

Modifying Lennard-Jones Parameters in the Amberff14SB force field

In this tutorial you will learn to:

- Modify the self-interaction Lennard-Jones parameters of oxygens of carboxylate groups in proteins.
- Modify the Lennard-Jones parameters defining the interaction between sodium ions and the oxygens of carboxylate groups in proteins.

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Introduction

- The Amber formulation of the 6-12 Lennard-Jones (LJ) potential, V_{ij} , between 2 atoms i and j is:

$$V_{i,j} = \epsilon_{i,j} \left(\left(\frac{R_{\min,i,j}}{r_{i,j}} \right)^{12} - 2 \left(\frac{R_{\min,i,j}}{r_{i,j}} \right)^6 \right) \quad (\text{eq. 1})$$

- Here $R_{\min,i,j}$ is the center-to-center distance between i and j at which the potential is at the minimum $\epsilon_{i,j}$.
- Van der Waals data in Amber force field files are given for each atom i as a single data pair: a radius $R_{\min,i,i}/2$ ('van der Waals' radius of atom i , in Å) and the energy $\epsilon_{i,i}$ (the minimum interaction energy between 2 atoms i , in kcal/mol). These parameters are also called the self-interaction parameters.
- For Amber force fields, cross terms involving different atom types i and j are typically evaluated according to the Lorentz/Berthelot mixing rules:

$$R_{\min,i,j} = 0.5(R_{\min,i,i} + R_{\min,j,j}) \quad (\text{eq. 2})$$

$$\epsilon_{i,j} = \sqrt{\epsilon_{i,i} \cdot \epsilon_{j,j}} \quad (\text{eq. 3})$$

- In the first part of this tutorial you will learn how to modify self-interaction LJ parameters, using as example the parameters for the oxygens of carboxylate groups in proteins. Notice that, by modifying the self-interaction parameters of these oxygens, you are in fact modifying the LJ interactions of *every atom type with these oxygens*, via the mixing rules given by eqs. 2,3.
- The mixing rules have been shown to poorly represent the van der Waals interactions in certain cases. In the second part of this tutorial you will learn how to override the mixing rules for specific pairs of atoms i and j , and to use instead values of $R_{\min,i,j}$ and/or ϵ_{ij} optimized for that interaction. Specifically, you will modify the LJ parameters for the interaction between Na^+ and the oxygens in the carboxylate groups of proteins.
- The new parameters are from Kashfolgheta, S. & Vila Verde, A. *PCCP*, **2017**, *19*, 20593-20607, doi: 10.1039/C7CP02557B. They yield better agreement with experiment for the hydration free energy of acetate and the solution activity derivative of 0.5 m sodium acetate in TIP3P water. The same paper also reports optimized parameters for the NH_3^+ group of lysine, which we recommend using to obtain a better description of salt bridges in proteins.

- The original parameter files for the AMBER force field are in a directory which in our system can be found via environmental variable \$AMBERHOME. The path to this directory is specific to each installation. If \$AMBERHOME is not defined in your system and you don't know the path, you will need to ask your local IT support for help. You will leave the original files unchanged, and you will do modifications on local copies.
- Create a directory Tutorial/ at a location of your choice. Copy the necessary files from \$AMBERHOME to Tutorial/ using the commands shown in fig. A.

```
Terminal - geraili@hot:/cluster/apps/amber18/gnu/amber18/dat/leap/cmd
File Edit View Terminal Tabs Help
[geraili@hot cmd]$ cd $AMBERHOME
[geraili@hot amber18]$ cd dat/leap/cmd
[geraili@hot cmd]$ ls
leaprc.conste      leaprc.gaff        leaprc.music       leaprc.protein.ff15ipq
leaprc.constph    leaprc.gaff2       leaprc.phosaa10   leaprc.protein.ff15ipq-vac
leaprc.DNA.bsc1   leaprc.GLYCAM_06EPb leaprc.protein.fb15 leaprc.RNA.OL3
leaprc.DNA.OL15   leaprc.GLYCAM_06j-1 leaprc.protein.ff03.r1 leaprc.RNA.ROC
leaprc.ff14SB.redq leaprc.lipid14     leaprc.protein.ff03ua leaprc.RNA.YIL
leaprc.ffAM1      leaprc.lipid17     leaprc.protein.ff14SB leaprc.water.fb3
leaprc.ffPM3      leaprc.modrna08    leaprc.protein.ff14SBonlysc leaprc.water.fb4
[geraili@hot cmd]$ cp leaprc.protein.ff14SB /usr/data/bgfs1/geraili/Simulation_area/Tutorial/
[geraili@hot cmd]$ cp leaprc.water.tip3p /usr/data/bgfs1/geraili/Simulation_area/Tutorial/
```

Fig. A

- Once this step is complete, your Tutorial/ directory should have the files shown in Fig. B.

