Summary of Previous Works

My research interests have been centered on the role of conformational dynamics of biological molecules on their functions using computer simulations and statistical mechanical theories, in close collaboration with experimental groups. I have been working mainly on two problems:

- Electron transfer reactions in protein molecules
- The fundamental nature of protein dynamics and the mechanisms of functional conformational change in proteins

Studies in each area are summarized below.

Electron Transfer Reactions in Protein Molecules

Introduction

Electron transfer reactions in protein molecules are the most basic chemical reaction used in biological functions, and they play fundamental role in energetics of biomolecules, such as photosynthesis and respiratory chains. The proteins are designed to carry electrons through the system and use energy to make chemical reactions (*1*). A key question is how the reactions are controlled by the protein dynamics to achieve very high efficiency. Electron transfer does not occur without conformational fluctuations of the protein and the solvent. The last decade has seen a tremendous growth in reliable molecular-level theories and models for biological electron transfer reactions. Utilizing these new approaches, and with the aid of new relevant protein structures, we are now able to explore the entire bioenergetics of photosynthesis and respiration.

New formalisms to understand protein electron transfer reactions

Long distance electron transfer reactions in proteins have a weak electronic coupling between the donor and acceptor sites, so the rate can be calculated in the non-adiabatic limit, as a product of two factors (Marcus theory (*2*)):

$$k_{\mathrm{ET}} = \frac{2\pi}{\hbar}\left|T_{\mathrm{DA}}\right|^2(\mathrm{F.C.})$$

where (F.C.) is the Frank Condon factor, which is the probability that the system is at the resonant state in the thermal fluctuation. $|T_{\mathrm{DA}}|^2$ is the coupling constant, which is the quantum probability that an electron transfers at resonant condition (see figure 1). My studies focused on the role of the protein dynamics in the control of electron transfer rate through the effects on these two factors.

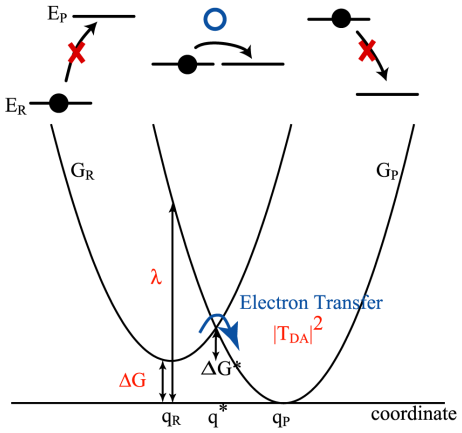

Figure 1: Schematic representation of Marcus theory. Electron transfer can occur only when two electronic states, Product (E_{P}) and Reactant (E_{R}), have the same energy. Reaction rate is the product of two probabilities (F.C.) and $|T_{\mathrm{DA}}|^2$. The Franck-Condon factor, (F.C.), is the probability that the system is at the resonant state in the thermal fluctuation. The coupling constant, $|T_{\mathrm{DA}}|^2$, is the quantum probability that an electron transfers at resonant condition. The reorganization energy, λ, and the reaction free energy, ΔG, are defined.

The Franck-Condon factor (F.C.) is determined by two energetic terms, "reaction free energy" and the "reorganization energy". Among them, of particular interest is the reorganization energy, which reflects the relaxation process of protein coupled to the electron transfer reaction. The reorganization energy is difficult to study experimentally due to its dynamical aspect, thus theoretical evaluations are valuable. I have developed a new formalism to analyze the reorganization energy from a classical molecular dynamics trajectory, with special emphasis on its structural and frequency signature that we applied to the intra-molecular electron transfer in cytochrome *c* (P1). Our study revealed that protein molecule is soft and thus has significant contribution to the Franck-Condon factor. Especially, large-scale conformational fluctuations of the protein were found to control the electron transfer rate.

Many experimental and theoretical studies on electron transfer have taken the temperature as a control parameter. Another important parameter is the *pressure*. By studying the pressure dependence of the electron transfer rate, we can obtain new information about protein structure dynamics. Therefore, I have also examined the effect of pressure on electron transfer reactions by developing a new formalism to analyze the experimental data (P2). We discussed experimental data with this new formalism, making a consistent explanation of the data, and identifying the contributions of the different factors (the coupling constant or the Franck-Condon factor). This formalism has been used by several groups to discuss their new experimental data (*3, 4*).

An inter-molecular electron transfer reaction

Electron transfer reactions do not always occur within a protein. Indeed, many important electron transfer processes involve *inter*-protein electron transfer. For example, water-soluble *c* type cytochromes shuttle electrons between specific membrane-proteins. However, inter-protein electron transfer has received limited theoretical attention. We have studied electron transfer between the photosynthetic reaction center (RC) and the electron shuttle protein, cytochrome c_2 (cyt c_2) by performing a molecular dynamics simulation that samples conformational space around the co-crystal structure by Axelrod (*5*), figure 2, and extended theoretical models we have developed for intra-molecular electron transfer to the analysis of this inter-molecular reaction (P3, P4).

