

4.3 Restraints

Special potentials are used for imposing restraints on the motion of the system, either to avoid disastrous deviations, or to include knowledge from experimental data. In either case they are not really part of the force field and the reliability of the parameters is not important. The potential forms, as implemented in GROMACS, are mentioned just for the sake of completeness. Restraints and constraints refer to quite different algorithms in GROMACS.

4.3.1 Position restraints

These are used to restrain particles to fixed reference positions \mathbf{R}_i . They can be used during equilibration in order to avoid drastic rearrangements of critical parts (*e.g.* to restrain motion in a protein that is subjected to large solvent forces when the solvent is not yet equilibrated). Another application is the restraining of particles in a shell around a region that is simulated in detail, while the shell is only approximated because it lacks proper interaction from missing particles outside the shell. Restraining will then maintain the integrity of the inner part. For spherical shells, it is a wise procedure to make the force constant depend on the radius, increasing from zero at the inner boundary to a large value at the outer boundary. This feature has not, however, been implemented in GROMACS.

The following form is used:

$$V_{pr}(\mathbf{r}_i) = \frac{1}{2} k_{pr} |\mathbf{r}_i - \mathbf{R}_i|^2 \quad (4.74)$$

The potential is plotted in Fig. 4.14.

The potential form can be rewritten without loss of generality as:

$$V_{pr}(\mathbf{r}_i) = \frac{1}{2} \left[k_{pr}^x (x_i - X_i)^2 \hat{\mathbf{x}} + k_{pr}^y (y_i - Y_i)^2 \hat{\mathbf{y}} + k_{pr}^z (z_i - Z_i)^2 \hat{\mathbf{z}} \right] \quad (4.75)$$

Now the forces are:

$$\begin{aligned} F_i^x &= -k_{pr}^x (x_i - X_i) \\ F_i^y &= -k_{pr}^y (y_i - Y_i) \\ F_i^z &= -k_{pr}^z (z_i - Z_i) \end{aligned} \quad (4.76)$$

Using three different force constants the position restraints can be turned on or off in each spatial dimension; this means that atoms can be harmonically restrained to a plane or a line. Position restraints are applied to a special fixed list of atoms. Such a list is usually generated by the `pdb2gmx` program.

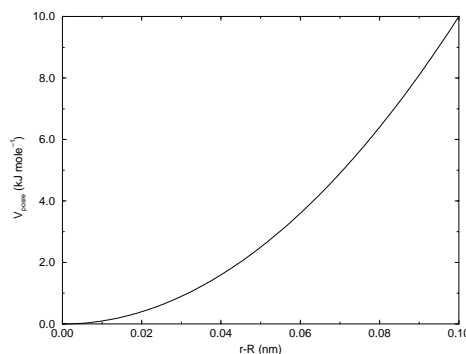


Figure 4.14: Position restraint potential.

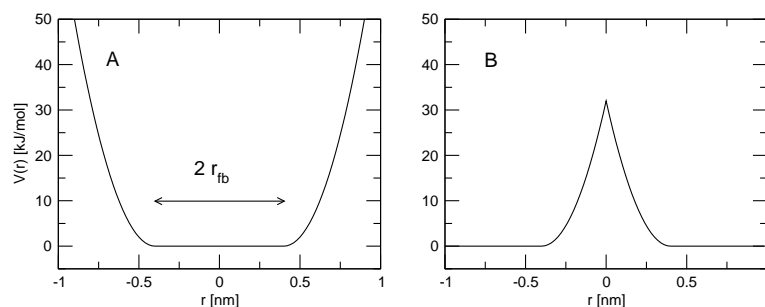


Figure 4.15: Flat-bottomed position restraint potential. (A) Not inverted, (B) inverted.

4.3.2 Flat-bottomed position restraints

Flat-bottomed position restraints can be used to restrain particles to part of the simulation volume. No force acts on the restrained particle within the flat-bottomed region of the potential, however a harmonic force acts to move the particle to the flat-bottomed region if it is outside it. It is possible to apply normal and flat-bottomed position restraints on the same particle (however, only with the same reference position \mathbf{R}_i). The following general potential is used (Figure 4.15A):

$$V_{\text{fb}}(\mathbf{r}_i) = \frac{1}{2} k_{\text{fb}} [d_g(\mathbf{r}_i; \mathbf{R}_i) - r_{\text{fb}}]^2 H[d_g(\mathbf{r}_i; \mathbf{R}_i) - r_{\text{fb}}], \quad (4.77)$$

where \mathbf{R}_i is the reference position, r_{fb} is the distance from the center with a flat potential, k_{fb} the force constant, and H is the Heaviside step function. The distance $d_g(\mathbf{r}_i; \mathbf{R}_i)$ from the reference position depends on the geometry g of the flat-bottomed potential.

The following geometries for the flat-bottomed potential are supported:

Sphere ($g = 1$): The particle is kept in a sphere of given radius. The force acts towards the center of the sphere. The following distance calculation is used:

$$d_g(\mathbf{r}_i; \mathbf{R}_i) = |\mathbf{r}_i - \mathbf{R}_i| \quad (4.78)$$

Cylinder ($g = 2$): The particle is kept in a cylinder of given radius parallel to the z -axis. The force from the flat-bottomed potential acts towards the axis of the cylinder. The z -component of the force is zero.

$$d_g(\mathbf{r}_i; \mathbf{R}_i) = \sqrt{(x_i - X_i)^2 + (y_i - Y_i)^2} \quad (4.79)$$

Layer ($g = 3, 4, 5$): The particle is kept in a layer defined by the thickness and the normal of the layer. The layer normal can be parallel to the x , y , or z -axis. The force acts parallel to the layer normal.

$$d_g(\mathbf{r}_i; \mathbf{R}_i) = |x_i - X_i|, \quad \text{or} \quad d_g(\mathbf{r}_i; \mathbf{R}_i) = |y_i - Y_i|, \quad \text{or} \quad d_g(\mathbf{r}_i; \mathbf{R}_i) = |z_i - Z_i|. \quad (4.80)$$

It is possible to apply multiple independent flat-bottomed position restraints of different geometry on one particle. For example, applying a cylinder and a layer in z keeps a particle within a disk. Applying three layers in x , y , and z keeps the particle within a cuboid.

