J Mol Model (2008) 14:995–1002
DOI 10.1007/s00894-008-0343-7

ORIGINAL PAPER

# Continuous metadynamics in essential coordinates as a tool for free energy modelling of conformational changes

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Received: 27 February 2008 / Accepted: 19 June 2008 / Published online: 17 July 2008


**Abstract** Modelling of conformational changes in biopolymers is one of the greatest challenges of molecular biophysics. Metadynamics is a recently introduced free energy modelling technique that enhances sampling of configurational (e.g. conformational) space within a molecular dynamics simulation. This enhancement is achieved by the addition of a history-dependent bias potential, which drives the system from previously visited regions. Discontinuous metadynamics in the space of essential dynamics eigenvectors (collective motions) has been proposed and tested in conformational change modelling. Here, we present an implementation of two continuous formulations of metadynamics in the essential subspace. The method was performed in a modified version of the molecular dynamics package GROMACS. These implementations were tested on conformational changes in cyclohexane, alanine dipeptide (terminally blocked alanine, Ace-Ala-Nme) and SH3 domain. The results illustrate that metadynamics in the space of essential coordinates can accurately model free energy surfaces associated with conformational changes.

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**Keywords** Conformational change · Essential dynamics · Free energy surface · Metadynamics · Molecular dynamics

## Introduction

One of the most important features of biopolymers is their ability to adopt different conformations to fulfil their catalytic, signalling, memory or mechanical roles. In addition, protein folding, unfolding, and misfolding can all be viewed as conformational changes. Accurate modelling of conformational changes using molecular modelling methods is one of the greatest challenges in this field. However, conformational families of a molecule are often separated by high free energy barriers. These barriers cause conformational changes to be slow and that the probability of overcoming these barriers in a short (e.g. nanosecond) molecular dynamics simulation is low or even negligible.

Solving this problem by using the brute force of supercomputer power is not always applicable. The idea of enhancement the sampling of configurational space is not new in molecular simulations [1]. Metadynamics [2, 3] is a recently introduced molecular dynamics-based technique, which enhances sampling and quantitatively evaluates a free energy surface. The initial step of metadynamics involves the choice of a few (typically two) collective variables. Collective variables are geometric parameters that are supposed to describe the progress of the studied process. Inter-atomic distances, valence and dihedral angles and coordination numbers have often been used as collective variables in recent applications of metadynamics [4–8]. In metadynamics, the system is simulated by a standard molecular dynamics simulation to which a history-dependent bias potential is added. The bias potential is usually formulated as the sum of Gaussian hills

along the trajectory. Each hill is located in the space of collective variables:

$$V_{bias}(\mathbf{s},t) = \sum_{t_i<t} \prod_j w_{t_i} \exp\left[\frac{-\left(s_j - s_j^{t_i}\right)^2}{2\delta s_j^{t_i 2}}\right] \quad (1)$$

where $s_j^{ti}$ is the $j$th collective variable of $i$th hill, $w$ and $\delta s$ are a height and width of a hill, respectively. These parameters can be chosen *ad hoc* and they can be further tuned to improve accuracy and performance of the metadynamics run [9]. The coupling between the bias and the microscopic potential (the meaning of the vector **s**) will be discussed later. This bias potential stepwise floods free energy minima and thus allows the system to explore the configurational space more efficiently. Moreover, when the flooding is complete, this bias potential approximates the free energy surface of the studied system. Metadynamics has been successfully applied in reaction mechanism modelling [4], protein-ligand [5] and protein-protein [6] docking, conformational change modelling [7] and crystal structure transition [8].

Several different formulations of metadynamics were proposed. The original formulation [2] is based on free energy gradient approximated by short runs of constrained molecular dynamics simulations. Another formulation [3] was proposed to combine metadynamics with Car-Parrinello dynamics. In this formulation a fictive particle (i.e. not located in the Cartesian space) is placed into the space of collective variables. This particle is characterized by its mass $M_j$ and its dynamics is driven by a harmonic restraint to the system, and by the bias potential (the vector **s** in Eq. 1 is the position of the particle **s**) as described by the Lagrangian:

$$L = L_0 + \frac{1}{2}\sum_j M_j\left(\frac{\partial s_j}{\partial \tau}\right)^2 + \frac{1}{2} \times \sum_j \lambda_j\left(S_j(\mathbf{x}) - s_j\right)^2 + V_{bias}(\mathbf{s},t) \quad (2)$$

where $L_0$ is the standard molecular dynamics Lagrangian and **S**(**x**) is the projection of Cartesian coordinates of the system onto the space of collective variables. This formulation (henceforth referred to as Lagrangian metadynamics) can obtain accurate free energy surfaces from very short simulations. Finally, a bias potential can also be added directly to the system (the vector **s** in Eq. 1 is a vector of parameters **S**(**x**)) as described by the Lagrangian:

$$L = L_0 + V_{bias}(\mathbf{S}(\mathbf{x}),t) \quad (3)$$

This formulation [9] is henceforth referred to as direct metadynamics.

