

## Supplementary information

### Why is the negative of the history dependent bias potential an estimate of the underlying free energy?

For the proof that the bias potential  $V_{\text{bias}}(\mathbf{s}, t)$  at the end of the metadynamics simulation an estimate of the free energy, we will closely follow the argument of the paper by Laio *et al.* in *Assessing the accuracy of metadynamics* (J. Phys. Chem. 109, 6714 (2005)).

The probability distribution of a canonical system at temperature  $\beta^{-1}$  as a function of a small number of collective variables  $\mathbf{s}(\mathbf{R})$  can be written as

$$P(\mathbf{s}) = \frac{\exp(-\beta F(\mathbf{s}))}{\int d\mathbf{s} \exp(-\beta F(\mathbf{s}))} \quad (1)$$

where  $\mathbf{R}$  are the atomic positions, and  $F(\mathbf{s})$  the free energy:

$$F(\mathbf{s}) = -\beta^{-1} \ln \left[ \int d\mathbf{R} \exp(-\beta V(\mathbf{R})) \delta(\mathbf{s}(\mathbf{R}) - \mathbf{s}) \right] \quad (2)$$

In principle the probability function and the free energy can be calculated from a very long, so called “brute force” simulation by taking a histogram of  $\mathbf{s}$ . However, if the free energy has a local minimum that acts as a stable basin of attraction, then the escape of the motion of  $\mathbf{s}$  can be a rare event on the time scale determined by the potential  $V(\mathbf{R})$  alone.

The metadynamics method uses a biasing potential  $V_{\text{bias}}(\mathbf{s}, t)$  to which a new Gaussian with height  $H$  and width  $w$  is added every time interval  $\delta t$ :

$$V_{\text{bias}}(\mathbf{s}, t) = \sum_{t_i} H \exp\left(-\frac{|\mathbf{s} - \mathbf{s}(t_i)|^2}{2w^2}\right) \quad (3)$$

to counteract the basin of attraction and reconstruct  $F(\mathbf{s})$ .

Starting from a zero biasing potential  $-V_{\text{bias}}(\mathbf{s}, t) = 0$ , the probability to deposit the first hill, after sufficient equilibration time, is largest in (the neighborhood of) the local minimum of the free energy

function, after which the new probability distribution becomes:

$$P(s, t) = P(s, 0) - \exp[-\beta V_{\text{bias}}(\mathbf{s}, t)] \quad (4)$$

For a sufficiently small Gaussian (*i.e.*  $H$  is smaller than the depth of the local minimum, and  $w$  is smaller than the width of the local minimum), the probability to find  $\mathbf{s}$  in the neighborhood of the local minimum has reduced and the local minimum in the function  $F(\mathbf{s}) - V_{\text{bias}}(\mathbf{s}, t)$  has also reduced. If the time interval  $\delta t$  of adding Gaussians to the biasing potential is larger than the equilibration time of the system (the time for the system to fall and thermalize in the new local minimum), repeating this procedure will lead to the minimization of the function  $F(\mathbf{s}) - V_{\text{bias}}(\mathbf{s}, t)$ , so that  $V_{\text{bias}}(\mathbf{s}, t)$  is an approximation of the free energy  $F(s)$ .

If instead the function  $F(\mathbf{s}) - V_{\text{bias}}(\mathbf{s}, t)$  is locally flat (and the probability distribution  $P(\mathbf{s}, t)$  therefore also), the next Gaussian added to  $V_{\text{bias}}(\mathbf{s}, t)$  will lead to an extra bump on the function  $F(\mathbf{s}) - V_{\text{bias}}(\mathbf{s}, t)$ , so that the only corrugations in the free energy estimation left are of the size of the Gaussians. By reducing the size of the Gaussians after every barrier recrossing to a previously flattened (initial) basin of attraction, the free energy function is reconstructed to arbitrary accuracy.

## Metadynamics working recipe

Here we briefly summarize the working recipe for obtaining the reaction mechanism and free energy profile for intrinsically multi-dimensional reactions, using metadynamics combined with Car-Parrinello molecular dynamics, as previously reported (Ensing, B.; Laio, A.; Parrinello, M.; Klein, M. L. A recipe for the computation of the free energy barrier and the lowest free energy path of concerted reactions. *J. Phys. Chem. B* **2005**, *109*, 6676.).