In addition, it is possible to invert the restrained region with the unrestrained region, leading to a potential that acts to keep the particle *outside* of the volume defined by \mathbf{R}_i , g , and r_{fb} . That feature is switched on by defining a negative r_{fb} in the topology. The following potential is used (Figure 4.15B):

$$V_{fb}^{inv}(\mathbf{r}_i) = \frac{1}{2} k_{fb} [d_g(\mathbf{r}_i; \mathbf{R}_i) - |r_{fb}|]^2 H[-(d_g(\mathbf{r}_i; \mathbf{R}_i) - |r_{fb}|)]. \quad (4.81)$$

4.3.3 Angle restraints

These are used to restrain the angle between two pairs of particles or between one pair of particles and the z -axis. The functional form is similar to that of a proper dihedral. For two pairs of atoms:

$$V_{ar}(\mathbf{r}_i, \mathbf{r}_j, \mathbf{r}_k, \mathbf{r}_l) = k_{ar}(1 - \cos(n(\theta - \theta_0))), \quad \text{where } \theta = \arccos\left(\frac{\mathbf{r}_j - \mathbf{r}_i}{\|\mathbf{r}_j - \mathbf{r}_i\|} \cdot \frac{\mathbf{r}_l - \mathbf{r}_k}{\|\mathbf{r}_l - \mathbf{r}_k\|}\right) \quad (4.82)$$

For one pair of atoms and the z -axis:

$$V_{ar}(\mathbf{r}_i, \mathbf{r}_j) = k_{ar}(1 - \cos(n(\theta - \theta_0))), \quad \text{where } \theta = \arccos\left(\frac{\mathbf{r}_j - \mathbf{r}_i}{\|\mathbf{r}_j - \mathbf{r}_i\|} \cdot \begin{pmatrix} 0 \\ 0 \\ 1 \end{pmatrix}\right) \quad (4.83)$$

A multiplicity (n) of 2 is useful when you do not want to distinguish between parallel and anti-parallel vectors. The equilibrium angle θ should be between 0 and 180 degrees for multiplicity 1 and between 0 and 90 degrees for multiplicity 2.

4.3.4 Dihedral restraints

These are used to restrain the dihedral angle ϕ defined by four particles as in an improper dihedral (sec. 4.2.12) but with a slightly modified potential. Using:

$$\phi' = (\phi - \phi_0) \text{ MOD } 2\pi \quad (4.84)$$

where ϕ_0 is the reference angle, the potential is defined as:

$$V_{dihr}(\phi') = \begin{cases} \frac{1}{2}k_{dihr}(\phi' - \phi_0 - \Delta\phi)^2 & \text{for } \phi' > \Delta\phi \\ 0 & \text{for } \phi' \leq \Delta\phi \end{cases} \quad (4.85)$$

where $\Delta\phi$ is a user defined angle and k_{dihr} is the force constant. **Note** that in the input in topology files, angles are given in degrees and force constants in kJ/mol/rad².

4.3.5 Distance restraints

Distance restraints add a penalty to the potential when the distance between specified pairs of atoms exceeds a threshold value. They are normally used to impose experimental restraints from, for instance, experiments in nuclear magnetic resonance (NMR), on the motion of the system. Thus, MD can be used for structure refinement using NMR data. In GROMACS there are three ways to impose restraints on pairs of atoms:

- Simple harmonic restraints: use [bonds] type 6 . (see sec. 5.4).
- Piecewise linear/harmonic restraints: [bonds] type 10.
- Complex NMR distance restraints, optionally with pair, time and/or ensemble averaging.

The last two options will be detailed now.

The potential form for distance restraints is quadratic below a specified lower bound and between two specified upper bounds, and linear beyond the largest bound (see Fig. 4.16).

$$V_{dr}(r_{ij}) = \begin{cases} \frac{1}{2}k_{dr}(r_{ij} - r_0)^2 & \text{for } r_{ij} < r_0 \\ 0 & \text{for } r_0 \leq r_{ij} < r_1 \\ \frac{1}{2}k_{dr}(r_{ij} - r_1)^2 & \text{for } r_1 \leq r_{ij} < r_2 \\ \frac{1}{2}k_{dr}(r_2 - r_1)(2r_{ij} - r_2 - r_1) & \text{for } r_2 \leq r_{ij} \end{cases} \quad (4.86)$$

The forces are

$$\mathbf{F}_i = \begin{cases} -k_{dr}(r_{ij} - r_0)\frac{\mathbf{r}_{ij}}{r_{ij}} & \text{for } r_{ij} < r_0 \\ 0 & \text{for } r_0 \leq r_{ij} < r_1 \\ -k_{dr}(r_{ij} - r_1)\frac{\mathbf{r}_{ij}}{r_{ij}} & \text{for } r_1 \leq r_{ij} < r_2 \\ -k_{dr}(r_2 - r_1)\frac{\mathbf{r}_{ij}}{r_{ij}} & \text{for } r_2 \leq r_{ij} \end{cases} \quad (4.87)$$

For restraints not derived from NMR data, this functionality will usually suffice and a section of [bonds] type 10 can be used to apply individual restraints between pairs of atoms, see 5.7.1. For applying restraints derived from NMR measurements, more complex functionality might be required, which is provided through the [distance_restraints] section and is described below.

