Applicability of Free Energy Calculations using High–Throughput Grid Approach

Jan Kmuníček[1], Petr Kulhánek[2], and Zora Střelcová[2]

[1] CESNET, z. s. p. o., Zikova 4, 160 00 Praha 6, Czech Republic
[2] National Centre for Biomolecular Research, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic
kmunicek@ics.muni.cz
{kulhanek, stre}@chemi.muni.cz

Abstract. Free energy calculations tightly bind together experimental observations and computer–aided design through corresponding simulations at atomic level. Nowadays, free energy calculations represent a cornerstone for obtaining deeper insight into any biomolecular system, its molecular structure and especially dynamic behaviour. Despite this fact, several challenges have to be considered before routine utilization of this type of calculations. At first one has to deal properly with so–called sampling problem. Secondly, the appropriate way how to treat their extreme time demands has to be applied for obtaining converged and reliable data comparable with the experimental ones. Here we present the experience obtained through design, implementation and subsequent application of Multiple Walkers Approach (MWA) connected with Adaptive Biasing Force (ABF) and corresponding advantages and added values of MWA ABF run within distributed, heterogeneous grid environment under the EUAsia virtual organization (VO).

Keywords: grid, distributed computing, virtual organization, Enabling Grids in E–SciencE, EUAsia VO, Multiple Walkers Approach, Adaptive Biasing Force.

1. Introduction

Nowadays, the grid phenomenon has influenced nearly any research area trying to tackle computationally demanding tasks and challenges. The origin of grid activities lies in the high-throughput approach used primarily for the purposes of large, international high energy physics experiments. However, there are a lot of other promising areas and application domains utilizing the available grid environment through their own high-throughput computations. One of such promising areas is free energy calculations within the computational chemistry domain. Here we describe our implementation of Multiple Walkers Approach (MWA) connected with Adaptive Biasing Force (ABF) used to estimate free energy for supramolecular chemical systems.

A generic overview of grid technologies currently available is provided in Chapter 2. Next section – Chapter 3 – is dedicated to description of methods used for estimation of free energy and a molecular system studied. Chapter 4 is devoted to detail the achieved results and in Chapter 5 we summarize the observations and derive plans for future research in this area.

2. Grid Technologies

The most promising technology emerging from the mature utilization of advanced high–speed networks is the grid [1]. Grid environments represent a central layer built on the network ubiquitous infrastructure and providing an access to desired network of worldwide scientific knowledge. During last few years the grid technology moved from experimental and prototype (testing) version into a unique, worldwide production service for thousands of users performing global scientific tasks. Nowadays, Grids are considered to be a base of global e–Infrastructures providing an easier access for small and distributed research groups interconnecting scientists from many different fields to completely new technologies thus allowing them to produce, store, access and manipulate massive amounts of data, to utilize large amounts of CPUs to their maximal computational potential and to access high–end facilities (i.e. supercomputers). Moreover, grids brought a set of previously unknown added values in facilitating distributed collaborations as well as in provisioning of new ways of community building through remote resource sharing across different administrative domains.

The term *grid* can be defined as a large, distributed system [2] composed of computational and storage capacities (eventually together with further instruments) of distinct owners interconnected by high–speed network. These resources create an illusion of uniform, computational and storage space without perception of boundaries [3] formed by proprietary or administrative relations.

Basically, several different types of grid environments can be recognized according to its primary function. Originally, at the very first were the *computational grids* trying to fulfil ever–growing request for computational power with low cost of activation barrier in their utilization. The computational grid is expected to provide a computational service (i.e. provide secured services pro running applications at distributed computational resources). Generally speaking, it represents a "virtual distributed computer" for solving computationally demanding tasks as it dynamically aggregates computational capacity of huge number of individual computers. Simultaneously with computational grids one could expect needs to manage all the data produced during massive computation campaigns and challenges. This led to huge effort investments into forming a logical extension of computational environments – *data grids* – serving for remote management and transparent access to millions of files. Data grids basically represent a processing of large datasets using the services of computational grids. The data grid can be characterized by sharing of vast amounts of data, by provision of secure access to those data and allowing their