In order to apply metadynamics to the field of modelling of conformational changes in biopolymers, we have introduced metadynamics in the space of essential coordinates (MTDEC) [10]. Essential coordinates are obtained by a principal component analysis of molecular trajectories (essential dynamics) [11]. Briefly, coordinates $\boldsymbol{x}$ of selected atoms are superimposed to a reference structure along the trajectory. Then a covariance matrix is calculated:

$$\mathbf{C} = \left\langle (\mathbf{x} - \langle\mathbf{x}\rangle)(\mathbf{x} - \langle\mathbf{x}\rangle)^T \right\rangle \quad (4)$$

where <·> denotes time averaging. The covariance matrix is diagonalized to obtain a set of eigenvectors and eigenvalues:

$$\mathbf{C} = \mathbf{E}\Lambda\mathbf{E}^{-1} \quad (5)$$

where **E** is a matrix of eigenvectors describing individual collective motions, and **Λ** is a diagonal matrix of eigenvalues describing the extent of these motions. Using this technique it is possible to dissect complicated motions of the studied biopolymer into a limited number of major collective motions. Essential coordinates (projections to eigenvectors) provide a dimensionally-reduced view of molecular structure and dynamics.

We have successfully performed MTDEC with the original (constraint-based) formulation [10] of metadynamics. Implementations of the Lagrangian and direct metadynamics in the space of essential coordinates in the GROMACS package are presented in this paper. The program was tested on three different molecular systems. The simplest example was a conformational change in a cyclohexane molecule in vacuum (Fig. 1). The second example is alanine dipeptide (Ace-Ala-Nme, Fig. 2), which serves as a simple model of conformational changes in peptides and proteins. Conformational changes in essential coordinates were previously studied [10] for this system using the original (constraint-based) metadynamics [2]. The third system was the SH3 domain (type 3 Src homology, Fig. 3) which is a regulatory (adaptor) domain present in numerous eukaryotic proteins and is involved in signalling events within a cell. Alanine dipeptide and SH3 domain were modelled in an explicit water.

The presented approach was implemented as an add-on to the popular molecular dynamics package GROMACS [12]. The code can be obtained free of charge on the author's web site.

## Methods

### Implementation

MTDEC was implemented in a similar manner as essential dynamic sampling [13, 14] or flooding [15], and many of