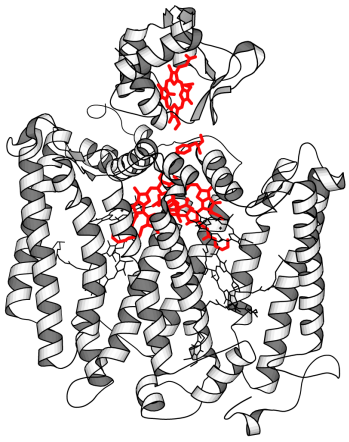

Figure 2: Structure of the reaction center/cytochrome c_2 complex obtained by Axelrod et al. (*5*). The groups involved in inter-protein electron transfer; heme in cytochrome, Tyr L162 and BChl dimer in the reaction center, are indicated in red.

First, we have investigated the Franck-Condon factor with the formalism described above. The calculated reorganization energy is small, because the binding interface is not solvent exposed, and contributions from cyt c_2 and RC are comparable. Decomposition of the reorganization energy at the residue level indicates that mutational effects on (F.C.) are likely to be small (P4). Dynamical influences on the coupling constant, $|T_{DA}|^2$, were also investigated. Using the *Pathway* model (*6*) that

predicts the dominant electron tunneling pathways, we found that they go though Tyr162 of unit L of the RC (P4), which is located midway between the redox sites (see figure 2) and had previously been argued to be important. Interestingly, tunneling pathways are occasionally water mediated because of large conformational fluctuations at the interface, which are a special feature of *inter*-molecular electron transfer (P4). From this, we expect that surface mutations would have a weak effect on the coupling constant as long as the overall complex structure is not changed. Water will move into any void at the interface if needed, providing alternate pathways. This can explain a puzzling experimental observation that Tyr162 mutation in *Rhodopseudomonas viridis* does not changes the electron transfer rate (*7*).

Binding and formation of protein-protein complex for electron transfer

These studies have focused on the electron transfer of the bound complex. However, in the inter-molecular electron transfers, the entire event involves slow binding process to form the active complex and following fast electron transfer reaction. The two molecules need to be bound correctly to make electron transfer efficiently. However still, our study indicates that water molecules can mediate the electron transfer reaction (P4), thus fully bound complex may not be necessary. We need to fully understand the binding kinetics of the two proteins to understand the entire reaction event.

Encounter complex formation by electrostatic interactions. To address these questions, we have been studying the binding process of cyt c_2 to the reaction center. Experimentally, the electrostatic interactions are known to be important for the binding processes of the RC (*8*). I generated electrostatic energy surfaces using a continuum electrostatics calculation parameterized to fit experimental data. The resulting energy surfaces show that the cyt c_2 is smoothly attracted toward the binding site (electrostatic steering), however, electrostatic interactions are not specific enough to guide the cyt to the final complex. Instead, a solvent-separated configuration is indicated that represents a pre-association encounter complex (figure 3a). Other short-range interactions are needed to form the final bound complex (P5). These observations are consistent with a two steps mechanism (*9, 10*): the formation of encounter complex is guided by electrostatic interactions followed by the formation of short-range interactions such as van der Waals interactions for rapid electron transfer.

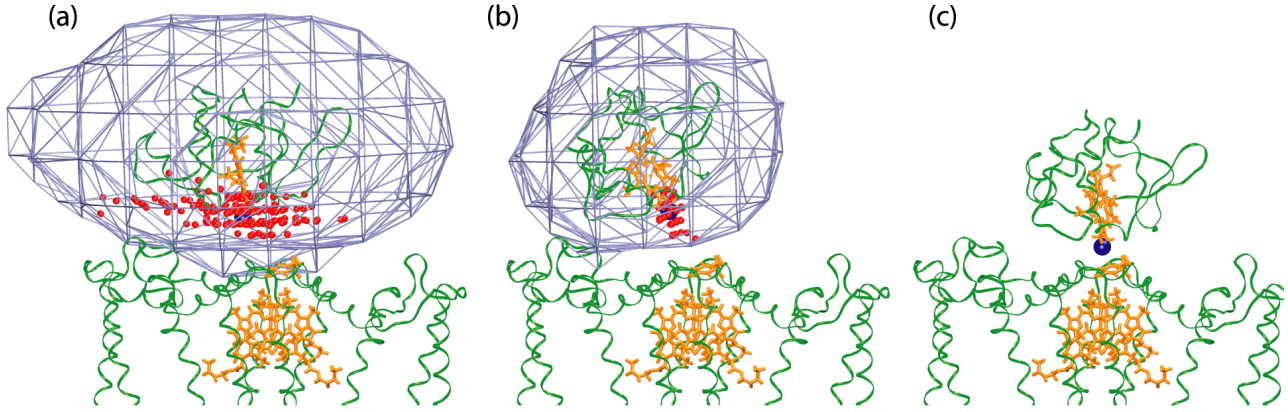

Figure 3: Representations of (a) the encounter complex, (b) the transition state and the (c) bound state for the reaction between cytochrome and the reaction center. The average structures are shown in green with the heme, TyrL162 and BChl dimer in orange. The blue envelope represents the region occupied by Cα atoms of the cyt in the ensemble. The blue circle shows the heme edge in the average structure. The red circles show representative positions of the heme edge in the ensemble. The figure shows the progression of the cyt from; (a) a delocalized distribution in the encounter complex through (b) a more localized transition state in which the heme edge points toward Tyr L162 to (c) the bound state in which the heme edge is in contact with the Tyr L162 for electron transfer.