subsequent management (solved using the form of replicated data catalogs creating of an illusion of uniform mass data storage). On the top of this hierarchy stays the third type of grid environments that is represented by *informational/knowledge* grids often also called as collaborative and/or application grids. This is an ultimate extension of grid approach into global sharing of resources of any kind at all. The knowledge grids are characterized by extension of data grid approach possibilities with provision of data categorization, ontology, knowledge sharing and workflow development. The collaborative added values including special cutting–edge services as the access to remote instruments, sensors and devices, smooth community/virtual laboratories building etc. are also standard part of the knowledge grids.

The principles driving the grid environment utilization can be summarized into following simple scheme: users should accept from the grid resources (CPUs or their power, respectively; disc space; transfer capacity of networks; special hardware) that they need, when they need and exactly where they need them. Grid is expected to provide unified access, security and reliable services on one hand and the necessary accounting on the other hand.

2.1. Worldwide Production Grid

Currently, all European grid computing projects are dominated by the Enabling Grids for E–sciencE (EGEE) [4] leading the way by providing a computing support infrastructure for over thousands of researchers from many distinct domains. The EGEE project brings together experts from more than 50 countries with the common aim of building on recent advances in Grid technology and developing a service Grid infrastructure which is available to scientists 24 hours–a–day (see infrastructure details in Table 1). The project provides researchers in academia and business with access to a production level Grid infrastructure, independent of their geographic location. The EGEE project also focuses on attracting a wide range of new users to the Grid. The main focus of the project is to expand and optimize Europe's largest production Grid infrastructure by continuous operation of the infrastructure, support for more user communities, and addition of further computational and data resources.

Table 1. EGEE infrastructure statistics.

Number of sites connected	260+
Number of countries connected	55
Number of CPUs available	24/7 cca 150 000
Storage capacity available	cca 28 PB disc space

Presently, EGEE worldwide production environment support quite a number of distinct application domains ranging from archaeology, astronomy and astrophysics, biotechnology, bioinformatics and biomedical sciences, civil protection,

computational chemistry, computer science, earth sciences, finances, fusion, geophysics, high–energy physics, life sciences up to multimedia and material sciences (Table 2). The end user work within EGEE is organized through appropriate sets of virtual organizations (VOs). The concept of virtual organization has been firstly introduced by I. Foster and C. Kesselman [1] defining the grid computing as "coordinated resource sharing and problem solving in dynamic, multi institutional virtual organizations". The virtual organization can be therefore defined as a sum of resources and persons usually spanning multiple administrative domains and even countries working on a joint project allowing seamless resource sharing and creating an abstraction of the user community (set of users and resources with a common goal in other words). Considerable number of EGEE virtual organizations (at least in the inital phase of EGEE project during which the appropriate user communities started to be engaged through formation and management of corresponding virtual organizations) have been set up and built as so–called catch–all virtual organizations. The catch–all virtual organizations represent an effective way for users to use grid environments. Catch–all VOs are provided as a service to users communities as part of user support activities of several projects. Catch–all VOs tie together resource providers and different end user communities, thus forming a crucial step towards routine production way of worldwide grid platform, which is easily available to users. Decreasing the entrance barrier is especially important for various regions with high heterogeneity and different grid knowledge of involved parties. Crucial advantages of catch–all VOs are especially their open environment nature (from the point of user access, application deployment, and high–level tools installation). The routine catch–all VO management is performed by dedicated administrators. In addition, the new user communities willing to test the grid environment are completely free from establishing their own VO before trying a Grid as the catch–all VO provide the initial outsourcing of all critical VO services. The suitable examples of such successes catch–all VO fulfilling regional needs are the Virtual Organization for Central Europe (VOCE) or EUAsia VO.

Table 2. EGEE end users statistics.