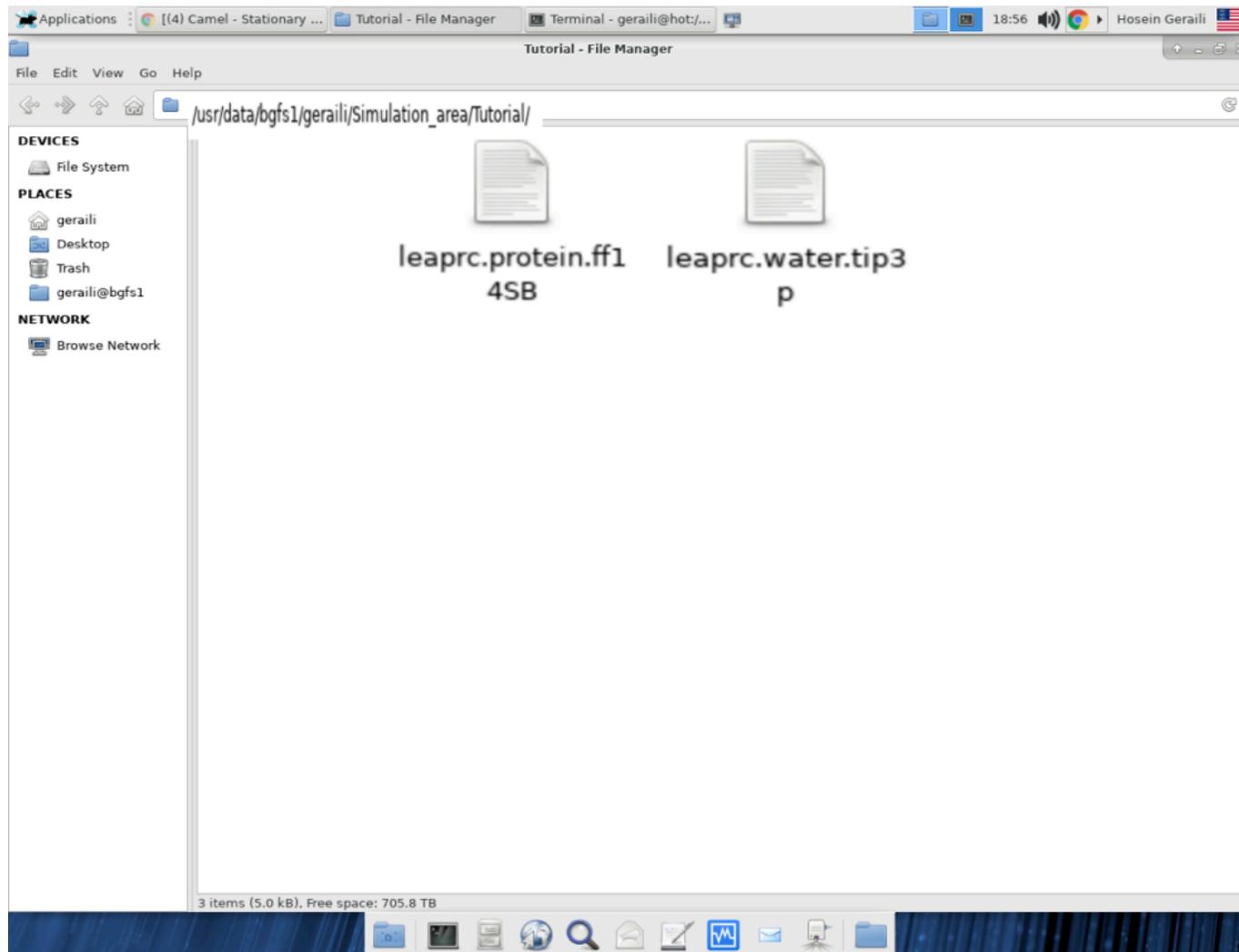


Fig. B: content of Tutorial/

- Copy the remaining necessary files following the commands in Fig. C.

```

Terminal - geraili@hot:/cluster/apps/amber18/gnu/amber18/dat/leap/lib
File Edit View Terminal Tabs Help
[geraili@hot lib]$ cd $AMBERHOME/dat/leap/parm
[geraili@hot parm]$ ls
all_modrna08.frcmod      frcmod.ions11m_126_tip3p      frcmod.parmbsc1      GLYCAM_06h.dat
frcmod.chcl3            frcmod.ions11m_126_tip4pew    frcmod.parmCHI_YIL   GLYCAM_06j.dat
frcmod.chi0L4          frcmod.ions11m_iod           frcmod.phmd          lipid11.dat
frcmod.conste          frcmod.ions234lm_1264_spce    frcmod.phosaa10     lipid14.dat
frcmod.constph        frcmod.ions234lm_1264_tip3p   frcmod.pol3         lipid17.dat
frcmod.dc4            frcmod.ions234lm_1264_tip4pew frcmod.protonated_nucleic lj_1264_pol.dat
frcmod.DNA.0L15       frcmod.ions234lm_126_spce     frcmod.qspcfw       music.dat
frcmod.fb15           frcmod.ions234lm_126_tip3p    frcmod.ROC-RNA      nucgen.dat
frcmod.ff02pol.r1     frcmod.ions234lm_126_tip4pew frcmod.ROC-RNA_const opls.info
frcmod.ff03           frcmod.ions234lm_hfe_spce     frcmod.spce         opls_parm.dat
frcmod.ff03ua         frcmod.ions234lm_hfe_tip3p    frcmod.spceb        parm10.dat
frcmod.ff12SB         frcmod.ions234lm_hfe_tip4pew  frcmod.spcfw        parm14ipq.dat
frcmod.ff14SB         frcmod.ions234lm_iod_spce     frcmod.tip3p        parm15ipq_10.3.dat
frcmod.ff99bsc0CG     frcmod.ions234lm_iod_tip3p    frcmod.tip3pf       parm91.dat
frcmod.ff99SB         frcmod.ions234lm_iod_tip4pew  frcmod.tip3pfb      parm91X.dat
frcmod.ff99SB14      frcmod.ionsff99_tip3p        frcmod.tip4p        parm91X.ua.dat
frcmod.ff99SBildn    frcmod.ionsjc_spce           frcmod.tip4pew     parm94.dat
frcmod.ff99SBnmr     frcmod.ionsjc_tip3p          frcmod.tip4pfb     parm96.dat
frcmod.ff99SP        frcmod.ionsjc_tip4pew        frcmod.tip5p       parm98.dat
frcmod.ions11m_1264_spce frcmod.meoh                  frcmod.urea         parm99.dat
frcmod.ions11m_1264_tip3p frcmod.nma                   frcmod.vdWall       parm99EP.dat
frcmod.ions11m_1264_tip4pew frcmod.opc                   frcmod.xFPchromophores parmAM1.dat
frcmod.ions11m_126_hfe_opc frcmod.opc3                   gaff2.dat           parmPM3.dat
frcmod.ions11m_126_iod_opc frcmod.parmbsc0               gaff.dat            toyrna.dat
frcmod.ions11m_126_spce frcmod.parmbsc0_ez0L1        GLYCAM_06EPb.dat   validate_torsions.py
[geraili@hot parm]$ cp parm10.dat /usr/data/bgfs1/geraili/Simulation_area/Tutorial/
[geraili@hot parm]$ cp frcmod.ff14SB /usr/data/bgfs1/geraili/Simulation_area/Tutorial/
[geraili@hot parm]$ cp frcmod.ionsjc_tip3p /usr/data/bgfs1/geraili/Simulation_area/Tutorial/
[geraili@hot parm]$ cd $AMBERHOME/dat/leap/lib
[geraili@hot lib]$ cp aminol12.lib /usr/data/bgfs1/geraili/Simulation_area/Tutorial/
[geraili@hot lib]$ cp aminoct12.lib /usr/data/bgfs1/geraili/Simulation_area/Tutorial/
[geraili@hot lib]$ cp aminont12.lib /usr/data/bgfs1/geraili/Simulation_area/Tutorial/
[geraili@hot lib]$ █

```

Fig. C

- Once you've copied all the files, Tutorial/ should have the content shown in Fig. D.
- The files *leaprc.water.tip3p* and *leaprc.protein.ff14SB* are loaded into “tleap” to build topology and coordination files for simulations of proteins in water. These files contain, among other things:
 - a list of atom types;
 - the path to the parameter files that will be loaded by “tleap”:
 - *leaprc.protein.ff14SB* calls parameter files *parm10.dat* and *frcmod.ff14SB* and topology files *amino12.lib*, *aminoc12.lib*, *aminont12.lib*;
 - *leaprc.water.tip3p* calls several parameter and topology files for TIP3P water and TIP3P-compatible ions; one of the files called is *frcmod.ionsjc_tip3p*, which we have copied. We will not change anything in *leaprc.water.tip3p* or in *frcmod.ionsjc_tip3p*. We copied these files because 1) we want to view the original Na⁺ parameters for the second part of the tutorial, and 2) it is convenient to have in a single directory all the files necessary to run a simulation in the AMBER MD software.
- Now that we have all the files necessary, we need to modify them one by one. **You will only modify files inside Tutorial/.**

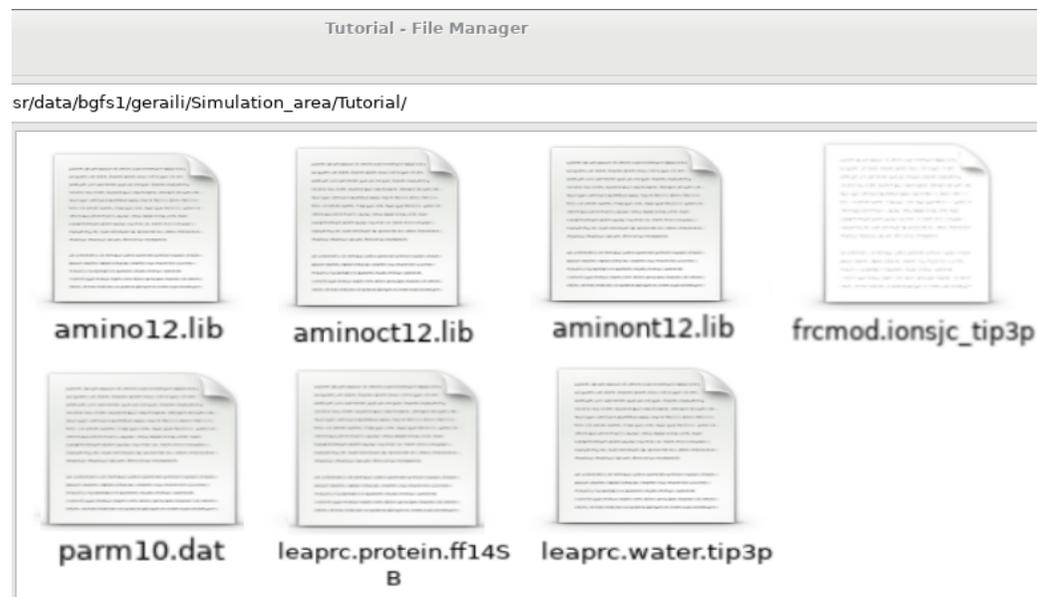


Fig. D: content of Tutorial/

- Our first, and most complex, task is to modify the self-interaction parameters of the oxygens in the carboxylate groups of proteins. Carboxylates exist in side chain of Asp (Aspartate) and Glu (Glutamate) residues, and in any uncapped amino acid forming the C-terminus of the protein.
- In general, the difficulty in creating new atom types for a specific functional group is to make sure that we change the parameters of only the atom type on those functional groups, while leaving oxygens with the same original atom type but not belonging to the same functional group with the original parameters. In our specific example this problem does not arise because we only have these type of oxygens on the carboxylates in the side chain of Asp (aspartate) and Glu (glutamate) residues, and in any uncapped amino acid forming the C-terminus of the protein. We nevertheless follow a standard procedure to create a general tutorial for any other kind of optimization that might need to consider this important point.

