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