Ambertools(https://ambermd.org/AmberTools.php):

For linux/windows-subsystem linux, install the requirements as follows:

```
mkdir amber
cp AmberTools24.tar.bz2 amber/
sudo apt-get install tcsh make
sudo apt-get install gcc gfortran
sudo apt-get install flex bison patch bc wget
sudo apt-get update --fix-mixing
apt-get install xorg-dev libz-dev libbz2-dev
```

Then start installing amber

```
cd amber/
ls
tar xvfj AmberTools24.tar.bz2
cd amber24_src/
ls
cd build/
ls
vi run_cmake
./run_cmake
make install
sudo apt-get install bison
./run_cmake
make install
ls
ll
cd AmberTools/
ls
cd ..
ls
cd build/
ls
cd ../../
ls
cd amber24 (creates two directories; one with amber24 and amber24_src; amber.sh directory is amber24 and source that)
ls
source /path/amber.sh
vi ~/.bashrc #give path of amber.sh
source ~/.bashrc (source it ; not bash it)
ls
make test.serial
```

For amber (only which is for md simulation; free for noncommercial use); download the pmemd.*.bz2 file , extract it and do the same installation as ambertools.

NAB is not included in ambertool recent versions. NAB is important to create any shape of rna dna molecules for md simulation. To install it, download the zip file from the github (https://github.com/dacase/nabc) and then do the following.

```
 unzip nabc-main.zip
 cd nabc-main/
 ls
 ./configure # configure
 make clean && make install #clean and install
 ls
 make nab #To compile nap
 nab
 ls
 cd test/
 ls
 cd nab/
 ls
 make test #to see if tests passed or failed
 cd ../shifts/
 make shifts
 make test # to see if shift is installed
 cd ..
 ls
 source nabc.sh #first source the nabc.sh file; then only use it
 nab bin/dna.nab The input is in bin and named as dna. nab
 ./a.out #to execute nab
 ls
 vi dna.pdb
 vmd dna.pdb #To visualize the output “dna.pdb” file
```

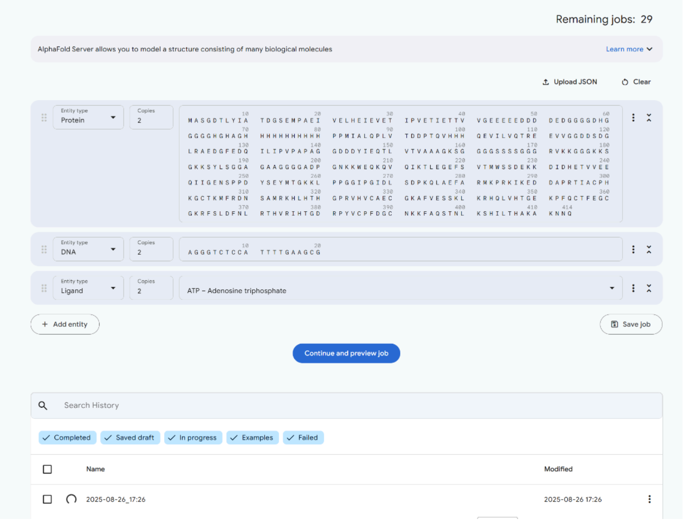
```
#Gromacs installation
tar xfz gromacs-2025.2.tar.gz
cd gromacs-2025.2
mkdir build
cd build
cmake .. -DGMX_BUILD_OWN_FFTW=ON -DREGRESSIONTEST_DOWNLOAD=ON (cmake error happens if not installed properly and install it properly with snap or apt get and should be in the path)
make
```

```
make check
sudo make install
source /usr/local/gromacs/bin/GMXRC
```

# Data file preparation for md simulation

1. AlphaFold structure file generation:

   1. Open AlphaFold server (https://alphafoldserver.com/)



Remaining jobs: 29

AlphaFold Server allows you to model a structure consisting of many biological molecules

