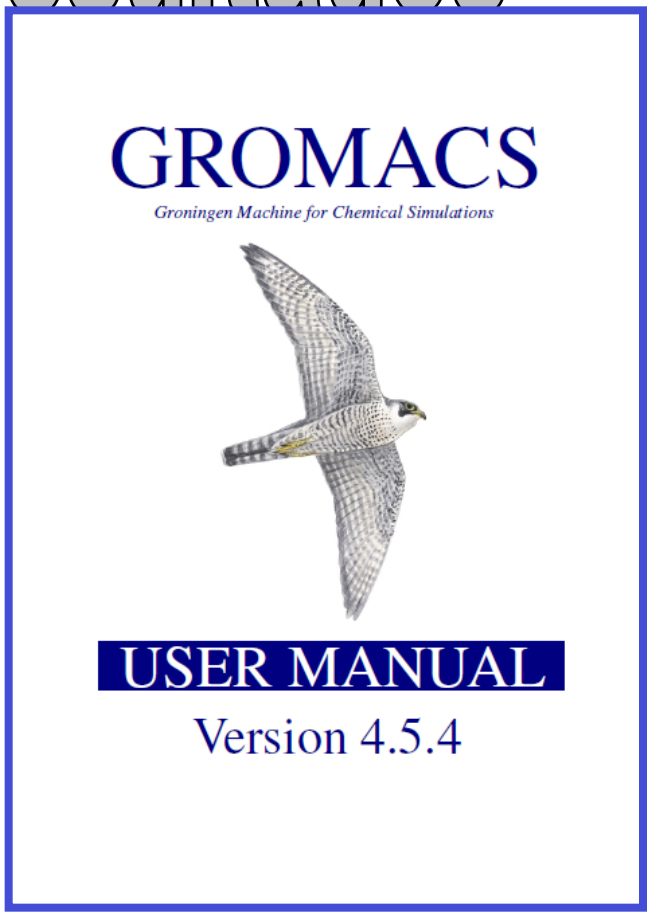
Simulações com o GROMACS e análise dos resultados



Estrutura do GROMACS:

1) Aplicabilidade

Realiza simulações de sistemas moleculares com método Dinâmica Molecular utilizando modelo de moléculas flexíveis.

2) Arquivos de entrada:

Para executar o Gromacs com o comando `mdrun` é necessário ter um arquivo binário com terminação tpr (`*.tpr`). Esse arquivo `*.tpr` só pode ser gerado pelo comando `grompp` que necessita de 3 arquivos de entrada:

`*.mdp` (contem as informações da simulação)

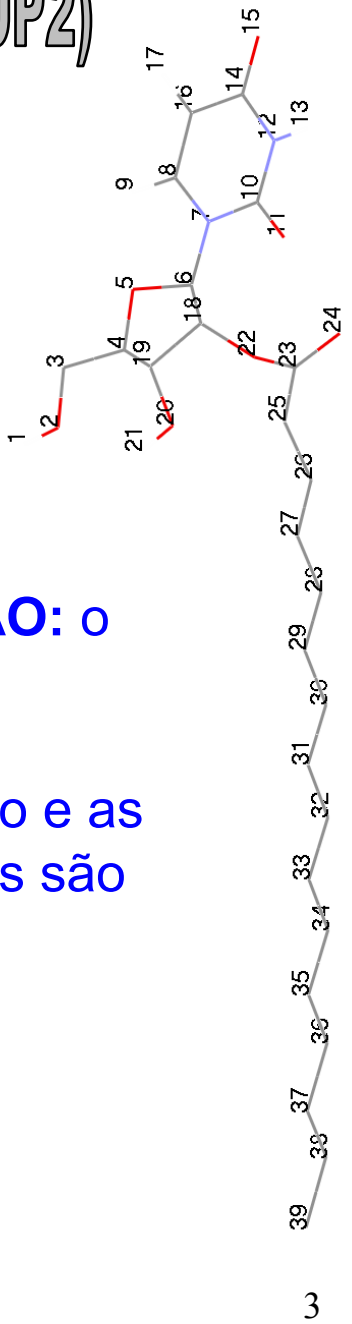
`*.gro` (contem numeração, coordenadas cartesianas e velocidades dos átomos do sistema)

`*.top` (contem informações da topologia das moléculas e campo de força)

Os arquivos `*.gro` e `*.top` são arquivos texto não formatados que podem ser gerados de várias formas. Uma dessas formas é através do comando `pdb2gmx` que usa um arquivo no formato pdb e as informações do campo de força. Neste tutorial vamos usar o campo de força gromos 53a6 que vem está no diretório `gromacs/share/gromacs/top/gromos53a6.ff` [J. Comp. Chem. 25 (2004) 1656].

Exemplo de aquivo PDF: molécula Uradinapalmitoil (UP2)

```
TITLE     Protein
REMARK    THIS IS A SIMULATION BOX
CRYST1   40.000   40.000   40.000  90.00  90.00  90.00 P 1           1
MODEL        1
ATOM      1  H5* UP2     1       8.937   1.721  19.728  1.00  0.00
ATOM      2  O5* UP2     1       8.057   1.314  19.995  1.00  0.00
ATOM      3  C5* UP2     1       7.699   1.255  21.392  1.00  0.00
ATOM      4  C4* UP2     1       6.619   0.207  21.669  1.00  0.00
ATOM      5  O4* UP2     1       6.239   0.093  23.047  1.00  0.00
ATOM      6  C1* UP2     1       5.096  -0.763  22.947  1.00  0.00
ATOM      7  N1  UP2     1       4.342  -0.799  24.230  1.00  0.00
ATOM      8  C6  UP2     1       4.054   0.378  24.934  1.00  0.00
ATOM      9  H6  UP2     1       4.417   1.333  24.561  1.00  0.00
ATOM     10  C2  UP2     1       3.940  -2.032  24.772  1.00  0.00
ATOM     11  O2  UP2     1       4.172  -3.076  24.161  1.00  0.00
ATOM     12  N3  UP2     1       3.255  -2.072  25.994  1.00  0.00
ATOM     13  H3  UP2     1       2.987  -2.951  26.381  1.00  0.00
ATOM     14  C4  UP2     1       2.968  -0.876  26.687  1.00  0.00
ATOM     15  O4  UP2     1       2.349  -0.903  27.746  1.00  0.00
ATOM     16  C5  UP2     1       3.371   0.343  26.148  1.00  0.00
ATOM     17  H5  UP2     1       3.156   1.266  26.690  1.00  0.00
ATOM     18  C2* UP2     1       4.277  -0.229  21.774  1.00  0.00
ATOM     19  C3* UP2     1       5.312   0.518  20.948  1.00  0.00
ATOM     20  O3* UP2     1       5.280   0.012  19.596  1.00  0.00
ATOM     21  H3* UP2     1       6.083   0.366  19.099  1.00  0.00
ATOM     22  O2* UP2     1       3.713  -1.293  20.962  1.00  0.00
ATOM     23  C21 UP2     1       2.476  -1.316  20.445  1.00  0.00
ATOM     24  O22 UP2     1       1.584  -1.901  21.055  1.00  0.00
ATOM     25  C22 UP2     1       2.256  -0.626  19.148  1.00  0.00
ATOM     26  C23 UP2     1       2.463  -1.530  17.929  1.00  0.00
ATOM     27  C24 UP2     1       2.249  -0.721  16.643  1.00  0.00
ATOM     28  C25 UP2     1       2.489  -1.571  15.394  1.00  0.00
ATOM     29  C26 UP2     1       2.308  -0.714  14.140  1.00  0.00
ATOM     30  C27 UP2     1       2.576  -1.521  12.874  1.00  0.00
ATOM     31  C28 UP2     1       2.415  -0.627  11.639  1.00  0.00
ATOM     32  C29 UP2     1       2.691  -1.398  10.348  1.00  0.00
ATOM     33 C210 UP2     1       2.530  -0.479   9.139  1.00  0.00
ATOM     34 C211 UP2     1       2.818  -1.233   7.836  1.00  0.00
ATOM     35 C212 UP2     1       2.665  -0.309   6.640  1.00  0.00
ATOM     36 C213 UP2     1       2.955  -1.051   5.332  1.00  0.00
ATOM     37 C214 UP2     1       2.803  -0.113   4.131  1.00  0.00
ATOM     38 C215 UP2     1       3.087  -0.849   2.817  1.00  0.00
ATOM     39 C216 UP2     1       2.941   0.083   1.622  1.00  0.00
TER
ENDMDL
```



ATENÇÃO: o arquivo *.pdb é formatado e as distâncias são em Å.