- Open file *amino12.lib* with your favorite text editor and go to the entry for aspartate (“ASP”). The carboxylate oxygen has type “O2”. An example section of the unmodified version of this file is in Fig. E.; the red boxes show you the lines containing “O2” for ASP.

```

!entry.ASP.unit.atoms table str name str type int typex int resx int flags int seq int el
mnt dbl chg
"N" "N" 0 1 131072 1 7 -0.516300
"H" "H" 0 1 131072 2 1 0.293600
"CA" "CX" 0 1 131072 3 6 0.038100
"HA" "H1" 0 1 131072 4 1 0.088000
"CB" "2C" 0 1 131072 5 6 -0.030300
"HB2" "HC" 0 1 131072 6 1 -0.012200
"HB3" "HC" 0 1 131072 7 1 -0.012200
"CG" "CO" 0 1 131072 8 6 0.799400
"OD1" "O2" 0 1 131072 9 8 -0.801400
"OD2" "O2" 0 1 131072 10 8 -0.801400
"C" "C" 0 1 131072 11 6 0.536600
"O" "O" 0 1 131072 12 8 -0.581900
!entry.ASP.unit.atoms pertinfo table str pname str ptype int ptypex int pelmnt dbl pchg
"N" "N" 0 -1 0.0
"H" "H" 0 -1 0.0
"CA" "CX" 0 -1 0.0
"HA" "H1" 0 -1 0.0
"CB" "2C" 0 -1 0.0
"HB2" "HC" 0 -1 0.0
"HB3" "HC" 0 -1 0.0
"CG" "CO" 0 -1 0.0
"OD1" "O2" 0 -1 0.0
"OD2" "O2" 0 -1 0.0
"C" "C" 0 -1 0.0
"O" "O" 0 -1 0.0
!entry.ASP.unit.boundbox array dbl
-1.000000
0.0

```

Fig. E: original *amino12.lib*

- In *amino12.lib*, change the atom type of the oxygens in the side chain of ASP from “O2” to a new type; we chose “9O”. See Fig. F for the modified entries for ASP.
 - It is indispensable that the new atom type: 1) has only 2 letters; 2) is not already used for other atoms. You can check whether your new atom type is not being used by searching through this file.
- Search through this file and double check that atom type “O2” is changed to “9O” for every entry for ASP. Repeat the procedure for glutamate (“GLU”). Save and close the file when you are done.

```

0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
!entry.ASP.unit.atoms table str name str type int typex int resx int flags int seq int el
mnt dbl chg
"N" "N" 0 1 131072 1 7 -0.516300
"H" "H" 0 1 131072 2 1 0.293600
"CA" "CX" 0 1 131072 3 6 0.038100
"HA" "H1" 0 1 131072 4 1 0.088000
"CB" "2C" 0 1 131072 5 6 -0.030300
"HB2" "HC" 0 1 131072 6 1 -0.012200
"HB3" "HC" 0 1 131072 7 1 -0.012200
"CG" "CO" 0 1 131072 8 6 0.799400
"OD1" "9O" 0 1 131072 9 8 -0.801400
"OD2" "9O" 0 1 131072 10 8 -0.801400
"C" "C" 0 1 131072 11 6 0.536600
"O" "O" 0 1 131072 12 8 -0.581900
!entry.ASP.unit.atoms pertinfo table str pname str ptype int ptypex int pelmnt dbl pchg
"N" "N" 0 -1 0.0
"H" "H" 0 -1 0.0
"CA" "CX" 0 -1 0.0
"HA" "H1" 0 -1 0.0
"CB" "2C" 0 -1 0.0
"HB2" "HC" 0 -1 0.0
"HB3" "HC" 0 -1 0.0
"CG" "CO" 0 -1 0.0
"OD1" "9O" 0 -1 0.0
"OD2" "9O" 0 -1 0.0
"C" "C" 0 -1 0.0
"O" "O" 0 -1 0.0
!entry.ASP.unit.boundbox array dbl
-1.000000

```

Attention!
Always make sure
you modify only
the entries you
want.

Fig. F: modified *amino12.lib*

- Amino acids forming the C- or N-terminus of a protein have separate entries in the AMBER force field. The C-terminus entries are in file *aminoc12.lib*; the N-terminus entries are in file *aminont12.lib*. You will need to modify these files as well.
- An example section of the unmodified version of *aminoc12.lib* is shown in Fig. G. Notice that you now have 2 carboxylates for CGLU (red boxes): one in the side-chain and one forming the C-terminus.

```

entry.CGLU.unit.atoms table str name str type int typex int resx int flags int seq int elmnt dbl chg
"N" "N" 0 1 131072 1 7 -0.519200
"H" "H" 0 1 131072 2 1 0.305500
"CA" "CX" 0 1 131072 3 6 -0.205900
"HA" "H1" 0 1 131072 4 1 0.139900
"CB" "2C" 0 1 131072 5 6 0.007100
"HB2" "HC" 0 1 131072 6 1 -0.007800
"HB3" "HC" 0 1 131072 7 1 -0.007800
"CG" "2C" 0 1 131072 8 6 0.067500
"HG2" "HC" 0 1 131072 9 1 -0.054800
"HG3" "HC" 0 1 131072 10 1 -0.054800
"CD" "CO" 0 1 131072 11 6 0.818300
"OE1" "O2" 0 1 131072 12 8 -0.822000
"OE2" "O2" 0 1 131072 13 8 -0.822000
"C" "C" 0 1 131072 14 6 0.742000
"O" "O2" 0 1 131072 15 8 -0.793000
"OXT" "O2" 0 1 131072 16 8 -0.793000
entry.CGLU.unit.atomsptinfo table str pname str ptype int ptypex int pelmnt dbl pchg
"N" "N" 0 -1 0.0
"H" "H" 0 -1 0.0
"CA" "CX" 0 -1 0.0
"HA" "H1" 0 -1 0.0
"CB" "2C" 0 -1 0.0
"HB2" "HC" 0 -1 0.0
"HB3" "HC" 0 -1 0.0
"CG" "2C" 0 -1 0.0
"HG2" "HC" 0 -1 0.0
"HG3" "HC" 0 -1 0.0
"CD" "CO" 0 -1 0.0
"OE1" "O2" 0 -1 0.0
"OE2" "O2" 0 -1 0.0
"C" "C" 0 -1 0.0
"O" "O2" 0 -1 0.0
"OXT" "O2" 0 -1 0.0
entry.CGLU.unit.boundbox array dbl
-1.000000
0.0

```

Fig. G: original *aminoc12.lib*

- For the C-terminus (*aminoc12.lib*):
 - Search for CGLU and change atom type “O2” to “9O”, similarly to what you did before. Fig H shows an example section of the modified *aminoc12.lib*.
 - Repeat for CASP.
 - Every other terminal amino acid has one carboxylate group; you will need to change “O2” to “9O” for all those carboxylates too (examples not shown).
 - Save and close the file when you are done.
- For the N-terminus (*aminont12.lib*):
 - Repeat the procedure to modify the side-chain oxygens in NASP and NGLU (images not shown). Only these amino acids need to be modified in this file, because N-terminus amino acids do not contain extra carboxylate groups.
 - Save and close the file when you are done.