Transition state ensemble. The binding process can be considered as a two steps mechanism (*9, 10*). The encounter complex is formed by weak electrostatic interactions, followed by the formation of full bound structure. The transition state, which precedes the final complex formation, is the rate limiting step of the RC/cyt binding process, and therefore of particular interest. I constructed an atomic model of the transition state ensemble by combining the theoretical computation and experimental data. Exploring the full rotational and translational conformational space of cyt c_2, we found a configuration that could reproduce the experimental data for the binding rate constant of several mutants (*11*). From these electrostatic calculations, we can construct a model for the transition state ensemble which positions the cytochrome ~5Å away from the reaction center (that may correspond to the water separation) and ~5Å aside from the center (figure 3b). More interestingly, the heme is oriented toward a Tyr residue, which is forming hydrophobic interactions at the fully bound structure (figure 3c). The similarity between the structures of the encounter complex, transition state and bound state can account for the rapid rate of association responsible for fast electron transfer (P6).

Fundamental Character of Protein Dynamics

Energy landscape of protein conformational change

Proteins often undergo large conformational change upon ligand binding. Allostery requires a biomolecule to have at least a pair or, more likely, a multiplicity of conformational states of nearly equal free energy. How can we describe movement between such states? Most of studies describe only the geometry of allosteric structural changes to estimate the energetics, however we need to go beyond the usual approaches, i.e., the description free energy barriers between different minima need to be described to understand the conformational change dynamics.

We have been developing a theoretical model to describe the energy landscape of protein conformational changes and applied it to conformational change of adenylate kinase due to ligand binding (P7, P8). Our goal is to describe how allosteric conformational switches function by using a theoretical framework that unites an energy landscape description with the elastic model based on normal modes. In this model, we consider two energy surfaces that correspond to different states, such as ligand bound and unbound states. In this study, the protein is modeled as an elastic object (*12*). Energy surfaces are extrapolated from each of the steady states (energy minima) using the normal mode. In the normal mode analysis, which is the simplest model to describe protein dynamics, the energy surface is approximated as a perfect parabolic, and so its dynamics is described by a superposition of harmonic oscillations. However, the harmonic approximation of the energy surface is not adequate by itself to describe large conformational changes. Therefore, we introduced a new model that describes the protein conformational change as a *non-linear* path, by iterative usage of normal mode calculations (P7).

The energy surface along the reaction coordinate, defined by the above method, shows a very high-energy barrier, which is 2 times higher than the total stability of the protein (figure 4). From an analysis of individual energy terms, we found that some particular residues are under high strain. Considering the protein folding theory, we propose that such locally high strained residues are partially unfolded, or ‘cracked’, i.e., biological molecules partially unfold in the middle of conformational change process and refold again during the conformational change (P7, P8). The model provides us a new picture for the mechanism of biological functions: unlike macroscopic machines, biological machinery can break during ordinary function and still complete its task, and then re-assemble as needed through its folding capabilities.

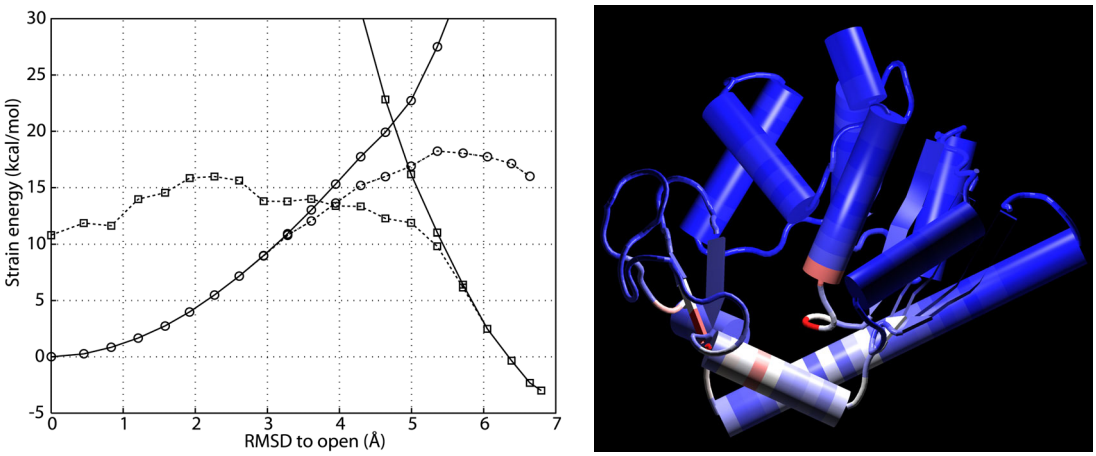

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Figure 4: The strain energy surfaces of the ligand unbound open state (○) and the ligand bound closed state (□) are shown in the solid lines. The closed state has energy minimum at RMSD~7Å. The energy with the "cracking" (partial unfolding) effect is shown in the dotted line. The cracking effect could lower the energy barrier significantly. An intermediate structure between open and closed form of adenylate kinase is shown in right. Each of residues is colored according to its strain energy. Blue is the residue under no strain energy. Red is the residue under high strain energy, which may partially unfold.

