3G0936 H-ras GTP 複合体と H-ras GDP 複体の分子動力学シミュレーション**Molecular dynamics simulations of the structures of H-ras GTP complex in solution and H-ras GDP complex in solutions****Takeshi Miyakawa¹**, Ryota Morikawa¹, Masako Takasu¹, Hiroaki Saito², Hidemi Nagao² (¹*Tokyo University of Pharmacy and Life Sciences*, ²*Kanazawa University*)

We study the structures of H-ras GTP complex and H-ras GDP complex in solution by Molecular dynamics simulations. We have derived the potentials and the atomic charges around Mg^{2+} in H-ras GTP complex and H-ras GDP complex by quantum chemical calculations. We will perform MD simulations and sample the structures of H-ras GTP complex and H-ras GDP complex in solution as NPT ensembles.

3G0948 天然構造のデータのみから構築されたタンパク質モデル構造の絶対的な質の評価法**An absolute quality evaluation method of protein model structures developed based only on the native structure data set****Matsuyuki Shirota¹**, Ishida Takashi², Kengo Kinoshita^{1,3} (¹*Grad. Sch. Info. Sci., Tohoku Univ.*, ²*Grad. Sch. Info. Sci. Eng., TITEch*, ³*IDAC, Tohoku Univ.*)

In protein structure prediction, it is important that the model structure predicted from amino acid sequence can be assessed for its absolute quality, i.e. its structural similarity to the corresponding native structure. Statistical pair potentials extracted from the native protein structure data set are widely used in quality assessment, but they have not been represented as the absolute quality scores. On the other hand, existing absolute quality estimation methods, such as machine learning ones, require a data set of model structures of known quality for training, in addition to the native structures. In this study, we developed a method that can evaluate the absolute quality of a model structure using only a set of native structures, by way of normalizing statistical pair potentials. This normalization was based on a natural extension of the idea of the native and reference states, which are the necessary components of statistical potentials, applied to each of the model structures to be evaluated. We tested this method using the model structures of various proteins developed in CASP (Critical Assessment of Techniques for Protein Structure Prediction) contests. As results, our method showed comparable performance with current state-of-art machine learning-based methods in terms of correlation with the true absolute quality and in terms of discrimination of native structures. These results show that statistical potentials can evaluate the absolute quality if they make full use of statistical information in the native structures.

3G1012 分子動力学シミュレーションによる Rad51 リコンビナーゼの分子モデリング**Molecular modeling of Rad51 recombinase using molecular dynamics simulations****Yuichi Kokabu**, Mitsunori Ikeguchi (*Yokohama City Univ*)

The exchange of strands between two homologous DNA molecules, which is a central reaction in homologous recombination, is catalyzed by Rad51 recombinase in eukaryotes. In the reaction, a filament comprised of Rad51 and ssDNA searches a homologous DNA strand, and exchanges those strands. In the presence of ATP, Rad51 can form active filaments even without ssDNA. Electron microscope studies have shown that the pitch of the active filament is ~100 Å. However, in the crystal structure, the pitch of the filament is ~130 Å, which is distinctly different from the pitch observed by electron microscope. Further, no ATP is bound to the crystal structure of Rad51.

To elucidate the atomic structure of the Rad51 active filament, homology modeling was carried out using the crystal structure of an archaeal homolog, RadA, in which the pitch of the filament is ~100 Å, and ATP is bound in the interface between protomers. Then, we performed an all-atom molecular dynamics (MD) simulation with explicit water for the modeled dimer that is a minimum unit of the filament.

During the MD simulation for the modeled structure, the dimer structure retained the initial arrangement, and the adenyl ring of ATP tightly interacted with both protomers of the dimer. The stable salt bridge between Arg in the P-loop of one protomer and Glu of the other protomer was observed, which is a unique interaction in Rad51, not in RadA.

3G1024 ハブ蛋白質のリガンド依存構造変化：ユビキチンの場合**Ligand-dependent conformational changes of the promiscuous protein: A case for Ubiquitin****Bhaskar Dasgupta^{1,2}**, Akira Kinjo¹, Haruki Nakamura¹ (¹*Osaka University, IPR*, ²*JBIC*)

Protein-protein interactions play dominant roles in proper functioning of the cell. In the network of such interactions, the proteins that bind multiple partners (or ligands) are critically important. Recognition of different ligands by these so-called hub proteins (or receptors) should be controlled by collective motions of the receptor and ligand chains. To demonstrate the dynamic nature of protein-protein recognition by hub proteins in atomic level, we have performed all-atom normal mode analysis of 11 protein-protein complexes, where Ubiquitin receptor is binding to diverse ligands. Specificity of recognition was analyzed by observing change in the internal and external motion of the receptor due to binding different ligands. Our results indicate that coupling of the external motions of the ligands to the motions of the receptor may be crucial for the protein-protein recognition. Moreover, we observed higher flexibility of residues and atoms in the common overlapping region relative to the total multi-binding interface (that bind to all types of ligands). Curiously, we also observed some residues (and atoms) that show large mean square fluctuation are distant from the protein-protein interface.