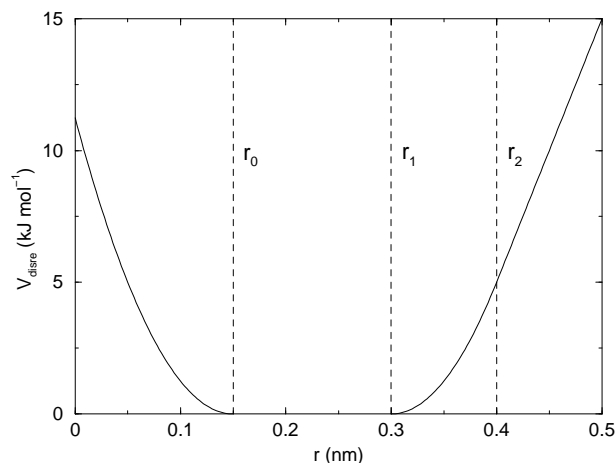


Figure 4.16: Distance Restraint potential.

Time averaging

Distance restraints based on instantaneous distances can potentially reduce the fluctuations in a molecule significantly. This problem can be overcome by restraining to a *time averaged* distance [91]. The forces with time averaging are:

$$\mathbf{F}_i = \begin{cases} -k_{dr}^a (\bar{r}_{ij} - r_0) \frac{\mathbf{r}_{ij}}{r_{ij}} & \text{for } \bar{r}_{ij} < r_0 \\ 0 & \text{for } r_0 \leq \bar{r}_{ij} < r_1 \\ -k_{dr}^a (\bar{r}_{ij} - r_1) \frac{\mathbf{r}_{ij}}{r_{ij}} & \text{for } r_1 \leq \bar{r}_{ij} < r_2 \\ -k_{dr}^a (r_2 - r_1) \frac{\mathbf{r}_{ij}}{r_{ij}} & \text{for } r_2 \leq \bar{r}_{ij} \end{cases} \quad (4.88)$$

where \bar{r}_{ij} is given by an exponential running average with decay time τ :

$$\bar{r}_{ij} = \langle r_{ij}^{-3} \rangle^{-1/3} \quad (4.89)$$

The force constant k_{dr}^a is switched on slowly to compensate for the lack of history at the beginning of the simulation:

$$k_{dr}^a = k_{dr} \left(1 - \exp \left(-\frac{t}{\tau} \right) \right) \quad (4.90)$$

Because of the time averaging, we can no longer speak of a distance restraint potential.

This way an atom can satisfy two incompatible distance restraints *on average* by moving between two positions. An example would be an amino acid side-chain that is rotating around its χ dihedral angle, thereby coming close to various other groups. Such a mobile side chain can give rise to multiple NOEs that can not be fulfilled by a single structure.

The computation of the time averaged distance in the `mdrun` program is done in the following fashion:

$$\begin{aligned} \overline{r_{ij}^{-3}}(0) &= r_{ij}(0)^{-3} \\ \overline{r_{ij}^{-3}}(t) &= \overline{r_{ij}^{-3}}(t - \Delta t) \exp \left(-\frac{\Delta t}{\tau} \right) + r_{ij}(t)^{-3} \left[1 - \exp \left(-\frac{\Delta t}{\tau} \right) \right] \end{aligned} \quad (4.91)$$

When a pair is within the bounds, it can still feel a force because the time averaged distance can still be beyond a bound. To prevent the protons from being pulled too close together, a mixed approach can be used. In this approach, the penalty is zero when the instantaneous distance is within the bounds, otherwise the violation is the square root of the product of the instantaneous violation and the time averaged violation:

$$\mathbf{F}_i = \begin{cases} k_{dr}^a \sqrt{(r_{ij} - r_0)(\bar{r}_{ij} - r_0)} \frac{\mathbf{r}_{ij}}{r_{ij}} & \text{for } r_{ij} < r_0 \text{ and } \bar{r}_{ij} < r_0 \\ -k_{dr}^a \min\left(\sqrt{(r_{ij} - r_1)(\bar{r}_{ij} - r_1)}, r_2 - r_1\right) \frac{\mathbf{r}_{ij}}{r_{ij}} & \text{for } r_{ij} > r_1 \text{ and } \bar{r}_{ij} > r_1 \\ 0 & \text{otherwise} \end{cases} \quad (4.92)$$

Averaging over multiple pairs

Sometimes it is unclear from experimental data which atom pair gives rise to a single NOE, in other occasions it can be obvious that more than one pair contributes due to the symmetry of the system, *e.g.* a methyl group with three protons. For such a group, it is not possible to distinguish between the protons, therefore they should all be taken into account when calculating the distance between this methyl group and another proton (or group of protons). Due to the physical nature of magnetic resonance, the intensity of the NOE signal is inversely proportional to the sixth power of the inter-atomic distance. Thus, when combining atom pairs, a fixed list of N restraints may be taken together, where the apparent “distance” is given by:

$$r_N(t) = \left[\sum_{n=1}^N \bar{r}_n(t)^{-6} \right]^{-1/6} \quad (4.93)$$

where we use r_{ij} or eqn. 4.89 for the \bar{r}_n . The r_N of the instantaneous and time-averaged distances can be combined to do a mixed restraining, as indicated above. As more pairs of protons contribute to the same NOE signal, the intensity will increase, and the summed “distance” will be shorter than any of its components due to the reciprocal summation.