Number of VOs using EGEE	~200
Number of registered VOs	~140
Number of registered users	~14 000
Number of jobs	~330k jobs/day

3. Free Energy Calculations

The free energy is a thermodynamical quantity that uniquely describes the state of chemical system. As it is a state function, its change is intimately connected with kinetics and equilibrium of chemical processes. Thus its knowledge can be helpful in computer aided rational drug design or in description of processes, which cannot be

directly studied by experimental means. For such outstanding features, free energy calculations by computer simulations have attracted a lot of interest in the past. Quite huge effort was put to the development of the Potential of Mean Force (PMF) methods [5] because they are able to describe kinetics of studied systems. Popular methods that belongs to PMF group are constrained dynamics [6], umbrella sampling [7], metadynamics [8] and Adaptive Biasing Force (ABF) method [9]. All of them *bias* studied system along prescribed set of Collective Variables (CVs) (also called reaction coordinates) in such a way that the free energy of *unbiased* system can be easily recovered either directly from underlying simulations or during post-processing of their results. Despite achieved success in this area, very long simulations are usually necessary to obtain converged and reliable results. To overcome this problem, several techniques were recently suggested and used on the top of mentioned PMF methods. Promising methods are replica exchange molecular dynamics [10], string method [11], and Multiple Walkers Approach (MWA) [12]. Their common feature is parallel simulation of several replicas of studied system. Since communication overhead among replicas is usually very small, all of these methods are suitable for large scale calculations. In this work, we will demonstrate the utilization of ABF method accelerated by MWA in the grid environment. Both methods were tested on the study of a molecular switch from a pseudorotaxane family [13,14].

Adaptive Biasing Force Method. [9,15] The method will be briefly reviewed in this section. As mentioned above, the success of every PMF method lies in proper way of system biasing. In ABF method, the system potential V is biased by underlying free energy A in such a way that a motion in the space of collective variables $\boldsymbol{\xi}$ is barrier-free. In other words, this motion is solely govern by diffusive motion, which guarantee that the system visits all places in CV space in reasonable time. The aim of ABF method is the calculation of free energy thus a simple question arises. How such unknown free energy can be applied? The answer is in adaptive application of continuously refined PMF. At the beginning, the molecular dynamics is started without any bias and PMF is calculated. Whenever the number of samples in any bin reaches predefined threshold, the calculated PMF is applied to the system. Such perturbed system has then tendency to visit places in CV space further from the starting point. As the number of samples in every bin increases, PMF is more precise and the barrier-free motion in an entire CV space is achieved very soon. The free energy is not direct outcome of ABF simulation but its derivative is. Thus the resulting free energy has to be obtained by numerical integration of PMF.

Multiple Walkers Approach. This idea was firstly used with metadynamics [12]. The utilization of MWA together with ABF is also very simple. The approach employs N independent molecular dynamic simulations biased by ABF (so called walkers) that feel the estimated PMF accumulated from all walkers. With this approach, the sampling of CV space is N-times faster than in conventional ABF run. A key essence of MWA is independence of the individual walkers. This condition is easily fulfilled, for example, when walkers start from entirely different configurations (reactants, product, and intermediates). But even, if these different starting configurations are not known in advance, one can start from the same configuration

with different starting velocities. In this case, all walkers become incoherent very soon. ABF is not the only method that can be accelerated by MWA.

3.1 Pseudorotaxane Based Molecular Switch

Pseudorotaxanes [16] are supramolecular complexes which are formed by a cyclic molecule (wheel) threaded on a string molecule (axle). A key property of such complexes is a limited motion of both components to each other but without prohibition of complex dissociation. Pseudorotaxanes have several unique properties therefore they are intensively studied both experimentally [13,14] and theoretically [17-19]. In our work, pseudorotaxane complexes composed of cucurbit[7]uril and 4,4'-bipyridinium based axle (1,1'-bis(4-carboxybutyl)-4,4'-bipyridinium) molecules (Fig. 1) were studied.

