学位論文の要約

題目　Theoretical investigation of protein functions related to electron and ion transports working in thermal fluctuation
(イオンと電子が関わる生体分子機能におけるタンパク質熱ゆらぎの役割の理論的解明)

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序論

Protein undergoes large conformational changes during its reactions, such as ligand bindings, hydrolysis, photoexcitation or redox reactions. These reactions were widely studied by several experimental methods such as X-ray crystallography, NMR spectroscopy, FTIR spectroscopy and so on. However, with technologies nowadays, it is hard for us to study chemical reactions in complicated protein systems at atomic level. This problem is partly resolved by a hybrid QM/MM method called QM/MM reweighting free-energy self-consist field (QM/MM RWFE-SCF), in which the fluctuation of environments is treated as a statistical procedure.

In this thesis, several applications of QM/MM RWFE-SCF in different fields of protein reactions contains photoexcitation and redox reactions are concluded, showing the power and potential of this new hybrid QM/MM method. These applications will be concluded in three parts:

1. Molecular Mechanism of Wide Photoabsorption Spectral Shifts of Color Variants of Human Cellular Retinol Binding Protein II

Color variants of human cellular retinol binding protein II (hCRBPII) created by protein engineering were recently shown to exhibit anomalously wide photoabsorption spectral shifts over ~200 nm across the visible region. The remarkable phenomenon provides a unique opportunity to gain insight into the molecular basis of the color tuning of retinal binding proteins for understanding of color vision as well as for engineering of novel color variants of retinal binding photoreceptor proteins employed in optogenetics. Here, we report a theoretical investigation of the molecular mechanism underlying the anomalously wide spectral shifts of the color variants of hCRBPII. Computational modeling of the color variants with hybrid molecular simulations of free energy geometry optimization succeeded in reproducing the experimentally observed wide spectral shifts, and revealed that protein flexibility, through which the active site

structure of the protein and bound water molecules is altered by remote mutations, plays a significant role in inducing the large spectral shifts.

2. An Atomistic Model of a Precursor State of Light-Induced Channel Opening of Channelrhodopsin

Channelrhodopsins (ChRs) are microbial light-gated ion channels with a retinal chromophore and are widely uti- lized in optogenetics to precisely control neuronal activity with light. Despite increasing understanding of their structures and photoactivation kinetics, the atomistic mechanism of light gating and ion conduction remains elusive. Here, we present an atomic structural model of a chimeric ChR in a precursor state of the channel opening determined by an accurate hybrid mo- lecular simulation technique and a statistical theory of internal water distribution. The photoactivated structure features exten- sive tilt of the chromophore accompanied by redistribution of water molecules in its binding pocket, which is absent in previously known photoactivated structures of analogous photoreceptors, and widely agrees with structural and spectroscopic experimental evidence of ChRs. The atomistic model manifests a photoactivated ion-conduction pathway that is markedly different from a previously proposed one and successfully explains experimentally observed mutagenic effects on key channel properties.

3. Ab initio evaluation of redox potential of metalloprotein

Redox processes are involved in various electro-chemical systems as fuel cell, photosynthesis, energy storage, and enzymes. Redox reactions which occur in complicated bio-systems consisting of flexible proteins, mobile solvent water molecules, and lipids are coupled to their thermal fluctuations, and provide electro-chemical properties different from that in water solutions. In this study, we evaluated the redox potential of cytochrome c by calculating free energy difference between the redox states. The calculated redox potential is 111.6 kcal/mol ,which is overestimated only by 4 kcal/mol compared to the experimental value of 108.1 kcal/mol. The ab initio approach thus allows one to readily evaluate redox potentials of metalloprotein systems without formidable molecular modeling of electronically complex reaction centers.