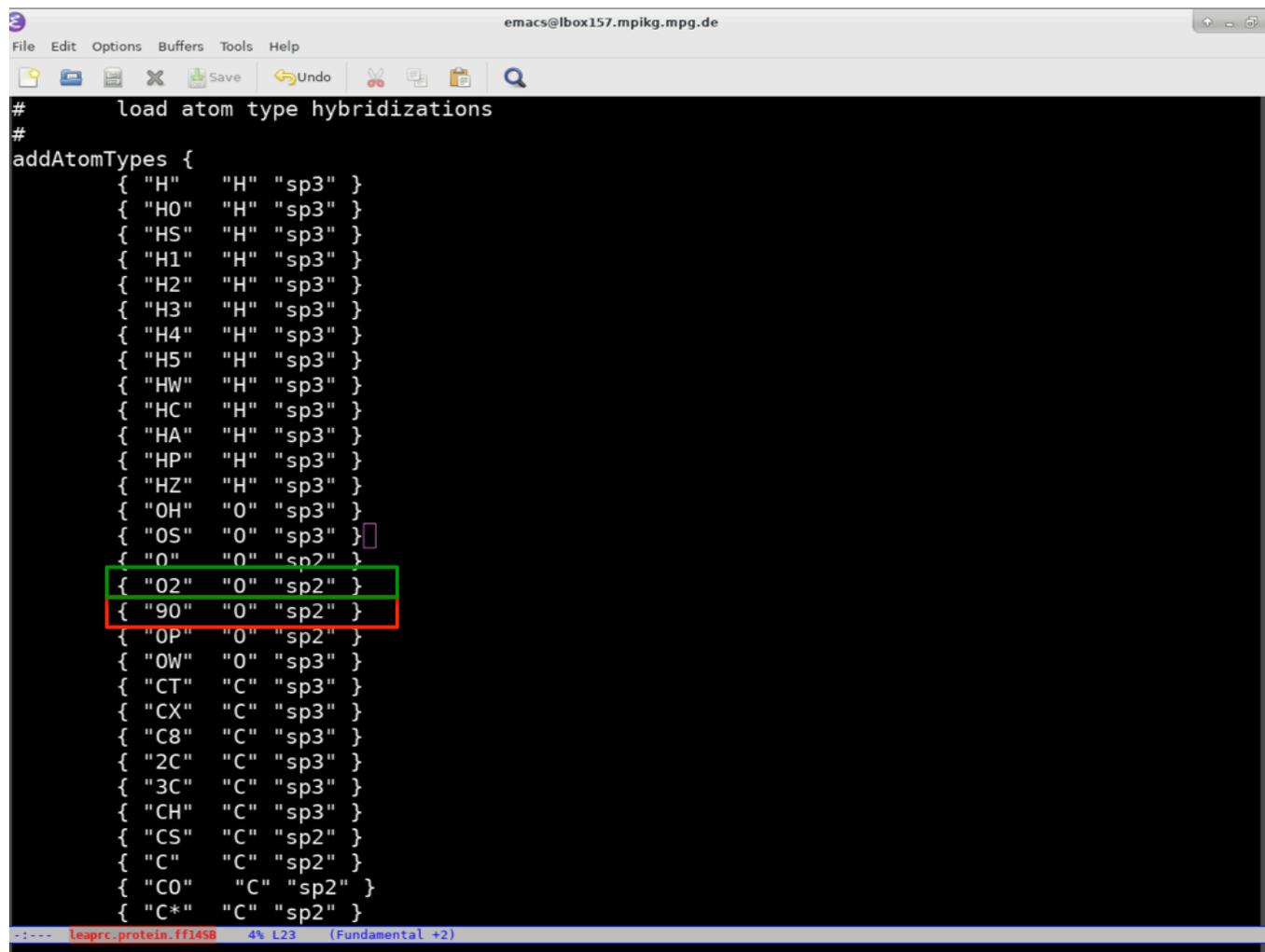
```

!entry.CGLU.unit.atoms table str name str type int typex int resx int flags int seq int elmnt dbl chg
"N" "N" 0 1 131072 1 7 -0.519200
"H" "H" 0 1 131072 2 1 0.305500
"CA" "CX" 0 1 131072 3 6 -0.205900
"HA" "H1" 0 1 131072 4 1 0.139900
"CB" "2C" 0 1 131072 5 6 0.007100
"HB2" "HC" 0 1 131072 6 1 -0.007800
"HB3" "HC" 0 1 131072 7 1 -0.007800
"CG" "2C" 0 1 131072 8 6 0.067500
"HG2" "HC" 0 1 131072 9 1 -0.054800
"HG3" "HC" 0 1 131072 10 1 -0.054800
"CD" "CO" 0 1 131072 11 6 0.818300
"OE1" "9O" 0 1 131072 12 8 -0.822000
"OE2" "9O" 0 1 131072 13 8 -0.822000
"C" "C" 0 1 131072 14 6 0.742000
"O" "9O" 0 1 131072 15 8 -0.793000
"OXT" "9O" 0 1 131072 16 8 -0.793000
!entry.CGLU.unit.atomsperinfo table str pname str ptype int ptypex int pelmnt dbl pchg
"N" "N" 0 -1 0.0
"H" "H" 0 -1 0.0
"CA" "CX" 0 -1 0.0
"HA" "H1" 0 -1 0.0
"CB" "2C" 0 -1 0.0
"HB2" "HC" 0 -1 0.0
"HB3" "HC" 0 -1 0.0
"CG" "2C" 0 -1 0.0
"HG2" "HC" 0 -1 0.0
"HG3" "HC" 0 -1 0.0
"CD" "CO" 0 -1 0.0
"OE1" "9O" 0 -1 0.0
"OE2" "9O" 0 -1 0.0
"C" "C" 0 -1 0.0
"O" "9O" 0 -1 0.0
"OXT" "9O" 0 -1 0.0
!entry.CGLU.unit.boundbox array dbl
-1.000000
0.0
0.0
0.0
0.0
!entry.CGLU.unit.childsequence single int
---- aminoc12.lib 26% L913 (Fundamental +1)
Wrote /usr/data/bgfs1/geraili/Simulation_area/Tutorial/aminoc12.lib

```

Fig. H: modified section of *aminoc12.lib*

- In the *leaprc.protein.ff14SB* file, copy the line where “O2” is defined (green box in Fig. I), and paste it directly below it as a new line.
- In the new line, change the “O2” to “YOUR NEW NAME” (in our example, the new name is “9O”; red box in Fig. I).
- Save and close the file.



```
emac@lbox157.mpikg.mpg.de
File Edit Options Buffers Tools Help
Save Undo
# load atom type hybridizations
#
addAtomTypes {
  { "H" "H" "sp3" }
  { "HO" "H" "sp3" }
  { "HS" "H" "sp3" }
  { "H1" "H" "sp3" }
  { "H2" "H" "sp3" }
  { "H3" "H" "sp3" }
  { "H4" "H" "sp3" }
  { "H5" "H" "sp3" }
  { "HW" "H" "sp3" }
  { "HC" "H" "sp3" }
  { "HA" "H" "sp3" }
  { "HP" "H" "sp3" }
  { "HZ" "H" "sp3" }
  { "OH" "O" "sp3" }
  { "OS" "O" "sp3" }
  { "O" "O" "sp2" }
  { "O2" "O" "sp2" }
  { "9O" "O" "sp2" }
  { "OP" "O" "sp2" }
  { "OW" "O" "sp3" }
  { "CT" "C" "sp3" }
  { "CX" "C" "sp3" }
  { "C8" "C" "sp3" }
  { "2C" "C" "sp3" }
  { "3C" "C" "sp3" }
  { "CH" "C" "sp3" }
  { "CS" "C" "sp2" }
  { "C" "C" "sp2" }
  { "CO" "C" "sp2" }
  { "C*" "C" "sp2" }
}
-:--- leaprc.protein.ff14SB 4% L23 (Fundamental +2)
```

