**Theoretical Molecular Science Laboratory**
**Chief Scientist: Yuji Sugita (D.Sci.)**

**(0) Research field**

CPR Subcommittee**:** Chemistry

**Keywords:** Molecular dynamics simulation, *ab initio* quantum chemistry, multi-scale simulation, crowded cellular environment, integrative dynamic structural biology

**(1) Long-term goal of laboratory and research background**

Our long-term goal is to understand molecular mechanisms for dynamical processes in chemical and biological systems using computational chemistry and biophysics. Multi-scale simulation methods, which include hybrid quantum mechanics/molecular mechanics (QM/MM), all-atom molecular dynamics (AAMD), and coarse-grained MD (CGMD) methods, are necessary to study the structure-dynamics-function relationships in various molecular systems. We develop multi-scale simulation methods in our MD software, GENESIS (GENeralized-Ensemble SImulation System) and optimize GENESIS to Fugaku supercomputer. The next step is to connect the results of QM/MM, AAMD, and CGMD using data science, such as machine learning and data assimilation. Using this approach, we investigate protein dynamics and function in crowded cellular environments and examine the roles of weak and non-specific molecular interactions on biomolecular functions in living cells. AAMD and CGMD are useful to explore large conformational changes in biomolecules, while QM/MM is required to compute enzymatic reactions based on the electronic structures. We carry out large-scale biomolecular simulations of various biological processes on Fugaku or other supercomputers. Such simulation results are integrated with single-molecule experiments and cryo-electron microscopy for our better understanding of molecular and cellular functions.

**(2) Current research activities (FY2020) and plan (until Mar. 2025)**

**(A) Development of multi-scale simulation methods for chemical and biological systems**

The shared usage of supercomputer Fugaku has started in March 2021. During the development of Fugaku, we optimized MD software GENESIS to Fugaku in collaboration with the system software and hardware developers. In the 'co-design' strategy, we accelerated MD simulations using GENESIS on Fugaku more than a hundred times faster than that on K computer, theoretically. In 2020, we confirmed that the expected performance of GENESIS is achieved on Fugaku [1]. In the new version of GENESIS (version 2.0beta or later), we implemented a lot of new functions. One of the most important developments is more accurate evaluations of system temperature and pressure in MD simulations. Using the accurate evaluations, stable MD simulations are possible even using a longer time step $\Delta t$ [2].

We have also developed enhanced conformational sampling methods to explore dynamic structures of biomacromolecules effectively in molecular dynamics simulations. In 2018, gREST (generalized Replica Exchange with Solute Tempering) was developed in the framework of REST2. In gREST (and REST2), the simulation system is decomposed into solute and solvent. In all replicas, solvent temperatures are kept fixed at room temperature, while solute temperatures are different in each replica. The solute temperatures in different replicas are exchanged according to the Metropolis criteria in a similar way to the replica-exchange molecular dynamics. gREST (or REST2) allows us to sample various conformers at higher solute temperatures and examine low-energy stable structures at lower solute temperatures. The advantage of gREST over REST2 is that a flexible selection of the solute regions is available in gREST: solute is defined as a part of a molecule and/or a part of the potential energy terms in the selected solute region. In 2020, we propose a new gREST scheme, which is useful in simulations of multi-domain proteins. The method, which we call gREST_SSCR (gREST with selective surface charged residues), selects charged amino-acid residues on the protein surface at the domain interface regions as the solute region. By controlling the interaction between these amino-acid residues, relative domain motions in a protein are enhanced greatly in gREST_SSCR simulations.

We applied this method to ribose-binding protein (RBP) to examine the conformational sampling efficiency of gREST_SSCR. Four simulations of RBP were performed: conventional MD and gREST_SSCR in Holo (with ribose) and Apo (without ribose) states, respectively. The free-energy landscapes of both Holo and Apo states in gREST_SSCR simulations are much wider than those from conventional MD simulations. We also investigated molecular mechanisms underlying the large conformational changes of RBP upon ligand binding. Generally, two different mechanisms underlying ligand-induced protein conformational changes have been proposed. In the "induced fit" mechanism,

protein conformational changes happen due to the interactions with ligand, while a protein is considered to have intrinsic large conformational fluctuations and the ligand binding just shifts the equilibrium of dynamic structures in "conformer selection". Multiple independent MD simulations of RBP were also performed from the representative structures observed in the free-energy landscapes from gREST_SSCR simulations. In our theoretical analysis, both conformer selection and induced fit mechanisms are necessary, while the former is emphasized in the open conformation and the latter is important to stabilize the closed structures with ribose [3].

