.994  13.541   7.388  1.00  0.00
ATOM   1279  CA  SER    81     -11.750  14.550   8.095  1.00  0.00
ATOM   1280  HA  SER    81     -12.735  14.529   7.629  1.00  0.00
ATOM   1281  CB  SER    81     -11.738  13.545   9.276  1.00  0.00
ATOM   1282  HB2 SER    81     -12.538  13.834   9.957  1.00  0.00
ATOM   1283  HB3 SER    81     -11.949  12.558   8.866  1.00  0.00
ATOM   1284  OG  SER    81     -10.506  13.511   9.980  1.00  0.00
ATOM   1285  HG  SER    81     -10.563  12.873  10.695  1.00  0.00
ATOM   1286  C   SER    81     -11.518  15.982   8.552  1.00  0.00
ATOM   1287  O   SER    81     -12.267  16.544   9.350  1.00  0.00
ATOM   1288  OXT SER    81     -10.554  16.622   8.135  1.00  0.00
TER
```

Simulating System: Minimization1

https://ambermd.org/tutorials/basic/tutorial13/index.php

体系准备好了，可以退出tleap，正式开始模拟了，首先仍然是能量最小化



```
pmemd -O -i 1min.in -o 1min.out -p RAMP.prmtop -c RAMP.inpcrd -r 1min.rst7 -inf 1min.info -ref RAMP.inpcrd -x mdcrd.1min
```

使用pmemd进行em

覆盖重名文件

控制文件为1min.in

Log输出

输入的拓扑文件

输入的坐标文件

Restart文件，记录了体系坐标等信息

模拟进度信息

参考坐标，给体系加限制的时候会用到

产生的MD轨迹

1min.in

```
minimization of solvent
 &cntrl
  imin = 1, maxcyc = 1000,
  ncyc = 20, ntx = 1,
  ntwe = 0, ntwr = 500, ntpr = 50,
  ntc = 2, ntf = 2, ntb = 1, ntp = 0,
  cut = 10.0,
  ntr=1, restraintmask = ':1-81',
  restraint_wt = 100.,
  ioutfm=1, ntxo=2,
 /
```

- ntr=1代表要对体系加限制
- restraintmask = ‘:1-81‘代表对残基1-81加限制，注意这是amber重排序过的编号，可以在上一页存出的pdb里确认
- restraint_wt = 100 限制大小100 kcal/mol*Ang^-2.

Simulating System: Heat2

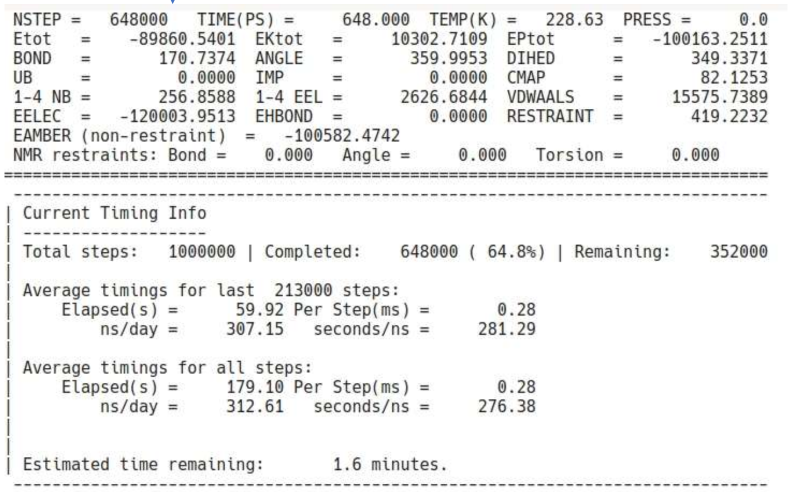
https://ambermd.org/tutorials/basic/tutorial13/index.php

能量最小化以后是体系加热

```
pmemd.cuda -O -i 2heat.in -o 2heat.out -p RAMP.prmtop -c 1min.rst7 -r 2heat.rst7 -inf 2heat.info -ref 1min.rst7 -x mdcrd.2heat
```

使用pmemd.cuda，
这是GPU加速的版本

上一步得到的坐标，
这一步的输入坐标



