3E1034 粗視化 Go モデルを用いた Ferredoxine-like fold タンパクのフォールディング機構の解析**Analyses of folding processes on the Ferredoxin-like fold proteins by means of a coarse grained Go model****Masatake Sugita**, Takeshi Kikuchi (*Dept. Bioinf., Coll. Life Sci., Ritsumeikan Univ.*)

It has been recognized that analysis of proteins with a same native topology is efficient strategy for comprehending protein folding mechanisms. In particular, proteins sharing Ferredoxin-like fold are widely studied experimentally. It has been reported that these proteins exhibit various ϕ value patterns. These profiles are interpreted that stability of the two competing foldons regulates folding mechanisms. We are currently studying Ferredoxine-like fold proteins by analyzing amino acid sequence information. Our data also indicate that a significant segment for a folding is different among Ferredoxin-like fold proteins. For detailed elucidation of folding mechanisms with higher resolution including extensive free energy profiles, we have to conduct simulation approach. In this study, we analyze the folding processes of Ferredoxin-like fold proteins using coarse grained Go model. We use protein S6, U1A and ADA2h as target proteins. While we couldn't distinguish individual folding pathway using a topology-only model, we discern the difference of heights of free energy barrier. On the other hand, we got inherent folding process respect to each target protein using the model including sequence colors.

3E1046 異なる参照状態を用いた統計ポテンシャルによるタンパク質モデル構造の最適化**Optimization of protein model structures according to statistical potentials with different reference states****Matsuyuki Shirota**^{1,2}, Kengo Kinoshita^{1,2,3} (¹*GSIS, Tohoku Univ.*, ²*ToMMo, Tohoku Univ.*, ³*IDAC, Tohoku Univ.*)

Prediction of the 3D structure of a protein molecule from its amino acid sequence is an important challenge in computational biophysics. One of the bottlenecks is to select the most native-like structure among the model structures generated in the prediction process. Statistical potentials derived from protein structure database are widely used for this purpose. In principle, they are developed by counting the conformations in the database of native protein structures under the assumption that frequently observed conformations are energetically favorable ones. To quantify the significance of the counts of conformations in the database, the reference state, a virtual state in which all atomic interactions are random, is required. Previously, we demonstrated that the definition of the reference state of distance-dependent atomic pair statistical potentials can be theoretically variable and in practice statistical potentials with various reference states show different advantages, such as specificity for the native structures or the correlation to the native-likeness. To further explore the problem of reference states, here we developed an optimization method of protein model structures under several statistical potentials based on distinct reference states. In addition, we also employed several statistical potentials in a linear combination to improve the performance. Our results showed that various reference states have different advantages in both model evaluation and model optimization.

3E1058 クーロンレプリカ交換法を用いた A β フラグメントに関する研究**Studies on a A β fragment by the Coulomb replica-exchange method****Satoru Itoh**^{1,2}, Hisashi Okumura^{1,2} (¹*Institute for Molecular Science*, ²*The Graduate University for Advanced Studies*)

Effective samplings in the conformational space are necessary to predict the native structures of proteins. The replica-exchange method (REM) is one of the most well-known methods among the generalized-ensemble algorithms which realize effective samplings in the conformational space for biomolecular systems. However, REM has a difficulty for large systems that many replicas are needed to realize such effective samplings. In other words, huge amount of computer time is required in the replica-exchange method for the large systems. In order to overcome this difficulty, we proposed a new replica-exchange method, which we refer to as the Coulomb replica-exchange method (CREM), recently. In this CREM, electrostatic charges are exchanged among replicas although the temperatures are exchanged in conventional replica-exchange method. By exchanging the electrostatic charges, the free-energy barrier come

from the electrostatic interactions are reduced. Moreover, the number of replicas can be decreased by exchanging the electrostatic charges only of solute atoms. Consequently, the CREM simulations realize effective samplings in the conformational space with small number of replicas. I will show the effectiveness of this method through applications to a amyloid-beta fragment.