Firstly, identify the relevant collective variables (bond distances, angles, torsion angles, coordination numbers) that describe the reaction and perform short metadynamics simulations without adding hills,

to obtain values for the mass and spring constants that guarantee a healthy (largely adiabatically decoupled) meta-dynamics. The amplitudes of the collective variables in the reactant well indicate the width of the well, allowing for a hill width to be chosen. Secondly, perform the hills method simulation, using a hill height of  $H = 0.2 - 2k_B T$  (depending on the desired accuracy versus sampling speed) and a displacement dependent stride  $\Delta t$  based on  $\frac{3}{2}$  hill width or a fixed stride based on  $\Delta t = (1.5w)^2/D$  (*i.e.* the average time to diffuse a distance of  $\frac{3}{2}$  hill width). Decrease the hills size after every recrossing for free energy convergence. Thirdly, locate the lowest free energy path to depict the reaction mechanism. Finally, perform one-dimensional umbrella sampling along the lowest free energy path to obtain the reaction free energy profile and reaction barrier to arbitrary accuracy.

## Computation details of the alanine peptide simulations

In the simulation of aqueous solution, an alanine dipeptide molecule is placed into a periodic cubic box ( $L = 18.8 \text{ \AA}$ ) with 216 water molecules. The electrostatic interactions are calculated by Ewald summation and the real space cut off is half of the cell dimension ( $9.4 \text{ \AA}$ ). Prior to the metadynamics runs, an NPT simulation at 1 atm and 298 K for at least 50 ps was carried out to equilibrate the cell volume. The metadynamics simulations are performed within the NVT ensembles at  $T = 298 \text{ K}$ , using a time step of 0.5 fs, hill dimensions of  $w = 0.2 \text{ rad}$  and  $H = 0.02 \text{ kcal/mol}$ . The total simulation times for the metadynamics runs were  $\approx 5 \text{ ns}$  for gas phase and  $\approx 3.5 \text{ ns}$  for aqueous solution phase, respectively.

## Error estimation of the alanine peptide metadynamics simulations

To make a valid comparison, we have estimated the resolutions of our results as following. In the metadynamics run, once the entire  $\phi$ - $\psi$  conformational space is filled with hills at time  $t$  ( $\approx 4 \text{ ns}$ ), the molecule moves freely afterward. Take  $t'$  to be the simulation time to add another layer of hills over the

entire  $\phi$ - $\psi$  conformational space (which should not affect the relative energy and positions of minima). Then, variations between the FES at  $t$  and the one at  $t' > t$  are brought by errors. By calculating the standard deviation of the free energy difference map between different simulation times, such as 4 ns vs 5 ns or 4.5 ns vs 5 ns, we have estimated the errors in relative energy to be 0.3-0.5 kcal/mol. In addition, comparing the minima positions at 4 ns, 4.5 ns and 5 ns gives an estimation of errors on the dihedral angles. The resolution for more clearly defined minima such as  $C7_{eq}$  is small, at about 3 degrees. The error in more shallow minima such as  $\alpha_R$  is larger, at about 10 degrees. Although the simulation in aqueous solution is not long enough to make a good estimation on errors, preliminary comparison of our findings with those of the gas phase indicates that the errors are very similar. Considering the variations observed from different studies, the precision of the metadynamics method is rather good.

### Is the gas phase $\alpha_R$ minimum a true minimum?

For the gas phase  $\alpha_R$  minimum, three of the four boundaries of the well (across  $\phi$  and on the smaller side of  $\psi$ ) are clearly defined. The depth of the well relative to the transition state linking to the  $C7_{eq}$  minimum is only  $\approx 0.3$  kcal/mol and thus at the same magnitude as our estimated error range. Note however that, even though our  $\alpha_R$  minimum may not be a true minimum, there is a clearly defined flat region at around  $-67^\circ < \phi < -98^\circ$  and  $-76^\circ < \psi < 0^\circ$  that encloses the  $\alpha_R$  conformation. This observation is in agreement with the QM optimization at the MP2/6-311++G\*\* level of theory which could not locate the exact position of  $\alpha_R$ , because the QM potential energy surface shows an extended flat valley from the  $C7_{eq}$  region up towards the helical region at around  $(-60^\circ, -45^\circ)$ . Further calculations are in progress to assess the results here presented.

