1.INITIAL SCREENS FOR INPUT

pdb4amber 1fko.pdb > 1fko_amber.pdb

```
==================================================
Summary of pdb4amber for: 1fko.pdb
==================================================

----------Chains
The following (original) chains have been found:
A
B

---------- Alternate Locations (Original Residues!))

The following residues had alternate locations:
None
-----------Non-standard-resnames
EFZ, CSD

---------- Gaps (Renumbered Residues!)
gap of 3.272103 A between LEU 279 and LYS 281
gap of 7.585162 A between PHE 628 and LEU 629
gap of 22.506030 A between GLY 750 and GLU 751

---------- Mising heavy atom(s)

None
```

(base) /Users/mfuxreiter/Courses/P2> pdb4amber hiv_rt.pdb > hivrt_amber.pdb

```
==================================================
Summary of pdb4amber for: hiv_rt.pdb
==================================================

----------Chains
The following (original) chains have been found:
A

---------- Alternate Locations (Original Residues!))

The following residues had alternate locations:
None
-----------Non-standard-resnames
```

CSD

---------- Gaps (Renumbered Residues!)
gap of 3.272103 A between LEU 279 and LYS 281

---------- Mising heavy atom(s)

None

(base) /Users/mfuxreiter/Courses/P2> pdb4amber hivrt_cys.pdb > hivrt_cys_amber.pdb

==
Summary of pdb4amber for: hivrt_cys.pdb
==

----------Chains
The following (original) chains have been found:
A

---------- Alternate Locations (Original Residues!))

The following residues had alternate locations:
None
----------Non-standard-resnames

---------- Mising heavy atom(s)

None

2A. Processing SUBSTRATE – adding hydrogens
(base) /Users/mfuxreiter/Courses/P2> reduce efz.pdb > efz_h.pdb
reduce: version 3.3 06/02/2016, Copyright 1997-2016, J. Michael Word
Processing file: "efz.pdb"
Database of HETATM connections:
"/Users/mfuxreiter/Courses/amber20//dat/reduce_wwPDB_het_dict.txt"
VDW dot density = 16/A^2
Orientation penalty scale = 1 (100%)
Eliminate contacts within 3 bonds.
Ignore atoms with |occupancy| <= 0.01 during adjustments.
Waters ignored if B-Factor >= 40 or |occupancy| < 0.66

Aromatic rings in amino acids accept hydrogen bonds.
Building or keeping OH & SH Hydrogens.
Rotating NH3 Hydrogens.
WARNINGS
Found 0 hydrogens (0 hets)
Standardized 0 hydrogens (0 hets)
Added 9 hydrogens (9 hets)
Removed 0 hydrogens (0 hets)
If you publish work which uses reduce, please cite:
Word, et. al. (1999) J. Mol. Biol. 285, 1735-1747.
For more information see http://kinemage.biochem.duke.edu

- cp efz_h.pdb efz_new.pdb

2B. Processing SUBSTRATE – compute charges (AM1), assign gaff

(base) /Users/mfuxreiter/Courses/P2> antechamber -i efz_new.pdb -fi pdb -o efz.mol2 -fo mol2 -c bcc -s 2

Welcome to antechamber 21.0: molecular input file processor.

acdoctor mode is on: check and diagnose problems in the input file.
The atom type is set to gaff; the options available to the -at flag are
gaff, gaff2, amber, bcc, and sybyl.
-- Check Format for pdb File --
Status: pass
Info: Determining atomic numbers from atomic symbols which are case sensitive.
-- Check Unusual Elements --
Status: pass
-- Check Open Valences --
Status: pass
-- Check Geometry --
for those bonded
for those not bonded
Status: pass
-- Check Weird Bonds --
Status: pass
-- Check Number of Units --
Status: pass
acdoctor mode has completed checking the input file.

Running: /Users/mfuxreiter/Courses/amber20/bin/bondtype -j full -i ANTECHAMBER_BOND_TYPE.AC0 -o ANTECHAMBER_BOND_TYPE.AC -f ac

Running: /Users/mfuxreiter/Courses/amber20/bin/atomtype -i ANTECHAMBER_AC.AC0 -o ANTECHAMBER_AC.AC -p gaff
Info: Total number of electrons: 160; net charge: 0

Running: /Users/mfuxreiter/Courses/amber20/bin/sqm -O -i sqm.in -o sqm.out

Running: /Users/mfuxreiter/Courses/amber20/bin/am1bcc -i ANTECHAMBER_AM1BCC_PRE.AC -o ANTECHAMBER_AM1BCC.AC -f ac -p /Users/mfuxreiter/Courses/amber20/dat/antechamber/BCCPARM.DAT -s 2 -j 1

Running: /Users/mfuxreiter/Courses/amber20/bin/atomtype -f ac -p bcc -o ANTECHAMBER_AM1BCC.AC -i ANTECHAMBER_AM1BCC_PRE.AC

Check the results

- vi sqm.out
- vi efz.mol2

Are all parameters available?

