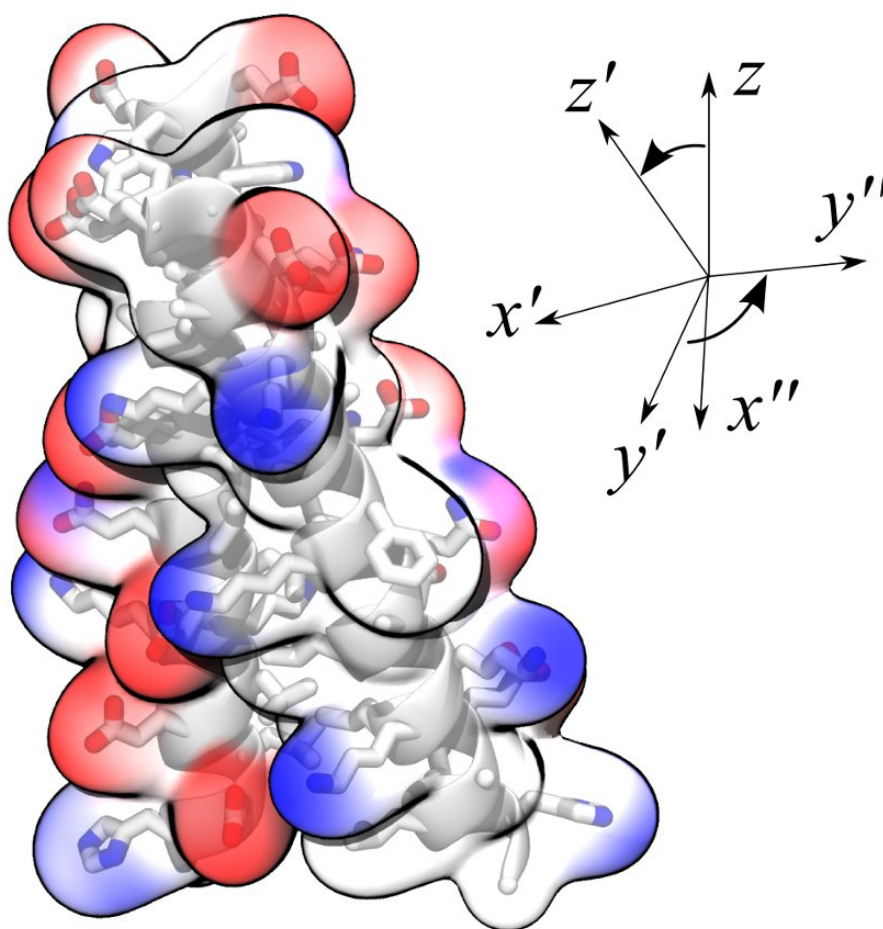# COLLECTIVE VARIABLES MODULE
Reference manual for Tinker-HP

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Updated versions of this manual: [GROMACS] [LAMMPS] [NAMD] [Tinker-HP] [VMD]

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(HTML version)

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1 Overview

In molecular dynamics simulations, it is often useful to reduce the large number of degrees of freedom of a physical system into few parameters whose statistical distributions can be analyzed individually, or used to define biasing potentials to alter the dynamics of the system in a controlled manner. These have been called 'order parameters', 'collective variables', '(surrogate) reaction coordinates', and many other terms.

Here we use primarily the term 'collective variable', often shortened to *colvar*, to indicate any differentiable function of atomic Cartesian coordinates, x_i, with i between 1 and N, the total number of atoms:

$$\xi(t) = \xi(X(t)) = \xi(x_i(t), x_j(t), x_k(t), \ldots) , \ \ 1 \le i, j, k \ldots \le N \tag{1}$$

This manual documents the collective variables module (**Colvars**), a software that provides an implementation for the functions $\xi(X)$ with a focus on flexibility, robustness and high performance. The module is designed to perform multiple tasks concurrently during or after a simulation, the most common of which are:

- apply restraints or biasing potentials to multiple variables, tailored on the system by choosing from a wide set of basis functions, without limitations on their number or on the number of atoms involved;

- calculate potentials of mean force (PMFs) along any set of variables, using different enhanced sampling methods, such as Adaptive Biasing Force (ABF), metadynamics, steered MD and umbrella sampling; variants of these methods that make use of an ensemble of replicas are supported as well;

- calculate statistical properties of the variables, such as running averages and standard deviations, correlation functions of pairs of variables, and multidimensional histograms: this can be done either at run-time without the need to save very large trajectory files, or after a simulation has been completed (post-processing).

Detailed explanations of the design of the Colvars module are provided in reference [1]. Please cite this reference whenever publishing work that makes use of this module, alongside any other publications for specific features being, according to the usage summary printed when running a Colvars-enabled MD simulation or analysis.

2 Writing a Colvars configuration: a crash course

The Colvars configuration is a plain text file or string that defines collective variables, biases, and general parameters of the Colvars module. It is passed to the module using back-end-specific commands documented in section 3.

Example: steering two groups of atoms away from each other. Now let us look at a complete, non-trivial configuration. Suppose that we want to run a steered MD experiment where a small molecule is pulled away from a protein binding site. In Colvars terms, this is done by applying a moving restraint to the distance between the two objects. The configuration will contain two blocks, one defining the distance variable (see section 4 and 4.3.1), and the other the moving harmonic restraint (6.8).

```
colvar {
  name dist
  distance {
    group1 { atomNumbersRange 42-55 }
    group2 { indexGroup C-alpha_15-30 }
  }
}

harmonic {
  colvars dist
  forceConstant 20.0
  centers 4.0          # initial distance
  targetCenters 15.0  # final distance
  targetNumSteps 500000
}
```

Reading this input in plain English: the variable here named *dist* consists in a distance function between the centers of two groups: the ligand (atoms 42 to 55) and the α-carbon atoms of residues 15 to 30 in the protein . To the "*dist*" variable, we apply a `harmonic` potential of force constant 20 kcal/mol/Å^2, initially centered around a value of 4 Å, which will increase to 15 Å over 500,000 simulation steps.

The atom selection keywords are detailed in section 5.

Example: using multiple variables and multiple biasing/analysis methods together. A more complex example configuration is included below, showing how a variable may be constructed by combining multiple existing functions, and how multiple variables or multiple biases may be used concurrently. The colvar indicated below as "d" is defined as the difference between two distances (see 4.3): the first distance (d_1) is taken between the center of mass of atoms 1 and 2 and that of atoms 3 to 5, the second (d_2) between atom 7 and the center of mass of atoms 8 to 10 (see 5). The difference $d = d_1 - d_2$ is obtained by multiplying the two by a coefficient $C = +1$ or $C = -1$, respectively (see 4.18). The colvar called "c" is the coordination number calculated between atoms

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1 to 10 and atoms 11 to 20. A harmonic restraint (see 6.8) is applied to both d and c: to allow using the same force constant K, both d and c are scaled by their respective fluctuation widths w_d and w_c. The values of "c" are also recorded throughout the simulation as a joint 2-dimensional histogram (see 6.13).

```
colvar {
  # difference of two distances
  name d
  width 0.2  # estimated fluctuation width
  distance {
    componentCoeff  1.0
    group1 { atomNumbers 1 2 }
    group2 { atomNumbers 3 4 5 }
  }
  distance {
    componentCoeff -1.0
    group1 { atomNumbers 7 }
    group2 { atomNumbers 8 9 10 }
  }
}

colvar {
  name c
  coordNum {
    cutoff 6.0
    group1 { atomNumbersRange  1-10 }
    group2 { atomNumbersRange 11-20 }
    tolerance 1.0e-6
    pairListFrequency 1000
  }
}

harmonic {
  colvars d c
  centers 3.0 4.0
  forceConstant 5.0
}

histogram {
  colvars c
}
```

3 Enabling and controlling the Colvars module in Tinker-HP

Here, we document the syntax of the commands and parameters used to set up and use the Colvars module in Tinker-HP. One of these parameters is the configuration file or the configuration text for the module itself, whose syntax is described in 3.3 and in the following sections.

3.1 Units in the Colvars module

The "internal units" of the Colvars module are the units in which values are expressed in the configuration file, and in which collective variable values, energies, etc. are expressed in the output and colvars trajectory files. Generally **the Colvars module uses internally the same units as its back-end MD engine, with the exception of VMD**, where different unit sets are supported to allow for easy setup, visualization and analysis of Colvars simulations performed with any simulation engine.

Note that **angles** are expressed in degrees, and derived quantities such as force constants are based on degrees as well. Some colvar components have default values, expressed in Ångström (Å) in this documentation. They are converted to the current length unit, if different from Å. Atomic coordinates read from **XYZ files** (and PDB files where applicable) are expected to be expressed in Ångström, no matter what unit system is in use by the back-end (Tinker-HP) or the Colvars Module. They are converted internally to the current length unit as needed. Note that force constants in `harmonic` and `harmonicWalls` biases (6.8) are rescaled according to the `width` parameter of colvars, so that they are formally in energy units, although if `width` is given its default value of 1.0, force constants are effectively expressed in kcal/mol/$(colvar\ unit)^2$.

To avoid errors due to reading configuration files written in a different unit system, it can be specified within the input:

- `units` — *Unit system to be used*

  [string, context: global]

  A string defining the units to be used internally by Colvars. In Tinker-HP, the only supported value is Tinker-HP's native units: *real* (Å, kcal/mol).

3.2 Running Colvars in Tinker-HP

To enable Colvars within a Tinker-HP run, just provide a Colvars configuration file with the same prefix as the `.key` file, and the extension `.colvars`. Tcl-scripted variables and biases may be defined by sourcing a script file using the `sourceTclFile` option.

3.3 Configuration syntax used by the Colvars module

Configuration for the Colvars module is passed using an external file. Configuration lines follow the format "`keyword value`" or "`keyword { ... }`", where the keyword and its value must be separated by one or more space characters. The following formatting rules apply:

- *Keywords are case-insensitive*; for example, `upperBoundary` is the same as `upperboundary` and `UPPERBOUNDARY`); note that their string values are however still case-sensitive (e.g. names of variables, file names).

- A long value, or a list of multiple values, can be distributed across multiple lines by using *curly braces*, "{" and "}": the opening brace "{" must occur on the same line as the keyword, following at least one space character; the closing brace "}" may be at any position after that; any keywords following the closing brace on the same line are not valid (they should appear instead on a different line).

- Many keywords are nested, and are only meaningful within the specific context of another keyword; for example, the keyword `name` is available inside the block of the keyword `colvar {...}`, but not outside of it; for every keyword documented in the following, the *"parent" keyword* that defines such context is also indicated.

- If a keyword requiring a boolean value (`yes|on|true` or `no|off|false`) is provided without an explicit value, it defaults to '`yes|on|true`'; for example, '`outputAppliedForce`' may be used as shorthand for '`outputAppliedForce on`'.

- The hash character "#" indicates a *comment*: all text in the same line following this character will be ignored.

- Outside of comments, *only ASCII characters* are allowed for defining keywords, and the only white-space characters supported are spaces, tabs and newlines: a warning will be printed upon detection of non-ASCII characters in a configuration line, which include both characters that are visibly "special", as well as those with a very similar appearance to ASCII ones (for instance, non-breaking spaces); common ways to identify/remove non-ASCII characters are using the Emacs text editor, or using `LC_ALL=C vi`.

3.4 Global keywords

The following keywords are available in the global context of the Colvars configuration, i.e. they are not nested inside other keywords:

- `colvarsTrajFrequency` — *Colvar value trajectory frequency*

  Default: 100 [positive integer, context: global]

  The values of each colvar (and of other related quantities, if requested) are written to the file *output*`.colvars.traj` every these many steps throughout the simulation. If the value is 0, such trajectory file is not written. For optimization the output is buffered, and synchronized with the disk only when the restart file is being written.

- `colvarsRestartFrequency` — *Colvar module restart frequency*

  Default: [positive integer, context: global]

  When this value is non-zero, a state file suitable for restarting will be written every these many steps. Additionally, any other output files produced by Colvars are written as well (except the trajectory file, which is written every `colvarsTrajFrequency` steps). It is generally a good idea to leave this parameter at its default value, unless needed for special cases or to disable automatic writing of output files altogether.

- `indexFile` — *Index file for atom selection (GROMACS "ndx" format)*

  [UNIX filename, context: global]

  This option reads an index file (usually with a `.ndx` extension) as produced by the `make_ndx` tool of GROMACS. This keyword may be repeated to load multiple index files. A group with the same name may appear multiple times, as long as it contains the same indices in identical order each time: an error is raised otherwise. The names of index groups contained in this file can then be used to define atom groups with the `indexGroup` keyword. Other supported methods to select atoms are described in 5.

- `smp` — *Whether SMP parallelism should be used*

  Default: `on` [boolean, context: global]

  If this flag is enabled (default), SMP parallelism over threads will be used to compute variables and biases, provided that this is supported by the Tinker-HP build in use.

- `sourceTclFile` — *Tcl file to be sourced at startup*

  [UNIX filename, context: global]

  This option causes the provided Tcl script file to be loaded at startup, e.g. to initialize the Tcl callback procedures for scripted variables or biases. This is the only way to define such callbacks in Tinker-HP.

3.5 Input state file

Several of the sampling methods implemented in Colvars are time- or history-dependent, i.e. they work by accumulating data as a simulation progresses, and use these data to determine their biasing forces. If the simulation engine uses a checkpoint or restart file (as GROMACS and LAMMPS do), any data needed by Colvars are embedded into that file. Otherwise, a dedicated *state file* can be loaded into Colvars directly.

When a dedicated Colvars state file is used, it may be in either one of two formats:

- Formatted, i.e. *"text"* format, which takes more space and is slower to to load/save but is also portable across different platforms and even different simulation engines (save for changes in physical units). This format is used by default, unless explicitly requested otherwise.

- Unformatted, i.e. *"binary"* format, which is both space-efficient and quick to load/save, but requires that the same Tinker-HP build was used to write the file and that the Colvars configuration remains the same. This format is supported by Colvars versions starting 2023-09-

25. Colvars state files can be written in binary format by setting the environment variable "`COLVARS_BINARY_RESTART`" to 1.

In either format, the state file contains accumulated data as well as the step number at the end of the run. The step number read from a state file overrides any value that Tinker-HP provides, and will be incremented if the simulation proceeds. This means that the step number used internally by Colvars may not always match the step number reported by Tinker-HP.

3.5.1 Changing configuration upon restarting.

In some cases, it is useful to modify the configuration of variables or biases between consecutive runs, for example by adding or removing a restraint. Some special provisions will happen in that case. When a state file is loaded, no information is available about any newly added variable or bias, which will thus remain uninitialized until the first compute step. Conversely, any information that the state file may contain about variables or biases that are no longer defined will be silently ignored. Please note that these checks are performed based only on the *names* of variables and biases: it is your responsibility to ensure that these names have *consistent definitions between runs.*

The flexibility just described carries some limitations: namely, it is only supported when reading *text-format* Colvars state files. Instead, restarting from binary files after a configuration change will trigger an error. It is also important to remind that when switching to a different build of Tinker-HP, the binary format may change slightly, even if the release version is the same.

To work around the potential issues just described, a text-format Colvars state file should be loaded.

3.6 Output files

When the output prefix *output* is defined, the following output files are written during a simulation run:

- A *state file*, named *output*`.colvars.state`, which is written at the end of the specified run. This file is in plain text format by default, or in binary format if the environment variable `COLVARS_BINARY_RESTART` is set to a non-zero integer. The state file can be used to continue a simulation: unless its contents are embedded in the checkpoint file of the MD engine itself (currently, GROMACS and LAMMPS support this), instructions for loading the Colvars state file will be required in the simulation script (see 3.5).

- If the parameter `colvarsRestartFrequency` is larger than zero and the *restart* prefix is defined, a *restart file* is written every that many steps: this file is fully equivalent to the final state file. The name of this file is *restart*`.colvars.state`.

- If the parameter `colvarsTrajFrequency` is greater than 0 (default value: 100 steps), a *trajectory file*, named *output*`.colvars.traj`, is written during the simulation. Unlike a state file, this file is not needed to restart a simulation, but can be used for post-processing and analysis. The format of this file is described in sec. 3.7.5.

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- Additionally, certain features, when enabled, can emit output files with a specific purpose: for example, potentials of mean force (PMFs) can be written to file to be analyzed or plotted. These files are described in the respective sections, but as a general rule they all use names beginning with the *output* prefix. Like the trajectory file, these additional files are needed only for analyzing a simulation's results, but not to continue it.

3.7   File formats

This section summarizes the file formats of various files that Colvars may be reading or writing.

3.7.1   Configuration and state files.

Configuration files are text files that are generally read as input by Tinker-HP. Starting from version 2017-02-01, changes in newline encodings are handled transparently, i.e. it is possible to typeset a configuration file in Windows (CR-LF newlines) and then use it with Linux or macOS (LF-only newlines).

Formatted state files, although not written manually, follow otherwise the same text format as configuration files. `Binary state files` can only be read by the Colvars code itself.

3.7.2   Index (NDX) files

For atom selections that cannot be specified only by using internal Colvars keywords, external *index files* may also be used following the NDX format used in GROMACS:

```
[ group_1_name ]
  i1  i2  i3  i4  ...
  ...            ... iN
[ group_2_name ]
  ...
```

where *i1* through *iN* are 1-based indices. Each group name may not contain spaces or tabs: otherwise, a parsing error will be raised.

Multiple index files may be provided to Colvars, each using the keyword `indexFile`. Two index files may contain groups with the same names, however these must also represent identical atom selections, i.e. the same sequence of indices including order.

Other than with GROMACS, an index group may also be generated from the VMD command-line interface, using the helper function `write_index_group` provided in the `colvartools` folder:

```
source colvartools/write_index_group.tcl
set sel [atomselect top "resname XXX and not hydrogen"]
write_index_group indexfile.ndx $sel "Ligand"
```

3.7.3 XYZ coordinate files

XYZ coordinate files are text files with the extension ".xyz". They are read by the Colvars module using an internal reader, and expect the following format:

$$N$$

$$Comment \quad line$$

$$
\begin{array}{llll}
E_1 & x_1 & y_1 & z_1 \\
E_2 & x_2 & y_2 & z_2 \\
\ldots \\
E_N & x_N & y_N & z_N
\end{array}
$$

where N is the number of atomic coordinates in the file and E_i is the chemical element of the i-th atom. Because E_i is not used in Colvars, any string that does not contain tabs or spaces is acceptable.

Note: all XYZ coordinates are assumed to be expressed in Å units.

An XYZ file may contain either one of the following scenarios:

(i) The file contains as many coordinates as the atoms that they are being read for: all coordinates will be read from the file following the same order as the atoms appear in the selection generated using the keywords listed in section 5. (Note that the order is guaranteed only if a single type of selection keyword is used one or more times, and not guaranteed when different types of selection keywords are used.)

(ii) The file contains more coordinates than needed, and it is assumed to contain coordinates for the entire system: only coordinates that match the numeric indices of the selected atoms are read, in order of increasing number.

XYZ-file coordinates are read directly by Colvars and stored internally as double-precision floating point numbers.

3.7.4 Grid files: multicolumn text format

Many simulation methods and analysis tools write files that contain functions of the collective variables tabulated on a grid (e.g. potentials of mean force or multidimensional histograms) for the purpose of analyzing results. Such files are produced by ABF (6.3), metadynamics (6.6), multidimensional histograms (6.13), as well as any restraint with optional thermodynamic integration support (6.2).

In some cases, these files may also be read as input of a new simulation. Suitable input files for this purpose are typically generated as output files of previous simulations, or directly by the user in the specific case of ensemble-biased metadynamics (6.6.5). This section explains the "multicolumn" format used by these files. For a multidimensional function $f(\xi_1, \xi_2, \ldots)$ the multicolumn grid format is defined as follows:

```
#   N_cv
#   min(ξ₁)      width(ξ₁)      npoints(ξ₁)      periodic(ξ₁)
#   min(ξ₂)      width(ξ₂)      npoints(ξ₂)      periodic(ξ₂)
#   ...          ...            ...              ...
#   min(ξ_{N_cv})  width(ξ_{N_cv})  npoints(ξ_{N_cv})  periodic(ξ_{N_cv})

     ξ₁¹          ξ₂¹            ...              ξ_{N_cv}¹           f(ξ₁¹, ξ₂¹, ..., ξ_{N_cv}¹)
     ξ₁¹          ξ₂¹            ...              ξ_{N_cv}²           f(ξ₁¹, ξ₂¹, ..., ξ_{N_cv}²)
     ...          ...            ...              ...                ...
```

Lines beginning with the character "#" are the header of the file. N_{cv} is the number of collective variables sampled by the grid. For each variable ξ_i, $\mathtt{min}(\xi_i)$ is the lowest value sampled by the grid (i.e. the left-most boundary of the grid along ξ_i), $\mathtt{width}(\xi_i)$ is the width of each grid step along ξ_i, $\mathtt{npoints}(\xi_i)$ is the number of points and $\mathtt{periodic}(\xi_i)$ is a flag whose value is 1 or 0 depending on whether the grid is periodic along ξ_i. In most situations:

- $\mathtt{min}(\xi_i)$ is given by the lowerBoundary keyword of the variable ξ_i;

- $\mathtt{width}(\xi_i)$ is given by the width keyword;

- $\mathtt{npoints}(\xi_i)$ is calculated from the two above numbers and the upperBoundary keyword;

- $\mathtt{periodic}(\xi_i)$ is set to 1 if and only if ξ_i is periodic and the grids' boundaries cover its period.

How the grid's boundaries affect the sequence of points depends on how the contents of the file were computed. In many cases, such as histograms and PMFs computed by metadynamics (6.6.5), the values of ξ_i in the first few columns correspond to the *midpoints* of the corresponding bins, i.e. $\xi_1^1 = \mathtt{min}(\xi_i) + \mathtt{width}(\xi_i)/2$. However, there is a slightly different format in PMF files computed by ABF (6.3) or other biases that use thermodynamic integration (6.2). In these cases, it is free-energy gradients that are accumulated on an (npoints)-long grid along each variable ξ: after these gradients are integrated, the resulting PMF is discretized on a slightly larger grid with (npoints+1) points along ξ (unless the interval is periodic). Therefore, the grid's outer edges extend by $\mathtt{width}(\xi_i)/2$ above and below the specified boundaries, so that for instance $\mathtt{min}(\xi_i)$ in the header appears to be shifted back by $\mathtt{width}(\xi_i)/2$ compared to what would be expected. *Please keep this difference in mind when comparing PMFs computed by different methods.*

After the header, the rest of the file contains values of the tabulated function $f(\xi_1, \xi_2, \dots \xi_{N_{cv}})$, one for each line. The first N_{cv} columns contain values of $\xi_1, \xi_2, \dots \xi_{N_{cv}}$ and the last column contains the value of the function f. Points are sorted in ascending order with the fastest-changing values at the right ("C-style" order). Each sweep of the right-most variable $\xi_{N_{cv}}$ is terminated by an empty line. For two dimensional grid files, this allows quick visualization by programs such as GNUplot.

Example 1: multicolumn text file for a one-dimensional histogram with lowerBoundary = 15, upperBoundary = 48 and width = 0.1.

```
#  1
#  15      0.1            330  0

   15.05  6.14012e-07
   15.15  7.47644e-07
   ...     ...
   47.85  1.65944e-06
   47.95  1.46712e-06
```

Example 2: multicolumn text file for a two-dimensional histogram of two dihedral angles (periodic interval with 6° bins):

```
#  2
#  -180.0  6.0     30          1
#  -180.0  6.0     30          1

   -177.0  -177.0  8.97117e-06
   -177.0  -171.0  1.53525e-06
   ...     ...     ...
   -177.0   177.0  2.442956-06

   -171.0  -177.0  2.04702e-05
   ...     ...     ...
```

3.7.5 Output trajectory files

The Colvars trajectory file (with a suffix `.colvars.traj`) is a plain text file (scientific notation with 14-digit precision) whose columns represent quantities such as colvar values, applied forces, or individual restraints' energies. Under most scenarios, plotting or analyzing this file is straight-forward: for example, the following contains a variable "A" and the energy of a restraint "rA":

```
#     step   A                        E_rA
         0    1.42467449615693e+01   6.30982865292123e+02
       100    1.42282559728026e+01   6.20640585041317e+02
...
```

Occasionally, if the Colvars configuration is changed mid-run certain quantities may be added or removed, changing the column layout. Labels in comment lines can assist in such cases: for example, consider the trajectory above with the addition of a second variable, "B", after 10,000 steps:

```
#     step   A                        E_rA
         0    1.42467449615693e+01   6.30982865292123e+02
       100    1.42282559728026e+01   6.20640585041317e+02
...
#     step   A                        B                        E_rA
```

15

```
        10000      1.38136915830229e+01  9.99574098859265e-01  4.11184644791030e+02
        10100      1.36437184346326e+01  9.99574091957314e-01  3.37726286543895e+02
```

Analyzing the above file with standard tools is possible, but laborious: a convenience script is provided for this and related purposes. It may be used either as a command-line tool or as a Python module:

```
>>> from plot_colvars_traj import Colvars_traj
>>> traj = Colvars_traj('test.colvars.traj')
>>> print(traj['A'].steps, traj['A'].values)
[    0   100  ...   10000 10100] [14.246745 14.228256 ... 13.813692 13.643718]
>>> print(traj['B'].steps, traj['B'].values)
[10000 10100] [0.999574  0.9995741]
```

4 Defining collective variables

A collective variable is defined by the keyword `colvar` followed by its configuration options contained within curly braces:

```
colvar {
  name xi
  <other options>
  function_name {
    <parameters>
    <atom selection>
  }
}
```

There are multiple ways of defining a variable:

- The *simplest and most common way* way is using one of the precompiled functions (here called "components"), which are listed in section 4.1. For example, using the keyword `rmsd` (section 4.6.1) defines the variable as the root mean squared deviation (RMSD) of the selected atoms.

- A new variable may also be constructed as a linear or polynomial combination of the components listed in section 4.1 (see 4.18 for details).

- A user-defined mathematical function of the existing components (see list in section 4.1), or of the atomic coordinates directly (see the `cartesian` keyword in 4.10.1). The function is defined through the keyword `customFunction` (see 4.19 for details).

- A user-defined Tcl function of the existing components (see list in section 4.1), or of the atomic coordinates directly (see the `cartesian` keyword in 4.10.1). The function is provided by a separate Tcl script, and referenced through the keyword `scriptedFunction` (see 4.20 for details).

Choosing a component (function) is the only parameter strictly required to define a collective variable. It is also highly recommended to specify a name for the variable:

- `name` — *Name of this colvar*

  Default: "`colvar`" + numeric id [string, context: `colvar`]

  The name is an unique case-sensitive string which allows the Colvars module to identify this colvar unambiguously; it is also used in the trajectory file to label to the columns corresponding to this colvar.

4.1 Choosing a function

In this context, the function that computes a colvar is called a *component*. A component's choice and definition consists of including in the variable's configuration a keyword indicating the type of function (e.g. `rmsd`), followed by a definition block specifying the atoms involved (see 5) and any additional parameters (cutoffs, "reference" values, . . .). *At least one component must be chosen to define a variable:* if none of the keywords listed below is found, an error is raised.

The following components implement functions with a scalar value (i.e. a real number):

- `distance`: distance between two groups;

- `distanceZ`: projection of a distance vector on an axis;

- `distanceXY`: projection of a distance vector on a plane;

- `distanceInv`: mean distance between two groups of atoms (e.g. NOE-based distance);

- `angle`: angle between three groups;

- `dihedral`: torsional (dihedral) angle between four groups;

- `dipoleAngle`: angle between two groups and dipole of a third group;

- `dipoleMagnitude`: magnitude of the dipole of a group of atoms;

- `polarTheta`: polar angle of a group in spherical coordinates;

- `polarPhi`: azimuthal angle of a group in spherical coordinates;

- `eulerPhi`: Roll angle of rotation from references coordinates;

- `eulerTheta`: Pitch angle of rotation from references coordinates;

- `eulerPsi`: Yaw angle of rotation from references coordinates;

- `coordNum`: coordination number between two groups;

- `selfCoordNum`: coordination number of atoms within a group;

- `hBond`: hydrogen bond between two atoms;

- `rmsd`: root mean square deviation (RMSD) from a set of reference coordinates;

- `eigenvector`: projection of the atomic coordinates on a vector;

- `orientationAngle`: angle of the best-fit rotation from a set of reference coordinates;

- `orientationProj`: cosine of `orientationProj`;

- `spinAngle`: projection orthogonal to an axis of the best-fit rotation from a set of reference coordinates;

- `tilt`: projection on an axis of the best-fit rotation from a set of reference coordinates;

- `gyration`: radius of gyration of a group of atoms;

- `inertia`: moment of inertia of a group of atoms;

- **inertiaZ**: moment of inertia of a group of atoms around a chosen axis;

- **alpha**: α-helix content of a protein segment.

- **dihedralPC**: projection of protein backbone dihedrals onto a dihedral principal component.

- **alchLambda**: alchemical lambda parameter (for controlling the back-end).

- **alchFlambda**: force along alchLambda.

Some components do not return scalar, but vector values:

- **distanceVec**: distance vector between two groups (length: 3);

- **distanceDir**: unit vector parallel to distanceVec (length: 3);

- **cartesian**: vector of atomic Cartesian coordinates (length: N times the number of Cartesian components requested, X, Y or Z);

- **distancePairs**: vector of mutual distances (length: $N_1 \times N_2$);

- **orientation**: best-fit rotation, expressed as a unit quaternion (length: 4).

The types of components used in a colvar (scalar or not) determine the properties of that colvar, and particularly which biasing or analysis methods can be applied.

What if "X" is not listed? If a function type is not available on this list, it may be possible to define it as a polynomial superposition of existing ones (see 4.18), a custom function (see 4.19).

In the rest of this section, all available component types are listed, along with their physical units and their ranges of values, if limited. Such ranges are often used to define automatically default sampling intervals, for example by setting the parameters **lowerBoundary** and **upperBoundary** in the parent colvar.

For each type of component, the available configurations keywords are listed: when two components share certain keywords, the second component references to the documentation of the first one that uses that keyword. The very few keywords that are available for all types of components are listed in a separate section 4.15.

4.2 Treatment of periodic boundary conditions

In all colvar components described below, the following rules apply concerning periodic boundary conditions (PBCs):

1. Distance vectors between two coordinates $\mathbf{d}_{i,j} = (\mathbf{x}_1 - \mathbf{x}_2)$, are calculated following the minimum-image convention by default, unless **forceNoPBC** is enabled. ($\mathbf{x}_1$ and $\mathbf{x}_2$ may be either individual atomic coordinates, or centers of mass of two groups.)

2. For all other functions of individual atomic coordinates, $f(\mathbf{x}_1, \mathbf{x}_2, \ldots)$, it is assumed that all atoms that are part of the same group are in the same periodic unit cell (see 5.3).

- `forceNoPBC` — *Use absolute rather than minimum-image distances?*

  Default: `no` [boolean, context: any component]

  If this keyword is set to `yes`, PBCs will be ignored when calculating this component. This is only useful in a limited number of special cases, e.g. to describe the distance between remote points of a single macromolecule, which cannot be split across periodic cell boundaries, and for which the minimum-image distance might give the wrong result because of a relatively small periodic cell.

