"Molecular dynamics (MD) simulations: a computational microscope to study biological processes at the atomic level"

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Introduction

The exact dynamics and structure at the atomic level of biological molecules such as proteins, DNA, lipids or carbohydrates as well as their complexes is often inaccessible using experiments, but important to understand the function. Here, molecular dynamics (MD) computer simulations can provide crucial insights that may explain and complement experimental studies, and in some cases even suggest and predict experimental results. MD simulations generate a time series of configurations of a system, i.e. a 3D movie of the molecules, which allows the study of dynamic processes. The aim of studying biomolecular systems of ever growing complexity and size with increasing accuracy necessitates a constant development and advancement of the methodology. The research in the Computational Chemistry Group at ETH Zürich focuses on method development for MD simulations (e.g. advanced free-energy techniques) as well as their application to important biological and chemical questions (e.g. the membrane permeability of cyclic peptides).

How can cyclic peptides cross cell membranes?

Peptides and peptidomimetics have recently attracted much attention as alternative chemotypes to interfere with key therapeutic targets such as class B G-protein coupled receptors and protein-protein interactions. The preferred route for delivering drugs is oral administration, for which the compound must be able to cross the gut wall by passive diffusion. Therefore, the successful development of peptide-type drugs requires a reliable determination of the membrane permeability of molecules. As experimental assays are time and cost intensive, computational approaches are particularly appealing for this purpose.

In our group, we employ kinetic models based on multi-microsecond molecular dynamics data obtained in polar and apolar environments to rationalize the membrane permeability of cyclic peptides. As a first example, we investigated the natural product cyclosporine A (CsA) [1], which is used in medicine as a potent immunosuppressive drug to prevent graft rejection. The conformational landscape of such peptides was found to be more complicated than previously assumed, and thus not only thermodynamics but also kinetics are important to understand the unusually good permeability of CsA. Due to the increased flexibility of cyclic peptides compared to small organic molecules, different conformations can exhibit largely different polarities. As a result, the populations and interconversion rates between metastable conformational states may become important for permeability (Figure 1). The permeability of cyclosporine E (CsE), a synthetic derivative of CsA, which differs structurally only in one missing backbone *N*-methylation, is an order of magnitude lower compared to CsA. In line with this, we found that the interconversion time scales in water were nearly an order of magnitude slower [2].

The approach was subsequently applied to a series of cyclic decapeptides [3]. Six peptides were selected that had all the same backbone methylation patterns and only differed in the two residues at the turns. Nevertheless, they show large differences in permeability and aqueous solubility. Here, the simulations showed that the population of the "closed" conformation (i.e. the conformation with the maximum number of intra-molecular hydrogen bonds) correlated with the experimentally observed passive permeability.

We are in the process of applying our computational approach to diverse cyclic peptides in order to extract the general principles leading to good passive membrane permeability, such that they can be used in the design of new orally available peptidic drugs.