3E1110 レプリカ交換インターフェースプログラム(REIN)**Replica-exchange interface program (REIN)****Naoyuki Miyashita**^{1,2,3}, Suyong Re⁴, Yuji Sugita^{1,2,3,4} (¹*RIKEN QBiC*, ²*RIKEN CSRC*, ³*RIKEN AICS*, ⁴*RIKEN ASI*)

In the recent decade, replica-exchange molecular dynamics (REMD) method [1] has attracted much attention as an enhanced conformational sampling method. In REMD, ensembles consist of non-interacting copies (replicas) of an original system with different conditions. The pair of replicas are exchanged every few steps by metropolis criteria to enhance the sampling efficiency.

We have developed replica-exchange Interface program (REIN) to facilitate REMD simulations. Currently, the multi-dimensional REMD can be performed [2] using the highly parallelized conventional MD (NAMD2 [3] and MARBLE [4]). The other conventional MD programs can be easily combined as well. REIN is also tested for use in supercomputer systems, enabling us to simulate substrate binding or conformational changes in large proteins with high precision. The detail of program and some recent applications will be presented.

[1] Y. Sugita and Y. Okamoto, *Chem. Phys. Lett.*, 314,141(1999)[2] Y. Sugita, A. Kitao, and Y. Okamoto, *J. Chem. Phys.*, 113, 6042 (2000)[3] J. C. Philip et al., *J. Comp. Chem.*, 26, 1781 (2005)[4] M. Ikeguchi, *J. Comp. Chem.*, 25, 529 (2004)**3F0900 サポート膜を用いた膜タンパク質の顕微鏡下でのイオン輸送活性計測****Evaluation of ion-pump activity of membrane proteins reconstituted on supported membrane under optical microscope****Jyunan Ki**, Rikiya Watanabe, Kazuhito Tabata, Hiroyuki Noji (*Department of Applied Chem., Univ. Tokyo*)

Supported membrane is the experimental platform in which a lipid bilayer is tethered on a solid surface. Owing to the mechanical stability, it allows us to investigate physicochemical properties of membranes and also membrane proteins reconstituted in the membrane. In this study, to enhance the versatility of this method, we developed a novel supported membrane system that enables to measure the ion-pump activity of membrane proteins under an optical microscope. As a model ion pump, we reconstituted F₀F₁-ATP synthase (F₀F₁) in liposomes. The supported membrane was prepared on a glass surface from the reconstituted liposome with a buffer containing a pH indicator dye (pHrodo) and caged-ATP. By introducing the evanescent illumination of 405 nm light for uncaging of ATP from the bottom of the glass surface, ATP was supplied only in the illumination area. The local pH of the membrane-surface space was monitored with the fluorescent signal of pHrodo excited with the evanescent illumination of 532 nm. Only after the illumination for uncaging, the intensity of pHrodo evidently increased, suggesting the acidification between the membrane-surface space. Furthermore, the degree of the acidification was proportional to the amount of uncaged ATP. These results showed that this systems allowed us to successfully monitor the H⁺-pumping activity of F₀F₁ reconstituted in the supported membrane.

3F0912 Catalytic activity of MsbA reconstituted in nanodisc particles is modulated by remote interactions with the bilayer**Takeaki Kawai**¹, Jose Caaveiro^{1,2}, Toyomasu Katagiri³, Hisashi Tadakuma¹, Takuya Ueda¹, Kouhei Tsumoto^{1,2,4} (¹*Grad. Sch. of Front. Sci., Univ. of Tokyo*, ²*Inst. Med. Sci., Univ. of Tokyo*, ³*Inst. Gen. Res., Univ. of Tokushima*, ⁴*Dep. Chem. Bio., Univ. of Tokyo*)