Example: ignoring PBCs in a distance between two groups. Using `forceNoPBC yes` when defining a `distance { ...  }` function lets Colvars ignore the minimum-image convention when computing the distance between the centers of mass of `group1` and `group2`. (Note that the centers of mass themselves are always calculated as weighted averages anyway).

```
colvar {
  name d
  distance {
    forceNoPBC yes
    group1 { ... }
    group2 { ... }
  }
}
```

4.3   Distances

4.3.1 `distance`: **center-of-mass distance between two groups.**

The `distance {...}` block defines a distance component between the two atom groups, `group1` and `group2`.

List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `group1` — *First group of atoms*

  [Atom group, context: `distance`]

  First group of atoms.

- `group2` — analogous to `group1`

- `oneSiteTotalForce` — *Measure total force on group 1 only?*

  Default: `no` [boolean, context: `angle`, `dipoleAngle`, `dihedral`]

  If this is set to `yes`, the total force is measured along a vector field (see equation (24) in section 6.3) that only involves atoms of `group1`. This option is only useful for ABF, or custom biases that compute total forces. See section 6.3 for details.

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

The value returned is a positive number (in Å), ranging from 0 to the largest possible interatomic distance within the chosen boundary conditions (with PBCs, the minimum image convention is used unless the `forceNoPBC` option is set).

4.3.2 `distanceZ`: **projection of a distance vector on an axis.**

The `distanceZ {...}` block defines a distance projection component, which can be seen as measuring the distance between two groups projected onto an axis, or the position of a group along such an axis. The axis can be defined using either one reference group and a constant vector, or dynamically based on two reference groups. One of the groups can be set to a dummy atom to allow the use of an absolute Cartesian coordinate.

List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `main` — *Main group of atoms*

  [Atom group, context: `distanceZ`]

  Group of atoms whose position r is measured.

- `ref` — *Reference group of atoms*

  [Atom group, context: `distanceZ`]

  Reference group of atoms. The position of its center of mass is noted r_1 below.

- `ref2` — *Secondary reference group*

  Default: none [Atom group, context: `distanceZ`]

  Optional group of reference atoms, whose position r_2 can be used to define a dynamic projection axis: $e = (\|r_2 - r_1\|)^{-1} \times (r_2 - r_1)$. In this case, the origin is $r_m = 1/2(r_1 + r_2)$, and the value of the component is $e \cdot (r - r_m)$.

- `axis` — *Projection axis*

  Default: `(0.0, 0.0, 1.0)` [`(x, y, z)` triplet, context: `distanceZ`]

  This vector will be normalized to define a projection axis e for the distance vector $r - r_1$ joining the centers of groups `ref` and `main`. The value of the component is then $e \cdot (r - r_1)$. The vector should be written as three components separated by commas and enclosed in parentheses.

- `oneSiteTotalForce` — same definition as `oneSiteTotalForce` (`distance` component)

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

This component returns a number (in Å) whose range is determined by the chosen boundary conditions. For instance, if the z axis is used in a simulation with periodic boundaries, the returned value ranges between $-b_z/2$ and $b_z/2$, where b_z is the box length along z (this behavior is disabled if `forceNoPBC` is set).

4.3.3 `distanceXY`: modulus of the projection of a distance vector on a plane.

The `distanceXY {...}` block defines a distance projected on a plane, and accepts the same keywords as the component `distanceZ`, i.e. `main`, `ref`, either `ref2` or `axis`, and `oneSiteTotalForce`. It returns the norm of the projection of the distance vector between `main` and `ref` onto the plane orthogonal to the axis. The axis is defined using the `axis` parameter or as the vector joining `ref` and `ref2` (see `distanceZ` above).

List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `main` — same definition as `main` (`distanceZ` component)

- `ref` — same definition as `ref` (`distanceZ` component)

- `ref2` — same definition as `ref2` (`distanceZ` component)

- `axis` — same definition as `axis` (`distanceZ` component)

- `oneSiteTotalForce` — same definition as `oneSiteTotalForce` (`distance` component)

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

4.3.4 `distanceVec`: distance vector between two groups.

The `distanceVec` component computes the 3-dimensional vector joining the centers of mass of `group1` and `group2`. Its values are therefore multi-dimensional and are subject to the restrictions listed in 4.17. Moreover, when computing differences between two different values of a `distanceVec` variable the minimum-image convention is assumed (unless `forceNoPBC` is enabled).

List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `group1` — same definition as `group1` (`distance` component)

- `group2` — analogous to `group1`

- `oneSiteTotalForce` — same definition as `oneSiteTotalForce` (`distance` component)

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

4.3.5 `distanceDir`: distance unit vector between two groups.

The `distanceDir {...}` block defines a distance unit vector component, which accepts the same keywords as the component `distance`: `group1`, `group2`, and `forceNoPBC`. It returns a 3-dimensional unit vector $\mathbf{d} = (d_x, d_y, d_z)$, with $|\mathbf{d}| = 1$.

This multi-dimensional variable has two intrinsic degrees of freedom: however, these cannot be sampled independently as one-dimensional variables. A decomposition in two dimensions can be done using `polarTheta` and `polarPhi` angles, with the caveat that the latter is ill-defined when the former approaches $0°$ or $180°$.

The distance between two values of `distanceDir` is calculated internally as the angle (in radians) between the two unit vectors: this definition adapts the standard Euclidean distance to the unit sphere, to ensure that restraint forces comply with the mathematical constraint.

List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `group1` — same definition as `group1` (`distance` component)

- `group2` — analogous to `group1`

- `oneSiteTotalForce` — same definition as `oneSiteTotalForce` (`distance` component)

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

4.3.6 `distanceInv`: **mean distance between two groups of atoms.**

The `distanceInv {...}` block defines a generalized mean distance between two groups of atoms 1 and 2, where each distance is taken to the power $-n$:

$$d_{1,2}^{[n]} = \left(\frac{1}{N_1 N_2} \sum_{i,j} d_{ij}^{-n} \right)^{-1/n} \tag{2}$$

where d_{ij} is the distance between atoms i and j in groups 1 and 2 respectively, and n is an even integer.

List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `group1` — same definition as `group1` (`distance` component)

- `group2` — analogous to `group1`

- `oneSiteTotalForce` — same definition as `oneSiteTotalForce` (`distance` component)

- `exponent` — *Exponent n in equation 2*

  Default: 6 [positive even integer, context: `distanceInv`]

  Defines the exponent to which the individual distances are elevated before averaging. The default value of 6 is useful for example to applying restraints based on NOE-measured distances.

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

This component returns a number ranging from 0 to the largest possible distance within the chosen boundary conditions.

4.4 Angles

4.4.1 `angle`: angle between three groups.

The `angle {...}` block defines an angle, and contains the three blocks `group1`, `group2` and `group3`, defining the three groups. It returns an angle (in degrees) within the interval $[0 : 180]$.
List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `group1` — same definition as `group1` (`distance` component)

- `group2` — analogous to `group1`

- `group3` — analogous to `group1`

- `oneSiteTotalForce` — same definition as `oneSiteTotalForce` (`distance` component)

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

4.4.2 `dipoleAngle`: angle between two groups and dipole of a third group.

The `dipoleAngle {...}` block defines an angle, and contains the three blocks `group1`, `group2` and `group3`, defining the three groups, being `group1` the group where dipole is calculated. It returns an angle (in degrees) within the interval $[0 : 180]$.
List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `group1` — same definition as `group1` (`distance` component)

- `group2` — analogous to `group1`

- `group3` — analogous to `group1`

- `oneSiteTotalForce` — same definition as `oneSiteTotalForce` (`distance` component)

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

4.4.3 `dihedral`: torsional angle between four groups.

The `dihedral {...}` block defines a torsional angle, and contains the blocks `group1`, `group2`, `group3` and `group4`, defining the four groups. It returns an angle (in degrees) within the interval $[-180 : 180]$. The Colvars module calculates all the distances between two angles taking into account periodicity. For instance, reference values for restraints or range boundaries can be defined by using any real number of choice.

List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `group1` — same definition as `group1` (`distance` component)

- `group2` — analogous to `group1`

- `group3` — analogous to `group1`

- `group4` — analogous to `group1`

- `oneSiteTotalForce` — same definition as `oneSiteTotalForce` (`distance` component)

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

4.4.4   `polarTheta`: **polar angle in spherical coordinates.**

The `polarTheta {...}` block defines the polar angle in spherical coordinates, for the center of mass of a group of atoms described by the block `atoms`. It returns an angle (in degrees) within the interval $[0 : 180]$. To obtain spherical coordinates in a frame of reference tied to another group of atoms, use the `fittingGroup` (5.2) option within the `atoms` block. An example is provided in file `examples/11_polar_angles.in` of the Colvars public repository.

List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `atoms` — *Group of atoms defining this function*

  [Atom group, context: `polarPhi`]

  Defines the group of atoms for the COM of which the angle should be calculated.

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

4.4.5   `polarPhi`: **azimuthal angle in spherical coordinates.**

The `polarPhi {...}` block defines the azimuthal angle in spherical coordinates, for the center of mass of a group of atoms described by the block `atoms`. It returns an angle (in degrees) within the interval $[-180 : 180]$. The Colvars module calculates all the distances between two angles taking into account periodicity. For instance, reference values for restraints or range boundaries can be defined by using any real number of choice. To obtain spherical coordinates in a frame of reference tied to another group of atoms, use the `fittingGroup` (5.2) option within the `atoms` block. An example is provided in file `examples/11_polar_angles.in` of the Colvars public repository.

List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `atoms` — *Group of atoms defining this function*

  [Atom group, context: `polarPhi`]

  Defines the group of atoms for the COM of which the angle should be calculated.

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

Note: `polarPhi` is ill-defined when the corresponding `polarTheta` component is close to $0°$ or $180°$; please take measures to avoid sampling these configurations in your simulations.

4.5 Contacts

4.5.1 `coordNum`: **coordination number between two groups.**

The `coordNum {...}` block defines a coordination number (or number of contacts), which calculates the function $(1 - (d/d_0)^n)/(1 - (d/d_0)^m)$, where d_0 is the "cutoff" distance, and n and m are exponents that can control its long range behavior and stiffness [2]. This function is summed over all pairs of atoms in `group1` and `group2`:

$$C(\texttt{group1}, \texttt{group2}) \;=\; \sum_{i \in \texttt{group1}} \sum_{j \in \texttt{group2}} \frac{1 - (|\mathbf{x}_i - \mathbf{x}_j|/d_0)^n}{1 - (|\mathbf{x}_i - \mathbf{x}_j|/d_0)^m} \tag{3}$$

List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `group1` — same definition as `group1` (`distance` component)

- `group2` — analogous to `group1`

- `cutoff` — *"Interaction" distance (Å)*

  Default: 4.0 Å [positive decimal, context: `coordNum`]

  This number defines the switching distance to define an interatomic contact: for $d \ll d_0$, the switching function $(1 - (d/d_0)^n)/(1 - (d/d_0)^m)$ is close to 1, at $d = d_0$ it has a value of n/m ($1/2$ with the default n and m), and at $d \gg d_0$ it goes to zero approximately like d^{m-n}. Hence, for a proper behavior, m must be larger than n.

- `cutoff3` — *Reference distance vector (Å)*

  Default: (4.0, 4.0, 4.0) Å ["(x, y, z)" triplet of positive decimals, context: `coordNum`]

  The three components of this vector define three different cutoffs d_0 for each direction. This option is mutually exclusive with `cutoff`.

- `expNumer` — *Numerator exponent*

  Default: 6 [positive even integer, context: `coordNum`]

  This number defines the n exponent for the switching function.

- **expDenom** — *Denominator exponent*

  Default: 12 [positive even integer, context: `coordNum`]

  This number defines the m exponent for the switching function.

- **group2CenterOnly** — *Use only `group2`'s center of mass*

  Default: `off` [boolean, context: `coordNum`]

  If this option is on, only contacts between each atoms in `group1` and the center of mass of `group2` are calculated (by default, the sum extends over all pairs of atoms in `group1` and `group2`). If `group2` is a `dummyAtom`, this option is set to `yes` by default.

- **tolerance** — *Pairlist control*

  Default: 0.0 [decimal, context: `coordNum`]

  This controls the pair list feature, dictating the minimum value for each summation element in Eq. 3 such that the pair that contributed the summation element is included in subsequent simulation timesteps until the next pai r list recalculation. For most applications, this value should be small (eg. 0.001) to avoid missing important contributions to the overall sum. Higher values will improve performance by reducing the number of pairs that contribute to the sum. Values above 1 will exclude all possible pair interactions. Similarly, values below 0 will never exclude a pair from consideration. To ensure continuous forces, Eq. 3 is further modified by subtracting the tolerance and then rescaling so that each pair covers the range $[0, 1]$.

- **pairListFrequency** — *Pairlist regeneration frequency*

  Default: 100 [positive integer, context: `coordNum`]

  This controls the pairlist feature, dictating how many steps are taken between regenerating pair lists if the tolerance is greater than 0.

- **forceNoPBC** — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

This component returns a dimensionless number, which ranges from approximately 0 (all interatomic distances are much larger than the cutoff) to $N_{\text{group1}} \times N_{\text{group2}}$ (all distances are less than the cutoff), or N_{group1} if group2CenterOnly is used. For performance reasons, at least one of `group1` and `group2` should be of limited size or `group2CenterOnly` should be used: the cost of the loop over all pairs grows as $N_{\text{group1}} \times N_{\text{group2}}$. Setting `tolerance` > 0 ameliorates this to some degree, although every pair is still checked to regenerate the pair list.

4.5.2 `selfCoordNum`: **coordination number between atoms within a group.**

The `selfCoordNum {...}` block defines a coordination number similarly to the component `coordNum`, but the function is summed over atom pairs within `group1`:

$$C(\text{group1}) = \sum_{i \in \text{group1}} \sum_{j > i} \frac{1 - (|\mathbf{x}_i - \mathbf{x}_j|/d_0)^n}{1 - (|\mathbf{x}_i - \mathbf{x}_j|/d_0)^m} \tag{4}$$

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The keywords accepted by `selfCoordNum` are a subset of those accepted by `coordNum`, namely `group1` (here defining *all* of the atoms to be considered), `cutoff`, `expNumer`, and `expDenom`.
List of keywords (see also for additional options):

- `group1` — same definition as `group1` (`coordNum` component)

- `cutoff` — same definition as `cutoff` (`coordNum` component)

- `cutoff3` — same definition as `cutoff3` (`coordNum` component)

- `expNumer` — same definition as `expNumer` (`coordNum` component)

- `expDenom` — same definition as `expDenom` (`coordNum` component)

- `tolerance` — same definition as `tolerance` (`coordNum` component)

- `pairListFrequency` — same definition as `pairListFrequency` (`coordNum` component)

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

This component returns a dimensionless number, which ranges from approximately 0 (all inter-atomic distances much larger than the cutoff) to $N_{\text{group1}} \times (N_{\text{group1}} - 1)/2$ (all distances within the cutoff). For performance reasons, `group1` should be of limited size, because the cost of the loop over all pairs grows as N_{group1}^2.

4.5.3 `hBond`: hydrogen bond between two atoms.

The `hBond {...}` block defines a hydrogen bond, implemented as a coordination number (eq. 3) between the donor and the acceptor atoms. Therefore, it accepts the same options `cutoff` (with a different default value of 3.3 Å), `expNumer` (with a default value of 6) and `expDenom` (with a default value of 8). Unlike `coordNum`, it requires two atom numbers, `acceptor` and `donor`, to be defined. It returns a dimensionless number, with values between 0 (acceptor and donor far outside the cutoff distance) and 1 (acceptor and donor much closer than the cutoff).
List of keywords (see also for additional options):

- `acceptor` — *Number of the acceptor atom*

  [positive integer, context: `hBond`]

  Number that uses the same convention as `atomNumbers`.

- `donor` — analogous to `acceptor`

- `cutoff` — same definition as `cutoff` (`coordNum` component)
  Note: default value is 3.3 Å.

- `expNumer` — same definition as `expNumer` (`coordNum` component)
  Note: default value is 6.

- `expDenom` — same definition as `expDenom` (`coordNum` component)
  Note: default value is 8.

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

4.6 Collective metrics

4.6.1 `rmsd`: root mean square displacement (RMSD) from reference positions.

The block `rmsd {...}` defines the root mean square replacement (RMSD) of a group of atoms with respect to a reference structure. For each set of coordinates $\{\mathbf{x}_1(t), \mathbf{x}_2(t), \ldots \mathbf{x}_N(t)\}$, the colvar component `rmsd` calculates the optimal rotation $U^{\{\mathbf{x}_i(t)\} \to \{\mathbf{x}_i^{(\mathrm{ref})}\}}$ that best superimposes the coordinates $\{\mathbf{x}_i(t)\}$ onto a set of reference coordinates $\{\mathbf{x}_i^{(\mathrm{ref})}\}$. Both the current and the reference coordinates are centered on their centers of geometry, $\mathbf{x}_{\mathrm{cog}}(t)$ and $\mathbf{x}_{\mathrm{cog}}^{(\mathrm{ref})}$. The root mean square displacement is then defined as:

$$\mathrm{RMSD}\left(\{\mathbf{x}_i(t)\}, \left\{\mathbf{x}_i^{(\mathrm{ref})}\right\}\right) = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left|U\left(\mathbf{x}_i(t) - \mathbf{x}_{\mathrm{cog}}(t)\right) - \left(\mathbf{x}_i^{(\mathrm{ref})} - \mathbf{x}_{\mathrm{cog}}^{(\mathrm{ref})}\right)\right|^2} \tag{5}$$

The optimal rotation $U^{\{\mathbf{x}_i(t)\} \to \{\mathbf{x}_i^{(\mathrm{ref})}\}}$ is calculated within the formalism developed in reference [3], which guarantees a continuous dependence of $U^{\{\mathbf{x}_i(t)\} \to \{\mathbf{x}_i^{(\mathrm{ref})}\}}$ with respect to $\{\mathbf{x}_i(t)\}$.
List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `atoms` — *Group of atoms defining this function*

  [Atom group, context: `rmsd`]

  Defines the group of atoms of which the RMSD should be calculated. Optimal fit options (such as `refPositions` and `rotateToReference`) should typically NOT be set within this block. Exceptions to this rule are the special cases discussed in the *Advanced usage* paragraph below.

- `refPositions` — *Reference coordinates*

  [space-separated list of (x, y, z) triplets, context: `rmsd`]

  This option (mutually exclusive with `refPositionsFile`) sets the reference coordinates for RMSD calculation, and uses these to compute the roto-translational fit. See the equivalent option `refPositions` within the atom group definition for details on acceptable formats and other features.

- `refPositionsFile` — *Reference coordinates file*

  [UNIX filename, context: `rmsd`]

  This option (mutually exclusive with `refPositions`) sets the reference coordinates for RMSD calculation, and uses these to compute the roto-translational fit. See the equivalent option `refPositionsFile` within the atom group definition for details on acceptable file formats and other features.

- `atomPermutation` — *Alternate ordering of atoms for RMSD computation*

  [List of atom numbers, context: `rmsd`]

  If defined, this parameter defines a re-ordering (permutation) of the 1-based atom numbers that can be used to compute the RMSD, typically due to molecular symmetry. This parameter can be specified multiple times, each one defining a new permutation: the returned RMSD value is the minimum over the set of permutations. For example, if the atoms making up the group are 6, 7, 8, 9, and atoms 7, 8, and 9 are invariant by circular permutation (as the hydrogens in a CH3 group), a symmetry-adapted RMSD would be obtained by adding:
  ```
  atomPermutation 6 8 9 7
  atomPermutation 6 9 7 8
  ```
  This will result in these 2 atom orders being considered *in addition to the order used when defining the atom group*. Note that this does not affect the least-squares roto-translational fit. Therefore, this feature is mostly useful when using custom fitting parameters within the atom group, such as `fittingGroup`, or when fitting is disabled altogether. For details, see reference [4].

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

This component returns a positive real number (in Å).

4.6.2 Advanced usage of the `rmsd` component.

In the standard usage as described above, the `rmsd` component calculates a minimum RMSD, that is, current coordinates are optimally fitted onto the same reference coordinates that are used to compute the RMSD value. The fit itself is handled by the atom group object, whose parameters are automatically set by the `rmsd` component. For very specific applications, however, it may be useful to control the fitting process separately from the definition of the reference coordinates, to evaluate various types of non-minimal RMSD values. This can be achieved by setting the related options (`refPositions`, etc.) explicitly in the atom group block. This allows for the following non-standard cases:

1. applying the optimal translation, but no rotation (`rotateToReference off`), to bias or restrain the shape and orientation, but not the position of the atom group;

2. applying the optimal rotation, but no translation (`centerToReference off`), to bias or restrain the shape and position, but not the orientation of the atom group;

3. disabling the application of optimal roto-translations, which lets the RMSD component describe the deviation of atoms from fixed positions in the laboratory frame: this allows for custom positional restraints within the Colvars module;

4. fitting the atomic positions to different reference coordinates than those used in the RMSD calculation itself (by specifying `refPositions` or `refPositionsFile` within the atom group as well as within the `rmsd` block);

5. applying the optimal rotation and/or translation from a separate atom group, defined through `fittingGroup`: the RMSD then reflects the deviation from reference coordinates in a separate, moving reference frame (see example in the section on `fittingGroup`).

4.6.3 `eigenvector`: **projection of the atomic coordinates on a vector.**

The block `eigenvector {...}` defines the projection of the coordinates of a group of atoms (or more precisely, their deviations from the reference coordinates) onto a vector in $\mathbb{R}^{3n}$, where n is the number of atoms in the group. The computed quantity is the total projection:

$$p\left(\left\{\mathbf{x}_i(t)\right\}, \left\{\mathbf{x}_i^{(\mathrm{ref})}\right\}\right) = \sum_{i=1}^{n} \mathbf{v}_i \cdot \left(U(\mathbf{x}_i(t) - \mathbf{x}_{\mathrm{cog}}(t)) - (\mathbf{x}_i^{(\mathrm{ref})} - \mathbf{x}_{\mathrm{cog}}^{(\mathrm{ref})})\right), \tag{6}$$

where, as in the `rmsd` component, U is the optimal rotation matrix, $\mathbf{x}_{\mathrm{cog}}(t)$ and $\mathbf{x}_{\mathrm{cog}}^{(\mathrm{ref})}$ are the centers of geometry of the current and reference positions respectively, and $\mathbf{v}_i$ are the components of the vector for each atom. Example choices for $(\mathbf{v}_i)$ are an eigenvector of the covariance matrix (essential mode), or a normal mode of the system. It is assumed that $\sum_i \mathbf{v}_i = 0$: otherwise, the Colvars module centers the $\mathbf{v}_i$ automatically when reading them from the configuration.
List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `atoms` — same definition as `atoms` (`rmsd` component)

- `refPositions` — same definition as `refPositions` (`rmsd` component)

- `refPositionsFile` — same definition as `refPositionsFile` (`rmsd` component)

- `vector` — *Vector components*

  [space-separated list of (x, y, z) triplets, context: `eigenvector`]

  This option (mutually exclusive with `vectorFile`) sets the values of the vector components.

- `vectorFile` — *file containing vector components*

  [UNIX filename, context: `eigenvector`]

  This option (mutually exclusive with `vector`) sets the name of an XYZ (3.7.3) coordinate file containing the vector components. **Note:** *Reading data from a coordinate file may entail an automatic unit conversion if the length unit currently used by the MD engine is not Å. If this is not the desired behavior, this can be remedied using the `normalizeVector` option described below.*

- `normalizeVector` — *Normalize the vector components when reading them?*

  Default: `off` [boolean, context: `eigenvector`]

  If this flag is activated, the norm of the vector $|\mathbf{v}| = \sqrt{\sum_i |\mathbf{v}_i|^2}$ is set equal to 1 by automatically rescaling all the components $\mathbf{v}_i$; alternatively, the value of $|\mathbf{v}|$ is printed.

- `differenceVector` — *The $3n$-dimensional vector is the difference between `vector` and `refPositions`*

  Default: `off` [boolean, context: `eigenvector`]

  If this option is `on`, the numbers provided by `vector` are interpreted as another set of positions, $\mathbf{x}_i'$: the vector $\mathbf{v}_i$ is then defined as $\mathbf{v}_i = \left(\mathbf{x}_i' - \mathbf{x}_i^{(\mathrm{ref})}\right)$. This allows to conveniently define

a colvar ξ as a projection on the linear transformation between two sets of positions, "A" and "B". If this flag is on, the vector is normalized so that $\xi = 0$ when the atoms are at the set of positions "A" and $\xi = 1$ at the set of positions "B". Setting `normalizeVector on` overrides this behavior.

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

4.6.4 `gyration`: radius of gyration of a group of atoms.

The block `gyration {...}` defines the parameters for calculating the radius of gyration of a group of atomic positions $\{\mathbf{x}_1(t), \mathbf{x}_2(t), \ldots \mathbf{x}_N(t)\}$ with respect to their center of geometry, $\mathbf{x}_{\mathrm{cog}}(t)$:

$$R_{\mathrm{gyr}} = \sqrt{\frac{1}{N} \sum_{i=1}^{N} \left| \mathbf{x}_i(t) - \mathbf{x}_{\mathrm{cog}}(t) \right|^2} \tag{7}$$

This component must contain one `atoms {...}` block to define the atom group, and returns a positive number, expressed in Å.
List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `atoms` — same definition as `atoms` (`rmsd` component)

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

4.6.5 `inertia`: total moment of inertia of a group of atoms.

The block `inertia {...}` defines the parameters for calculating the total moment of inertia of a group of atomic positions $\{\mathbf{x}_1(t), \mathbf{x}_2(t), \ldots \mathbf{x}_N(t)\}$ with respect to their center of geometry, $\mathbf{x}_{\mathrm{cog}}(t)$:

$$I = \sum_{i=1}^{N} \left| \mathbf{x}_i(t) - \mathbf{x}_{\mathrm{cog}}(t) \right|^2 \tag{8}$$

Note that all atomic masses are set to 1 for simplicity. This component must contain one `atoms {...}` block to define the atom group, and returns a positive number, expressed in Å^2.
List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `atoms` — same definition as `atoms` (`rmsd` component)

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

4.6.6 `dipoleMagnitude`: **dipole magnitude of a group of atoms.**

The `dipoleMagnitude {...}` block defines the dipole magnitude of a group of atoms (norm of the dipole moment's vector), being `atoms` the group where dipole magnitude is calculated. It returns the magnitude in elementary charge e times Å.
List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `atoms` — same definition as `atoms` (`rmsd` component)

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

4.6.7 `inertiaZ`: **total moment of inertia of a group of atoms around a chosen axis.**

The block `inertiaZ {...}` defines the parameters for calculating the component along the axis $\mathbf{e}$ of the moment of inertia of a group of atomic positions $\{\mathbf{x}_1(t), \mathbf{x}_2(t), \ldots \mathbf{x}_N(t)\}$ with respect to their center of geometry, $\mathbf{x}_{\text{cog}}(t)$:

$$I_{\mathbf{e}} = \sum_{i=1}^{N} \left(\left(\mathbf{x}_i(t) - \mathbf{x}_{\text{cog}}(t) \right) \cdot \mathbf{e} \right)^2 \tag{9}$$

Note that all atomic masses are set to 1 for simplicity. This component must contain one `atoms {...}` block to define the atom group, and returns a positive number, expressed in $Å^2$.
List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `atoms` — same definition as `atoms` (`rmsd` component)

- `axis` — *Projection axis*

  Default: `(0.0, 0.0, 1.0)` [(x, y, z) triplet, context: `inertiaZ`]

  The three components of this vector define (when normalized) the projection axis $\mathbf{e}$.

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

4.7 Rotations

The variables discussed in this section quantify the rotations of macromolecules (or other quasi-rigid objects) from a given set of reference coordinates to the current coordinates. Such rotations are computed following the same method used for best-fit RMSDs (see `rmsd` and `fittingGroup`). The underlying mathematical formalism is described in reference [3], and the implementation in reference [1].

The first of the functions described is the `orientation`, which describes the full rotation as a unit quaternion q = (q_0, q_1, q_2, q_3), i.e. 4 numbers with one constraint (3 degrees of freedom). The quaternion q is *one of only two representations that are both complete and accurate*, the other being a 3×3 unit matrix with 3 independent parameters. Although q is used internally in the Colvars module for features such as the `rmsd` function and the `fittingGroup` option, its direct use as a collective variable is more difficult, and mostly limited to fixed or moving restraints.

The two functions `orientationAngle` and `orientationProj`, with the latter being the cosine of the former, represent the amplitude of the full rotation described by q, regardless of the direction of its axis. As one-dimensional scalar variables, both `orientationAngle` and `orientationProj` are a much reduced simplification of the full rotation. However, they can be used in a variety of methods including both restraints and PMF computations.

A slightly more complete parametrization is achieved by decomposing the full rotation into the two parameters, `tilt` and `spinAngle`. These quantify the amplitudes of two independent sub-rotations away from a certain axis **e**, and around the same axis **e**, respectively. The axis **e** is chosen by the user, and is by default the Z axis: under that choice, `tilt` is equivalent to the sine of the Euler "pitch" angle θ, and `spinAngle` to the sum of the other two angles, $(\phi + \psi)$. This parameterization is mathematically well defined for almost all full rotations, including small ones when the current coordinates are almost completely aligned with the reference ones. However, a mathematical singularity prevents using the `spinAngle` function near configurations where the value of tilt `tilt` is -1 (i.e. a -180° rotation around an axis orthogonal to **e**). For these reasons, `tilt` and `spinAngle` are useful when the allowed rotations are known to have approximately the same axis, and differ only in the magnitude of the corresponding angle. In this use case, `spinAngle` measures the angle of the sub-rotation around the chosen axis **e**, whereas `tilt` measures the dot product between **e** and the actual axis of the full rotation.

Lastly, the traditional Euler angles are also available as the functions `eulerPhi`, `eulerTheta` and `eulerPsi`. Altogether, these are sufficient to represent all three degrees of freedom of a full rotation. However, they also suffer from the potential "gimbal lock" problem, which emerges whenever $\theta \simeq \pm 90°$, which includes also the case where the full rotation is small. Under such conditions, the angles ϕ and ψ are both ill-defined and cannot be used as collective variables. For these reasons, it is highly recommended that Euler angles are used only in simulations where their range of applicability is *known ahead of time*, and excludes configurations where $\theta \simeq \pm 90°$ altogether.

4.7.1 `orientation`: **orientation from reference coordinates.**

The block `orientation {...}` returns the same optimal rotation used in the `rmsd` component to superimpose the coordinates $\{\mathbf{x}_i(t)\}$ onto a set of reference coordinates $\{\mathbf{x}_i^{(\mathrm{ref})}\}$. Such component returns a four dimensional vector q = (q_0, q_1, q_2, q_3), with $\sum_i q_i^2 = 1$; this *quaternion* expresses the optimal rotation $\{\mathbf{x}_i(t)\} \rightarrow \{\mathbf{x}_i^{(\mathrm{ref})}\}$ according to the formalism in reference [3]. The quaternion (q_0, q_1, q_2, q_3) can also be written as $(\cos(\theta/2), \sin(\theta/2)\mathbf{u})$, where θ is the angle and **u** the normalized axis of rotation; for example, a rotation of 90° around the z axis is expressed as "(0.707, 0.0, 0.0, 0.707)". The script `quaternion2rmatrix.tcl` provides Tcl functions for converting to and from a 4×4 rotation matrix in a format suitable for usage in VMD.