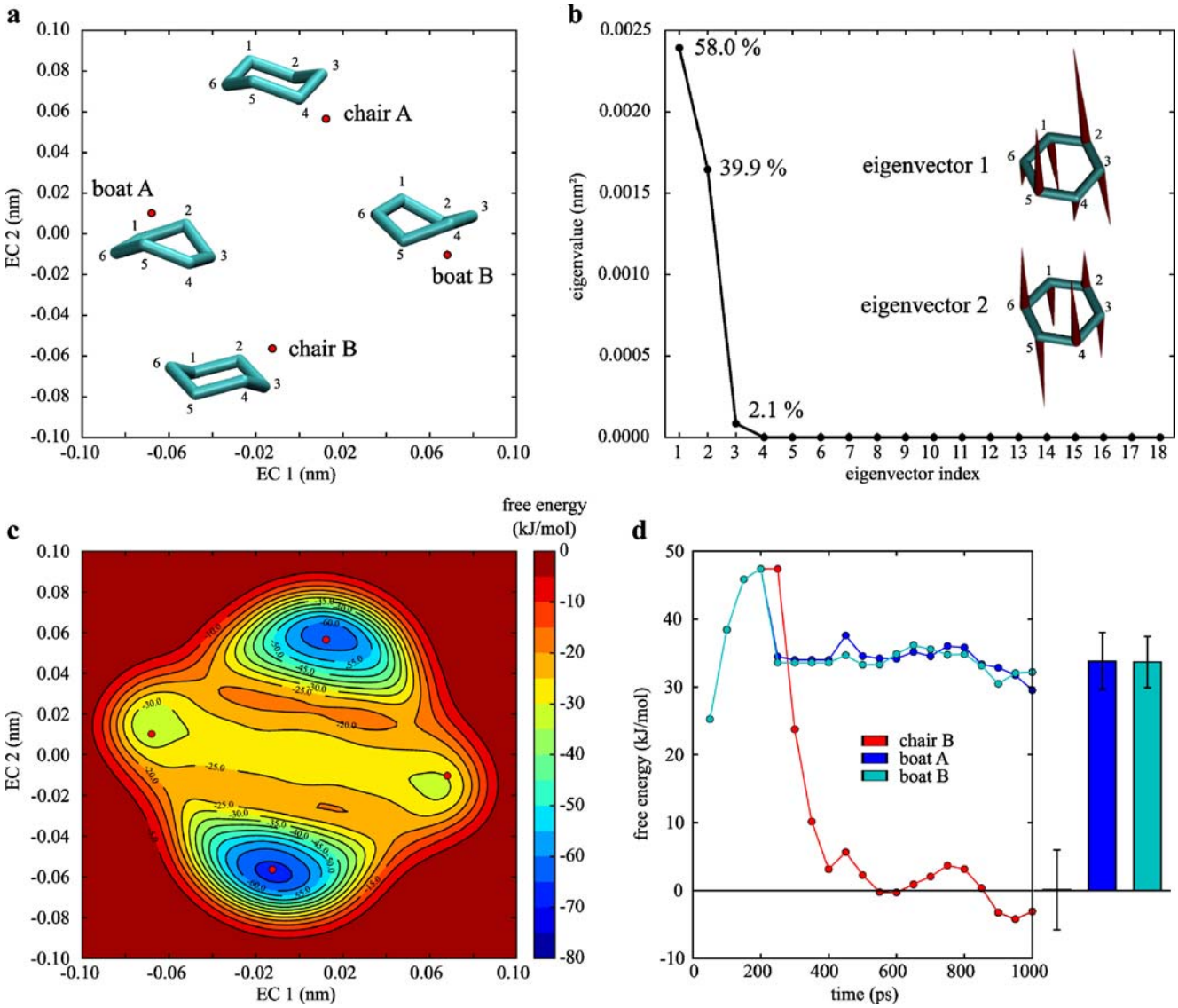


**Fig. 1** Free energy surface of cyclohexane in vacuum. (**a**) the training set composed of four conformations projected to the subspace of essential coordinates, (**b**) the scree plot, together with a graphical illustration of eigenvectors, (**c**) the FES calculated using direct MTDEC, (**d**) development of free energies of free energy minima (chair A was taken as zero)

the functions already implemented in GROMACS were also used in the implementation of MTDEC. Reference and average structures, eigenvectors and other parameters are read from the edi file, similar to the essential dynamic sampling or flooding in GROMACS. This file can be written/edited manually or created using the modified program make_edi by typing, e.g.:

```
make_edi -s before_mtd -f eigenvec -o sam -n index
  -mtdec 1,2 -mtdss 0.05,0.05
```

This creates the file sam.edi for a direct metadynamics in the space of the first two eigenvectors. Widths of a hill in both coordinates are 0.05 nm. MTDEC is run by typing:

```
grompp -f mtd -s before_mtd -o mtdl
mdrun -s mtdl -mtd hills -ei sam -eo sam
```

where hills is the name of the file in which centres of Gaussian hills are stored (with .mtd suffix). The program searches for the file hills.mtd and if present it continues adding new hills (even with different width(s) and height).

Several keywords were added to the edi file. Hills are added and read in/from the file every MTDPERSTEP steps. Height and width(s) of a hill are set by MTDW and MTDDS, respectively. Parameter LAGRANGE switches between