We also addressed other biochemical questions. For example, adenylate kinase has a core domain and two flexible domains, LID and NMP_{bind}, thus its conformational change is rather complex. Several experimental studies have discussed the order for the closure of the two domains. Using our model, we can address this issue by studying the energy landscape of the conformational change as a function of domains closure (P8).

The strain energy analysis in the above studies can be used to measure the change in local environment in the protein upon conformational dynamics. In the carboxyl-terminal Src kinase (CSK), it has been observed experimentally that a mutation affects the dynamics of apparently distant part of the protein. By applying our strain energy analysis to CSK, we found that the mutated residue and this distant part of the protein are coupled through large concerted conformational fluctuations, which provides an explanation for this observation (P9).

Vibrational energy relaxation in protein

The theoretical description of complex protein dynamics is still debatable. It is clear that anharmonicity of the energy surface is significant in protein dynamics and probably important for protein function. We attempted to describe the anharmonicity of protein dynamics as a perturbation to the harmonic energy surface using the myoglobin molecule as a target system (P10, P11).

We found that the energy was transferred by two distinct mechanisms depending on temperature:

1) At low temperature, anharmonicity is rather weak, and the energy transfer occurs to follow a certain rule of relation between mode frequencies, i.e., the vibrational energy is transferred when modes satisfies the Fermi resonance rule.

2) As temperature increases, near room temperature, the anharmonicity is so large that such a frequency rule does not appear significant. Energy transfer occurs between modes that are structurally similar and indirect processes through intermediate modes.

Volume fluctuation of proteins

From molecular dynamics simulation, we calculated the volume fluctuation of human lysozyme. Spectral analysis shows that low-frequency dynamics dominate the total volume fluctuation. The same

aspect is found in the study using principal component analysis. This low-frequency region is related to large and slow motions of proteins, which indicates that a long time dynamics simulation is necessary to describe the volume fluctuations of proteins (P12).

Normal mode analysis for fitting atomic structures to low resolution electron microscopy data

As an application of normal mode analysis, a novel method was introduced for the docking of a high-resolution structure into low-resolution maps of macromolecular complexes from electron microscopy that takes into account the conformational flexibility of biological systems. This method uses a linear combination of low frequency normal modes obtained from elastic network model in an iterative manner to deform the structure optimally to conform to the low-resolution electron density map. Using only the low frequency normal modes, the proteins are deformed in a mechanically realistic way (P13, P14).

Research Plans

My research interests have been centered on the role of conformational dynamics of biological molecules on their functions using computer simulations and statistical mechanical theories, in close collaboration with experimental groups. In structural biology, the atomic structure obtained from X-ray crystallography is the starting point for understanding the mechanism of biological molecules. The structure provides a basic idea of what is necessary for functions. However, the structure is a static picture and cannot explain how the biological molecules perform their functions. The role of conformational dynamics is evident especially in allosteric transitions of proteins and motor proteins, however it is still critical even for some simple biochemical reactions such as electron transfer.

Computer simulations have been helpful to understand mechanisms of biological functions. Using computers, we could simulate how the molecules are actually moving. However, even with the most powerful computer, it is difficult to simulate the entire time course of the functions. Moreover, the biological molecules are not working in deterministic way as our macro machines do. Since the forces driving biological functions are comparable to thermal forces, we need to describe the biological events in a statistical manner. In my researches, I aim to develop theoretical frameworks to describe biological functions in terms of statistical physics, and use computational approaches to simulate the real biological systems. Results need to be compared to experimental data. Different level of calculations such as molecular dynamics simulation, normal mode analysis and dielectric continuum model in combination with different level of models from simple elastic network model to all atom detail potentials are being used.

I have been working mainly on two problems: 1) Electron transfer reactions in protein molecules, 2) The fundamental nature of protein dynamics and the mechanisms of functional conformational change in proteins. Both topics share the same basic interest, i.e. the role of protein conformational dynamics on the protein functions. I intend to extend my previous works through development of new theoretical frameworks and models. I first describe some new directions aimed to obtain a further understanding of protein dynamics-function relationship through the studies on the inter-protein electron transfer reaction and protein allosteric conformational changes. I also describe my long-term research plans. I will focus on allosteric transitions of proteins with special emphasis of the control of the transition by substrate binding. In addition, I would like to answer questions related to the evolution of protein architecture.

New directions

Description of the Complete Process of Inter-Protein Electron Transfer: From Binding to Electron Transfer

Introduction. Electron transfer reactions are the most basic chemical reaction used in biological functions, and they play fundamental role in bioenergetics, such as photosynthesis and respiratory chains. In these bioenergetic systems, several proteins carry electrons through the system and use energy to make chemical reactions. Many important electron transfer processes involve *inter*-protein electron transfer (*1*). For example, water-soluble *c* type cytochromes shuttle electrons between specific membrane-proteins (see figure 5). The last decade has seen a tremendous growth in reliable molecular-level theories and experimental data for biological electron transfer reactions. However, inter-protein

electron transfer has received limited theoretical attention. Compared to intra-protein reactions, it is more complex, since there is an additional protein-protein association step. Even the uni-molecular electron transfer step after the protein complex formation is likely to have a different character from intra-protein electron transfer. The electron has to go through the protein-protein interface, which tends to be more flexible than the protein core, and water molecules may profoundly affect the interface.