As for the component `rmsd`, the available options are `atoms`, `refPositionsFile` and `refPositions`.

Note: `refPositions` and `refPositionsFile` define the set of positions *from which* the optimal rotation is calculated, but this rotation is not applied to the coordinates of the atoms involved: it is

used instead to define the variable itself.

List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `atoms` — same definition as `atoms` (`rmsd` component)

- `refPositions` — same definition as `refPositions` (`rmsd` component)

- `refPositionsFile` — same definition as `refPositionsFile` (`rmsd` component)

- `closestToQuaternion` — *Reference rotation*

  Default: (1.0, 0.0, 0.0, 0.0) ("null" rotation) ["(q0, q1, q2, q3)" quadruplet, context: `orientation`]

  Between the two equivalent quaternions (q_0, q_1, q_2, q_3) and $(-q_0, -q_1, -q_2, -q_3)$, the closer to (1.0, 0.0, 0.0, 0.0) is chosen. This simplifies the visualization of the colvar trajectory when sampled values are a smaller subset of all possible rotations. **Note:** *this only affects the output, never the dynamics.*

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

Example: stopping the rotation of a protein. To stop the rotation of an elongated macromolecule in solution (and use an anisotropic box to save water molecules), it is possible to define a colvar with an `orientation` component, and restrain it through the `harmonic` bias around the identity rotation, (1.0, 0.0, 0.0, 0.0). Only the overall orientation of the macromolecule is affected, and *not* its internal degrees of freedom.

```
colvar {
  name Orient
  orientation {
    atoms { ... }
    refPositionsFile reference.pdb
  }
}

harmonic {                # Define a harmonic restraint
  colvars Orient        # acting on colvar "Orient"
  centers  (1.0, 0.0, 0.0, 0.0)  # center the unit quaternion (no rotation)
  forceConstant 500.0           # unit is energy: quaternions are dimensionless
}
```

4.7.2 `orientationAngle`: **angle of rotation from reference coordinates.**

The block `orientationAngle {...}` accepts the same base options as the component `orientation`: `atoms`, `refPositions`, `refPositionsFile`. The returned value is the angle of rotation θ between

the current and the reference positions. This angle is expressed in degrees within the range [0°:180°].

List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `atoms` — same definition as `atoms` (rmsd component)

- `refPositions` — same definition as `refPositions` (rmsd component)

- `refPositionsFile` — same definition as `refPositionsFile` (rmsd component)

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

4.7.3 `orientationProj`: cosine of the angle of rotation from reference coordinates.

The block `orientationProj {...}` accepts the same base options as the component `orientation`: `atoms`, `refPositions`, `refPositionsFile`. The returned value is the cosine of the angle of rotation θ between the current and the reference positions. The range of values is [-1:1].

List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `atoms` — same definition as `atoms` (rmsd component)

- `refPositions` — same definition as `refPositions` (rmsd component)

- `refPositionsFile` — same definition as `refPositionsFile` (rmsd component)

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

4.7.4 `spinAngle`: angle of rotation around a given axis.

The complete rotation described by `orientation` can optionally be decomposed into two sub-rotations: one is a "*spin*" rotation around **e**, and the other a "*tilt*" rotation around an axis orthogonal to **e**. The component `spinAngle` measures the angle of the "spin" sub-rotation around **e**.

List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `atoms` — same definition as `atoms` (rmsd component)

- `refPositions` — same definition as `refPositions` (rmsd component)

- `refPositionsFile` — same definition as `refPositionsFile` (rmsd component)

- `axis` — *Special rotation axis*

  Default: (0.0, 0.0, 1.0) [(x, y, z) triplet, context: `tilt`]

  The three components of this vector define (when normalized) the special rotation axis used to calculate the `tilt` and `spinAngle` components.

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- **`forceNoPBC`** — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

The component `spinAngle` returns an angle (in degrees) within the periodic interval $[-180 : 180]$.

    Note: the value of `spinAngle` is a continuous function almost everywhere, with the exception of configurations with the corresponding "tilt" angle equal to $180°$ (i.e. the `tilt` component is equal to -1): in those cases, `spinAngle` is undefined. If such configurations are expected, consider defining a `tilt` colvar using the same axis **e**, and restraining it with a lower wall away from -1.

4.7.5   `tilt`: **cosine of the rotation orthogonal to a given axis.**

    The component `tilt` measures the cosine of the angle of the "tilt" sub-rotation, which combined with the "spin" sub-rotation provides the complete rotation of a group of atoms. The cosine of the tilt angle rather than the tilt angle itself is implemented, because the latter is unevenly distributed even for an isotropic system: consider as an analogy the angle θ in the spherical coordinate system. The component `tilt` relies on the same options as `spinAngle`, including the definition of the axis **e**. The values of `tilt` are real numbers in the interval $[-1 : 1]$: the value 1 represents an orientation fully parallel to **e** (tilt angle = $0°$), and the value -1 represents an anti-parallel orientation.

List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- **`atoms`** — same definition as `atoms` (`rmsd` component)

- **`refPositions`** — same definition as `refPositions` (`rmsd` component)

- **`refPositionsFile`** — same definition as `refPositionsFile` (`rmsd` component)

- **`axis`** — same definition as `axis` (`spinAngle` component)

- **`forceNoPBC`** — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

4.7.6   `eulerPhi`: **Roll angle from references coordinates.**

    Assuming the axes of the original frame are denoted as *x, y, z* and the axes of the rotated frame as *X, Y, Z*, the line of nodes, *N*, can be defined as the intersection of the plane *xy* and *XY*. The axis perpendicular to *N* and *z* is defined as *P*. In this case, as illustrated in the figure below, the complete rotation described by `orientation` can optionally be decomposed into three Euler angles:

   (i) the "roll" angle ϕ, i.e. the rotation angle from the *x* axis to the *N* axis;

   (ii) the "pitch" angle θ, i.e. the rotation angle from the *P* axis to the *Z* axis, and

   (iii) the "yaw" angle ψ, i.e. the rotation angle from the *N* axis to the *X* axis.
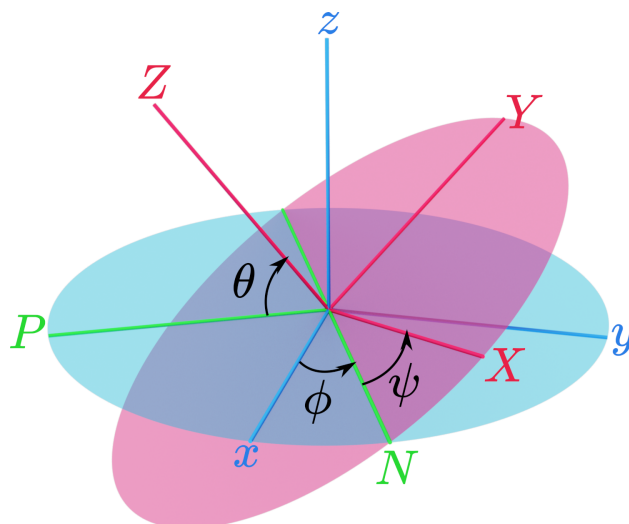
Although Euler angles are more straightforward to use than quaternions, they are also potentially subject to the "gimbal lock" problem:

https://en.wikipedia.org/wiki/Gimbal_lock

which arises whenever $\theta \simeq \pm 90°$, including the common case when *the simulated coordinates are near the reference coordinates*. Therefore, a safe use of Euler angles as collective variables requires the use of restraints to avoid such singularities, such as done in reference [5] and in the protein-ligand binding NAMD tutorial.

The `eulerPhi` component accepts exactly the same options as `orientation`, and measures the rotation angle from the *x* axis to the *N* axis. This angle is expressed in degrees within the periodic range $[-180° : 180°]$.

List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `atoms` — same definition as `atoms` (`rmsd` component)

- `refPositions` — same definition as `refPositions` (`rmsd` component)

- `refPositionsFile` — same definition as `refPositionsFile` (`rmsd` component)

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

4.7.7 `eulerTheta`**: Pitch angle from references coordinates.**

This component accepts exactly the same options as `orientation`, and measures the rotation angle from the *P* axis to the *Z* axis. This angle is expressed in degrees within the range $[-90° : 90°]$.

Warning: When this angle reaches $-90°$ or $90°$, the definition of orientation by euler angles suffers from the gimbal lock issue. You may need to apply a restraint to keep `eulerTheta` away from the singularities.

List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `atoms` — same definition as `atoms` (`rmsd` component)

- `refPositions` — same definition as `refPositions` (`rmsd` component)

- refPositionsFile — same definition as refPositionsFile (rmsd component)

- forceNoPBC — *Use absolute rather than minimum-image distances?* (see description)
  Default: no

This component accepts exactly the same options as orientation, and measures the rotation angle from the *N* axis to the *X* axis. This angle is expressed in degrees within the periodic range $[-180° : 180°]$.

List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- atoms — same definition as atoms (rmsd component)

- refPositions — same definition as refPositions (rmsd component)

- refPositionsFile — same definition as refPositionsFile (rmsd component)

- forceNoPBC — *Use absolute rather than minimum-image distances?* (see description)
  Default: no

4.8   Protein structure descriptors

4.8.1   alpha**: α-helix content of a protein segment.**

The block alpha {...} defines a measure of the helical content of a segment of protein residues, as a tunable combination of an angle term between alpha carbon atoms, and a 1-4 hydrogen-bond term. To create a colvar that computes the helical content of a protein with several, non-contiguous helical segments, just define several alpha blocks inside a single colvar block, and give them linear combination coefficients that sum to 1 (see componentCoeff in section 4.18).

The α-helical content across the $N+1$ residues N_0 to N_0+N is calculated by the formula:

$$\alpha\left(C_\alpha^{(N_0)}, O^{(N_0)}, C_\alpha^{(N_0+1)}, O^{(N_0+1)}, \dots N^{(N_0+5)}, C_\alpha^{(N_0+5)}, O^{(N_0+5)}, \dots N^{(N_0+N)}, C_\alpha^{(N_0+N)}\right) = \tag{10}$$

$$\frac{(1-C_{\text{hb}})}{N-1} \sum_{n=N_0}^{N_0+N-2} \text{angf}\left(C_\alpha^{(n)}, C_\alpha^{(n+1)}, C_\alpha^{(n+2)}\right) + \frac{C_{\text{hb}}}{N-3} \sum_{n=N_0}^{N_0+N-4} \text{hbf}\left(O^{(n)}, N^{(n+4)}\right),$$

$$\tag{11}$$

where C_{hb} is defined by hBondCoeff, the score function angf for the $C_\alpha - C_\alpha - C_\alpha$ angle is defined as:

$$\text{angf}\left(C_\alpha^{(n)}, C_\alpha^{(n+1)}, C_\alpha^{(n+2)}\right) = \frac{1 - \left(\theta(C_\alpha^{(n)}, C_\alpha^{(n+1)}, C_\alpha^{(n+2)}) - \theta_0\right)^2 / (\Delta\theta_{\text{tol}})^2}{1 - \left(\theta(C_\alpha^{(n)}, C_\alpha^{(n+1)}, C_\alpha^{(n+2)}) - \theta_0\right)^4 / (\Delta\theta_{\text{tol}})^4}, \tag{12}$$

and the score function `hbf` for the $O^{(n)} \leftrightarrow N^{(n+4)}$ hydrogen bond is defined through a `hBond` colvar component on the same atoms.

List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `prefix` — *Prefix of atom groups to be used for computing the α-helix content*

  Default: `alpha_` [string, context: `alpha`]

  Atoms involved in an alpha-helix component are based on groups defined within a GROMACS-style index file specified with `indexFile` in the global Colvars configuration, i.e. outside of a `colvar` block. The group names must use a common prefix followed by atom names, e.g. `alpha_N`, `alpha_CA`, and `alpha_O` when using the default prefix `alpha_`. The CA group is only required when the angle term is enabled, that is when `hBondCoeff` is less than 1. This option lets users set a different prefix, for example, when several α-helical segments must be tracked.

- `hBondCoeff` — *Coefficient for the hydrogen bond term*

  Default: 0.5 [positive between 0 and 1, context: `alpha`]

  This number specifies the contribution to the total value from the hydrogen bond terms. 0 disables the hydrogen bond terms, 1 disables the angle terms.

- `angleRef` — *Reference $C_\alpha - C_\alpha - C_\alpha$ angle*

  Default: 88° [positive decimal, context: `alpha`]

  This option sets the reference angle used in the score function (12).

- `angleTol` — *Tolerance in the $C_\alpha - C_\alpha - C_\alpha$ angle*

  Default: 15° [positive decimal, context: `alpha`]

  This option sets the angle tolerance used in the score function (12).

- `hBondCutoff` — *Hydrogen bond cutoff*

  Default: 3.3 Å [positive decimal, context: `alpha`]

  Equivalent to the `cutoff` option in the `hBond` component.

- `hBondExpNumer` — *Hydrogen bond numerator exponent*

  Default: 6 [positive integer, context: `alpha`]

  Equivalent to the `expNumer` option in the `hBond` component.

- `hBondExpDenom` — *Hydrogen bond denominator exponent*

  Default: 8 [positive integer, context: `alpha`]

  Equivalent to the `expDenom` option in the `hBond` component.

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

This component returns positive values, always comprised between 0 (lowest α-helical score) and 1 (highest α-helical score).

4.8.2 `dihedralPC`: **protein dihedral principal component**

The block `dihedralPC {...}` defines the parameters of the projection of backbone dihedral angles within a protein segment onto a *dihedral principal component*, following the formalism of dihedral principal component analysis (dPCA) proposed by Mu et al.[6] and documented in detail by Altis et al.[7]. Given a peptide or protein segment of N residues, each with Ramachandran angles ϕ_i and ψ_i, dPCA rests on a variance/covariance analysis of the $4(N-1)$ variables $\cos(\psi_1), \sin(\psi_1), \cos(\phi_2), \sin(\phi_2) \cdots \cos(\phi_N), \sin(\phi_N)$. Note that angles ϕ_1 and ψ_N have little impact on chain conformation, and are therefore discarded, following the implementation of dPCA in the analysis software Carma.[8]

For a given principal component (eigenvector) of coefficients $(k_i)_{1 \le i \le 4(N-1)}$, the projection of the current backbone conformation is:

$$\xi = \sum_{n=1}^{N-1} k_{4n-3} \cos(\psi_n) + k_{4n-2} \sin(\psi_n) + k_{4n-1} \cos(\phi_{n+1}) + k_{4n} \sin(\phi_{n+1}) \tag{13}$$

The atoms involved in `dihedralPC` are defined by the same parameters as the `alpha` component.

List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `prefix` — *Prefix of atom groups to be used for computing a dihedralPC projection*

  Default: `dihed_` [string, context: `dihedralPC`]

  Backbone atoms involved in a `dihedralPC` component are based on groups defined within a GROMACS-style index file specified with `indexFile` in the global Colvars configuration, i.e. outside of a `colvar` block. The group names must use a common prefix followed by atom names CA, N, and C, e.g. `dihed_CA`, `dihed_N`, and `dihed_C` when using the default prefix `dihed_`.

- `vectorFile` — *File containing dihedral PCA eigenvector(s)*

  [file name, context: `dihedralPC`]

  A text file containing the coefficients of dihedral PCA eigenvectors on the cosine and sine coordinates. The vectors should be arranged in columns, as in the files output by Carma.[8]

- `vectorNumber` — *File containing dihedralPCA eigenvector(s)*

  [positive integer, context: `dihedralPC`]

  Number of the eigenvector to be used for this component.

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

4.9 Alchemical variables for lambda-dynamics

4.9.1 `alchLambda`: **alchemical lambda parameter.**

The `alchLambda` block defines a component returning the alchemical lambda parameter of the back-end simulation in Tinker-HP, provided that it is enabled within that software. The current lambda value is obtained from the back-end at the beginning of a simulation, and synchronized from Colvars to Tinker-HP at every following time step. This enables lambda-dynamics simulations as documented in detail in [9]. The `alchLambda` keyword itself takes no parameters.

To run lambda-dynamics, the enclosing `colvar` block should contain extended-system parameters (4.23) as in the following example:

```
colvar {
  name lambda
  extendedLagrangian on
  extendedMass 150000
  extendedLangevinDamping 1000

  lowerBoundary 0
  upperBoundary 1
  reflectingLowerBoundary
  reflectingUpperBoundary

  alchLambda
}
```

These parameter values have been found to give stable dynamics for a wide range of applications[9]. Reflecting boundary conditions are recommended for lambda-dynamics to ensure that the alchemical variable remains precisely within its physical range.

4.9.2 `alchFlambda`: **Force on the alchemical lambda parameter.**

The `alchFlambda {}` block defines a component returning the force exterted on the alchemical lambda parameter of the back-end simulation in Tinker-HP, provided that it is enabled using the appropriate options of Tinker-HP. This coordinate is obtained from the back-end at each time step of a simulation. The `alchFlambda` block itself takes no parameters, it should be left empty.

4.10 Raw data: building blocks for custom functions

4.10.1 `cartesian`: **vector of atomic Cartesian coordinates.**

The `cartesian {...}` block defines a component returning a flat vector containing the Cartesian coordinates of all participating atoms, in the order $(x_1, y_1, z_1, \cdots, x_n, y_n, z_n)$.
List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `atoms` — *Group of atoms*

  [Atom group, context: `cartesian`]

  Defines the atoms whose coordinates make up the value of the component. If `rotateToReference`, `centerToReference`, or `centerToOrigin` are defined, coordinates are evaluated within the moving frame of reference.

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

4.10.2 `distancePairs`: set of pairwise distances between two groups.

The `distancePairs {...}` block defines a $N_1 \times N_2$-dimensional variable that includes all mutual distances between the atoms of two groups. This can be useful, for example, to develop a new variable defined over two groups, by using the `scriptedFunction` feature.
List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `group1` — same definition as `group1` (`distance` component)

- `group2` — analogous to `group1`

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

This component returns a $N_1 \times N_2$-dimensional vector of numbers, each ranging from 0 to the largest possible distance within the chosen boundary conditions.

4.11 Geometric path collective variables

The geometric path collective variables define the progress along a path, s, and the distance from the path, z. These CVs are proposed by Leines and Ensing[10] , which differ from that[11] proposed by Branduardi et al., and utilize a set of geometric algorithms. The path is defined as a series of frames in the atomic Cartesian coordinate space or the CV space. s and z are computed as

$$s = \frac{m}{M} \pm \frac{1}{2M} \left(\frac{\sqrt{(\mathbf{v}_1 \cdot \mathbf{v}_3)^2 - |\mathbf{v}_3|^2(|\mathbf{v}_1|^2 - |\mathbf{v}_2|^2)} - (\mathbf{v}_1 \cdot \mathbf{v}_3)}{|\mathbf{v}_3|^2} - 1 \right) \tag{14}$$

$$z = \sqrt{\left(\mathbf{v}_1 + \frac{1}{2} \left(\frac{\sqrt{(\mathbf{v}_1 \cdot \mathbf{v}_3)^2 - |\mathbf{v}_3|^2(|\mathbf{v}_1|^2 - |\mathbf{v}_2|^2)} - (\mathbf{v}_1 \cdot \mathbf{v}_3)}{|\mathbf{v}_3|^2} - 1 \right) \mathbf{v}_4 \right)^2} \tag{15}$$

where $\mathbf{v}_1 = \mathbf{s}_m - \mathbf{z}$ is the vector connecting the current position to the closest frame, $\mathbf{v}_2 = \mathbf{z} - \mathbf{s}_{m-1}$ is the vector connecting the second closest frame to the current position, $\mathbf{v}_3 = \mathbf{s}_{m+1} - \mathbf{s}_m$ is the vector connecting the closest frame to the third closest frame, and $\mathbf{v}_4 = \mathbf{s}_m - \mathbf{s}_{m-1}$ is the vector connecting the second closest frame to the closest frame. m and M are the current index of the closest frame and the total number of frames, respectively. If the current position is on the left of the closest reference frame, the $\pm$ in s turns to the positive sign. Otherwise it turns to the negative sign.

The equations above assume: (i) the frames are equidistant and (ii) the second and the third closest frames are neighbouring to the closest frame. When these assumptions are not satisfied, this set of path CV should be used carefully.

4.11.1 `gspath`: progress along a path defined in atomic Cartesian coordinate space.

In the `gspath {...}` and the `gzpath {...}` block all vectors, namely $\mathbf{z}$ and $\mathbf{s}_k$ are defined in atomic Cartesian coordinate space. More specifically, $\mathbf{z} = [\mathbf{r}_1, \cdots, \mathbf{r}_n]$, where $\mathbf{r}_i$ is the i-th atom specified in the `atoms` block. $\mathbf{s}_k = [\mathbf{r}_{k,1}, \cdots, \mathbf{r}_{k,n}]$, where $\mathbf{r}_{k,i}$ means the i-th atom of the k-th reference frame.

List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `atoms` — *Group of atoms*

  [Atom group, context: `gspath` and `gzpath`]

  Defines the atoms whose coordinates make up the value of the component.

- `refPositionsCol` — *PDB column containing atom flags*

  [O, B, X, Y, or Z, context: `gspath` and `gzpath`]

  If `refPositionsFileN` is a PDB file that contains all the atoms in the topology, this option may be provided to set which PDB field is used to flag the reference coordinates for `atoms`.

- `refPositionsFileN` — *File containing the reference positions for fitting*

  [UNIX filename, context: `gspath` and `gzpath`]

  The path is defined by multiple `refPositionsFiles` which are similiar to `refPositionsFile` in the `rmsd` CV. If your path consists of 10 nodes, you can list the coordinate file (in PDB or XYZ format) from `refPositionsFile1` to `refPositionsFile10`.

- `useSecondClosestFrame` — *Define $\mathbf{s}_{m-1}$ as the second closest frame?*

  Default: `on` [boolean, context: `gspath` and `gzpath`]

  The definition assumes the second closest frame is neighbouring to the closest frame. This is not always true especially when the path is crooked. If this option is set to `on` (default), $\mathbf{s}_{m-1}$ is defined as the second closest frame. If this option is set to `off`, $\mathbf{s}_{m-1}$ is defined as the left or right neighbouring frame of the closest frame.

- `useThirdClosestFrame` — *Define $\mathbf{s}_{m+1}$ as the third closest frame?*

  Default: `off` [boolean, context: `gspath` and `gzpath`]

  The definition assumes the third closest frame is neighbouring to the closest frame. This is

not always true especially when the path is crooked. If this option is set to $\texttt{on}$, $\mathbf{s}_{m+1}$ is defined as the third closest frame. If this option is set to $\texttt{off}$ (default), $\mathbf{s}_{m+1}$ is defined as the left or right neighbouring frame of the closest frame.

- $\boxed{\texttt{fittingAtoms}}$ — *The atoms that are used for alignment*

  [Group of atoms, context: $\texttt{gspath}$ and $\texttt{gzpath}$]

  Before calculating $\mathbf{v}_1$, $\mathbf{v}_2$, $\mathbf{v}_3$ and $\mathbf{v}_4$, the current frame need to be aligned to the corresponding reference frames. This option specifies which atoms are used to do alignment.

- $\boxed{\texttt{forceNoPBC}}$ — *Use absolute rather than minimum-image distances?* (see description)
  Default: $\texttt{no}$

4.11.2 $\texttt{gzpath}$: distance from a path defined in atomic Cartesian coordinate space.

List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- $\boxed{\texttt{useZsquare}}$ — *Compute z^2 instead of z*

  Default: $\texttt{off}$ [boolean, context: $\texttt{gzpath}$]

  z is not differentiable when it is zero. This implementation workarounds it by setting the derivative of z to zero when $z = 0$. Another workaround is set this option to $\texttt{on}$, which computes z^2 instead of z, and then z^2 is differentiable when it is zero.

- $\boxed{\texttt{forceNoPBC}}$ — *Use absolute rather than minimum-image distances?* (see description)
  Default: $\texttt{no}$

The usage of $\texttt{gzpath}$ and $\texttt{gspath}$ is illustrated below:

```
colvar {
  # Progress along the path
  name gs
  # The path is defined by 5 reference frames (from string-00.pdb to string-04.pdb)
  # Use atomic coordinate from atoms 1, 2 and 3 to compute the path
  gspath {
    atoms {atomnumbers { 1 2 3 }}
    refPositionsFile1 string-00.pdb
    refPositionsFile2 string-01.pdb
    refPositionsFile3 string-02.pdb
    refPositionsFile4 string-03.pdb
    refPositionsFile5 string-04.pdb
  }
}
colvar {
  # Distance from the path
  name gz
  # The path is defined by 5 reference frames (from string-00.pdb to string-04.pdb)
  # Use atomic coordinate from atoms 1, 2 and 3 to compute the path
```

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```
  gzpath {
    atoms {atomnumbers { 1 2 3 }}
    refPositionsFile1 string-00.pdb
    refPositionsFile2 string-01.pdb
    refPositionsFile3 string-02.pdb
    refPositionsFile4 string-03.pdb
    refPositionsFile5 string-04.pdb
  }
}
```

4.11.3 `linearCombination`: **Helper CV to define a linear combination of other CVs**

This is a helper CV which can be defined as a linear combination of other CVs. It maybe useful when you want to define the `gspathCV {...}` and the `gzpathCV {...}` as combinations of other CVs. Total forces (required by ABF) of this CV are not available.

4.11.4 `customColvar`: **Helper CV to define a mathematical expression as CV from other CVs**

This is a helper CV which can be defined as a mathematical expression (see 4.19) of other CVs by using `customFunction`. Currently only the scalar type of `customFunction` is supported. If `customFunction` is not provided, this component falls back to `linearCombination`. It maybe useful when you want to define the `gspathCV {...}`, the `gzpathCV {...}` and `NeuralNetwork {...}` as combinations of other CVs. Total forces (required by ABF) of this CV are not available.

4.11.5 `gspathCV`: **progress along a path defined in CV space.**

In the `gspathCV {...}` and the `gzpathCV {...}` block all vectors, namely $\mathbf{z}$ and $\mathbf{s}_k$ are defined in CV space. More specifically, $\mathbf{z} = [\xi_1, \cdots, \xi_n]$, where ξ_i is the i-th CV. $\mathbf{s}_k = [\xi_{k,1}, \cdots, \xi_{k,n}]$, where $\xi_{k,i}$ means the i-th CV of the k-th reference frame. It should be note that these two CVs requires the `pathFile` option, which specifies a path file. Each line in the path file contains a set of space-seperated CV value of the reference frame. The sequence of reference frames matches the sequence of the lines.
List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `useSecondClosestFrame` — *Define $\mathbf{s}_{m-1}$ as the second closest frame?*

  Default: `on` [boolean, context: `gspathCV` and `gzpathCV`]

  The definition assumes the second closest frame is neighbouring to the closest frame. This is not always true especially when the path is crooked. If this option is set to `on` (default), $\mathbf{s}_{m-1}$ is defined as the second closest frame. If this option is set to `off`, $\mathbf{s}_{m-1}$ is defined as the left or right neighbouring frame of the closest frame.

- `useThirdClosestFrame` — *Define $\mathbf{s}_{m+1}$ as the third closest frame?*

  Default: `off` [boolean, context: `gspathCV` and `gzpathCV`]

  The definition assumes the third closest frame is neighbouring to the closest frame. This is not always true especially when the path is crooked. If this option is set to `on`, $\mathbf{s}_{m+1}$ is defined as the third closest frame. If this option is set to `off` (default), $\mathbf{s}_{m+1}$ is defined as the left or right neighbouring frame of the closest frame.

- `pathFile` — *The file name of the path file.*

  [UNIX filename, context: `gspathCV` and `gzpathCV`]

  Defines the nodes or images that constitutes the path in CV space. The CVs of an image are listed in a line of `pathFile` using space-seperated format. Lines from top to button in `pathFile` corresponds images from initial to last.

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

4.11.6 `gzpathCV`: distance from a path defined in CV space.

List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `useZsquare` — *Compute z^2 instead of z*

  Default: `off` [boolean, context: `gzpathCV`]

  z is not differentiable when it is zero. This implementation workarounds it by setting the derivative of z to zero when $z = 0$. Another workaround is set this option to `on`, which computes z^2 instead of z, and then z^2 is differentiable when it is zero.

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

The usage of `gzpathCV` and `gspathCV` is illustrated below:

```
colvar {
  # Progress along the path
  name gs
  # Path defined by the CV space of two dihedral angles
  gspathCV {
    pathFile ./path.txt
    dihedral {
      name 001
      group1 {atomNumbers {5}}
      group2 {atomNumbers {7}}
      group3 {atomNumbers {9}}
      group4 {atomNumbers {15}}
    }
    dihedral {
```

```
      name 002
      group1 {atomNumbers {7}}
      group2 {atomNumbers {9}}
      group3 {atomNumbers {15}}
      group4 {atomNumbers {17}}
    }
  }
}

colvar {
  # Distance from the path
  name gz
  gzpathCV {
    pathFile ./path.txt
    dihedral {
      name 001
      group1 {atomNumbers {5}}
      group2 {atomNumbers {7}}
      group3 {atomNumbers {9}}
      group4 {atomNumbers {15}}
    }
    dihedral {
      name 002
      group1 {atomNumbers {7}}
      group2 {atomNumbers {9}}
      group3 {atomNumbers {15}}
      group4 {atomNumbers {17}}
    }
  }
}
```

4.12 Arithmetic path collective variables

The arithmetic path collective variable in CV space uses a similar formula as the one proposed by Branduardi[11] et al., except that it computes s and z in CV space instead of RMSDs in Cartesian space. Moreover, this implementation allows different coefficients for each CV components as described in [12]. Assuming a path is composed of N reference frames and defined in an M-dimensional CV space, then the equations of s and z of the path are

$$s = \frac{1}{N-1} \frac{\sum_{i=0}^{N-1} i \exp\left(-\lambda \sum_{j=1}^{M} c_j^2 (x_j - x_{i,j})^2\right)}{\sum_{i=0}^{N-1} \exp\left(-\lambda \sum_{j=1}^{M} c_j^2 (x_j - x_{i,j})^2\right)} \tag{16}$$

$$z = -\frac{1}{\lambda} \ln\left(\sum_{i=0}^{N-1} \exp\left(-\lambda \sum_{j=1}^{M} c_j^2 (x_j - x_{i,j})^2\right)\right) \tag{17}$$

where c_j is the coefficient(weight) of the j-th CV, $x_{i,j}$ is the value of j-th CV of i-th reference frame and x_j is the value of j-th CV of current frame. λ is a parameter to smooth the variation of s and z. It should be noted that the index i ranges from 0 to $N-1$, and the definition of s is normalized by $1/(N-1)$. Consequently, the scope of s is $[0:1]$.

4.12.1 `aspathCV`: progress along a path defined in CV space.

This colvar component computes the s variable.

List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `weights` — *Coefficients of the collective variables*

  Default: `{1.0 ...}` [space-separated numbers in a `{...}` block, context: `aspathCV` and `azpathCV`]

  Define the coefficients. The j-th value in the `{...}` block corresponds the c_j in the equations.

- `lambda` — *Smoothness of the variation of s and z*

  Default: inverse of the mean square displacements of successive reference frames [decimal, context: `aspathCV` and `azpathCV`]

  The value of λ in the equations.

- `pathFile` — *The file name of the path file.*

  [UNIX filename, context: `aspathCV` and `azpathCV`]

  Defines the nodes or images that constitutes the path in CV space. The CVs of an image are listed in a line of `pathFile` using space-separated format. Lines from top to button in `pathFile` corresponds images from initial to last.

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

4.12.2 `azpathCV`: distance from a path defined in CV space.

This colvar component computes the z variable. Options are the same as in 4.12.1.
The usage of `azpathCV` and `aspathCV` is illustrated below:

```
colvar {
  # Progress along the path
  name as
  # Path defined by the CV space of two dihedral angles
  aspathCV {
    pathFile ./path.txt
    weights {1.0 1.0}
    lambda 0.005
    dihedral {
```

```
        name 001
        group1 {atomNumbers {5}}
        group2 {atomNumbers {7}}
        group3 {atomNumbers {9}}
        group4 {atomNumbers {15}}
      }
      dihedral {
        name 002
        group1 {atomNumbers {7}}
        group2 {atomNumbers {9}}
        group3 {atomNumbers {15}}
        group4 {atomNumbers {17}}
      }
    }
  }
}

colvar {
  # Distance from the path
  name az
  azpathCV {
    pathFile ./path.txt
    weights {1.0 1.0}
    lambda 0.005
    dihedral {
      name 001
      group1 {atomNumbers {5}}
      group2 {atomNumbers {7}}
      group3 {atomNumbers {9}}
      group4 {atomNumbers {15}}
    }
    dihedral {
      name 002
      group1 {atomNumbers {7}}
      group2 {atomNumbers {9}}
      group3 {atomNumbers {15}}
      group4 {atomNumbers {17}}
    }
  }
}
```

4.12.3 `aspath`: progress along a path defined in atomic Cartesian coordinate space.

This CV computes a special case of Eq. 16, where x_j is the j-th atomic position, $x_{i,j}$ is the j-th atomic position of the i-th reference frame. The subtraction $x_j - x_{i,j}$ is actually calculated as $\mathbf{x}_j - R_i \mathbf{x}_{i,j}$, where R_i is a 3x3 rotation matrix that minimizes the RMSD between the current atomic

positions of simulation and the i-th reference frame. Bold $\mathbf{x}_j$ is used since an atomic position is a vector.

List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `atoms` — *Group of atoms*

  [Atom group, context: `aspath` and `azpath`]

  Defines the atoms whose coordinates make up the value of the component.

- `refPositionsCol` — *PDB column containing atom flags*

  [O, B, X, Y, or Z, context: `aspath` and `azpath`]

  If `refPositionsFileN` is a PDB file that contains all the atoms in the topology, this option may be provided to set which PDB field is used to flag the reference coordinates for `atoms`.

- `refPositionsFileN` — *File containing the reference positions for fitting*

  [UNIX filename, context: `aspath` and `azpath`]

  The path is defined by multiple `refPositionsFile`s which are similiar to `refPositionsFile` in the `rmsd` CV. If your path consists of 10 nodes, you can list the coordinate file (in PDB or XYZ format) from `refPositionsFile1` to `refPositionsFile10`.

- `fittingAtoms` — *The atoms that are used for alignment*

  [Group of atoms, context: `aspath` and `azpath`]

  Before calculating $\mathbf{v}_1$, $\mathbf{v}_2$, $\mathbf{v}_3$ and $\mathbf{v}_4$, the current frame need to be aligned to the corresponding reference frames. This option specifies which atoms are used to do alignment.

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

4.12.4 `azpath`: distance from a path defined in atomic Cartesian coordinate space.

Similar to `aspath`, this CV computes a special case of Eq. 17, and shares the same options as `aspath`.

The usage of `azpath` and `aspath` is illustrated below:

```
colvar {
  # Progress along the path
  name as
  # The path is defined by 5 reference frames (from string-00.pdb to string-04.pdb)
  # Use atomic coordinate from atoms 1, 2 and 3 to compute the path
  aspath {
    atoms {atomnumbers { 1 2 3 }}
    refPositionsFile1 string-00.pdb
    refPositionsFile2 string-01.pdb
    refPositionsFile3 string-02.pdb
    refPositionsFile4 string-03.pdb
```

```
        refPositionsFile5 string-04.pdb
    }
}

colvar {
  # Distance from the path
  name az
  # The path is defined by 5 reference frames (from string-00.pdb to string-04.pdb)
  # Use atomic coordinate from atoms 1, 2 and 3 to compute the path
  azpath {
    atoms {atomnumbers { 1 2 3 }}
    refPositionsFile1 string-00.pdb
    refPositionsFile2 string-01.pdb
    refPositionsFile3 string-02.pdb
    refPositionsFile4 string-03.pdb
    refPositionsFile5 string-04.pdb
  }
}
```

4.13 Dense neural network in CV space (MLCV)

This colvar component computes a non-linear combination of other scalar colvar components, where the transformation is defined by a dense neural network.[13] The network can be optimized using any framework, and its parameters are provided to Colvars in plain text files, as detailed below. An example Python script to export the parameters of a TensorFlow model is provided in `colvartools/extract_weights_biases.py` in the Colvars source tree.
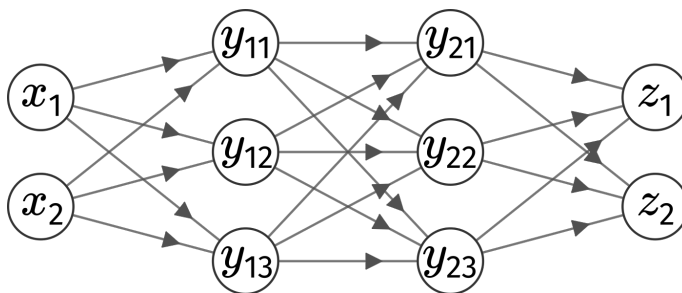
Figure 1: Graphical representation of an example dense neural network with two hidden layers y_1 and y_2. Both the input layer x and the output layer z have two nodes. The input nodes can be any existing scalar CVs.

The output of the j-th node of a k-th layer that has N_k nodes is computed by

$$y_{k,j} = f_k \left(\sum_{i=1}^{N_{k-1}} w_{(k,j),(k-1,i)} y_{k-1,i} + b_{k,j} \right), \tag{18}$$

where f_k is the activation function of the k-th layer, $w_{(k,j),(k-1,i)}$ is the weight of j-th node with respect to the i-th output of previous layer, and $b_{k,j}$ is the bias of j-th node of k-th layer.

List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `output_component` — *The j-th node of the output or the last layer*

  [integer starting from 0, context: `NeuralNetwork`]

  The value of this option specifies the output node to be used as the value of this CV.

- `layeri_WeightsFile` — *The weights from layer $i-1$ to layer i*

  [UNIX filename, context: `NeuralNetwork`]

  The letter i in this option needs to be replaced with the indexing number starting from 1, for example, `layer1_WeightsFile` and `layer2_WeightsFile`. The value of this option specifies a plain text file containing the weights from layer $i-1$ to layer i. In the file, the number at k-th column and l-th row represents the weight from node k at layer $i-1$ to node l at layer i.

- `layeri_BiasesFile` — *The biases from layer $i-1$ to layer i*

  [UNIX filename, context: `NeuralNetwork`]

  The letter i in this option needs to be replaced with the indexing number starting from 1, for example, `layer1_BiasesFile` and `layer2_BiasesFile`. The value of this option specifies a plain text file containing the weights of layer i. The file should have only one column, where the number at l-th row represents the bias of node l from layer $i-1$ to layer i.

- `layeri_activation` — *The activation function from layer $i-1$ to layer i*

  [`tanh, sigmoid, linear, relu, lrelu100, elu` , context: `NeuralNetwork`]

  The letter i in this option needs to be replaced with the indexing number starting from 1, for example, `layer1_activation` and `layer2_activation`. The activation function from layer $i-1$ to layer i. Available choices are `tanh`, `sigmoid`, `linear` (identity), `relu`, `lrelu100` (a leaky rely with coefficients 10^{-2} and 1), and `elu` (with coefficient 1).

- `layeri_custom_activation` — *An alternative custom expression as the activation function from layer $i-1$ to layer i*

  [string, context: `NeuralNetwork`]

  Mathematical expression to define the activation function from layer $i-1$ to layer i. The input value must be written as `x`. For example, the ELU activation function can be expressed as `select(step(x), alpha*(exp(x)-1), x)`. For details of the expression syntax, see customFunction. This option is mutually exclusive with `colvar|NeuralNetwork|layeri_activation`

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

An example of configuration using `NeuralNetwork` is shown below:

```
colvar {
  # Define a neural network with 2 layers
  # The inputs are two torsion angles
  # and the first node at the output layer is used as the final CV
  name nn_output_1
  NeuralNetwork {
```

```
      output_component 0
      layer1_WeightsFile      dense_1_weights.txt
      layer1_BiasesFile       dense_1_biases.txt
      layer1_activation       tanh
      layer2_WeightsFile      dense_2_weights.txt
      layer2_BiasesFile       dense_2_biases.txt
      layer2_activation       tanh
      # The component coefficient is used for normalization
      componentCoeff 180.0
      dihedral {
        name 001
        # normalization factor 1.0/180.0
        componentCoeff 0.00555555555555555556
        group1 {atomNumbers {5}}
        group2 {atomNumbers {7}}
        group3 {atomNumbers {9}}
        group4 {atomNumbers {15}}
      }
      dihedral {
        name 002
        # normalization factor 1.0/180.0
        componentCoeff 0.00555555555555555556
        group1 {atomNumbers {7}}
        group2 {atomNumbers {9}}
        group3 {atomNumbers {15}}
        group4 {atomNumbers {17}}
      }
    }
  }
}
```

4.14   Torch-based collective variables

The `torchANN` keyword defines a scalar component based on an artificial neural network (ANN) model previously trained using PyTorch. Similarly to the `linearCombination` and `neuralNetwork` components, the ANN model is expressed as a function of collective variable components. The model is loaded from a PyTorch-formatted file, defined by the keyword `modelFile`.

This component requires that Tinker-HP is linked against the libTorch library; see 8 for more details.

List of keywords (see also 4.2, 4.15, 4.16 and 4.18 for additional options):

- `modelFile` — *Name of the file containing the model (PyTorch format)*

  [filename, context: `torchANN`]

  This file contains the PyTorch model, as saved for example by the `torch.jit.save()` function. The model is assumed to take a one-dimensional tensor (excluding batch dimension) as input.

- `output_component` — *Index of the output node to use*

  Default: 0 [0-based integer, context: `torchANN`]

  This option specifies the index of the output node to be used as the value of the collective variable (see also the equivalent keyword `output_component` in the built-in neural network implementation).

- `doubleInputTensor` — *Specify that the input model is in double precision*

  Default: `off` [boolean, context: `torchANN`]

  This flags controls the precision of the PyTorch model contained in `modelFile`.

- `forceNoPBC` — *Use absolute rather than minimum-image distances?* (see description)
  Default: `no`

An example of creating a model file and defining a collective variable component using `torchANN` is shown below:

```
import torch
import torch.nn as nn

# The neural network model is a function:
#       f = (f₁, f₂, f₃) : ℝ⁸ → ℝ³ .
model = nn.Sequential(nn.Linear(8, 100),
                      nn.ReLU(),
                      nn.Linear(100, 3))
# Train the model
...
# Save the trained model
torch.jit.script(model).save("./model.pt")
```

```
colvar {
  # Define a scalar CV as ξ = f₁(x₂,y₂,z₂,x₄,y₄,z₄,φ,ψ),
  # i.e. the function represented by the first component of the Torch model,
  # evaluated at the coordinates of the two atoms with atomNumbers 2 and 4,
  # and the two torsion angles φ,ψ.
  name nn_output_1
  torchann {
    modelFile model.pt
    output_component 0
    cartesian {
      atoms {
        atomNumbers {{2 4}}
      }
    }
    dihedral {
      name 001
      group1 {atomnumbers {5}}
```

```
        group2 {atomnumbers {7}}
        group3 {atomnumbers {9}}
        group4 {atomnumbers {15}}
      }
     dihedral {
        name 002
        group1 {atomnumbers {7}}
        group2 {atomnumbers {9}}
        group3 {atomnumbers {15}}
        group4 {atomnumbers {17}}
      }
    }
  }
}
```

4.15 Shared keywords for all components

The following options can be used for any of the above colvar components in order to obtain a polynomial combination or any user-supplied function provided by `scriptedFunction`.

- `name` — *Name of this component*

  Default: type of component + numeric id [string, context: any component]

  The name is an unique case-sensitive string which allows the Colvars module to identify this component. This is useful, for example, when combining multiple components via a `scriptedFunction`.It also defines the variable name representing the component's value in a `customFunction` expression.

- `scalable` — *Attempt to calculate this component in parallel?*

  Default: `on`, if available [boolean, context: any component]

  If set to `on` (default), the Colvars module will attempt to calculate this component in parallel to reduce overhead. Whether this option is available depends on the type of component: currently supported are `distance`, `distanceZ`, `distanceXY`, `distanceVec`, `distanceDir`, `angle` and `dihedral`. This flag influences computational cost, but does not affect numerical results: therefore, it should only be turned off for debugging or testing purposes.

4.16 Periodic components

Certain components, such as `dihedral` or `dihedral`, compute angles that lie in a periodic interval between $-180°$ and $180°$. When computing pairwise distances between values of those angles (e.g. for the sake of computing restraint potentials, or sampling PMFs), periodicity is taken into account by following the minimum-image convention.

Additionally, several other components, such as `distanceZ`, support optional periodicity if this is provided in the configuration.

The following keywords can be used within periodic components, or within custom variables (4.19)).

- `period` — *Period of the component*

  Default: 0.0, unless otherwise stated [positive decimal, context: components with optional periodicity]

  Setting this number enables the treatment of as a periodic component: by default, `distanceZ` is not considered periodic. The keyword is supported, but irrelevant within `dihedral` or `spinAngle`, because their period is always 360 degrees.

- `wrapAround` — *Center of the wrapping interval for periodic variables*

  Default: 0.0 [decimal, context: periodic components]

  By default, values of the periodic components are centered around zero, ranging from $-P/2$ to $P/2$, where P is the period. Setting this number centers the interval around this value. This can be useful for convenience of output, or to set the walls for a `harmonicWalls` in an order that would not otherwise be allowed.

Note: using linear/polynomial combinations of periodic components (see 4.18), or other custom or scripted function may invalidate the periodicity. Use such combinations carefully: estimate the range of possible values of each component in a given simulation, and make use of `wrapAround` to limit this problem whenever possible.

4.17   Non-scalar components

When one of the following components are used, the defined colvar returns a value that is not a scalar number:

- `distanceVec`: 3-dimensional vector of the distance between two groups;

- `distanceDir`: 3-dimensional unit vector of the distance between two groups;

- `orientation`: 4-dimensional unit quaternion representing the best-fit rotation from a set of reference coordinates.

The distance between two 3-dimensional unit vectors is computed as the angle between them. The distance between two quaternions is computed as the angle between the two 4-dimensional unit vectors: because the orientation represented by q is the same as the one represented by $-q$, distances between two quaternions are computed considering the closest of the two symmetric images.

Non-scalar components carry the following restrictions:

- Calculation of total forces (`outputTotalForce` option) is currently not implemented.

- Each colvar can only contain one non-scalar component.

- Binning on a grid (`abf`, `histogram` and `metadynamics` with `useGrids` enabled) is currently not implemented for colvars based on such components.

Note: while these restrictions apply to individual colvars based on non-scalar components, no limit is set to the number of scalar colvars. To compute multi-dimensional histograms and PMFs, use sets of scalar colvars of arbitrary size.

4.17.1   Calculating total forces

In addition to the restrictions due to the type of value computed (scalar or non-scalar), a final restriction can arise when calculating total force (`outputTotalForce` option or application of a `abf` bias). total forces are available currently only for the following components: `distance`, `distanceZ`, `distanceXY`, `angle`, `dihedral`, `rmsd`, `eigenvector` and `gyration`.

4.18   Linear and polynomial combinations of components

To extend the set of possible definitions of colvars $\xi(\mathbf{r})$, multiple components $q_i(\mathbf{r})$ can be summed with the formula:

$$\xi(\mathbf{r}) = \sum_i c_i [q_i(\mathbf{r})]^{n_i} \tag{19}$$

where each component appears with a unique coefficient c_i (1.0 by default) the positive integer exponent n_i (1 by default).

Any set of components can be combined within a colvar, provided that they return the same type of values (scalar, unit vector, vector, or quaternion). By default, the colvar is the sum of its components. Linear or polynomial combinations (following equation (19)) can be obtained by setting the following parameters, which are common to all components:

- `componentCoeff` — *Coefficient of this component in the colvar*

  Default: `1.0` [decimal, context: any component]

  Defines the coefficient by which this component is multiplied (after being raised to `componentExp`) before being added to the sum.

- `componentExp` — *Exponent of this component in the colvar*

  Default: `1` [integer, context: any component]

  Defines the power at which the value of this component is raised before being added to the sum. When this exponent is different than 1 (non-linear sum), total forces and the Jacobian force are not available, making the colvar unsuitable for ABF calculations (eABF remains possible).

Example: To define the *average* of a colvar across different parts of the system, simply define within the same colvar block a series of components of the same type (applied to different atom groups), and assign to each component a `componentCoeff` of $1/N$.

4.19   Custom functions

Collective variables may be defined by specifying a custom function of multiple components, i.e. an analytical expression that is more general than the linear combinations described in 4.18. Such expression is parsed and calculated by Lepton, the lightweight expression parser written by Peter Eastman (https://simtk.org/projects/lepton) that produces efficient evaluation routines for both the expression and its derivatives. Although Lepton is generally available in most applications and builds where Colvars is included, it is best to check section 8 to confirm.

- `customFunction` — *Compute colvar as a custom function of its components*

  [string, context: `colvar`]

  Mathematical expression to define the colvar as a closed-form function of its colvar components. See below for the detailed syntax of Lepton expressions. Multiple mentions of this keyword can be used to define a vector variable (as in the example below).

- `customFunctionType` — *Type of value returned by the custom function*

  Default: `scalar` [string, context: `colvar`]

  With this flag, the user may specify whether the colvar is a scalar or one of the following vector types: `vector3` (a 3D vector), `unit_vector3` (a normalized 3D vector), or `unit_quaternion` (a normalized quaternion), or `vector`. Note that the scalar and vector cases are not necessary, as they are detected automatically.

The expression may use the collective variable components as variables, referred to by their user-defined `name`. Scalar elements of vector components may be accessed by appending a 1-based index to their `name`, as in the example below. When implementing generic functions of Cartesian coordinates rather than functions of existing components, the `cartesian` component may be particularly useful. A scalar-valued custom variable may be manually defined as periodic by providing the keyword `period`, and the optional keyword `wrapAround`, with the same meaning as in periodic components (see 4.16 for details). A vector variable may be defined by specifying the `customFunction` parameter several times: each expression defines one scalar element of the vector colvar, as in this example:

```
colvar {
  name custom

  # A 2-dimensional vector function of a scalar x and a 3-vector r
  customFunction cos(x) * (r1 + r2 + r3)
  customFunction sqrt(r1 * r2)

  distance {
    name x
    group1 { atomNumbers 1 }
    group2 { atomNumbers 50 }
  }
  distanceVec {
    name r
    group1 { atomNumbers 10 11 12 }
    group2 { atomNumbers  20 21 22 }
```

```
    }
}
```

Numeric constants may be given in either decimal or exponential form (e.g. 3.12e-2). An expression may be followed by definitions for intermediate values that appear in the expression, separated by semicolons. For example, the expression:

`aˆ2 + a*b + bˆ2; a = a1 + a2; b = b1 + b2`

is exactly equivalent to:

`(a1 + a2)ˆ2 + (a1 + a2) * (b1 + b2) + (b1 + b2)ˆ2.`

The definition of an intermediate value may itself involve other intermediate values. All uses of a value must appear *before* that value's definition.

Lepton supports the usual arithmetic operators +, -, *, /, and ˆ (power), as well as the following functions:

sqrt	Square root
exp	Exponential
log	Natural logarithm
erf	Error function
erfc	Complementary error function
sin	Sine (angle in radians)
cos	Cosine (angle in radians)
sec	Secant (angle in radians)
csc	Cosecant (angle in radians)
tan	Tangent (angle in radians)
cot	Cotangent (angle in radians)
asin	Inverse sine (in radians)
acos	Inverse cosine (in radians)
atan	Inverse tangent (in radians)
atan2	Two-argument inverse tangent (in radians)
sinh	Hyperbolic sine
cosh	Hyperbolic cosine
tanh	Hyperbolic tangent
abs	Absolute value
floor	Floor
ceil	Ceiling
min	Minimum of two values
max	Maximum of two values
delta	$\mathrm{delta}(x) = 1$ if $x = 0$, 0 otherwise
step	$\mathrm{step}(x) = 0$ if $x < 0$, 1 if $x >= 0$
select	$\mathrm{select}(x, y, z) = z$ if $x = 0$, y otherwise

4.20 Scripted functions

When scripting is supported, a colvar may be defined as a scripted function of its components, rather than a linear or polynomial combination. When implementing generic functions of Cartesian coordinates rather than functions of existing components, the `cartesian` component may be

particularly useful. A scalar-valued scripted variable may be manually defined as periodic by providing the keyword `period`, and the optional keyword `wrapAround`, with the same meaning as in periodic components (see 4.16 for details).

An example of elaborate scripted colvar is given in example 10, in the form of path-based collective variables as defined by Branduardi et al[11] (Section **??**).

- `scriptedFunction` — *Compute colvar as a scripted function of its components*

  [string, context: `colvar`]

  If this option is specified, the colvar will be computed as a scripted function of the values of its components. To that effect, the user should define two Tcl procedures: `calc_<scriptedFunction>` and `calc_<scriptedFunction>_gradient`, both accepting as many parameters as the colvar has components. Values of the components will be passed to those procedures in the order defined by their sorted `name` strings. Note that if all components are of the same type, their default names are sorted in the order in which they are defined, so that names need only be specified for combinations of components of different types. `calc_<scriptedFunction>` should return one value of type <scriptedFunctionType>, corresponding to the colvar value. `calc_<scriptedFunction>_gradient` should return a Tcl list containing the derivatives of the function with respect to each component. If both the function and some of the components are vectors, the gradient is really a Jacobian matrix that should be passed as a linear vector in row-major order, i.e. for a function $f_i(x_j)$: $\nabla_x f_1 \nabla_x f_2 \cdots$.

- `scriptedFunctionType` — *Type of value returned by the scripted colvar*

  Default: `scalar` [string, context: `colvar`]

  If a colvar is defined as a scripted function, its type is not constrained by the types of its components. With this flag, the user may specify whether the colvar is a scalar or one of the following vector types: `vector3` (a 3D vector), `unit_vector3` (a normalized 3D vector), or `unit_quaternion` (a normalized quaternion), or `vector` (a vector whose size is specified by `scriptedFunctionVectorSize`). Non-scalar values should be passed as space-separated lists.

- `scriptedFunctionVectorSize` — *Dimension of the vector value of a scripted colvar*

  [positive integer, context: `colvar`]

  This parameter is only valid when `scriptedFunctionType` is set to `vector`. It defines the vector length of the colvar value returned by the function.

4.21   Defining grid parameters for a colvar

Many algorithms require the definition of two boundaries and a bin width for each colvar, which are necessary to compute discrete "states" for a collective variable's otherwise continuous values. The following keywords define these parameters for a specific variable, and will be used by all bias that refer to that variable unless otherwise specified. Some of these parameters can be set directly within a bias configuration by inserting a `grid` block (6.1).

- `lowerBoundary` — *Lower boundary of the colvar*

  Default: natural boundary of the function [decimal, context: `colvar`]

  Defines the lowest end of the interval of "relevant" values for the variable. This number can be, for example, a true physical boundary imposed by the choice of function (e.g. the `distance` function is always larger than zero): if this is the case, and only one function is used to define the variable, the default value of this number is set to the lowest end of the range of values of that function, if available (see Section 4.1). Alternatively, this value may be provided by the user, to represent for example the left-most point of a PMF calculation along this variable. In the latter case, it is the user's responsibility to either *(a)* ensure the variable does not go significantly beyond the boundary (for example by adding a `harmonicWalls` restraint, 6.10), or *(b)* instruct the code that this is a true physical boundary by setting `hardLowerBoundary`.

- `upperBoundary` — *Upper boundary of the colvar*

  Default: natural boundary of the function [decimal, context: `colvar`]

  Similarly to `lowerBoundary`, defines the highest of the "relevant" values of the variable.

- `width` — *grid spacing, or unit of the variable*

  Default: 1.0 [positive decimal, context: `colvar`]

  This number defines the width of a discrete "state" for a collective variable, and is used by the many biasing methods to achieve different purposes. Histograms (6.13), ABF (6.3) and metadynamics (6.6) all use this number as the initial choice for the grid spacing along this variable. As a typical rule of thumb, `width` should be no larger than the standard deviation of the colvar in an unbiased simulation (to characterize a local free-energy minimum with at least two points).

  Further, many restraints such as harmonic potentials (6.8), harmonic walls (6.10) and linear restraints (6.11) also use this parameter to define the *expected fluctuations* of the colvar, allowing to express the force constant in terms of this unit. This is most useful with multi-dimensional restraints acting on variables that have very different units (for examples, working with Å and degrees $^\circ$ simultaneously): a single force constant can be used for all, which is converted to the respective unit of each variable when forces are applied (the are printed at initialization time.

- `hardLowerBoundary` — *Whether the lower boundary is the physical lower limit*

  Default: provided by the component [boolean, context: `colvar`]

  When the colvar has a "natural" boundary (for example, a `distance` colvar cannot go below 0) this flag is automatically enabled. For more complex variable definitions, or when `lowerBoundary` is provided directly by the user, it may be useful to set this flag explicitly. This option does not affect simulation results, but enables some internal optimizations by letting the code know that the variable is unable to cross the lower boundary, regardless of whether restraints are applied to it.

- `hardUpperBoundary` — *Whether the upper boundary is the physical upper limit of the colvar's values*

  Default: provided by the component [boolean, context: `colvar`]

Analogous to `hardLowerBoundary`.

- `expandBoundaries` — *Allow to expand the two boundaries if needed*

  Default: `off` [boolean, context: `colvar`]

  If defined, `lowerBoundary` and `upperBoundary` may be automatically expanded to accommodate colvar values that do not fit in the initial range. Currently, this option is used by the metadynamics bias (6.6) to keep all of its hills fully within the grid. Enabling this option does not affect any boundaries that are defined as "hard" (see above), or any boundaries that span the full period of a periodic colvar.

4.22   Trajectory output

- `outputValue` — *Output a trajectory for this colvar*

  Default: `on` [boolean, context: `colvar`]

  If `colvarsTrajFrequency` is non-zero, the value of this colvar is written to the trajectory file every `colvarsTrajFrequency` steps in the column labeled "<name>".

- `outputVelocity` — *Output a velocity trajectory for this colvar*

  Default: `off` [boolean, context: `colvar`]

  If `colvarsTrajFrequency` is defined, the finite-difference calculated velocity of this colvar are written to the trajectory file under the label "v_<name>".

- `outputEnergy` — *Output an energy trajectory for this colvar*

  Default: `off` [boolean, context: `colvar`]

  This option applies only to extended Lagrangian colvars. If `colvarsTrajFrequency` is defined, the kinetic energy of the extended degree and freedom and the potential energy of the restraining spring are are written to the trajectory file under the labels "Ek_<name>" and "Ep_<name>".

- `outputTotalForce` — *Output a total force trajectory for this colvar*

  Default: `off` [boolean, context: `colvar`]

  If `colvarsTrajFrequency` is defined, the total force on this colvar (i.e. the projection of all atomic total forces onto this colvar — see equation (24) in section 6.3) are written to the trajectory file under the label "fs_<name>". For extended Lagrangian colvars, the "total force" felt by the extended degree of freedom is simply the force from the harmonic spring. **Note:** not all components support this option. The physical unit for this force is kcal/mol/*(colvar unit)*.

- `outputAppliedForce` — *Output an applied force trajectory for this colvar*

  Default: `off` [boolean, context: `colvar`]

  If `colvarsTrajFrequency` is defined, the total force applied on this colvar by Colvars biases

are written to the trajectory under the label "`fa_<name>`". For extended Lagrangian colvars, this force is actually applied to the extended degree of freedom rather than the geometric colvar itself. The physical unit for this force is kcal/mol/*(colvar unit)*.

4.23   Extended Lagrangian

The following options enable extended-system dynamics, where a colvar is coupled to an additional degree of freedom (fictitious particle) by a harmonic spring. This extended coordinate masks the colvar and replaces it transparently from the perspective of biasing and analysis methods. Biasing forces are then applied to the extended degree of freedom, and the actual geometric colvar (function of Cartesian coordinates) only feels the force from the harmonic spring. This is particularly useful when combined with an `abf` bias to perform eABF simulations (6.4).

Note that for some biases (`harmonicWalls`, `histogram`), this masking behavior is controlled by the keyword `bypassExtendedLagrangian`. Specifically for `harmonicWalls`, the default behavior is to bypass extended Lagrangian coordinates and act directly on the actual colvars.

- `extendedLagrangian` — *Add extended degree of freedom*

  Default: `off` [boolean, context: `colvar`]

  Adds a fictitious particle to be coupled to the colvar by a harmonic spring. The fictitious mass and the force constant of the coupling potential are derived from the parameters `extendedTimeConstant` and `extendedFluctuation`, described below. Biasing forces on the colvar are applied to this fictitious particle, rather than to the atoms directly. This implements the extended Lagrangian formalism used in some metadynamics simulations [2]. .

- `extendedFluctuation` — *Standard deviation between the colvar and the fictitious particle (colvar unit)*

  [positive decimal, context: `colvar`]

  Defines the spring stiffness for the `extendedLagrangian` mode, by setting the typical deviation between the colvar and the extended degree of freedom due to thermal fluctuation. The spring force constant is calculated internally as $k_B T / \sigma^2$, where σ is the value of `extendedFluctuation`.

- `extendedTimeConstant` — *Oscillation period of the fictitious particle (fs)*

  Default: `200` [positive decimal, context: `colvar`]

  Defines the inertial mass of the fictitious particle, by setting the oscillation period of the harmonic oscillator formed by the fictitious particle and the spring. The period should be much larger than the MD time step to ensure accurate integration of the extended particle's equation of motion. The fictitious mass is calculated internally as $k_B T (\tau / 2\pi\sigma)^2$, where τ is the period and σ is the typical fluctuation (see above).

- `extendedMass` — *Fictitious mass of the alchemical particle ([E]×fs^2)*

  [positive decimal, context: `colvar`]

  This parameter specifies the fictitious mass in the case of an alchemical variable used in

lambda-dynamics (`alchLambda`). Note that the dimension is not that of a usual mass: its unit is the energy unit (3.1) times fs^2.

- `extendedTemp` — *Temperature for the extended degree of freedom (K)*

  Default: thermostat temperature [positive decimal, context: `colvar`]

  Temperature used for calculating the coupling force constant of the extended variable (see `extendedFluctuation`) and, if needed, as a target temperature for extended Langevin dynamics (see `extendedLangevinDamping`). This should normally be left at its default value.

- `extendedLangevinDamping` — *Damping factor for extended Langevin dynamics (ps^{-1})*

  Default: `1.0` [positive decimal, context: `colvar`]

  If this is non-zero, the extended degree of freedom undergoes Langevin dynamics at temperature `extendedTemp`. The friction force is minus `extendedLangevinDamping` times the velocity. This is useful because the extended dynamics coordinate may heat up in the transient non-equilibrium regime of ABF. Use moderate damping values, to limit viscous friction (potentially slowing down diffusive sampling) and stochastic noise (increasing the variance of statistical measurements). In doubt, use the default value.

- `reflectingLowerBoundary` — *Whether the lower boundary reflects the extended Lagrangian particle*

  Default: `off` [boolean, context: `colvar`]

  This turns the specified `lowerBoundary` into a reflecting wall for the extended particle: upon collision, the particle is reflected with opposite momentum.

- `reflectingUpperBoundary` — *Whether the upper boundary reflects the extended Lagrangian particle*

  Default: `off` [boolean, context: `colvar`]

  This turns the specified `upperBoundary` into a reflecting wall for the extended particle: upon collision, the particle is reflected with opposite momentum.

4.24 Multiple time-step variables

- `timeStepFactor` — *Compute this colvar once in a certain number of timesteps*

  Default: `1` [positive integer, context: `colvar`]

  Instructs this colvar to activate at a time interval equal to the base (MD) timestep times `timeStepFactor`.[14] At other time steps, the value of the variable is not updated, and no biasing forces are applied. Any forces exerted by biases are accumulated over the given time interval, then applied as an impulse at the next update.

4.25 Backward-compatibility

- `subtractAppliedForce` — *Do not include biasing forces in the total force for this colvar*

  Default: `off` [boolean, context: `colvar`]

  If the colvar supports total force calculation (see 4.17.1), all forces applied to this colvar by biases will be removed from the total force. This keyword allows to recover some of the "system force" calculation available in the Colvars module before version 2016-08-10. Please note that removal of *all* other external forces (including biasing forces applied to a different colvar) is *no longer supported*, due to changes in the underlying simulation engines (primarily NAMD). This option may be useful when continuing a previous simulation where the removal of external/applied forces is essential. *For all new simulations, the use of this option is not recommended.*

4.26 Statistical analysis

Run-time calculations of statistical properties that depend explicitly on time can be performed for individual collective variables. Currently, several types of time correlation functions, running averages and running standard deviations are implemented. For run-time computation of histograms, please see the histogram bias (6.13).

- `corrFunc` — *Calculate a time correlation function?*

  Default: `off` [boolean, context: `colvar`]

  Whether or not a time correlaction function should be calculated for this colvar.

- `corrFuncWithColvar` — *Colvar name for the correlation function*

  [string, context: `colvar`]

  By default, the auto-correlation function (ACF) of this colvar, ξ_i, is calculated. When this option is specified, the correlation function is calculated instead with another colvar, ξ_j, which must be of the same type (scalar, vector, or quaternion) as ξ_i.

- `corrFuncType` — *Type of the correlation function*

  Default: `velocity` [`velocity`, `coordinate` or `coordinate_p2`, context: `colvar`]

  With `coordinate` or `velocity`, the correlation function $C_{i,j}(t) = \langle \Pi(\xi_i(t_0), \xi_j(t_0 + t)) \rangle$ is calculated between the variables ξ_i and ξ_j, or their velocities. $\Pi(\xi_i, \xi_j)$ is the scalar product when calculated between scalar or vector values, whereas for quaternions it is the cosine between the two corresponding rotation axes. With `coordinate_p2`, the second order Legendre polynomial, $(3\cos(\theta)^2 - 1)/2$, is used instead of the cosine.

- `corrFuncNormalize` — *Normalize the time correlation function?*

  Default: `on` [boolean, context: `colvar`]

If enabled, the value of the correlation function at $t = 0$ is normalized to 1; otherwise, it equals to $\langle O(\xi_i, \xi_j) \rangle$.

- `corrFuncLength` — *Length of the time correlation function*

  Default: `1000` [positive integer, context: `colvar`]

  Length (in number of points) of the time correlation function.

- `corrFuncStride` — *Stride of the time correlation function*

  Default: `1` [positive integer, context: `colvar`]

  Number of steps between two values of the time correlation function.

- `corrFuncOffset` — *Offset of the time correlation function*

  Default: `0` [positive integer, context: `colvar`]

  The starting time (in number of steps) of the time correlation function (default: $t = 0$). **Note:** *the value at $t = 0$ is always used for the normalization.*

- `corrFuncOutputFile` — *Output file for the time correlation function*

  Default: *output*.$<$`name`$>$.`corrfunc.dat` [UNIX filename, context: `colvar`]

  The time correlation function is saved in this file.

- `runAve` — *Calculate the running average and standard deviation*

  Default: `off` [boolean, context: `colvar`]

  Whether or not the running average and standard deviation should be calculated for this colvar.

- `runAveLength` — *Length of the running average window*

  Default: `1000` [positive integer, context: `colvar`]

  Length (in number of points) of the running average window.

- `runAveStride` — *Stride of the running average window values*

  Default: `1` [positive integer, context: `colvar`]

  Number of steps between two values within the running average window.

- `runAveOutputFile` — *Output file for the running average and standard deviation*

  Default: *output*.$<$`name`$>$.`runave.traj` [UNIX filename, context: `colvar`]

  The running average and standard deviation are saved in this file.

5   Selecting atoms

To define collective variables, atoms are usually selected as groups. Each group is defined using an identifying keyword that is unique in the context of the specific colvar component (e.g. for a distance component, the two groups are identified by the `group1` and `group2` keywords).

The group's identifying keyword is followed by a brace-delimited block containing selection keywords and other parameters, one of which is `name`:

- `name` — *Unique name for the atom group*

  [string, context: atom group]

  This parameter defines a globally unique name for this atom group, which can be referred to in the definition of other atom groups (including in other colvars) by invoking `atomsOfGroup` as a selection keyword. This has two benefits: (1) it can make the configuration shorter and more legible, and (2) several related atom groups can be defined using a common set of atoms, so that this selection can be changed in one place, keeping all related groups synchronized with the same atom set.

Other keywords are documented in the following sections.

In the example below, the `gyration` component uses the identifying keyword `atoms` to define its associated group, which is defined based on the index group named "`Protein-H`". Optionally, the group is also given the unique name "`my_protein`", so that atom groups defined later in the Colvars configuration may refer to it.

