Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Informatin for "Solvation free energies via alchemical simulations: let's get honest about sampling, once more"

Piero Procacci*

Department of Chemistry, University of Florence, Via Lastruccia n. 3, Sesto Fiorentino, I-50019 Italy

E-mail: procacci@unifi.it

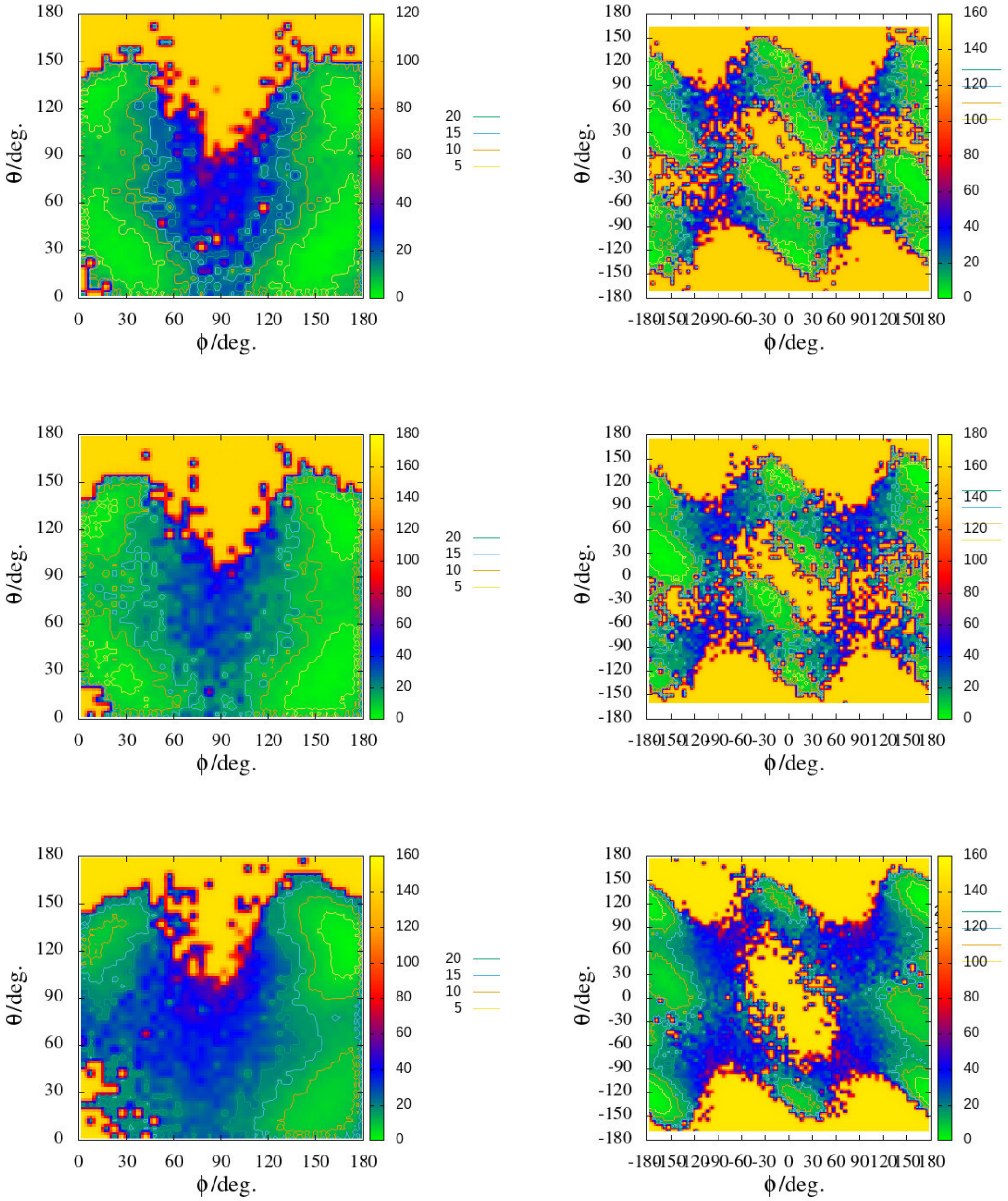

Figure S1: Free energy surfaces at T=300 K with respect to the two dihedral angles (see main paper) of SM02 in water (upper panels), 1-octanol (central panel) and in the gas-phase (lower panel), obtained using the MBAR analysis (see main tpaper) on the HREM data. Panels on the left and right refer to the FES for $0 \leq |\theta| \leq 180^\circ,\ 0 \leq |\phi| \leq 180^\circ$ and in the full dihedral ranges $-180^\circ \leq |\theta| \leq 180^\circ,\ -180^\circ \leq |\phi| \leq 180^\circ$, respectively. The color-coded z-energy scale is in kJ mol^{-1}