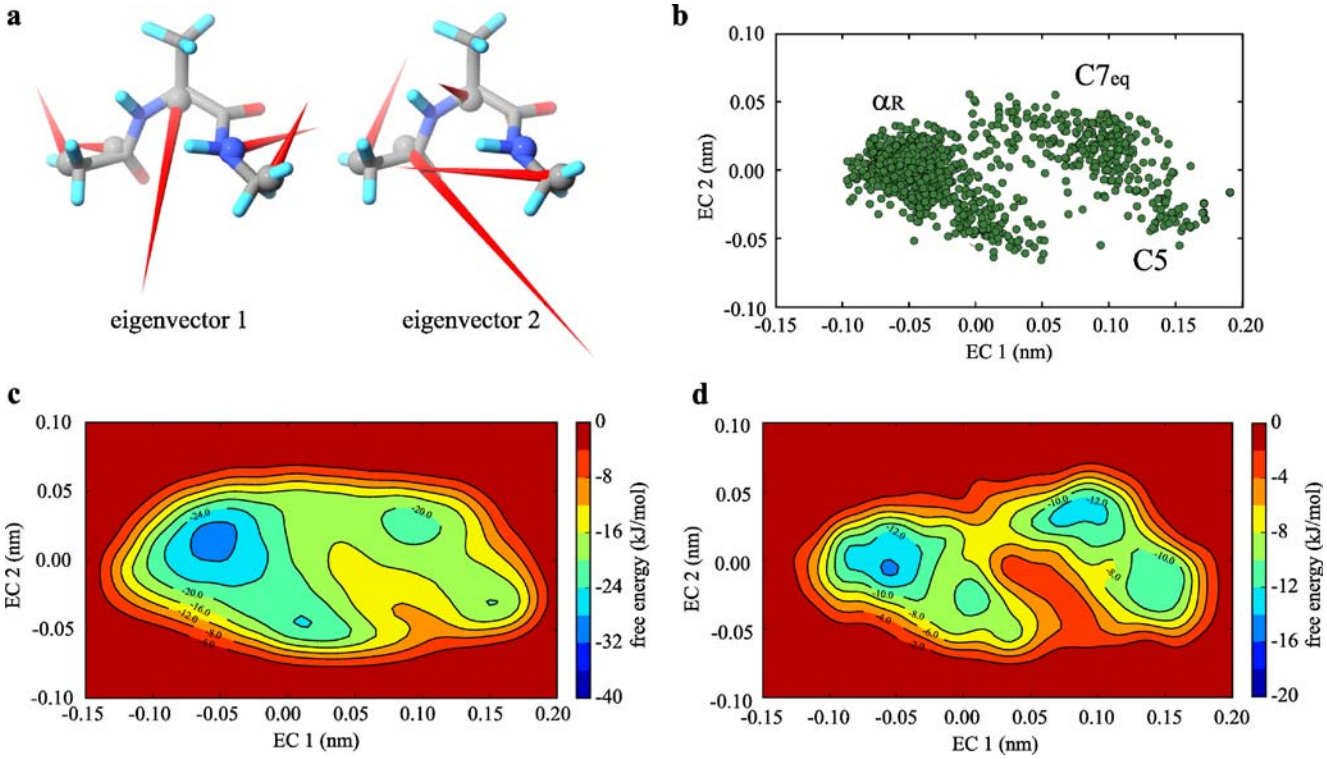


**Fig. 2** Free energy surface of alanine dipeptide in water. (**a**) illustration of eigenvectors, (**b**) projection of the training set (1 ns molecular dynamics trajectory) to the space of essential coordinates. FES calculated using 10 ns direct (**c**) and 200 ps Lagrangian (**d**) MTDEC

Lagrangian (1) and direct (0) metadynamics. If the parameter RESTART (only for Lagrangian metadynamics) is set to 0, initial values of collective variables are set explicitly by the MTDS parameter. Otherwise they are obtained as $s(t=0) = S(x(t=0))$. Multiple walker [16] MTDEC was successfully tested with 10 walkers, by simply running 10 independent mdrun jobs with a common hills file.

## Umbrella sampling/WHAM

Umbrella sampling (US) combined with weighted histogram analysis method (WHAM) [17, 18] were used as reference techniques to provide a comparison with the FES of metadynamics for SH3 domain. The system was simulated in 36 molecular dynamics simulations (each

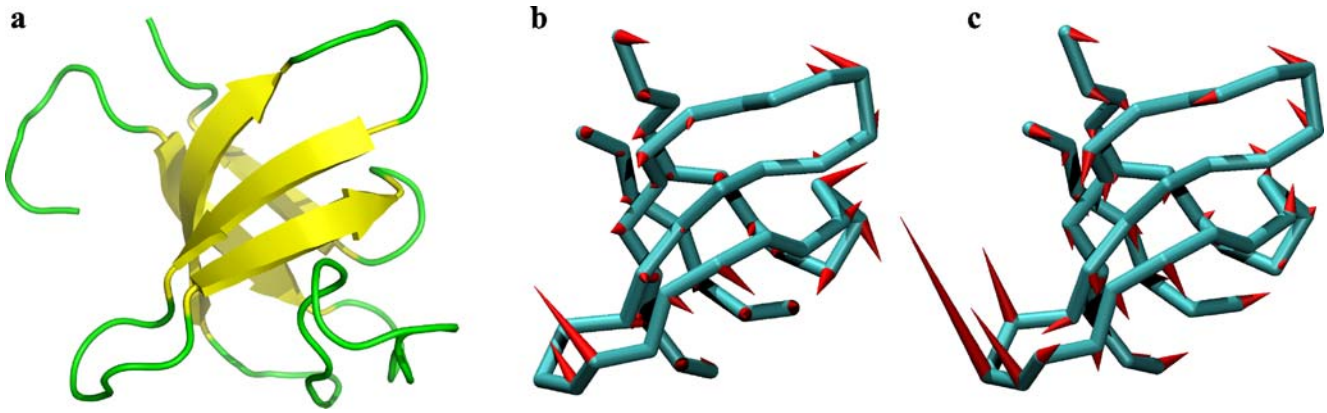


**Fig. 3** The structure of SH3 domain (**a**, PDB-ID: 2O9S) and a graphical representation of the first (**b**) and the second (**c**) eigenvector