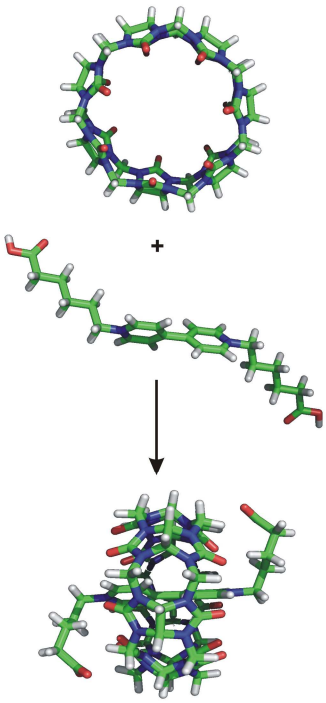

Fig. 1. Schematic representation of pseudorotaxane formation: (top) cucurbit[7]uril; (middle) axle component; (bottom) formed pseudorotaxane complex.

Since both axle terminals bear carboxylic groups, their protonation states can be changed by different pH conditions. At low pH (~3), both terminals are protonated (COOH) and the axle has +2 charge, which originates from the viologen core (State

I). On contrary, at high pH (~9), both terminals loose two protons (COO^-) and the axle then becomes electro neutral (State II). According to reported NMR, thermodynamic, and kinetic studies [13,14] it was found that both states differ in the wheel position on the axle. The wheel prefers to bind to aliphatic sidearm (side position A and A') of the axle in State I rather than around the nucleus of the axle (central position B) in State II (see Fig. 2).

This pseudorotaxane system can be considered as a molecular switch, which is switched on and off by different pH conditions. Unfortunately, the molecular basis of this process is not fully understood. Thus, we used the free energy calculations to obtain more information about this interesting phenomenon.

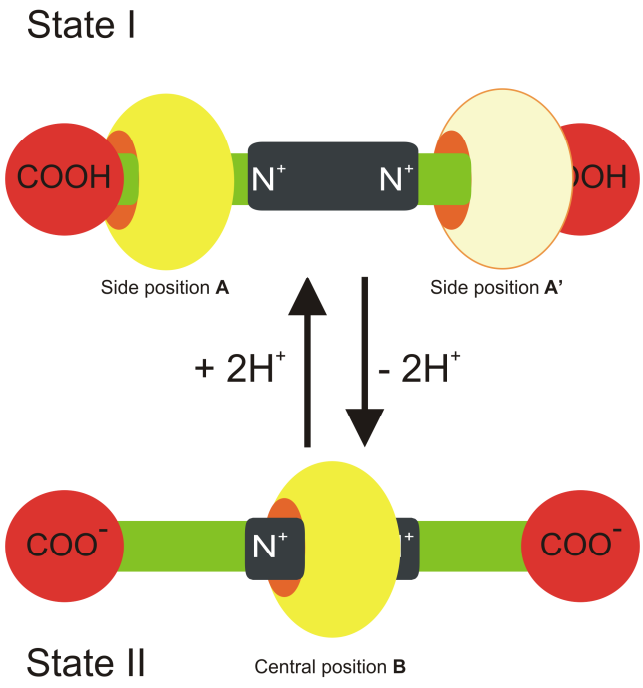

Fig. 2. Principle of molecular switch based on pseudorotaxane complex.

4. Results and Discussion

Two distinct approaches accelerating free energy calculations employing ABF method were tested. One relies on the utilization of MPI parallel version of pmemd program. The second one uses MWA. Their comparison towards simple sequential run is shown in Fig. 3.

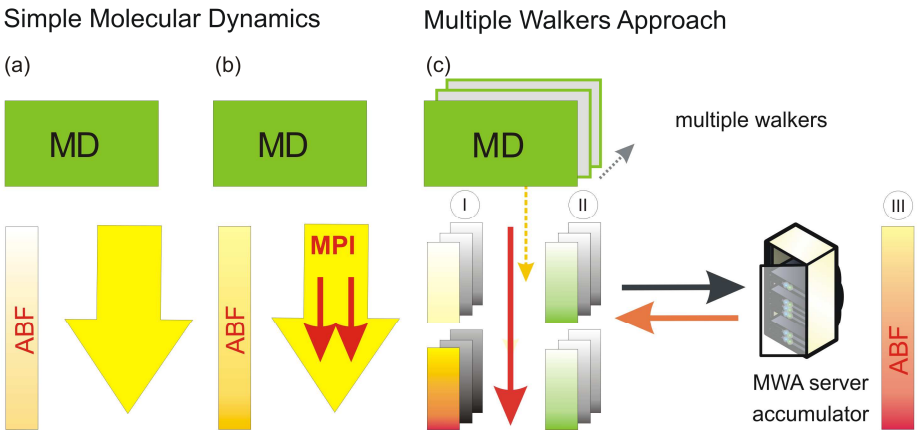

Fig. 3. Tested variants of ABF method: (a) simple ABF run; (b) MPI parallelized ABF run; and (c) MWA accelerated ABF run.