## List of publications on alanine dipeptide

Below follows a more complete list of publications on the alanine dipeptide case than we could provide in the paper due to size limitations:

1. Mezei, M. Adaptive umbrella sampling - self-consistent determination of the non-Boltzmann bias. *J. Comp. Phys.* **1987**, *68*, 237-248.
2. Roux, B. The calculation of the potential of mean force using computer simulations. *Comput. Phys. Commun.* **1995**, *91*, 275-282.
3. Olender, R.; Elber, R. Calculation of classical trajectories with a very large time step: formalism and numerical examples. *J. Chem. Phys.* **1996**, *105*, 9299-9315.
4. Anderson, A. G.; Hermans, J. Microfolding: Conformational probability map for the alanine dipeptide in water from molecular dynamics simulations. *Proteins: Struct. Funct. Genet.* **1988**, *3*, 262-265.
5. Tobias, D. J.; Brooks III, C. L. Conformational equilibrium in the alanine dipeptide in the gas phase and aqueous solution: a comparison of theoretical results. *J. Phys. Chem.* **1992**, *96*, 3864-3870.
6. Chipot, C.; Pohorille, A. Conformational equilibria of terminally blocked single amino acids at the water-hexane interface. a molecular dynamics study. *J. Phys. Chem. B* **1998**, *102*, 281-290.
7. Pettit, B. M.; Karplus, M. Conformational free energy of hydration for the alanine dipeptide: thermodynamic analysis. *J. Phys. Chem.* **1988**, *92*, 3994-3997.
8. Smith, P. E. The alanine dipeptide free energy surface in solution. *J. Chem. Phys.* **1999**, *111*, 5568-5579.
9. Apostolakis, J.; Ferrara, P.; Cafish, A. Calculation of conformational transitions and barriers in solvated systems: Application to the alanine dipeptide in water. *J. Chem. Phys.* **1999**, *110*, 2099-2108.
10. Schmidt, A. B.; Fine, R. M. A CFF91-based continuum solvation model: solvation free energies of small molecules and conformations of the alanine dipeptide in solution. *Mol. Simul.* **1994**, *13*, 347-365.

11. Smart, J. L.; Marrone, T. J.; McCammon, J. A. Conformational sampling with Poisson-Boltzmann forces and a stochastic dynamics/monte carlo method: Application to alanine dipeptide. *J. Comp. Chem.* **1997**, *18*, 1750-1759.
12. Pellegrini, M.; Doniach, S. Modeling solvation contributions to conformational free energy changes of biomolecules using a potential of mean force expansion. *J. Chem. Phys.* **1995**, *103*, 2696-2702.
13. Bolhuis, P. G.; Dellago, C.; Chandler, D. Reaction coordinates of biomolecular isomerization. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 5877-5882.
14. Rosso, L.; Abrams, J. B.; Tuckerman, M. E. Mapping the backbone dihedral free-energy surfaces in small peptides in solution using adiabatic free-energy dynamics. *J. Phys. Chem. B* **2005**, *109*, 4162-4167.
15. Jr., A. D. M.; Feig, M.; Brooks III, C. L. Extending the treatment of backbone energetics in protein force fields: Limitations of gas-phase quantum mechanics in reproducing protein conformational distributions in molecular dynamics simulations. *J. Comp. Chem.* **2004**, *25*, 1400-1415.

## List of publications using metadynamics

List of publications from studies on or using the metadynamics method, at the time this review was submitted:

1. Laio, A.; Parrinello, M. Escaping free-energy minima. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 12562-12566.
2. Ceccarelli, M.; Danelon, C.; Laio, A.; Parrinello, M. Microscopic mechanism of antibiotics translocation through a porin. *Biophys. J.* **2004**, *87*, 58-64.
3. Wu, Y.; Schmitt, J. D.; Car, R. Mapping potential energy surfaces. *J. Chem. Phys.* **2004**, *121*, 1193-1200.