```
NSTEP =   648000   TIME(PS) =     648.000  TEMP(K) =   228.63  PRESS =     0.0
Etot   =    -89860.5401  EKtot   =     10302.7109  EPtot      =   -100163.2511
BOND   =       170.7374  ANGLE   =       359.9953  DIHED      =       349.3371
UB     =         0.0000  IMP     =         0.0000  CMAP       =        82.1253
1-4 NB =       256.8588  1-4 EEL =      2626.6844  VDWAALS    =     15575.7389
EELEC  =   -120003.9513  EHBOND  =         0.0000  RESTRAINT  =       419.2232
EAMBER (non-restraint)  =   -100582.4742
NMR restraints: Bond =    0.000   Angle =     0.000   Torsion =     0.000
==============================================================================
 -----------------------------------------------------------------------------
| Current Timing Info
| -------------------
| Total steps:   1000000 | Completed:    648000 ( 64.8%) | Remaining:    352000
|
| Average timings for last  213000 steps:
|     Elapsed(s) =      59.92 Per Step(ms) =       0.28
|         ns/day =     307.15   seconds/ns =     281.29
|
| Average timings for all steps:
|     Elapsed(s) =     179.10 Per Step(ms) =       0.28
|         ns/day =     312.61   seconds/ns =     276.38
|
|
| Estimated time remaining:       1.6 minutes.
 -----------------------------------------------------------------------------
```

2heat.in

```
&cntrl
 imin = 0, nstlim = 2000000, dt = 0.001,
 irest = 0, ntx = 1, ig = -1,
 tempi = 100.0, temp0 = 298.0,
 ntc = 2, ntf = 2, tol = 0.00001,
 ntwx = 10000, ntwe = 0, ntwr = 1000, ntpr = 1000,
 cut = 8.0, iwrap = 0,
 ntt =3, gamma_ln=1., ntb = 1, ntp = 0,
 nscm = 0,
 ntr=1, restraintmask=':1-81', restraint_wt=100.0
 nmropt=1,
 ioutfm=1, ntxo=2,
 /
&wt TYPE="TEMP0", istep1=0, istep2=1000000, value1=100., value2=298., /
&wt TYPE="END", /
```

2ns

ntb=1，恒体积

从0到1000000步将温度从100K升到298K

Simulating System: NPT平衡3

https://ambermd.org/tutorials/basic/tutorial13/index.php

NPT平衡

```
pmemd.cuda -O -i 3md.in -o 3md.out -p RAMP.prmtop -c 2heat.rst7 -r 3md.rst7 -inf 3md.info -ref 2heat.rst7 -x mdcrd.3md
```

3md.in

```
&cntrl
 imin = 0, nstlim = 1000000, dt = 0.001,
 irest = 1, ntx = 5, ig = -1,
 temp0 = 298.0,
 ntc = 2, ntf = 2, tol = 0.00001,
 ntwx = 10000, ntwe = 0, ntwr = 1000, ntpr = 1000,
 cut = 8.0, iwrap = 0,
 ntt =3, gamma_ln=1.0, ntb = 2, ntp = 1, barostat = 2,
 nscm = 0,
 ntr=1, restraintmask=':1-81', restraint_wt=100.
 ioutfm=1, ntxo=2,
/
```

1ns

ntb=2，恒压
ntp=1，各向同性压浴
barostat=2，蒙特卡洛压浴

Simulating System: NPT平衡4

https://ambermd.org/tutorials/basic/tutorial13/index.php

```
pmemd.cuda -O -i 4md.in -o 4md.out -p RAMP.prmtop -c 3md.rst7 -r 4md.rst7 -inf 4md.info -ref 3md.rst7 -x mdcrd.4md
```

4md.in

```
&cntrl
 imin = 0, nstlim = 1000000, dt = 0.001,
 irest = 1, ntx = 5, ig = -1,
 temp0 = 298.0,
 ntc = 2, ntf = 2, tol = 0.00001,
 ntwx = 10000, ntwe = 0, ntwr = 1000, ntpr = 1000,
 cut = 8.0, iwrap = 0,
 ntt =3,  gamma_ln=1.0, ntb = 2, ntp = 1,
 nscm = 0, barostat = 2,
 ntr=1, restraintmask=':1-81', restraint_wt=10.
 ioutfm=1, ntxo=2,
/
```

1ns

放松限制到10

Simulating System: Minimization5

https://ambermd.org/tutorials/basic/tutorial13/index.php

```
pmemd -O -i 5min.in -o 5min.out -p RAMP.prmtop -c 4md.rst7 -r 5min.rst7 -inf 5min.info -ref 4md.rst7 -x mdcrd.5min
```

5min.in

```
Minimization of everything excluding backbone
 &cntrl
  imin = 1, maxcyc = 1000,
  ncyc = 30, ntx = 1,
  ntwe = 0, ntwr = 500, ntpr = 50,
  ntc = 2, ntf = 2, ntb = 1, ntp = 0,
  cut = 8.0,
  ntr=1, restraintmask="@CA,N,C", restraint_wt=10.
  ioutfm=1, ntxo=2,
 /
```

之前约束了残基1-81的所有原子，此时只约束名为CA，N，C的原子，由于我们的体系里除了蛋白就是水和离子，所以这里实际上只约束了蛋白质的主链原子

Simulating System: NPT平衡6

https://ambermd.org/tutorials/basic/tutorial13/index.php