```
colvar {
  name rgyr
  gyration {
    atoms {
      name my_protein
      indexGroup Protein-H
    }
  }
}
```

5.1   Atom selection keywords

Selection keywords may be used individually or in combination with each other, and each can be repeated any number of times. Selection is incremental: each keyword adds the corresponding atoms to the selection, so that different sets of atoms can be combined. However, atoms included by multiple keywords are only counted once. Below is an example configuration for an atom group called "`atoms`". **Note:** *this is an unusually varied combination of selection keywords, demonstrating how they can be combined together: most simulations only use one of them.*

```
atoms {

  # add atoms 1 and 3 to this group (note: first atom in the system is 1)
  atomNumbers {
    1 3
  }

  # add atoms starting from 20 up to and including 50
  atomNumbersRange  20-50
  # add index group (requires a .ndx file to be provided globally)
  indexGroup Water
}
```

The resulting selection includes atoms 1 and 3, those between 20 and 50, and those in the index group called "Water". The indices of this group are read from the file provided by the global keyword `indexFile`.

The complete list of selection keywords available in Tinker-HP is:

- `atomNumbers` — *List of atom numbers*

  [space-separated list of positive integers, context: atom group]

  This option adds to the group all the atoms whose numbers are in the list. *The number of the first atom in the system is 1: to convert from a VMD selection, use "atomselect get serial".*

- `indexGroup` — *Name of index group to be used (GROMACS format)*

  [string, context: atom group]

  If the name of an index file has been provided by `indexFile`, this option allows to select one index group from that file: the atoms from that index group will be used to define the current group.

- `atomsOfGroup` — *Name of group defined previously*

  [string, context: atom group]

  Refers to a group defined previously using its user-defined `name`. This adds all atoms of that named group to the current group.

- `atomNumbersRange` — *Atoms within a number range*

  [<Starting number>-<Ending number>, context: atom group]

  This option includes in the group all atoms whose numbers are within the range specified. *The number of the first atom in the system is 1.*

- `dummyAtom` — *Dummy atom position (Å)*

  [(x, y, z) triplet, context: atom group]

  Instead of selecting any atom, this option makes the group a virtual particle at a fixed position in space. This is useful e.g. to replace a group's center of geometry with a user-defined position.

5.2 Moving frame of reference.

The following options define an automatic calculation of an optimal translation (`centerToReference`) or optimal rotation (`rotateToReference`), that superimposes the positions of this group to a provided set of reference coordinates. Alternately, `centerToOrigin` applies a translation to place the geometric center of the group at (0, 0, 0). This can allow, for example, to effectively remove from certain colvars the effects of molecular tumbling and of diffusion. Given the set of atomic positions $\mathbf{x}_i$, the colvar ξ can be defined on a set of roto-translated positions $\mathbf{x}'_i = R(\mathbf{x}_i - \mathbf{x}^{\mathrm{C}}) + \mathbf{x}^{\mathrm{ref}}$. $\mathbf{x}^{\mathrm{C}}$ is the geometric center of the $\mathbf{x}_i$, R is the optimal rotation matrix to the reference positions and $\mathbf{x}^{\mathrm{ref}}$ is the geometric center of the reference positions.

Components that are defined based on pairwise distances are naturally invariant under global roto-translations. Other components are instead affected by global rotations or translations: however, they can be made invariant if they are expressed in the frame of reference of a chosen group of atoms, using the `centerToReference` and `rotateToReference` options. Finally, a few components are defined by convention using a roto-translated frame (e.g. the minimal RMSD): for these components, `centerToReference` and `rotateToReference` are enabled by default. In typical applications, the default settings result in the expected behavior.

Warning on rotating frames of reference and periodic boundary conditions. `rotateToReference` affects coordinates that depend on minimum-image distances in periodic boundary conditions (PBC). After rotation of the coordinates, the periodic cell vectors become irrelevant: the rotated system is effectively non-periodic. A safe way to handle this is to ensure that the relevant inter-group distance vectors remain smaller than the half-size of the periodic cell. If this is not desirable, one should avoid the rotating frame of reference, and apply orientational restraints to the reference group instead, in order to keep the orientation of the reference group consistent with the orientation of the periodic cell.

Warning on rotating frames of reference and ABF. Note that `centerToReference` and `rotateToReference` may affect the Jacobian derivative of colvar components in a way that is not taken into account by default. Be careful when using these options in ABF simulations or when using total force values.

- `centerToReference` — *Implicitly remove translations for this group*

  Default: `off` [boolean, context: atom group]

  If this option is `on`, the center of geometry of the group will be aligned with that of the reference positions provided by `refPositions` or `refPositionsFile`. Colvar components will only have access to the aligned positions. **Note**: unless otherwise specified, `rmsd` and `eigenvector` set this option to `on` *by default*.

- `centerToOrigin` — *Implicitly remove translations for this group by keeping its center at the origin*

  Default: `off` [boolean, context: atom group]

  This option implies `centerToReference`. If this option is `on`, coordinates from the group will be translated so that the center of geometry of the group remains at (0, 0, 0), except if

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`fittingGroup` is enabled. In that case, the translation applied is the translation that brings the center of geometry of the fitting group to (0, 0, 0).

- `rotateToReference` — *Implicitly remove rotations for this group*

  Default: `off` [boolean, context: atom group]

  If this option is `on`, the coordinates of this group will be optimally superimposed to the reference positions provided by `refPositions` or `refPositionsFile`. The rotation will be performed around the center of geometry if `centerToReference` is `on`, or around the origin otherwise. The algorithm used is the same employed by the `orientation` colvar component [3]. Forces applied to the atoms of this group will also be implicitly rotated back to the original frame. **Note**: unless otherwise specified, `rmsd` and `eigenvector` set this option to `on` *by default*.

- `refPositions` — *Reference positions for fitting (Å)*

  [space-separated list of (x, y, z) triplets, context: atom group]

  This option provides a list of reference coordinates for `centerToReference` and/or `rotateToReference`, and is mutually exclusive with `refPositionsFile`. If only `centerToReference` is `on`, the list may contain a single (x, y, z) triplet; if also `rotateToReference` is `on`, the list should be as long as the atom group, and *its order must match the order in which atoms were defined*.

- `refPositionsFile` — *File containing the reference positions for fitting*

  [UNIX filename, context: atom group]

  This option provides a list of reference coordinates for `centerToReference` and/or `rotateToReference`, and is mutually exclusive with `refPositions`. The acceptable file format is XYZ (3.7.3), which is read in double precision. .

- `fittingGroup` — *Use an alternate set of atoms to define the roto-translation*

  Default: This atom group itself [Atom group, context: atom group]

  If either `centerToReference` or `rotateToReference` is defined, this keyword defines an alternate atom group to calculate the optimal roto-translation. Use this option to define a continuous rotation if the structure of the group involved changes significantly (a typical symptom would be the message "Warning: discontinuous rotation!"). **Performance considerations:** note that enabling this option will result in projecting each of the atomic gradients of the colvar (e.g. the RMSD) onto each the gradients of the roto-translation, which may be a computationally expensive operation: see the closely related `enableFitGradients` for details.

The following options have default values appropriate for the vast majority of applications, and are only provided to support rare, special cases.

- `enableFitGradients` — *Include the roto-translational contribution to colvar gradients*

  Default: `on` [boolean, context: atom group]

  When either `centerToReference` or `rotateToReference` is on, the gradients of some colvars include terms proportional to $\partial R/\partial \mathbf{x}_i$ (rotational gradients) and $\partial \mathbf{x}^C/\partial \mathbf{x}_i$ (translational gradients). In practice, these terms are calculated as follows,

$$\nabla_k \zeta(\mathbf{X}) = \sum_{l,\text{main}} \left[\nabla_l f \cdot \nabla_k R \left(\mathbf{x}_l - \bar{\mathbf{x}}^{(\text{fit})} \right) - \frac{1}{n^{(\text{fit})}} \nabla_l f \cdot R \mathbb{1} \right]$$

$$= \left(\sum_{l,\text{main}} \nabla_l f \cdot \frac{\partial R}{\partial q_0} \left(\mathbf{x}_l - \bar{\mathbf{x}}^{(\text{fit})} \right), \sum_{l,\text{main}} \nabla_l f \cdot \frac{\partial R}{\partial q_1} \left(\mathbf{x}_l - \bar{\mathbf{x}}^{(\text{fit})} \right), \sum_{l,\text{main}} \nabla_l f \cdot \frac{\partial R}{\partial q_2} \left(\mathbf{x}_l - \bar{\mathbf{x}}^{(\text{fit})} \right), \right.$$

$$\left. \sum_{l,\text{main}} \nabla_l f \cdot \frac{\partial R}{\partial q_3} \left(\mathbf{x}_l - \bar{\mathbf{x}}^{(\text{fit})} \right) \right) \cdot (\nabla_k q_0, \nabla_k q_1, \nabla_k q_2, \nabla_k q_3) - \frac{1}{n^{(\text{fit})}} R^\mathsf{T} \sum_{l,\text{main}} \nabla_l f$$

By default, these terms are calculated and included in the total gradients; if this option is set to `off`, they are neglected. In the case of a minimum RMSD component, this flag is automatically disabled because the contributions of those derivatives to the gradients cancel out; other types of variable will require projecting each of the gradients of the variable onto each of the gradients of the roto-translation. When `fittingGroup` is enabled, the computation is a $O(n^{(\text{fit})} + n^{(\text{main})})$ loop for all variables, including RMSDs.

5.3 Treatment of periodic boundary conditions.

In simulations with periodic boundary conditions (PBCs), Colvars computes all distances between two points following the nearest-image convention, using PBC parameters provided by Tinker-HP. However, many common variables rely on a consistent definition of the center of mass or geometry of a group of atoms. This requires the use of *unwrapped* coordinates, which are not subject to "jumps" when they diffuse across periodic boundaries.

In general, internal coordinate wrapping by Tinker-HP does not affect the calculation of colvars if each atom group satisfies one or more of the following:

i) it is composed by only one atom;

ii) it is used by a colvar component which does not make use of its center of geometry, but only of pairwise distances (`distanceInv`, `coordNum`, `hBond`, `alpha`, `dihedralPC`);

iii) it is used by a colvar component that ignores the ill-defined Cartesian components of its center of mass (such as the x and y components of a membrane's center of mass modeled with `distanceZ`).

5.4 Performance of a Colvars calculation based on group size.

In simulations performed with MD simulation engines such as GROMACS, LAMMPS or NAMD, the computation of energy and forces is distributed (i.e., parallelized) over multiple nodes, as well as over the CPU/GPU cores of each node. When Colvars is enabled, atomic coordinates are collected on a single CPU core, where collective variables and their biases are computed. This means that in the case of simulations that are already being run over large numbers of nodes, or inside a GPU, a Colvars calculation may produce a significant overhead. This overhead comes from the combined cost of two operation: transmitting atomic coordinates, and computing functions of the same.

Performance can be improved in multiple ways:

- As a general rule, the size of atom groups should be kept relatively small (up to a few thousands of atoms, depending on the size of the entire system in comparison). For example, restraining a protein through a RMSD colvar defined over all of its atoms is only marginally different than one defined over only the α carbon atoms, but the difference in computational cost is much higher. To gain an estimate of the computational cost of a specific Colvars configuration, one may use a test calculation of the same colvar in VMD (hint: use the `time` Tcl command to measure the cost of running `cv update`).

- The calculation of variables, components and biases can be distributed over the processor cores of the node where the Colvars module is executed. Currently, an equal weight is assigned to each colvar, or to each component of those colvars that include more than one component. The performance of simulations that use many colvars or components is improved automatically. For simulations that use a single large colvar, it may be advisable to partition it in multiple components, which will be then distributed across the available cores. The messages "SMP parallelism is available" or "SMP parallelism is enabled", printed by Colvars at initialization time, indicate the availability or status of this feature. If available, the option is turned on by default, but may be disabled using the keyword `smp` if required for debugging or troubleshooting.

6 Biasing and analysis methods

A biasing or analysis method can be applied to existing collective variables by using the following configuration:

```
<biastype> {
  name <name>
  colvars <xi1> <xi2> ...
  <parameters>
}
```

The keyword `<biastype>` indicates the method of choice. There can be multiple instances of the same method, e.g. using multiple `harmonic` blocks allows defining multiple restraints.

All biasing and analysis methods implemented recognize the following options:

- `name` — *Identifier for the bias*

  Default: `<type of bias><bias index>` [string, context: colvar bias]

  This string is used to identify the bias or analysis method in the output, and to name some output files. **Tip:** although a unique name is assigned automatically, *you are strongly encouraged to give a name to a bias that you may want to analyze later*. For example, processing a trajectory file (sec. 3.7.5) containing a harmonic restraint is much simpler with a clearly identifiable name, like "`smd`" for a moving harmonic restraint or "`us`" for a static one.

- `colvars` — *Collective variables involved*

  [space-separated list of colvar names, context: colvar bias]

  This option selects by name all the variables to which this bias or analysis will be applied.

- `outputEnergy` — *Write the current bias energy to the trajectory file*

  Default: `off` [boolean, context: colvar bias]

  If this option is chosen and `colvarsTrajFrequency` is not zero, the current value of the biasing energy will be written to the trajectory file during the simulation.

- `outputFreq` — *Frequency (number of steps) at which output files are written*

  Default: `colvarsRestartFrequency` [positive integer, context: colvar bias]

  If this bias produces aggregated data that needs to be written to disk (for example, a PMF), this number specifies the number of steps after which these data are written to files. A value of zero disables writing files for this bias during the simulation (except for `outputEnergy`, which is controlled by `colvarsTrajFrequency`). All output files are also written at the end of a simulation run, regardless of the value of this number.

- `bypassExtendedLagrangian` — *Apply bias to actual colvars, bypassing extended coordinates*

  Default: `off` [boolean, context: colvar bias]

This option is implemented by the `harmonicWalls` and `histogram` biases. It is only relevant if the bias is applied to one or several extended-Lagrangian colvars (4.23), for example within an eABF (6.4) simulation. Usually, biases use the value of the extended coordinate as a proxy for the actual colvar, and their biasing forces are applied to the extended coordinates as well. If `bypassExtendedLagrangian` is enabled, the bias behaves as if there were no extended coordinates, and accesses the value of the underlying colvars, applying any biasing forces along the gradients of those variables.

- `stepZeroData` — *Accumulate data starting at step 0 of a simulation run*

  Default: `off` [boolean, context: colvar bias]

  This option is meaningful for biases that record and accumulate data during a simulation, such as ABF (6.3), metadynamics (6.6), histograms (6.13) and in general any bias that accumulates free-energy samples with thermodynamic integration, or TI (6.2). When this option is disabled (default), data will only be recorded into the bias after the first coordinate update: this is generally the correct choice in simulation runs. Biasing energy and forces will always be computed for all active biases, regardless of this option.

- `scaledBiasingForce` — *Scale biasing force by a factor in an external histogram*

  Default: `off` [boolean, context: colvar bias]

  If this option is set to `on`, the biasing force at each step will be scaled by a factor provided in the grid of an external histogram. The histogram file is provided by `scaledBiasingForceFactorsGrid`. It ought to be noted that if the variables are not in any grids of the histogram, the scaling factor is 1.0.

- `scaledBiasingForceFactorsGrid` — *A histogram file with the scaling factor of biasing force in each bin*

  [string, context: colvar bias]

  If `scaledBiasingForce` is set to `on`, this option accepts the filename of the histogram file that contains the scaling factors. The histogram file is expected to be in "multicolumn" format (3.7.4), similar to the low-dimensional the PMF files written by `metadynamics` and `ABF`.

6.1 Defining grids for biasing and analysis methods

Several methods (histograms, ABF and eABF, optionally metadynamics) rely on discretizing colvars on regular grids to represent functions such as probability distributions or free energy surfaces.

Grid parameters are specific to each variable, and some of them may even be defined automatically: for instance, periodic angles have default boundaries of -180° and 180°. When not given by default, boundaries may be also specified when defining the collective variable itself (4.21).

Each bias will use by default any colvar boundaries that are automatically defined or explicitly given. Optionally, the bias may also contain a dedicated configuration block `grid { ...}` inside its configuration. The options supported within the `grid { ...}` block are:

- `lowerBoundary` — *Lower boundaries of the grid*

[list of space-separated decimals, context: `grid`]

This option is specified once, and takes as many parameters as there are colvars associated with this bias. It defines the lower boundaries of the grid, overriding any values defined by the `lowerBoundary` keyword of each colvar.

- `upperBoundary` — analogous to `lowerBoundary`

- `width` — *Bin widths of the grid*

  [list of space-separated decimals, context: `grid`]

  This option is specified once, and takes as many parameters as there are colvars associated with this bias. It defines the bin widths of the grid along each dimension, overriding any values defined by the `width` keyword of each colvar.

Example: Definition of custom grid parameters for a histogram. Note how some of the boundaries of the variables are automatically defined, but this histogram's grid is custom-defined to also specify a finer width and narrower boundaries.

```
colvar {
  name r
  # lowerBoundary 0.0 is automatically defined
  upperBoundary 20.0
  distance { ... }
}

colvar {
  name theta
  # lowerBoundary -180.0 is automatically defined
  # upperBoundary 180.0 is automatically defined
  dihedral { ... }
}

