Supporting Information for

BFEE: A User-Friendly Graphical Interface Facilitating Absolute Binding Free-energy Calculations

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Readme of example files

`bfee.tcl`: the source code of BFEE plug-in.

Installation guide:

(Windows)

1) Create a "…\VMD\plugins\noarch\tcl\bfee" directory
2) Extract the "bfee.tcl" file inside
3) Create in the "…\bfee" a file named "pkgIndex.tcl" with the following line as content:

```
package ifneeded BFEEstimator 0.4 [list source [file join $dir bfee.tcl]]
```

4) Run VMD, open Tk Console, type:

```
vmd_install_extension BFEEstimator bfee_tk "BFEE"
```

(Linux and OSX)

1) Create a "$HOME/vmdplugins" directory
2) Create in the "vmdplugins directory" a "bfee0.4" directory and extract the "bfee.tcl" file inside
3) Create in the "vmdplugins/bfee0.4" a file named "pkgIndex.tcl" with the following line as content:

```
package ifneeded BFEEstimator 0.4 [list source [file join $dir bfee.tcl]]
```

4) Create a "$HOME/.vmdrc" file (or edit it if existing) containing the following 3 lines:

```
menu main on
set auto_path [linsert $auto_path 0 [file join $env(HOME) vmdplugins]]
vmd_install_extension BFEEstimator bfee_tk "My Plugins/BFEE"
```

5) Run VMD, the plugin is accessible via the following Menu sequence:

```
Extensions -> My Plugins -> BFEE
```

One can also see http://www.ks.uiuc.edu/Research/vmd/plugins/doxygen/tcltkplugins.html or http://physiology.med.cornell.edu/faculty/hweinstein/vmdplugins/installation.html for more information about the installation of a VMD plug-in.

`complex_structure/p41-abl/`: the `psf`, `coor`, `vel`, `xsc` and force field files for p41:Abl-SH3 system as an example. One can use them as inputs to set up a binding free energy calculation. In principle, the results of *J. Chem. Theory Comput.* **2013**, *9*, 794-802 or *J. Chem. Theory Comput*. **2017**, *13*, 5173-5178 can be reproduced.

`complex_structure/CD-guest/`: the `psf`, `coor`, `vel`, `xsc` and force field files for cyclodextrin (CD)-progesterone system as a toy model. Since achieving convergence for the

p41-abf example requeres extensive computational simulations, we recommend using this case as a start point of applying BFEE. Note: in this example, $r^* \leq 25$ Å (by default, 30 Å) must be used in the post-treatment since the largest distance between CD and guest molecule in the separation simulation is 25 Å.

`example_pmf/p41-abl/default`: the `pmf` files in a binding free energy calculation of p41:Abl-SH3. In this case, the default settings of BFEE were used, that is, using a single window for each process without changing any force constant. We got ΔG = -6.9 kcal/mol in this case (compare to -7.99 kcal/mol in experiment), due to the inherent difficulties in the convergence of steps 1, 7 and 8. Using a window-stratified strategy for such complex cases, therefore, is highly recommended.

`example_pmf/p41-abl/window-stratified`: the `pmf` files generated in *J. Chem. Theory Comput*. **2017**, 13, 5173-5178. A calculated binding free energy of -8.0 kcal/mol shows a good agreement with experiment, suggestive of the importance of using windowing strategy to accelerate convergence of free-energy calculations.

`example_pmf/CD-guest/`: the `pmf` files of CD-progesterone toy model. This example converges easily using the default settings of BFEE plug-in. The calculated binding free energy of progesterone to CD is -5.3 kcal/mol, agreeing well with experimental value (-5.6 kcal/mol).

Difference between pmf, UI.pmf and czar.pmf files

BFEE uses the extended adaptive biasing force (eABF) method to perform potential of mean force calculation. By default, NAMD will output `pmf` and `czar.pmf` files, which describes the results of naïve and czar estimator, respectively. The latter is usually much more rigorous than the former. The umbrella-integration based estimator (corresponding to `UI.pmf`), which can be enabled by

using "`UIestimator on`" in the Colvars config file, can be used as an alternative of czar estimator. See *J. Phys. Chem. B* **2017**, *121*, 3676–3685 for more information about the estimators.

In practice, metadynamics and umbrella sampling can be also used in estimating free-energy change with respect to each coarse variable. One can see the latest version of Colvars reference manual for more information (http://colvars.github.io/).