```
pmemd.cuda -O -i 6md.in -o 6md.out -p RAMP.prmtop -c 5min.rst7 -r 6md.rst7 -inf 6md.info -ref 5min.rst7 -x mdcrd.6md
```

6md.in

```
&cntrl
 imin = 0, nstlim = 1000000, dt = 0.001,
 irest = 0, ntx = 1, ig = -1,
 tempi = 298.0, temp0 = 298.0,
 ntc = 2, ntf = 2, tol = 0.00001,
 ntwx = 10000, ntwe = 0, ntwr = 1000, ntpr = 1000,
 cut = 8.0, iwrap = 0,
 ntt =3, gamma_ln=1.0, ntb = 2, ntp = 1,
 nscm = 0, barostat = 2,
 ntr=1, restraintmask="@CA,N,C", restraint_wt=10.
 ioutfm=1, ntxo=2,
/
```

1ns

NPT

Simulating System: NPT平衡7

https://ambermd.org/tutorials/basic/tutorial13/index.php

```
pmemd.cuda -O -i 7md.in -o 7md.out -p RAMP.prmtop -c 6md.rst7 -r 7md.rst7 -inf 7md.info -ref 6md.rst7 -x mdcrd.7md
```

7md.in

```
&cntrl
 imin = 0, nstlim = 1000000, dt = 0.001,
 irest = 1, ntx = 5, ig = -1,
 temp0 = 298.0,
 ntc = 2, ntf = 2, tol = 0.00001,
 ntwx = 10000, ntwe = 0, ntwr = 1000, ntpr = 1000,
 cut = 8.0, iwrap = 0,
 ntt =3, gamma_ln=1.0, ntb = 2, ntp = 1,
 nscm = 0, barostat = 2,
 ntr=1, restraintmask="@CA,N,C", restraint_wt=1.
 ioutfm=1, ntxo=2,
/
```

1ns

放松约束到1

Simulating System: NPT平衡8

https://ambermd.org/tutorials/basic/tutorial13/index.php

```
pmemd.cuda -O -i 8md.in -o 8md.out -p RAMP.prmtop -c 7md.rst7 -r 8md.rst7 -inf 8md.info -ref 7md.rst7 -x mdcrd.8md
```

8md.in

```
&cntrl
 imin = 0, nstlim = 1000000, dt = 0.001,
 irest = 1, ntx = 5, ig = -1,
 temp0 = 298.0,
 ntc = 2, ntf = 2, tol = 0.00001,
 ntwx = 10000, ntwe = 0, ntwr = 1000, ntpr = 1000,
 cut = 8.0, iwrap = 0,
 ntt =3, gamma_ln=1.0, ntb = 2, ntp = 1,
 nscm = 0, barostat = 2,
 ntr=1, restraintmask="@CA,N,C", restraint_wt=0.1
 ioutfm=1, ntxo=2,
/
```

1ns

放松约束到0.1

Simulating System: NPT平衡9

https://ambermd.org/tutorials/basic/tutorial13/index.php

```
pmemd.cuda -O -i 9md.in -o 9md.out -p RAMP.prmtop -c 8md.rst7 -r 9md.rst7 -inf 9md.info -ref 8md.rst7 -x mdcrd.9md
```

9md.in