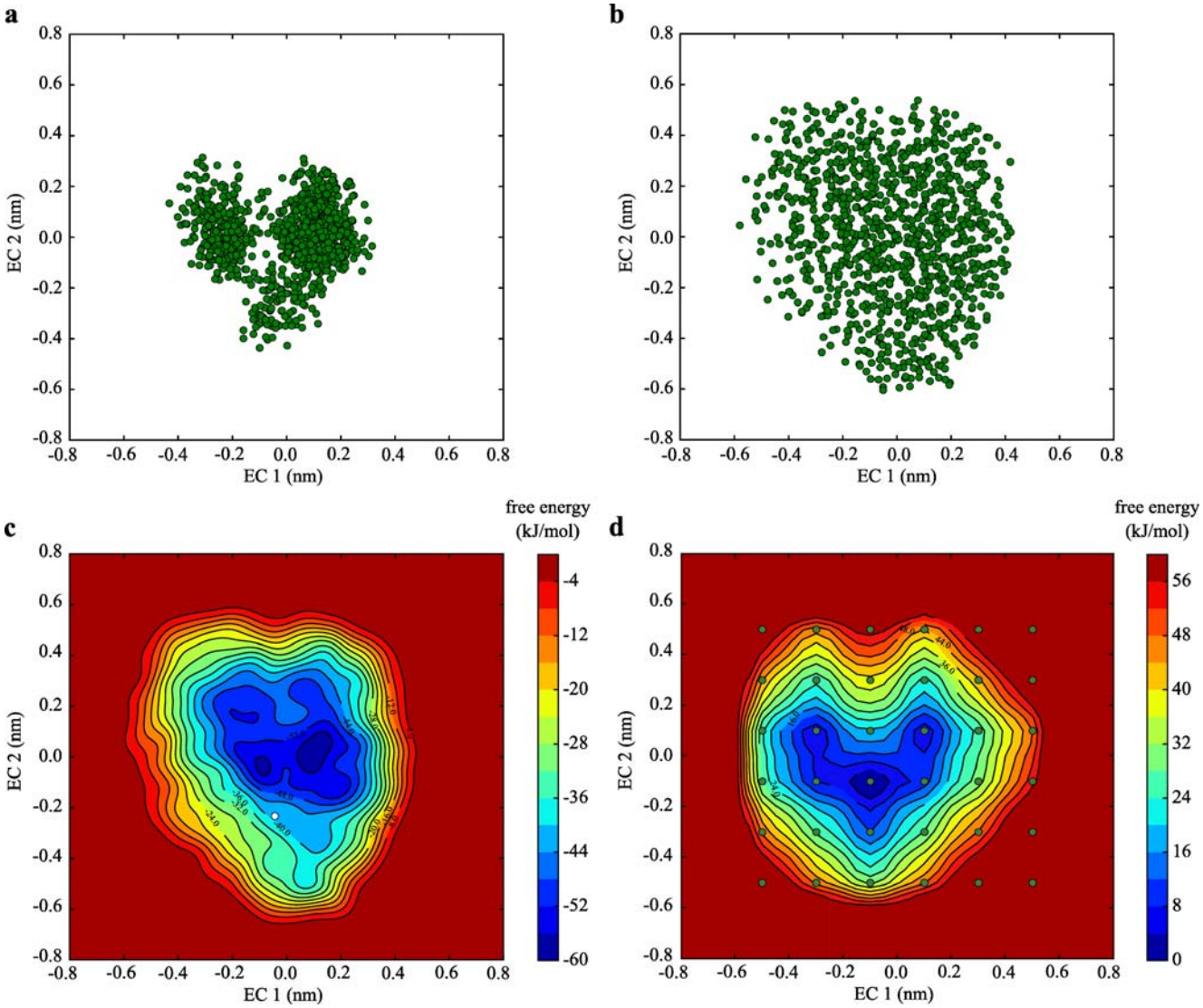


**Fig. 4** MTDEC of SH3 domain. (**a**) the training set (classical 1 ns dynamics) projected to the space of essential coordinates. (**b**) projection of MTDEC of the same duration (1 ns) to the space of essential coordinates. Free energy surface calculated by MTDEC (**c**) and by umbrella sampling (**d**) centres of harmonic restraints are shown as green points)

100 ps). In each simulation the system was restrained to one of points depicted in Fig. 4d by a harmonic restraint (1,000 kJ/mol.nm$^2$). Projections of resulting trajectories were analysed by 2D-WHAM (program wham-2d) [19].

## Results

### Cyclohexane

Cyclohexane was selected as one of simplest models of conformational changes. It is possible to describe conformational changes in ring molecules by Cremer's and Pople's puckering coordinates as already tested within the metadynamics machinery [20]. However, in this study we tested essential coordinates as parameters describing the ring puckering. The molecule of cyclohexane was modelled *in vacuo* with parameters of $CH_2$ groups taken from the GROMOS 53a5 force field (the united atom model) [21].

