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学位論文題目　Development and Application of the Free Energy Based Screening Methods

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論文内容の要旨

Owing to the dramatically increased number of 3D structures of pharmaceutical targets, structure based approaches play an important role in drug design. Especially, rapid and accurate estimation of binding affinities is essential for efficient screening of drugs. For this purpose, the free energy based screen methods have been developed by some groups. These methods are much faster than the free energy perturbation (FEP) method or thermodynamic integration method, and give more accurate relative free energies than the empirical scoring function methods.

The λ-dynamics method, one of these methods, has been recently developed by Brooks and co-workers. The λ-dynamics method is an extension of the FEP method. It differs from the FEP method in the following aspects: (1) In the FEP method, a single coupling parameter λ is used to transform one ligand to another. While in the λ-dynamics method, multiple λs, each corresponding to a given ligand, are used. Because of this feature, the binding free energies of multiple ligands are evaluated simultaneously. (2) In the FEP method λ is fixed during the simulation. While in λ-dynamics, the λs evolve according to their equations of motion via use of an extended system. The free energy difference can be obtained from the probability of the ligand i having the dominant state (i.e. $\lambda_i=1$, $\{\lambda_{m\neq i}=0\}$). In this method, the total computation time is not expected to increase with the total number of ligands because only the few favorable binders are able to compete for the $\lambda=1$ state. This is in contrast to the conventional free energy calculation methods.

The λ-dynamics method was used to study the binding of 10 heterocycle derivatives to an artificial cavity created inside cytochrome c peroxidase and the host guest system of β-cyclodextrin-monosubstituted benzene derivatives. Straightforward application of λ-dynamics using a multiple topology approach resulted in trapping in local minima. To extend the λ-dynamics method to multiple topology model, a new restraining potential, which keeps the ligands in lower-energy states, is added to the λ-dynamics Hamiltonian. Relatively short time λ-dynamics simulations with the restraining potential successfully identified the best binders as compared with both experimental data and FEP calculations. Using the iterative procedure with biasing potentials to enhance convergence, λ-dynamics method successfully yielded reasonable estimates of the binding affinity of all ligands. Furthermore, long time λ-dynamics simulations revealed that better ligands tend to have small statistical errors, which is appropriate for screening out the plausible ligands from all candidates. This methodology also provides a means to explore the binding orientations and conformations of the ligands inside the binding pocket much better than does conventional MD. A λ-dynamics simulation starting from random initial orientations, in which some ligands take significantly different orientations as compared with those from the X-ray structure, successfully sample the X-ray crystallographic orientations in all ligands, even though conventional MD starting from the same initial structures remain trapped in the local minima from which they start. Such an efficient sampling of ligand orientation and conformation is expected to diminish the limitation that an initial ligand structure must be close to its true bound orientation in order to get a reasonable estimate of binding free energy.

The incorporation of the generalized Born (GB) approach into free energy simulation methods (e.g. FEP or λ-dynamics) using thermodynamic cycles was introduced and applied to the trypsin-benzamidine derivatives system and seven mono-substituted benzene derivatives bound to β-cyclodextrin. Since the GB model is fully analytical continuum solvation representation, derivatives of the energy with respect to individual atoms are available and allow the effect of solvation to be efficiently included in molecular dynamics. The GB energies for the intermediate states, in which more than one ligand obtained competitive λ values, were defined by two ways. Both GB coupling schemes have been incorporated into the program CHARMM. The free energy simulations using both definitions of the GB implicit solvent model gave consistent binding free energy differences (ΔΔG) as compared to those using an explicit solvation model. Non-electrostatic solvation energy contributions, which are not included in the GB energies and approximately related to the solvent-accessible surface area, were successfully included using umbrella sampling techniques. Furthermore, a variant of the λ-dynamics approach, Chemical Monte Carlo / molecular dynamics method (CMC/MD), was implemented in CHARMM and compared with FEP and λ-dynamics methods. In the CMC/MD method, the Metropolis Monte Carlo criterion is used to evolve the λ-space and molecular dynamics is used to evolve the atomic coordinates. Free energy differences calculated using the GB energy agreed well among FEP, λ-dynamics, and CMC/MD. The λ dependent partial charge model was also introduced for the incorporation of hybrid topology model into the λ-dynamics method. In this model, the invariable ligand atoms are represented by the single topology and their partial charges are altered according to the movement of the coupling parameters as they would be the same as those of the end points. The hybrid topology λ-dynamics/GB simulations successfully converged without any restraining potential, however, the sampling configurational space was restricted as compared with that of multiple topology model.

They have also applied the λ-dynamics method for the stability analysis of the DNA-binding domain of the Myb transcriptional regulator. In this case, seven different side-chain mutants simultaneously compete to make the protein stabilize, whereas, multiple ligands compete in the previous λ-dynamics simulations. A single short (300 ps) λ-dynamics simulation successfully identified the best stabilized mutant. Furthermore, a series of λ-dynamics trajectories generated by the iterative techniques based on Weighted Histogram Analysis Method (WHAM) showed excellent correlation with data obtained from the conventional FEP simulations or experiments. The additional biasing potentials along λ coordinates successfully increased the ratio of the end points without wasting time by sampling unphysical intermediate states in the λ-dynamics simulations.