From profiling of modified pmemd program, it follows that almost 95 % of execution time is spent by the computation of potential of *V(x)*. The rest is spent by integrating equations of motions, computing PMF, and constraining bonds including hydrogen atoms (via SHAKE procedure [20]). Thus it is straightforward to speed up an entire calculation by parallel computation of potential of *V(x)*. Luckily, pmemd was already parallelized using MPI and used MPI implementation was not in conflict with our code modification due to ABF method.

We tested scaling of the MPI code on SMP (symmetric multiprocessing) system containing two Intel Quad-Core Xeon processors (E5472) clocked at 3 GHz (Fig. 4). Reasonable scaling was achieved in the range of 1 to 4 CPUs. For higher number of CPUs, the scaling drops down rapidly. This bad behaviour is probably due to very small size of simulated system (about 21000 atoms), because significantly better scaling results were reported for larger systems [21]. Thus our results indicate that the free energy calculation acceleration by MPI parallelization is only moderate and reasonable only on SMP systems. To achieve higher acceleration a different strategy was tested. It is called Multiple Walkers Approach and its implementation will be described in detail in the following section.

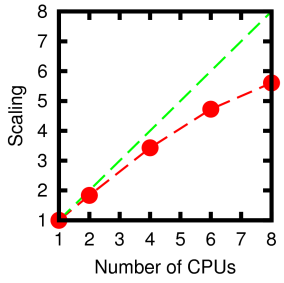

Fig. 4. (dark, red) Parallel scaling of MPI version of modified pmemd program tested on dual Intel Quad-Core Xeon SMP system; (gray, green) ideal scaling.

4.1 MWA Implementation

As it was mentioned in the introduction, MWA employs *N* independent molecular dynamic simulations so-called walkers that feel the estimated PMF accumulated from all walkers. This behavior can be achieved by simple client-server architecture. In our implementation, a client (a single walker) keeps two PMF accumulators. One accumulator (Fig. 3c – I) contains full set of PMF data, which are used to bias the system, and the other one (Fig. 3c – II) contains only those PMF data that were collected from the last data exchange with MWA server. Every accumulator is composed of three arrays. One array contains numbers of samples in every bin. Two other arrays contain sums and sums of squares of derivative part. Two first arrays are used to calculate PMF in every bin. The last array is only used to express basic statistical characterization of PMF such as standard deviation and error.

Data flow between clients and the server is as follows. Every specified period, which is 250 time steps in our case, newly accumulated data from accumulator II are sent by a client to MWA server. Received data are added by MWA server to its PMF accumulator III. Whole transaction is ended by sending accumulator III back to a client. Finally, received data are put by a client to accumulator I (previous data are discarded) and accumulator II is reset. From this moment, a client feels the PMF accumulated by all walkers so far plus newly accumulated data, which will be sent to the server during the next transaction. An advantage of our implementation is that the constant amount of data is sent back and forth between a client and the server. In the study presented here, it is about 35 kB.

MWA server is entirely written in C++ and is fully multithreaded using POSIX threads. Clients are written partially in Fortran 90 and partially in C++. The part responsible for communication with MWA server is written in C++. Data are transmitted in binary form over simple TCP/IP connection. This simplification was only used to test the MWA concept. For real application in the grid environment, it will be necessary to use secure connection and other than binary format to avoid possible problems arising from the utilizations of clients executed on different CPU architectures (little/big endian, different real number representation). Since the communication with the server is not necessarily required to be synchronized it is possible to run clients on machines with different CPU speed. Thus MWA approach is well suited for grid environments, which are usually highly heterogeneous from this point of view. Moreover, clients can also be run in parallel as described above. This speeds up further the entire free energy calculations.