Formato do arquivo PDB dentro do bloco MODEL ... ENDMDL:

Record Format

COLUMNS	DATA TYPE	FIELD	DEFINITION
1 - 6	Record name	"ATOM "	
7 - 11	Integer	serial	Atom serial number.
13 - 16	Atom	name	Atom name.
17	Character	altLoc	Alternate location indicator.
18 - 20	Residue name	resName	Residue name.
22	Character	chainID	Chain identifier.
23 - 26	Integer	resSeq	Residue sequence number.
27	AChar	iCode	Code for insertion of residues.
31 - 38	Real(8.3)	x	Orthogonal coordinates for X in Angstroms.
39 - 46	Real(8.3)	y	Orthogonal coordinates for Y in Angstroms.
47 - 54	Real(8.3)	z	Orthogonal coordinates for Z in Angstroms.
55 - 60	Real(6.2)	occupancy	Occupancy.
61 - 66	Real(6.2)	tempFactor	Temperature factor.
77 - 78	LString(2)	element	Element symbol, right-justified.
79 - 80	LString(2)	charge	Charge on the atom.

```
          1         2         3         4         5         6         7         8
12345678901234567890123456789012345678901234567890123456789012345678901234567890
MODEL        1
ATOM      1  N   ALA A   1      11.104   6.134  -6.504  1.00  0.00           N
ATOM      2  CA  ALA A   1      11.639   6.071  -5.147  1.00  0.00           C
…
…
…
ATOM    293 1HG  GLU A  18     -14.861  -4.847   0.361  1.00  0.00           H
ATOM    294 2HG  GLU A  18     -13.518  -3.769   0.084  1.00  0.00           H
TER     295      GLU A  18
ENDMDL
```

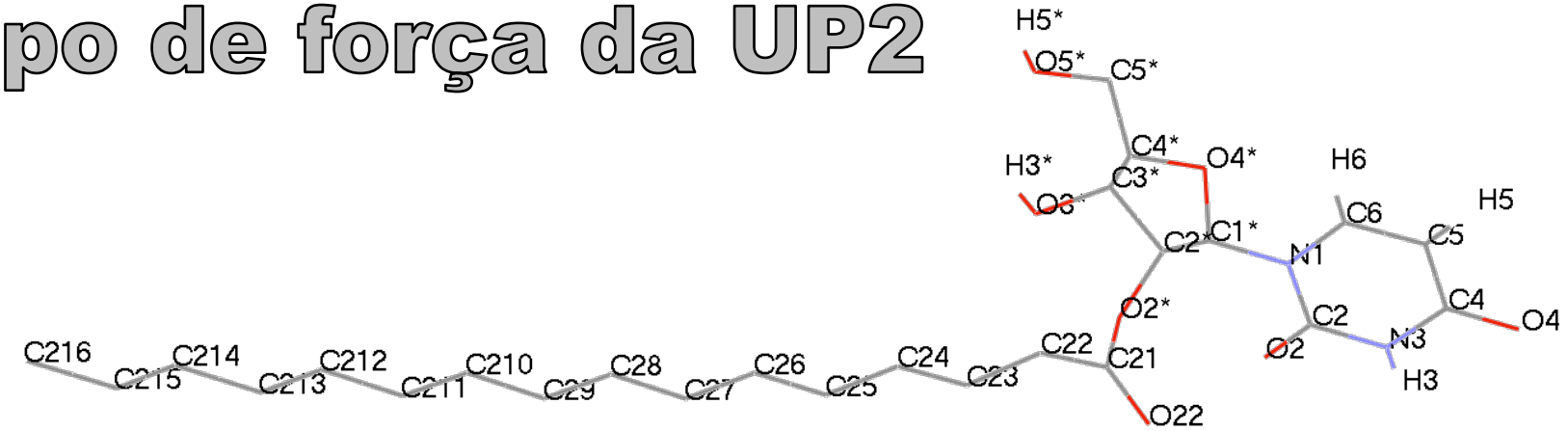
1) Como gerar o arquivo `*.gro` e `*.top` a partir do PDB

Copie para o seu diretório de trabalho o diretório do campo de força.
Exemplo: `gromacs/share/gromacs/top/gromos53a6.ff`

Neste doretório existem algums arquivo importante que precisaremos paradefinir a topologia da molécula que vamos simular:

- `atomtypes.atp`: neste arquivo estão definidos os tipos dos átomos que estão parametrizados nos campos de força. Se exitir algum átomo na sua molécula que não esteja definido neste arquivo, então é necessário editá-lo e também o arquivo ffnonbonded.itp para colocar os valores de C6 e C12 dos átomos novos no bloco `[ atomtypes ]` e das combinações com outros átomos no bloco `[ nonbond_params ]`.
- aminoacids.atp: neste arquivos você precisa editar e construir a topologia da sua molécula que deve ter um rótulo de 3 letras, já utilizado 4a. coluna do PDF (exemplo: UP2). Este arquivo já tem a topologia dos aminiácidos e ácidos nucleicos e alguma terminações de aminoácidos. Ele é composto de 6 blocos para cada molecula `[ atoms ]`, `[ bonds ]`, `[ exclusions ]`, `[ angles ]`, `[ impropers ]` e `[ dihedrals ]`.

Campo de força da UP2

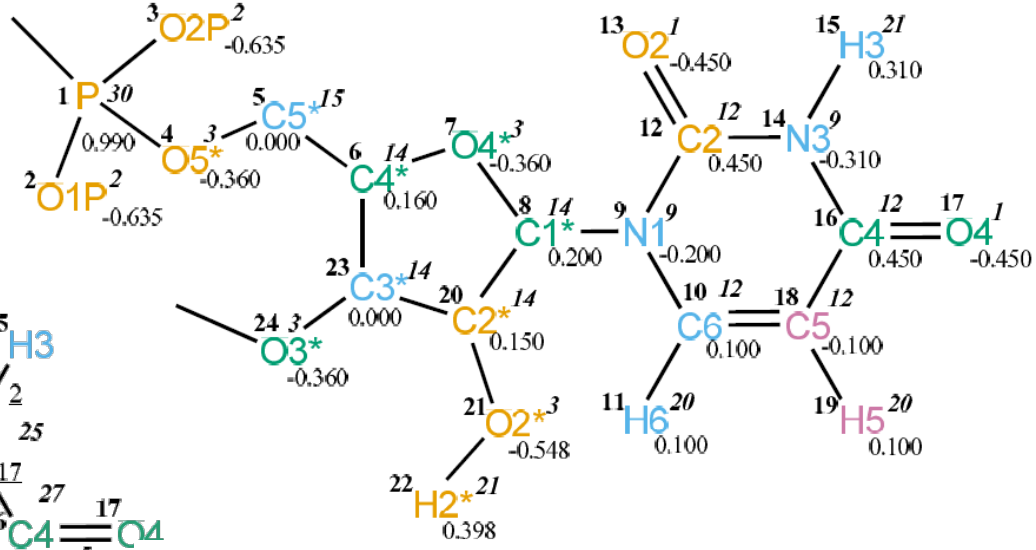


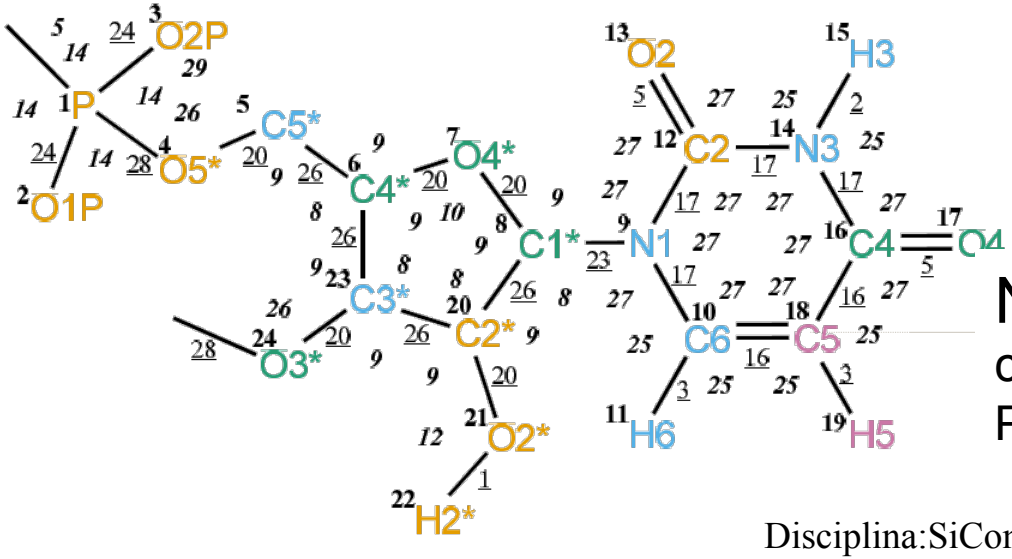
The GROMOS Software for (Bio)Molecular Simulation, vol. 3, pp.384

Bonded prameter for URA

bond 24 (gb_24) entre átomos 1 e 2

angle *14* (ga_14) entre átomos 2 1 4





Non-bonded prameter for URA

charge 0.990 no átomo 1

P tipo 30

FIGURE 4.182. URA bonded parameters.

Incluir o [UP2] no arquivo gromos53a6.ff/aminoacids.rtp

```
[ UP2 ]
 [ atoms ]
;  a    type     charge cgroup GROMOS pp.3-32 and pp.3-384
  H5*      H     0.36000      0
  O5*     OA     0.36000      0
  C5*    CH2     0.00000      1
  C4*    CH1     0.16000      2
  O4*     OA    -0.36000      2
  C1*    CH1     0.20000      2
   N1     NR    -0.20000      3
   C6      C     0.10000      3
   H6     HC     0.10000      3
   C2      C     0.45000      4
   O2      O    -0.45000      4
   N3     NR    -0.31000      5
   H3      H     0.31000      5
   C4      C     0.45000      6
   O4      O    -0.45000      6
   C5      C    -0.10000      7
   H5     HC     0.10000      7
  C2*    CH1     0.15000      8
  C3*    CH1     0.16000      9
  O3*     OA    -0.54800      8
  H3*      H     0.39800      8
;  a    type    charge  cgroup GROMOS pp.3-32 and pp.3-471
  O2*     OA    -0.36000      9
  C21    CH0     0.58000      9
  O22      O    -0.38000      9
  C22    CH2     0.00000     10
  C23    CH2     0.00000     10
  C24    CH2     0.00000     11
  C25    CH2     0.00000     11
  C26    CH2     0.00000     12
  C27    CH2     0.00000     12
  C28    CH2     0.00000     13
  C29    CH2     0.00000     13
 C210    CH2     0.00000     14
 C211    CH2     0.00000     14
 C212    CH2     0.00000     15
 C213    CH2     0.00000     15
 C214    CH2     0.00000     16
 C215    CH2     0.00000     16
 C216    CH3     0.00000     17
```