There are two options for distributing the forces over the atom pairs. In the conservative option, the force is defined as the derivative of the restraint potential with respect to the coordinates. This results in a conservative potential when time averaging is not used. The force distribution over the pairs is proportional to r^{-6} . This means that a close pair feels a much larger force than a distant pair, which might lead to a molecule that is “too rigid.” The other option is an equal force distribution. In this case each pair feels $1/N$ of the derivative of the restraint potential with respect to r_N . The advantage of this method is that more conformations might be sampled, but the non-conservative nature of the forces can lead to local heating of the protons.

It is also possible to use *ensemble averaging* using multiple (protein) molecules. In this case the bounds should be lowered as in:

$$\begin{aligned} r_1 &= r_1 * M^{-1/6} \\ r_2 &= r_2 * M^{-1/6} \end{aligned} \quad (4.94)$$

where M is the number of molecules. The GROMACS preprocessor `grompp` can do this automatically when the appropriate option is given. The resulting “distance” is then used to calculate

the scalar force according to:

$$\mathbf{F}_i = \begin{cases} 0 & r_N < r_1 \\ k_{dr}(r_N - r_1) \frac{\mathbf{r}_{ij}}{r_{ij}} & r_1 \leq r_N < r_2 \\ k_{dr}(r_2 - r_1) \frac{\mathbf{r}_{ij}}{r_{ij}} & r_N \geq r_2 \end{cases} \quad (4.95)$$

where i and j denote the atoms of all the pairs that contribute to the NOE signal.

Using distance restraints

A list of distance restrains based on NOE data can be added to a molecule definition in your topology file, like in the following example:

```
[ distance_restraints ]
; ai   aj   type   index   type'   low   up1   up2   fac
10    16    1      0       1       0.0   0.3   0.4   1.0
10    28    1      1       1       0.0   0.3   0.4   1.0
10    46    1      1       1       0.0   0.3   0.4   1.0
16    22    1      2       1       0.0   0.3   0.4   2.5
16    34    1      3       1       0.0   0.5   0.6   1.0
```

In this example a number of features can be found. In columns `ai` and `aj` you find the atom numbers of the particles to be restrained. The `type` column should always be 1. As explained in 4.3.5, multiple distances can contribute to a single NOE signal. In the topology this can be set using the `index` column. In our example, the restraints 10-28 and 10-46 both have index 1, therefore they are treated simultaneously. An extra requirement for treating restraints together is that the restraints must be on successive lines, without any other intervening restraint. The `type'` column will usually be 1, but can be set to 2 to obtain a distance restraint that will never be time- and ensemble-averaged; this can be useful for restraining hydrogen bonds. The columns `low`, `up1`, and `up2` hold the values of r_0 , r_1 , and r_2 from eqn. 4.86. In some cases it can be useful to have different force constants for some restraints; this is controlled by the column `fac`. The force constant in the parameter file is multiplied by the value in the column `fac` for each restraint.

4.3.6 Orientation restraints

This section describes how orientations between vectors, as measured in certain NMR experiments, can be calculated and restrained in MD simulations. The presented refinement methodology and a comparison of results with and without time and ensemble averaging have been published [92].

Theory

In an NMR experiment, orientations of vectors can be measured when a molecule does not tumble completely isotropically in the solvent. Two examples of such orientation measurements are

residual dipolar couplings (between two nuclei) or chemical shift anisotropies. An observable for a vector \mathbf{r}_i can be written as follows:

$$\delta_i = \frac{2}{3} \text{tr}(\mathbf{S}\mathbf{D}_i) \quad (4.96)$$

where \mathbf{S} is the dimensionless order tensor of the molecule. The tensor \mathbf{D}_i is given by:

$$\mathbf{D}_i = \frac{c_i}{\|\mathbf{r}_i\|^\alpha} \begin{pmatrix} 3xx - 1 & 3xy & 3xz \\ 3xy & 3yy - 1 & 3yz \\ 3xz & 3yz & 3zz - 1 \end{pmatrix} \quad (4.97)$$

$$\text{with: } x = \frac{r_{i,x}}{\|\mathbf{r}_i\|}, \quad y = \frac{r_{i,y}}{\|\mathbf{r}_i\|}, \quad z = \frac{r_{i,z}}{\|\mathbf{r}_i\|} \quad (4.98)$$

For a dipolar coupling \mathbf{r}_i is the vector connecting the two nuclei, $\alpha = 3$ and the constant c_i is given by:

$$c_i = \frac{\mu_0}{4\pi} \gamma_1^i \gamma_2^i \frac{\hbar}{4\pi} \quad (4.99)$$

where γ_1^i and γ_2^i are the gyromagnetic ratios of the two nuclei.

The order tensor is symmetric and has trace zero. Using a rotation matrix \mathbf{T} it can be transformed into the following form:

$$\mathbf{T}^T \mathbf{S} \mathbf{T} = s \begin{pmatrix} -\frac{1}{2}(1 - \eta) & 0 & 0 \\ 0 & -\frac{1}{2}(1 + \eta) & 0 \\ 0 & 0 & 1 \end{pmatrix} \quad (4.100)$$

where $-1 \leq s \leq 1$ and $0 \leq \eta \leq 1$. s is called the order parameter and η the asymmetry of the order tensor \mathbf{S} . When the molecule tumbles isotropically in the solvent, s is zero, and no orientational effects can be observed because all δ_i are zero.

