Free Energy Perturbation Method in GENESIS

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Accurate predictions of protein-ligand binding affinities have been a central challenge in in-silico drug design to reduce the total cost and time required for drug development. The free energy perturbation (FEP) method based on the all-atom molecular dynamics (MD) simulation is one of the most essential tools to predict the binding affinity and solubility of ligands with high accuracy. We implemented the FEP method to MD software "GENESIS" [1]. FEP in GENESIS can predict relative and absolute binding affinities as well as relative and absolute solvation free energies with high accuracy [2,3]. We also proposed a modified FEP scheme by introducing non-uniform scaling parameters into Hamiltonian [4]. Modified FEP greatly improves the computational performance, which is marked for large biomolecular systems. Here we introduce the FEP functions in GENESIS and discuss about the applicability of modified FEP to drug discovery on supercomputer "Fugaku".

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Lipid composition is critical for accurate membrane permeability prediction of large cyclic peptides by molecular dynamics simulations

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Keywords: Cyclic peptides, Membrane permeability, Molecular dynamics

Poor membrane permeability is the biggest bottleneck hindering successful drug discovery based on cyclic peptides. Therefore, the development of computational methods that can predict membrane permeability and support elucidation of the membrane permeation mechanism of drug candidate peptides is eagerly awaited. In this study, we developed a protocol to simulate the behavior in membrane permeation steps and estimate the membrane permeability, especially for large cyclic peptides which are prone to achieve high affinity with the target.[1] This protocol requires the use of more realistic membrane model than a single-lipid phospholipid bilayer. To select a membrane model, we first analyzed the effect of cholesterol concentration in the model membrane on the potential of mean force and hydrogen bonding networks along the direction perpendicular to the membrane surface as predicted by molecular dynamics simulations using cyclosporine A. These results suggest that a membrane model containing 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine and 40 or 50 mol% cholesterol was suitable for predicting the permeation process. To validate the efficiency of our protocol the membrane permeability of 18 10-residue peptides were predicted. The total calculation in this study took approximately 860 thousand GPU hour using NVIDIA P100 GPUs. Correlation coefficients of $R > 0.8$ between the experimental and calculated permeability values were obtained. The results of this study demonstrate that the lipid membrane is not just a medium, but also among the main factors determining the membrane permeability of molecules. The computational protocol proposed in this study and the findings obtained on the effect of membrane model composition will contribute to build a schematic view on the membrane permeation process. Furthermore, the results of this study will eventually aid the elucidation of design rules for peptide drugs with high membrane permeability.

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Molecular Dynamics Study of 3D-DS dimerization process of Cyt *c*

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Keywords: Molecular Dynamics, 3D-DS, Free-energy analysis

The 3-dimensional domain swapping (3D-DS) is a phenomenon where multiple proteins form unique complexes by donating part of their domains each other [1,2]. One of the characteristic points of 3D-DS is that the formed 3D-DS complexes are rather stable because the inter-protein interaction is the same as the intra-protein one in their monomer structure. Then, 3D-DS is possibly utilized to make an artificial protein complex to regulate, combine, encourage or discourage their functions and activities. 3D-DS formation occurs when denatured monomers by alcohol or salt refold by dilution. However, its detailed formation process is not so well understood.

In this study, 3D-DS formation process of the cytochrome *c* (cyt *c*) complex as shown in Fig. 1 was studied by molecular dynamics simulations with a coarse-grained (CG) model and the smoothed Wang-Landau (SWL) method [3]. As the distance of the two cyt *c* monomer plays a critical role in forming their complex, the 1- (energy) and 2-dimensional SWL-MD (energy and gyration radius) are applied to enhance the conformational sampling. In Fig. 2, obtained trajectories by the conventional CG simulation and 1D and 2D SWL simulations are mapped onto the subspace spanned by root-mean-square deviations (RMSDs) from monomer A/B structures indicating the sampling efficiency of each method. Although 3D-DS region is the lower left corner, the conventional CG did not sample this region. On the other hand, 1D and 2D SWL simulations can sample the 3D-DS structures. As an example, the sampled CG model was superimposed with the crystal structure (see figure legend in detail). The model fit well with the crystal structure, indicating the robustness of our SWL method. The results of a free-energy analysis will be explained in the presentation.

