Biomolecular machines, such as molecular motors and transporters in the cell, are known to change their structure when they function. For example, ATP synthase, which synthesizes ATP in mitochondria, is a molecular motor that uses chemical energy to rotate unidirectionally. Transporters, which transport substrate molecules across the cell membrane, perform substrate transport by changing their structure between an inwardly and outwardly open structure relative to the membrane. Our goal is to elucidate the mechanism of these elaborate and dynamic nanomachines created by nature at the atomic and molecular level, and to control their functions based on our findings.

We would like to understand the mechanism of biomolecular machines by “seeing” the motion of biomolecular machines at the moment they function at the molecular level, on a computer. However, this is not an easy task, because biomolecular machines are huge molecules, and their functioning time scale is slow (for a molecular scale) at millisecond or longer. Conventional atomistic molecular dynamics (MD) simulations cannot cover millisecond-long functional dynamics, especially for a large system like typical biomolecular machines. Therefore, we have developed and applied methods such as coarse-grained modeling, enhanced sampling and importance sampling to capture the motion at the moment of function.

We have been working on biomolecular motors such as ATP synthase. ATP synthase is a rotary motor that produces most of ATP required in the cell. It is composed of two rotary motors: $F_o$ and $F_1$. $F_o$ motor is embedded in the membrane and driven by proton gradient, while $F_1$ motor is driven by ATP hydrolysis reaction. We clarified how the rotation of $F_1$ motor is driven by a key chemical step, $P_i$ release after ATP hydrolysis reaction, by accelerating atomistic MD simulations with external forces.

Transporters are membrane proteins that transport their substrates across the membrane. We have studied Na$^+$/H$^+$ antiporter, which exchanges sodium ions and protons inside and outside the cell. The ion transport process by the Na$^+$/H$^+$ antiporter was simulated in atomic detail with transition path sampling technique to capture the moment of the ion transports. The simulations predicted the mutation that can speed up the ion transport. The mutation was tested in experiments and shown to speed up the ion transport twice faster than the wild type. Therefore, we succeeded in controlling the function of the transporter based on mechanism obtained from simulations.

Selected Publications
1. Mechanism of \( \text{Na}^+/\text{H}^+ \) Antiporter and Engineering of a Faster Transporter

\( \text{Na}^+/\text{H}^+ \) antiporters control pH and \( \text{Na}^+ \) concentration in the cell by exchanging sodium ions and protons across lipid membranes. They belong to the cation/proton antiporter (CPA) superfamily, and prevail in all domains of life. The archaeal \( \text{Na}^+/\text{H}^+ \) antiporters \( \text{PaNhaP} \) and \( \text{MjNhaP1} \) as well as human NHE1, which is linked to a wide spectrum of diseases from heart failure to autism and has no structure solved yet, are electroneutral antiporters of the CPA1 family, exchanging one proton against one sodium ion. As a model system in mechanistic studies of electroneutral \( \text{Na}^+/\text{H}^+ \) exchange, we studied the transport mechanism of \( \text{PaNhaP} \).

\( \text{Na}^+/\text{H}^+ \) antiporters use the gradient of either sodium ion or proton to drive the uphill transport of the other ion (Figure 1A). The conformational transition of the transporter makes the ion-binding site accessible from either side of the membrane in the alternating manner. For \( \text{PaNhaP} \), the inward-open conformation was obtained by X-ray crystallography, while the outward-open conformation is not known experimentally. We modelled the outward-open conformation by MDFF flexible fitting to the low-resolution outward-open structure of the homologous \( \text{MjNhaP1} \) from cryo-EM, followed by the long equilibrium MD simulations. It was shown that the transporter domain moves ~3.5 Å in the direction normal to the membrane to take the outward-open state.

By applying the transition path sampling technique, we sampled unbiased transition paths between the inward- and outward-open states. In analysis of the transition paths, we found hydrophobic gates above and below the ion-binding site, which open and close in response to the domain motions (Figure 1B). From the reaction coordinate analysis, it was shown that open-close motion of the outside gate (Ile163-Tyr255) is a rate-limiting step of the alternating-access conformational change. Based on this result, we weakened the outside gate by mutating the residues to both alanine. It was expected that this mutation lowers the barrier and makes the ion transport faster. It was confirmed by experiments that the ion-transport speed of the mutant is indeed twice faster than the wild-type transporter.

2. Machine Learning of Reaction Coordinates

It is a challenging task to identify reaction coordinates for biomolecular systems with many degrees of freedom. Unlike order parameters or collective variables, a reaction coordinate should describe progress of a reaction between two metastable states. We have developed a machine learning method to identify reaction coordinates based on the committor function. Assuming a linear combination of many collective variables, reaction coordinates are optimized via likelihood maximization or cross-entropy minimization. From coefficients of the optimized reaction coordinates, we can also identify rate-limiting variables, which play an important role in transition state area. We have also applied a deep neural network and Explainable Artificial Intelligence (XAI) for this problem.

3. Mechanism of Membrane Remodeling by F-BAR Protein Pacsin1

F-Bin/Amphiphysin/Rvs (F-BAR) domain proteins play essential roles in biological processes that involve membrane remodelling, such as endocytosis and exocytosis. Notably, Pacsin1 from the Pacsin/Syndapin subfamily has the ability to transform the membrane into various morphologies: Striated tubes, featureless wide and thin tubes, and pearling vesicles. We clarified the membrane curvature induction and sensing characteristics of Pacsin1 by combining all-atom (AA) and coarse-grained (CG) MD simulations. By matching structural fluctuations between AA and CG simulations, a CG protein model called “Gō-MARTINI” was developed and optimized. The model should prove useful for describing protein dynamics that are involved in membrane remodeling processes.

References