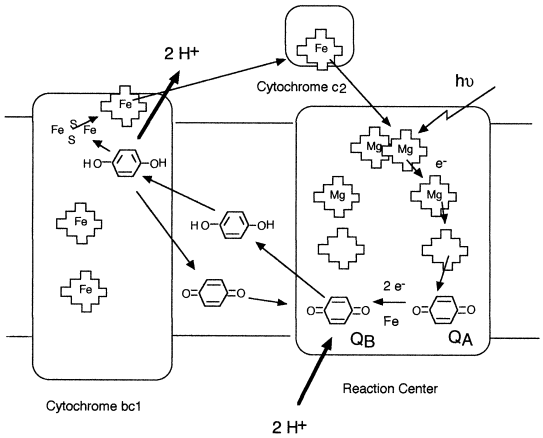

Figure 5: Simplified representation of electron and proton transfer in photosynthetic membranes. Cytochrome c_2 shuttles an electron from cytochrome bc1 to the reaction center. The reaction center couples light-induced electron transfer to proton transfer by reducing ubiquinone at the Q_B site through several steps of electron transfer reactions. In the reaction center, the reactions only occur through the pigments in the A branch. Figure from (*13*).

A key question is how the reactions are controlled by the protein dynamics to achieve very high efficiency. In the inter-molecular electron transfers, the entire event involves slow binding process to form the active complex and following fast electron transfer reaction. The two molecules need to be bound correctly to make electron transfer efficiently.

I have been studying the binding process of photosynthetic reaction center (RC) and cytochrome c_2 (cyt) and following electron transfer reactions. The binding process can be considered as a two steps mechanism (*9, 10*). The encounter complex is formed by weak electrostatic interactions, followed by the formation of full bound structure. A transition state from the encounter complex to the final complex is the rate-limiting step of the binding process. In our previous studies, we have identified the encounter complex and the transition state by combination of experimental data and electrostatic calculations (P5, P6). Still, we just obtained snapshots of the binding process. Complete understanding of entire binding coupled with electron transfer process needs further investigations. While the following studies center on photoreaction center, in the future I also intend to extend such studies to other important bioenergetics systems such as cyt bc_1 complex.

Final complex formation. I intend to study the kinetics of final binding process from the transition state to the fully bound complex. It is of great interest to understand how the system forms the reaction active complex to allow the efficient electron transport. For the final binding stage, short-range van der Waals interactions need to be considered explicitly. These interactions include the small rearrangements between residues as well as the desolvation process of water molecules. Formation of such short-range contacts are also important for electron transfer, since it could create an electron tunneling path that could allow the transfer of the electron from cyt c_2 to the RC.

These questions could be approached by molecular dynamics (MD) simulations. Since the process leading from the transition state to the final complex is downhill, it should occur fast, which would be approachable by MD simulation. From the MD trajectory, we could analyze the development of the binding interactions and the electron coupling to obtain useful insights into the final stages of bound structure formation and the following or concurrent electron transfer reactions.

Inter-protein electron transfer through loosely bound proteins. In our previous study on the electron transfer reaction from cyt to the RC, we assumed that the proteins are tightly bound at the configuration obtained by the X-ray co-crystal structure. However, inter-protein electron transfer reaction without tight bound structure formation has also been recently proposed. In this model, the electron transfer could occur probabilistically at different binding configurations (*14*). Combining the above analyses on the binding process and the electron tunneling pathway calculations, we may observe a character of inter-protein electron transfers much more fuzzy and dynamical than previous images. In addition, such loose-binding model was invoked for a different pair of electron transfer proteins. It would be interesting to compare these results to the reaction in the pair of the RC and cyt. Different inter-protein electron transfer mechanism may be adopted for different functions.

Origin of the transition state. The transition state of the binding process is of particular interest, since it is the rate-limiting step, and thus closely related the efficiency of the electron shuttling function of cytochrome. We have already succeeded to construct a model of the transition state ensemble using the experimental data (P5, P6). However, the origin of the transition state is not explained. Here we could draw a parallel with the protein folding funnel theories (*15*). Indeed, the formation of the bound complex, which is very confined by short range interactions, from the encounter complex, which is loosely formed by electrostatic interactions, would be unfavorable in terms of entropy but at the same time energetically favorable since the strong short-range interactions are being formed. The balance between entropy and energy could be the origin of the transition state of the binding process. Using a model similar to the Go-model (*16*), which consists of the simple chain connectivity and the native contacts, we could simulate the binding process and derive the free energy surface to identify the cause of the transition state.