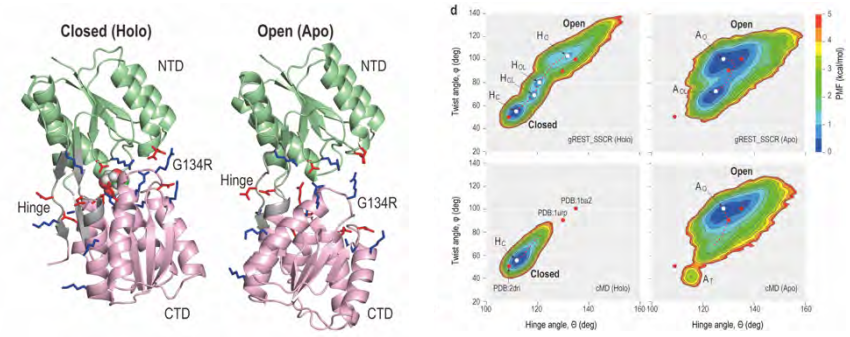


Fig. 1. (left) Ribose Binding Protein (RBP) in Holo and Apo states. Side chain atoms treated as solute in gREST are shown in blue and red. (right) Free-energy landscapes from MD (bottom) and gREST_SSCR (top) for Holo (left) and Apo (right).

**(B) Biomolecular structure, dynamics, and function in crowded cellular environments**

Our research activity was significantly restricted due to the Covid-19 in 2020. Therefore, we considered what we can do for this social issue using GENESIS on Fugaku and decided to investigate dynamic structures of spike protein on the surface of SARS-CoV-2. This protein plays important functional roles when the virus infects a human cell. Cryo-EM spectroscopy has shown that there are at least two distinct structures of spike protein: the active Up form which is able to infect with a human cell and the Down form which is inactive in the virus infection. However, experimental studies cannot understand molecular mechanisms underlying the large conformational changes between the inactive Down and active Up forms. It is also difficult to understand functional roles of glycans on the surface of spike protein. By performing MD simulations from the Up and Down structures, we aimed to understand the conformational changes and the glycan functional roles. In 1 μs MD simulations, we could not observe any transitions between the Up and Down forms, while we observed that three glycans stabilize conformations of the Up and Down forms [4]. We also performed gREST_SSCR simulations of spike protein on Fugaku and analyzed the conformational changes between the two forms.

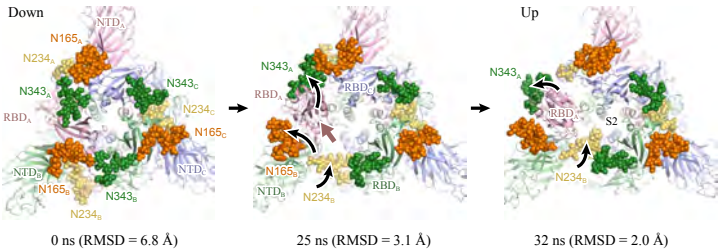


Fig. 2. Essential protein-glycan interactions that stabilize the Down or Up structures of SARS-CoV-2 spike protein.

## (3) Members

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**(Senior Technical Scientist)**
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**(Special Postdoctoral Researcher)**
Ai Niitsu, Mao Oide, Yosuke Sumiya

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**(Trainee)**
Masahiro Motohashi

**(Assistant)**
Machiko Ishigaki, Hiromi Kano

**(4) Representative research achievements**


1. "New parallel computing algorithm of molecular dynamics for extremely huge scale biological systems", J. Jung, C. Kobayashi, K. Kasahara, C. Tan, A. Kuroda, K. Minami, S. Ishiduki, T. Nishiki, H. Inoue, Y. Ishikawa, M. Feig, Y. Sugita, *J. Comput. Chem.* 42 (2021) 231-241.
2. "Group-based evaluation of temperature and pressure for molecular dynamics simulation with a large time step", J. Jung, Y. Sugita, *J. Chem. Phys.* 153 (2020) 234115.
3. "Unraveling the Coupling between Conformational Changes and Ligand-Binding in Ribose Binding Protein Using Multiscale Molecular Dynamics and Free-Energy Calculations", W. Ren, H. M. Dokainish, A. Shinobu, H. Oshima, Y. Sugita, *J. Phys. Chem. B* 125 (2021) 2898-2909.
4. "Elucidation of Interactions Regulating Conformational Stability and Dynamics of SARS-CoV-2 S-Protein", T. Mori, J. Jung, C. Kobayashi, H. M. Dokainish, S. Re, Y. Sugita, *Biophys. J.* 120 (2021) 1060-1071.
5. "Atg9 is a lipid scramblase that mediates autophagosomal membrane expansion", K. Matoba, T. Kotani, A. Tsutsumi, T. Tsuji, T. Mori, D. Noshiro, Y. Sugita, N. Nomura, S. Iwata, Y. Ohsumi, T. Fujimoto, H. Nakatogawa, M. Kikkawa, N. N Noda, *Nat. Struct. Mol. Biol.* 27 (2020) 1185-1193.


**Supplementary**

Group Photo

Laboratory Homepage

https://www.riken.jp/en/research/labs/chief/theor_mol_sci/index.html

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