

Multiscale Aspects of Molecular Motions: From Molecular Vibrations, Conformational Changes of Biomolecules to Cellular Dynamics

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Molecular aspects of living systems are important because it is the most basic aspects of life as exemplified in biochemistry and structural biology. Since molecules move due to interactive forces between atoms, physics plays an important role to understand the dynamic phenomena of living systems. Here we review our multiscale approaches for computationally treating different levels of molecular motions: vibrational dynamics of molecules, conformational change of biomolecules, and cellular dynamics using statistical-mechanics-based models. (J Nippon Med Sch 2022; 89: 9–15)

Key words: computer simulations, molecular vibration, quantum dynamics, conformational change, cellular dynamics

Introduction

Since the discoveries of DNA structure by Watson and Crick¹ and protein structures by Kendrew² and Perutz³, it has been recognized that the molecular levels of understanding is the most fundamental in biology and life science. It is now possible to determine the 3D structures of large protein/RNA/DNA complexes including virus such as SARS-Cov-2 using X-ray crystallography, NMR spectroscopy, or cryo-EM microscopy. However, resolving the 3D structure of a whole cell is still out of reach in terms of molecular levels. On the other hand, for medical applications, it is important to understand more “macroscopic” aspects of life such as multi-cells, organs, and whole body. For both levels, molecular or macroscopic, physics can play a role because “everything” (stability and dynamics) is determined by the forces between components. At a molecular level, there are forces between atoms (covalent, electrostatic, or subtle types of forces such as hydrophobic forces), and because of such forces, atoms move according to Newton’s laws of motions:

$$m_i \frac{d^2 r_i}{dt^2} = F_i \quad (1)$$

where m_i is the mass of the i -th particle, r_i is the Cartesian coordinate of the i -th particle, F_i is the force applied

to the i -th particle. Molecular dynamics (MD) simulations⁴ are such methods to simulate molecular levels of dynamics for biomolecules including proteins, DNA, RNA, where we numerically solve Eq. (1) using (super) computers. Now we can even simulate the conformational change of the spike protein of SARS-Cov-2⁵, but it is still not efficient enough to simulate and describe the signaling processes or other more complicated processes in a whole cell⁶. This motivates us to develop “multiscale” methods⁷, combining different hierarchical levels of methods to simulate a whole cell or multi-cells. QM/MM (it is an abbreviation of Quantum Mechanics/Molecular Mechanics) method⁸ is such a method, which combines quantum mechanics and classical mechanics to treat, for example, catalytic reactions in enzyme proteins, and has been successfully applied to many biological systems since the seminal work of Warshel and Levitt⁹. The enzymatic reaction is a basic component of biochemistry or biology, and the reaction occurs only around the “active” sites of a protein, validating the use of such a multiscale method. In addition to the QM/MM method, there are a lot of multiscale methods, and we here review some of our recent attempts to devise multiscale methods in molecular science, chemical physics, or biophysics, hoping

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them to connect to biochemistry and biology of a cell.

Vibrational Dynamics of Molecules

Atoms are often connected by strong covalent interactions in molecules, which is approximated by harmonic springs, inducing *molecular vibrations*. When a hydrogen atom is attached to another atom, its vibrational period is $\sim 10 \times 10^{-15}$ second (~ 10 femtosecond). By combination of different types of atoms, molecular vibrations become complex and have longer timescales, reflecting in optical (IR or UV) spectroscopy¹⁰. It is quite important to understand and interpret optical spectroscopy experiments because molecular structures and dynamics are embedded in them. Many experimental techniques and theoretical methods have been devised over 100 years, and here we focus on the latter. It is known that molecular vibrations are mathematically well described by independent oscillators, called *normal modes*, in molecules¹¹. Each normal mode vibration has a kinetic energy and a spring energy, so the total energy for N normal modes is written as

$$E = \sum_{i=1}^N \left(\frac{p_i^2}{2} + \frac{\omega_i^2}{2} q_i^2 \right) \quad (2)$$

where p_i is the i -th momentum, ω_i is the i -th frequency, q_i is the i -th coordinate of a normal mode. By adding the information of oscillator strength, this energy representation can well describe the spectroscopic features of molecules. However, there remains residual interactions between the coordinates, which will be written as