Fig. I: modified section of *leaprc.protein.ff14SB*

- The actual parameter values - atomic masses, charges, Lennard-Jones, bonds, angles, dihedral and improper potentials - for each interaction between 2 or more atoms are in files *parm10.dat* and *frcmod.ff14SB*.
 - *parm10.dat* is the main file; it contains the parameters for most interactions, and it is **never** modified.
 - *frcmod.ff14SB* supplements *parm10.dat* in two different ways: 1) it contains parameters for any new interaction; 2) it may also contain new parameters for any interaction that is already defined in *parm10.dat* but which we wish to override.
 - “tleap” reads *parm10.dat* first and *frcmod.ff14SB* afterwards; if it finds parameters for the same interaction in both files, it will use those from *frcmod.ff14SB* only.
 - In the next few slides we will illustrate the procedure to include in *frcmod.ff14SB* parameters for all interactions involving our new atom type “9O”. In general, the steps in the procedure are (do not worry if you do not understand the procedure at this point; the examples will clarify it):
 - step 1: for each reference to carboxylate-“O2” in *parm10.dat*, check if the same interaction has been defined in *frcmod.ff14SB*.
 - If yes, duplicate the line in *frcmod.ff14SB* and past the duplicate directly below the original line; in the duplicate line, change “O2” to “9O”.
 - if no, copy the line in *parm10.dat*, paste it at the end of the corresponding section in *frcmod.ff14SB*; in the copy, change “O2” to “9O”.
 - by following these instructions, you make sure that all parameters in *parm10.dat* for carboxylate-“O2” are put into *frcmod.ff14SB*, without overwriting newer parameters for the same interaction if they exist in *frcmod.ff14SB*.
 - step 2: once you’re done with step 1, you need to search for all references to carboxylate-“O2” in *frcmod.ff14SB*, and make sure that equivalent information is defined for “9O”. This step is necessary because some interactions may only be defined in *frcmod.ff14SB*.

- Entering mass information for “9O”:
 - Look for the mass of “O2” in *parm10.dat* (Fig. J, red box).
 - Inspect the MASS section in *frcmmod.ff14SB*. There is no entry for “O2” there.
 - Copy the line containing the mass information from *parm10.dat*, paste it at the end of the MASS section in *frcmmod.ff14SB* and change the atom type from O2 to “9O” (Fig. K, red box).

```

NC 14.01      0.530      sp2 N in 6 memb.ring w/LP (ADE,GUA)
N2 14.01      0.530      sp2 N in amino groups
N3 14.01      0.530      sp3 N for charged amino groups (Lys, etc)
NT 14.01      0.530      sp3 N for amino groups amino groups
N* 14.01      0.530      sp2 N
NY 14.01      0.530      nitrile N (Howard et al.JCC,16,243,1995)
O  16.00      0.434      carbonyl group oxygen
O2 16.00      0.434      carboxyl and phosphate group oxygen
OW 16.00      0.000      oxygen in TIP3P water
OH 16.00      0.465      oxygen in hydroxyl group
OS 16.00      0.465      ether and ester oxygen
OP 16.00      0.465      2- phosphate oxygen
P  30.97      1.538      phosphate,pol:JACS,112,8543,90,K.J.Miller
S  32.06      2.900      S in disulfide linkage,pol:JPC,102,2399,98
SH 32.06      2.900      S in cystine
CU 63.55
FE 55.00
Zn 65.4
EP  0.00      0.000      extra point

C  H  HO  N  NA  NB  NC  N2  NT  N2  N3  N*  O  OH  OS  P  O2
OW-HW 553.0  0.9572  ! TIP3P water
HW-HW 553.0  1.5136  TIP3P water
C -C  310.0  1.525   Junmei et al, 1999
C -CA 469.0  1.409   JCC,7,(1986),230; (not used any more in TYR)
C -CB 447.0  1.419   JCC 7 (1986) 230: GUA

```

Fig. J: Original parm10.dat

```

ff14SB protein backbone and sidechain parameters
MASS
CO 12.01      0.616  !      sp2 C carboxylate group
2C 12.01      0.878      sp3 aliphatic C with two (duo) heavy atoms
3C 12.01      0.878      sp3 aliphatic C with three (tres) heavy atoms
C8 12.01      0.878      sp3 aliphatic C basic AA side chain
9O 16.00      0.434      carboxyl group sp2 oxygen, PCCP 2017, 19, 20593

BOND
C -2C 317.0    1.5220
C* -2C 317.0    1.4950
C8-C8 310.0    1.5260
C8-CX 310.0    1.5260
C8-H1 340.0    1.0900
C8-HC 340.0    1.0900
C8-HP 340.0    1.0900
C8-N2 337.0    1.4630
C8-N3 367.0    1.4710
CA-2C 317.0    1.5100
CC-2C 317.0    1.5040

```

Fig. K: modified section of *frcmmod.ff14SB*

- Entering parameters for bonds involving “9O”:
 - locate bond parameters involving “O2” in *parm10.dat*. Here is the relevant line:
 - C -O2 656.0 1.250 JCC,7,(1986),230; GLU,ASP (line 81)
 - O2-P 525.0 1.480 JCC,7,(1986),230; NA PHOSPHATES (line 173)
 - locate bond parameters involving “O2” in *frcmod.ff14SB*. There is only one line:
 - CO-O2 656.0 1.2500 (line 20)
 - Interactions “C -O2” and “O2-P” are only defined in *parm10.dat*. Copy the line for “C -O2” from *parm10.dat*, paste it at the end of the bond section in *frcmod.ff14SB* and change “O2” to “9O” (Fig. L, green box).
 - Note that you should **not** insert a line for the “9O-P” bond in *frcmod.ff14SB*. We developed these oxygen parameters specifically for carboxylates. Because of their high specificity, these parameters should not be used for anything other than the intended functional groups.
 - Interaction “CO-O2” only exists in *frcmod.ff14SB*. Duplicate this line, paste it directly below the original and change “O2” to “9O” (Fig. L, red box).
 - Note: if “CO-O2” had simultaneously existed in *parm10.dat*, you would have ignored the *parm10.dat* data and would have done exactly the same.

```

ff14SB protein backbone and sidechain parameters
MASS
CO 12.01      0.616  !           sp2 C carboxylate group
2C 12.01      0.878             sp3 aliphatic C with two (duo) heavy atoms
3C 12.01      0.878             sp3 aliphatic C with three (tres) heavy atoms
C8 12.01      0.878             sp3 aliphatic C basic AA side chain
9O 16.00      0.434             carboxyl group sp2 oxygen, PCCP, 2017, 19, 20593

BOND
C -2C 317.0    1.5220
C* -2C 317.0    1.4950
C8 -C8 310.0    1.5260
C8 -CX 310.0    1.5260
C8 -H1 340.0    1.0900
C8 -HC 340.0    1.0900
C8 -HP 340.0    1.0900
C8 -N2 337.0    1.4630
C8 -N3 367.0    1.4710
CA -2C 317.0    1.5100
CC -2C 317.0    1.5040
CO -O2 656.0    1.2500
CO -9O 656.0    1.2500      new parameter
CO -2C 317.0    1.5220
CT -2C 310.0    1.5260
CT -3C 310.0    1.5260
CX -2C 310.0    1.5260
CX -3C 310.0    1.5260
H1 -2C 340.0    1.0900
H1 -3C 340.0    1.0900
HC -2C 340.0    1.0900
HC -3C 340.0    1.0900
OH -2C 320.0    1.4100
OH -3C 320.0    1.4100
S -2C 227.0     1.8100
SH -2C 237.0     1.8100
2C -2C 310.0    1.5260
2C -3C 310.0    1.5260
C -9O 656.0    1.250      new parameter
  