Rótulos dos átomos no arquivo PDF

Rótulos dos átomos no campo de força listados no arquivo gromos53a6.ff/ atomtypes.atp

```
 [ bonds ]
;  ai    aj  gromos type
  H5*   O5*    gb_1
  O5*   C5*    gb_20
  C5*   C4*    gb_26
  C4*   O4*    gb_20
  C4*   C3*    gb_26
  O4*   C1*    gb_20
  C1*    N1    gb_23
  C1*   C2*    gb_26
   N1    C6    gb_17
   N1    C2    gb_17
   C6    H6    gb_3
   C6    C5    gb_16
   C2    O2    gb_5
   C2    N3    gb_17
   N3    H3    gb_2
   N3    C4    gb_17
   C4    O4    gb_5
   C4    C5    gb_16
   C5    H5    gb_3
  C2*   O2*    gb_20
  C2*   C3*    gb_26
  O3*   H3*    gb_1
  C3*   O3*    gb_20
;  ai    aj  gromos type
  O2*   C21    gb_10
  C21   O22    gb_5
  C21   C22    gb_23
  C22   C23    gb_27
  C23   C24    gb_27
  C24   C25    gb_27
  C25   C26    gb_27
  C26   C27    gb_27
  C27   C28    gb_27
  C28   C29    gb_27
  C29  C210    gb_27
 C210  C211    gb_27
 C211  C212    gb_27
 C212  C213    gb_27
 C213  C214    gb_27
 C214  C215    gb_27
 C215  C216    gb_27
```

```
[ exclusions ]
;  ai    aj
  C1*    H6
  C1*    O2
  C1*    N3
  C1*    C5
   N1    H3
   N1    C4
   N1    H5
   C6    O2
   C6    N3
   C6    O4
   H6    C2
   H6    C4
   H6    H5
   C2    O4
   C2    C5
   O2    H3
   O2    C4
   N3    H5
   H3    O4
   H3    C5
   O4    H5
   H5   -O3*
  O2*   O3*
  H3*   O2*
```

Rótulos dos parâmetros de ligações no campo de força listados no arquivo gromos53a6.ff/ ffbonded.itp

```
[ angles ]
;  ai    aj    ak    gromos type
   H5*   O5*   C5*      ga_26
   O5*   C5*   C4*      ga_9
   C5*   C4*   O4*      ga_9
   C5*   C4*   C3*      ga_8
   O4*   C4*   C3*      ga_9
   C4*   O4*   C1*      ga_10
   O4*   C1*    N1      ga_9
   O4*   C1*   C2*      ga_9
    N1   C1*   C2*      ga_8
   C1*    N1    C6      ga_27
   C1*    N1    C2      ga_27
    C6    N1    C2      ga_27
    N1    C6    H6      ga_25
    N1    C6    C5      ga_27
    H6    C6    C5      ga_25
    N1    C2    O2      ga_27
    N1    C2    N3      ga_27
    O2    C2    N3      ga_27
    C2    N3    H3      ga_25
    C2    N3    C4      ga_27
    H3    N3    C4      ga_25
    N3    C4    O4      ga_27
    N3    C4    C5      ga_27
    O4    C4    C5      ga_27
    C6    C5    C4      ga_27
    C6    C5    H5      ga_25
    C4    C5    H5      ga_25
   C1*   C2*   O2*      ga_9
   C1*   C2*   C3*      ga_8
   O2*   C2*   C3*      ga_9
   C3*   O3*   H3*      ga_12
   C4*   C3*   C2*      ga_8
   C4*   C3*   O3*      ga_9
   C2*   C3*   O3*      ga_9
; C3*   O3*    +P      ga_26
;  ai    aj    ak    gromos type
;  C4*   C3*   O3*      ga_13
   O3*   C3*   C2*      ga_13
   C2*   O2*   C21      ga_22
   O2*   C21   O22      ga_31
   O2*   C21   C22      ga_16
   O22   C21   C22      ga_35
   C21   C22   C23      ga_15
   C22   C23   C24      ga_15
   C23   C24   C25      ga_15
   C24   C25   C26      ga_15
```

```
 [ impropers ]
;  ai    aj    ak    al    gromos type
    N1    C6    C2   C1*      gi_1
    N1    C6    C5    C4      gi_1
    N1    C2    N3    C4      gi_1
    C6    N1    C2    N3      gi_1
    C6    N1    C5    H6      gi_1
    C2    N1    C6    C5      gi_1
    C2    N3    C4    C5      gi_1
    O2    N1    N3    C2      gi_1
    N3    C4    C5    C6      gi_1
    H3    C2    C4    N3      gi_1
    O4    N3    C5    C4      gi_1
    C5    C6    C4    H5      gi_1
   C2*   O4*    N1   C1*      gi_2
   C2*   O2*   C3*   C1*      gi_2
   C3*   C5*   O4*   C4*      gi_2
   C3*   C2*   O3*   C4*      gi_2
;  ai    aj    ak    al    gromos type
   C21   O2*   C22   O22      gi_1
```

```
 [ dihedrals ]
;  ai    aj    ak    al    gromos type
   H5*   O5*   C5*   C4*      gd_7
   O5*   C5*   C4*   O4*      gd_8
   O5*   C5*   C4*   O4*      gd_25
   O5*   C5*   C4*   C3*      gd_17
   O5*   C5*   C4*   C3*      gd_34
   C3*   C4*   O4*   C1*      gd_29
   C5*   C4*   C3*   C2*      gd_34
   C5*   C4*   C3*   O3*      gd_17
   O4*   C4*   C3*   C2*      gd_17
   O4*   C4*   C3*   O3*      gd_18
   C4*   O4*   C1*   C2*      gd_29
   O4*   C1*    N1    C2      gd_90
   O4*   C1*    N1    C2      gd_91
   O4*   C1*   C2*   O2*      gd_18
   O4*   C1*   C2*   C3*      gd_17
   O4*   C1*   C2*   C3*      gd_34
    N1   C1*   C2*   O2*      gd_17
   C1*   C2*   O2*   C21      gd_23
   C1*   C2*   C3*   C4*      gd_34
   C1*   C2*   C3*   O3*      gd_17
   O2*   C2*   C3*   C4*      gd_17
   O2*   C2*   C3*   O3*      gd_18
   C4*   C3*   O3*   H3*      gd_29
;  ai    aj    ak    al    gromos type
   C2*   O2*   C21   C22      gd_13
   O2*   C21   C22   C23      gd_40
   C21   C22   C23   C24      gd_34
   C22   C23   C24   C25      gd_34
   C23   C24   C25   C26      gd_34
   C24   C25   C26   C27      gd_34
   C25   C26   C27   C28      gd_34
   C26   C27   C28   C29      gd_34
   C27   C28   C29  C210      gd_34
   C28   C29  C210  C211      gd_34
   C29  C210  C211  C212      gd_34
  C210  C211  C212  C213      gd_34
  C211  C212  C213  C214      gd_34
  C212  C213  C214  C215      gd_34
  C213  C214  C215  C216      gd_34
```

Incluído no gromos53a6.ff/ffbonded.itp

```
;ANGULOS ADICIONAIS PARA UP
#define gd_90    8.7500        1.0          1
; -CHn-NT-       0.9
;
#define gd_91    5.0300        1.0          2
; -CHn-NT-       0.9
```

min.mdp

```
integrator              = steep
tinit                   = 0.0
dt                      = 0.001
nsteps                  = 25000
emtol                   = 1
nstcomm                 = 5
comm-grps               = UP2
nstxout                 = 10
nstvout                 = 10
nstfout                 = 0
nstlog                  = 10
nstenergy               = 10
nstxtcout               = 10
xtc_precision           = 10
xtc-grps                =
energygrps              = UP2
nstlist                 = 5
ns_type                 = grid
pbc                     = xyz
rlist                   = 1.4
coulombtype             = reaction-field
rcoulomb                = 1.4
epsilon_rf              = 1.5
vdw_type                = cut-off
rvdw                    = 1.4
DispCorr                = No
constraints             = none
```

nvt.mdp

```
integrator              = md
tinit                   = 0.0
dt                      = 0.001
nsteps                  = 10000000
emtol                   = 1
comm-mode               = Angular
nstcomm                 = 5
comm-grps               = UP2
nstxout                 = 1000
nstvout                 = 1000
nstfout                 = 0
nstlog                  = 1000
nstenergy               = 1000
nstxtcout               = 1000
xtc_precision           = 1000
xtc-grps                =
energygrps              = UP2
nstlist                 = 5
ns_type                 = grid
pbc                     = xyz
rlist                   = 1.4
coulombtype             = reaction-field
rcoulomb                = 1.4
epsilon_rf              = 1.5
vdw_type                = cut-off
rvdw                    = 1.4
DispCorr                = No
tcoupl                  = v-rescale
tc-grps                 = UP2
tau_t                   = 0.1
ref_t                   = 300
constraints             = none
```

O comando comm-mode = Angular, retira a rotação do centro de massa para evitar que todo o movimento cinético seja transferido para a rotação do centro de massa. Esse comando gera um *warning* e deve ser usado apenas para a dinâmica de uma molécula em vácuo com a utilização da opção de -maxwarn 1 (veja na linha de comando do grompp)

Minimização e simulação em vácuo:

3) Execução:

```
> mkdir simula
> cp -r /usr/local/gromacs/share/gromcas/top/gromos53a6.ff * ./simula/.
> ls
gromos53a6.ff   min.mdp   nvt.mdp   up2.pdb
```

No diretório gromos53a6.ff lembre de modificar os arquivos: `aminoacids.rtp` e `ffbonded.itp`

Gerar o up2.top e up2.gro:

```
> pdb2gmx -f up2.pdb -o up2.gro -p up2.top -ff gromos53a6 -water none
```

Centralizar a molécula no meio da caixa de simulação de 4x4x4nm:

```
> editconf -c -f up2.gro -o up2.gro -box 4 4 4
```

Gerar o arquivo binário up2_vac_min.tpr para minimização:

```
> grompp -f min.mdp -c up2.gro -p up2.top -o up2_vac_min.tpr -pp top_all.top
```

Executar a minimização e gerar o arquivo up2_vac_min-run.gro:

```
> mdrun -s  up2_vac_min.tpr -deffnm  up2_vac_min-run -v >&  up2_vac_min.out
> editconf -f up2_vac_min-run.gro -o up2_vac_min-run.pdb
```

(transforma em PDB caso não tenha o VMD para visualizar o GRO)

Gerar o arquivo binário up2_nvt.tpr para a simulação:

```
> grompp -f nvt.mdp -c up2_vac_min-run.gro -p up2.top -o up2_nvt.tpr -maxwarn 1
```

Executar a simulação no vácuo:

```
> mdrun -s  up2 nvt.tpr -deffnm  up2 nvt-run -v >&  up2 nvt.out
```

Outras opções dos comandos: `pdb2gmx`, `editconf`, `grompp` e `mdrun` podem ser visualizadas com a opção `-h`.

4) Adição de Hs da cadeia carbônica na última configuração da minimização ou simulação (no arquivo `*.gro` de saída):

Copiar o campo de força `gmx.ff` para o diretório ./simula e modificar os arquivos no diretório `./simula/gmx.ff` para incluir o parâmetros dos Hs do soluto no campo de força gmx:

- `aminoacids.rtp` (inclui as informações dos Hs no [UP2])
- `aminoacids.hdb` (inclui as informações dos átomos nos quais os Hs estão ligados)

Usar o arquivo de saída da minimização: `up2_vac_min-run.gro` para gerar o arquivo `up2_vac_min-run+h.pdb` com os Hs:

```
> pdb2gmx -f up2_vac_min-run.gro -o up2_vac_min-run+h.pdb -ff gmx -water
none -p up2_vac_min-run+h.top -i up2_vac_min-run+h.itp
```

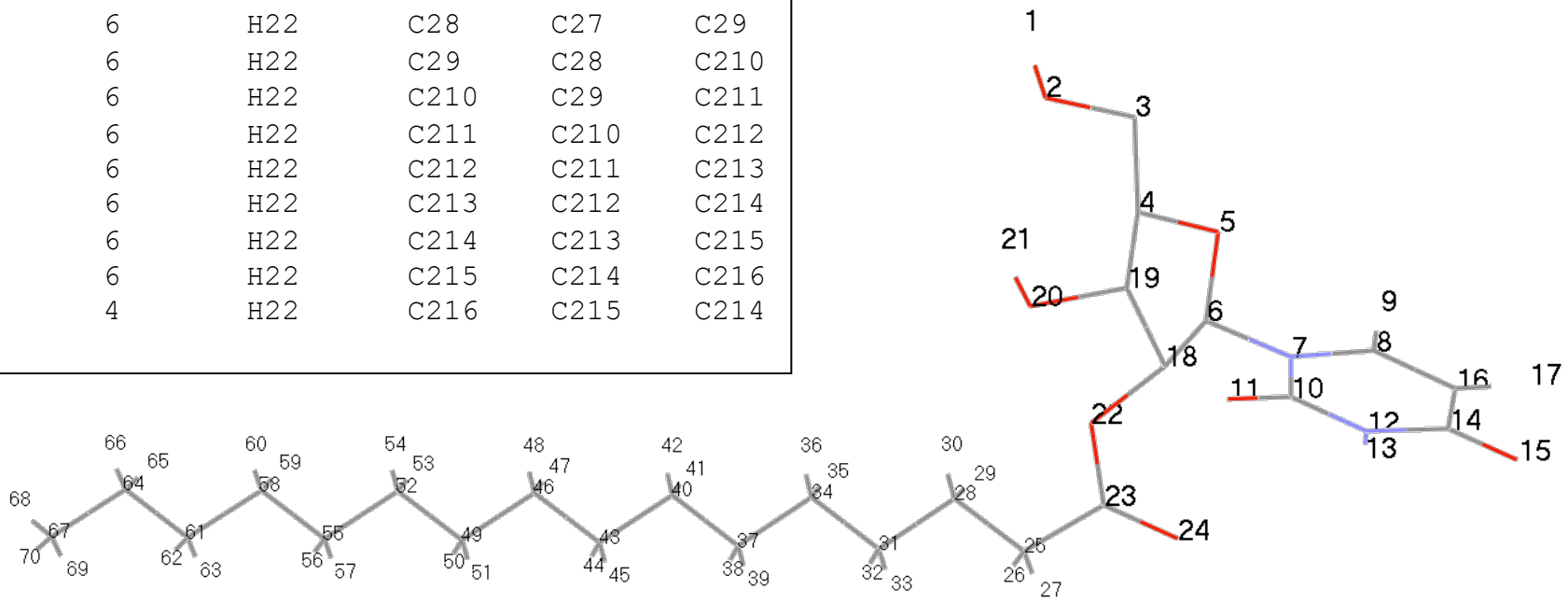
Incluir os H221, H222 e H223 nos [atoms] do [UP2] do `gromos53a6.ff/aminoacids.rtp` e copiar para `gmf.ff/aminoacids.rtp`

`gmf.ff/aminoacids.hdb`

```
UP2      15
2        6        H22      C22      C21      C23
2        6        H22      C23      C22      C24
2        6        H22      C24      C23      C25
2        6        H22      C25      C24      C26
2        6        H22      C26      C25      C27
2        6        H22      C27      C26      C28
2        6        H22      C28      C27      C29
2        6        H22      C29      C28      C210
2        6        H22      C210     C29      C211
2        6        H22      C211     C210     C212
2        6        H22      C212     C211     C213
2        6        H22      C213     C212     C214
2        6        H22      C214     C213     C215
2        6        H22      C215     C214     C216
3        4        H22      C216     C215     C214
```

`gmf.ff/aminoacids.rtp`

```
[ UP2 ]
 [ atoms ]
;   a    type      charge    cgroup
 H221      H      0.00000     18
 H222      H      0.00000     18
 H223      H      0.00000     18
```



Etapas de análise:

1) Conferir dados de entrada no arquivo padrão de saída: output.out

```
                      :-)  G  R  O  M  A  C  S  (-:
                           Grunge ROck MAChoS
                         :-)  VERSION 4.5.4  (-:
.
Getting Loaded...
Reading file up2_vac_min.tpr, VERSION 4.5.4 (single precision)
NOTE: Parallelization is limited by the small number of atoms,
      only starting 1 threads.
      You can use the -nt option to optimize the number of threads.
Loaded with Money

Steepest Descents:
   Tolerance (Fmax)   =  1.00000e+00
   Number of steps    =        25000
Step=    0, Dmax= 1.0e-02 nm, Epot=  2.80109e+02 Fmax= 7.31658e+02, atom= 23
Step=    1, Dmax= 1.0e-02 nm, Epot=  5.24442e+02 Fmax= 1.05546e+04, atom= 23
Step=    2, Dmax= 5.0e-03 nm, Epot=  3.26337e+02 Fmax= 4.65193e+03, atom= 23
Step=    3, Dmax= 2.5e-03 nm, Epot=  2.84779e+02 Fmax= 1.91171e+03, atom= 23
Step=    4, Dmax= 1.2e-03 nm, Epot=  2.77897e+02 Fmax= 6.63492e+02, atom= 22
Step=    5, Dmax= 1.5e-03 nm, Epot=  2.78821e+02 Fmax= 1.33307e+03, atom= 23
Step=    6, Dmax= 7.5e-04 nm, Epot=  2.76608e+02 Fmax= 4.02456e+02, atom= 23
.
Step=16077, Dmax= 1.5e-06 nm, Epot=  2.16197e+02 Fmax= 9.87983e+00, atom= 14
Step=16078, Dmax= 1.8e-06 nm, Epot=  2.16199e+02 Fmax= 7.61529e+00, atom= 14
Stepsize too small, or no change in energy.
Converged to machine precision,
but not to the requested precision Fmax < 1

Double precision normally gives you higher accuracy.

writing lowest energy coordinates.

Steepest Descents converged to machine precision in 16079 steps,
but did not reach the requested Fmax < 1.
Potential Energy  =  2.1619743e+02
Maximum force     =  9.8798285e+00 on atom 14
Norm of force     =  3.1266160e+00
```

Etapas de análise:

2) O principal arquivos de saída é o arquivo binário com terminação trr (*.trr). Esse arquivo *.trr contem as trajetórias dos átomos do sistema e pode ser analisado por vários programas de análise.