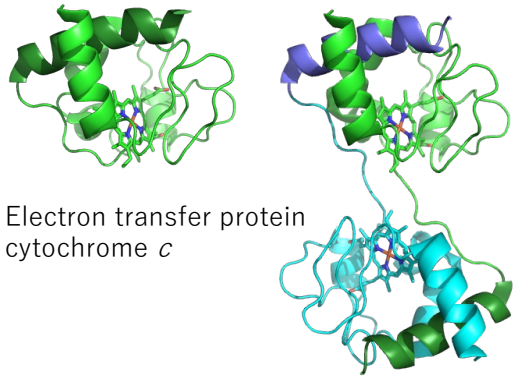

Fig. 1 Monomer and 3D-DS dimer structure of cyt *c*, where blue and cyan colors indicate chain A and light and deep green ones chain B, respectively.

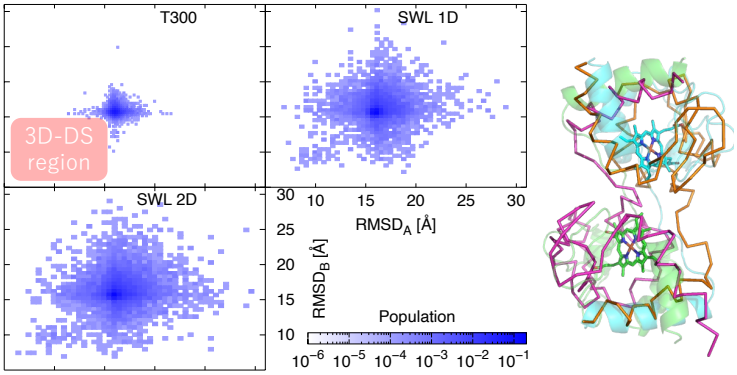

Fig. 2 Population of the samples on the RMSD$_{A/B}$ plane; RMSD$_{A/B}$ is RMSD of the monomer A/B with respect to the 3D-DS structure (PDB ID: 3NBS). Right panel shows the most similar structure to the 3D-DS structure obtained from MD simulation.

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O3-4

Complex model of the bile acid transporter NTCP and binding peptides involved in the HBV infection explored by molecular dynamics simulation

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Keywords: hepatitis B virus, sodium taurocholate cotransporting polypeptide, preS1, Mycludex B, cyclosporine A, molecular dynamics simulation

Hepatic diseases involved in the infection of hepatitis B virus (HBV) are one of the worldwide issues of health [1]. In the cellular entry process of HBV, the N-terminal myristoylated preS1 domain (myr-preS1) of the viral large protein interacts with sodium taurocholate cotransporting polypeptide (NTCP), i.e., NTCP acts as both a bile acid transporter and a receptor of the HBV entry [2]. Therefore, NTCP is a pharmaceutical target, and its inhibitors, such as small compounds, a peptide drug mimicking myr-preS1 called MyrcludexB, and a cyclic peptide cyclosporine A (CsA) and CsA derivatives, have been approved and developed [1,3]. In 2022, the structure of human NTCP has been solved for the first time using cryo-EM technique [4,5]. The structure adopts an outward-facing state, and a hole is formed. However, in complexes with myr-preS1 or Myrcludex B, the binding peptide were unresolved, and therefore, the complex structure and interaction modes were still unknown. Since the binding peptide is flexible and is presumed to be non-resolving due to its dynamic binding mode, a method that is capable for searching dynamic binding modes, such as molecular dynamics (MD) simulation, will be effective. In this study, using MD simulation, complex structure model of NTCP and binding peptides was explored. The regions of NTCP in contact with myr-preS1, Myrcludex B, and CsA were analyzed from the complex model. The NTCP residues with high frequency of contacts were essentially identical to the regions experimentally shown to be important for the HBV infection.