histogram {
  name hist2d
  colvars r theta
  grid {
    width   0.1 1.0
    lowerBoundary   2.0 30.0
    upperBoundary  10.0 90.0
  }
}
```

6.2   Thermodynamic integration

The methods implemented here provide a variety of estimators of conformational free-energies. These are carried out at run-time, or with the use of post-processing tools over the generated output files. The specifics of each estimator are discussed in the documentation of each biasing or analysis method.

A special case is the traditional thermodynamic integration (TI) method, used for example to compute potentials of mean force (PMFs). Most types of restraints (6.8, 6.10, 6.11, ...) as well as metadynamics (6.6) can optionally use TI alongside their own estimator, based on the keywords documented below.

- `writeTIPMF` — *Write the PMF computed by thermodynamic integration*

  Default: `off` [boolean, context: colvar bias]

  If the bias is applied to a variable that supports the calculation of total forces (see `outputTotalForce` and 4.17.1), this option allows calculating the corresponding PMF by thermodynamic integration, and writing it to the file *output*.<name>.ti.pmf, where <name> is the name of the bias and the contents of the file are in multicolumn text format (3.7.4). The total force includes the forces applied to the variable by all bias, except those from this bias itself. If any bias applies time-dependent forces besides the one using this option, an error is raised.

- `writeTISamples` — *Write the free-energy gradient samples*

  Default: `off` [boolean, context: colvar bias]

  This option allows to compute total forces for use with thermodynamic integration as done by the keyword `writeTIPMF`. The names of the files containing the variables' histogram and mean thermodynamic forces are *output*.<name>.ti.count and *output*.<name>.ti.force, respectively: these can be used by `abf_integrate` (see 6.3.5) or similar utility. Note that because the `.force` file contains mean forces instead of free-energy gradients, `abf_integrate` <filename> -s -1.0 should be used. This option is on by default when `writeTIPMF` is on, but can be enabled separately if the bias is applied to more than one variable, making not possible the direct integration of the PMF at runtime. If any bias applies time-dependent forces besides the one using this option, an error is raised.

In adaptive biasing force (ABF) (6.3) the above keywords are not recognized, because their functionality is either included already (conventional ABF) or not available (extended-system ABF).

6.3 Adaptive Biasing Force

For a full description of the Adaptive Biasing Force method, see reference [15]. For details about this implementation, see references [16] and [17]. **When publishing research that makes use of this functionality, please cite references [15] and [17].**

An alternate usage of this feature is the application of custom tabulated biasing potentials to one or more colvars. See `inputPrefix` and `updateBias` below.

Combining ABF with the extended Lagrangian feature (4.23) of the variables produces the extended-system ABF variant of the method (6.4).

ABF is based on the thermodynamic integration (TI) scheme for computing free energy profiles. The free energy as a function of a set of collective variables $\xi = (\xi_i)_{i \in [1,n]}$ is defined from the canonical distribution of ξ, $\mathscr{P}(\xi)$:

$$A(\xi) = -\frac{1}{\beta} \ln \mathscr{P}(\xi) + A_0 \tag{20}$$

In the TI formalism, the free energy is obtained from its gradient, which is generally calculated in the form of the average of a force F_ξ exerted on ξ, taken over an iso-ξ surface:

$$\nabla_\xi A(\xi) = \langle -F_\xi \rangle_\xi \tag{21}$$

Several formulae that take the form of (21) have been proposed. This implementation relies partly on the classic formulation [18], and partly on a more versatile scheme originating in a work by Ruiz-Montero et al. [19], generalized by den Otter [20] and extended to multiple variables by Ciccotti et al. [21]. Consider a system subject to constraints of the form $\sigma_k(x) = 0$. Let $(v_i)_{i \in [1,n]}$ be arbitrarily chosen vector fields ($\mathbb{R}^{3N} \to \mathbb{R}^{3N}$) verifying, for all i, j, and k:

$$v_i \cdot \nabla_x \xi_j = \delta_{ij} \tag{22}$$
$$v_i \cdot \nabla_x \sigma_k = 0 \tag{23}$$

then the following holds [21]:

$$\frac{\partial A}{\partial \xi_i} = \langle v_i \cdot \nabla_x V - k_B T \nabla_x \cdot v_i \rangle_\xi \tag{24}$$

where V is the potential energy function. v_i can be interpreted as the direction along which the force acting on variable ξ_i is measured, whereas the second term in the average corresponds to the geometric entropy contribution that appears as a Jacobian correction in the classic formalism [18]. Condition (22) states that the direction along which the total force on ξ_i is measured is orthogonal to the gradient of ξ_j, which means that the force measured on ξ_i does not act on ξ_j.

Equation (23) implies that constraint forces are orthogonal to the directions along which the free energy gradient is measured, so that the measurement is effectively performed on unconstrained degrees of freedom.

In the framework of ABF, $\mathbf{F}_\xi$ is accumulated in bins of finite size $\delta\xi$, thereby providing an estimate of the free energy gradient according to equation (21). The biasing force applied along the collective variables to overcome free energy barriers is calculated as:

$$\mathbf{F}^{\text{ABF}} = \alpha(N_\xi) \times \nabla_x \widetilde{A}(\xi) \tag{25}$$

where $\nabla_x \widetilde{A}$ denotes the current estimate of the free energy gradient at the current point ξ in the collective variable subspace, and $\alpha(N_\xi)$ is a scaling factor that is ramped from 0 to 1 as the local number of samples N_ξ increases to prevent non-equilibrium effects in the early phase of the simulation, when the gradient estimate has a large variance. See the `fullSamples` parameter below for details.

As sampling of the phase space proceeds, the estimate $\nabla_x \widetilde{A}$ is progressively refined. The biasing force introduced in the equations of motion guarantees that in the bin centered around ξ, the forces acting along the selected collective variables average to zero over time. Eventually, as the underlying free energy surface is canceled by the adaptive bias, evolution of the system along ξ is governed mainly by diffusion. Although this implementation of ABF can in principle be used in arbitrary dimension, a higher-dimension collective variable space is likely to be difficult to sample and visualize. Most commonly, the number of variables is one or two, sometimes three.

6.3.1 ABF requirements on collective variables

The following conditions must be met for an ABF simulation to be possible and to produce an accurate estimate of the free energy profile. Note that these requirements do not apply when using the extended-system ABF method (6.4).

1. *Only linear combinations* of colvar components can be used in ABF calculations.

2. *Availability of total forces* is necessary. The following colvar components can be used in ABF calculations: `distance`, `distance_xy`, `distance_z`, `angle`, `dihedral`, `gyration`, `rmsd` and `eigenvector`. Atom groups may not be replaced by dummy atoms, unless they are excluded from the force measurement by specifying `oneSiteTotalForce`, if available.

3. *Mutual orthogonality of colvars*. In a multidimensional ABF calculation, equation (22) must be satisfied for any two colvars ξ_i and ξ_j. Various cases fulfill this orthogonality condition:

   - ξ_i and ξ_j are based on non-overlapping sets of atoms.
   - atoms involved in the force measurement on ξ_i do not participate in the definition of ξ_j. This can be obtained using the option `oneSiteTotalForce` of the `distance`, `angle`, and `dihedral` components (example: Ramachandran angles ϕ, ψ).
   - ξ_i and ξ_j are orthogonal by construction. Useful cases are the sum and difference of two components, or `distance_z` and `distance_xy` using the same axis.

4. *Mutual orthogonality of components*: when several components are combined into a colvar, it is assumed that their vectors v_i (equation (24)) are mutually orthogonal. The cases described for colvars in the previous paragraph apply.

5. *Orthogonality of colvars and constraints*: equation 23 can be satisfied in two simple ways, if either no constrained atoms are involved in the force measurement (see point 3 above) or pairs of atoms joined by a constrained bond are part of an *atom group* which only intervenes through its center (center of mass or geometric center) in the force measurement. In the latter case, the contributions of the two atoms to the left-hand side of equation 23 cancel out. For example, all atoms of a rigid TIP3P water molecule can safely be included in an atom group used in a `distance` component.

6.3.2 Parameters for ABF

ABF depends on parameters from each collective variable to define the grid on which free energy gradients are computed: see 4.21 for details. Alternately, grid parameters can be set directly inside the `abf` block by inserting a `grid` sub-block, as explained in 6.1. Other parameters to control the ABF runtime can be set in the ABF configuration block:

- `name` — same definition as `name` (biasing and analysis methods)

- `colvars` — same definition as `colvars` (biasing and analysis methods)

- `outputEnergy` — same definition as `outputEnergy` (biasing and analysis methods)

- `outputFreq` — same definition as `outputFreq` (biasing and analysis methods)

- `stepZeroData` — same definition as `stepZeroData` (biasing and analysis methods)

- `fullSamples` — *Number of samples in a bin prior to full application of the ABF*

  Default: 200 [positive integer, context: `abf`]

  To avoid non-equilibrium effects due to large fluctuations of the force exerted along the colvars, it is recommended to apply a biasing force only after a the estimate has started converging. If `fullSamples` is non-zero, the applied biasing force is scaled by a factor $\alpha(N_\xi)$ between 0 and 1. If the number of samples N_ξ in the current bin is higher than `fullSamples`, the factor is one. If it is less than `minSamples` (by default, half of `fullSamples`), the factor is zero and no bias is applied. Between those two thresholds, the factor follows a linear ramp from 0 to 1: $\alpha(N_\xi) = (N_\xi - \mathrm{minSamples})/(\mathrm{fullSamples} - \mathrm{minSamples})$.

- `minSamples` — *Number of samples in a bin prior to application of the ABF*

  Default: fullSamples / 2 [positive integer, context: `abf`]

  See `fullSamples`.

- `maxForce` — *Maximum magnitude of the ABF force*

  Default: disabled [positive decimals (one per colvar), context: `abf`]

  This option enforces a cap on the magnitude of the biasing force effectively applied by this ABF bias on each colvar. This can be useful in the presence of singularities in the PMF such as hard walls, where the discretization of the average force becomes very inaccurate, causing the colvar's diffusion to get "stuck" at the singularity. To enable this cap, provide one non-negative value for each colvar. The unit of force is kcal/mol/*(colvar unit)*.

- `hideJacobian` — *Remove geometric entropy term from calculated free energy gradient?*

  Default: `no` [boolean, context: `abf`]

  In a few special cases, most notably distance-based variables, an alternate definition of the potential of mean force is traditionally used, which excludes the Jacobian term describing the effect of geometric entropy on the distribution of the variable. This results, for example, in particle-particle potentials of mean force being flat at large separations. The Jacobian term is exactly represented in equation (24) by the second term of the average, $-k_B T \nabla_x \cdot v_i$. Enabling the `hideJacobian` option causes the output data to follow the traditional potential of mean force convention, by omitting this contribution from the measured free energy gradients. To ensure uniform sampling despite the incomplete description of the free energy, an additional biasing force that counteracts the Jacobian force is applied internally by the colvar. **Warning:** using this option without detailed knowledge of the terms of equation (24) is *not recommended*. **Warning:** a bug affecting Colvars version until October 2020 leads to this term to be subtracted twice in the PMFs reported by conventional ABF. The bug does not affect the simulated trajectory, and the correct PMF can be recovered by adding back the term manually. Other than conventional ABF, the PMFs computed by other sampling methods were never affected.

- `historyFreq` — *Frequency (in timesteps) at which ABF history files are accumulated*

  Default: 0 [positive integer, context: `abf`]

  If this number is non-zero, the free energy gradient estimate and sampling histogram (and the PMF in one-dimensional calculations) are written to files on disk at the given time interval. History file names use the same prefix as output files, with ".`hist`" appended (*output*.`hist.pmf`). `historyFreq` must be a multiple of `outputFreq`.

- `inputPrefix` — *Filename prefix for reading ABF data*

  [list of strings, context: `abf`]

  If this parameter is set, for each item in the list, ABF tries to read a gradient and a sampling files named <`inputPrefix`>.`grad` and <`inputPrefix`>.`count`. This is done at startup and sets the initial state of the ABF algorithm. The data from all provided files is combined appropriately. Also, the grid definition (min and max values, width) need not be the same that for the current run. This command is useful to piece together data from simulations in different regions of collective variable space, or change the colvar boundary values and widths. Note that it is not recommended to use it to switch to a smaller width, as that will leave some bins empty in the finer data grid. This option is NOT compatible with reading the data from a restart file.

- `applyBias` — *Apply the ABF bias?*

  Default: `yes` [boolean, context: `abf`]

  If this is set to no, the calculation proceeds normally but the adaptive biasing force is not applied. Data is still collected to compute the free energy gradient. This is mostly intended for testing purposes, and should not be used in routine simulations.

- `updateBias` — *Update the ABF bias?*

  Default: `yes` [boolean, context: `abf`]

  If this is set to no, the initial biasing force (e.g. read from a restart file or through `inputPrefix`) is not updated during the simulation. As a result, a constant bias is applied. This can be used to apply a custom, tabulated biasing potential to any combination of colvars. To that effect, one should prepare a gradient file containing the gradient of the potential to be applied (negative of the bias force), and a count file containing only values greater than `fullSamples`. These files must match the grid parameters of the colvars.

6.3.3 Multiple-walker ABF

This implements the multiple-walker ABF scheme described in [22]. The references for this implementation are [23] and [24]. This feature requires that Tinker-HP be compiled and executed with multiple-replica support.

If `shared` is enabled, the total force samples will be synchronized among all replicas at intervals defined by `sharedFreq`. Each replica maintains a separate buffer of total force samples that determine the biasing force. Every `sharedFreq` steps, the replicas communicate the samples that

have been gathered since the last synchronization time, ensuring all replicas apply a similar biasing force. Thus, it is as if total force samples among all replicas are gathered in a single shared buffer. Shared ABF allows all replicas to benefit from the sampling done by other replicas and can lead to faster convergence of the biasing force.

Output files of multiple-walker ABF. In multiple-walker ABF runs, since Colvars version 2024-01-06, each walker outputs gradient and count files containing only data collected locally. In addition, the first walker outputs the collected data using the common prefix, with an additional ".all" string (i.e. file names ending with ".all.count", ".all.grad" etc.).

- `shared` — *Apply multiple-walker ABF, sharing force samples among the replicas?*

  Default: `no` [boolean, context: `abf`]

  Enable sharing of ABF data between replicas, in a multi-replica simulation.

- `sharedFreq` — *Frequency (in timesteps) at which force samples are synchronized among the replicas*

  Default: `outputFreq` [positive integer or zero, context: `abf`]

  Every `sharedFreq` steps, the replicas communicate the samples that have been gathered since the last synchronization time. Because this forces the replicas to synchronize, small values of this parameter may slightly reduce the overall throughput, especially on systems with unequal performance.

6.3.4   Output files

The ABF bias produces the following files, all in multicolumn text format (3.7.4):

- *output*.`grad`: current estimate of the free energy gradient (grid), in multicolumn;

- *output*.`count`: histogram of samples collected, on the same grid;

- *output*.`pmf`: integrated free energy profile or PMF (for dimension 1, and dimension 2 or 3).

Also in the case of one-dimensional calculations, the ABF bias can report its current energy via `outputEnergy`; in higher dimensions, such computation is not implemented and the energy reported is zero.

If several ABF biases are defined concurrently, their name is inserted to produce unique filenames for output, as in *output*.`abf1.grad`. This should not be done routinely and could lead to meaningless results: only do it if you know what you are doing!

If the colvar space has been partitioned into sections (*windows*) in which independent ABF simulations have been run, the resulting data can be merged using the `inputPrefix` option described above (a run of 0 steps is enough).

6.3.5 Multidimensional free energy surfaces

The ABF method only produces an estimate of the free energy *gradient*. The free energy surface itself can be computed depending on the value of `integrate` and related options.

- `integrate` — *Integrate free energy surface from ABF gradients (dim < 3)*

  Default: `yes` [boolean, context: `abf`]

  This option, active by default when the dimension of the colvar space is 3 or less, enables the calculation of an integrated free energy surface every time ABF output files are written. In dimension 2 or 3, integration is performed by solving a Poisson equation: [25]

$$\nabla^2 A_t = \nabla \cdot G_t \qquad (26)$$

  wehere G_t is the estimated gradient at time t, and A_t is corresponding free energy surface. The free energy surface is written under the file name `<outputName>.pmf`, in a plain text format (see 3.7.4) that can be read by most data plotting and analysis programs (e.g. Gnuplot). Periodic boundary conditions are applied to periodic coordinates, and Neumann boundary conditions otherwise (imposed free energy gradient at the boundary of the domain). The grid used for free energy discretization is extended by one point along non-periodic coordinates, but not along periodic coordinates. See ref. [25] for details.

- `integrateTol` — *Tolerance for free energy integration*

  Default: 1e-6 [positive decimal, context: `abf`]

  The conjugate gradients algorithm used to integrate the free energy surface is stopped when the RMS error reaches `integrateTol`.

- `integrateMaxIterations` — *Maximum iterations for free energy integration*

  Default: 10000 [integer, context: `abf`]

  The conjugate gradients algorithm used to integrate the free energy surface is stopped when the number of iterations reaches `integrateMaxIterations`, unless the RMS error has reached `integrateTol` before.

In dimension 4 or greater, integrating the discretized gradient becomes non-trivial. The standalone utility `abf_integrate` is provided to perform that task. Because 4D ABF calculations are uncommon, **this tool is practically deprecated** by the Poisson integration described above.

`abf_integrate` reads the gradient data and uses it to perform a Monte-Carlo (M-C) simulation in discretized collective variable space (specifically, on the same grid used by ABF to discretize the free energy gradient). By default, a history-dependent bias (similar in spirit to metadynamics) is used: at each M-C step, the bias at the current position is incremented by a preset amount (the *hill height*). Upon convergence, this bias counteracts optimally the underlying gradient; it is negated to obtain the estimate of the free energy surface.

`abf_integrate` is invoked using the command-line:

```
abf_integrate <gradient_file> [-n <nsteps>] [-t <temp>] [-m (0|1)] [-h <hill_height>] [-f <factor>]
```

The gradient file name is provided first, followed by other parameters in any order. They are described below, with their default value in square brackets:

- `-n`: number of M-C steps to be performed; by default, a minimal number of steps is chosen based on the size of the grid, and the integration runs until a convergence criterion is satisfied (based on the RMSD between the target gradient and the real PMF gradient)

- `-t`: temperature for M-C sampling; expects kcal/mol as energy unit (unrelated to the simulation temperature) [500 K]

- `-s`: scaling factor for the gradients; when using a histogram of total forces obtained from `outputTotalForce` or the `.force` file written by `writeTISamples`, a scaling factor of -1 should be used [1.0]

- `-m`: use metadynamics-like biased sampling? (0 = false) [1]

- `-h`: increment for the history-dependent bias ("hill height") [0.01 kcal/mol]

- `-f`: if non-zero, this factor is used to scale the increment stepwise in the second half of the M-C sampling to refine the free energy estimate [0.5]

Using the default values of all parameters should give reasonable results in most cases.

`abf_integrate` produces the following output files:

- `<gradient_file>.pmf`: computed free energy surface

- `<gradient_file>.histo`: histogram of M-C sampling (not usable in a straightforward way if the history-dependent bias has been applied)

- `<gradient_file>.est`: estimated gradient of the calculated free energy surface (from finite differences)

- `<gradient_file>.dev`: deviation between the user-provided numerical gradient and the actual gradient of the calculated free energy surface. The RMS norm of this vector field is used as a convergence criterion and output periodically during integration.

Note: Typically, the "deviation" vector field does not vanish as the integration converges. This happens because the numerical estimate of the gradient does not exactly derive from a potential, due to numerical approximations used to obtain it (finite sampling and discretization on a grid). See Ref.[25] for details.

6.4 Extended-system Adaptive Biasing Force (eABF)

Extended-system ABF (eABF) is a variant of ABF (6.3) where the bias is not applied directly to the collective variable, but to an extended coordinate ("fictitious variable") λ that evolves dynamically according to Newtonian or Langevin dynamics. Such an extended coordinate is enabled for a given colvar using the `extendedLagrangian` and associated keywords (4.23). The theory of eABF and the present implementation are documented in detail in reference [26].

Defining an ABF bias on a colvar wherein the `extendedLagrangian` option is active will perform eABF automatically; there is no dedicated option.

The extended variable λ is coupled to the colvar $z = \xi(q)$ by the harmonic potential $(k/2)(z - \lambda)^2$. Under eABF dynamics, the adaptive bias on λ is the running estimate of the average spring force:

$$F^{\text{bias}}(\lambda^*) = \langle k(\lambda - z) \rangle_{\lambda^*} \qquad (27)$$

where the angle brackets indicate a canonical average conditioned by $\lambda = \lambda^*$. At long simulation times, eABF produces a flat histogram of the extended variable λ, and a flattened histogram of ξ, whose exact shape depends on the strength of the coupling as defined by `extendedFluctuation` in the colvar.

Coupling should be strong enough that the bias helps overcome barriers along the colvar.[26] A value of `extendedFluctuation` equal to the bin width is often reasonable. In the limit of strong coupling (small `extendedFluctuation`), the eABF free energy surface (`.pmf` file) becomes close to the true one, making the asymptotically more accurate but noisier CZAR estimator (`.czar.pmf` file) unnecessary. In practice, we recommend comparing these two free energy surfaces to decide which one to use.

Distribution of the colvar may be assessed by plotting its histogram, which is written to the *output*`.zcount` file in every eABF simulation. Note that a `histogram` bias (6.13) applied to an extended-Lagrangian colvar will access the extended degree of freedom λ, not the original colvar ξ; however, the joint histogram may be explicitly requested by listing the name of the colvar twice in a row within the `colvars` parameter of the `histogram` block.

The eABF PMF is that of the coordinate λ, it is not exactly the free energy profile of ξ. That quantity can be calculated based on the CZAR estimator.

6.4.1 CZAR estimator of the free energy

The *corrected z-averaged restraint* (CZAR) estimator is described in detail in reference [26]. It is computed automatically in eABF simulations, regardless of the number of colvars involved. Note that ABF may also be applied on a combination of extended and non-extended colvars; in that case, CZAR still provides an unbiased estimate of the free energy gradient.

CZAR estimates the free energy gradient as:

$$A'(z) = -\frac{1}{\beta}\frac{d\ln\tilde{\rho}(z)}{dz} + k(\langle\lambda\rangle_z - z).$$ (28)

where $z = \xi(q)$ is the colvar, λ is the extended variable harmonically coupled to z with a force constant k, and $\tilde{\rho}(z)$ is the observed distribution (histogram) of z, affected by the eABF bias.

Parameters for the CZAR estimator are:

- `CZARestimator` — *Calculate CZAR estimator of the free energy?*

  Default: `yes` [boolean, context: `abf`]

  This option is only available when ABF is performed on extended-Lagrangian colvars. When enabled, it triggers calculation of the free energy following the CZAR estimator.

- `writeCZARwindowFile` — *Write internal data from CZAR to a separate file?*

  Default: `no` [boolean, context: `abf`]

  When this option is enabled, eABF simulations will write a file containing the z-averaged restraint force under the name *output*`.zgrad`. The same information is always included in the colvars state file, which is sufficient for restarting an eABF simulation. These separate file is only useful when joining adjacent windows from a stratified eABF simulation, either to continue the simulation in a broader window or to compute a CZAR estimate of the PMF over the full range of the coordinate(s). **Important warning.** Unbiased free-energy estimators

from eABF dynamics rely on some form of sampling histogram. When running stratified (windowed) calculations this histogram becomes discontinuous, and as a result the free energy gradient estimated by CZAR is inaccurate at the window boundary, resulting in visible "blips" in the PMF. As a workaround, we recommend manually replacing the two free energy gradient values at the boundary, either with the ABF values from .grad files (accurate in the limit of tight coupling), or with values interpolated for the neighboring values of the CZAR gradient.

Similar to ABF, the CZAR estimator produces two output files in multicolumn text format (3.7.4):

- *output*.czar.grad: current estimate of the free energy gradient (grid), in multicolumn;

- *output*.czar.pmf: only for one-dimensional calculations, integrated free energy profile or PMF.

The sampling histogram associated with the CZAR estimator is the z-histogram, which is written in the file *output*.zcount.

6.5   Adiabatic Bias Molecular Dynamics (ABMD)

This implements the Adiabatic Bias Molecular Dynamics (ABMD) method of Marchi and Ballone [27], sometimes referred to as *ratchet-and-pawl* or *ratcheted MD*. ABMD is a non-equilibrium process that enhances the motion of a scalar colvar in a given direction. For simplicity, the case of an increasing value is described below, but enhancing downward motion of the variable is also supported via the decreasing flag.

ABMD does not directly push the variable forward, but prevents it from backtracking by applying a time-dependent half-harmonic potential V_t, the center of which is the highest value attained by the variable so far (its high-water mark). This design implies that the bias is conservative at all times and therefore exerts zero net work, hence the "adiabatic" qualifier:

$$V_t(\xi_t) = \begin{cases} \dfrac{1}{2}k\left(\xi_t - \xi_t^{\text{ref}}\right)^2 & \text{if } \xi_t < \xi_t^{\text{ref}} \\ 0 & \text{otherwise} \end{cases} \tag{29}$$

where ξ_t^{ref} is the high-water mark at time t, bounded by a user-defined stopping value ξ^{stop}:

$$\xi_t^{\text{ref}} = \min\left(\max_{s=0}^{t} \xi_s, \xi^{\text{stop}}\right). \tag{30}$$

Note: because the ABMD potential in eq. 29 is never defined for more than one variable, no internal unit conversion is applied to k: this behavior is different from other restraints available in Colvars, such as the harmonic wall restraints in 6.10.

Besides the name of the biased variable specified by the colvars keyword, the tunable parameters of ABMD are the force constant k and the stopping value ξ^{stop}, set by the following user keywords:

- forceConstant — *Force constant for the half-harmonic bias*

  [positive decimal, context: abmd]

  The value of k. Stronger values result if faster motion of the coordinate, along more biased pathways.

- `stoppingValue` — *Value beyond which the reference value is not updated*

  [decimal, context: `abmd`]

  This sets the value of ξ^{stop}. It is useful to prevent the variable from reaching unrealistic values after the relevant range has been covered.

- `decreasing` — *Should the variable decrease over time?*

  Default: no [boolean, context: `abmd`]

  This is used to make ABMD enhance motion toward lower colvar values. When enabled, it reverses the shape of the half-harmonic potential, and makes the reference value follow the minimum of the variable.

ABMD also supports the following common bias parameters:

- `name` — same definition as `name` (biasing and analysis methods)

- `colvars` — same definition as `colvars` (biasing and analysis methods)

- `outputEnergy` — same definition as `outputEnergy` (biasing and analysis methods)

- `outputFreq` — same definition as `outputFreq` (biasing and analysis methods)

6.6 Metadynamics

The metadynamics method uses a history-dependent potential [28] that generalizes to any type of colvars the conformational flooding [29] and local elevation [30] methods, originally formulated to use as colvars the principal components of a covariance matrix or a set of dihedral angles, respectively. The metadynamics potential on the colvars $\xi = (\xi_1, \xi_2, \ldots, \xi_{N_{\text{cv}}})$ is defined as:

$$V_{\text{meta}}(\xi(t)) = \sum_{t'=\delta t, 2\delta t, \ldots}^{t' < t} W \prod_{i=1}^{N_{\text{cv}}} \exp\left(-\frac{(\xi_i(t) - \xi_i(t'))^2}{2\sigma_{\xi_i}^2}\right), \tag{31}$$

where V_{meta} is the history-dependent potential acting on the *current* values of the colvars ξ, and depends only parametrically on the *previous* values of the colvars. V_{meta} is constructed as a sum of N_{cv}-dimensional repulsive Gaussian "hills", whose height is a chosen energy constant W, and whose centers are the previously explored configurations $(\xi(\delta t), \xi(2\delta t), \ldots)$.

During the simulation, the system evolves towards the nearest minimum of the "effective" potential of mean force $\tilde{A}(\xi)$, which is the sum of the "real" underlying potential of mean force $A(\xi)$ and the the metadynamics potential, $V_{\text{meta}}(\xi)$. Therefore, at any given time the probability of observing the configuration ξ^* is proportional to $\exp\left(-\tilde{A}(\xi^*)/\kappa_{\text{B}}T\right)$: this is also the probability that a new Gaussian "hill" is added at that configuration. If the simulation is run for a sufficiently long time, each local minimum is canceled out by the sum of the Gaussian "hills". At that stage the "effective" potential of mean force $\tilde{A}(\xi)$ is constant, and $-V_{\text{meta}}(\xi)$ is an estimator of the "real" potential of mean force $A(\xi)$, save for an additive constant:

$$A(\xi) \simeq -V_{\text{meta}}(\xi) + K \tag{32}$$

Such estimate of the free energy can be provided by enabling `writeFreeEnergyFile`. Assuming that the set of collective variables includes all relevant degrees of freedom, the predicted error of the estimate is a simple function of the correlation times of the colvars τ_{ξ_i}, and of the user-defined parameters W, σ_{ξ_i} and δt [31]. In typical applications, a good rule of thumb can be to choose the ratio $W/\delta t$ much smaller than $\kappa_B T/\tau_\xi$, where τ_ξ is the longest among ξ's correlation times: σ_{ξ_i} then dictates the resolution of the calculated PMF.

If the metadynamics parameters are chosen correctly, after an equilibration time, t_e, the estimator provided by eq. 32 oscillates on time around the "real" free energy, thereby a better estimate of the latter can be obtained as the time average of the bias potential after t_e [32, 33]:

$$A(\xi) = -\frac{1}{t_{tot} - t_e} \int_{t_e}^{t_{tot}} V_{\mathrm{meta}}(\xi, t) dt \tag{33}$$

where t_e is the time after which the bias potential grows (approximately) evenly during the simulation and t_{tot} is the total simulation time. The free energy calculated according to eq. 33 can thus be obtained averaging on time multiple time-dependent free energy estimates, that can be printed out through the keyword `keepFreeEnergyFiles`. An alternative is to obtain the free energy profiles by summing the hills added during the simulation; the hills trajectory can be printed out by enabling the option `writeHillsTrajectory`.

6.6.1 Treatment of the PMF boundaries

In typical scenarios the Gaussian hills of a metadynamics potential are interpolated and summed together onto a grid, which is much more efficient than computing each hill independently at every step (the keyword `useGrids` is `on` by default). This numerical approximation typically yields negligible errors in the resulting PMF [1]. However, due to the finite thickness of the Gaussian function, the metadynamics potential would suddenly vanish each time a variable exceeds its grid boundaries.

To avoid such discontinuity the Colvars metadynamics code will keep an explicit copy of each hill that straddles a grid's boundary, and will use it to compute metadynamics forces outside the grid. This measure is taken to protect the accuracy and stability of a metadynamics simulation, except in cases of "natural" boundaries (for example, the $[0:180]$ interval of an `angle` colvar) or when the flags `hardLowerBoundary` and `hardUpperBoundary` are explicitly set by the user. Unfortunately, processing explicit hills alongside the potential and force grids could easily become inefficient, slowing down the simulation and increasing the state file's size.

In general, it is a good idea to *define a repulsive potential to avoid hills from coming too close to the grid's boundaries*, for example as a `harmonicWalls` restraint (see 6.10).

Example: Using harmonic walls to protect the grid's boundaries.

```
colvar {
  name r
  distance { ... }
  upperBoundary 15.0
  width 0.2
}
```

```
metadynamics {
  name meta_r
  colvars r
  hillWeight 0.001
  hillWidth 2.0
}

harmonicWalls {
  name wall_r
  colvars r
  upperWalls 13.0
  upperWallConstant 2.0
}
```

In the colvar `r`, the `distance` function used has a `lowerBoundary` automatically set to 0 by default, thus the keyword `lowerBoundary` itself is not mandatory and `hardLowerBoundary` is set to `yes` internally. However, `upperBoundary` does not have such a "natural" choice of value. The metadynamics potential `meta_r` will individually process any hill whose center is too close to the `upperBoundary`, more precisely within fewer grid points than 6 times the Gaussian σ parameter plus one. It goes without saying that if the colvar `r` represents a distance between two freely-moving molecules, it will cross this "threshold" rather frequently.

In this example, where the value of `hillWidth` (2σ) amounts to 2 grid points, the threshold is 6+1 = 7 grid points away from `upperBoundary`. In explicit units, the `width` of `r` is $w_r = 0.2$ Å, and the threshold is 15.0 - 7×0.2 = 13.6 Å.

The `wall_r` restraint included in the example prevents this: the position of its `upperWall` is 13 Å, i.e. 3 grid points below the buffer's threshold (13.6 Å). For the chosen value of `upperWallConstant`, the energy of the `wall_r` bias at $r = r_{\mathrm{upper}}$ = 13.6 Å is:

$$E^* = \frac{1}{2}k\left(\frac{r - r_{\mathrm{upper}}}{w_r}\right)^2 = \frac{1}{2}2.0\,(-3)^2 = 9 \text{ kcal/mol}$$

which results in a relative probability $\exp(-E^*/\kappa_B T) \simeq 3 \times 10^{-7}$ that `r` crosses the threshold. The probability that `r` exceeds `upperBoundary`, which is further away, has also become vanishingly small. At that point, you may want to set `hardUpperBoundary` to `yes` for `r`, and let `meta_r` know that no special treatment near the grid's boundaries will be needed.

What is the impact of the wall restraint onto the PMF? Not a very complicated one: the PMF reconstructed by metadynamics will simply show a sharp increase in free-energy where the wall potential kicks in ($r > 13$ Å). You may then choose between using the PMF only up until that point and discard the rest, or subtracting the energy of the `harmonicWalls` restraint from the PMF itself. Keep in mind, however, that the statistical convergence of metadynamics may be less accurate where the wall potential is strong.

In summary, although it would be simpler to set the wall's position `upperWall` and the grid's boundary `upperBoundary` to the same number, the finite width of the Gaussian hills calls for setting the former strictly within the latter.

6.6.2   Required metadynamics keywords

To enable a metadynamics-based calculation, a `metadynamics {...}` block must be included in the Colvars configuration file.

By default, metadynamics bias energy and forces will be recorded onto a grid, the parameters of which can be defined within the definition of each `colvar`, as described in 4.21. Alternately, grid parameters can be set directly inside the `metadynamics` block by inserting a `grid` sub-block, as explained in 6.1.

Other required keywords will be specified within the `metadynamics` block: these are `colvars` (the names of the variables involved), `hillWeight` (the weight parameter W), and the widths 2σ of the Gaussian hills in each dimension that can be given either as the single dimensionless parameter `hillWidth`, or explicitly for each colvar with `gaussianSigmas`.

- `name` — same definition as `name` (biasing and analysis methods)

- `colvars` — same definition as `colvars` (biasing and analysis methods)

- `outputEnergy` — same definition as `outputEnergy` (biasing and analysis methods)

- `outputFreq` — same definition as `outputFreq` (biasing and analysis methods)

- `writeTIPMF` — same definition as `writeTIPMF` (biasing and analysis methods)

- `writeTISamples` — same definition as `writeTISamples` (biasing and analysis methods)

- `stepZeroData` — same definition as `stepZeroData` (biasing and analysis methods)

- `hillWeight` — *Height of each hill (kcal/mol)*

  [positive decimal, context: `metadynamics`]

  This option sets the height W of the Gaussian hills that are added during this run. Lower values provide more accurate sampling of the system's degrees of freedom at the price of longer simulation times to complete a PMF calculation based on metadynamics.

- `hillWidth` — *Width 2σ of a Gaussian hill, measured in number of grid points*

  [positive decimal, context: `metadynamics`]

  This keyword sets the Gaussian width $2\sigma_{\xi_i}$ for all colvars, expressed in *number of grid points*, with the grid spacing along each colvar ξ determined by the respective value of `width`. Values between 1 and 3 are recommended for this option: smaller numbers will fail to adequately interpolate each Gaussian function [1], while larger values may be unable to account for steep free-energy gradients. The values of each half-width σ_{ξ_i} in the physical units of ξ_i are also printed by Tinker-HP at initialization time; alternatively, they may be set explicitly via `gaussianSigmas`.

- `gaussianSigmas` — *Half-widths σ of the Gaussian hill (one for each colvar)*

  [space-separated list of decimals, context: `metadynamics`]

This option sets the parameters σ_{ξ_i} of the Gaussian hills along each colvar ξ_i, expressed in *the same unit of ξ_i*. No restrictions are placed on each value, but a warning will be printed if `useGrids` is on and the Gaussian width $2\sigma_{\xi_i}$ is smaller than the corresponding grid spacing, `width`(ξ_i). If not given, default values will be computed from the dimensionless number `hillWidth`.

- `newHillFrequency` — *Frequency of hill creation*

  Default: `1000` [positive integer, context: `metadynamics`]

  This option sets the number of steps after which a new Gaussian hill is added to the metadynamics potential. The product of this number and the integration time-step defines the parameter δt in eq. 31. Higher values provide more accurate statistical sampling, at the price of longer simulation times to complete a PMF calculation.

6.6.3 Output files

When interpolating grids are enabled (default behavior), the PMF is written by default every `colvarsRestartFrequency` steps to the file *output*`.pmf` in multicolumn text format (3.7.4). The following two options allow to disable or control this behavior and to track statistical convergence:

- `writeFreeEnergyFile` — *Periodically write the PMF for visualization*

  Default: `on` [boolean, context: `metadynamics`]

  When `useGrids` and this option are on, the PMF is written every `outputFreq` steps.

- `keepFreeEnergyFiles` — *Keep all the PMF files*

  Default: `off` [boolean, context: `metadynamics`]

  When `writeFreeEnergyFile` and this option are on, the step number is included in the file name, thus generating a series of PMF files. Activating this option can be useful to follow more closely the convergence of the simulation, by comparing PMFs separated by short times.

- `writeHillsTrajectory` — *Write a log of new hills*

  Default: `off` [boolean, context: `metadynamics`]

  If this option is on, a file containing the Gaussian hills written by the `metadynamics` bias, with the name:
  "*output*`.colvars.<name>.hills.traj`",
  which can be useful to post-process the time series of the Gassian hills. Each line is written every `newHillFrequency`, regardless of the value of `outputFreq`. When `multipleReplicas` is on, its name is changed to:
  "*output*`.colvars.<name>.<replicaID>.hills.traj`".
  The columns of this file are the centers of the hills, $\xi_i(t')$, followed by the half-widths, σ_{ξ_i}, and the weight, W. **Note:** prior to version 2020-02-24, the full-width 2σ of the Gaussian was reported in lieu of σ.

6.6.4 Performance optimization

The following options control the computational cost of metadynamics calculations, but do not affect results. Default values are chosen to minimize such cost with no loss of accuracy.

- `useGrids` — *Interpolate the hills with grids*

  Default: `on` [boolean, context: `metadynamics`]

  This option discretizes all hills for improved performance, accumulating their energy and their gradients on two separate grids of equal spacing. Grids are defined by the values of `lowerBoundary`, `upperBoundary` and `width` for each colvar. Currently, this option is implemented for all types of variables except the non-scalar types (`distanceDir` or `orientation`). If `expandBoundaries` is defined in one of the colvars, grids are automatically expanded along the direction of that colvar.

- `rebinGrids` — *Recompute the grids when reading a state file*

  Default: `off` [boolean, context: `metadynamics`]

  When restarting from a state file, the grid's parameters (boundaries and widths) saved in the state file override those in the configuration file. Enabling this option forces the grids to match those in the current configuration file.

- `keepHills` — *Write each individual hill to the state file*

  Default: `off` [boolean, context: `metadynamics`]

  When `useGrids` and this option are `on`, all hills are saved to the state file in their analytic form, alongside their grids. This makes it possible to later use exact analytic Gaussians for `rebinGrids`. To only keep track of the history of the added hills, `writeHillsTrajectory` is preferable.

6.6.5 Ensemble-Biased Metadynamics

The ensemble-biased metadynamics (EBMetaD) approach [34] is designed to reproduce a target probability distribution along selected collective variables. Standard metadynamics can be seen as a special case of EBMetaD with a flat distribution as target. This is achieved by weighing the Gaussian functions used in the metadynamics approach by the inverse of the target probability distribution:

$$V_{\text{EBmetaD}}\left(\xi\left(t\right)\right) = \sum_{t'=\delta t, 2\delta t, \dots}^{t' < t} \frac{W}{\exp\left(S_\rho\right)\rho_{exp}(\xi(t'))} \prod_{i=1}^{N_{\text{cv}}} \exp\left(-\frac{(\xi_i(t) - \xi_i(t'))^2}{2\sigma_{\xi_i}^2}\right), \tag{34}$$

where $\rho_{exp}(\xi)$ is the target probability distribution and $S_\rho = -\int \rho_{exp}(\xi)\log\rho_{exp}(\xi)\,d\xi$ its corresponding differential entropy. The method is designed so that during the simulation the resulting distribution of the collective variable ξ converges to $\rho_{exp}(\xi)$. A practical application of EBMetaD is to

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reproduce an "experimental" probability distribution, for example the distance distribution between spectroscopic labels inferred from Förster resonance energy transfer (FRET) or double electron-electron resonance (DEER) experiments [34].

The PMF along ξ can be estimated from the bias potential and the target ditribution [34]:

$$A(\xi) \simeq -V_{\text{EBmetaD}}(\xi) - \kappa_{\text{B}}T \log \rho_{exp}(\xi) \tag{35}$$

and obtained by enabling `writeFreeEnergyFile`. Similarly to eq. 33, a more accurate estimate of the free energy can be obtained by averaging (after an equilibration time) multiple time-dependent free energy estimates (see `keepFreeEnergyFiles`).

The following additional options define the configuration for the ensemble-biased metadynamics approach:

- `ebMeta` — *Perform ensemble-biased metadynamics*

  Default: `off` [boolean, context: `metadynamics`]

  If enabled, this flag activates the ensemble-biased metadynamics as described by Marinelli et al.[34]. The target distribution file, `targetdistfile`, is then required. The keywords `lowerBoundary`, `upperBoundary` and `width` for the respective variables are also needed to set the binning (grid) of the target distribution file.

- `targetDistFile` — *Target probability distribution file for ensemble-biased metadynamics*

  [multicolumn text file, context: `metadynamics`]

  This file provides the target probability distribution, $\rho_{exp}(\xi)$, reported in eq. 34. The latter distribution must be a tabulated function provided in a multicolumn text format (see 3.7.4). The provided distribution is then normalized.

- `ebMetaEquilSteps` — *Number of equilibration steps for ensemble-biased metadynamics*

  [positive integer, context: `metadynamics`]

  The EBMetaD approach may introduce large hills in regions with small values of the target probability distribution (eq. 34). This happens, for example, if the probability distribution sampled by a conventional molecular dynamics simulation is significantly different from the target distribution. This may lead to instabilities at the beginning of the simulation related to large biasing forces. In this case, it is useful to introduce an equilibration stage in which the bias potential gradually switches from standard metadynamics (eq. 31) to EBmetaD (eq. 34) as $\lambda V_{\text{meta}}(\xi) + (1-\lambda)V_{\text{EBmetaD}}(\xi)$, where $\lambda = (\texttt{ebMetaEquilSteps} - \texttt{step})/\texttt{ebMetaEquilSteps}$ and `step` is the current simulation step number.

- `targetDistMinVal` — *Minimum value of the target distribution in reference to its `maximum value`*

  [positive decimal, context: `metadynamics`]

  It is useful to set a minimum value of the target probability distribution to avoid values of the latter that are nearly zero, leading to very large hills. This parameter sets the minimum value of the target probability distribution that is expressed as a fraction of its `maximum value`: `minimum value = maximum value X targetDistMinVal`. This implies that $0 < \texttt{targetDistMinVal} < 1$ and its default value is set to 1/1000000. To avoid divisions by zero (see eq. 34), if `targetDistMinVal` is set as zero, values of ρ_{exp} equal to zero are replaced by the smallest positive value read in the same file.

As with standard metadynamics, multidimensional probability distributions can be targeted using a single `metadynamics` block using multiple `colvars` and a multidimensional target distribution file (see 3.7.4). Instead, multiple probability distributions on different variables can be targeted separately in the same simulation by introducing multiple `metadynamics` blocks with the `ebMeta` option.

Example: EBmetaD configuration for a single variable.