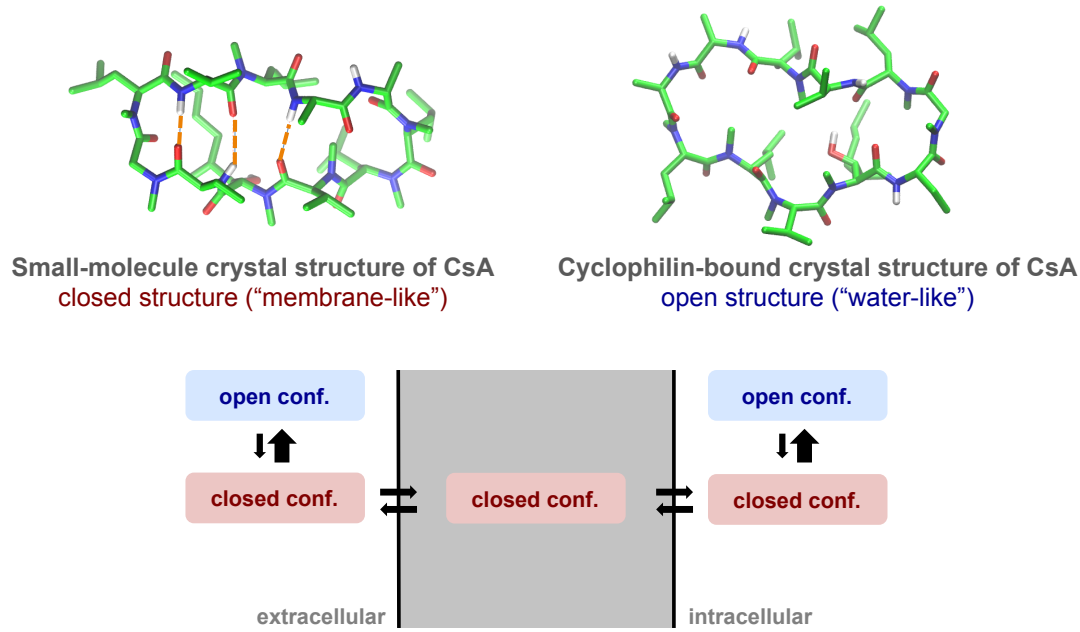

Figure 1. The known crystal structures of cyclosporine A (CsA) with the "closed" and "open" conformation and the current hypothesis for the conformational change involved in the passive membrane permeation. The orange dashed lines indicate the intra-molecular hydrogen bonds.

How can we calculate free-energy differences more efficiently and accurately?

The estimation of free-energy differences using computational tools is an important task in computational chemistry with applications in medicinal chemistry, environmental chemistry, biology and material science. We have proposed an improved method for free-energy calculations, replica-exchange enveloping distributions sampling (RE-EDS), which allows the efficient estimation of free-energy differences between multiple states simultaneously [4, 5] (Figure 2). This is achieved by combining the Hamiltonians of the individual states into a reference Hamiltonian, which can be modified by two kinds of parameters (i.e. energy offsets and smoothness parameter) to ensure sufficient sampling of all states. Two key ingredients had to be developed for estimating these parameters in a robust and automatic fashion: a scheme to efficiently obtain the energy offsets for multiple states [4] and an optimization algorithm to distribute the replicas optimally in the smoothness-parameter space [5].

One of the most important applications of free-energy methods is the *in silico* prediction of binding free energies of new drug candidates. For this task, accurate and at the same time efficient approaches are needed in order to process the large number of potentially interesting molecules. With RE-EDS, multiple molecules can be simulated simultaneously, which increases the computational efficiency by up to one order of magnitude while providing a low statistical uncertainty at the same time. In addition, the RE-EDS methodology has great potential due to its high versatility. Theoretically, any kind of Hamiltonian can be combined through the EDS reference Hamiltonian. Thus, not only the calculation of relative binding free energies between structurally unrelated compounds is possible, which is extremely challenging for other methods, but also the estimation of free-energy differences between molecules treated at the quantum level. The latter would open up opportunities in catalysis and materials design.

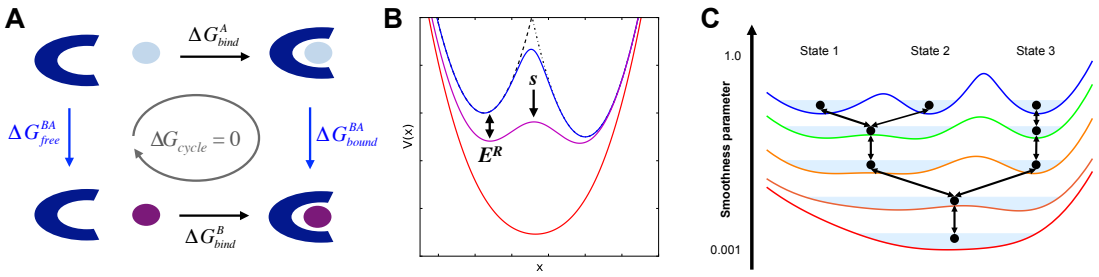

Figure 2. (**A**): Schematic illustration of the thermodynamic cycle used to calculate relative binding free energies (**B**): EDS reference-state Hamiltonian HR (colored solid lines) for two harmonic oscillators A (black dashed line) and B (black dotted-dashed line) with different values for the smoothness parameter and energy offsets. The blue line corresponds to a reference-state Hamiltonian without modulation. (**C**): Pictorial representation of the RE-EDS approach with three end-states (harmonic oscillators) where the M replicas are associated with different values of the smoothness parameter of HR (colors). The black circles denote the energetic minima of the different HR and the black arrows illustrate configuration exchanges between the replicas.

Summary and Outlook

Although the methodological advances during the past decades have transformed MD simulations into a valuable tool to study biological processes, many challenges remain open, requiring the development of even more efficient and more accurate approaches. At the same time, exciting new possibilities arise due to influences from quantum chemistry and computer sciences such as machine learning and parallel computing.

References

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