A primeira análise deve ser de energia com o comando:

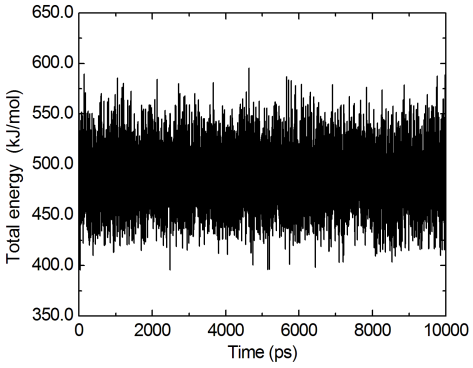
```
g_energy -f up2_nvt-run.edr -s up2_nvt.tpr
```

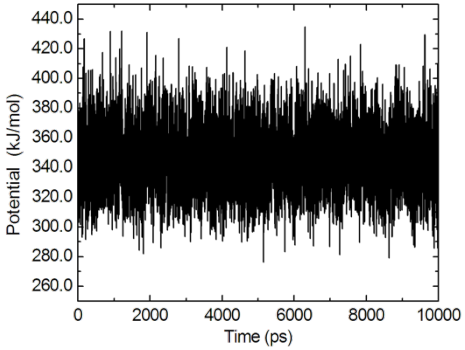
```
Select the terms you want from the following list by
selecting either (part of) the name or the number or a combination.
End your selection with an empty line or a zero.
-------------------------------------------------------------------
  1  G96Bond          2  G96Angle         3  Proper-Dih.      4  Improper-Dih.
  5  LJ-14            6  Coulomb-14       7  LJ-(SR)          8  Coulomb-(SR)
  9  RF-excl.        10  Potential       11  Kinetic-En.     12  Total-Energy
 13  Conserved-En.   14  Temperature     15  Pressure        16  Vir-XX
 17  Vir-XY          18  Vir-XZ          19  Vir-YX          20  Vir-YY
 21  Vir-YZ          22  Vir-ZX          23  Vir-ZY          24  Vir-ZZ
 25  Pres-XX         26  Pres-XY         27  Pres-XZ         28  Pres-YX
 29  Pres-YY         30  Pres-YZ         31  Pres-ZX         32  Pres-ZY
 33  Pres-ZZ         34  #Surf*SurfTen   35  Mu-X            36  Mu-Y
 37  Mu-Z            38  T-UP2           39  Lamb-UP2
```

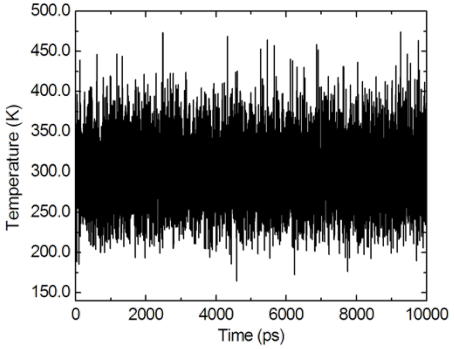
```
10 11 12 14 15 0

Last energy frame read 10000 time 10000.000

Statistics over 10000001 steps [ 0.0000 through 10000.0000 ps ], 6 data sets
All statistics are over 2000001 points
```

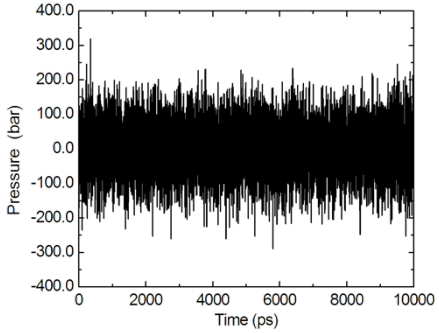
Energy	Average	Err.Est.	RMSD	Tot-Drift	
Potential	345.066	0.41	21.0214	-1.18202	(kJ/mol)
Kinetic En.	142.132	0.055	18.8013	0.178617	(kJ/mol)
Total Energy	487.199	0.43	28.2425	-1.00341	(kJ/mol)
Temperature	299.903	0.12	39.6712	0.376888	(K)
Pressure	0.00440807	0.0033	72.9946	0.0127406	(bar)

A saída é um arquivo energy.xvg pronto para ser graficado no xmgrace.









Em seguida é interessante fazer a construção de grupos de análise no arquivo `index.ndx`, pois pode ser necessário para outras análises, com o comando:

```
make_ndx -f up2.pdb -o index.ndx
```

```
Reading structure file
Going to read 0 old index file(s)
Analysing residue names:
There are:     1      Other residues
Analysing residues not classified as Protein/DNA/RNA/Water and splitting into groups...
  0 System              :    39 atoms
  1 Other               :    39 atoms
  2 UP2                 :    39 atoms
 nr : group       !   'name' nr name   'splitch' nr    Enter: list groups
 'a': atom        &   'del' nr         'splitres' nr   'l': list residues
 't': atom type   |   'keep' nr        'splitat' nr    'h': help
 'r': residue         'res' nr         'chain' char
 "name": group        'case': case sensitive           'q': save and quit
 'ri': residue index
> del 1
Removed group 1 'Other'
> del 1
Removed group 1 'UP2'
>q
```

index.ndx

```
[ System ]
   1    2    3    4    5    6    7    8    9   10   11   12   13   14   15
  16   17   18   19   20   21   22   23   24   25   26   27   28   29   30
  31   32   33   34   35   36   37   38   39
```

Adição de Hs da cadeia carbônica na trajetória toda:

Tendo editado os arquivos `aminoacids.rtp` e `aminoacids.hdb` do diretório `./simula/gmx.ff` é necessário transformar o arquivo de trajetória em pdb num dado intervalo (-skip 100) com o comando:

```
trjconv -f up2_nvt-run.trr -o up2_nvt-run-trr.pdb -t0 500 -s
up2_nvt.tpr -n index.ndx –skip 100 -ndec 5
```

e separar as configurações com o comando:

```
split –l 46 –d –a 3 up2_nvt-run-trr.pdb
```

`-l 46` (indica que cada configuração tem 46 linhas)
`-d` (os arquivo de saída terão diferenciador numérico)
`-a 3` (o diferenciador numérico terá 3 dígitos)
Usar o comando: `sh add.sh`

```
#!/bin/sh
for i in x???
do
    echo $i
    cp $i $i.pdb
    pdb2gmx -f $i.pdb -o $i+h.pdb -ff gmx -water none -p $i.top -i $i.itp
    \rm $i.pdb $i.top $i.itp
done
cat x???+h.pdb > traj+h.pdb
```

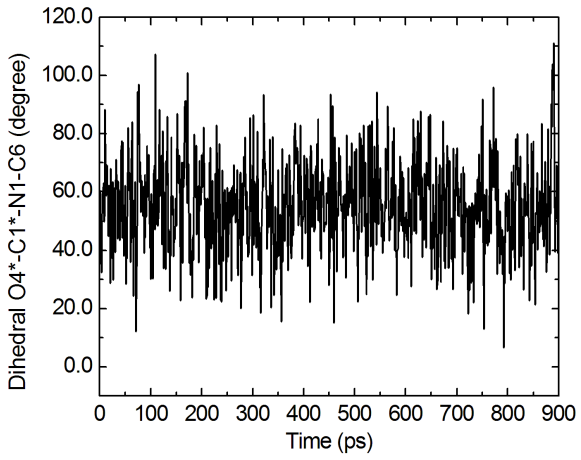
Analisar ângulos de torção:

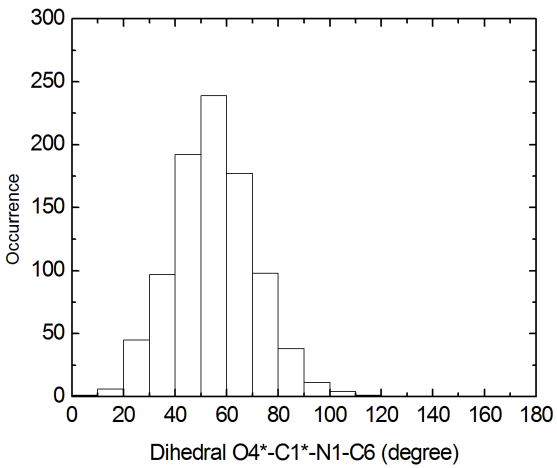
Criar vário arquivo, dihedral.ndx, com linhas que definem os nomes dos grupos e os conjuntos dos 4 átomos que formar o ângulo de torção:

```
[ O4C1N1C6 ]
5 6 7 8
[ C1C2O2C21 ]
6 18 22 23
```

Executar o comando para gerar o arquivo do4c1n1c6.xvg, com os dados no formato do xmgrace:

```
> g_angle -f up2_npt-run.trr -n dihedral.ndx -ov do4c1n1c6 -type dihedral
Group     0 (       O4C1N1C6) has     4 elements
Group     1 (      C1C2O2C21) has     4 elements
Select a group: 0
Selected 0: 'O4C1N1C6'
trn version: GMX_trn_file (single precision)
Reading frame     900 time  900.000
Found points in the range from 186 to 292 (max 360)
 < angle >  = 55.2178
< angle^2 > = 3294.02
Std. Dev.   = 15.6529
Order parameter S^2 = 0.928323
```





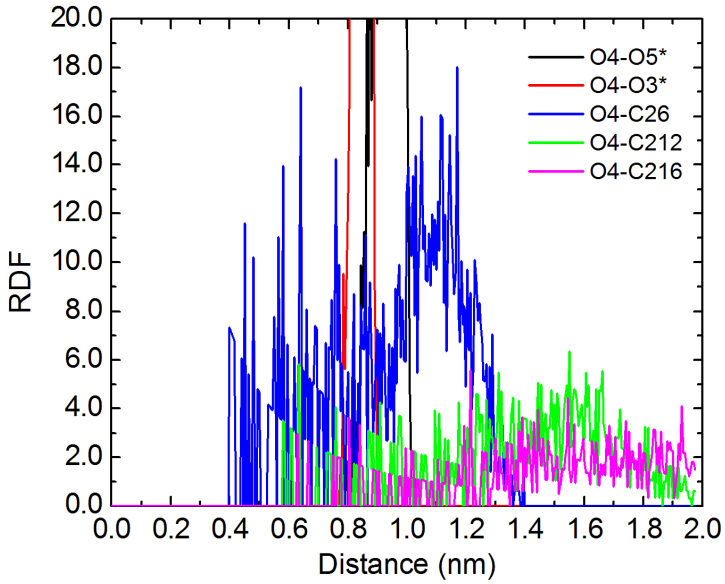
Analisar funções de distribuição radial de pares (intramolecular):