Calculating orientations in a simulation

For reasons which are explained below, the \mathbf{D} matrices are calculated which respect to a reference orientation of the molecule. The orientation is defined by a rotation matrix \mathbf{R} , which is needed to least-squares fit the current coordinates of a selected set of atoms onto a reference conformation. The reference conformation is the starting conformation of the simulation. In case of ensemble averaging, which will be treated later, the structure is taken from the first subsystem. The calculated \mathbf{D}_i^c matrix is given by:

$$\mathbf{D}_i^c(t) = \mathbf{R}(t) \mathbf{D}_i(t) \mathbf{R}^T(t) \quad (4.101)$$

The calculated orientation for vector i is given by:

$$\delta_i^c(t) = \frac{2}{3} \text{tr}(\mathbf{S}(t) \mathbf{D}_i^c(t)) \quad (4.102)$$

The order tensor $\mathbf{S}(t)$ is usually unknown. A reasonable choice for the order tensor is the tensor which minimizes the (weighted) mean square difference between the calculated and the observed orientations:

$$MSD(t) = \left(\sum_{i=1}^N w_i \right)^{-1} \sum_{i=1}^N w_i (\delta_i^c(t) - \delta_i^{exp})^2 \quad (4.103)$$

To properly combine different types of measurements, the unit of w_i should be such that all terms are dimensionless. This means the unit of w_i is the unit of δ_i to the power -2 . **Note** that scaling all w_i with a constant factor does not influence the order tensor.

Time averaging

Since the tensors \mathbf{D}_i fluctuate rapidly in time, much faster than can be observed in an experiment, they should be averaged over time in the simulation. However, in a simulation the time and the number of copies of a molecule are limited. Usually one can not obtain a converged average of the \mathbf{D}_i tensors over all orientations of the molecule. If one assumes that the average orientations of the \mathbf{r}_i vectors within the molecule converge much faster than the tumbling time of the molecule, the tensor can be averaged in an axis system that rotates with the molecule, as expressed by equation (4.101). The time-averaged tensors are calculated using an exponentially decaying memory function:

$$\mathbf{D}_i^a(t) = \frac{\int_{u=t_0}^t \mathbf{D}_i^c(u) \exp\left(-\frac{t-u}{\tau}\right) du}{\int_{u=t_0}^t \exp\left(-\frac{t-u}{\tau}\right) du} \quad (4.104)$$

Assuming that the order tensor \mathbf{S} fluctuates slower than the \mathbf{D}_i , the time-averaged orientation can be calculated as:

$$\delta_i^a(t) = \frac{2}{3} \text{tr}(\mathbf{S}(t) \mathbf{D}_i^a(t)) \quad (4.105)$$

where the order tensor $\mathbf{S}(t)$ is calculated using expression (4.103) with $\delta_i^c(t)$ replaced by $\delta_i^a(t)$.

Restraining

The simulated structure can be restrained by applying a force proportional to the difference between the calculated and the experimental orientations. When no time averaging is applied, a proper potential can be defined as:

$$V = \frac{1}{2} k \sum_{i=1}^N w_i (\delta_i^c(t) - \delta_i^{exp})^2 \quad (4.106)$$

where the unit of k is the unit of energy. Thus the effective force constant for restraint i is $k w_i$. The forces are given by minus the gradient of V . The force \mathbf{F}_i working on vector \mathbf{r}_i is:

$$\begin{aligned} \mathbf{F}_i(t) &= -\frac{dV}{d\mathbf{r}_i} \\ &= -k w_i (\delta_i^c(t) - \delta_i^{exp}) \frac{d\delta_i(t)}{d\mathbf{r}_i} \\ &= -k w_i (\delta_i^c(t) - \delta_i^{exp}) \frac{2c_i}{\|\mathbf{r}\|^{2+\alpha}} \left(2\mathbf{R}^T \mathbf{S} \mathbf{R} \mathbf{r}_i - \frac{2+\alpha}{\|\mathbf{r}\|^2} \text{tr}(\mathbf{R}^T \mathbf{S} \mathbf{R} \mathbf{r}_i \mathbf{r}_i^T) \mathbf{r}_i \right) \end{aligned}$$

Ensemble averaging

Ensemble averaging can be applied by simulating a system of M subsystems that each contain an identical set of orientation restraints. The systems only interact via the orientation restraint potential which is defined as:

$$V = M \frac{1}{2} k \sum_{i=1}^N w_i \langle \delta_i^c(t) - \delta_i^{exp} \rangle^2 \quad (4.107)$$

The force on vector $\mathbf{r}_{i,m}$ in subsystem m is given by:

$$\mathbf{F}_{i,m}(t) = -\frac{dV}{d\mathbf{r}_{i,m}} = -k w_i \langle \delta_i^c(t) - \delta_i^{exp} \rangle \frac{d\delta_{i,m}^c(t)}{d\mathbf{r}_{i,m}} \quad (4.108)$$

Time averaging

When using time averaging it is not possible to define a potential. We can still define a quantity that gives a rough idea of the energy stored in the restraints:

$$V = M \frac{1}{2} k^a \sum_{i=1}^N w_i \langle \delta_i^a(t) - \delta_i^{exp} \rangle^2 \quad (4.109)$$

The force constant k_a is switched on slowly to compensate for the lack of history at times close to t_0 . It is exactly proportional to the amount of average that has been accumulated:

$$k^a = k \frac{1}{\tau} \int_{u=t_0}^t \exp\left(-\frac{t-u}{\tau}\right) du \quad (4.110)$$