2C Generate input for tleap

- parmchk2 -i efz.mol2 -f mol2 -o efz.frcmod

Check the results

- vi efz.frcmod

3. Load the generated parameters into tleap

3A Start tleap and read i/protein parameter library ff99SB and ii/ gaff library

tleap
-I: Adding /Users/mfuxreiter/Courses/amber20/dat/leap/prep to search path.
-I: Adding /Users/mfuxreiter/Courses/amber20/dat/leap/lib to search path.
-I: Adding /Users/mfuxreiter/Courses/amber20/dat/leap/parm to search path.
-I: Adding /Users/mfuxreiter/Courses/amber20/dat/leap/cmd to search path.

Welcome to LEaP!
(no leaprc in search path)
> source oldff/leaprc.ff99SB
----- Source: /Users/mfuxreiter/Courses/amber20/dat/leap/cmd/oldff/leaprc.ff99SB
----- Source of /Users/mfuxreiter/Courses/amber20/dat/leap/cmd/oldff/leaprc.ff99SB done
Log file: ./leap.log
Loading parameters: /Users/mfuxreiter/Courses/amber20/dat/leap/parm/parm99.dat
Reading title:
PARM99 for DNA,RNA,AA, organic molecules, Polariz.& LP incl.02/04/99

Loading parameters: /Users/mfuxreiter/Courses/amber20/dat/leap/parm/frcmod.ff99SB
Reading force field modification type file (frcmod)
Reading title:
Modification/update of parm99.dat (Hornak & Simmerling)
Loading library: /Users/mfuxreiter/Courses/amber20/dat/leap/lib/all_nucleic94.lib
Loading library: /Users/mfuxreiter/Courses/amber20/dat/leap/lib/all_amino94.lib
Loading library: /Users/mfuxreiter/Courses/amber20/dat/leap/lib/all_aminoct94.lib
Loading library: /Users/mfuxreiter/Courses/amber20/dat/leap/lib/all_aminont94.lib
Loading library: /Users/mfuxreiter/Courses/amber20/dat/leap/lib/ions94.lib
Loading library: /Users/mfuxreiter/Courses/amber20/dat/leap/lib/solvents.lib
> source leaprc.gaff
----- Source: /Users/mfuxreiter/Courses/amber20/dat/leap/cmd/leaprc.gaff
----- Source of /Users/mfuxreiter/Courses/amber20/dat/leap/cmd/leaprc.gaff done
Log file: ./leap.log
Loading parameters: /Users/mfuxreiter/Courses/amber20/dat/leap/parm/gaff.dat
Reading title:
AMBER General Force Field for organic molecules (Version 1.81, May 2017)

3B. generate a new entry for the substrate EFZ

> EFZ = loadmol2 efz.mol2
Loading Mol2 file: ./efz.mol2
Reading MOLECULE named EFZ

3C. check whether EFZ entry exists

> list
ACE ALA ARG ASH ASN ASP CALA CARG
CASN CASP CCYS CCYX CGLN CGLU CGLY CHCL3BOX
CHID CHIE CHIP CHIS CILE CIO CLEU CLYS
CMET CPHE CPRO CSER CTHR CTRP CTYR CVAL
CYM CYS CYX Cl- Cs+ DA DA3 DA5
DAN DC DC3 DC4 DC5 DCN DG DG3
DG5 DGN DT DT3 DT5 DTN EFZ FB3
FB3BOX FB4 FB4BOX GLH GLN GLU GLY HID
HIE HIP HIS HOH IB ILE K+ LEU
LYN LYS Li+ MEOHBOX MET MG2 NALA NARG
NASN NASP NCYS NCYX NGLN NGLU NGLY NHE
NHID NHIE NHIP NHIS NILE NLEU NLYS NMABOX
NME NMET NPHE NPRO NSER NTHR NTRP NTYR
NVAL Na+ OP3 OPC OPC3BOX OPCBOX PHE PL3
POL3BOX PRO QSPCFWBOX RA RA3 RA5 RAN RC
RC3 RC5 RCN RG RG3 RG5 RGN RU
RU3 RU5 RUN Rb+ SER SPC SPCBOX SPCFWBOX

SPF SPG T4E THR TIP3PBOX TIP3PFBOX TIP4PBOX TIP4PEWBOX
TIP5PBOX TP3 TP4 TP5 TPF TRP TYR VAL
WAT frcmod99SBgaff parm99

3D. check whether there are missing parameters for EFZ

> check EFZ
Checking 'EFZ'....
Checking parameters for unit 'EFZ'.
Checking for bond parameters.
Checking for angle parameters.
Unit is OK.

3E. if there are missing parameters load the ones you have generated

> loadamberparams efz.frcmod
Loading parameters: ./efz.frcmod
Reading force field modification type file (frcmod)
Reading title:
Remark line goes here

3F. if the unit is OK (check EFZ) save this library entry

> saveoff EFZ efz.lib
Creating efz.lib
Building topology.
Building atom parameters.

Also the save parameters and the geometry in restart format

> saveamberparm EFZ efz.prmtop efz.rst7
Checking Unit.
Building topology.
Building atom parameters.
Building bond parameters.
Building angle parameters.
Building proper torsion parameters.