Criar vário arquivo, atup2.ndx, com linhas que definem os nomes dos átomos e os números dos átomos:

```
> make_ndx -f up2.gro -o atup2.ndx
Analysing residues not classified as Protein/DNA/RNA/Water and splitting into groups...
  0 System              :  6498 atoms
  1 Other               :    39 atoms
  2 UP2                 :    39 atoms
 nr : group       !   'name' nr name   'splitch' nr    Enter: list groups
 'a': atom        &   'del' nr         'splitres' nr   'l': list residues
 't': atom type   |   'keep' nr        'splitat' nr    'h': help
 'r': residue         'res' nr         'chain' char
 "name": group        'case': case sensitive           'q': save and quit
 'ri': residue index
> del 1
Removed group 1 'Other'
> del 0
Removed group 3 'System'
> a O2
Found 1 atoms with name O2
  1 O2                  :     1 atoms
> a O4
Found 1 atoms with name O4
  2 O4                  :     1 atoms
> a O2*
Found 1 atoms with name O2*
  3 O2*                 :     1 atoms
```

Executar o comando para gerar o arquivo rdfo4o3.xvg:

```
> g_rdf -f up2-agua_npt-run.trr -s up2_npt.tpr -o rdf-o4o3 -cn rdf_cn-o4o3 -n
atup2.ndx -bin 0.005
Select a reference group and 1 group
Group     0 (             UP2) has    39 elements
Group     1 (              O2) has     1 elements
Group     2 (              O4) has     1 elements
Group     3 (             O3*) has     1 elements
Group     4 (             O4*) has     1 elements
Group     5 (             O5*) has     1 elements
Group     6 (             O22) has     1 elements
Group     7 (             C26) has     1 elements
Group     8 (            C212) has     1 elements
Group     9 (            C216) has     1 elements
Select a group: 2
Selected 2: 'O4'
Select a group: 3
Selected 3: 'O3*'
trn version: GMX_trn_file (single precision)
Reading frame     900 time  900.000
```




```
> g_rdf -f up2-agua_npt-run.trr -s up2_npt.tpr -o rdf-o4o5 -cn rdf_cn-o4o5 -n
atup2.ndx -bin 0.005
Select a group: 2
Selected 2: 'O4'
Select a group: 5
Selected 5: 'O5*'
trn version: GMX_trn_file (single precision)
Reading frame     900 time  900.000
```

Minimização e simulação em solvente (água)

Gerar um diretório para fazer a simulação:

```
> mkdir simulaagua
> cd simulaagua
> cp -r /usr/local/gromacs/share/gromcas/top/spc216.gro .
> cp -r ../simula/gromos53a6.ff * .
> cp ../simula/up2_vac_min-run.gro .
> ls
gromos53a6.ff  min-agua.mdp   npt-agua.mdp   spc216.gro  up2_vac_min-run.gro
```

Gerar o up2-agua.top e up2-agua.pdb a partir da conformação minimizada up2_vac_min.gro informando que o solvente será água SPC:

```
> pdb2gmx -f up2_vac_min-run.gro -o up2_min.pdb -p up2-agua.top -ff gromos53a6
-water spc

--------- PLEASE NOTE ------------
You have successfully generated a topology from: up2_vac_min-run.gro.
The Gromos53a6 force field and the spc water model are used.
--------- ETON ESAELP ------------
```

Gerar o up2-agua.top e up2-agua.gro a partir do up2_min.pdb (que já tem o tamanho da caixa) e da caixa com 216 moléculas de água SPC:

```
> genbox -cp up2_min.pdb -cs  spc216.gro -p up2-agua.top -o up2-agua.gro

Output configuration contains 6498 atoms in 2154 residues
Volume                 :           64 (nm^3)
Density                :      1017.98 (g/l)
Number of SOL molecules:    2153
```

Gerar o arquivo binário up2-agua_min.tpr para minimização:

```
> grompp -f min-agua.mdp -c up2-agua.gro -p up2-agua.top -o up2-agua_min.tpr
```

Executar a minimização e gerar o arquivo up2-agua_min-run.gro:

```
> mdrun -s  up2-agua_min.tpr -deffnm  up2-agua_min-run -v >&  up2-agua_min.out
```

Gerar o arquivo binário up2-agua_nvt.tpr para a simulação:

```
> grompp -f npt-agua.mdp -c up2-agua_min-run.gro -p up2-agua.top -o up2-agua_npt.tpr
```

Executar a simulação em água:

```
> mdrun -s  up2-agua_npt.tpr -deffnm  up2-agua_npt-run -v >&  up2-agua_npt.out
```

ATENÇÃO COM A MENSAGEM ABAIXO:

```
NOTE 1 [file up2-agua.top, line 297]:
  The bond in molecule-type Other between atoms 12 N3 and 13 H3 has an
  estimated oscillational period of 1.0e-02 ps, which is less than 10 times
  the time step of 1.0e-03 ps.
  Maybe you forgot to change the constraints mdp option.
```

min-agua.mdp

```
integrator               = steep
tinit                    = 0.0
dt                       = 0.001
nsteps                   = 25000
emtol                    = 1
nstcomm                  = 5
comm-grps                = UP2 SOL
nstxout                  = 10
nstvout                  = 10
nstfout                  = 0
nstlog                   = 10
nstenergy                = 10
nstxtcout                = 10
xtc_precision            = 10
xtc-grps                 =
energygrps               = UP2 SOL
nstlist                  = 5
ns_type                  = grid
pbc                      = xyz
rlist                    = 1.4
coulombtype              = reaction-field
rcoulomb                 = 1.4
epsilon_rf               = 78.5
vdw_type                 = cut-off
rvdw                     = 1.4
DispCorr                 = No
constraints              = none
```

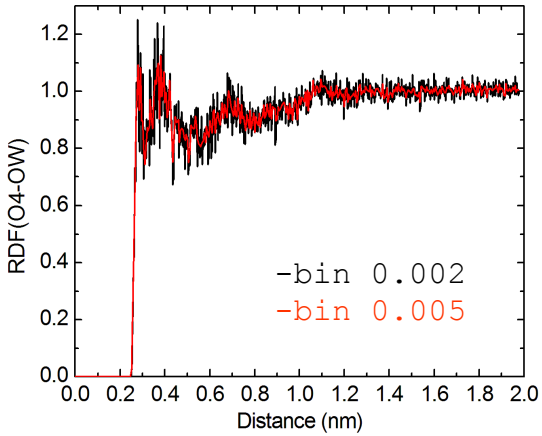
npt-agua.mdp

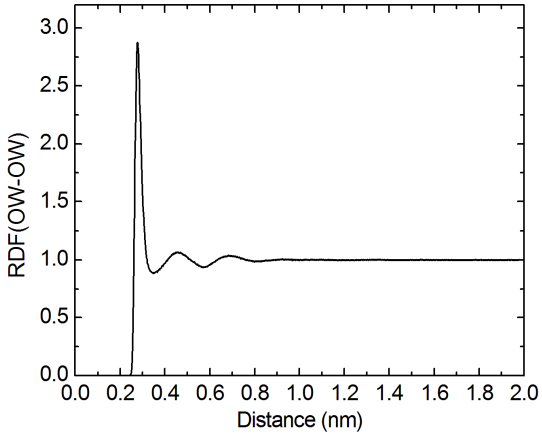
```
integrator               = md
tinit                    = 0.0
dt                       = 0.001
nsteps                   = 5000000
emtol                    = 1
comm-mode                = Angular
nstcomm                  = 5
comm-grps                = UP2 SOL
nstxout                  = 1000
nstvout                  = 1000
nstfout                  = 0
nstlog                   = 1000
nstenergy                = 1000
nstxtcout                = 1000
xtc_precision            = 1000
xtc-grps                 =
energygrps               = UP2 SOL
nstlist                  = 5
ns_type                  = grid
pbc                      = xyz
rlist                    = 1.4
coulombtype              = reaction-field
rcoulomb                 = 1.4
epsilon_rf               = 78.5
vdw_type                 = cut-off
rvdw                     = 1.4
DispCorr                 = No
tcoupl                   = v-rescale
tc-grps                  = UP2 SOL
tau_t                    = 0.1 0.1
ref_t                    = 300 300
Pcoupl                   = berendsen
Pcoupltype               = isotropic
tau_p                    = 0.1
compressibility          = 4.51e-5
ref_p                    = 1.0
constraints              = none
```

