Protocol

1. **Download or create structure**
    1. Folded - AA sequence to AlphaFold server for hairpin structure, randomly choose one of 5 structures
    2. Unfolded - Build AA sequence in PyMol, drag to linear form

2. **Add Hydrogens**
    1. Chimera —> structure editing —> Add H
    2. `tleap pdb4amber -i pdbin.pdb -o pdbout.pdb --reduce --dry`

3. **tleap system setup**
    1. Must calculate required ions with https://www.phys.ksu.edu/personal/schmit/SLTCAP/SLTCAP.html

```
# Template RNA Hairpin FOLDED PREP
# MUST REMOVE TERMINAL PHOSPHATES
# Run with tleap -s -f leap.in on CL

source leaprc.RNA.OL3
source leaprc.water.tip3p
mol = loadpdb in.pdb
addions mol Na+ 0
**solvateoct mol TIP3PBOX 12.0
addionsrand mol Na+ 000 Cl- 000**
savepdb mol out.pdb
saveamberparm mol out.prmtop out.inpcrd
saveamberparm mol out.prmtop out.rst7
check mol
quit
```

4. **md 1 Initial minimization:** Let solvent relax around restrained solute
    1. 1000 steps (500 steepest descents/500 conjugate gradient)
    2. 500 kcal/mol-Å2 restraints (solute)

5. **md2 Second minimization:** Let everything relax
    1. 2500 steps (1000 steepest descents/1500 conjugate gradient)

6. **Defrost (md1):** Begin constant volume to warm to proper temperature with restrained solute
    1. 100 ps NVT
    2. Langevin temperature control 0 -> 300K
    3. 25 kcal/mol-Å2 restraints (solute)

7. **Equilibration (md2):** Switch to constant pressure to get proper density while gradually releasing restraints on solute (5-stage release with strong restraints for first 40ps while density is changing most rapidly)
    1. 250 ps NPT
    2. Langevin temperature control 300K
    3. “Weak-coupling” pressure control 1.0bar (~1 atm)
    4. 25 -> 5 kcal/mol-Å2 restraints(solute)
        1. md2a 25 kcal 50ps
        2. md2b 20 kcal 50ps
        3. md2c 15 kcal 50ps
        4. md2d 10 kcal 50ps
        5. md2e 5 kcal 50ps

8. **Equilibration (md3):** Release solute restraints and collect data to isotropically scale box size to reflect average volume
    1. 200 ps NPT
    2. Langevin temperature control 300K
    3. “Weak-coupling” pressure control 1.0bar(~1.0atm)

9. **Calculate new volume and replace x,y,z in restart file**
    1. New flag: ntxo=1

10. **Equilibration (md4):** Switch to constant volume and equilibrate with scaled box size
    1. 1 ns NVT
    2. Remove ntxo=1 flag

11. **Production run: keep the same conditions as the equilibration run**
    1. 1 microsecond
    2. Copy over md4.rst to production folder, rename as md.rst
    3. Keep running production for however long is needed for your system until it equilibrates completely.

```
Production run NVT
&cntrl
imin=0,                         ## No minimization
irest=1, ntx=5,                 ## Restart MD with coordinates
ntb=1,                          ## Periodic boundaries, constant volume
ig=-1,                          ## Random seed
cut=10.0,                       ## Non-bonded interactions cutoff 10A
ntc=2, ntf=2,                   ## ntc=2 Hydrogen bond constraints SHAKE for TIP3P
temp1=310.0, temp0=310.0,       ## Temp controlled 310K intial, 310k hold
ntt=3, gamma_ln=1.0,            ## Langevin thermostat, collision freq, 1.0^-1 ps
nstlim=50000000, dt=0.002,      ## ntslim=50,000,000 steps; dt=0.002 ps (2 fs) timestep; total time = 100 ns
ntpr=1000 ntwx=500, ntwr=10000, ## ntpr=Every 1000 steps energy printed to human readable .mdout and .mdinfo
/                               ## ntwr=Every 10000 steps restrt file written ## ntwx=Every 500 steps coordinates written to .mdcrd
```

FOR UNFOLDED

1. **Production**
    1. **300 ns**
    2. Save only last frame
    3. Generate average structure, remove all ions and water to send back into min_eq to re-equilibrate system

2. **Re-Set up System**
    1. Add H
    2. Add ions, water, set up with tleap

3. **Repeat Min_Eq and full production run**