```
colvar {
  name r
  distance {
    group1 { atomNumbers 991 992 }
    group2 { atomNumbers 1762 1763 }
  }
  upperBoundary  100.0
  width          0.1
}

metadynamics {
  name              ebmeta
  colvars           r
  hillWeight        0.01
  hillWidth         3.0
  ebMeta            on
  targetDistFile    targetdist1.dat
  ebMetaEquilSteps  500000
}
```

where `targetdist1.dat` is a text file in "multicolumn" format (3.7.4) with the same `width` as the variable `r` (0.1 in this case):

```
#  1
#  0.0    0.1      1000  0

   0.05  0.0012
   0.15  0.0014
   ...    ...
  99.95  0.0010
```

Tip: Besides setting a meaningful value for `targetDistMinVal`, the exploration of unphysically low values of the target distribution (which would lead to very large hills and possibly numerical instabilities) can be also prevented by restricting sampling to a given interval, using e.g. `harmonicWalls` restraint (6.10).

6.6.6 Well-tempered metadynamics

The following options define the configuration for the "well-tempered" metadynamics approach [35]:

- `wellTempered` — *Perform well-tempered metadynamics*

  Default: `off` [boolean, context: `metadynamics`]

  If enabled, this flag causes well-tempered metadynamics as described by Barducci et al.[35] to be performed, rather than standard metadynamics. The parameter `biasTemperature` is then required. This feature was contributed by Li Li (Luthey-Schulten group, Department of Chemistry, UIUC).

- `biasTemperature` — *Temperature bias for well-tempered metadynamics*

  [positive decimal, context: `metadynamics`]

  When running metadynamics in the long time limit, collective variable space is sampled to a modified temperature $T + \Delta T$. In conventional metadynamics, the temperature "boost" ΔT would constantly increases with time. Instead, in well-tempered metadynamics ΔT must be defined by the user via `biasTemperature`. The written PMF includes the scaling factor $(T + \Delta T)/\Delta T$ [35]. A careful choice of ΔT determines the sampling and convergence rate, and is hence crucial to the success of a well-tempered metadynamics simulation.

Note: usage of well-tempered metadynamics in combination with multiple walkers (6.6.7) is discouraged. Should you want to use the two features, keep in mind that in Colvars, the well-tempered scaling factor is applied independently of the communication between replicas, i.e. each walker has its Gaussian hills scaled independently of the other walkers.

6.6.7 Multiple-walker metadynamics

Metadynamics calculations can be performed concurrently by multiple replicas that share a common history. This variant of the method is called multiple-walker metadynamics [36]: the Gaussian hills of all replicas are periodically combined into a single biasing potential, intended to converge to a single PMF.

In the implementation here described [1], replicas communicate through files. This arrangement allows launching the replicas either (1) as a bundle (i.e. a single job in a cluster's queueing system) or (2) as fully independent runs (i.e. as separate jobs for the queueing system). One advantage of the use case (1) is that an identical Colvars configuration can be used for all replicas (otherwise, `replicaID` needs to be manually set to a different string for each replica). However, the use case (2) is less demanding in terms of high-performance computing resources: a typical scenario would be a computer cluster (including virtual servers from a cloud provider) where not all nodes are connected to each other at high speed, and thus each replica runs on a small group of nodes or a single node.

Whichever way the replicas are started (coupled or not), a shared filesystem is needed so that each replica can read the files created by the others: paths to these files are stored in the shared file `replicasRegistry`. This file, and those listed in it, are read every `replicaUpdateFrequency` steps. Each time the Colvars state file is written (for example, `colvarsRestartFrequency` steps), the file named:

output.`colvars`.*name*.*replicaID*.`state`

is written as well; this file contains only the state of the metadynamics bias, which the other replicas will read in turn. In between the times when this file is modified/replaced, new hills are also temporarily written to the file named:

output.`colvars`.*name*.*replicaID*.`hills`
Both files are only used for communication, and may be deleted after the replica begins writing files with a new *output*.

Example: Multiple-walker metadynamics with file-based communication.

```
metadynamics {
  name mymtd
  colvars x
  hillWeight 0.001
  newHillFrequency 1000
  hillWidth 3.0

  multipleReplicas       on
  replicasRegistry       /shared-folder/mymtd-replicas.txt
  replicaUpdateFrequency 50000  # Best if larger than newHillFrequency
}
```

The following are the multiple-walkers related options:

- `multipleReplicas` — *Enable multiple-walker metadynamics*

  Default: `off` [boolean, context: `metadynamics`]

  This option turns on multiple-walker communication between replicas.

- `replicasRegistry` — *Multiple replicas database file*

  [UNIX filename, context: `metadynamics`]

  If `multipleReplicas` is `on`, this option sets the path to the replicas' shared database file. It is best to use an absolute path (especially when running individual replicas in separate folders).

- `replicaUpdateFrequency` — *How often hills are shared between replicas*

  [positive integer, context: `metadynamics`]

  If `multipleReplicas` is `on`, this option sets the number of steps after which each replica tries to read the other replicas' files. On a networked file system, it is best to use a number of steps that corresponds to at least a minute of wall time.

- `replicaID` — *Set the identifier for this replica (required only for independent jobs)*

  Default: replica index (only if MPI is used) [string, context: `metadynamics`]

  If `multipleReplicas` is `on`, this option sets a unique identifier for this replicas. Specifying this option is thus only required when the replicas are launched as independent computations: when the replicas share a common parallel communication framework (i.e. they are all launched together as a single message-passing computation via MPI) the default value of this keyword is the replica's numeric index (zero-based).

- `writePartialFreeEnergyFile` — *Periodically write the contribution to the PMF from this replica*

Default: off [boolean, context: metadynamics]

If multipleReplicas is on, enabling this option produces an additional file *output*.partial.pmf, which can be useful to monitor the contribution of each replica to the total PMF (which is written to the file *output*.pmf). **Note:** the name of this file is chosen for consistency and convenience, *but its content is not a PMF* and it is not expected to converge, even if the total PMF does.

6.7 On-the-fly probability enhanced sampling (OPES)

This biasing method implements the on-the-fly probability enhanced sampling (OPES) with metadynamics-like target distribution.[37] The bias samples target distributions defined via their marginal distribution $p^{\mathrm{tg}}(\xi)$ over some CVs, $\xi = \xi(x)$. By default opes_metad targets the well-tempered distribution, $p^{\mathrm{WT}}(\xi) = [P(\xi)]^{1/\gamma}$, where γ is known as the biasfactor. Similarly to metadynamics, opes_metad optimizes the bias on-the-fly, with a given newHillfrequency. It does so by reweighting via kernel density estimation of the unbiased distribution in the CV space, $P(\xi)$. A compression algorithm is used to prevent the number of kernels from growing linearly with the simulation time. The bias at step n is

$$V_n(\xi) = -k_{\mathrm{B}}T\left(1 - 1/\gamma\right)\ln\left(\frac{P_n(\xi)}{Z_n} + \varepsilon\right), \tag{36}$$

where the probability $P_n(\xi)$ and the normalization factor Z_n are computed as

$$P_n(\xi) = \frac{\sum_k^n w_k G(\xi, \xi_k)}{\sum_k^n w_k} \tag{37}$$

$$Z_n = \frac{1}{|\Omega_n|}\int_{\Omega_n} P_n(\xi)\,\mathrm{d}\xi, \tag{38}$$

where the weights w_k are given by $w_k = \exp(\beta V_{k-1}(\xi_k))$, and the Gaussian kernels $G(\xi, \xi') = h\exp\left[-1/2\left(\xi - \xi'\right)^T \Sigma^{-1}\left(\xi - \xi'\right)\right]$ have a diagonal covariance matrix $\Sigma_{ij} = \sigma^2\delta_{ij}$ and fixed height $h = \Pi_i(\sigma_i\sqrt{2\pi})^{-1}$ (see Ref.[37] for a complete description of the method).

If the exploration mode (keyword explore) is on, then the on-the-fly target probability distribution $p^{\mathrm{WT}}(\xi)$ is used to define the biasing energy:

$$V_n(\xi) = -k_{\mathrm{B}}T\left(\gamma - 1\right)\ln\left(\frac{p_n^{\mathrm{WT}}(\xi)}{Z_n} + \varepsilon\right), \tag{39}$$

(See Ref.[38] for a complete description of the exploration mode.)

The implementation of opes_metad and its documentation are largely based on the OPES module of the PLUMED package.

6.7.1 Implementation notes

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Compared to the the OPES module in PLUMED [39], this implementation currently has the following limitations:

- Colvars does not support chaining bias actions in PLUMED, so the Colvars OPES implementation cannot take the biasing energies of other biases, such as harmonic restraints, into account for the internal reweighting, which means that there is no way to achieve the same functionality as the `EXTRA_BIAS` option in the OPES module in PLUMED. This means that it is not possible to sample a custom target distribution in the Colvars OPES implementation.

- Due to the same limitation of Colvars as described above, you cannot collect samples on-the-fly by chaining `opes_metad` with a weighted histogram for PMF calculations. Although the PMF calculation via reweighting is implemented into the `pmf` option, the functionality is quite limited. The CVs in `pmfColvars` must be among the biased ones. In other words, you cannot apply bias on (ξ_1, ξ_2) and compute the PMF along ξ_3 by the on-the-fly reweighting using `pmf` (you can only compute the PMF along ξ_1, ξ_2 or (ξ_1, ξ_2)). In addition, you can only specify one set of CVs for estimating the PMF (you cannot apply the OPES bias on (ξ_1, ξ_2, ξ_3) and compute two 2D PMFs along (ξ_1, ξ_2) and (ξ_2, ξ_3) simultaneously using the `pmf` option). However, you can always bypass this limitation by post-hoc reweighting with the trajectories.

- Colvars computes the biases in parallel with SMP, so it cannot use multiple threads again for computing the probability and the normalization factor, searching the kernels that are able to compress and updating the neighbor list. As a result, the CPU performance of this implementation may be slower than the PLUMED OPES module.

The following table summarizes the differences of the option names in Colvars and the corresponding option names in the PLUMED OPES module:

Colvars keyword	PLUMED keyword
barrier	BARRIER
newHillFrequency	PACE
gaussianSigma	SIGMA
gaussianSigmaMin	SIGMA_MIN
kernelCutoff	KERNEL_CUTOFF
compressionThreshold	COMPRESSION_THRESHOLD
adaptiveSigma	(use SIGMA=ADAPTIVE)
adaptiveSigmaStride	ADAPTIVE_SIGMA_STRIDE
neighborList	NLIST
neighborListNewHillReset	NLIST_PACE_RESET
neighborListParameters	NLIST_PARAMETERS
noZed	NO_ZED
fixedGaussianSigma	FIXED_SIGMA
recursiveMerge	(the opposite of RECURSIVE_MERGE_OFF)
calcWork	CALC_WORK
multipleReplicas	WALKERS_MPI

The PLUMED options for restarting (`STATE_RFILE`, `STATE_WFILE` and `STATE_WSTRIDE`) are not necessary in Colvars, since Colvars has a unified mechanism to save the information required for restarting in the `.colvars.state` file.

6.7.2 General options

To enable a OPES-based calculation, a `opes_metad {...}` block must be included in the Colvars configuration file. The `opes_metad` block supports the following options:

- `name` — same definition as `name` (biasing and analysis methods)

- `colvars` — same definition as `colvars` (biasing and analysis methods)

- `outputEnergy` — same definition as `outputEnergy` (biasing and analysis methods)

- `barrier` — *The highest free-energy barrier you wish to overcome (kcal/mol)*

  [positive decimal, context: `opes_metad`]

  This parameter should be set to be at least equal to the highest free energy barrier you wish to overcome. If it is much lower than that, you will not cross the barrier, if it is much higher, convergence might take a little longer. If the system has a basin that is clearly more stable than the others, it is better to start the simulation from there.

- `newHillFrequency` — *Frequency of hill creation*

  [positive integer, context: `opes_metad`]

  The frequency for kernel deposition.

- `biasfactor` — *The γ biasfactor used for the well-tempered target distribution*

  Default: $\mathrm{barrier}/k_\mathrm{B}T$ [positive decimal or "inf", context: `opes_metad`]

  This option sets the γ in Eq.(36). If setting to "inf", the target distribution is uniform. It cannot be "inf" in the exploration mode.

- `gaussianSigma` — *The initial widths of the kernels (divided by the square root of gamma in exploration mode)*

  [positive decimal, context: `opes_metad`]

  This option sets the initial σ values of the Gaussian kernels. The number of `gaussianSigma` values should match the number of CVs used by `colvars`. If `adaptiveSigma` is set, then this option can be ignored.

- `gaussianSigmaMin` — *The minimum widths of the kernels (divided by the square root of gamma in exploration mode)*

  [positive decimal (optional), context: `opes_metad`]

  This option sets the minimum σ values of the Gaussian kernels. The number of `gaussianSigmaMin` values should match the number of CVs used by `colvars`. The OPES method scales down the σ during the simulation, and will never shrink σ under `gaussianSigmaMin` if this option is set.

- `explore` — *Exploration mode*

  Default: `off` [boolean, context: `opes_metad`]

If enabled, the OPES exploration mode will be used (see Ref.[38] for more information about the exploration mode).

- `epsilon` — *The value of the regularization constant for the probability*

  Default: $\exp(-\text{barrier}/k_\text{B}T/(1-1/\gamma))$ [positive decimal, context: `opes_metad`]

  This option sets the ε in Eq.(36).

- `kernelCutoff` — *Truncate kernels at this distance, in units of σ*

  Default: $\sqrt{2 * \text{barrier}/k_\text{B}T/(1-1/\gamma)}$ [positive decimal, context: `opes_metad`]

  If the value of $(\xi - \xi')^T\Sigma^{-1}(\xi - \xi')$ is larger than the square of this value, then $G(\xi, \xi')$ is evaluated as zero.

- `compressionThreshold` — *Merge kernels if closer than this threshold, in units of σ*

  Default: 1.0 [positive decimal, context: `opes_metad`]

  Kernels will be merged the distances of their centers (scaled by the square of σ) are closer than this threshold.

- `adaptiveSigma` — *Adaptively compute the bandwidths*

  Default: `off` [boolean, context: `opes_metad`]

  If enabled, the values of σ of CVs are computed adaptively based on their variances. See the Supporting Information of Ref.[38] for more details.

- `adaptiveSigmaStride` — *Number of steps for measuring adaptive sigma*

  Default: $10 \times \text{newHillFrequency}$ [positive integer, context: `opes_metad`]

  This option sets the number of steps for measuring the rolling variances of CVs that are used by the adaptive kernel bandwidth algorithm. See the Supporting Information of Ref.[38] for more details.

- `neighborList` — *Use neighbor list for kernels summation (experimental)*

  Default: `off` [boolean, context: `opes_metad`]

  If enabled, a neighbor list will be used for kernels summation.

- `neighborListNewHillReset` — *Force the reset of the neighbor list at each time when depositing a new hill*

  Default: `off` [boolean, context: `opes_metad`]

  If enabled, the neighbor list will be rebuilt at each time when depositing a new hill.

- `neighborListParameters` — *The two cutoff parameters of the neighbor list*

  Default: 3.0 0.5 [positive decimals, context: `opes_metad`]

  The first parameter is the maximum distance of neighboring kernels. The second parameters is the threshold for rebuilding.

- `noZed` — *Do not normalize the probability over the explored CV space*

Default: `off` [boolean, context: `opes_metad`]

If enabled, Z_n is always 1.

- `fixedSigma` — *Do not scale down* σ

  Default: `off` [boolean, context: `opes_metad`]

  If enabled, σ will not be decreased as the simulation proceeds.

- `recursiveMerge` — *Merge the kernels recursively*

  Default: `on` [boolean, context: `opes_metad`]

  If enabled, the kernels will be compressed recursively when a new kernel is added.

- `calcWork` — *Calculate the work done by the bias*

  Default: `off` [boolean, context: `opes_metad`]

  If enabled, the total accumulated work will be computed.

6.7.3 Multiple-walker options

The following options can be used for the multiple-walker OPES, and require the direct communication between replicas through MPI:

- `multipleReplicas` — *Enable multiple-walker OPES*

  Default: `off` [boolean, context: `opes_metad`]

  This option turns on multiple-walker communication between replicas.

- `replicaID` — *Set the identifier for this replica (required only for independent jobs)*

  Default: replica index) [string, context: `opes_metad`]

  If `multipleReplicas` is `on`, this option sets a unique identifier for this replicas. Specifying this option is thus only required when the replicas are launched as independent computations: when the replicas share a common parallel communication framework (i.e. they are all launched together as a single message-passing computation via MPI) the default value of this keyword is the replica's numeric index (zero-based).

- `sharedFreq` — *Frequency (in number of steps) at which force samples are synchronized among the replicas*

  Default: `outputFreq` [positive integer or zero, context: `opes_metad`]

  Every `sharedFreq` steps, the replicas communicate the samples that have been gathered since the last synchronization time. Because this forces the replicas to synchronize, small values of this parameter may slightly reduce the overall throughput, especially on systems with unequal performance.

6.7.4 Output options

The following options are used for setting the PMF and trajectory output. The PMF is calculated based on reweighting the CVs specified by `pmfColvars`, which collects on-the-fly the biasing energy $V(\xi)$ and the values of CVs of every step, and builds a weighted histogram to calculate the unbiased probability of ξ

$$\langle \mathscr{P}(\xi') \rangle = \frac{\sum_{t=0}^{N} \delta\left(\xi(\mathbf{x}) - \xi'\right) \exp\left(\frac{V(\xi)}{k_\mathrm{B}T}\right)}{\sum_{t=0}^{N} \exp\left(\frac{V(\xi)}{k_\mathrm{B}T}\right)} \tag{40}$$

where N is the total number of simulation steps, ξ' is the grid center in the histogram, and δ is the dirac delta function. The PMF is then obtained by

$$A(\xi') = -k_\mathrm{B}T \ln \langle \mathscr{P}(\xi') \rangle + A_0 \tag{41}$$

where A_0 is a constant to ensure $A(\xi')$ on every grid of the histogram is not negative.

The grid used for discretizing the free energy surface (PMF) computated by OPES is defined by the relevant options of the colvars. Alternately, grid parameters can be set directly inside the `opes_metad` block by inserting a `grid` sub-block, as explained in 6.1.

- `pmf` — *Output the PMF*

  Default: `off` [boolean, context: `opes_metad`]

  This option turns on the PMF output based on reweighting.

- `pmfColvars` — *The CVs used for estimating the PMF*

  [positive decimal, context: `opes_metad`]

  This option sets the CVs used for the PMF. The CVs specified in this option must have appeared in `colvars` and had the boundaries and widths.

- `pmfHistoryFrequency` — *The frequency of saving the PMF history file*

  Default: 0 [positive integer, context: `opes_metad`]

  If this option is larger than 0 and `pmf` is on, then every `pmfHistoryFrequency` steps the current PMF will also be saved into "*output*.colvars.<name>.<replicaID>.hist.pmf".

- `pmfShared` — *Output a global PMF for multiple-walker OPES*

  Default: `on` [boolean, context: `opes_metad`]

  If `multipleReplicas` is on and this option is also on, then a global reweighting histogram will be built to collect samples from all replicas, and the corresponding global PMF is written into "*output*.colvars.<name>.<replicaID>.global.pmf" by replica 0. The history global PMF is also written to "*output*.colvars.<name>.<replicaID>.global.hist.pmf" by replica 0.

- `printTrajectoryFrequency` — *The frequency of saving the trajectories*

  Default: `colvarsTrajFrequency` [positive integer, context: `opes_metad`]

If this option is larger than 0, then every `printTrajectoryFrequency`, the simulation time in ps, the values of CVs, the biasing energy, $c(t)$, Z_n (if `noZed` is off), the effective sample size (N_{eff} in Ref.[37]), the work (if `calcWork` is on), the total number of compressed kernels, the number of kernels in the neighbor list (if `neighborList` is on), and the number of steps from the last neighbor list update (if `neighborList` is on) will be written to "*output*`.colvars.<name>.<replicaID>.misc.traj`".

Similar to the output file specified by the `FILE` option in the PLUMED implementation, the output file "*output*`.colvars.<name>.<replicaID>.kernels.dat`" includes all deposited uncompressed kernels (the step number of deposition, the kernel center, the Gaussian kernel σ, the height of the kernel, and the biasing energy in $k_{\text{B}}T$). The format of `.kernels.dat` files manages to be compatible with the post-processing tools provided in the PLUMED OPES tutorial, so that it is possible to run `State_from_Kernels.py` and then `FES_from_State.py` to get the PMF along the biased CVs. Besides the `pmf` option, there are two ways to manually estimate the PMF, namely (i) reweighting the trajectories using the biasing energy either in the `.colvars.traj` file (with `outputEnergy`) or the `.misc.traj` file (the `<name>.bias` column), and (ii) summing up all the kernels (see Ref.[37] for more information).

6.7.5 Example input

Example: OPES with adaptive kernel bandwidth, neighbor list and PMF based on reweighting.

```
colvarsTrajFrequency     500

colvar {
  name phi
  lowerBoundary -180
  upperBoundary 180
  width 5.0
  dihedral {
    group1 {atomNumbers { 5 }}
    group2 {atomNumbers { 7 }}
    group3 {atomNumbers { 9 }}
    group4 {atomNumbers { 15}}
  }
}

colvar {
  name psi
  lowerBoundary -180
  upperBoundary 180
  width 5.0
  dihedral {
    group1 {atomNumbers { 7 }}
    group2 {atomNumbers { 9 }}
```

```
        group3 {atomNumbers { 15 }}
        group4 {atomNumbers { 17 }}
    }
}