4.2 Molecular Shuttle

The ABF MWA method was used to investigate in atomistic details a technologically important chemical problem – the evaluation of the free energy profile of the molecular shuttle process in the pseudorotaxane complexes. To be able to perform desired computations using ABF method, CVs have to be defined properly. Their appropriate selection is crucial for the free energy results accuracy and

the ability to describe the system correctly from the free energy point of view. The selection of CVs has to be done in such a way that they allow maximal sampling of the conformation space while in parallel they are defined in accordance with the specific reaction mechanism.

In our case, one CV (ξ_1) was specified as the distance from the central plane of the wheel (formed by carbon atoms only), and the centre of mass of axle bipyridinium core. Since the system showed the unfavorable bending of the axle, the system was stacked in some states and the whole conformational space was not sampled properly. Consequently, we were forced to add the second CV (ξ_2) to ensure the sampling of all available system conformations. The second CV is defined as the angle between carboxyl group on the axle terminus, and the centers of both pyridinium rings given by their heavy atoms. Generally speaking, the utilization of multidimensional CVs requires an extensive usage of computational resources. The necessity to use 2D CVs during our exploration was a key motivating factor for trying to exploit advantages of current grid technologies enabling to solve challenging, computationally-demanding tasks in fast and reliable way.

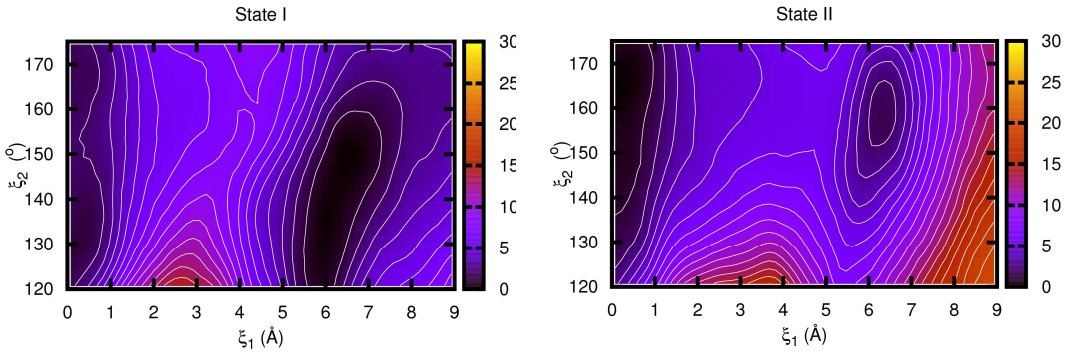

Fig. 5. Free energy profiles (in kcal/mol) for state I and II in water.

The ABF MWA simulations were performed in two different environments – water and NaCl solution. Obtained results from both sets of the free energy calculations are presented in the form of 2D plots (Fig. 5 and 6), where *x* and *y* axes represent the used CVs (ξ_1 and ξ_2). The resulted plots show the complete free energy surface. Simultaneously, they indicate the most important points on the surface – the energy minima and the transition states. Knowledge of these points enables the definition of the reaction pathways and evaluation of the final free energy profiles.

In water media (Fig. 5), both studied systems (State I and State II, see Fig. 2) showed two energy minima: the first one (position A, see Fig. 2) in the central position of the axle and the second (central position B, see Fig. 2) in the distance about 6.1 Å (protonated system), 6.5 Å (neutral system) respectively. These two systems, (Fig. 5a and 5b) differ in the global energy minima position which is located

on the axle terminus in the protonated system (State I), whereas this minimum lies in the central position in neutral system (State II). Since the global energy minimum represents the favorable system state, we can conclude that in State I the wheel is preferably located on the axle terminus while the central position will be the preferred one in State II.

