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Drug design: Insights from atomistic simulations

F. Collu, E. Spiga, A. Kumar, E. Hajjar, A. V. Vargiu, M. Ceccarelli and P. Ruggerone

CNR-INFM SLACS and Dipartimento di Fisica, Università di Cagliari Monserrato (CA), Italy

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Summary. — Computer simulations have become a widely used and powerful tool to study the behaviour of many-particle and many-interaction systems and processes such as nucleic acid dynamics, drug-DNA interactions, enzymatic processes, membrane, antibiotics. The increased reliability of computational techniques has made possible to plane a bottom-up approach in drug design, *i.e.* designing molecules with improved properties starting from the knowledge of the molecular mechanisms. However, the *in silico* techniques have to face the fact that the number of degrees of freedom involved in biological systems is very large while the time scale of several biological processes is not accessible to standard simulations. Algorithms and methods have been developed and are still under construction to bridge these gaps. Here we review the activities of our group focussed on the time-scale bottleneck and, in particular, on the use of the metadynamics scheme that allows the investigation of rare events in reasonable computer time without reducing the accuracy of the calculation. In particular, we have devoted particular attention to the characterization at microscopic level of translocation of antibiotics through membrane pores, aiming at the identification of structural and dynamical features helpful for a rational drug design.

PACS 87.15.A- – Theory, modeling, and computer simulation.
PACS 87.16.dp – Transport, including channels, pores, and lateral diffusion.

1. – Introduction

Several alarming features indicate that the strategy to develop new drugs should be revised and reconsidered. An example is the problem of the bacterial resistance to antibiotics. In the second half of the last century, infectious diseases caused by bacteria seemed to be definitely defeated because of the timely production of efficient antibacterial drugs. This picture went hand in hand with a progressive decrease of investment and research within the pharmaceutical industry. However, bacteria have been able to develop several mechanisms of resistance that acting simultaneously make the use of any antibiotics weak or ineffective. This phenomenon is known as bacterial multidrug resistance (MDR)

and is responsible for more deaths from infection, longer hospital stays and an increase of more toxic and expensive drugs [1, 2]. The spread of MDR bacteria and re-emerging pathogens, both in hospitals and in the community, appears in a moment where no truly novel active antibacterial compounds are in clinical trials, that means no new drugs for at least 6/8 years [3]. Today to respond to the emergency call of new drugs we need to strongly reinforce antibacterial research and reinvent a new way to search drugs [4, 5], moving from the traditional screening on large amount of molecules to a rational and microscopically well-founded drug design. A key role in development of this bottom-up strategy can be played by computer simulations and, in particular, by Molecular Dynamics (MD) simulations [6]. This technique has the potentiality to provide detailed information, *i.e.*, at the microscopic scale, regarding the structure, dynamics and energetics of the system of interest. All the mentioned properties, often barely detectable in experiments, represent a necessary starting point for the rational strategy that can rescue drug design from a dangerous stalemate. However, typical MD simulations have to face very often limitations associated with the reduced time and size scales ($\sim 100\,\text{ns}$ and $\sim 100000\,\text{atoms}$) of the systems that are simulated. Several algorithms have been developed to tackle these limits, and promising results have been obtained in the study of biological problems. In our group we have concentrated our attention on the time bottleneck, using metadynamics [7], a technique that allows the investigation of rare events (see sect. **2**). Recently, metadynamics has been successfully used to investigate various biology-oriented problems (see refs. [8-14] for some examples), but in the present paper we will briefly overview only some results obtained in our group concerning the translocation of antibiotics through bacterial pores.

2. – Metadynamics

Standard MD simulations cannot afford the typical biological time scale without important approximations in the system. Either the system is described at a lower level, the so-called coarse-grained description, or one can drastically approximate the interaction energies, producing systematic errors. However, to provide insights into biology-oriented questions often the system cannot be handled at the approximate levels mentioned above but must be treated at an all-atom level. Among the different methods proposed to accelerate simulations [15-25], the metadynamics [7, 26] seems extremely powerful. The algorithm requires that the process under investigation can be represented using a small set of reaction coordinates, called also collective variables (CVs), to be defined *a priori* and be process-dependent. When the slow evolution of the process can be attributed principally to these few CVs, the artifice is to accelerate the time evolution of the CVs.

During standard MD simulations many conformations of the same free-energy minimum are sampled, but the same conformer can be visited many times, *i.e.*, memory effects are absent. The idea of metadynamics is to take advantage of the previously visited conformers, moving from an algorithm with zero-memory effects to a history-dependent algorithm, the so-called non-Markovian dynamics. Within metadynamics, only the defined CVs are forced to evolve with a non-Markovian dynamics, during the simulation adding with a given frequency penalty terms in order to avoid, or to penalize, if the same conformer is visited many times. All the other coordinates are maintained near equilibrium. With this scheme it is possible, in the range of a typical simulation (tens of nanoseconds), to observe a process whose time scale is several microseconds. The core of metadynamics is to identify *a priori* the few CVs that better describe the process under investigation at the microscopic scale. They are complex functions of selected