$$E = \sum_{i=1}^N \left(\frac{p_i^2}{2} + \frac{\omega_i^2}{2} q_i^2 \right) + \sum_{ijk} C_{ijk} q_i q_j q_k + \dots \quad (3)$$

where C_{ijk} is the coefficients of (3rd order) *anharmonic interactions* between three coordinates q_i, q_j, q_k , inducing the interaction among normal modes. Without these anharmonic terms, the molecular motions are superposition of different frequencies, and the resulting motion is called quasi-periodic. By adding the anharmonic interaction in Eq. (3), the *energy transfer (flow)* between normal modes can take place. This is a very important process in molecular dynamics because this phenomenon of energy transfer is related to the ergodicity problem in statistical mechanics¹², assuring the equilibrium ensemble of many-degrees of freedom systems including molecules. (Based on this concept, we can derive a very useful formula for a chemical reaction rate called *transition state theory* first derived by Eyring, Evans, and Polanyi¹³.) The energy transfer efficiently occurs through the following *resonance condition* when the 3rd order coupling is effective as in Eq. (3):

$$|\omega_i - \omega_j - \omega_k| \approx O(C_{ijk}) \quad (4)$$

In this case, if the i -th mode is excited and all the other modes are unexcited (with zero energy), the excess energy tends to flow to modes j and k efficiently.

This is a well-known process in classical mechanics and this resonance structure Eq. (4) is also present in protein dynamics¹⁴. However, some vibrational modes (such as bonds containing hydrogen atoms) in (bio)molecules have high frequencies, which energy is a few times higher than the thermal energy (~ 0.6 kcal/mol), and as such quantum effects might play a role. Quantum mechanics is described by the following Schrödinger equation:

$$i\hbar \frac{\partial}{\partial t} \Psi(r_1, r_2, \dots, r_N, t) = H\Psi(r_1, r_2, \dots, r_N, t) \quad (5)$$

where \hbar is the Planck's constant, H is the Hamiltonian operator corresponding to the energy of the system, and $\Psi(r_1, r_2, \dots, r_N, t)$ is the wavefunction of the system. The wavefunction has all the information of the system, and we can extract positions and velocities of a system as an expectation value using the absolute square of the wavefunction as a probability density. Solving this equation is, however, completely different from solving Newton's equation Eq. (1), and much harder (if we can build a quantum computer, the situation will be totally different though). Many researchers have been looking for efficient but approximate methods for solving Eq. (5).

Previously, we addressed this issue of how to solve the Schrödinger equation for molecular vibration problems. For small (~ 10 atoms) molecules, we devised several "exact" methods, VSCF/VCI method¹⁵ and molecular tier method¹⁶, and successfully applied them to N-methylacetamide and acetylbenzotrionitrile in gas phase, respectively. For larger systems, we devised a perturbative method, which treats the anharmonic interaction in Eq. (3) as a perturbation in Eq. (5), and applied them to amide-I modes in proteins¹⁷ and some vibrational modes in porphyrin¹⁸. We also carried out the corresponding classical dynamics calculations, which use Newton's equations of motion, Eq. (1), and compared them with the numerical results using quantum mechanics. The comparison is often good, validating the use of more economical classical methods. In Ref.¹⁹, we combined the quantum mechanics and classical mechanics, where we solve the Schrödinger equation Eq. (5) and Newton's equations of motion Eq. (1) simultaneously. This is a kind of multiscale methods, which is useful for treating a