What really matters is the definition of the force. It is chosen to be proportional to the square root of the product of the time-averaged and the instantaneous deviation. Using only the time-averaged deviation induces large oscillations. The force is given by:

$$\mathbf{F}_{i,m}(t) = \begin{cases} 0 & \text{for } a b \leq 0 \\ k^a w_i \frac{a}{|a|} \sqrt{a b} \frac{d\delta_{i,m}^c(t)}{d\mathbf{r}_{i,m}} & \text{for } a b > 0 \end{cases} \quad (4.111)$$

$$\begin{aligned} a &= \langle \delta_i^a(t) - \delta_i^{exp} \rangle \\ b &= \langle \delta_i^c(t) - \delta_i^{exp} \rangle \end{aligned}$$

Using orientation restraints

Orientation restraints can be added to a molecule definition in the topology file in the section [orientation_restraints]. Here we give an example section containing five N-H residual dipolar coupling restraints:

```
[ orientation_restraints ]
; ai   aj   type   exp.   label   alpha   const.   obs.   weight
;                               Hz       nm^3      Hz      Hz^-2
  31   32     1     1     3       3       6.083   -6.73    1.0
  43   44     1     1     4       3       6.083   -7.87    1.0
  55   56     1     1     5       3       6.083   -7.13    1.0
  65   66     1     1     6       3       6.083   -2.57    1.0
  73   74     1     1     7       3       6.083   -2.10    1.0
```

The unit of the observable is Hz, but one can choose any other unit. In columns `ai` and `aj` you find the atom numbers of the particles to be restrained. The `type` column should always be 1. The `exp.` column denotes the experiment number, starting at 1. For each experiment a separate order tensor \mathbf{S} is optimized. The label should be a unique number larger than zero for each restraint. The `alpha` column contains the power α that is used in equation (4.97) to calculate the orientation. The `const.` column contains the constant c_i used in the same equation. The constant should have the unit of the observable times nm^α . The column `obs.` contains the observable, in any unit you like. The last column contains the weights w_i ; the unit should be the inverse of the square of the unit of the observable.

Some parameters for orientation restraints can be specified in the `grompp.mdp` file, for a study of the effect of different force constants and averaging times and ensemble averaging see [92].

4.4 Polarization

Polarization can be treated by GROMACS by attaching shell (Drude) particles to atoms and/or virtual sites. The energy of the shell particle is then minimized at each time step in order to remain on the Born-Oppenheimer surface.

4.4.1 Simple polarization

This is merely a harmonic potential with equilibrium distance 0.

4.4.2 Water polarization

A special potential for water that allows anisotropic polarization of a single shell particle [43].

4.4.3 Thole polarization

Based on early work by Thole [93], Roux and coworkers have implemented potentials for molecules like ethanol [94, 95, 96]. Within such molecules, there are intra-molecular interactions between shell particles, however these must be screened because full Coulomb would be too strong. The potential between two shell particles i and j is:

$$V_{thole} = \frac{q_i q_j}{r_{ij}} \left[1 - \left(1 + \frac{\bar{r}_{ij}}{2} \right) \exp^{-\bar{r}_{ij}} \right] \quad (4.112)$$

Note that there is a sign error in Equation 1 of Noskov *et al.* [96]:

$$\bar{r}_{ij} = a \frac{r_{ij}}{(\alpha_i \alpha_j)^{1/6}} \quad (4.113)$$

where a is a magic (dimensionless) constant, usually chosen to be 2.6 [96]; α_i and α_j are the polarizabilities of the respective shell particles.

4.5 Free energy interactions

This section describes the λ -dependence of the potentials used for free energy calculations (see sec. 3.12). All common types of potentials and constraints can be interpolated smoothly from state A ($\lambda = 0$) to state B ($\lambda = 1$) and vice versa. All bonded interactions are interpolated by linear interpolation of the interaction parameters. Non-bonded interactions can be interpolated linearly or via soft-core interactions.

Starting in GROMACS 4.6, λ is a vector, allowing different components of the free energy transformation to be carried out at different rates. Coulomb, Lennard-Jones, bonded, and restraint terms can all be controlled independently, as described in the `.mdp` options.

Harmonic potentials

The example given here is for the bond potential, which is harmonic in GROMACS. However, these equations apply to the angle potential and the improper dihedral potential as well.

$$V_b = \frac{1}{2} \left[(1 - \lambda)k_b^A + \lambda k_b^B \right] \left[b - (1 - \lambda)b_0^A - \lambda b_0^B \right]^2 \quad (4.114)$$

$$\begin{aligned} \frac{\partial V_b}{\partial \lambda} &= \frac{1}{2} (k_b^B - k_b^A) \left[b - (1 - \lambda)b_0^A + \lambda b_0^B \right]^2 + \\ &\quad (b_0^A - b_0^B) \left[b - (1 - \lambda)b_0^A - \lambda b_0^B \right] \left[(1 - \lambda)k_b^A + \lambda k_b^B \right] \end{aligned} \quad (4.115)$$

GROMOS-96 bonds and angles

Fourth-power bond stretching and cosine-based angle potentials are interpolated by linear interpolation of the force constant and the equilibrium position. Formulas are not given here.

Proper dihedrals

For the proper dihedrals, the equations are somewhat more complicated:

$$V_d = \left[(1 - \lambda)k_d^A + \lambda k_d^B \right] \left(1 + \cos \left[n_\phi \phi - (1 - \lambda)\phi_s^A - \lambda \phi_s^B \right] \right) \quad (4.116)$$

$$\begin{aligned} \frac{\partial V_d}{\partial \lambda} &= (k_d^B - k_d^A) \left(1 + \cos \left[n_\phi \phi - (1 - \lambda)\phi_s^A - \lambda \phi_s^B \right] \right) + \\ &\quad (\phi_s^B - \phi_s^A) \left[(1 - \lambda)k_d^A - \lambda k_d^B \right] \sin \left[n_\phi \phi - (1 - \lambda)\phi_s^A - \lambda \phi_s^B \right] \end{aligned} \quad (4.117)$$

Note: that the multiplicity n_ϕ can not be parameterized because the function should remain periodic on the interval $[0, 2\pi]$.