Same sets of calculations were performed using the same systems in NaCl solution, which better represents the experimental conditions. Obtained free energy profiles show important differences in comparison with water environment. On one hand the protonated system (State I) contains two equal minima in the same positions as in water environment while the neutral complex (State II) shows only one global minimum in the axle centre. The free energy barrier which has to be overcome to exchange between two equal minima in the protonated system is about 8 kcal/mol. Such barrier is reasonably low to allow the fast interchanging between these two states. The difference in both states corresponds well to the previously experimentally suggested shuttle mechanism. In the neutral complex the central position seems to be clearly preferred.

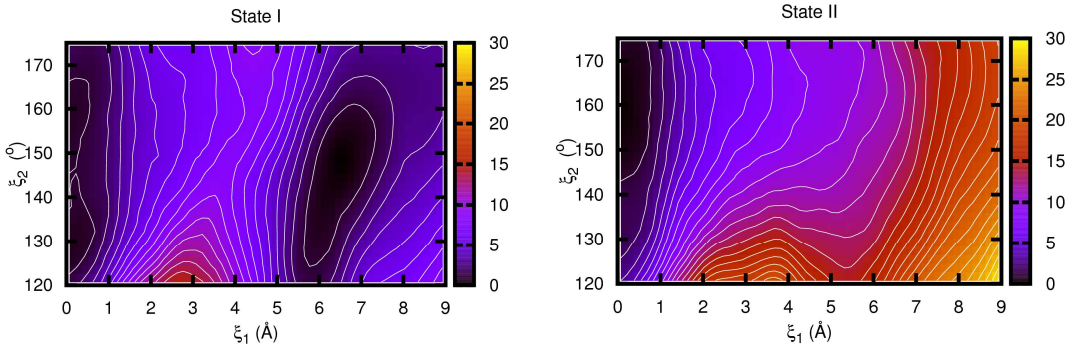

Fig. 6. Free energy profiles (in kcal/mol) for state I and II in NaCl solution ($c_{(NaCl)}$ ~ 0.1 M).

5. Conclusions and Future Perspectives

We successfully performed the free energy calculations of the molecular shuttle process using the Adaptive Biasing Force method accelerated by the Multiple Walkers Approach in two different grid computational environments: EUAsia VO and VOCE. The results were obtained nearly 40 times faster compared to a conventional run due to employing 40 independent walkers utilized for every single job. Currently, we are applying this approach to various complexes differed by cucurbit[n]uril sizes and axle lengths.

Our implementation of Adaptive Biasing Force method using Multiple Walkers Approach clearly indicated that grid environments - EGEE worldwide Grid in particular - create a unique medium for performing large scale, massive computations due to its nature well suited for execution of huge number of computational jobs. This evaluation study further motivates us to improve the current implementation of MWA. There are several issues in current implementation that need to be improved. Generally speaking, the security of communication between the individual clients and the server should be corrected. Current utilization of plain password authentication and unencrypted data transfer in the grid environment is clearly very risky, because potential intruder can easily change transferred data or he can easily connect a malicious client injecting poisoned data to a MWA server. Thus we plan to use an SSL encrypted communication with authentication via X.509 user proxy certificate. Another bottleneck of the current implementation lies in the utilization of the only server. If this server fails due to work node malfunction or communication problems, all accumulated data would be lost. To prevent such undesired behaviour we plan to utilize more mutually interconnected servers. We expect that this approach can potentially bring several benefits. Firstly, the required communication bandwidth will be smaller as only proportion of the clients will communicate with one of the servers. Secondly, this approach should minimize the risk of data loss as the accumulated data will be duplicated among the servers.

In conclusion, our study showed the applicability and usability of the worldwide grid environments represented by EGEE Grid. Taking into account suggested improvements and new planned features of currently tested and implemented approach one can expect in near future more intensive usage and wider acceptance of EGEE Grid within the field of free energy calculations and computational chemistry domain in general.

Acknowledgements. The presented work is supported by following grants: The Ministry of Education of the Czech Republic (contracts LC06030, MSM0021622413 and MSM6383917201) and by EGEE III project funded by European Commission (contract number INFSO–RI–222667). The research leading to these results has also received funding from the European Community's Seventh Framework Programme under grant agreement n° 205872.