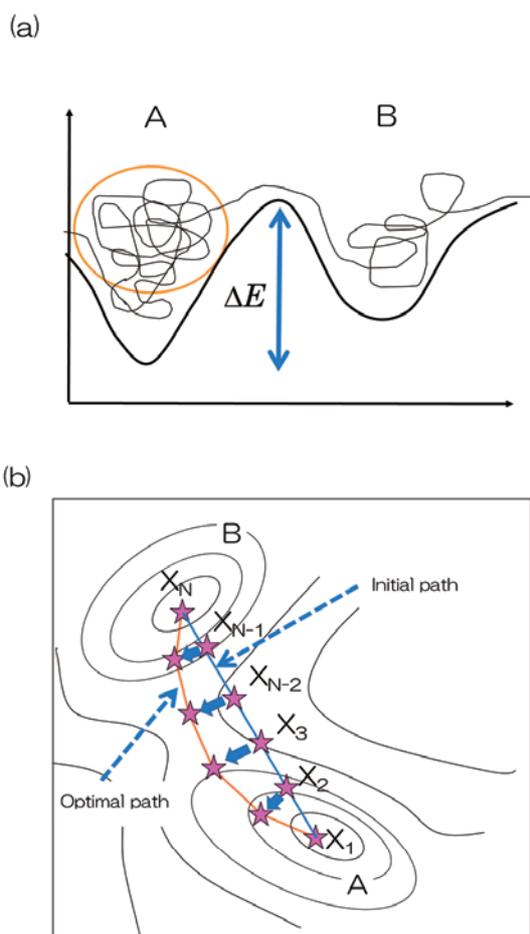


Fig. 1 (a) Schematic picture of chemical reaction or conformational change along a reaction coordinate. The barrier energy from the left basin is ΔE . (b) a path search strategy in a two dimensional model energy landscape. First we assume a straight but energetic path and according to some criterion, we can relax the initial path into an optimal one, traversing a transition state.

quantum system with time-varying parameters.

Conformational Changes of Biomolecules

Vibrational dynamics in the last section is assumed to occur in a single metastable basin of energy landscape. However, when a molecule reacts or conformational change takes place, there occurs a “hopping” or a transition between metastable basins (see **Figure 1(a)**). This is a famous activation process in chemistry, and the transition rate is often described by the Arrhenius law:

$$k = \nu \exp(-\Delta E/k_B T) \quad (6)$$

where ΔE is an activation energy (energy barrier), T is the absolute temperature, k_B is the Boltzmann constant, ν is an attempting frequency. This formula shows that the activation is very slow or rare when $\Delta E \gg k_B T$, which is

often the case in chemical reactions and conformational change of molecules.

Here two problems appear for computing this kinetic rate. One is that this kind of process takes place very slowly so it is very hard to directly simulate it using MD simulations. When we solve Eq. (1) for molecules, we discretize it as (this is the simplest Euler scheme though we usually use more sophisticated schemes)

$$\begin{aligned} r_i(t + \Delta t) &\approx r_i(t) + v_i(t)\Delta t \\ v_i(t + \Delta t) &\approx v_i(t) + F_i(t)\Delta t/m_i \end{aligned} \quad (7)$$

using a small time step $\Delta t \sim 1$ femtosecond ($\sim 1 \times 10^{-15}$ second). The activation processes including chemical reactions, protein folding, conformational changes, ligand (un)binding can take place with micro- to milli-second timescales ($10^{-6} \sim 10^{-3}$ second), so we need $10^9 \sim 10^{12}$ iterations of Eq. (7), which is not feasible for large molecules even using supercomputers. The other problem is that the Arrhenius formula itself is not accurate enough for various types of activation processes, so we need more sophisticated formulas to understand and estimate the transition rate.

For attacking these fundamental problems, many methods have been recently introduced and we explain several examples from our own studies. The first method is the *string method* devised by E, Ren, and Vanden-Eijnden²⁰, which is a useful method for finding a path connecting two metastable states. This is a path-based method, and as shown in **Figure 1(b)**, we assume an initial path and according to some criterions, we relax such a path into an optimized one. When we use on-the-fly string method²¹, such a path becomes a minimum free energy path, which can be thermally most populated path. We applied this method to conformational changes of two biomolecular systems, adenylate kinase (AdK)²² and AcrB transporter (membrane protein)²³. For each system, two crystal structures were taken from PDB (protein data bank), which are regarded as an initial and a final state for the string calculations. Our purpose is to connect these two states along a most plausible path.

However, if we use all the coordinates (all the atomic positions in biomolecules), the string method would fail, that is, the path does not converge to the optimized one and gets trapped somewhere not in optimized positions because the energy landscape of biomolecules is very rugged²⁴. The most straightforward approach to this problem is to rely on powerful sampling methods²⁵, but sampling huge path space is much harder than configurational sampling (and configurational sampling itself be-

comes intractable for large biomolecules). We thus usually attempt the other approaches, where we use collective variables (CVs) to restrict our sampling space and obtain the result more efficiently. CVs are some variables in configurational space, and for biomolecules, they might be some heavy atom coordinates, or angles among them, or hydrogen-bond distances, which are physically or chemically motivated or determined by machine learning (artificial intelligence) techniques. For the above examples, AdK and AcrB, we used several principal components (extracted from *principal component analysis (PCA)* for the heavy atom fluctuations calculated by equilibrium MD simulations of the system) and heavy atoms in the membrane region, respectively.