Entity type: Protein  Copies: 2

```
        10         20         30         40         50         60
MASGDTLYIA TDGSEMPAEI VELHEIEVET IPVETIETTV VGEEEEEDDD DEDGGGGDHG
        70         80         90        100        110        120
GGGGHGHAGH HHHHHHHHHH PPMIALQPLV TDDPTQVHHH QEVILVQTRE EVVGGDDSDG
       130        140        150        160        170        180
LRAEDGFEDQ ILIPVPAPAG GDDDYIEQTL VTVAAAGKSG GGGSSSSGGG RVKKGGGKKS
       190        200        210        220        230        240
GKKSYLSGGA GAAGGGGADP GNKKWEQKQV QIKTLEGEFS VTMWSSDEKK DIDHETVVEE
       250        260        270        280        290        300
QIIGENSPPD YSEYMTGKKL PPGGIPGIDL SDPKQLAEFA RMKPRKIKED DAPRTIACPH
       310        320        330        340        350        360
KGCTKMFRDN SAMRKHLHTH GPRVHVCAEC GKAFVESSKL KRHQLVHTGE KPFQCTFEGC
       370        380        390        400        410   414
GKRFSLDFNL RTHVRIHTGD RPYVCPFDGC NKKFAQSTNL KSHILTHAKA KNNQ
```

Entity type: DNA  Copies: 2

```
        10         20
AGGGTCTCCA TTTTGAAGCG
```

Entity type: Ligand  Copies: 2  ATP – Adenosine triphosphate

| □ | Name | Modified |
|---|---|---|
| □ | 2025-08-26_17:26 | 2025-08-26 17:26 |

   2. Paste sequence from fasta file from rcsb site pdb file and put it here. For DNA give 1 copy for 5` to 3` sequence from fasta file and then give another DNA and give one copy for that with the chain B sequence. And submit job. PTM with 0.8 is confident. 0.6 to 0.8 is grey area.
   3. The file can be downloaded in zip folder and .cif file is present in that folder and can be converted to .pdb file using vmd.
   4. Then use ambertools to create the initial structure for simulation.

2. Ambertool data file preparation:

   1. Download yy1 pdb file from rcsb
   2. Open the structure in VMD and see what non-standard residue is there.
   3. 1udb.pdb file: there are 4 ZN and some HOH HETATMs. Since we are modeling explicite water solven, we can keep these water molecules. replace HETATM to "ATOM" so that the sequence will be continuous. Be sure to keep the two spaces after the ATOM so the rest of the columns will be lined up (space is important). Sometimes, other solvents or phosphates will be present. These do not affect the function of the protein, so they can be removed.
   4. H++ web server (virginia tech) gives protonation state and also solvate it. But we may not use this solvate method. Use this only to know protonation state and charge residue.
   5. Remove the connects from pdb file.

3. tleap usage to create amber topo and coordinate file:

# Protein and DNA force fields (example)

```
source leaprc.protein.ff19SB
source leaprc.DNA.bsc1

# TIP3P water force field
source leaprc.water.tip3p
ramp=loadpdb RAMP1.pdb
saveAmberParm ramp RAMP1_gas.prmtop RAMP1_gas.inpcrd

addionsrand ramp Na+ 0 (add this according to H++ generated residue charge (if -24 , ten add
24 + Na ions)
addionsrand ramp Cl- 0
#solvate
solvateoct ramp TIP3PBOX 10.0
addIonsRand ramp Na+ 19 Cl- 19
saveamberparm ramp RAMP1_ion.prmtop RAMP1_ion.inpcrd
```

3. Visualization of amber topo and crd file in vmd (tkconsole):
   ```
   (amber_simulation) 49 % mol new RAMP1_ion.prmtop type parm7
   (amber_simulation) 50 % mol addfile RAMP1_ion.inpcrd type rst7
   ```