Error analysis on relative free energies between conformational states using data from standard and HREM simulations

In Table S1, I report the relative free energy differences between the four rotameric states (GG, GA, AG, AA) of SM02 in water, octanol and gas-phase computed from the HREM and standard simulations. I also report the errors obtained with bootstrap and using four block averages of 2 ns each. It is a common belief in FEP studies that bootstrapping the data provides a reliable estimate of the confidence interval. This only applies, however, if the sample data are uncorrelated, as it happens in the HREM simulations. If this is the case, given that N is the total number of sampled data points for free energy determination, we expect that the error obtained using four block averages with $N_b = N/4$ data points to be roughly the double of the bootstrap error. When in Table S1 the bootstrap error is compared to the error using block averages, while HREM behaves as expected, for the standard simulations the differences of the two errors are indeed striking. By limiting the "convergence analysis" to bootstrapping without duly checking for data correlation, one may get the wrong sentiment that the simulation is "well converged" and that errors are acceptably small. For time records such as those shown in the lower panel of Figure 3 of the main paper, bare bootstrap with resampling yields comparable dihedral distributions and hence comparable relative free energies with a seemingly small error. By double-checking using block averages, it may well be found that in one of blocks anti or gauche state are never visited hence getting an infinite free energy for that rotameric state. When this happens (as for the standard simulation in 1-octanol), in Table S1 the free energy of this state is arbitrarily set to $-RT\log(1/N_b)$ with N_b being the number of data points in the block. Block averages for unconverged or excessively short standard simulations may be strongly affected by these pitfalls, producing huge errors and hence unreliable relative free energies.

Table S1: Relative free energy differences, ΔG, between the four rotameric states of SM02 (see main text) in gas-phase, water and 1-octanol. The most probable state is taken to be the arbitrary zero of the free energy. Err(BS) is 95% confidence interval evaluated using bootstrap with resampling. Err (BL) is the root mean square error (RMSD) computed using 4 block averages. All reported values are in kcal mol^{-1}.

Gas-phase

H-REM				Standard			
type	ΔG	Err(BS)	Err(BL)	type	ΔG	Err(BS)	Err(BL)
GG	2.66	0.202	0.307	GG	1.50	0.089	2.325
GA	1.56	0.104	0.218	GA	0.33	0.053	3.393
AG	0.62	0.054	0.092	AG	0.93	0.077	1.079
AA	0.00	0.000	0.000	AA	0.00	0.000	0.498

Water

H-REM				Standard			
type	ΔG	Err(BS)	Err(BL)	type	ΔG	Err(BS)	Err(BL)
GG	0.29	0.084	0.103	GG	1.95	0.195	2.217
GA	0.90	0.137	0.265	GA	2.48	0.273	1.988
AG	0.00	0.000	0.000	AG	0.00	0.000	0.000
AA	0.71	0.123	0.068	AA	0.52	0.083	0.324

1-octanol

H-REM				Standard			
type	ΔG	Err(BS)	Err(BL)	type	ΔG	Err(BS)	Err(BL)
GG	0.46	0.070	0.245	GG	7.13	n/a	n/a
GA	0.89	0.109	0.246	GA	7.13	n/a	n/a
AG	0.00	0.000	0.000	AG	0.00	0.000	0.343
AA	0.45	0.080	0.189	AA	0.20	0.072	1.485