References

1. Foster, I., Kesselman, C.: Computational grids – Invited talk (Reprinted from The Grid: Blueprint for a new computing infrastructure, 1998). Vector and Parallel Processing – Vecpar. 1981, 3–37 (2001)
2. Foster, I.: The anatomy of the grid: Enabling scalable virtual organizations. First Ieee/Acm International Symposium on Cluster Computing and The Grid. 6–7 (2001)
3. Foster, I., Kesselman, C., Nick, J.M., Tuecke, S.: The Physiology of the Grid: An Open Grid Services Architecture for Distributed Systems Integration. Available via http://citeseerx.ist.psu.edu/viewdoc/summary?doi=10.1.1.14.8105.
4. EGEE Portal: Enabling Grids for E–sciencE. Available via http://eu–egee.org/

5. Kirkwood, J.: Statistical mechanics of fluid mixtures. J. Chem. Phys. 300–313 (1935)
6. Mulders, T., Kruger, P., Swegat, W., Schlitter, J.: Free energy as the potential of mean constraint force. J. Chem. Phys. 104, 4869-4870 (1996)
7. Torrie, G., Valleau, J.: Non-physical sampling distributions in monte-carlo free-energy estimation - umbrella sampling. J. Comput. Phys. 23, 187-199 (1977)
8. Laio, A., Parrinello, M.: Escaping free-energy minima. P. Natl. Acad. Sci. USA 99, 12562-12566 (2002)
9. Darve, E., Pohorille, A.: Calculating free energies using average force. J. Chem. Phys. 115, 9169-9183 (2001)
10. Sugita, Y., Okamoto, Y.: Replica-exchange molecular dynamics method for protein folding. Chem. Phys. Lett. 314, 141-151 (1999)
11. E, W., Ren, W., Vanden-Eijnden, E.: String method for the study of rare events. Phys. Rev. B 66, 052301 (2002)
12. Raiteri, P., Laio, A., Gervasio, F., Micheletti, C., Parrinello, M.: Efficient reconstruction of complex free energy landscapes by multiple walkers metadynamics. J. Phys. Chem. B 110, 3533-3539 (2006)
13. Sindelar, V., Silvi, S., Kaifer, A.E.: Switching a molecular shuttle on and off: simple, pH-controlled pseudorotaxanes based on cucurbit[7]uril. Chem. Commun. 2185–2187 (2006).
14. Sindelar, V., Silvi, S., Parker, S.E., Sobransingh, D., Kaifer, A.E.: Proton and electron transfer control of the position of cucurbit[n]uril wheels in pseudorotaxanes. Adv. Funct. Mater. 17, 694–701 (2007)
15. Darve, E., Rodriguez-Gomez, D., Pohorille, A.: Adaptive biasing force method for scalar and vector free energy calculations. J. Chem. Phys. 128, 144120 (2008)
16. Kim, K.: Mechanically interlocked molecules incorporating cucurbituril and their supramolecular assemblies. Chem. Soc. Rev. 31, 96-107 (2002)
17. Grabuleda, X., Ivanov, P., Jaime, C.: Computational studies on pseudorotaxanes by molecular dynamics and free energy perturbation simulations. J. Org. Chem. 68, 1539-1547 (2003)
18. Grabuleda, X., Jaime, C.: Molecular shuttles. A computational study (MM and MD) on the translational isomerism in some [2]rotaxanes. J. Org. Chem. 63, 9635-9643 (1998)
19. Grabuleda, X., Ivanov, P., Jaime, C.: Shuttling process in [2]rotaxanes. Modeling by molecular dynamics and free energy perturbation simulations. J. Phys. Chem. B 107, 7582-7588 (2003)
20. Ryckaert, J.P., Ciccotti, G., Berendsen, H.J.C.: Numerical-integration of cartesian equations of motion of a system with constraints - molecular-dynamics of n-alkanes. J. Comput. Phys. 23, 327–341 (1977)
21. Duke, B.: PMEMD benchmarks for Amber 9. Available via http://ambermd.org/amber9.bench2.html Cited 2 Aug 2009