ATP-binding cassette (ABC) transporters couple hydrolysis of ATP with vectorial transport across the cell membrane. We have reconstituted ABC transporter MsbA in nanodiscs of various sizes and lipid compositions to test whether ATPase activity is modulated by the properties of the bilayer. ATP hydrolysis rates, Michaelis-Menten parameters, and dissociation constants of substrate analog ATP- γ -S demonstrated that physicochemical properties of the bilayer modulated binding and ATPase activity. This is remarkable when considering that the catalytic unit is located ~50 angstrom from the transmembrane region. Our results validated the use of nanodiscs as an effective tool to reconstitute MsbA in an active catalytic state, and highlighted the close

relationship between otherwise distant transmembrane and ATPase modules.

3F0924 線虫イネキシシン 6 ギャップ結合チャネルの単離と機能特性

Isolation and characterization of *C. elegans* innexin-6 gap junction channels

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Invertebrates possess gap junction channels composed of innexins that evolved independently from vertebrate connexins. Only limited information is, however, available on the innexin channel structure. Here we successfully expressed and purified *C. elegans* innexin-6 (INX-6, 45kDa) gap junction channels, and characterized the structural properties and channel permeability using electron microscopy and microinjection of fluorescent dye tracers, respectively. The INX-6 proteins formed gap junction plaques with a loosely packed hexagonal lattice. Channel distance, based on the reflection spots of the INX-6 gap junction plaques, was longer than that of connexin-26 (Cx26, 26 kDa). Thin-section electron microscopy revealed that the INX-6 junction was thicker than the Cx26 junction. The purified INX-6 channels in dodecylmaltoside solution generally ran slower than Cx26 channels in gel filtration analysis, suggesting that the purified INX-6 channels were mostly hemichannels, which was supported by electron microscopy and native gel electrophoresis. Dye transfer experiments showed that 3-kDa dextran-Texas red and 10-kDa dextran-Texas red passed through INX-6 channels at rates of approximately 50% and 10%, respectively, while no significant amounts passed through connexin-43 channels. These results have implications regarding the dimensions and overall structure of the INX-6 channels, and provide insight into the wider pore diameter of INX-6 channels compared with that of connexin channels.

3F0936 ATR-FTIR 法を用いた KcsA 野生型と不活化しない変異体に対するカチオン誘起構造変化の赤外分光分析

Cation-induced structural changes in the WT and non-inactivating mutant KcsA studied by ATR-FTIR

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The bacterial potassium channel KcsA is a membrane protein complex of homotetramer. The channel is gated by high concentration of intracellular protons, and transports cations through the filter that permeates K⁺ selectively over Na⁺. In the wild type (WT) KcsA, the filter can be in both open and inactivated (collapsed) structures depending on ionic/pH conditions, whereas the filter remains open in the non-inactivating mutants such as E71A, E71V and F103A. In this study, we investigate the mechanism of ion selectivity coupled with gating process using attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy. WT and mutant proteins of KcsA of *Streptomyces lividans* were expressed in *E. coli*, and purified proteins were reconstituted in liposomes and deposited on an ATR-prism. K⁺- or Na⁺-induced difference FTIR spectra were measured by switching perfusion buffers with and without cation. At pH 7, the gate of the WT was closed and the selectivity filter was presumably open. K⁺-induced spectrum showed different IR features from Na⁺-induced one, indicating different ionic interactions of K⁺ and Na⁺ to the protein. The IR spectra obtained at pH 4 were different from those at pH 7. The corresponding spectra measured in the non-inactivating mutants were compared to those in the WT. Conformational changes in peptides upon the binding of cations to KcsA are discussed based on the obtained IR data in the light of atomic structures available from X-ray crystallography.

3F0948 黄色ブドウ球菌の 2 成分性膜孔形成毒素の分子機構

Molecular basis of staphylococcal bi-component pore forming toxin

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Pathogenic bacteria secrete various pore-forming toxins (PFTs) to kill host cells. PFTs are expressed as water-soluble monomeric proteins that oligomerize into nonlytic prepore intermediates on the membrane of the target cells, and then form bilayer-spanning pores.