In CV space, we first assume an initial path, which is mathematically continuous but for numerical computation, it is discretized using “segments” or “beads” as shown in **Figure 1(b)**. And each bead moves according to the free energy gradient (on that point) calculated by the on-the-fly MD simulations using the mean-force dynamics²⁶. If we only use this procedure, every bead converges to the basins of attraction, and the path image is gone. So we add another procedure: the distances between the neighboring beads should be the same, and this is called equidistance criterion. Because of this, the path always looks continuous until the end of calculations. From thus obtained path, we can extract the information of a transition state because it should be located in the middle of the path. Transition states give us the mechanism and bottleneck of the reactions, which will be important for further elaborations (one such analysis is the committor test¹³) or mutation studies.

The minimum free energy path explained above is a nice representation of a reaction including conformational change, but it does not necessarily capture the kinetic aspects of the reaction. For example, substituting thus obtained value of the free energy barrier ΔE into the Arrhenius formula Eq. (6), it does not often predict the reaction rate with sufficient accuracy even if we evaluate the prefactor correctly. The most common approach to obtain kinetic information without using the Arrhenius formula is *Markov state model (MSM)*^{13,27}, where we run short-time multiple MD simulations from different initial conditions, and calculate the so-called “transition matrix” from thus obtained huge trajectory data, and evaluate the *mean-first passage times (MFPT)*¹³ between “states”, which is defined by some clustering algorithm. From MFPT, we can calculate the transition rate from state A to B as $1/\text{MFPT}(A \rightarrow B)$. This method is now very popular for ana-

lyzing the kinetic properties of trajectories, but there is always concern about how Markovianity (memory-loss) properties hold for the trajectory data, which are intermixed with the choice of “states” or CVs and the length of trajectories.

The most general and legitimate approach for extracting kinetic properties and CVs is *transition path sampling (TPS)* method introduced by Chandler and coworkers^{13,28}. In this method, a path itself (not a configuration) is regarded as a quantity to be examined, and Monte Carlo sampling of path space is realized by moving a whole path using some algorithms. After the calculation, a path ensemble is generated, from which we can extract the transition states and kinetic properties and even the most plausible CVs using the so-called committor test. Though TPS is conceptually beautiful and general (for overdamped Langevin dynamics, the Onsager-Machlup action method^{29,30}, which is a variant of TPS, can be used), moving a whole path and generating a path ensemble is much harder than configurational sampling (which is mainly used for calculating free energy landscapes), especially for larger biomolecules and slower processes. This is why many researchers are trying to develop more efficient and less expensive methods for path sampling, including transition interface sampling²⁸, forward flux sampling³¹, and nonequilibrium umbrella sampling³² etc.

Recently we have been using the *weighted ensemble (WE) method* introduced by Kim and Huber, and further elaborated by Zuckerman and coworkers³³. In the WE method, we first assume some CV space where state A and B are defined. We then divide CV space into several segments, which are called cells, so that the short-time MD simulation can fully explore a single cell. In each cell, we prepare several trajectories or particles, and each particle has a weight, where the sum of weights in each cell can approximate the population in the cell. There is another procedure: birth and death processes. We predefine the number of particles N in a cell, and if a particle moves into an empty cell, the particle is then divided into N particles there. During this process, the weight is also appropriately divided so that the population in a cell becomes constant. If there are more than N particles in a cell, we need to “kill” some of them so that the number in a cell becomes the same N .

We applied this WE method to several biomolecular systems. The first example is folding/misfolding transitions of a small artificial protein, chignolin³⁴. With the conventional amber force field, this system has two metastable regions, one is the folded region and the other is

misfolded one. For easiness of calculations, Mitsutake and Takano elevated the temperature up to 420 K (near folding temperature), and carried out brute-force MD simulations to observe the folding/misfolding transitions of chignolin³⁵. But they needed to simulate at least ~ 1 microsecond to calculate the relaxation modes and the transition matrix. By using the WE method, we can simulate the transition with much shorter timescale MD simulations though there is a burden that we have to rely on multiple MD simulations with many replicas ($\sim 1,000$ particles). However, this burden will be soon overcome because of the advance of multiple core technology. In this case of chignolin dynamics, we used two different types of CVs: one is hydrogen-bond distances and the other is diffusion map coordinates³⁴, but the result does not depend much on the choice of CVs.