```

Fig. L: modified section of *frcmod.ff14SB*

- Entering parameters for angles, dihedrals or impropers involving carboxylate-“O2”:
- To make these changes, you follow the same procedure we have exemplified for inserting parameters for bonds involving “9O”, with one difference: when you are changing the carboxylate-“O2” angle, dihedral, or improper, you must change them in a way to be able to consider any combination of interactions in the future.
 - search for carboxylate angles with “O2” in *parm10.dat*. There are multiple lines:
 - CT-C -O2 70.0 117.00 (line 237)
 - CX-C -O2 70.0 117.00 (was CT-C -O2) (line 238)
 - O2-C -O2 80.0 126.00 AA GLU (SCH JPC 79,2379) (line 254)
 - O2-P -OH 45.0 108.23 (line 539)
 - O2-P -O2 140.0 119.90 (line 540)
 - O2-P -OS 100.0 108.23 (line 543)
 - do the same thing in *frmod.ff14SB*. Here are the lines:
 - O2-CO-O2 80.0 126.00
 - O2-CO-2C 70.0 117.00
 - Notice that each angle interaction is defined in only one file.
 - Duplicate the line with the “O2-CO-2C “ interaction in *frmod.ff14SB* and paste it directly below the original. In the duplicate, change “O2” to “9O” (green box in Fig. M)
 - Duplicate the line with the “O2-CO-O2 “ interaction in *frmod.ff14SB* and paste it twice directly below the original. In the duplicates, change “O2” to “9O” to allow all possible combinations of “O2” and “9O” (red box in Fig. M)
 - For our specific application, it would have been sufficient to insert only a line with “9O-CO-9O “ in *frmod.ff14SB* because the *lib files we modified do not have an “O2-CO-O9” angle. We nevertheless suggest always including the combinations in *frmod.ff14SB* to avoid unexpected problems if an angle “9O-CO-O2” ever becomes necessary.
 - Copy each of the lines from *parm10.dat* with angle interactions involving carboxylate-“O2” and paste them one or more times (as necessary) at the end of the ANGLE section in *frmod.ff14SB*. Make the necessary changes from “O2” to “9O” (images not shown).
 - Angle potentials involving the “O2-P” bond (in red above) should **not** be updated to “9O” in *frmod.ff14SB*, for the reasons explained in the previous slide.
 - Follow the same procedure to update dihedral and impropers (images not shown).

```

emac
File Edit Options Buffers Tools Help
Save Undo
NB-CC-2C 70.0 120.00
O2-CO-O2 80.0 126.00
9O-CO-9O 80.0 126.00 new parameter
O2-CO-9O 80.0 126.00 new parameter
O2-CO-2C 70.0 117.00
9O-CO-2C 70.0 117.00 new parameter
HC-CT-2C 50.0 109.50
HC-CT-3C 50.0 109.50
C -CX-C8 63.0 111.10
C -CY-2C 63.0 111.10
frmod.ff14SB 5% L53 (Fundamental +1)

```

Fig. M: modified angle section of *frmod.ff14SB*

- We will now input the new Lennard-Jones (LJ) parameters for atom type “9O”. LJ parameters exist in *parm10.dat* (Fig. N; red box shows parameters for “O2”), and in *frcmod.ff14SB*, in the “NONB” and “LJEDIT” sections (Fig. O, red boxes).
- The “NONB” section will contain the new self-interaction parameters.
- the “LJEDIT” will contain LJ parameters for those pairs of atoms for which we want to override the Lorentz-Berthelot combination rules.

The figure consists of two side-by-side screenshots of an Emacs editor window. The left window shows the file *parm10.dat* with a table of parameters for atom types. A red box highlights the row for atom type O2. The right window shows the file *frcmod.ff14SB* with two sections highlighted by red boxes: the NONB section and the LJEDIT section.

Fig. N: *parm10.dat*

N	NA	N2	N*	NC	NB	NT	NY	
C*	CA	CB	CC	CD	CK	CM	CN	CQ CR CV CW CY CZ CP CS
MOD4	RE							
H		0.6000	0.0157					!Ferguson base pair geom.
H0		0.0000	0.0000					OPLS Jorgensen, JACS,110,(1988),1657
HS		0.6000	0.0157					W. Cornell CH3SH --> CH30H FEP
HC		1.4870	0.0157					OPLS
H1		1.3870	0.0157					Veenstra et al JCC,8,(1992),963
H2		1.2870	0.0157					Veenstra et al JCC,8,(1992),963
H3		1.1870	0.0157					Veenstra et al JCC,8,(1992),963
HP		1.1000	0.0157					Veenstra et al JCC,8,(1992),963
HA		1.4590	0.0150					Spellmeyer
H4		1.4090	0.0150					Spellmeyer, one electrowithdr. neighbor
H5		1.3590	0.0150					Spellmeyer, two electrowithdr. neighbor
HW		0.0000	0.0000					TIP3P water model
HZ		1.4590	0.0150					H bonded to sp C (Howard et al JCC 16)
O		1.6612	0.2100					OPLS
O2		1.6612	0.2100					OPLS
OW		1.7683	0.1520					TIP3P water model
OH		1.7210	0.2104					OPLS
OS		1.6837	0.1700					OPLS ether
OP		1.8500	0.1700					Steinbrecher/Latzer for 2- phosphate
C*		1.9080	0.0860					Spellmeyer
CI		1.9080	0.1094					parmbsc0
C5		1.9080	0.0860					Spellmeyer
C4		1.9080	0.0860					Spellmeyer
CT		1.9080	0.1094					Spellmeyer

Fig. O: unmodified *frcmod.ff14SB*

Pair	1	2	3	4	5	6
CX-2C-S	-S	1	0.666	0.0	-2.	
CX-2C-S	-S	1	0.056	0.0	1.	
2C-S	-S -2C	1	0.379	0.0	-4.	
2C-S	-S -2C	1	0.682	0.0	-3.	
2C-S	-S -2C	1	4.480	0.0	-2.	
2C-S	-S -2C	1	0.420	0.0	1.	
IMPR						
X	-O2-CO-O2		10.5	180.	2.	
2C-O	-C -OH		10.5	180.	2.	
CA-CA-CA	-2C		1.1	180.	2.	
NONB						
2C			1.9080	0.1094		Spellmeyer
3C			1.9080	0.1094		Spellmeyer
C8			1.9080	0.1094		Spellmeyer
CO			1.9080	0.0860		OPLS
LJEDIT						

Fig. N: *parm10.dat*

Fig. O: unmodified *frcmod.ff14SB*

- Download the *zip file that you'll find as supporting information of the article we mentioned in the introduction (doi: 10.1039/C7CP02557B). Open file Parameters/Acetate.top. This file contains the LJ parameter information in gromacs format; amber format parameters are given as comments. The relevant lines for this tutorial are:
 - ;name bond_type mass charge ptype sigma (nm) epsilon (kJ/mol) r(AMBER; A) epsilon(AMBER; kcal/mol)
 - OACE OACE 0.00000 0.00000 A 2.95992e-01 6.76553e-01 ; 1.6612 0.1617 ; 0.77x Original epsilon
- ;i j func sigma epsilon Rij(AMBER; A) epsilon_ij(AMBER, kcal/mol)
- OACE NA+ 1 2.75899e-1 4.97508e-1 ; 3.0969 0.1189 ; 1.022 times Original sigma (Rij in AMBER) ;
OACE self interaction correction for epsilon included

- “OACE” corresponds to our “9O”. “r(AMBER)” and “epsilon (AMBER)” are the optimized self-interaction parameters. “r(AMBER)” is $R_{\min,ij}/2$ and “epsilon(AMBER)” is ϵ_{ij} in the notation of eq. 1 of this tutorial.
- “Rij(AMBER)” and “epsilon_ij(AMBER)” are the optimized LJ parameters for the interaction between “O9” and Na⁺. Notice that “Rij(AMBER)” is $R_{\min,ij}$ in the notation of eq. 1.
- Notice that “r(AMBER)” is the same as in *parm10.dat* (Fig. N) but that “epsilon (AMBER)” is different: the optimized “r(AMBER)” is 0.77 times the original “r(AMBER)”.
- Insert the new LJ self-interaction parameters (“r(AMBER)” and “epsilon (AMBER)”) for “9O” into *frcmod.ff14SB* as shown in Fig. P.