4. Yu, J.; Ha, T.; Schulten, K. Conformational model of the holliday junction transition deduced from molecular dynamics simulations. *Nucl. Acids Research* **2004**, *32*, 6683-6695.
5. Gervasio, F. L.; Laio, A.; Parrinello, M. Flexible docking in solution using metadynamics. *J. Am. Chem. Soc.* **2005**, *127*, 2600-2607.
6. Iannuzzi, M.; Laio, A.; Parrinello, M. Efficient exploration of reactive potential energy surfaces using car-parrinello molecular dynamics. *Phys. Rev. Lett.* **2003**, *90*, 238302.
7. Stirling, A.; Iannuzzi, M.; Laio, A.; Parrinello, M. Azuleze-to-napthalene rearrangement: the Car-Parrinello metadynamics method explores various reaction mechanisms. *ChemPhysChem* **2004**, *5*, 1558-1568.
8. Churakov, S. V.; Iannuzzi, M.; Parrinello, M. Ab initio study of dehydroxylation-carbonation reaction on brucite surface. *J. Phys. Chem. B* **2004**, *108*, 11567-11574.
9. Ensing, B.; Laio, A.; Gervasio, F. L.; Parrinello, M.; Klein, M. L. Minimum free energy reaction path for the E2 reaction between fluoro ethane and a fluoride ion. *J. Am. Chem. Soc.* **2004**, *126*, 9492-9493.
10. Ensing, B.; Klein, M. L. Perspective of the reactions between  $F^-$  and  $CH_3CH_2F$ : the free energy landscape of the E2 and  $S_N2$  reaction channels. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 6755-6759.
11. Gervasio, F. L.; Laio, A.; Iannuzzi, M.; Parrinello, M. Influence of DNA structure on the reactivity of the guanine radical cation. *Chem. Eur. J.* **2004**, *10*, 4846-4852.
12. Boero, M.; Ikeshoji, T.; Liew, C. C.; Terakura, K.; Parrinello, M. Hydrogen bond driven chemical reactions: Beckmann rearrangement of cyclohexanone oxime into  $\epsilon$ -caprolactam in supercritical water. *J. Am. Chem. Soc.* **2004**, *126*, 6280-6286.
13. Zipoli, F.; Bernasconi, M.; Martoňák, R. Constant pressure reactive molecular dynamics simulations of phase transitions under pressure: The graphite to diamond conversion revisited. *Eur. Phys. J. B* **2004**, *39*, 41-47.

14. Iannuzzi, M.; Parrinello, M. Proton transfer in heterocycle crystals. *Phys. Rev. Lett.* **2004**, *93*, 025901.
15. Donadio, D.; Bernasconi, M. Ab initio simulation of photoinduced transformation of small rings in amorphous silica. *Phys. Rev. B* **2005**, *71*, 073307.
16. Martoňák, R.; Laio, A.; Parrinello, M. Predicting crystal structures: the Parrinello-Rahman method revisited. *Phys. Rev. Lett.* **2003**, *90*, 075503.
17. Ceriani, C.; Laio, A.; Fois, E.; Gamba, A.; Martoňák, R.; Parrinello, M. Molecular dynamics simulation of reconstructive phase transitions on an anhydrous zeolite. *Phys. Rev. B* **2004**, *70*, 113403.
18. Donadio, D.; Raiteri, P.; Parrinello, M. Topological defects and bulk melting of hexagonal ice. *J. Phys. Chem. B* **2005**, *109*, 5421-5424.
19. Micheletti, C.; Laio, A.; Parrinello, M. Reconstructing the density of states by history-dependent metadynamics. *Phys. Rev. Lett.* **2004**, *92*, 170601.
20. Ensing, B.; Laio, A.; Parrinello, M.; Klein, M. L. A recipe for the computation of the free energy barrier and the lowest free energy path of concerted reactions. *J. Phys. Chem. B* **2005**, *109*, 6676-6687.
21. Laio, A.; Rodriguez-Forte, A.; Gervasio, F. L.; Ceccarelli, M.; Parrinello, M. Assessing the accuracy of metadynamics. *J. Phys. Chem. B* **2005**, *109*, 6714-6721.
22. Asciutto, E.; Sagui, C. Exploring intramolecular reactions in complex systems with metadynamics: The case of the malonate anions. *J. Phys. Chem. A* **2005**, *109*, 7682-7687.
23. Ikeda, T.; Hirata, M.; Kimura, T. Hydration structure of Y<sup>3+</sup> and La<sup>3+</sup> compared: An application of metadynamics. *J. Chem. Phys.* **2005**, *122*, 244507.
24. Martonak, R.; Laio, A.; Bernasconi, M.; et al. Simulation of structural phase transitions by metadynamics. *Zeitschrift für Kristallographie* **2005**, *220*, 489-498