Four minimized conformations (two chairs and two boats) were used as a model "trajectory" to describe the conformational change in essential coordinates (Fig. 1a). This four-snapshot "trajectory" was analysed using the essential dynamics tool of GROMACS. It must be noted that, strictly speaking, the resulting coordinates should not be referred to as essential because they have not been determined from a canonically sampled trajectory. Nevertheless, we refer to them as essential coordinates to highlight the principle of their determination. These four conformations, represented in the space of two essential

coordinates, are depicted in Fig. 1a. As expected, only two coordinates were necessary to describe the studied change (Fig. 1b). One nanosecond direct metadynamics was performed at 300 K. The size of a step was 1 fs. Gaussian hills with a width of 0.01 nm and a height of 1.0 kJ/mol were added every 1,000 steps. Metadynamics started from the chair A conformation (see Fig. 1a), and after approx. 170 metadynamics steps the system jumped to boat conformations. During the next approx. 100 metadynamics steps the system explored boats A and B by transitions via the twisted boat conformation (in the centre of Fig. 1c). During the rest of the metadynamics run, multiple transitions were observed. This led to the flooding of all free energy minima. Free energy values (Fig. 1d) of both chairs were entirely the same (0±6 kJ/mol) and boat conformations were modelled as less favourable (34±5 and 34±4 kJ/mol for boat A and boat B, respectively). Free energies of boat and twisted boat conformations were almost the same. It must be noted that the free energy surface could be deformed by a symmetry of the system and a detailed study would be required to address this point. Nevertheless, the predicted activation free energy of the conformational change (approx. 40 kJ/mol, starting from the chair) is in good agreement with experimental value (41.7 kJ/mol [22]), and these results illustrate that a physically relevant free energy surface of this text-book conformational change can be obtained using only a 3-minute calculation on a personal computer.

## Alanine dipeptide

Alanine dipeptide (Ace-Ala-Nme) is a popular model of conformational changes in peptides and proteins. Its conformation is determined by Ramachandran dihedral angles $\varphi$ and $\psi$, similar to peptides and proteins. In the study, which introduced the metadynamics in essential coordinates [10], we used alanine dipeptide in explicit solvent as a model of conformational change. A classical, 1 ns molecular dynamics simulation can explore three different conformational families, namely $\alpha_R$, $C7_{eq}$ and C5 (Fig. 2). This nanosecond trajectory of five atoms was used to determine the essential coordinates. As reported in reference [9], two eigenvectors are capable of describing 92% of motions in the selected atoms. Figure 2b shows a projection of the classical MDS trajectory to the first two essential coordinates.

Two different formulations of metadynamics in essential coordinates were tested. In both runs, temperature was set to 300 K and size of a step was 1 fs. Electrostatics was treated by a single cut-off set to 1 nm. First, direct metadynamics was tested with the height and the width of a hill set to 0.1 kJ/mol and 0.01 nm, respectively. Hills were added every 1,000 steps. In totall, 10,000 hills were deposited, corresponding to a 10 ns molecular dynamics simulation. The resulting FES is illustrated in Fig. 2c. The second tested metadynamics formulation was Lagrangian. This method was originally developed to be used within *ab initio* machinery in order to obtain a reasonably accurate FES from much shorter MDS runs. The mass and coupling constant of the fictive particle were set to $10^3$ $(kJ.ps^2)/(mol.nm^2)$ and $10^5$ $kJ/(mol.nm^2)$, respectively. The resulting FES coming from 200 ps MTDEC is illustrated in Fig. 2d.

Free energy surfaces calculated by direct (Fig. 2c), Lagrangian (Fig. 2d) and constraint-based [10] metadynamics where very similar. The FES calculated using Lagrangian MTDEC was rougher, which is the price paid for the fact that the simulation was significantly (50 times) shorter. When a long Lagrangian MTDEC was performed with slightly different setup, it produced a FES almost identical to the FES from the direct MTDEC (data not shown). Both continuous metadynamics (direct and Lagrangian) turned out to be more efficient than discontinuous. All three major free energy minima ($\alpha_R$, $C7_{eq}$ and C5) were resolved. Calculated free energy values were in qualitative as well as quantitative agreement between different formulations of metadynamics. Conformation $\alpha_R$ was modelled as the most favourable, whereas $C7_{eq}$ and C5 were almost isoenergetic and less favourable than $\alpha_R$.

