We proposed a new method termed **MBAR+wTP** (**M**ultistate **B**ennett **A**cceptance **R**atio + **w**eighted **T**hermodynamic **P**erturbation), with which the computation of the ab initio (ai) free energy (FE) profile can be accelerated by several orders of magnitude via a three-step procedure:

1. an umbrella sampling (US) using a semi-empirical (SE) QM/MM Hamiltonian is performed;
2. the FE profile is generated using the Multistate Bennett Acceptance Ratio (MBAR) analysis;
3. a weighted Thermodynamic Perturbation (wTP) from the SE Hamiltonian to the ai Hamiltonian is performed to obtain the ai QM/MM FE profile using weight factors from the MBAR analysis.

In the MBAR+wTP method, there are two key factors that are critical to the reliability of the results.

1. The similarity between the Hamiltonians for neighboring windows in the US simulations. It can be characterized by the overlap of the samples in the phase space, which can be quantitatively measured by, for instance, the **overlap matrix** proposed by Klimovich et al. The overlap between neighboring windows are larger than 0.03, which is the lower-limit suggested by Klimovich et al. It indicates that the phase space overlap is sufficient for the subsequent MBAR analysis. Therefore, all the FE profiles calculated by the MBAR method are statistically reliable.
2. The similarity between the low level (e.g. PM3/MM) Hamiltonian and the high level (B3LYP/MM) Hamiltonian, which determines the reliability of the weighted TP and can be measured by **reweighting entropy**. Because of the statistical noise in the wTP correction process, Gaussian processes regression (GPR) method was used to smooth the FE profiles after the wTP correction.

We will introduce some details as follows:

The reliability of MBAR analysis of umbrella sampling trajectories depends on the overlap in the phase space for the samples from neighboring windows. A strong biasing potential may limit the samples within a small region around the center of the biasing potential. In order to guarantee sufficient overlap in phase space, a proper number of windows must be chosen. On the other hand, a weak biasing potential may lead to wide distribution of the samples along the RC, and hence increase the overlap in phase space. However, it cannot guarantee sufficient sampling around the center of that window.

We used different values for different windows, which are determined by the gradient of the free energy profile. The number of windows and the centers of the biasing potential are properly chosen, so that the first off-diagonal elements of the overlap matrix are larger than 0.03, as suggested in J. Comput. Aid. Mol. Des. 2015, 29, 397-411.

Here, we choose a Claisen Rearrangement reaction as 1D example and all related scripts and codes have been collected in *“Claisen-Rearrangement”* directory which contains two subdirectories called *“MBAR”* and *“wTP”*. All python scripts are based on Anaconda2 and pymbar program whose tar file is contained in software directory. Please install pymbar program using Anaconda2 before performing our MBAR+wTP scripts.

*In directory “Claisen-Rearrangement/MBAR/data”,* the conformations from umbrella samplings are saved. Here, files *kvalue* and *windows* in the folder *“Claisen-Rearrangement/window\_info”* contain the stiffnesses and the center positions of the biasing potentials, respectively. File *meta.dat*, which will be used in subsequent US analysis, contains a list of the US windows sampling filenames, the center position of US windows and the stiffness\*2.

*In directory “Claisen-Rearrangement/MBAR/pymbar/bin-110”,* the files *pymbar.py and umbrella-sampling.py* are the main programs for generating the potential of mean force and the weight for each configuration (saved in *./weights/weights\*.dat*) under the unbiased Hamiltonian. Meanwhile, the overlap matrix as mentioned above is plotted (*overlap-matrix.pdf*) All the overlap matrix elements needed (*PM3-1D-om.pdf*) are listed into *OverlapMatrixReal.dat* (which is processed based on *OverlapMatrix.dat* by script *overlap-matrix.py*). Here, *bindexs/bindexs\*.dat* contain the bin index for all the conformations. The *./bindexs/bindexs\*.dat* and *./weights/weights\*.dat* files will be used in wTP calculations. Here, *pmf.dat* contains the FE profile result at PM3 level.

*In directory “Claisen-Rearrangement/wTP/reweight-from-PM3-to-PM6”,* the wTP is performed from PM3 to PM6, where the reweighting result at PM6 (*AH-pmf.dat*) level can be compared with the exact PM6 result (*PM6-pmf.dat*) which can verify the reliability of our method. Here, the python script called *reweighting.py* is used to perform wTP calculation which needs the single point energies for all conformations at PM3 and PM6 level (saved in *./single-point-energy/PM3/energy\_\*.dat and ./single-point-energy/PM6/energy\_\*.dat*). Here, in *./Check/*, the reweighting entropy as mentioned as above is contained in *reweighting-entropy.dat* by using the python script called *reweighting-entropy.py*. Then, in *./python-fitting/GPR-RE,* Gaussian Process Regression is performed to smooth the fluctuated FE profiles at PM3 or PM6 level. Before true GPR is done, an optimal parameter needed to be determined in *./python-fitting/GPR-RE/scale.*

*In directory “Claisen-Rearrangement/wTP/reweight-from-PM3-to-B3LYP”,* the wTP is performed from PM3 to B3LYP, where the reweighting result at PM6 (*AH-pmf.dat*) level can be compared with the exact B3LYP result (*B3LYP-pmf.dat*) which can verify the reliability of our method.

*In directory “Claisen-Rearrangement/Plot”,* all results are plotted.