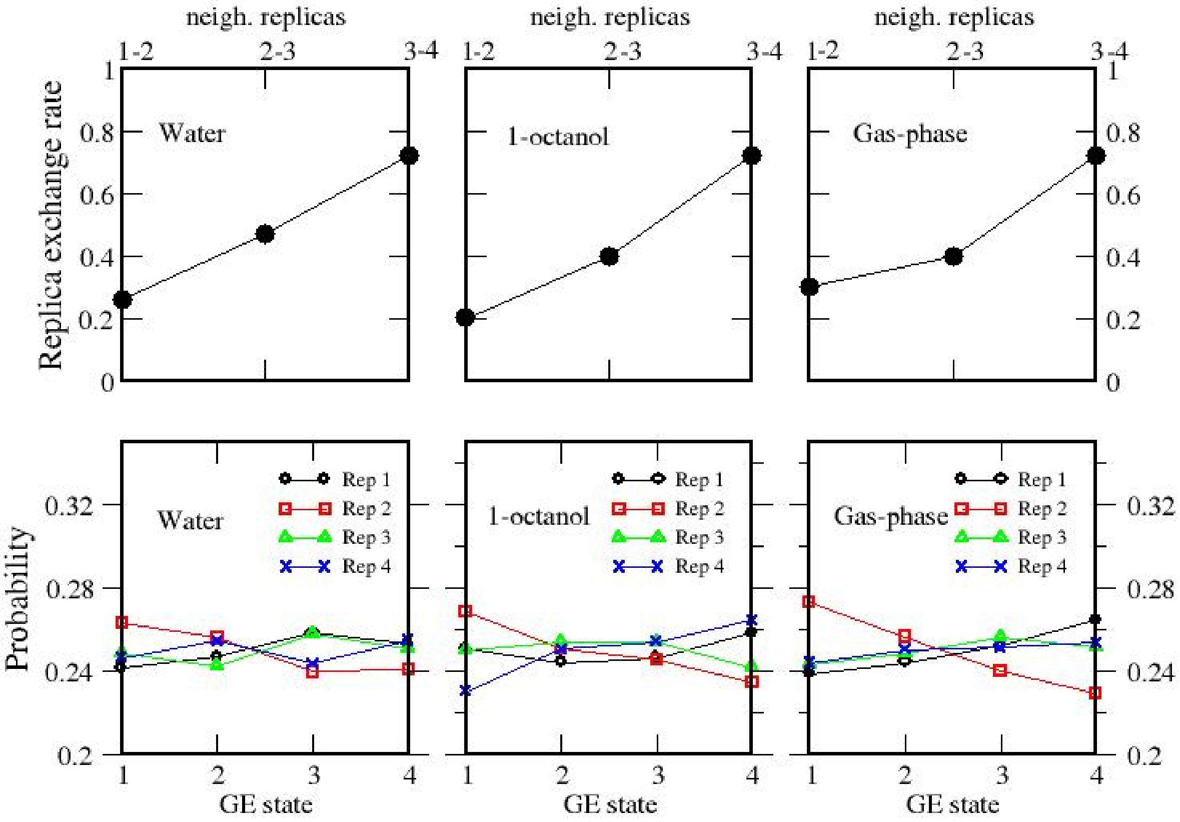

Figure S2: Upper panels: replica exchange rates bewteen neighboring replicas in water, 1-octanol and gas-phase for the HREM 8 ns simulation on 4 GE states. Lower panels: GE state probability distributions for all replica walkers in water, 1-octanol and gas-phase, recorded in a 8 ns HREM simulation on 4 GE states. In all cases the “torsional temperature” of the four GE states, (defined as $T_t = 300/c$ with c being the torsional scaling factor, see main text) were set to 300 K (target state), 646.5 K (GE state 2), 1395.3 K (GE state 3), 3000 K (GE state 4).