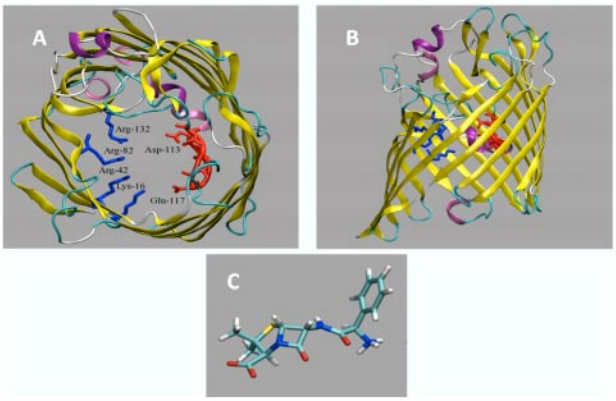

Fig. 1. – (Colour online) (A) Top view of the OmpF structure with the key charged residues of the constriction zone highlighted and labeled (Arg-42-82-132, Lys-16 in blue and Glu-117, Asp-113 in red); (B) side view of OmpF; (C) structure of ampicillin. The central four-members ring is the active part that binds to the bacterial target, the peptidoglycan.

degrees of freedom, such as a geometrical coordinate (distances, angles, dihedrals) or an interactions-based coordinate, such as the coordination number of a group of atoms, the number of hydrogen bonds, or hydrophobic contacts between different groups. The first advantage of metadynamics is the capability to accelerate events. However, the choice of the CVs represents implicitly a way to test whether that process can be described by those CVs, or implicitly it is assumed as a mechanism for that process. In the case of their failure, any evolution of the system can be observed, and more appropriate coordinates should be considered, or a different mechanism should be assumed for that process. Therefore, the procedure is implicitly a speculation on the mechanism governing the process. Another advantage of this algorithm is the possibility to quantify the process through a free-energy description [26]. Finally, with respect to other acceleration algorithms metadynamics does not require to define the range of variation of the CVs *a priori* or the predefined grid, instead letting the system evolve toward the lowest transition state, thus obtaining the minimum free-energy landscape along the path connecting the two minima. This prevents sampling of uninteresting regions and, in principle, allows the introduction of a high number of CVs [12].

3. – Translocation of antibiotics

Our investigation strategy was based on the use of metadynamics to find a reactive path for the escape of ampicillin down from the constriction region of OmpF [27]. OmpF is considered one of the main entry points for different classes of antibiotics to pass through the outer membrane and is a trimeric porin with 16 beta-barrels that also shows a small cation selectivity, with each monomer charged at -10 at neutral pH (see fig. 1 A and B). The high-resolution double-funnel-shape crystal structure of OmpF [28] is characterized by a narrow central region created by the internal fold of loop L3, the longest in OmpF. This region, whose sizes are roughly 7×11 Å, is supposed to be the bottleneck for transport properties, as the loop L3 prevents the passage of large molecules. The starting structure for ampicillin in the constriction region essentially coincides with

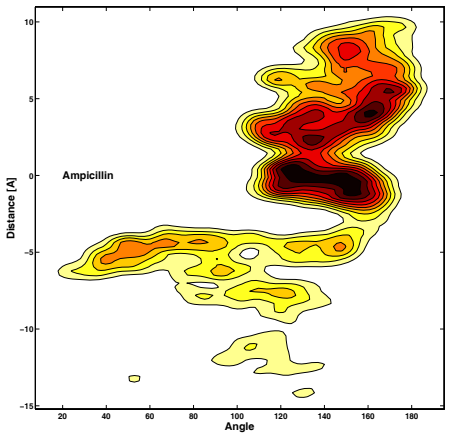

Fig. 2. – The free energy surface associated with the reaction pathway along the subspace of the two variables, displacement of the center of mass of moxifloxacin along the z-axis of the porin ($z = 0$ corresponds to the constriction zone), and orientation angle. Each isoline corresponds to 1 kcal/mol.

the one proposed by Bezrukov and co-workers [29]. As stated above, metadynamics has allowed considering in a natural and not computer-time–demanding way more than one relevant coordinate for the process. The coordinates we assume to be accelerated in our simulations are inspired by the analysis of the initial structure. Since the orientation of the compound with respect to the translocation axis has to be appropriate to facilitate the passage, the angle between the axis of the molecule and the pore axis represents one rate-limiting slow coordinate for the translocation and hence is a natural collective variable to consider and to accelerate. The second natural coordinate is the position of the ampicillin along the z-axis of diffusion, which must change in order to observe translocation. Accelerated MD simulations proved the passage of ampicillin through the constriction region with a simulation lasting a few nanoseconds. The obtained free energy surface is reported in fig. 2. The main results obtained can be cast in four points:

– During translocation, no large conformational change of the pore is observed. In particular, the constriction zone keeps its structure practically unaffected by the presence of the antibiotic.

– The barrier encountered by ampicillin in exiting from the binding-site is on the order of 10/12 kcal/mol. Using the transition state theory, this corresponds to a typical time of hundreds of microseconds, which agrees well with experiments.

– The flexibility of the molecule seems to help translocation through the central region. The same simulation performed with an internal dihedral angle of ampicillin constrained to a fixed value yielded still a translocation but with a higher energy barrier to be overcome. It is tempting to postulate, therefore, that the low permeability of OmpF toward other compounds of larger size, like azlocillin and piperacillin, probably originates from their bulky lateral chains.

– The charged residues are important in locations other than near the constriction region, as for example the role of Lys-16, located on the opposite side across the

pore, in the so-called anti-loop 3, in the escape process via an interaction with the COO group of the antibiotic. The fingerprints of this role confirmed the experimental findings of an altered permeability of the pore as a consequence of mutation of the Lys-16 residue [30, 31].

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