# BFEE v2.1 Documentation

**"File" → "Settings" → "Third party software" → "VMD".** If linked with VMD before input generation, BFEE can generate the required ligand-only system automatically during the process. Otherwise, scripts will be provided and users should execute them before simulations.

**"Pre-treatment" → "NAMD" → "Inputs for complex" → "psf/parm file".** CHARMM psf file or Amber parm file, depending on which force field being used.

**"Pre-treatment" → "NAMD" → "Inputs for complex" → "pdb/rst file".** pdb (CHARMM/Amber) or rst (Amber) file.

**"Pre-treatment" → "NAMD" → "Force fields" → "Force field type".** Specify whether CHARMM or Amber force field are used.

**"Pre-treatment" → "NAMD" → "Force fields" → "Force field files".** CHARMM force field files.

**"Pre-treatment" → "NAMD" → "Other parameters" → "Temperature".** The temperature under which the free-energy calculation will be carried out.

**"Pre-treatment" → "NAMD" → "Other parameters" → "Select protein".** Specify the protein. MDAnalysis selection language is used.

**"Pre-treatment" → "NAMD" → "Other parameters" → "Select ligand".** Specify the ligand. MDAnalysis selection language is used.

**"Pre-treatment" → "NAMD" → "Other parameters" → "Select strategy".** Specify whether the Geometrical or the Alchemical route is adopted for the free-energy calculation.

**"Pre-treatment" → "NAMD" → "Other parameters" → "Advanced settings (Geometrical)" → "User-defined pulling direction".** By default, the pulling direction is along the distance vector between the center of masses (COMs) of the protein and the ligand. When a reference group is set (under MDAnalysis selection language), the pulling direction will be along the distance vector between the COMs of the reference group and the ligand.

**"Pre-treatment" → "NAMD" → "Other parameters" → "Advanced settings (Geometrical)" → "User-provided large box".** A large solvation box should be provided to ensure enough space to allow the separation simulation (step 7) of the geometrical route. If the CHARMM + TIP3P model is used, BFEE will automatically generate the topology and coordinate files of the enlarged water box. Otherwise, users should provide these files.

**"Pre-treatment" → "NAMD" → "Other parameters" → "Advanced settings (Geometrical)"**

→ **"Stratification windows".** Number of stratification windows in PMF calculations.

**"Pre-treatment" → "NAMD" → "Other parameters" → "Advanced settings (Geometrical)" → "Pinning down the protein".** Pinning down the protein in free-energy calculations.

**"Pre-treatment" → "NAMD" → "Other parameters" → "Advanced settings (Geometrical)" → "Membrane protein".** BFEE2 will try to recognize membrane-protein system and generate corresponding scripts and configurational files. The end-user, however, should provide the topology and coordinate files for steps 7 and 8 if the Amber force field is used.

**"Pre-treatment" → "NAMD" → "Other parameters" → "Advanced settings (Geometrical)" → "Parallel runs".** Generate files for parallel runs, through which the errors can be calculated.

**"Pre-treatment" → "NAMD" → "Other parameters" → "Advanced settings (Alchemical)" → "Stratification windows".** Number of stratification windows in alchemical calculations.

**"Pre-treatment" → "NAMD" → "Other parameters" → "Advanced settings (Alchemical)" → "Double-wide simulation".** Generate input files for double-wide FEP simulations. The users are required to know how to analyze the results.

**"Pre-treatment" → "NAMD" → "Other parameters" → "Advanced settings (Alchemical)" → "Minimization before sampling".** Do minimization before sampling in each window of alchemical runs.

**"Pre-treatment" → "NAMD" → "Other parameters" → "Advanced settings (Alchemical)" → "Pinning down the protein".** Pinning down the protein in free-energy calculations.

**"Pre-treatment" → "NAMD" → "Other parameters" → "Advanced settings (Alchemical)" → "Membrane protein".** BFEE2 will try to recognize membrane-protein system and generate corresponding scripts and configurational files. The end-user, however, should provide the topology and coordinate files for steps 3 and 4 if the Amber force field is used.

**"Pre-treatment" → "Gromacs" → "Input for complex" → "top file".** Gromacs file for the protein-ligand complex.

**"Pre-treatment" → "Gromacs" → "Input for complex" → "pdb file".** pdb file for the protein-ligand complex.

**"Pre-treatment" → "Gromacs" → "Input for ligand-only system" → "top file".** Gromacs file for the ligand-only system.

**"Pre-treatment" → "Gromacs" → "Input for ligand-only system" → "pdb file".** pdb file for the ligand-only system.

**“Pre-treatment” → ”Gromacs” → ”Other parameters”.** Same as those for NAMD, while the Alchemical route and Advanced settings are currently not supported for Gromacs.

**“Post-treatment” → ”Geometrical” → ”PMF inputs”.** The pmf files of steps 1-8.

**“Post-treatment” → ”Geometrical” → ”Force constants”.** The force constants used to restrain RMSD, Euler theta, Euler phi, Euler psi, spherical theta and spherical phi, respectively. It should be noted that the Colvars units are different for NAMD and Gromacs. Use the value in the colvars.in file.

**“Post-treatment” → ”Geometrical” → ”Other parameters” → “temperature”.** The temperature under which the free-energy calculation will be carried out.

**“Post-treatment” → ”Geometrical” → ”Other parameters” → “r*”.** In step 7, $r=r^*$ guarantees that the ligand is far from the protein. The choice of this value should not impact the results, provided that $r^*$ is large enough.

**“Post-treatment” → ”Geometrical” → ”Other parameters” → “PMF type”.** Either the PMFs are calculated using NAMD or Gromacs.

**“Post-treatment” → ”Alchemical” → ”Other parameters” → “Inputs for alchemical simulations”.** The forward/backward simulation results obtained from steps 1-4 of alchemical simulations.

**“Post-treatment” → ” Alchemical” → ”Force constants”.** The force constants used to restrain Euler theta, Euler phi, Euler psi, spherical theta, spherical phi and COM distance between the protein and the ligand respectively.

**“Post-treatment” → ” Alchemical” → ”Restraint centers”.** The restraining centers used to restrain Euler theta, spherical theta and COM distance between the protein and the ligand respectively.

**“Quick plot” → ” Plot (stratified) PMFs”.** Plot the 1D PMF. .czar.pmf files from stratified calculations are supported.

**“Quick plot” → ” Merge (stratified) PMFs”.** Merge .czar.pmf files from stratified calculations.

**“Quick plot” → ” Calculate PMF RMSD convergence”.** Plot the time evolution of PMF RMSD with respect to a zero vector.