## SH3 domain

SH3 domains are present in numerous multidomain eukaryotic proteins such as protein kinases, protein phosphatases, phospholipases, binding proteins and others. Biological function of these proteins is regulated by an equilibrium between a target-bound and the Pro-X-X-Pro-bound form of SH3 domain. Because of its compact structure and medicinal importance, it is a popular object of molecular modelling studies. The SH3 domain from ponsin (PDB ID: 2O9S, Fig. 3a) [23] was selected for this study because of its high resolution. The protein was placed into a periodic box of solvent (3,683 TIP3P water molecules). Electrostatics was modelled using the particle-mesh Ewald method, with a cut-off set to 0.9 nm. A one nanosecond molecular dynamics simulation in the AMBER 99 force field [24, 25] was performed. The trajectory $(C^{\alpha}$ – atoms$)$ was analysed by essential dynamics analysis. Two highly flexible regions (residues 818–822 and 830–844) were excluded from the essential dynamics analysis and biasing by metadynamics. Contrary to applications of essential dynamics on cyclohexane and alanine dipeptide, in the SH3 domain there was a higher number of essential coordinates necessary to describe major motions. The first and the second eigenvectors (Fig. 3b and c) describe 22% and 13%, respectively, of the motions in the selected atoms.

Direct metadynamics was performed in the space of the two first eigenvectors. Heights and sizes of hills were 2 kJ/mol and 0.05 nm, respectively. Hills were added every 1,000 steps. Metadynamics (1 ns, 1,000 hills) in these essential coordinates yielded a funnel-shaped free energy surface. The space explored by metadynamics (Fig. 4b) was wider than that explored by classical molecular dynamics (Fig. 4a). Depth of the free energy surface obtained by metadynamics was 59 kJ/mol (unexplored regions set to zero, Fig. 4c), which corresponds roughly to microsecond dynamics. The studied protein remained in its folded state during the metadynamics run. The resulting free energy lies in the range of activation free energies of unfolding usual for mesophilic proteins (50–120 kJ/mol at a given temperature) [26]. It is therefore not possible to say whether the protein remained folded due to the fact that its unfolding free energy was not reached. Another explanation is that the essential coordinates obtained using analysis of a nanosecond normal temperature trajectory are not capable of describing the unfolding process. The resulting FES was also compared by the FES calculated by US/WHAM (Fig. 4d). The FES obtained from 36×100 ps US/WHAM calculation is in quantitative agreement to the FES obtained by MTDEC.

## Discussion

Metadynamics provides two great advantages compared to a classical molecular dynamics simulation, namely free energy modelling and the enhancement of configurational sampling. Both of these advantages are relevant in modelling conformational changes. Numerous examples of metadynamics application illustrate the great potential of this method to be applied to different types of chemical processes and at different levels of theory [4–8]. Results of metadynamics are influenced by the choice of collective variables. Choice of collective variables represents a dimensionality reduction of the studied process. Collective variables must be chosen to maximally describe the studied process and simultaneously, their number must be kept to a minimum (typically two). If the collective variables are not capable of separating two important free energy minima, these minima are not resolved in the resulting free energy surface. Here we present metadynamics in the space of essential coordinates. Principal component analysis is a general purpose dimensionality reduction method and is also widely applied in the description of molecular structures. Therefore essential coordinates could be determined automatically, contrary to the selection of other types of collective variables, which require certain experience. Essential coordinates enhance the scope of collective variables that can be applied in metadynamics.

Essential subspace modelling has proved to be very useful in tracing collective motions in biopolymers. Roles of collective motions in protein dynamics, activity regulation or enzymatic activity were studied using this approach [27–29]. Here, essential coordinates were applied within the metadynamics framework on relatively small systems, such as cyclohexane (this study) or alanine dipeptide (this study and reference [9]). The first real protein (SH3 domain in this study) was modelled only within the vicinity of its native structure. Future application of metadynamics in the space of essential coordinates will show whether these coordinates can accurately describe conformational free energy changes in large proteins, including large conformational changes, folding and unfolding processes etc. Examples of the application of essential dynamics sampling methods [13, 14] are encouraging.

The essential coordinates of a protein or other biopolymer are usually determined for all atoms, backbone atoms or $C^{\alpha}$ atoms. However, in order to be applied in metadynamics, they can also be determined for some selected "hot spot" regions in the studied biopolymer. In addition, alternative methods which provide eigenvectors in a similar fashion as essential dynamics analysis could be applied. Such methods include normal mode analysis [30], Gaussian network model [31] or full correlation analysis [32].

The results presented in this study show some practical aspects of combining metadynamics with essential dynamics, including its coding, comparison of different metadynamics formulations and its application to systems of different sizes. The presented code implements MTDEC into the popular GROMACS code [12] and can be obtained free of charge and further extended due to its open source nature.

**Acknowledgements** This work was supported by REVCAT (Marie Curie RTN CT-2006–35866–2) and Czech Ministry of Education, Youth and Sport (MSM 6046137305). We would like to thank Dr. Oliver Lange for helpful discussions. The code can be obtained at *http://web.vscht.cz/spiwokv/mtdec/*. Videos illustrating simulations performed in this study can be viewed at *http://www.youtube.com/spiwokv*.