Tabulated bonded interactions

For tabulated bonded interactions only the force constant can be interpolated:

$$V = ((1 - \lambda)k^A + \lambda k^B) f \quad (4.118)$$

$$\frac{\partial V}{\partial \lambda} = (k^B - k^A) f \quad (4.119)$$

Coulomb interaction

The Coulomb interaction between two particles of which the charge varies with λ is:

$$V_c = \frac{f}{\varepsilon_{rf} r_{ij}} \left[(1 - \lambda) q_i^A q_j^A + \lambda q_i^B q_j^B \right] \quad (4.120)$$

$$\frac{\partial V_c}{\partial \lambda} = \frac{f}{\varepsilon_{rf} r_{ij}} \left[-q_i^A q_j^A + q_i^B q_j^B \right] \quad (4.121)$$

where $f = \frac{1}{4\pi\varepsilon_0} = 138.935\,485$ (see chapter 2).

Coulomb interaction with reaction field

The Coulomb interaction including a reaction field, between two particles of which the charge varies with λ is:

$$V_c = f \left[\frac{1}{r_{ij}} + k_{rf} r_{ij}^2 - c_{rf} \right] \left[(1 - \lambda) q_i^A q_j^A + \lambda q_i^B q_j^B \right] \quad (4.122)$$

$$\frac{\partial V_c}{\partial \lambda} = f \left[\frac{1}{r_{ij}} + k_{rf} r_{ij}^2 - c_{rf} \right] \left[-q_i^A q_j^A + q_i^B q_j^B \right] \quad (4.123)$$

Note that the constants k_{rf} and c_{rf} are defined using the dielectric constant ε_{rf} of the medium (see sec. 4.1.4).

Lennard-Jones interaction

For the Lennard-Jones interaction between two particles of which the *atom type* varies with λ we can write:

$$V_{LJ} = \frac{(1 - \lambda)C_{12}^A + \lambda C_{12}^B}{r_{ij}^{12}} - \frac{(1 - \lambda)C_6^A + \lambda C_6^B}{r_{ij}^6} \quad (4.124)$$

$$\frac{\partial V_{LJ}}{\partial \lambda} = \frac{C_{12}^B - C_{12}^A}{r_{ij}^{12}} - \frac{C_6^B - C_6^A}{r_{ij}^6} \quad (4.125)$$

It should be noted that it is also possible to express a pathway from state A to state B using σ and ϵ (see eqn. 4.5). It may seem to make sense physically to vary the force field parameters σ and ϵ rather than the derived parameters C_{12} and C_6 . However, the difference between the pathways in parameter space is not large, and the free energy itself does not depend on the pathway, so we use the simple formulation presented above.

Kinetic Energy

When the mass of a particle changes, there is also a contribution of the kinetic energy to the free energy (note that we can not write the momentum \mathbf{p} as $m\mathbf{v}$, since that would result in the sign of $\frac{\partial E_k}{\partial \lambda}$ being incorrect [97]):

$$E_k = \frac{1}{2} \frac{\mathbf{p}^2}{(1-\lambda)m^A + \lambda m^B} \quad (4.126)$$

$$\frac{\partial E_k}{\partial \lambda} = -\frac{1}{2} \frac{\mathbf{p}^2(m^B - m^A)}{((1-\lambda)m^A + \lambda m^B)^2} \quad (4.127)$$

after taking the derivative, we *can* insert $\mathbf{p} = m\mathbf{v}$, such that:

$$\frac{\partial E_k}{\partial \lambda} = -\frac{1}{2} \mathbf{v}^2 (m^B - m^A) \quad (4.128)$$

Constraints

The constraints are formally part of the Hamiltonian, and therefore they give a contribution to the free energy. In GROMACS this can be calculated using the LINCS or the SHAKE algorithm. If we have a number of constraint equations g_k :

$$g_k = \mathbf{r}_k - d_k \quad (4.129)$$

where \mathbf{r}_k is the distance vector between two particles and d_k is the constraint distance between the two particles, we can write this using a λ -dependent distance as

$$g_k = \mathbf{r}_k - ((1-\lambda)d_k^A + \lambda d_k^B) \quad (4.130)$$

the contribution C_λ to the Hamiltonian using Lagrange multipliers λ :

$$C_\lambda = \sum_k \lambda_k g_k \quad (4.131)$$

$$\frac{\partial C_\lambda}{\partial \lambda} = \sum_k \lambda_k (d_k^B - d_k^A) \quad (4.132)$$

4.5.1 Soft-core interactions

In a free-energy calculation where particles grow out of nothing, or particles disappear, using the simple linear interpolation of the Lennard-Jones and Coulomb potentials as described in Equations 4.125 and 4.123 may lead to poor convergence. When the particles have nearly disappeared, or are close to appearing (at λ close to 0 or 1), the interaction energy will be weak enough for particles to get very close to each other, leading to large fluctuations in the measured values of $\partial V / \partial \lambda$ (which, because of the simple linear interpolation, depends on the potentials at both the endpoints of λ).