Analisar funções de distribuição radial de pares:

```
> make_ndx -f up2-agua.gro -o atup2w.ndx
Analysing residues not classified as Protein/DNA/RNA/Water and splitting into groups...
  0 System              :  6498 atoms
  1 Other               :    39 atoms
  2 UP2                 :    39 atoms
  3 Water               :  6459 atoms
  4 SOL                 :  6459 atoms
  5 non-Water           :    39 atoms
 nr : group       !   'name' nr name   'splitch' nr    Enter: list groups
 'a': atom        &   'del' nr         'splitres' nr   'l': list residues
 't': atom type   |   'keep' nr        'splitat' nr    'h': help
 'r': residue         'res' nr         'chain' char
 "name": group        'case': case sensitive           'q': save and quit
 'ri': residue index
> del 1
Removed group 1 'Other'
> del 3
Removed group 3 'SOL'
> del 4
Removed group 3 'non-Water'
> a O2
Found 1 atoms with name O2
  4 O2                  :     1 atoms
> a O4
Found 1 atoms with name O4
  5 O4                  :     1 atoms
.
> a OW
Found 2153 atoms with name OW
  10 OW                 :     2153 atoms
```

```
> g_rdf -f up2-agua_npt-run.trr -s up2-agua_npt.tpr -o rdf-o4o5 -cn rdf_cn-o4o5
-n atup2w.ndx -bin 0.005 -b 100
Select a reference group and 1 group
Group     0 (         System) has  6498 elements
Group     1 (            UP2) has    39 elements
Group     2 (          Water) has  6459 elements
Group     3 (             O2) has     1 elements
Group     4 (             O4) has     1 elements
Group     5 (            O3*) has     1 elements
Group     6 (            O5*) has     1 elements
Group     7 (            C26) has     1elements
Group     8 (           C212) has     1 elements
Group     9 (           C216) has     1 elements
Group    10 (             OW) has  2153 elements
Select a group: 4
Selected 4: 'O4'
Select a group: 10
Selected 10: 'OW'
trn version: GMX_trn_file (single precision)
Reading frame     900 time  900.000
```



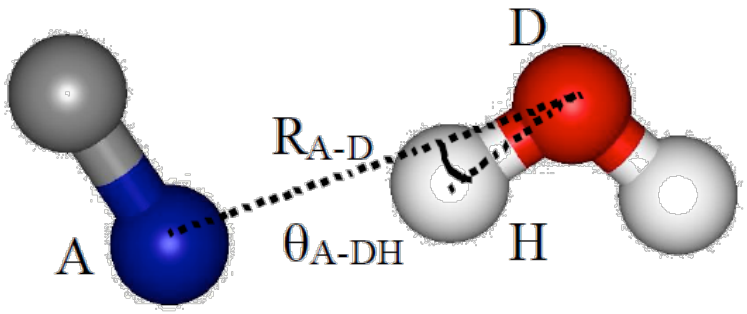



```
> g_rdf -f up2-agua_npt-run.trr -s up2-agua_npt.tpr -o rdf-owow -cn rdf_cn-owow
-n atup2w.ndx -bin 0.005 -b 100
Select a group: 10
Selected 10: 'OW'
Select a group: 10
Selected 10: 'OW'
```

Analisar de ligações de hidrogênio:

Critérios: -a 30.0 Cutoff angle (degrees, A-D-H); -r 0.35 Cutoff radius (nm, X - Acceptor); -da yes Donor-Acceptor (if TRUE) or Hydrogen-Acceptor (FALSE)

```
> g_hbond -f up2-agua_npt-run.trr -s up2-agua_npt.tpr -n -num -g -ac -dist -
ang -hbn -don -dan -life -nhbdist -da yes -r 0.35 -a 35.0
Specify 2 groups to analyze:
Group     0 (         System) has  6498 elements
Group     1 (            UP2) has    39 elements
Group     2 (          Water) has  6459 elements
Select a group: 1
Selected 1: 'UP2'
Select a group: 2
Selected 2: 'Water'
Checking for overlap in atoms between UP2 and Water
Calculating hydrogen bonds between UP2 (39 atoms) and Water (6459 atoms)
Found 2156 donors and 2162 acceptors
Making hbmap structure...done.
trn version: GMX_trn_file (single precision)
Reading frame       0 time    0.000
Will do grid-seach on 9x9x9 grid, rcut=0.35
Reading frame     900 time  900.000
Found 1445 different hydrogen bonds in trajectory
Found 3258 different atom-pairs within hydrogen bonding distance
Merging hbonds with Acceptor and Donor swapped
2156/2156
- Reduced number of hbonds from 1445 to 1374
- Reduced number of distances from 3258 to 3258
Average number of hbonds per timeframe 7.654 out of 2.33064e+06 possible
++++ PLEASE READ AND CITE THE FOLLOWING REFERENCE ++++
D. van der Spoel, P. J. van Maaren, P. Larsson and N. Timneanu
Thermodynamics of hydrogen bonding in hydrophilic and hydrophobic media
J. Phys. Chem. B 110 (2006) pp. 4393-4398
-------- -------- --- Thank You --- -------- --------
```



```
Doing autocorrelation according to the theory of Luzar and Chandler.
ACF 1374/1374
Normalization for c(t) = 0.130511 for gh(t) = 1
Hydrogen bond thermodynamics at T = 298.15 K
Fitting parameters chi^2 = 0.00982687
Q =          0
-------------------------------------------------
Type      Rate (1/ps) Time (ps)  DG (kJ/mol)  Chi^2
Forward         1.012      0.988       4.498  0.00982687
Backward        0.000   2725.160      24.137
One-way         0.387      2.581       6.879
Integral        0.145      6.891       9.313
Relaxation      0.254      3.937       7.925

HB lifetime = 1.93 ps
Note that the lifetime obtained in this manner is close to useless
Use the -ac option instead and check the Forward lifetime

++++ PLEASE READ AND CITE THE FOLLOWING REFERENCE ++++
D. van der Spoel, P. J. van Maaren, P. Larsson and N. Timneanu
Thermodynamics of hydrogen bonding in hydrophilic and hydrophobic media
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-------- -------- --- Thank You --- -------- --------
```

Arquivos gerados: danum.xvg hbac.xvg hbdist.xvg hbnum.xvg hbond.ndx nhbdist.xvg donor.xvg hbang.xvg hblife.xvg hbond.log

hbond.ndx

```
[ UP2 ]
    1     2     3     4     5     6     7     8     9    10    11    12    13    14    15
   16    17    18    19    20    21    22    23    24    25    26    27    28    29    30
   31    32    33    34    35    36    37    38    39
[ donors_hydrogens_UP2 ]
    2    1
   12   13
   20   21
[ acceptors_UP2 ]
    2     5     7    11    12    15    20    22    24
[ Water ]
   40    41    42    43    44    45 . . .
[ donors_hydrogens_Water ]
   40   41   40   42
   43   44   43   45
. . .
[ acceptors_Water ]
   40    43    46    49    52    55 . . .
[ hbonds_UP2-Water ]
      2      1    115
      2      1    145
      2      1    181 . . .
```

hbond.log

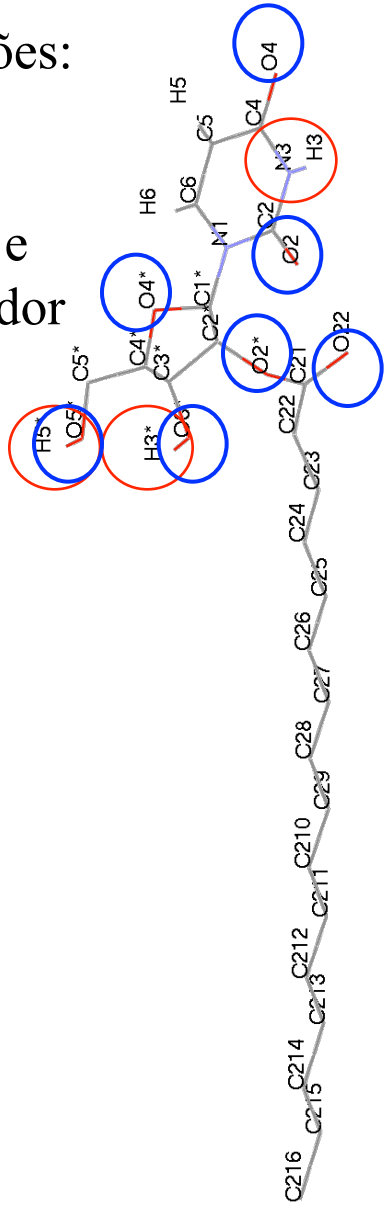
# Donor	Hydrogen	Acceptor
UP21O5*	UP21H5*	SOL27OW
UP21O5*	UP21H5*	SOL37OW
UP21O5*	UP21H5*	SOL49OW
UP21O5*	UP21H5*	SOL76OW
UP21O5*	UP21H5*	SOL94OW
UP21O5*	UP21H5*	SOL103OW
UP21O5*	UP21H5*	SOL125OW
UP21O5*	UP21H5*	SOL136OW
UP21O5*	UP21H5*	SOL138OW
. . .		
UP21N3	UP21H3	SOL25OW
UP21N3	UP21H3	SOL27OW
UP21N3	UP21H3	SOL37OW
UP21N3	UP21H3	SOL44OW
UP21N3	UP21H3	SOL79OW
UP21N3	UP21H3	SOL120OW
UP21N3	UP21H3	SOL123OW
. . .		
UP21O3*	UP21H3*	SOL83OW
UP21O3*	UP21H3*	SOL92OW
UP21O3*	UP21H3*	SOL94OW
UP21O3*	UP21H3*	SOL182OW
. . .		
SOL3OW	SOL3HW1	UP21O4
SOL6OW	SOL6HW1	UP21O3*
SOL6OW	SOL6HW1	UP21O2*
SOL9OW	SOL9HW1	UP21O4
SOL17OW	SOL17HW1	UP21O4*
SOL17OW	SOL17HW1	UP21O3*
SOL21OW	SOL21HW1	UP21O5*

Análise de 910 configurações:

1374 H bonds:

322 como doador e

1052 como aceitador



UP21N3-UP21H3 = 136
UP21O5*-UP21H5*= 131
UP21O3*-UP21H3* = 55

UP21O4 = 266
UP21O2 = 203
UP21O22 = 173
UP21O4* = 130
UP21O3* = 117
UP21O2* = 59
UP21O5* = 90
UP21N1 = 10
UP21N3 = 4

`hbnum.xvg` = H Bonds

Time, t (ps) x Hydrogen bonds

Time, t (ps) x Pairs within 0.35 nm

`danum.xvg` = Donors and Acceptors

Time, t (ps) x Donors UP2

Time, t (ps) x Acceptors UP2

Time, t (ps) x Donors Water

Time, t (ps) x Acceptors Water

`donor.xvg` = Donor properties:

Time, t (ps) x Nbound

Time, t (ps) x Nfree

`hblife.xvg` = Uninterrupted H bond lifetime

Time, t (ps) x p(t)

Time, t (ps) x t p(t)

`hbdist.xvg` = H Bond Distribution:

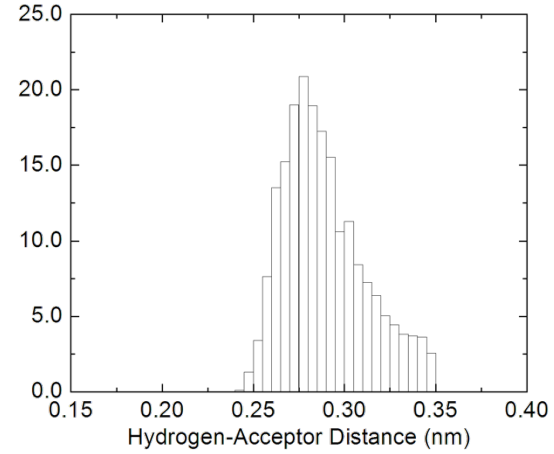
Hydrogen - Acceptor Distance (nm)

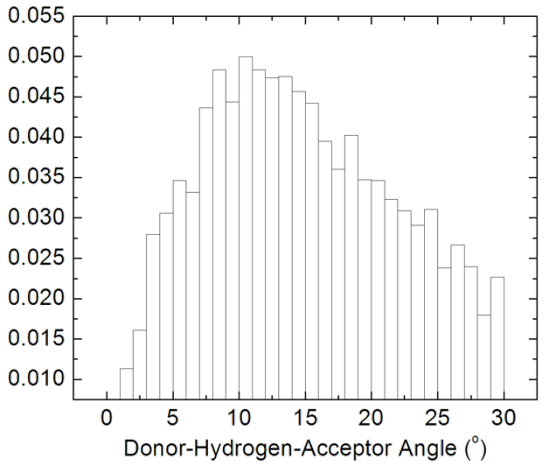
`hbang.xvg` = H Bond Distribution:

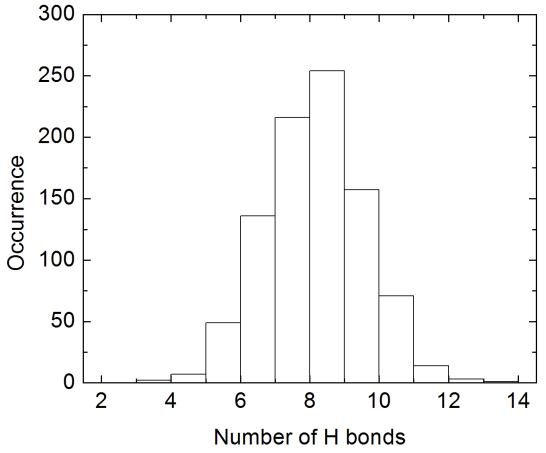
Donor - Hydrogen - Acceptor Angle (º)

`hbac.xvg` = H Bond Autocorrelation:

Time, t (ps) x C(t)







Minimização e simulação em solvente (outro)

Gerar um diretório para fazer a simulação:

```
> mkdir simulachcl3
> cd simulachcl3
> cp -r ../simula/gromos53a6.ff * .
> cp ../simula/up2_vac_min-run.gro .
> ls
clorof.gro  min-clorof.mdp  up2_vac_min-run.gro
gromos53a6.ff    npt-clorof.mdp
```

No diretório gromos53a6.ff lembre de modificar o arquivo watermodels.dat para incluir a linha (`clorof  Cloroformio as solvent`) e criar o cloroformio.ipt

Gerar o up2-clorof.top e up2-clorof.pdb a partir da conformação minimizada up2_vac_min.gro informando que o solvente será clorofórmio clorof:

```
> pdb2gmx -f up2_vac_min-run.gro -o up2_min.pdb -p up2-clorof.top -ff
gromos53a6

Using the Gromos53a6 force field in directory ./gromos53a6.ff
Opening force field file ./gromos53a6.ff/watermodels.dat
Select the Water Model:
 1: SPC    simple point charge, recommended
 2: SPC/E  extended simple point charge
 3: Cloroformio as solvent
 4: None
3
```

```
--------- PLEASE NOTE ------------
You have successfully generated a topology from: up2_vac_min-run.gro.
The Gromos53a6 force field and the clorof water model are used.
--------- ETON ESAELP ------------
```

Gerar o up2-clorof.top e up2-clorof.gro a partir do up2_min.pdb (que já tem o tamanho da caixa) e da caixa com 216 moléculas de clorofórmio:

```
> genbox -cp up2_min.pdb -cs  clorof.gro -p up2-clorof.top -o up2-clorof.gro

Output configuration contains 1874 atoms in 368 residues
Volume                 :           64 (nm^3)
Density                :      1148.34 (g/l)
Number of SOL molecules:    367
```

Gerar o arquivo binário up2-clorof_min.tpr para minimização:

```
> grompp -f min-clorof.mdp -c up2-clorof.gro -p up2-clorof.top -o up2-clorof_min.tpr
```

Executar a minimização e gerar o arquivo up2-clorof_min-run.gro:

```
> mdrun -s up2-clorof_min.tpr -deffnm up2-clorof_min-run -v >& up2-clorof_min.out
```

Gerar o arquivo binário up2-clorof_nvt.tpr para a simulação:

```
> grompp -f npt-clorof.mdp -c up2-clorof_min-run.gro -p up2-clorof.top -o up2-clorof_npt.tpr
```

Executar a simulação em clorofórmio:

```
> mdrun -s up2-clorof_npt.tpr -deffnm up2-clorof_npt-run -v >& up2-clorof_npt.out
```

cloroformio.itp

```
[ moleculetype ]
; molname       nrexcl
SOL              3

[ atoms ]
     1    CChl 1 SOL   CChl 1  0.17900 12.0000
     2    HChl 1 SOL   HChl 1  0.08200  1.00790
     3   CLChl 1 SOL  CLCh1 1 -0.08700 35.45300
     4   CLChl 1 SOL  CLCh2 1 -0.08700 35.45300
     5   CLChl 1 SOL  CLCh3 1 -0.08700 35.45300

[ exclusions ]
1       3       4
1       4       5
1       3       2
1       5       3
2       3       4
2       5       4
2       5       1
2       3       1
3       4       5
3       1       5
5       1       4
5       1       3

[ bonds ]
; i     j     funct     length     force.c.
1       3       1       0.1758   8.1200e+06
1       4       1       0.1758   8.1200e+06
1       5       1       0.1758   8.1200e+06
2       3       1       0.233839 2.6800e+06
2       4       1       0.233839 2.6800e+06
2       5       1       0.233839 2.6800e+06
3       4       1       0.290283 2.9800e+06
3       5       1       0.290283 2.9800e+06
4       5       1       0.290283 2.9800e+06
```

cloroformio.gro

```
216 CHCl3 generated from DICE
 1080
    1SOL    CChl    1   1.560   1.557   1.508
    1SOL    HChl    2   1.640   1.606   1.451
    1SOL   CLCh1    3   1.633   1.513   1.662
    1SOL   CLCh2    4   1.512   1.418   1.411
    1SOL   CLCh3    5   1.433   1.678   1.523
    2SOL    CChl    6   1.259   1.248   1.643
    2SOL    HChl    7   1.281   1.336   1.580
    2SOL   CLCh1    8   1.108   1.183   1.580
    2SOL   CLCh2    9   1.244   1.311   1.807
    2SOL   CLCh3   10   1.396   1.140   1.623
    3SOL    CChl   11   1.671   1.792   1.148
    3SOL    HChl   12   1.579   1.731   1.149
    3SOL   CLCh1   13   1.657   1.897   1.288
    3SOL   CLCh2   14   1.668   1.879   0.995
    3SOL   CLCh3   15   1.803   1.676   1.161
    4SOL    CChl   16   1.682   2.046   1.758
    4SOL    HChl   17   1.614   2.128   1.785
    4SOL   CLCh1   18   1.723   2.073   1.589
    4SOL   CLCh2   19   1.591   1.898   1.785
    4SOL   CLCh3   20   1.820   2.062   1.866
.
.
.
  215SOL    CChl 1071   2.594   2.997   0.032
  215SOL    HChl 1072   2.534   3.081   0.069
  215SOL   CLCh1 1073   2.698   3.066  -0.092
  215SOL   CLCh2 1074   2.478   2.881  -0.032
  215SOL   CLCh3 1075   2.682   2.937   0.172
  216SOL    CChl 1076   2.989   2.891   3.015
  216SOL    HChl 1077   2.932   2.933   2.931
  216SOL   CLCh1 1078   3.141   2.837   2.946
  216SOL   CLCh2 1079   2.889   2.759   3.076
  216SOL   CLCh3 1080   3.007   3.022   3.130
   3.07000   3.07000   3.07000
```

Restart

```
mdrun -s up2-clorof_npt.tpr -deffnm  up2-clorof_npt-run -cpi up2-clorof_npt-run.cpt
```