opes_metad {
    colvars phi psi
    newHillFrequency 500
    barrier 11.950286806883364
    adaptiveSigma on
    neighborList on
    printTrajectoryFrequency 500
    pmf on
    pmfColvars phi psi
    pmfHistoryFrequency 1000
    outputEnergy on
}
```

6.8 Harmonic restraints

The harmonic biasing method may be used to enforce fixed or moving restraints, including variants of Steered and Targeted MD. Within energy minimization runs, it allows for restrained minimization, e.g. to calculate relaxed potential energy surfaces. In the context of the Colvars module, harmonic potentials are meant according to their textbook definition:

$$V(\xi) = \frac{1}{2}k\left(\frac{\xi - \xi_0}{w_\xi}\right)^2 \tag{42}$$

There are two noteworthy aspects of this expression:

1. Because the *standard coefficient of* $1/2$ of the harmonic potential is included, this expression differs from harmonic bond and angle potentials historically used in common force fields, where the factor was typically omitted resulting in a non-standard definition of the force constant.

2. The variable ξ is not only centered at ξ_0, but is also *scaled by its characteristic length scale* w_ξ (keyword `width`). The resulting dimensionless variable $z = (\xi - \xi_0)/w_\xi$ is typically easier to treat numerically: for example, when the forces typically experienced by ξ are much smaller than k/w_ξ and k is chosen equal to $\kappa_B T$ (thermal energy), the resulting probability distribution of z is approximately a Gaussian with mean equal to 0 and standard deviation equal to 1.

   This property can be used for setting the force constant in umbrella-sampling ensemble runs: if the restraint centers are chosen in increments of w_ξ, the resulting distributions of ξ are most often optimally overlapped. In regions where the underlying free-energy landscape induces highly skewed distributions of ξ, additional windows may be added as needed, with spacings finer than w_ξ.

Beyond one dimension, the use of a scaled harmonic potential also allows a standard definition of a multi-dimensional restraint with a unified force constant:

$$V(\xi_1, \ldots, \xi_M) = \frac{1}{2} k \sum_{i=1}^{M} \left(\frac{\xi_i - \xi_0}{w_\xi} \right)^2 \qquad (43)$$

If one-dimensional or homogeneous multi-dimensional restraints are defined, and there are no other uses for the parameter w_ξ, width *can be left at its default value of* 1.

A harmonic restraint is defined by a `harmonic {...}` block, which may contain the following keywords:

- `name` — same definition as `name` (biasing and analysis methods)

- `colvars` — same definition as `colvars` (biasing and analysis methods)

- `outputEnergy` — same definition as `outputEnergy` (biasing and analysis methods)

- `writeTIPMF` — same definition as `writeTIPMF` (biasing and analysis methods)

- `writeTISamples` — same definition as `writeTISamples` (biasing and analysis methods)

- `stepZeroData` — same definition as `stepZeroData` (biasing and analysis methods)

- `forceConstant` — *Scaled force constant (kcal/mol)*

  Default: `1.0` [positive decimal, context: `harmonic`]

  This option defines a *scaled* force constant k for the harmonic potential (eq. 43). To ensure consistency for multidimensional restraints, it is divided internally by the square of the specific `width` of each variable (which is 1 by default). This makes all values effectively dimensionless and of commensurate size. For instance, if this force constant is set to the thermal energy $\kappa_B T$ (equal to RT if molar units are used), then the amplitude of the thermal fluctuations of each variable ξ will be on the order of its `width`, w_ξ. This can be used to estimate the optimal spacing of umbrella-sampling windows (under the assumption that the force constant is larger than the curvature of the underlying free energy). *The values of the actual force constants k/w_ξ^2 are always printed when the restraint is defined.*

- `centers` — *Initial harmonic restraint centers*

  [space-separated list of colvar values, context: `harmonic`]

  The centers (equilibrium values) of the restraint, ξ_0, are entered here. The number of values must be the number of requested colvars. Each value is a decimal number if the corresponding colvar returns a scalar, a "(x, y, z)" triplet if it returns a unit vector or a vector, and a "(q0, q1, q2, q3)" quadruplet if it returns a rotational quaternion. If a colvar has periodicities or symmetries, its closest image to the restraint center is considered when calculating the harmonic potential.

Tip: A complex set of restraints can be applied to a system, by defining several colvars, and applying one or more harmonic restraints to different groups of colvars. In some cases, dozens of colvars can be defined, but their value may not be relevant: to limit the size of the colvars trajectory file, it may be wise to disable `outputValue` for such "ancillary" variables, and leave it enabled only for "relevant" ones.

6.8.1   Moving restraints: steered molecular dynamics

The following options allow to change gradually the centers of the harmonic restraints during a simulations. When the centers are changed continuously, a steered MD in a collective variable space is carried out.

- `targetCenters` — *Steer the restraint centers towards these targets*

  [space-separated list of colvar values, context: `harmonic`]

  When defined, the current `centers` will be moved towards these values during the simulation. By default, the centers are moved over a total of `targetNumSteps` steps by a linear interpolation, in the spirit of Steered MD. If `targetNumStages` is set to a nonzero value, the change is performed in discrete stages, lasting `targetNumSteps` steps *each*. This second mode may be used to sample successive windows in the context of an Umbrella Sampling simulation. When continuing a simulation run, the `centers` specified in the configuration file <colvarsConfig> are overridden by those saved in the restart file <colvarsInput>. To perform Steered MD in an arbitrary space of colvars, it is sufficient to use this option and enable `outputAccumulatedWork` and/or `outputAppliedForce` within each of the colvars involved.

- `targetNumSteps` — *Number of steps for steering*

  [positive integer, context: `harmonic`]

  In single-stage (continuous) transformations, defines the number of MD steps required to move the restraint centers (or force constant) towards the values specified with `targetCenters` or `targetForceConstant`. After the target values have been reached, the centers (resp. force constant) are kept fixed. In multi-stage transformations, this sets the number of MD steps *per stage*.

- `outputCenters` — *Write the current centers to the trajectory file*

  Default: `off` [boolean, context: `harmonic`]

  If this option is chosen and `colvarsTrajFrequency` is not zero, the positions of the restraint centers will be written to the trajectory file during the simulation. This option allows to conveniently extract the PMF from the Colvars trajectory files in a steered MD calculation.

Note on restarting moving restraint simulations: Information about the current step and stage of a simulation with moving restraints is stored in the restart file (state file). Thus, such simulations can be run in several chunks, and restarted directly using the same colvars configuration file. In case of a restart, the values of parameters such as `targetCenters`, `targetNumSteps`, etc. should not be changed manually.

6.8.2   Moving restraints: umbrella sampling

The centers of the harmonic restraints can also be changed in discrete stages: in this cases a one-dimensional umbrella sampling simulation is performed. The sampling windows in simulation are calculated in sequence. The colvars trajectory file may then be used both to evaluate the correlation times between consecutive windows, and to calculate the frequency distribution of the colvar of interest in each window. Furthermore, frequency distributions on a predefined grid can be automatically obtained by using the `histogram` bias (see 6.13).

To activate an umbrella sampling simulation, the same keywords as in the previous section can be used, with the addition of the following:

- `targetNumStages` — *Number of stages for steering*

  Default: `0` [non-negative integer, context: `harmonic`]

  If non-zero, sets the number of stages in which the restraint centers or force constant are changed to their target values. If zero, the change is continuous. Each stage lasts `targetNumSteps` MD steps. To sample both ends of the transformation, the simulation should be run for `targetNumSteps` $\times$ (`targetNumStages` + 1).

6.8.3 Changing force constant

The force constant of the harmonic restraint may also be changed over time, either to introduce or remove a restraint smoothly, or to estimate a restraint free energy[40].

- `targetForceConstant` — *Change the force constant towards this value*

  [positive decimal, context: `harmonic`]

  When defined, the current `forceConstant` will be moved towards this value during the simulation. Time evolution of the force constant is dictated by the `lambdaExponent` parameter (see below). By default, the force constant is changed smoothly over a total of `targetNumSteps` steps. This is useful to introduce or remove restraints in a progressive manner. If `targetNumStages` is set to a nonzero value, the change is performed in discrete stages, lasting `targetNumSteps` steps *each*. This second mode may be used to compute the conformational free energy change associated with the restraint, within the FEP or TI formalisms. For convenience, the code provides an estimate of the free energy derivative for use in TI, with the format:
  `colvars:  Lambda= ***.** dA/dLambda= ***.**`
  A more complete free energy calculation (particularly with regard to convergence analysis), while not handled by the Colvars module, can be performed by post-processing the colvars trajectory, if `colvarsTrajFrequency` is set to a suitably small value. It should be noted, however, that restraint free energy calculations may be handled more efficiently by an indirect route, through the determination of a free energy profile for the restrained coordinate.[40]

- `lambdaExponent` — *Exponent in the time-dependence of the force constant*

  Default: `1.0` [decimal equal to or greater than 1.0, context: `harmonic`]

  Sets the exponent, α, in the function used to vary the force constant as a function of time. The force is varied according to a coupling parameter λ, raised to the power α: $k_\lambda = k_0 + \lambda^\alpha(k_1 - k_0)$, where k_0, k_λ, and k_1 are the initial, current, and final values of the force constant.

The parameter λ evolves linearly from 0 to 1, either smoothly, or in `targetNumStages` equally spaced discrete stages, or according to an arbitrary schedule set with `lambdaSchedule`. When the initial value of the force constant is zero, an exponent greater than 1.0 distributes the effects of introducing the restraint more smoothly over time than a linear dependence, and ensures that there is no singularity in the derivative of the restraint free energy with respect to lambda. A value of 4 has been found to give good results in some tests. To remove the restraint over time rather than introduce it, use the `decoupling` option.

- `decoupling` — *Perform a restraint decoupling transformation*

  Default: `off` [boolean, context: `harmonic`]

  When this option is enabled, the restraint is decoupled by changing the force constant from `forceConstant` to 0.0 over the course of the simulation, as specified by `targetNumSteps` and, optionally, `targetNumStages`. The force constant is then varied as: $k_\lambda = (1 - \lambda)^\alpha k$, where α is the value of `lambdaExponent`. Setting `lambdaExponent` to a value greater than 1 (e.g. 4) ensures a smoother dependence of the energy on λ around $k_\lambda = 0$.

- `targetEquilSteps` — *Number of steps discarded from TI estimate*

  Default: 0 [positive integer, context: `harmonic`]

  Defines the number of steps within each stage that are considered equilibration and discarded from the restraint free energy derivative estimate reported reported in the output.

- `lambdaSchedule` — *Schedule of lambda-points for changing force constant*

  [list of real numbers between 0 and 1, context: `harmonic`]

  If specified together with targetForceConstant, sets the sequence of discrete λ values that will be used for different stages.

6.9 Computing the work of a changing restraint

If the restraint centers or force constant are changed continuosly (`targetNumStages` undefined) it is possible to record the net work performed by the changing restraint:

- `outputAccumulatedWork` — *Write the accumulated work of the changing restraint to the Colvars trajectory file*

  Default: `off` [boolean, context: `harmonic`]

  If `targetCenters` or `targetForceConstant` are defined and this option is enabled, the accumulated work from the beginning of the simulation will be written to the trajectory file (`colvarsTrajFrequency` must be non-zero). When the simulation is continued from a state file, the previously accumulated work is included in the integral. This option allows to conveniently extract the estimated PMF of a steered MD calculation (when `targetCenters` is used), or of other simulation protocols.

6.10 Harmonic wall restraints

The `harmonicWalls {...}` bias is closely related to the harmonic bias (see 6.8), with the following two differences: *(i)* instead of a center a *lower wall* and/or an *upper wall* are defined, outside of which the bias implements a half-harmonic potential;

$$V(\xi) = \begin{cases} \frac{1}{2}k\left(\frac{\xi - \xi_{upper}}{w_\xi}\right)^2 & \text{if } \xi > \xi_{upper} \\ 0 & \text{if } \xi_{lower} \leq \xi \leq \xi_{upper} \\ \frac{1}{2}k\left(\frac{\xi - \xi_{lower}}{w_\xi}\right)^2 & \text{if } \xi < \xi_{lower} \end{cases} \tag{44}$$

where ξ_{lower} and ξ_{upper} are the lower and upper wall thresholds, respectively; *(ii)* because an interval between two walls is defined, only scalar variables can be used (but any number of variables can be defined, and the wall bias is intrinsically multi-dimensional).

Note: this bias replaces the keywords `lowerWall`, `lowerWallConstant`, `upperWall` and `upperWallConstant` defined in the `colvar` context. Those keywords are deprecated.

The `harmonicWalls` bias implements the following options:

- `name` — same definition as `name` (biasing and analysis methods)

- `colvars` — same definition as `colvars` (biasing and analysis methods)

- `outputEnergy` — same definition as `outputEnergy` (biasing and analysis methods)

- `writeTIPMF` — same definition as `writeTIPMF` (biasing and analysis methods)

- `writeTISamples` — same definition as `writeTISamples` (biasing and analysis methods)

- `stepZeroData` — same definition as `stepZeroData` (biasing and analysis methods)

- `lowerWalls` — *Position of the lower wall(s)*

  [Space-separated list of decimals, context: `colvar`]

  Defines the values ξ_{lower} below which a confining restraint on the colvar is applied to each colvar ξ.

- `upperWalls` — *Position of the upper wall(s)*

  [Space-separated list of decimals, context: `colvar`]

  Defines the values ξ_{upper} above which a confining restraint on the colvar is applied to each colvar ξ.

- `forceConstant` — same definition as `forceConstant` (Harmonic restraints)

- `lowerWallConstant` — *Force constant for the lower wall*

  Default: `forceConstant` [positive decimal, context: `harmonicWalls`]

  When both sets of walls are defined (lower and upper), this keyword allows setting different

force constants for them. As with `forceConstant`, the specified constant is divided internally by the square of the specific `width` of each variable (see also the equivalent keyword for the harmonic restraint, `forceConstant`). The force constant reported in the output as "k", and used in the change of force constant scheme, is the geometric mean of `upperWallConstant` and `upperWallConstant`.

- `upperWallConstant` — analogous to `lowerWallConstant`

- `targetForceConstant` — same definition as `targetForceConstant` (harmonic restraints)

- `targetForceConstant` — *Change the force constant(s) towards this value*

  [positive decimal, context: `harmonicWalls`]

  This keyword allows changing either one or both of the wall force constants over time. In the case that `lowerWallConstant` and `upperWallConstant` have the same value, the behavior of this keyword is identical to the corresponding keyword in the `harmonic` restraint; otherwise, the change schedule is applied to the geometric mean of the two constant. When only one set of walls is defined (`lowerWall` or `upperWalls`), only the respective force constant is changed. **Note:** if only one of the two force constants is meant to change over time, it is possible to use two instances of `harmonicWalls`, and apply the changing schedule only to one of them.

- `targetLowerWalls` — *Change the lower wall value(s) towards these values*

  [positive decimal, context: `harmonicWalls`]

  This keyword allows changing the lower wall position over time. By default, this change is continuous over `targetEquilSteps`. In that case, the non-equilibrium work performed can be obtained by enabling `outputAccumulatedWork`. If `targetNumStages` is provided, the change happens in the given number of increments. See `targetNumStages` for details.

- `targetUpperWalls` — *Change the upper wall value(s) towards these values*

  [positive decimal, context: `harmonicWalls`]

  Analogous to `targetLowerWalls`.

- `targetNumSteps` — same definition as `targetNumSteps` (harmonic restraints)

- `lambdaExponent` — same definition as `lambdaExponent` (harmonic restraints)

- `targetEquilSteps` — same definition as `targetEquilSteps` (harmonic restraints)

- `targetNumStages` — same definition as `targetNumStages` (harmonic restraints)

- `lambdaSchedule` — same definition as `lambdaSchedule` (harmonic restraints)

- `outputAccumulatedWork` — same definition as `outputAccumulatedWork` (harmonic restraints)

- `bypassExtendedLagrangian` — *Apply bias to actual colvars, bypassing extended coordinates*

  Default: `on` [boolean, context: `harmonicWalls`]

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This option behaves as `bypassExtendedLagrangian` for other biases, but **it defaults to** `on`, **unlike in the general case**. Thus, by default, the `harmonicWalls` bias applies to the actual colvars, so that the distribution of the colvar between the walls is unaffected by the bias, which then applies a flat-bottom potential *as a function of the colvar value*. This bias will affect the extended coordinate distribution near the walls. If `bypassExtendedLagrangian` is disabled, `harmonicWalls` applies a flat-bottom potential *as a function of the extended coordinate*. Conversely, this bias will then modify the distribution of the actual colvar value near the walls.

Example 1: harmonic walls for one variable with two different force constants.

```
harmonicWalls {
  name   mywalls
  colvars     dist
  lowerWalls  22.0
  upperWalls  38.0
  lowerWallConstant  2.0
  upperWallConstant 10.0
}
```

Example 2: harmonic walls for two variables with a single force constant.

```
harmonicWalls {
  name   mywalls
  colvars        phi    psi
  lowerWalls -180.0    0.0
  upperWalls    0.0  180.0
  forceConstant 5.0
}
```

6.11   Linear restraints

The `linear` keyword defines a linear potential:

$$V(\xi) = k\left(\frac{\xi - \xi_0}{w_\xi}\right) \tag{45}$$

whose force is simply given by the constant k/w_ξ itself:

$$f(\xi) = k/w_\xi \tag{46}$$

This type of bias is therefore most useful in situations where a *constant force* is desired. As all other restraints, it can be defined on one or more CVs, with each contribution added to the total potential and the parameters w_ξ determining the relative magnitude for each.

Example: A possible use case of the `linear` bias is mimicking a constant electric field acting on a specific particle, or the center of mass of many particles. In the following example, a linear restraint is applied on a `distanceZ` variable (4.3.2), generating a constant force parallel to the Z axis of magnitude 5 kcal/mol/Å:

```
colvar {
  name z
  distanceZ {
    ...
  }
}

linear {
  colvars z
  forceConstant 5.0
  centers 0.0
}
```

Another useful application of a linear restraint is to enforce experimental constraints in a simulation, with a lower non-equilibrium work than e.g. harmonic restraints [41]. There is generally a unique strength of bias for each CV center, which means you must know the bias force constant specifically for the center of the CV. This force constant may be found by using experiment directed simulation described in section 6.12.

- `name` — same definition as `name` (biasing and analysis methods)

- `colvars` — same definition as `colvars` (biasing and analysis methods)

- `outputEnergy` — same definition as `outputEnergy` (biasing and analysis methods)

- `forceConstant` — *Scaled force constant (kcal/mol)*

  Default: 1.0 [Decimal, context: `linear`]

  This option defines a *scaled* force constant k for the linear bias. To ensure consistency for multidimensional restraints, it is divided internally by the specific `width` of each variable (which is 1 by default), so that all variables are effectively dimensionless and of commensurate size. *The values of k/w_ξ are always printed when the restraint is defined.*

- `centers` — *Initial linear restraint centers*

  [space-separated list of colvar values, context: `linear`]

  These are analogous to the `centers` keyword of the harmonic restraint. Although they do not affect dynamics, they are here necessary to ensure a well-defined energy for the linear bias.

- `writeTIPMF` — same definition as `writeTIPMF` (biasing and analysis methods)

- `writeTISamples` — same definition as `writeTISamples` (biasing and analysis methods)

- `targetForceConstant` — same definition as `targetForceConstant` (Harmonic restraints)

- `targetNumSteps` — same definition as `targetNumSteps` (Harmonic restraints)

- `lambdaExponent` — same definition as `lambdaExponent` (Harmonic restraints)

- `targetEquilSteps` — same definition as `targetEquilSteps` (Harmonic restraints)

- **targetNumStages** — same definition as targetNumStages (Harmonic restraints)

- **lambdaSchedule** — same definition as lambdaSchedule (Harmonic restraints)

- **outputAccumulatedWork** — same definition as outputAccumulatedWork (Harmonic restraints)

6.12 Adaptive Linear Bias/Experiment Directed Simulation

Experiment directed simulation applies a linear bias with a changing force constant. Please cite White and Voth [42] when using this feature. As opposed to that reference, the force constant here is scaled by the `width` corresponding to the biased colvar. In White and Voth, each force constant is scaled by the colvars set center. The bias converges to a linear bias, after which it will be the minimal possible bias. You may also stop the simulation, take the median of the force constants (ForceConst) found in the colvars trajectory file, and then apply a linear bias with that constant. All the notes about units described in sections 6.11 and 6.8 apply here as well. **This is not a valid simulation of any particular statistical ensemble and is only an optimization algorithm until the bias has converged**.

- **name** — same definition as name (biasing and analysis methods)

- **colvars** — same definition as colvars (biasing and analysis methods)

- **centers** — *Collective variable centers*

  [space-separated list of colvar values, context: `alb`]

  The desired center (equilibrium values) which will be sought during the adaptive linear biasing. The number of values must be the number of requested colvars. Each value is a decimal number if the corresponding colvar returns a scalar, a "(x, y, z)" triplet if it returns a unit vector or a vector, and a "q0, q1, q2, q3" quadruplet if it returns a rotational quaternion. If a colvar has periodicities or symmetries, its closest image to the restraint center is considered when calculating the linear potential.

- **updateFrequency** — *The duration of updates*

  [An integer, context: `alb`]

  This is, N, the number of simulation steps to use for each update to the bias. This determines how long the system requires to equilibrate after a change in force constant ($N/2$), how long statistics are collected for an iteration ($N/2$), and how quickly energy is added to the system (at most, $A/2N$, where A is the `forceRange`). Until the force constant has converged, the method as described is an optimization procedure and not an integration of a particular statistical ensemble. It is important that each step should be uncorrelated from the last so that iterations are independent. Therefore, N should be at least twice the autocorrelation time of the collective variable. The system should also be able to dissipate energy as fast as $N/2$, which can be done by adjusting thermostat parameters. Practically, N has been tested successfully at significantly shorter than the autocorrelation time of the collective variables being biased and still converge correctly.

- `forceRange` — *The expected range of the force constant in units of energy*

  Default: 3 $k_b T$ [A space-separated list of decimal numbers, context: `alb`]

  This is largest magnitude of the force constant which one expects. If this parameter is too low, the simulation will not converge. If it is too high the simulation will waste time exploring values that are too large. A value of 3 $k_b T$ has worked well in the systems presented as a first choice. This parameter is dynamically adjusted over the course of a simulation. The benefit is that a bad guess for the forceRange can be corrected. However, this can lead to large amounts of energy being added over time to the system. To prevent this dynamic update, add `hardForceRange yes` as a parameter

- `rateMax` — *The maximum rate of change of force constant*

  [A list of space-separated real numbers, context: `alb`]

  This optional parameter controls how much energy is added to the system from this bias. Tuning this separately from the `updateFrequency` and `forceRange` can allow for large bias changes but with a low `rateMax` prevents large energy changes that can lead to instability in the simulation.

6.13 Multidimensional histograms

The `histogram` feature is used to record the distribution of a set of collective variables in the form of a N-dimensional histogram. Defining such a histogram is generally useful for analysis purposes, but it has no effect on the simulation.

Example: the two-dimensional histogram of a distance and an angle can be generated using the configuration below. The histogram code requires that each variable is a scalar number that is confined within a pre-defined interval. The interval's boundaries may be specified by hand (e.g. through `lowerBoundary` and `upperBoundary` in the variable definition), or auto-detected based on the type of function. In this example, the lower boundary for the distance variable "r" is automatically set to zero, and interval for the three-body angle "`theta`" is $0°$ and $180°$: however, that an upper boundary for the distance "r" still needs to be specified manually. The grid spacings for the two variables are 0.2 Åand $3.0°$, respectively.

```
colvar {
  name r
  width 0.2
  upperBoundary 20.0
  distance { ... }
}

colvar {
  name theta
  width 3.0
  dihedral { ... }
}
```

```
histogram {
  name hist2d
  colvars r theta
}
```

The standard keywords below are used to control the histogram's computation and to select the variables that are sampled. See also 6.1 for keywords used to define the grid, 6.13.1 for output parameters and 6.13.2 for more advanced keywords.

- `name` — same definition as `name` (biasing and analysis methods)

- `colvars` — same definition as `colvars` (biasing and analysis methods)

- `stepZeroData` — same definition as `stepZeroData` (biasing and analysis methods)

Grid parameters for the histogram may be provided at the level of the individual variables, or via a dedicated configuration block `grid { ...}` inside the configuration of this `histogram`. The options supported *inside this block* are described in 6.1.

Note that when `gatherVectorColvars` is `on`, each vector variable is automatically treated as a scalar, and a single value of each grid parameter should be provided for it.

6.13.1 Output options for multi-dimensional histograms

The accumulated histogram is written in the Colvars state file, allowing for its accumulation across continued runs. Additionally, the following files are written depending on the histogram's dimensionality:

- `outputFreq` — same definition as `outputFreq` (biasing and analysis methods)

- `outputFile` — *Write the histogram to a file*

  Default: *output*.<name>.dat [UNIX filename, context: `histogram`]

  Name of the file containing histogram data (multicolumn format, see 3.7.4), which is written every `outputFreq` steps. If `outputFile` is set to `none`, the file is not written.

- `outputFileDX` — *Write the histogram to a file*

  Default: *output*.<name>.dx [UNIX filename, context: `histogram`]

  Name of the file containing histogram data (OpenDX format), which is written every `outputFreq` steps. For the special case of 3 variables, VMD may be used to visualize this file. This file is written by default if the dimension is 3 or more (you cannot visualize it easily for dimensions 4 and above, but the DX format is still more compact than the multicolumn format). If `outputFileDX` is set to `none`, the file is not written.

6.13.2 Histogramming vector variables

- `gatherVectorColvars` — *Treat vector variables as multiple observations of a scalar variable?*

  Default: `off` [UNIX filename, context: `histogram`]

  When this is set to `on`, the components of a multi-dimensional colvar (e.g. one based on `cartesian`, `distancePairs`, or a vector of scalar numbers given by `scriptedFunction`) are treated as multiple observations of a scalar variable. This results in the histogram being accumulated multiple times for each simulation step). When multiple vector variables are included in `histogram`, these must have the same length because their components are accumulated together. For example, if ξ, λ and τ are three variables of dimensions 5, 5 and 1, respectively, for each iteration 5 triplets (ξ_i, λ_i, τ) $(i = 1, \ldots 5)$ are accumulated into a 3-dimensional histogram.

- `weights` — *Relative contributions of each vector component to the histogram*

  Default: all weights equal to 1 [list of space-separated decimals, context: `histogram`]

  When `gatherVectorColvars` is `on`, the components of each multi-dimensional colvar are accumulated with a different weight. For example, if x and y are two distinct `cartesian` variables defined on the same group of atoms, the corresponding 2D histogram can be weighted on a per-atom basis in the definition of `histogram`.

As with any other biasing and analysis method, when a histogram is applied to an extended-system colvar (4.23), it accesses the value of the extended coordinate rather than that of the actual colvar. This can be overridden by enabling the `bypassExtendedLagrangian` option. A *joint histogram* of the actual colvar and the extended coordinate may be collected by specifying the colvar name twice in a row in the `colvars` parameter (e.g. `colvars myColvar myColvar`): the first instance will be understood as the actual colvar, and the second, as the extended coordinate.

- `bypassExtendedLagrangian` — same definition as `bypassExtendedLagrangian` (biasing and analysis methods)

6.14 Probability distribution-restraints

The `histogramRestraint` bias implements a continuous potential of many variables (or of a single high-dimensional variable) aimed at reproducing a one-dimensional statistical distribution that is provided by the user. The M variables $(\xi_1, \ldots, \xi_M)$ are interpreted as multiple observations of a random variable ξ with unknown probability distribution. The potential is minimized when the histogram $h(\xi)$, estimated as a sum of Gaussian functions centered at $(\xi_1, \ldots, \xi_M)$, is equal to the reference histogram $h_0(\xi)$:

$$V(\xi_1, \ldots, \xi_M) = \frac{1}{2} k \int (h(\xi) - h_0(\xi))^2 \, d\xi \tag{47}$$

$$h(\xi) = \frac{1}{M\sqrt{2\pi\sigma^2}} \sum_{i=1}^{M} \exp\left(-\frac{(\xi - \xi_i)^2}{2\sigma^2}\right) \tag{48}$$

When used in combination with a `distancePairs` multi-dimensional variable, this bias implements the refinement algorithm against ESR/DEER experiments published by Shen *et al* [43].

This bias behaves similarly to the `histogram` bias with the `gatherVectorColvars` option, with the important difference that *all* variables are gathered, resulting in a one-dimensional histogram. Future versions will include support for multi-dimensional histograms.

The list of options is as follows:

- `name` — same definition as `name` (biasing and analysis methods)

- `colvars` — same definition as `colvars` (biasing and analysis methods)

- `outputEnergy` — same definition as `outputEnergy` (biasing and analysis methods)

- `lowerBoundary` — *Lower boundary of the colvar grid*

  [decimal, context: `histogramRestraint`]

  Defines the lowest end of the interval where the reference distribution $h_0(\xi)$ is defined. Exactly one value must be provided, because only one-dimensional histograms are supported by the current version.

- `upperBoundary` — analogous to `lowerBoundary`

- `width` — *Width of the colvar grid*

  [positive decimal, context: `histogramRestraint`]

  Defines the spacing of the grid where the reference distribution $h_0(\xi)$ is defined.

- `gaussianSigma` — *Standard deviation of the approximating Gaussian*

  Default: $2 \times$ `width` [positive decimal, context: `histogramRestraint`]

  Defines the parameter σ in eq. 48.

- `forceConstant` — *Force constant (kcal/mol)*

  Default: `1.0` [positive decimal, context: `histogramRestraint`]

  Defines the parameter k in eq. 47.

- `refHistogram` — *Reference histogram $h_0(\xi)$*

  [space-separated list of M positive decimals, context: `histogramRestraint`]

  Provides the values of $h_0(\xi)$ consecutively. The mid-point convention is used, i.e. the first point that should be included is for $\xi =$ `lowerBoundary+width`/2. If the integral of $h_0(\xi)$ is not normalized to 1, $h_0(\xi)$ is rescaled automatically before use.

- `refHistogramFile` — *Reference histogram $h_0(\xi)$*

  [UNIX file name, context: `histogramRestraint`]

  Provides the values of $h_0(\xi)$ as contents of the corresponding file (mutually exclusive with `refHistogram`). The format is that of a text file, with each line containing the space-separated values of ξ and $h_0(\xi)$. The same numerical conventions as `refHistogram` are used.

- `writeHistogram` — *Periodically write the instantaneous histogram $h(\xi)$*

  Default: `off` [boolean, context: `metadynamics`]

  If `on`, the histogram $h(\xi)$ is written every `colvarsRestartFrequency` steps to a file with the name *output*.`<name>`.`hist.dat`This is useful to diagnose the convergence of $h(\xi)$ against $h_0(\xi)$.

6.15 Defining scripted biases

Rather than using the biasing methods described above, it is possible to apply biases provided at run time as a Tcl script.

- `scriptedColvarForces` — *Enable custom, scripted forces on colvars*

  Default: `off` [boolean, context: global]

  If this flag is enabled, a Tcl procedure named `calc_colvar_forces` accepting one parameter should be defined by the user. It is executed at each timestep, with the current step number as parameter, between the calculation of colvars and the application of bias forces. This procedure may use the `cv` command to access the values of colvars (e.g. `cv colvar xi value`), apply forces on them (`cv colvar xi addforce $F`) or add energy to the simulation system (`cv addenergy $E`), effectively defining custom collective variable biases.

6.16 Performance of scripted biases

If concurrent computation over multiple threads is available (this is indicated by the message "SMP parallelism is available." printed at initialization time), it is useful to take advantage of the scripting interface to combine many components, all computed in parallel, into a single variable.

The default SMP schedule is the following:

1. distribute the computation of all components across available threads;

2. on a single thread, collect the results of multi-component variables using polynomial combinations (see 4.18), or custom functions (see 4.19);

3. distribute the computation of all biases across available threads;

4. compute on a single thread any scripted biases implemented via the keyword `scriptedColvarForces`.

5. communicate on a single thread forces to Tinker-HP.

The following options allow to fine-tune this schedule:

- `scriptingAfterBiases` — *Scripted colvar forces need updated biases?*

  Default: `on` [boolean, context: global]

This flag specifies that the `calc_colvar_forces` procedure (last step in the list above) is executed only after all biases have been updated (next-to-last step) For example, this allows using the energy of a restraint bias, or the force applied on a colvar, to calculate additional scripted forces, such as boundary constraints. When this flag is set to `off`, it is assumed that only the values of the variables (but not the energy of the biases or applied forces) will be used by `calc_colvar_forces`: this can be used to schedule the calculation of scripted forces and biases concurrently to increase performance.

7   Syntax changes from older versions

The following is a list of syntax changes in Colvars since its first release. Many of the older keywords are still recognized by the current code, thanks to specific compatibility code. *This is not a list of new features:* its primary purpose is to make you aware of those improvements that affect the use of old configuration files with new versions of the code.

Note: if you are using any of the NAMD and VMD tutorials:

https://www.ks.uiuc.edu/Training/Tutorials/

please be aware that *several of these tutorials are not actively maintained*: for those cases, this list will help you reconcile any inconsistencies.

- **Colvars version 2016-06-09 or later .**
  The legacy keyword refPositionsGroup has been renamed fittingGroup for clarity (the legacy version is still supported).

- **Colvars version 2016-08-10 or later .**
  "System forces" have been replaced by "total forces" (see for example outputTotalForce). See the following page for more information:
  https://colvars.github.io/README-totalforce.html

- **Colvars version 2017-01-09 or later .**
  A new type of restraint, harmonicWalls (see 6.10), replaces and improves upon the legacy keywords lowerWall and upperWall: these are still supported as short-hands.

- **Colvars version 2018-11-15 or later .**
  The global analysis keyword has been discontinued: specific analysis tasks are controlled directly by the keywords corrFunc and runAve, which continue to remain off by default.

- **Colvars version 2020-02-25 or later.**
  The parameter hillWidth, expressing the Gaussian width 2σ in relative units (number of grid points), does not have a default value any more. A new alternative parameter gaussianSigmas allows setting the σ parameters explicitly for each variable if needed.
  Furthermore, to facilitate the use of other analysis tools such as for example sum_hills:
  https://www.plumed.org/doc-v2.6/user-doc/html/sum_hills.html
  the format of the file written by writeHillsTrajectory has also been changed to use σ instead of 2σ. This change does not affect how the biasing potential is written in the state file, or the simulated trajectory.

- **Colvars version 2020-02-25 or later.**
  The legacy keywords lowerWall and upperWall of a colvar definition block do not have default values any longer, and need to be set explicitly, preferably as part of the harmonicWalls restraint. When using an ABF bias, it is recommended to set the two walls equal to lowerBoundary and upperBoundary, respectively. When using a metadynamics bias, it is recommended to set the two walls strictly *within* lowerBoundary and upperBoundary; see 6.6.1 for details.

- **Colvars version 2020-11-09 or later.**
  The legacy keyword disableForces for atom groups is now deprecated and will be discon-

tinued in a future release. Atom groups now have an automated way to save computation if forces are not used, and enabling this option otherwise would lead to incorrect behavior.

8 Compilation notes

The Colvars module is typically built using the recipes of each supported software package: for this reason, no installation instructions are needed, and the vast majority of the features described in this manual are supported in the most common builds of each package.

This section lists the few cases where the choice of compilation settings affects the availability features in the Colvars module.

- Scripting commands using the Tcl language (`https://www.tcl.tk`) are supported in VMD, NAMD, and Tinker-HP. All precompiled builds of NAMD and VMD include Tcl, and it is highly recommended to enable Tcl support in any custom build, using precompiled Tcl libraries from the UIUC website.

- The Lepton library (`https://simtk.org/projects/lepton`) used to implement the `customFunction` feature is currently included only in NAMD (always on), in LAMMPS (on by default) and in the Colvars-patched GROMACS releases (but currently, not in the standard releases). For VMD, a patch that allows to link Lepton is available.

- The Torch library is required to support the `torchANN` variable type, which is conditionally compiled if a Torch library is present. In most engines, patching will be required to support this.

- Colvars requires the C++11 language standard or higher, which is either supported (VMD) or required (GROMACS, LAMMPS, NAMD) by all the engines. However, many of the VMD official builds are produced on very old architectures, where C++11 features are disabled at build time, thus limiting functionality. For details please see:
  `https://colvars.github.io/README-c++11.html`

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