Energy Landscape of Allosteric Protein Conformational Transitions Examination by Models with Different Levels of Approximations

Introduction. Proteins undergo large conformational change upon ligand binding. Although such large conformational change can be identified from the comparison of X-ray structures with different form, it does not provide information on the kinetics of the functional transitions. From analyses of structure-function relationship, we can identify some motifs that are necessary of the function. However still, to understand the remarkable efficiency of biomolecular functions, we need to study the dynamics of biomolecules, i.e., the relation between energy landscape and function needs to be considered.

We have been developing a theoretical model to describe the energy landscape of protein conformational changes and applied it to the conformational change of adenylate kinase due to ligand binding (P7, P8). In this model, we consider two energy surfaces that correspond to ligand bound and unbound state. Normal mode was used to describe the conformational change path, and energy surface of each state is obtained from strain energy calculations of the molecule. From the superposition of these two surfaces, the entire energy surface of the conformational transition can be obtained. I intend to pursue research in this area by addressing the following points.

Survey study of other systems. From our theoretical models, we have proposed new hypotheses that could be tested experimentally. In particular, the cracking mechanism (P7) that we proposed is a novel idea that should be investigated in a more detail since it might change our view of conformational change processes. In this model, some parts of protein partially unfold, or ‘crack’

during conformational transitions. In other words, protein molecules are marginally stable to function efficiently by allowing partial unfolding if necessary. One of our predictions from this theory is that a small amount of urea can lower the energy barrier, and thus enhances the conformational change rate. Only a few studies have examined such possibility, however it is known that the thermophilic enzyme activity is low at low temperature, and interestingly their activity can be partially restored by a small amount of denaturant (*17*). Thus, enzymes from thermophiles would be interesting systems to study; in particular if one can compare results with the same enzyme from mesophiles. More generally, by undertaking a survey for a large number of biological systems, with different functions, ranging from small proteins to macromolecular complexes, we could correlate the character of the energy landscape to a particular class of biomolecular functions.

Strain energy analysis as a measure to identify change in local environment. In our study of the adenylate kinase, we have analyzed how each residue is strained during the conformational change, and found that only a fraction of residues is under high strain. Such strain energy analysis can also be used to identify changes in local environment in the protein upon conformational dynamics. Recently, effects of a mutation on the conformational dynamics of c-terminal Src kinase (CSK) were studied using hydrogen exchange mass spectroscopy by the group of Patricia Jennings (UCSD). The result indicates that a small perturbation to a domain of CSK can affect the dynamics of other domains through some kind of long-range communications. I intend to study also this interesting finding by performing normal mode analysis and comparing strain energy distribution over the protein with and without peptide. Increase in the strain energy could indicate the increase of local fluctuations, which could provide a possible explanation for such long-range communication.

Semi-atomic model. Another interesting direction to pursue is the development of models with different levels of description. In our original study, we used the elastic network model, in which atoms are connected by spring without any distinction of atom type, covalent bond or non-bond interactions. Although the model has proven to be adequate to describe large conformational dynamics (*12*), energy calculations would greatly benefit from a more complex potential where covalent bonds and non-bond interactions could be treated in different way. One could imagine using the Go-model that has been very successful for folding studies (*16, 18*). In a simple version of the Go-model, residues in a given protein are represented as single beads centered in their Cα positions. Adjacent beads are strung together into a polymer chain by means of bond and angle interactions, while the geometry of the native state is encoded in the dihedral angle potential and non-local interactions. We could use the Go-model to describe energy landscape in conjunction with normal mode analysis, since with Go-model one can easily explore large conformational spaces. These studies could be complemented with MD simulation using detailed all-atom potentials such as AMBER or CHARMM to explicitly observe predicted partial unfolding.

Long Term Research Plans

Energetics and Kinetics of Coupled Ligand Binding and Protein Conformational Change Processes

Dynamical picture of binding process. The regulation of biological machinery through allostery is a dominant theme in our modern molecular understanding of life. In the long term, I aim to study allosteric transitions of proteins. Proteins often undergo large conformational change upon ligand binding. The coupling between the conformational change and the substrate binding has been considered as a deterministic picture, i.e., the protein switches from one conformation to the other

upon ligand binding. Recently, a more dynamical picture has emerged where allosteric proteins have two coexisting states and the substrate binding makes a shift in the population between the states (*19, 20*). However, these discussions focus on the system at the equilibrium. Dynamics of protein conformational change coupled to substrate binding is still not well understood. One of the fundamental questions is when does the binding occur? Some studies assume that the binding occurs first and that the bound substrate induces the conformational change of the protein, which implies that the conformational change is fast. However, the relative kinetics of ligand binding and protein conformational change will vary depending on the system and the environmental condition such as temperature and ligand concentration. The coupling of ligand binding and protein conformational change is also important in the mechanism of the F_1 motor protein. It was suggested that its high efficiency can be achieved by converting ATP binding energy into elastic strain energy of the proteins without energy loss (*21*). However, the actual energy conversion process remains unclear. I intend to study the energetics and the kinetics of allosteric conformational changes caused by ligand binding.