Furthermore, a hybrid Monte Carlo and Langevin dynamics method (MC/LD) was introduced to study the binding orientations of toluene in β-cyclodextrin. In this method, the guest atoms are replicated. One of the replicas is sampled with the full force of the host, while the rest of the replicas are sampled with scaled "ghost force" to find the other local minima. In constant time intervals of Langevin dynamics (LD) simulation, Monte Carlo method is applied to choose a new guest among all replicas, which helps to jump the barrier quickly. The trajectory of

the MC/LD simulation successfully gave the broader set of binding orientations as compared with the conventional MD trajectory. The potential of mean force calculated using its trajectory gave the good agreement with that calculated by intensive computational use of umbrella sampling method and WHAM. Therefore, MC/LD can succeed in both exploring the free energy surface much more efficiently and yielding the canonical ensemble of the system.

In conclusion, they have developed and applied the free energy based screening methods such as λ-dynamics and CMC/MD. These methods will be used either to rapidly identify ligands with favorable binding free energy or to estimate specific change in free energy using the iterative procedure with WHAM. Since they screen the binding free energy of the ligands instead of interaction energy, they provide accurate assessment of binding affinity. The restraining potential is very effective and important for the application of the λ-dynamics method to a multiple topology model. It successfully enhanced both sampling of λ-space and binding configurations of the ligands. The combination of the GB model with λ-dynamics or CMC/MD has a great potential in the application for drug lead optimization. These combinations may fill the gap between the empirical methods using a single minimized complex structure and the theoretically rigorous methods like FEP or thermodynamic integration. The hybrid topology λ-dynamics representation with λ dependent partial charge model will be the promising representation when one investigates free energy changes for an ensemble of slightly varying ligands. Moreover, the MC/LD method can be applied efficiently to explore the free energy surface which will be useful in many purposes such as the protein folding studies, the loop search, or the conformational search of side chains.

論文の審査結果の要旨

創薬においてはデータベース上の何百万という化学物質から、ある目的を持った薬剤として有望な候補物質を計算科学的手法を用いたスクリーニングにより選び出し、その上で実験的研究を開始するというスタイルがとられつつある。しかしながら、現在のところこのスクリーニングは経験則に基づいて行われており、候補物質の絞込みの精度は低いものでしかない。本研究は薬剤設計において本質的に重要となるタンパク質とリガンドとの結合安定性の予測を、自由エネルギー計算に基づいて精度よく行うための実用的な計算科学的手法の確立を目指したものである。

研究の第一歩として、実際のいくつかの系に対して、λ-ダイナミックス法と呼ばれる既存の手法を適用して計算を実行し、この方法の有効性や限界について評価を加えている。その結果、実用レベルでの方法論を確立するためにはより効率的なサンプリング法の開発が不可欠であるとの結論に至っている。そこでこの問題の解決のために、λ-ダイナミックスにアンブレラサンプリングの手法を取り入れ、重み関数を導入することによりあまり安定でないリガンドの出現確率も人為的に高くする方法を提案している。これにより、全空間、全物質を効率的にサンプリングすることができるようになり、多数のリガンドの結合安定性、自由エネルギー変化を一度の計算で精度よく求めることに成功している。

一方、溶媒である水の存在が、このようなリガンドとタンパク質の結合自由エネルギー変化に本質的に重要な働きをすることはよく知られていることである。この溶媒効果を実用計算のレベルで、極端な計算負荷の増大を伴なわずに取り扱う方法として、一般化 Born モデルをλ-ダイナミックスに適用した計算手法の提案を行っている。λ-ダイナミックスにおけるこの新しい計算方法をまずは比較的単純な系に適用し、その結果をポアソン方程式に基づいたモデルやあらわに水を取り扱う方法と比較することにより、質的に同等の精度を維持しながらも計算時間を大幅に削減できることを実証している。そしてこの方法をより複雑な実際のタンパク質-リガンド系に対して適用し、リガンドとタンパク質の結合自由エネルギー変化に対して計算の有効性を確認している。そしてさらには、溶媒との相互作用にランダム力を仮定するランジュバンダイナミックスをλ-ダイナミックスに用い、一層の計算時間の短縮を図っている。

これらの研究成果は、3編の原著論文としてすでに J. Chem. Phys. および J. Phys. Chem. に掲載され、さらに3報が投稿中である。

以上のことから、本論文は博士(理学)の学位論文として十分であると判断する。

口述試験においては、出願者による研究発表も含めて約 3 時間の質疑応答を行った。研究発表は、高度なシミュレーションの方法論が他分野の審査員にもよく理解できるように整理され、計算の本質に対する理解をうながすレベルの高いものであった。質疑も活発に数多くなされたが、これらに対する応答も意をそらすことなく的を射たものであり、一方で現実社会における本研究成果の適用に関しても今後解決すべき問題点を明確に提示するなど、理解の深さをうかがわせるものであった。

語学力に関しては、博士論文が的確な英文で書かれており、既に発表済みの英文論文等からも十分な水準に達していると判断された。さらに、8月 30 日の公開発表会でも、時間内に論文内容の要を得た説明がなされ、質疑に対する応答も適正なものであった。

以上の結果より、審査委員全員の一致した評価として出願者が本学の博士(理学)として充分な学力を備えており、適格であると判定した。