## References

1. Levitt M (1976) J Mol Biol 104:59–107 doi:10.1016/0022-2836(76)90004-8
2. Laio A, Parrinello M (2002) Proc Natl Acad Sci USA 99:12562–12566 doi:10.1073/pnas.202427399
3. Iannuzzi M, Laio A, Parrinello M (2003) Phys Rev Lett 90:238302 doi:10.1103/PhysRevLett.90.238302
4. Stirling A, Iannuzzi M, Laio A, Parrinello M (2004) Chem Phys Chem 5:1558–1568 doi:10.1002/cphc.200400063
5. Gervasio FL, Laio A, Parrinello M (2005) J Am Chem Soc 127:2600–2607 doi:10.1021/ja0445950
6. Fiorin G, Pastore A, Carloni P, Parrinello M (2006) Biophys J 91:2768–2777 doi:10.1529/biophysj.106.086611
7. Barducci A, Chelli R, Procacci P, Schettino V, Gervasio FL, Parrinello M (2006) J Am Chem Soc 128:705–2710 doi:10.1021/ja057076l

8. Oganov AR, Martoňák R, Laio A, Raiteri P, Parrinello M (2005) Nature 438:1142–1144 doi:10.1038/nature04439
9. Laio A, Rodriguez-Fortea A, Gervasio FL, Ceccarelli M, Parrinello M (2005) J Phys Chem B 109:6714–6721 doi:10.1021/jp045424k
10. Spiwok V, Lipovová P, Králová B (2007) J Phys Chem B 111:3073–3076 doi:10.1021/jp068587c
11. Amadei A, Linssen AB, Berendsen HJ (1993) Proteins 17:412–425 doi:10.1002/prot.340170408
12. Lindahl E, Hess B, van der Spoel D (2001) J Mol Model 7:306–317 doi:10.1007/s008940100045
13. Amadei A, Linssen AB, de Groot BL, van Aalten DM, Berendsen HJ (1996) J Biomol Struct Dyn 13:615–625
14. de Groot BL, Amadei A, van Aalten DM, Berendsen HJ (1996) J Biomol Struct Dyn 13:741–751
15. Lange OF, Schafer LV, Grubmuller H (2006) J Comput Chem 27:1693–1702 doi:10.1002/jcc.20473
16. Raiteri P, Laio A, Gervasio FL, Micheletti C, Parrinello M (2006) J Phys Chem B 110:3533–3539 doi:10.1021/jp054359r
17. Kumar S, Rosenberg JM, Bouzida D, Swendsen RH, Kollman PA (1995) J Comput Chem 16:1339–1350 doi:10.1002/jcc.540161104
18. Roux B (1995) Comput Phys Commun 91:275–282 doi:10.1016/0010-4655(95)00053-I
19. Grossfield A (2004) An implementation of WHAM: the Weighted Histogram Analysis Method (http://membrane.urmc.rochester.edu/wham/)
20. Biarnés X, Ardévol A, Planas A, Rovira C, Laio A, Parrinello M (2007) J Am Chem Soc 129:10686–10693 doi:10.1021/ja068411o
21. Oostenbrink C, Villa A, Mark AE, van Gunsteren WF (2004) J Comput Chem 25:1656–1676 doi:10.1002/jcc.20090
22. Anet FAL, Bourn AJR (1967) J Am Chem Soc 89:760–768
23. Gehmlich K, Pinotsis N, Hayess K, van der Ven PF, Milting H, El Banayosy A, Wilmanns M, Ehler E (2007) J Mol Biol 369:665–682 doi:10.1016/j.jmb.2007.03.050
24. Wang J, Cieplak P, Kollman PA (2000) J Comp Chem 21:1049–1074 doi:10.1002/1096–987X(200009)21:12<1049::AID-JCC3>3.0.CO;2-F
25. Sorin EJ, Rhee YM, Pande VS (2005) Biophys J 88:2516–2524 doi:10.1529/biophysj.104.055087
26. Jaswal SS, Truhlar SM, Dill KA, Agard DA (2005) J Mol Biol 347:355–366 doi:10.1016/j.jmb.2005.01.032
27. Vanaalten DMF, Amadei A, Linssen ABM, Eijsink VGH, Vriend G, Berendsen HJC (1995) Proteins 22:45–54 doi:10.1002/prot.340220107
28. Young MA, Gonfloni S, Superti-Furga G, Roux B, Kuriyan J (2001) Cell 105:115–126
29. Agarwal PK (2005) J Am Chem Soc 127:15248–15256 doi:10.1021/ja055251s
30. Tama F (2003) Protein Pept Lett 10:119–132
31. Haliloglu T, Bahar I, Erman B (1997) Phys Rev Lett 79:3090–3093 doi:10.1103/PhysRevLett.79.3090
32. Lange OF, Grubmüller H (2008) Proteins 70:1294–1312 doi:10.1002/prot.21618