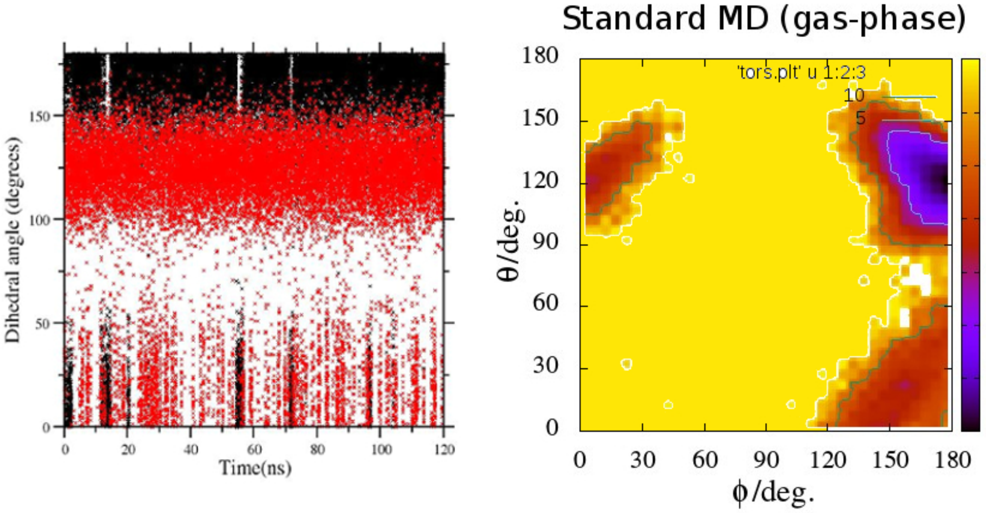

Figure S3: Time record (left) and corresponding free energy surface (right) of the dihedral angles of the decoupled ($\lambda = 0$) SM02 (see main text) at 300 K computed using a standard 100 ns MD simulation.

Potential of mean force as a function of λ

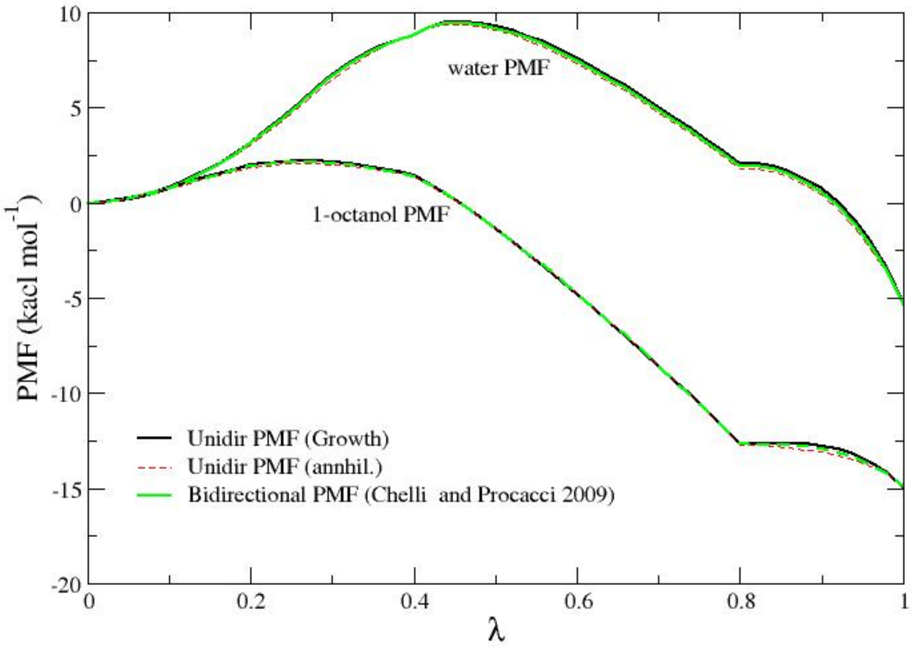

Figure S4: Unidirectional (black and red trait) and bidirectional (in green) PMF's for the growth/annihilation of SM02 in water and octanol. The bidirectional PMF's have been computed using the estimate of Ref.[1] For more detail see discusson near Fig 6 of the main text.

Nutshell tutorials for FEP and NEW

FEP

Freely accessible and excellent FEP tutorials for solvation free energy calculations are available on the internet.[2] According to the gromacs Sander Pronk tutorial

`http://www.gromacs.org/Documentation/Tutorials#Free_Energy_Calculations`

a FEP calculation of the solvation free energy is made of three distinct and sequential computational tasks, namely

- **Global equilibration** The global equilibration stage is a standard equilibrium simulation of one of the end-states of the system based on an user-prepared gromacs input file (e.g. `equil.mpd`). Typically, the selected state corresponds the fully coupled solute, but any other λ state can be (in principle) selected.