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MAXS for 3D molecular visualization of human antibody conformational changes

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Keywords: solution scattering, MAXS, conformational change, flexibility

Biopharmaceuticals, especially antibody drugs, have been increasing their use and presence. While they have the advantage of higher specificity and lesser side effects, they have the disadvantage of expensive development and production costs. Moreover, maintaining and controlling their quality is more difficult since they consist of extensive protein moieties with a complex molecular nature. The complexities come from both chemical and physical characteristics and also their heterogeneities. The chemical heterogeneities come from spontaneous degradation; charge variants are one of them, and biochemical heterogeneity is like sugar chain variation. Such chemical characteristics have been well assigned and controlled; however, the rapid expansion of novel modalities is becoming more challenging to develop analytical methods for novel antibody-based drugs.

In contrast, most analytical methods for physical characterizations tolerate the varieties in those novel modalities. However, many of those are time and sample-requiring processes. Moreover, the results are challenging to manage and limited in use, such as monitoring the identities because the essential information for physical properties, the three-dimensional structures, are unavailable. X-ray crystallography and single particle cryo-EM observation are the established methods to explore the tertiary structure of biomolecules. However, those results are from some extreme conditions, may be affected by crystallization or freezing, and are experimentally selected from a specific group of the original ensemble. Those limitations come from the flexible nature of biomolecules. Especially antibodies are highly flexible, mainly between Fab-Fc domains and essential sugar chains, making crystallization of whole antibody molecules unrealistic and/or limited insight from frozen snapshots.

It is undoubtedly of great help in developing novel modalities, their CMC studies, and quality control; once we had a method to visualize the flexible biomolecules avoiding prerequisites and specific requirements. X-ray solution scattering experiments have been utilized to analyze structures and conformational changes. In those experiments, scattering data within a small-angle region are widely used and thus called SAXS (Small Angle X-ray Scattering). The SAXS experiments often focus on macroscopic shapes and sizes of the molecules. In principle, the scattering data among the higher region contains detailed structural information; thus, we could obtain much information; for example, q values around 0.30 to 0.65 $Å^{-1}$ reflect distances between domains, secondary structure components, and/or adjunct chemical groups in ADCs. The experiments should give us a novel picture of complex molecular behaviors as well as conformational changes of flexible biomolecules.

In the above background, we are substantially working on the molecular visualization of medicinal macromolecules utilizing extensive SAXS data reaching the sub-WAXS region. We abbreviate it as MAXS, Middle Angle X-ray Scattering. As shown in our presentation, MAXS experiments give us novel and valuable information that could lead to the molecular design and processing of antibody drugs. In this presentation, as the first step of our work, we will discuss the solution structure analysis of human IgG that revealed significant differences between the solution and crystalline states, as well as a novel observation of its flexibility. We also discuss the effects of the sugar chain moiety in the Fc region on the structural nature of human IgG.

CrotBiopsy: New methods for evaluation of lung inflammation

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Keywords: CrotBiopsy, serum, lung inflammation, bleomycin

In general, laboratory methods for assessing health effects rely heavily on blood tests. However, the amount of sample used in the test is limited, and since it is only for the liquid factor, the data obtained is also limited. Furthermore, there are often various limitations when collecting samples, and repeated sample collection from the same patient should be avoided. Therefore, we proposed a testing method that focuses on clots that are discarded as precipitates during serum preparation. An advantage of using a clot biopsy is that both the protein contained in the clot and the industrial waste discarded during serum preparation can be used.

In this study, we succeeded in detecting NF-κB as an activation marker in a mouse model of bleomycin-induced pulmonary inflammation by preparing clot lysates. These results strongly suggested that the clotbiopsy method would be a useful method for assessing biological effects.