The second example is the isomerization of a substrate in PIN1 enzyme protein³⁶. In this case, a torsional angle (omega angle), representing the rigidity of the peptide plane of proline in the substrate, is the target of calculations and naturally a candidate for CVs, and the isomerization barrier is rather high (~ 10 kcal/mol) even though the catalytic interaction between the substrate and the enzyme lowers the original barrier (~ 20 kcal/mol) in water. We can estimate and characterize the kinetic properties of the forward and backward transitions of isomerization, and also carried out some mutation studies for further comparison with free energy landscape calculations³⁶.

Cellular Dynamics Based on Statistical Mechanics

The MD simulation methods explained above have been developing because of the advances of hardware, software, and algorithms, and the target system is now as big as virus⁵ or a very crowded environment in a cell⁶, but it is still not efficient for simulating a whole cell. Incidentally there is famous Moore's law, predicting that the computing power becomes $\times 1.5$ every two years. Assuming this law and that the number of atoms in a cell is 1,014, we can execute 1 second MD simulation of a whole cell within 100 days in 75 years³⁷. This is beyond the patience of current researchers, and we need more efficient and approximate methods to circumvent this situation.

A traditional approach in physics for this problem is *coarse-graining*. For example, in fluid dynamics of water, we consider the density and velocity fields for water not the positions and velocities of individual water molecules, making it possible to simulate large-scale hydrody-

namic phenomena such as atmosphere-ocean dynamics on earth. Another coarse-graining procedure applies to biomolecular simulations, where each residue is treated as a ball, neglecting the details of side chains etc.³⁸. In MD simulations, we usually employ empirical force fields, which is determined by some quantum chemistry calculations and experiment, but its validity is limited. Recently many researchers use machine learning (ML) or artificial intelligence to improve the accuracy of force fields³⁸, and ML can be considered as a systematic way to coarse-grain a molecular system.

To simulate cell dynamics, coarse-graining is still difficult because a cell is a strongly heterogeneous system, and we need to rely on more empirical and approximate approaches. Though there are a lot of such approaches to computationally treat a cell, we chose to use the *Cellular Potts Model (CPM)*, which is based on statistical mechanics³⁹. A cell consists of many small "segments" which is placed on a (two-dimensional or three-dimensional) lattice, and the occurring frequency of the segment configuration is determined by the "energy" of a cell. The motion of the segment is determined by a Metropolis procedure often employed in Monte Carlo methods [4]: We first choose a segment to move in a random way, and then calculate the energies before and after the movement, and the movement is accepted or rejected using a criterion related to the energy difference.

This model itself has nothing to do with the molecular picture of a cell, and is regarded as an emulator of a cell (like cellular automata, e.g., life game). However, there is a connection to a molecular picture: in an actual situation, there are chemicals such as Ca ion or ATP generated and coupled to the cellular dynamics. As such, in the simulation, we need to set up the equation of motion for such chemicals, which are basically reaction-diffusion (RD) equations⁴⁰ used in modeling pattern dynamics for biological systems (a typical example is the Turing pattern). To simulate cellular dynamics with these chemical details, we need to combine CPM and the RD equations, which is another type of multiscale modeling.

We applied this combined method to wound healing process for a model tissue, which was experimentally set up and examined by Takada and coworkers⁴¹. In the experiment, they applied stretching forces to a model tissue with a wound, and the healing process turned out to be faster compared to the case without such forces. We modeled this situation with the above method, and successfully reproduced the experimental finding⁴². We are now trying to use the same strategy for simulating angi-

ogenesis, which is most important for wound healing for real human systems, to further elucidate the basic mechanisms of mechanotherapy⁴³.

In this review, we started with a discussion of the vibrational motion of molecules, then protein conformational changes, and finally cellular dynamics. While there are “subtle” connections between molecular vibrations and conformational changes, or between molecular conformational changes and cellular dynamics, the “exact” connections are lost and difficult to characterize. We hope to uncover these connections by combining new experiments with more sophisticated algorithms, theories, and computations.

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