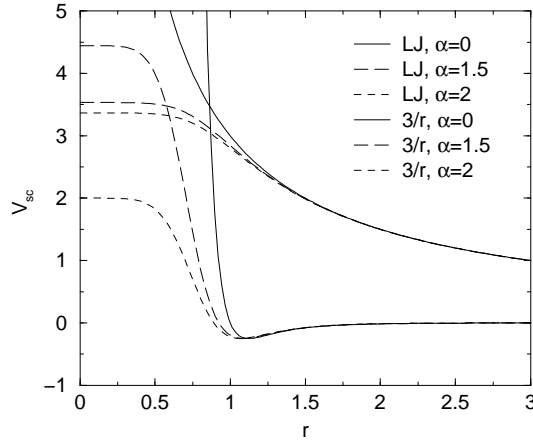


Figure 4.17: Soft-core interactions at $\lambda = 0.5$, with $p = 2$ and $C_6^A = C_{12}^A = C_6^B = C_{12}^B = 1$.

To circumvent these problems, the singularities in the potentials need to be removed. This can be done by modifying the regular Lennard-Jones and Coulomb potentials with “soft-core” potentials that limit the energies and forces involved at λ values between 0 and 1, but not at $\lambda = 0$ or 1.

In GROMACS the soft-core potentials V_{sc} are shifted versions of the regular potentials, so that the singularity in the potential and its derivatives at $r = 0$ is never reached:

$$V_{sc}(r) = (1 - \lambda)V^A(r_A) + \lambda V^B(r_B) \quad (4.133)$$

$$r_A = \left(\alpha \sigma_A^6 \lambda^p + r^6 \right)^{\frac{1}{6}} \quad (4.134)$$

$$r_B = \left(\alpha \sigma_B^6 (1 - \lambda)^p + r^6 \right)^{\frac{1}{6}} \quad (4.135)$$

where V^A and V^B are the normal “hard core” Van der Waals or electrostatic potentials in state A ($\lambda = 0$) and state B ($\lambda = 1$) respectively, α is the soft-core parameter (set with `sc_alpha` in the `.mdp` file), p is the soft-core λ power (set with `sc_power`), σ is the radius of the interaction, which is $(C_{12}/C_6)^{1/6}$ or an input parameter (`sc_sigma`) when C_6 or C_{12} is zero.

For intermediate λ , r_A and r_B alter the interactions very little for $r > \alpha^{1/6}\sigma$ and quickly switch the soft-core interaction to an almost constant value for smaller r (Fig. 4.17). The force is:

$$F_{sc}(r) = -\frac{\partial V_{sc}(r)}{\partial r} = (1 - \lambda)F^A(r_A) \left(\frac{r}{r_A} \right)^5 + \lambda F^B(r_B) \left(\frac{r}{r_B} \right)^5 \quad (4.136)$$

where F^A and F^B are the “hard core” forces. The contribution to the derivative of the free energy is:

$$\begin{aligned} \frac{\partial V_{sc}(r)}{\partial \lambda} &= V^B(r_B) - V^A(r_A) + (1 - \lambda) \frac{\partial V^A(r_A)}{\partial r_A} \frac{\partial r_A}{\partial \lambda} + \lambda \frac{\partial V^B(r_B)}{\partial r_B} \frac{\partial r_B}{\partial \lambda} \\ &= V^B(r_B) - V^A(r_A) + \\ &\quad \frac{p\alpha}{6} \left[\lambda F^B(r_B) r_B^{-5} \sigma_B^6 (1 - \lambda)^{p-1} - (1 - \lambda) F^A(r_A) r_A^{-5} \sigma_A^6 \lambda^{p-1} \right] \end{aligned} \quad (4.137)$$

The original GROMOS Lennard-Jones soft-core function [98] uses $p = 2$, but $p = 1$ gives a smoother $\partial H/\partial \lambda$ curve.

Another issue that should be considered is the soft-core effect of hydrogens without Lennard-Jones interaction. Their soft-core σ is set with `sc-sigma` in the `.mdp` file. These hydrogens produce peaks in $\partial H/\partial \lambda$ at λ is 0 and/or 1 for $p = 1$ and close to 0 and/or 1 with $p = 2$. Lowering `sc-sigma` will decrease this effect, but it will also increase the interactions with hydrogens relative to the other interactions in the soft-core state.

When soft core potentials are selected (by setting `sc-alpha > 0`), and the Coulomb and Lennard-Jones potentials are turned on or off sequentially, then the Coulombic interaction is turned off linearly, rather than using soft core interactions, which should be less statistically noisy in most cases. This behavior can be overwritten by using the mdp option `sc-coul` to `yes`. Additionally, the soft-core interaction potential is only applied when either the A or B state has zero interaction potential. If both A and B states have nonzero interaction potential, default linear scaling described above is used. When both Coulombic and Lennard-Jones interactions are turned off simultaneously, a soft-core potential is used, and a hydrogen is being introduced or deleted, the sigma is set to `sc-sigma-min`, which itself defaults to `sc-sigma-default`.

Recently, a new formulation of the soft-core approach has been derived that in most cases gives lower and more even statistical variance than the standard soft-core path described above. [99, 100] Specifically, we have:

$$V_{sc}(r) = (1 - \lambda)V^A(r_A) + \lambda V^B(r_B) \quad (4.138)$$

$$r_A = \left(\alpha \sigma_A^{48} \lambda^p + r^{48} \right)^{\frac{1}{48}} \quad (4.139)$$

$$r_B = \left(\alpha \sigma_B^{48} (1 - \lambda)^p + r^{48} \right)^{\frac{1}{48}} \quad (4.140)$$

This “1-1-48” path is also implemented in GROMACS. Note that for this path the soft core α should satisfy $0.001 < \alpha < 0.003$, rather than $\alpha \approx 0.5$.