Staphylococcus aureus, a ubiquitous and pernicious human pathogen, secretes

several PFTs including α -hemolysin (α HL), γ -hemolysin (γ HL), leukocidin (LUK), and Panton-Valentine leukocidin (PVL). α HL consists of a single polypeptide, whereas the others are bi-component PFTs that require the synergistic association of a class F component and a class S component. Extensive experiments have been carried out for more than two decades, and the crystal structures of the monomeric forms of bi-component PFTs have been determined. However, the structures of the prepore and pore forms have not been reported at atomic resolution, which has hindered detailed discussion of the complicated molecular mechanism.

In the present study, we determined the crystal structure of the prepore and pore form of bi-component PFT, γ HL. This is the first report of the crystal structure of a heterocomponent β -barrel-type transmembrane protein. This is also the first PFT of which all of monomer-, prepore-, and pore-form structures have been determined by X-ray crystallography. Based on the structural differences between pore and monomer forms in combination with biological data accumulated over the past two decades, we propose a mechanism of pore formation by PFTs.

3F1010 分子動力学法によるグラミシジン A を含んだ脂質二重層膜の構造と圧力特性

Structure and lateral pressure profile of lipid bilayer containing gramicidin A by molecular dynamics simulation

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The molecular dynamics (MD) simulations of DMPC (diC14:0-PC) and DSPC (di18:0-PC) lipid bilayers in the absence and presence of gramicidin A (GA) peptide dimer were carried out to investigate the GA effect on these membrane structure and the lateral structure. The observed hydrophobic thickness and order parameter increased, and the gauche conformation reduced in the presence of GA, and these results are consistent with the experimental measurements. Due to the hydrophobic mismatch between the GA and surrounding lipid bilayer, the hydrophobic thickness of the DSPC membrane largely decreased to fit the length of hydrophobic core region of the GA peptide dimer, resulting in a distortion of the membrane surface around the GA. The surface tension around the polar headgroup region decreased due to the decrease of electrostatic interaction between headgroups. Since the DSPC lipid bilayer has a larger hydrophobic length, the larger positive lateral pressure appears around the GA channel entrance region in the membrane. The larger lateral pressure in the hydrophobic region of the DSPC/GA membrane results in the reduction of pore radius around the GA channel entrance, suggesting the decrease of ion accessibility to the GA channel entrance.

3F1022 代謝型グルタミン酸受容体の膜貫通領域における 1 アミノ酸変異がアゴニストをインバースアゴニストに変える

Single amino acid substitution in the transmembrane domain of metabotropic glutamate receptor changes an agonist into an inverse agonist

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Metabotropic glutamate receptors (mGluRs) are prototypical family 3 G protein-coupled receptors (GPCRs) that function as a constitutive homo dimer. They have a large extracellular ligand-binding domain (ECD) on the N-terminal side of seven-transmembrane domain (TMD). Upon binding of an agonist, an intersubunit relocation of the dimeric ECDs induces a mutual rearrangement of TMDs, which was observed by our previous fluorescence resonance energy transfer (FRET) analyses. However, the conformational change within each protomer of mGluR is largely unknown. Here we report that a single amino acid mutation at a threonine residue conserved in helix VI of mGluRs caused elevation of a basal activity, whereas the glutamate binding to the mutant partially suppressed the basal activity. Namely, original "agonist" works as an "inverse agonist" on this mutant. The comparative FRET analysis of wild-type and the mutant demonstrated that the glutamate-dependent dimeric rearrangement of the mutant is similar to that of wild-type. Therefore, alteration of the ligand character in the mutant should be originated from a conformational change within each protomer. Double mutation analyses revealed that an additional mutation in helix VII restored the "agonist"-dependent increase of the activity. In conclusion, the proper packing between helices VI and VII around the threonine residue within each protomer is a key determinant of the activation of mGluRs.