N3-CX-2C-S	1	0.323	0.0	-3.
N3-CX-2C-S	1	0.021	180.0	-2.
N3-CX-2C-S	1	0.469	0.0	1.
CX-2C-S-S	1	0.135	180.0	-4.
CX-2C-S-S	1	0.302	0.0	-3.
CX-2C-S-S	1	0.666	0.0	-2.
CX-2C-S-S	1	0.056	0.0	1.
2C-S-S-2C	1	0.379	0.0	-4.
2C-S-S-2C	1	0.682	0.0	-3.
2C-S-S-2C	1	4.480	0.0	-2.
2C-S-S-2C	1	0.420	0.0	1.
IMPR				
X-O2-C0-O2		10.5	180.	2.
2C-O-C-OH		10.5	180.	2.
CA-CA-CA-2C		1.1	180.	2.
NONB				
2C	1.9080	0.1094		Spellmeyer
3C	1.9080	0.1094		Spellmeyer
CB	1.9080	0.1094		Spellmeyer
CO	1.9080	0.0860		OPLS
9O	1.6612	0.1617		PCCP, 2017, 19, 20593

Fig. P: modified *frcmod.ff14SB*

- Now for the second (and shorter) part of this tutorial: modifying the LJ interactions between “9O” and Na⁺:
- We will start by examining the original ion parameters (from JPCB 2008, 112, 9020-9041) typically used with TIP3P water. Open file Tutorial/*frcmod.ionsjc_tip3p*. The values under the NONBON section are $R_{\min,i,i}/2$ (in Å) and $\epsilon_{i,i}$ (in kcal/mol). This file is loaded in “tleap” following the indications in *leaprc.water.tip3p*; to confirm this information, open *leaprc.water.tip3p* and search for the text string “frcmod.ionsjc_tip3p”.

```

Rb+ 85.47 rubidium
Cs+ 132.91 cesium
F- 19.00 0.320 fluorine
Cl- 35.45 1.910 chlorine (Applequist)
Br- 79.90 2.880 bromine (Applequist)
I- 126.9 4.690 iodine (Applequist)

NONBON
Li+ 1.025 0.0279896
Na+ 1.369 0.0874393
K+ 1.705 0.1936829
Rb+ 1.813 0.3278219
Cs+ 1.976 0.4065394
F- 2.303 0.0033640
Cl- 2.513 0.0355910
Br- 2.608 0.0586554
I- 2.860 0.0536816

```

Fig. Q: original *frcmod.ionsjc_tip3p*

- Entries in the “LJEDIT” section in *frcmmod.ff14SB* have the following (unexpected!) format:
 - atom_type_A atom_type_B d_A E_A d_B E_B ; “d” in angstrom; E in kcal/mol
 - these parameters are *exclusively* used to define the interactions between atom type A and atom type B as

$$R_{\min,A,B} = d_A + d_B \qquad \epsilon_{A,B} = \sqrt{E_A E_B}$$

- Notice that *any* combination of d_A and d_B, and of E_A and E_B, that yields the correct values R_{min,A,B} and ε_{A,B} is allowed. d_A,E_A and e_B,E_B are, by themselves, meaningless; only R_{min,A,B} and ε_{A,B} have meaning.
- In the “LJEDIT” section of *frcmmod.ff14SB*, add the new parameters for the “9O”...”Na+” interaction (red box, Fig. R).
- Notice that you could have also written:


```
9O Na+ 1.54845 0.1189 1.54845 0.1189
```

 or


```
9O Na+ 0 1 3.0969 0.01414
```

 where 0.01414=0.1189*0.1189
- Save and close *frcmmod.ff14SB* when you are done.

```

2C-0 -C -OH      10.5      180.      2.
CA-CA-CA-2C     1.1       180.      2.

NONB
 2C      1.9080  0.1094      Spellmeyer
 3C      1.9080  0.1094      Spellmeyer
 C8      1.9080  0.1094      Spellmeyer
 C0      1.9080  0.0860      OPLS
 9O      1.6612  0.1617      PCCP, 2017, 19, 20593

LJEDIT
 9O Na+      3.0969  0.1189  0  0.1189
  
```

Fig. R

- The final step is to update the paths to your modified parameter and topology files so they are loaded by “tleap”.
 - Open `Tutorial/leaprc.protein.ff14SB` and update the paths of `parm10.dat`, `frmod.ff14SB`, `amino12.lib`, `aminoct12.lib` and `aminont12.lib` to your `Tutorial/` directory, as exemplified in Fig. S. We did not modify `parm10.dat` but it is convenient to call it also from `Tutorial/` rather than from `$AMBERHOME`. Save and close `leaprc.protein.ff14SB` when you are done.
 - Create a new file (we called it `ff14_tleap_-f_ThisName.in`; Fig T) and add the following command:
 - `source /your/path/to/Tutorial/leaprc.protein.ff14SB`
 - if you had made modifications in any of the water files, you could also add the command:
 - `source /your/path/to/Tutorial/leaprc.water.tip3p`
 - save and close `ff14_tleap_-f_ThisName.in`.
 - To build topology (`.prmtop`) and coordination (`.inpcrd`) files using the new parameters, open “tleap” with the following command:


```
$ tleap -f "YOUR DIRECTORY"/ ff14_tleap_-f_ThisName.in
```

```

{ "SH" "S" "sp3" }
{ "P" "P" "sp3" }
{ "LP" "" "sp3" }
{ "EP" "" "sp3" }
{ "F" "F" "sp3" }
{ "Cl" "Cl" "sp3" }
{ "Br" "Br" "sp3" }
{ "I" "I" "sp3" }
}
#
# Load the main parameter set.
parm10 = loadamberparams /usr/data/bgfs1/geraili/Simulation_area/Tutorial/parm10.dat
frmod14SB = loadamberparams /usr/data/bgfs1/geraili/Simulation_area/Tutorial/frmod.ff14SB
#
# Load main chain and terminating amino acid libraries
#
loadOff /usr/data/bgfs1/geraili/Simulation_area/Tutorial/amino12.lib
loadOff /usr/data/bgfs1/geraili/Simulation_area/Tutorial/aminoct12.lib
loadOff /usr/data/bgfs1/geraili/Simulation_area/Tutorial/aminont12.lib