```
&cntrl
 imin = 0, nstlim = 1000000, dt = 0.001,
 irest = 1, ntx = 5, ig = -1,
 temp0 = 298.0,
 ntc = 2, ntf = 2, tol = 0.00001,
 ntwx = 10000, ntwe = 0, ntwr = 1000, ntpr = 1000,
 cut = 8.0, iwrap = 0,
 ntt =3, gamma_ln=1.0, ntb = 2, ntp = 1,
 nscm = 1000, barostat = 2,
 ioutfm=1, ntxo=2,
/
```

1ns

无约束平衡

Simulating System: Running MD

https://ambermd.org/tutorials/basic/tutorial14/index.php

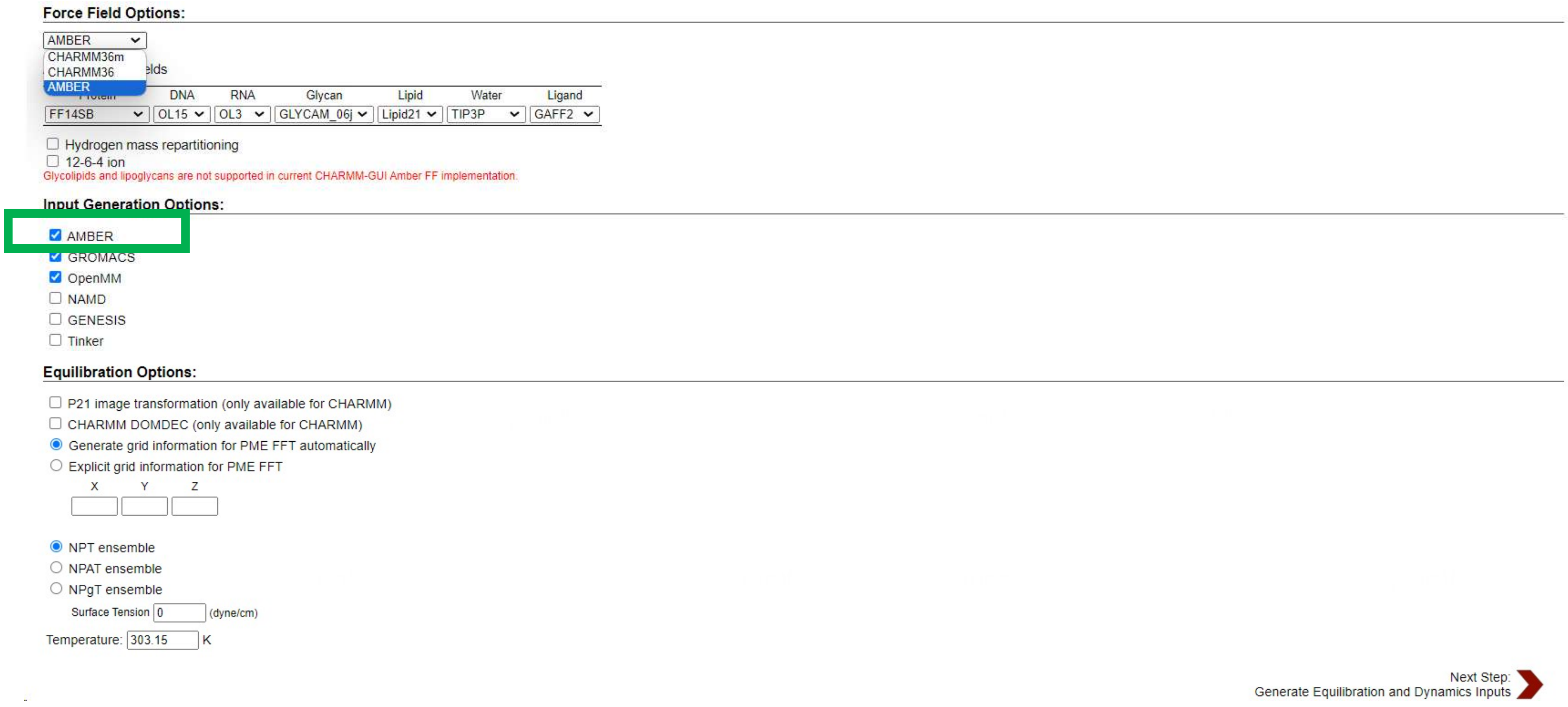
```
pmemd.cuda -O -i md.in -o md.out -p RAMP.prmtop -c 9md.rst7 -r md.rst7 -inf md.info -ref 9md.rst7 -x mdcrd.md
```

md.in

```
Explicit solvent molecular dynamics constant pressure 50 ns MD
 &cntrl
   imin=0, irest=1, ntx=5,
   ntpr=500000, ntwx=500000, ntwr=500000, nstlim=25000000,
   dt=0.002, ntt=3, tempi=300,
   temp0=300, gamma_ln=1.0, ig=-1,
   ntp=1, ntc=2, ntf=2, cut=9,
   ntb=2, iwrap=1, ioutfm=1,
/
```

Membrane Protein Simulation?

Charmm-gui



Force Field Options:

AMBER ⌄
CHARMM36m
CHARMM36
AMBER

elds

Protein	DNA	RNA	Glycan	Lipid	Water	Ligand
FF14SB ⌄	OL15 ⌄	OL3 ⌄	GLYCAM_06j ⌄	Lipid21 ⌄	TIP3P ⌄	GAFF2 ⌄

□ Hydrogen mass repartitioning
□ 12-6-4 ion

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

Input Generation Options:

☑ AMBER
☑ GROMACS
☑ OpenMM
□ NAMD
□ GENESIS
□ Tinker

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