Coupling between substrate binding and protein conformational change. Theoretical frameworks to describe the coupling of substrate binding and allosteric conformational change need to be developed. In our previous work for the adenylate kinase, we have considered two energy surfaces that correspond to ligand bound and unbound states, and binding occurs at the intersection of two energy surfaces. This approximation is valid when the binding process is fast compare to the conformational fluctuations. This model could be extended to describe situations that are more general. Several energy surfaces that would correspond to ligand bound/unbound and open/closed states could be considered. The ligand binding process could be described in a stochastic manner, i.e., the binding occurs around the intersections of energy surfaces probabilistically depending on the energy difference between the states (see figure 6). Such formalism could be used to predict the kinetic dependency on the environment such as the ligand concentration and temperature.

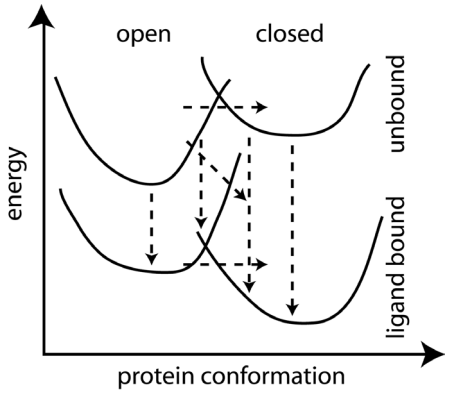

Figure 6: Generalized energy scheme of conformational change coupled to ligand binding. Four energy surfaces, open form with or without the ligand bound and closed form with or without the ligand, can be considered. The ligand binding process and the conformational transition could occur probabilistically in several different orders or concurrently.

I also intend to study the coupling of binding and allosteric transition using computation. The binding process could be considered as two step mechanism: the formation of encounter complex followed by final complex formation (3). The encounter complex could be modeled as the complex stabilized by electrostatic interactions, and then final complex is formed by short-range interactions. Here, the effects of large conformational fluctuations of the protein on these binding energy landscapes need to be considered. Short-range interactions could be very sensitive to the conformational fluctuations, while the electrostatic interactions are not. The binding process needs to be discussed as a stochastic process on such highly dynamical energy landscape.

Practically these studies on the coupling of binding and conformational change could also contribute to the ligand bound structure predictions. In the field of ligand binding predictions,

flexibility of protein is a challenging problem (*22*). By studying the general scheme of the binding process, it could help to develop better algorithm for bound structure predictions.

Simulation of Evolution Process to Understand the Protein Architecture

Evolution as another first principal. To complement studies on protein dynamics and functions, I intend to extend my researches to studies on protein evolution. The "evolution" has been used as a magic word to explain the remarkable character of biological systems. Biological molecules usually seem to have been optimally designed through evolution, however they sometimes include elements to which one cannot give a rational explanation. Biological molecules may not have been designed perfectly, which might be due to neutral evolution (*23*) or to the fact that all trial mutations are not examined during evolution. Therefore, evolution needs to be considered as another kind of fundamental principal to understand functions of biomolecules. The simulation of evolutionary process is a new area. Studies will be improved dramatically with information from the recent genomic revolution and will be quite necessary to understand the nature of evolutionary processes.

Nature exerts pressure on three essential factors of proteins in the course of evolution, their thermodynamics stability, folding kinetics, and function. Thus, the standard procedure for simulating evolutionary process is as following. 1) A trial mutant is generated by genetic or amino acid substitutions. 2) The mutant is tested to examine if it can survive under evolutionary pressure; if it survives, next mutation is tested. For computational studies of protein evolutionary processes, the thermodynamics stability is the main evolutionary pressure implemented in the existing models. Several studies have given insight into "mutational robustness of protein molecules" (*24, 25*), "hierarchical organization of proteins in fold families" (*26*). For the majority of these studies, protein molecules are modeled as a quite simple lattice model. For example, a protein molecule is often represented by 25 points, which are seating on 5×5 square lattice.

In the following, I describe some research interests aiming to understand evolution of protein architecture.

How does a specific interaction between proteins emerge from evolution spontaneously? Specificity of protein-protein or protein-ligand interaction is critically important for any kind of protein functions. The origin of such specific interactions between proteins is an interesting point. Some proteins are known to be unstable without forming oligomer with other proteins. Here such marginal protein stability may be the key to explain how protein specificity emerged from evolutionary processes. As we discussed, in the evolutionary processes of protein molecules, stability of the native structure is the strongest evolutionary pressure. If a sequence cannot form a stable native structure, it does not have any function and will not survive. However, such unstable sequences can "rescue" each other, i.e., they can form a stable structure by forming homo or hetero dimer, and then survive to the next generation. In this way, specific interactions between proteins could emerge from such evolutionary process.

To test such hypothesis, I will simulate co-evolution of a large network of protein interactions. Starting from a pool of model proteins, the evolutionary process will be simulated. After the mutation, we allow sequences to survive if they form stable native form alone OR if they can form a stable homo or hetero dimer with another sequence. I will start simulations with the simple lattice model. From these simulations, we can examine the hypothesis. Especially, we will focus on what type of proteins and interactions emerges from evolutionary processes.

Another interesting question is how a variety of protein interactions emerged as the result of evolution. In studies of gene sequence, it is known that interacting protein pair has same type of evolutionary tree (*27*), i.e. interacting protein pair could co-evolved. I aim to simulate such co-evolution in the structural space. By extensive evolutionary dynamics, we could generate the evolutionary neutral networks (i.e., the set of sequences connected by structure conserving binding specificity), and examine how specific residues pairs have evolved and need to be conserved.