Note: 1-4: angle 17 19 duplicates bond ('triangular' bond) or angle ('square' bond)

Note: 1-4: angle 17 18 duplicates bond ('triangular' bond) or angle ('square' bond)

Note: 1-4: angle 18 19 duplicates bond ('triangular' bond) or angle ('square' bond)

Building improper torsion parameters.
 total 8 improper torsions applied
Building H-Bond parameters.
Incorporating Non-Bonded adjustments.
Not Marking per-residue atom chain types.
Marking per-residue atom chain types.
  (Residues lacking connect0/connect1 -
   these don't have chain types marked:

        res   total affected

        EFZ 1
)
 (no restraints)

4. Prepare the whole complex protein + substrate

> cat hivrt_cys.pdb efz.pdb **>** hivrt_efz.pdb

4A generate input for tleap (tleap_protefz.in)

```
source oldff/leaprc.ff99SB
source leaprc.gaff
loadamberparams efz.frcmod
loadoff efz.lib
hivcompl = loadpdb hivrt_efz.pdb
saveamberparm hivcompl hivrt_efz.prmtop hivrt_efz.rst7
savepdb hivcompl hivrt_efz_amber.pdb
quit
```

4B run tleap for the complex

```
tleap -f tleap_protefz.in
```

```
Log file: ./leap.log
Loading parameters: /Users/mfuxreiter/Courses/amber20/dat/leap/parm/gaff.dat
Reading title:
AMBER General Force Field for organic molecules (Version 1.81, May 2017)
Loading parameters: ./efz.frcmod
Reading force field modification type file (frcmod)
Reading title:
Remark line goes here
```

```
Loading library: ./efz.lib
Loading PDB file: ./hivrt_efz.pdb
  Added missing heavy atom: .R<CGLY 543>.A<OXT 8>
  total atoms in file: 4432
  Leap added 4478 missing atoms according to residue templates:
       1 Heavy
       4477 H / lone pairs
Checking Unit.

/Users/mfuxreiter/Courses/amber20/bin/teLeap: Warning!
The unperturbed charge of the unit (2.000000) is not zero.

/Users/mfuxreiter/Courses/amber20/bin/teLeap: Note.
Ignoring the warning from Unit Checking.

Building topology.
Building atom parameters.
Building bond parameters.
Building angle parameters.
Building proper torsion parameters.
Building improper torsion parameters.
 total 1708 improper torsions applied
Building H-Bond parameters.
Incorporating Non-Bonded adjustments.
Not Marking per-residue atom chain types.
Marking per-residue atom chain types.
  (Residues lacking connect0/connect1 -
   these don't have chain types marked:

        res    total affected

        CGLY        1
        NPRO        1
  )
 (no restraints)
Writing pdb file: hivrt_efz_amber.pdb

/Users/mfuxreiter/Courses/amber20/bin/teLeap: Warning!
 Converting N-terminal residue name to PDB format: NPRO -> PRO

/Users/mfuxreiter/Courses/amber20/bin/teLeap: Warning!
 Converting C-terminal residue name to PDB format: CGLY -> GLY
        Quit
```

```
Exiting LEaP: Errors = 0; Warnings = 3; Notes = 1.
(base) /Users/mfuxreiter/Courses/P2> more tleap_protefz.in
source oldff/leaprc.ff99SB
source leaprc.gaff
loadamberparams efz.frcmod
loadoff efz.lib
hivcompl = loadpdb hivrt_efz.pdb
saveamberparm hivcompl hivrt_efz.prmtop hivrt_efz.rst7
savepdb hivcompl hivrt_efz_amber.pdb
quit
```

4C check the results

leap.log

hivrt_efz_amber.pdb in pymol

5 optimise the structure

5A generate an input file for minimisation

min_1.in

```
cat > mdin <<EOF
 &cntrl
   imin=1, maxcyc=200, ncyc=50, cut=16, ntb=0, igb=1,
 /
EOF

set output = minout
```

considerations

input – output format

steps for human readable data

restraints harmonic/not harmonic

minimisation method (switch between methods)

convergence criteria

cutoff

solvent (explicit, implicit)

5B run minimisation

sander -O -i min_1.in -o hivrt_efz_min.out -p hivrt_efz.prmtop -c hivrt_efz.rst7 -r hivrt_efz_min.ncrst &

5C analyze results

hivrt_efz_min.out

Maximum number of minimization cycles reached.

FINAL RESULTS

NSTEP	ENERGY	RMS	GMAX	NAME	NUMBER
200	-1.8014E+04	9.5384E-01	3.0060E+01	CD	6677

BOND = 267.8737 ANGLE = 1057.9599 DIHED = 5308.0281
VDWAALS = -4628.8386 EEL = -39183.2742 EGB = -8109.5363
1-4 VDW = 1838.2671 1-4 EEL = 25435.5720 RESTRAINT = 0.0000

Is gmax OK?

5D Visualise the results, compare with input

ambpdb -p hivrt_efz.prmtop -c hivrt_efz_min.ncrst > hivrt_efz_min.pdb