- **Creating the lambda points**

  In the second step, a simple application script processes a user-prepared template FEP input, producing as many directory as the λ states specified in the template. In each of these directories, the script generates the input files for running an equilibrium simulation at a fixed λ value, collecting the λ energies in gromacs-defined files (`dhdl.xvg`). The end-user must `cd` in this directories and run each simulation independently, i.e.

  ```
  $ cd lambda_00
  $ gmx grompp
  $ cd ../lambda_01
  $ gmx grompp
  ....
  ```

- **Post-processing the data, delivering the Free Energy**

  In the last step, a gromacs ancillary program, `bar`, must be launched prom the parent main directory:

```
$ gmx bar -b 100 -f lambda_*/dhdl.xvg}
```

The output yields the free energy change in each λ window and the total solvation free energy. The error is estimates using block averages (the default is 5).

The λ stratification (number of points and λ values in the range [0,1] must be provided by the end-user in the FEP input template. It should be best practice to repeat all the above three computational steps by starting from the other end state of the system (i.e the fully decoupled solute), in order to check whether the adopted stratification produces the same results. The preparation of the input files can be facilitated by the usage of libraries of application (typically bash or python) scripts.

NEW

For ORAC users, a detailed documentation for the preparation of the relevant input files in NEW can be found at

`www.chim.unifit.it/orac.`

Here we only briefly outsketch, as done for FEP, the main computational steps. As in FEP, also in NEW the computation of the solvation free energy must be done in three distinct and sequential computational steps, that must be repeated starting from both end states. As in FEP, also in NEW the end user must prepare two input files. As for FEP, the set-up of these input file can be automated using python or bash script libraries. No processing of templates input is however needed in NEW. For each starting end-state, the NEW steps are as follows

- **HREM stage for the end state**

  From a working directory, an indpendent HREM equilibrium simulations with solute tempering is launched by reading a user-prepared input file. Such simulation produces a series of starting phase points for the end-state, collected in a user specified directory in the input file. The typical ORAC command is

```
$ mpirun -n 4 orac < hrem.inp > hrem.out
```

In the above example, we have used 4 replicas, spaced according to the default scaling factor $c_m = c^{(m-1)/3}$, $m = 1..4$. The job is a hybrid one. The number openMP threads depends on the hardware and are specified in the end-user input file `hrem.inp` using the `#& NTHREADS` directive.

- **Fast swicthing annihilation/growth stage**

  From the same working directory, the end-user launches a fast-switching simulation reading a second user-prepared input file. The code reads the phase-space points in the user specified directory producing as many NE trajectories as the number of specifid MPI instances:

  ```
  $ mpirun -n 400 orac < fs.inp > fs.out
  ```

  In the example above, each MPI istance reads a different phase space point produced in the preceding HREM step, printing out in a program generated `PAR$IPROC` subdirectory at regulat time interval the corresponding alchemical work file (e.g. `workfile`).

- **Post-processing the data, delivering the Free Energy**

  From the same working directory, the free energy is recovered by launching an application bash script that reads the final work values from the `PAR$IPROC` directories that istantly yields the Jarzynsky and Gaussian estimate according to Eqs. 3,4 or 5,6 in the main paper), e.g.

  ```
  $ for i in PAR*/workfile; do tail -1 $i | awk '{print $6} ; done >
               works_forward
  $ Free_bs.bash works_forward
  ```

The first command builds a work file `works_forward` using the end-state work values from each of the `PAR$IPROC` subdirectories.

In the second command, the ORAC application script `Free.bash` prints to the standard output the free energy estimates providing a 95% confidence interval using bootstrap with resampling.

The free solvation energy provided by the application script `Free_bs.bash` are *unidirectional* estimates. As in FEP, it is best practice to repeat the whole calculation starting from the other end-state using **an inverted time schedule** (provided in the `fs.inp` user-prepared input file). The Bennett Acceptance ratio estimate can be computed once the two files, `works_forward` and `works_reverse`, are generated using the ORAC application program `bennett`:

```
$ bennett
```

References

(1) Chelli, R.; Procacci, P. A potential of mean force estimator based on nonequilibrium work exponential averages. *Phys. Chem. Chem. Phys.* **2009**, *11*, 1152.

(2) See for example the GROMACS manual and the tutorial for alchemical calculations: *Hands-on tutorial Solvation free energy of ethanol* available at http://www.gromacs.org (accessed date 04/04.2014). For NAMD, See the tutorial: *In silico alchemy: A tutorial for alchemical free-energy perturbation calculations with NAMD* available at http://www.ks.uiuc.edu (accessed date 04/04.2014).