How did the shape of biological macromolecule evolve? Recent studies on the global large-scale dynamics of macromolecules such as the ribosome indicate that the shape of molecules is designed to promote specific motion, which is essential for the function (*28*). It is often remarkable that each macromolecular machine adopts its shape to achieve the function. How do they adopt such shape? I would like to discuss this point from the evolutionary point of view. Since one of the evolutionary pressures is the stability of the molecule, the shape of a molecule could lay in some limitations. However, by forming complexes of a large number of proteins, these machines present special shapes. Considering the multiple complex formations in the evolutionary simulation, we could discuss how the mechanical architecture of macromolecules such as the ribosome has evolved to adopt a specific shape (Figure 7).

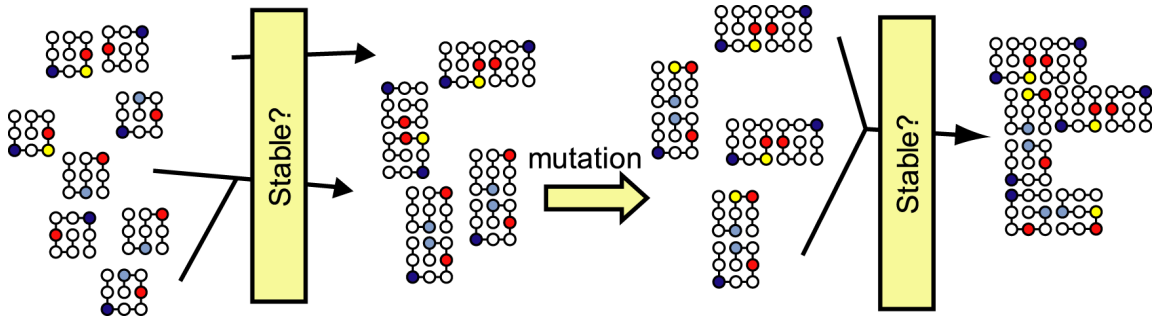

Figure 7: Scheme of evolutionary simulation for shape adaptation. We start an evolutionary simulation from a pool of model proteins. After mutation, enforcing the natural selection, even if a protein is unstable as a monomer, if it is stable by forming a complex, it survives to the next generation. By repeating such evolutionary processes, specific protein interactions could emerge, and therefore assembly complexes with specific shape could be generated. Number of components in the complex and energetic factor to determine the shape could be discussed by comparing to real macromolecular complexes.

Simulation of evolutionary process with semi-atomic model. In the research of complex systems such as biomolecules, simple minimalist model and detailed model need to complement each others. Due to the additional complexity of the evolutionary process, most studies on protein structure evolution have been done with lattice models. However, these models need to be improved to adopt a more realistic protein structure. Thus, although it is challenging, the development of off-lattice model is important. The off-lattice models for folding and structure prediction studies, such as associative memory Hamiltonian (*29*), could be used for this purpose. A new algorithm to sample different structures needs to be developed. These methods could be used to access the stability of a test sequence by feasible computational costs. By combining the knowledge from the simple lattice model and detailed model, we could lay the foundation to understand the evolution of protein architecture.

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Statement of Teaching Philosophy for Dr Osamu Miyashita

My teaching experience goes back to the time where I was an undergraduate student in Kyoto University, Japan. During this time I took a training course to teach a number of science classes in a high school. The process of transmitting my enthusiasm, knowledge and experience to young students fascinated me. As a result of this training, I received a license for teaching science in the high school. During graduate school, I also did some teaching assistance for a class of computational simulations, for which I provided course materials and grading examinations.

Does science education help a student's life later, for example, as a lawyer, a politician, an engineer, or even as a researcher? I believe it is beneficial for the students' life no matter what occupation they will have. However, it would not be in an explicit way. Even for my life as a scientist, the knowledge from my education does not directly solve problems in my researches. Rather, it is the scientific attitude that is helping my researches. It is important to keep logical, critical and skeptical attitude. This way of tackling problems is essential for achieving any kind of goals in our life.

Therefore, I will try to emphasize my teaching on the underlying ideas of a subject, and not simply transferring a vast amount of knowledge. Although I think some memorizations are always necessary for biology, computation, and even for physics, it can be best achieved by student's active participation in the learning process, i.e. doing homework. The task of an educator is to enable students to catch the idea, the concept and the logics of the subject. Then, by challenging non-trivial problems, students can understand the concept of the subject deeper, and expand their knowledge related to the subject.

I believe I am well qualified to teach several undergraduate courses, including introductory physics and chemistry, statistical physics, physical chemistry and introductory programming. My training in Japanese high school science course will be useful, since it overlaps with the courses in first grades in US Universities. I am also willing to provide physics educations to biological and medical students. At the graduate level, I would like to teach courses and seminars in statistical physics, biological physics, bioinformatics, and computer simulation methods. In these courses, I will gradually mix some specialized topics in biophysics. I will demonstrate how simple physical concepts can be applied to understand the complex biophysical problems.