4. Convert the modified topology and coordinate file to pdb file.
   Create a pdb.in file in a text editor and put
   ```
   trajin RAMP1_ion.inpcrd
   trajout RAMP1_wions_water.pdb PDB
   run
   ```
   There.
   Then do `$AMBERHOME/bin/cpptraj -p RAMP1_ion.prmtop -i pdb.in>pdb.out`

5. Md simulation:
   Create .in files for amber input and put them in a .scr file and do ./all_relax.scr in the terminal and the scr file contains:
   ```
   —-----------------------------------------------------------
   #!/bin/bash -l

   export AMBERHOME=/home/swayamshree/Desktop/amber/pmemd24/bin
   export CUDA_VISIBLE_DEVICES=0

   cd /home/swayamshree/Desktop/amber_simulation/test/all_relax; echo "starting 1min at
   'date'"

   $AMBERHOME/pmemd -O -i 1min.in\
             -o 1min.out -p RAMP1_ion.prmtop -c RAMP1_ion.inpcrd -r 1min.rst7\
             -inf 1min.info -ref RAMP1_ion.inpcrd -x mdcrd.1min
   ```

```
echo "ending 1min at 'date'"

echo "starting 2mdheat at 'date'"

$AMBERHOME/pmemd -O -i 2mdheat.in\
        -o 2mdheat.out -p RAMP1_ion.prmtop -c 1min.rst7 -r 2mdheat.rst7\
        -inf 2mdheat.info -ref 1min.rst7 -x mdcrd.2mdheat
echo "ending 2mdheat at 'date'"

echo "starting 3md at 'date'"

$AMBERHOME/pmemd -O -i 3md.in\
        -o 3md.out -p RAMP1_ion.prmtop -c 2mdheat.rst7 -r 3md.rst7\
        -inf 3md.info -ref 2mdheat.rst7 -x mdcrd.3md
echo "ending 3md at 'date'"

echo "starting 4md at 'date'"

$AMBERHOME/pmemd -O -i 4md.in\
        -o 4md.out -p RAMP1_ion.prmtop -c 3md.rst7 -r 4md.rst7\
        -inf 4md.info -ref 3md.rst7 -x mdcrd.4md
echo "ending 4md at 'date'"

echo "starting 5min at 'date'"

$AMBERHOME/pmemd -O -i 5min.in\
        -o 5min.out -p RAMP1_ion.prmtop -c 4md.rst7 -r 5min.rst7\
        -inf 5min.info -ref 4md.rst7 -x mdcrd.5min
echo "ending 5min at 'date'"

echo "starting 6md at 'date'"

$AMBERHOME/pmemd -O -i 6md.in\
        -o 6md.out -p RAMP1_ion.prmtop -c 5min.rst7 -r 6md.rst7\
        -inf 6md.info -ref 5min.rst7 -x mdcrd.6md
echo "ending 6md at 'date'"

echo "starting 7md at 'date'"

$AMBERHOME/pmemd -O -i 7md.in\
        -o 7md.out -p RAMP1_ion.prmtop -c 6md.rst7 -r 7md.rst7\
        -inf 7md.info -ref 6md.rst7 -x mdcrd.7md
echo "ending 7md at 'date'"
echo "starting 8md at 'date'"

$AMBERHOME/pmemd -O -i 8md.in\
        -o 8md.out -p RAMP1_ion.prmtop -c 7md.rst7 -r 8md.rst7\
        -inf 8md.info -ref 7md.rst7 -x mdcrd.8md
echo "ending 8md at 'date'"

echo "starting 9md at 'date'"

$AMBERHOME/pmemd -O -i 9md.in\
        -o 9md.out -p RAMP1_ion.prmtop -c 8md.rst7 -r 9md.rst7\
        -inf 9md.info -ref 8md.rst7 -x mdcrd.9md
echo "ending 9md at 'date'"

—---------------------------------------------------------
```

Note: pmemd.cuda is not running in local system. Use only pmemd; remove .cuda from the command.

6.