```

Fig. S: updated `leaprc.protein.ff14SB`

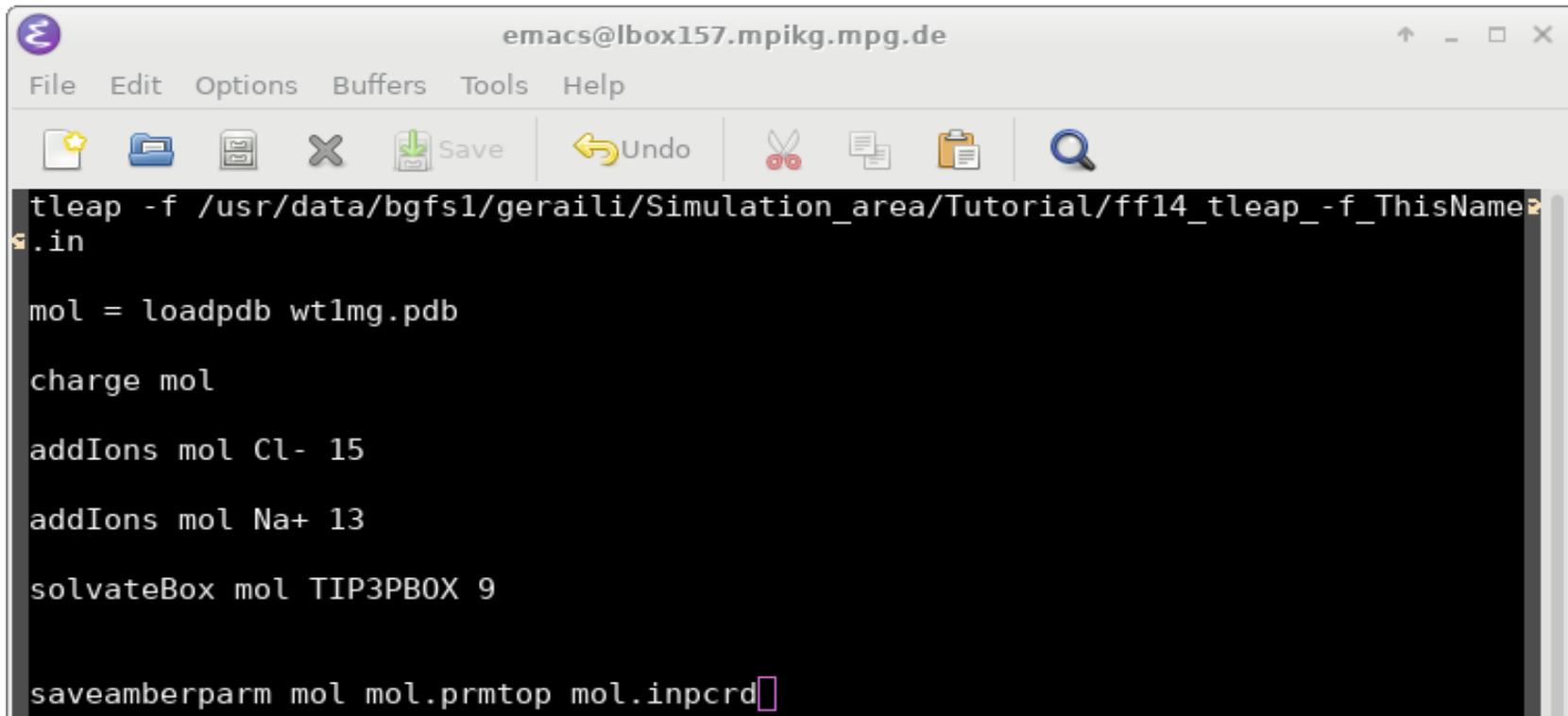
```

# sourcing the Amber protein ff14Sb and water tip3p
source /usr/data/bgfs1/geraili/Simulation_area/Tutorial/leaprc.protein.ff14SB
source /usr/data/bgfs1/geraili/Simulation_area/Tutorial/leaprc.water.tip3p

```

Fig. T: new file `ff14_tleap_-f_ThisName.in`

- It is always useful to verify if you've made the correct the modifications. To do so, download *wt1mg.pdb* from “ambermd.org” (<http://ambermd.org/tutorials/advanced/tutorial8/files/wt1mg.pdb>). This is a protein that has already been prepared for simulation. Put the pdb file in a “new_folder”.
- Go to “new_folder”, open a terminal there, and type all the commands in Fig. U (use your own path to Tutorial/, where you've saved all the modified topology and parameter files). After all the commands are executed, type “quit”.



The image shows a terminal window within the Emacs editor. The window title is "emacs@lbox157.mpikg.mpg.de". The menu bar includes "File", "Edit", "Options", "Buffers", "Tools", and "Help". The toolbar contains icons for file operations like "Save", "Undo", "Cut", "Copy", and "Paste". The terminal content shows the following commands:

```
tLeap -f /usr/data/bgfs1/geraili/Simulation_area/Tutorial/ff14_tLeap_-f_ThisName
.in

mol = loadpdb wt1mg.pdb

charge mol

addIons mol Cl- 15

addIons mol Na+ 13

solvateBox mol TIP3PBOX 9

saveamberparm mol mol.prmtop mol.inpcrd
```

Fig. U

- Now we will use “Parmed” (<https://parmed.github.io/ParmEd/html/index.html>) to inspect the “mol.prmtop” topology file. Type the following commands in the terminal

```
$ parmed -p mol.prmtop
$ printLJMatrix :ASP
$ printLJMatrix :Na+
```

– The last column shows $\epsilon_{i,j}$ in kcal/mol; the second-from-last column shows $R_{\min,i,j}$ in angstrom.

- Take a look at the self-interaction parameters involving “90” (Fig. V, red box) and at the interaction parameters between “90” and “Na+” (Fig. V, green box). Compare them with those in file acetate.top, and with the parameters for “O2”. Make sure there are no mistakes.

```
Terminal - geraili@lbox157:/usr/people/home/geraili/Documents/Tutorial/new/Tutorial
File Edit View Terminal Tabs Help
HC [11] OW [19] 0.000000 0.000000 0.000000 0.000000
HC [11] HW [20] 0.000000 0.000000 0.000000 0.000000
N, N2, N3, NA, NB [1] 90 [14] 532490.587000 594.258905 3.485200 0.165798
H, HS [2] 90 [14] 900.269543 13.470034 2.261200 0.050385
2C, 3C, C8, CT, CX [3] 90 [14] 568478.812000 549.944639 3.569200 0.133004
HP [4] 90 [14] 9896.444940 44.660339 2.761200 0.050385
H1 [5] 90 [14] 32420.995100 80.834282 3.048200 0.050385
S, SH [6] 90 [14] 1166289.750000 968.491319 3.661200 0.201060
C, C*, CA, CB, CC, CN, CO, CR, CV, CW [7] 90 [14] 504028.214000 487.595330 3.569200 0.117925
O [8] 90 [14] 333340.187000 495.685439 3.322400 0.184274
OH [9] 90 [14] 413303.707000 552.209132 3.382200 0.184450
HO [10] 90 [14] 0.000000 0.000000 0.000000 0.000000
HC [11] 90 [14] 47758.712600 98.108971 3.148200 0.050385
H4 [12] 90 [14] 34546.229000 82.495572 3.070200 0.049249
HA [13] 90 [14] 41036.272000 90.891910 3.120200 0.049249
90 [14] 90 [14] 292504.827000 434.962208 3.322400 0.161700
90 [14] H5 [15] 28368.023500 74.755793 3.020200 0.049249
90 [14] Mg2+ [16] 23488.714900 61.774949 3.021200 0.040617
90 [14] Cl- [17] 212275.910000 802.591625 4.174200 0.075862
90 [14] Na+ [18] 92535.428300 209.785247 3.096900 0.118900
90 [14] OW [19] 414998.405000 510.142660 3.429500 0.156775
90 [14] HW [20] 0.000000 0.000000 0.000000 0.000000
> printLJMatrix :Na+
Atom Type 1 Atom Type 2 A coefficient B coefficient R i,j Eps i,j
-----
N, N2, N3, NA, NB [1] Na+ [18] 136919.347000 258.405233 3.193000 0.121921
H, HS [2] Na+ [18] 125.820601 4.318247 1.969000 0.037051
2C, 3C, C8, CT, CX [3] Na+ [18] 149995.867000 242.242668 3.277000 0.097805
HP [4] Na+ [18] 1901.324490 16.786482 2.469000 0.037051
H1 [5] Na+ [18] 7114.652960 32.471955 2.756000 0.037051
S, SH [6] Na+ [18] 316107.989000 432.373832 3.369000 0.147851
C, C*, CA, CB, CC, CN, CO, CR, CV, CW [7] Na+ [18] 132990.267000 214.778698 3.277000 0.086717
O [8] Na+ [18] 81211.682800 209.807371 3.030200 0.135507
OH [9] Na+ [18] 102772.745000 236.133213 3.090000 0.135636
HO [10] Na+ [18] 0.000000 0.000000 0.000000 0.000000
HC [11] Na+ [18] 10911.930700 40.214472 2.856000 0.037051
H4 [12] Na+ [18] 7650.430290 33.290660 2.778000 0.036216
HA [13] Na+ [18] 9476.584230 37.051466 2.828000 0.036216
90 [14] Na+ [18] 92535.428300 209.785247 3.096900 0.118900
H5 [15] Na+ [18] 6152.212920 29.853493 2.728000 0.036216
Mg2+ [16] Na+ [18] 5096.198070 24.674882 2.729000 0.029868
Cl- [17] Na+ [18] 653416.059000 381.844565 3.882000 0.055786
Na+ [18] Na+ [18] 15520.581800 73.677916 2.738000 0.087439
Na+ [18] OW [19] 104820.861000 219.857570 3.137300 0.115286
Na+ [18] HW [20] 0.000000 0.000000 0.000000 0.000000
```

Fig. V

Well done; you're good to go!