3G1036 カメレオンモデルによる DHFR の構造転移の研究**Conformational transition of DHFR simulated with the chameleon model****Toru Kimura¹**, Tomoki Terada², Masaki Sasai² (¹*Dept. of Applied Physics, Grad. Sch. of Engineering, Nagoya Univ.*, ²*Dept. of Computational Science and Engineering, Grad. Sch. of Engineering, Nagoya Univ.*)

Most proteins undergo the conformational changes in their functioning. Single-molecule observations and NMR experiments have revealed the existence of the conformational transition within the preexisting equilibria, which is related to their functions. Furthermore, conformational transitions can correspond to the rate-limiting steps in the catalytic cycle, which is experimentally suggested in some proteins (and may be more general). A well-characterized protein dihydrofolate reductase (DHFR) is one of such proteins. Although their conformational change is relatively small, NMR relaxation dispersion analysis has suggested that three (out of five) steps in the catalytic cycle are accompanied by the two-state conformational transition with a free energy barrier. To obtain further insight into these transitions, we have performed coarse-grained molecular dynamics simulation using the chameleon model. In this model, the single energy minimum of local interaction is assumed to move between two limits defined by two crystal structures, depending on the local structure around the interaction sites. Calculated free energy landscape has indicated that the two basins are stabilized energetically whereas the free energy barrier between them is stabilized entropically, which is consistent with the loop motions expected from the visual comparison of two crystal structures. This suggests that the free energy barrier height is decreased at higher temperature, which can be tested experimentally.

3G1048 機能に関する選択圧による蛋白質の進化シミュレーション**Simulated evolution of protein by a selective pressure on function****Masahiro Yamamoto¹**, Takeshi N. Sasaki², Kengo Sawada¹, Shintaro Minami³, George Chikenji³, Tomoki P. Terada³, Masaki Sasai³ (¹*Dept. of Apply. Phy., Univ. of Nagoya*, ²*Dept. of Human Info., Fac. of Human Info., Aichi Shukutoku Univ.*, ³*Dept. of Comp. Sci. Eng., Univ. of Nagoya*)

[Background] Features which distinguish proteins from other polymers are their specific functions. To have some function, active site of each protein needs to take a definite structure upon functioning. A plausible scenario is that the primitive protein-like polymers which did not have any specific functions nor definite 3D structures have evolved to acquire some configuration at their active residues to interact with other molecules. We may be able to expect that the other features such as the globular shape of the whole structure and the local orders at the other sites have appeared as consequences of such evolution in functionality. [Purpose] To examine this hypothesis, we computationally simulated the process that random polypeptides evolve to have a function. [Method and Results] A DNA binding protein, the λ phage repressor, was chosen as a model protein. The original first amino acid sequence of the length

equal to the λ phage repressor was randomly generated. The point mutagenesis was introduced at each generation, and with thus generated sequence, conformation was calculated with a combined method of the fragment assembly and Langevin dynamics calculations. The mutated sequence was selected according to the criterion whether the calculated conformation has a local structure of the active residues more similar to that of the λ phage repressor. We discuss the numerical results of this simulated evolution.

3G1112 C型肝炎ウイルスにおけるNS3 セリンプロテアーゼの阻害剤 cVH 種に関する分子動力学シミュレーション

Molecular Dynamics Simulations of cVH domain antibody inhibitor of HCV NS3 protease

Masashi Iwayama, Kazutomo Kawaguchi, Hiroaki Saito, Hidemi Nagao (*Division of Mathematical and Physical Science, Graduate School of Natural Science and Technology, Kanazawa University*)

Antibodies exist in blood or body fluid, and they recognize and bind a variety of bacterium and viruses which invade the body and the cell as antigens. Antibodies generally form Y-shaped tetramer composed of two light chains and two heavy chains. That is, the dimerization of the VH domain and the VL domain, both of which are variable regions of antibodies are required for function as an antibody. On the other hand, many camelid antibodies composed of only two heavy chains have been known that only VH domain play a role as antibodies. This single domain antigen, so called nanobody, is much simpler than a conventional antibody in point of chemical composition and form. And it is expected as a various applications for its specificity in the field of medicine and biophysics. Especially according to the report, cVH-E2 antibody which has high-affinity and variant of hypervariable regions for antigen binding site is an effective inhibitor of NS3 serine protease which is key enzyme of the hepatitis C virus.

In this study, we carried out the molecular dynamics simulations of cVH-E2 monomer obtained from X-ray crystallography and solution NMR structure VH-P8. The VH-P8 is a precursor to cVH-E2, and different in the amino acid sequences of hypervariable regions and VL-binding face. From these calculations, we will discuss the conformational fluctuations of hypervariable regions and difference of conformational stability of VL-binding face in solution.

3G1124 アミノ酸ペア傾向を用いた β -sheet 形成予測法の開発 (2)

Development of prediction method for β -sheet formation using amino acid pairing propensity (2)

Hiromi Suzuki (*School of Agri., Meiji Univ*)

We have been developing a prediction method for β -sheet formation using statistical scores computed from the frequency of the inter-strand amino acid residue pairs based on experimental protein structure data, taking the existence of hydrogen bond formation into account. These scores showed that residues on a strand tend to avoid inconvenient residues on the other strand as partners (negative selection) rather than to select convenient residues (positive selection). Prediction accuracies for strand order in the β -sheets using these scores were 58, 35, 20 and 14% for 3-, 4-, 5- and 6-stranded sheets, respectively, and up to 77, 57, 39 and 22% by adding two restrictions: i) Strands form anti-parallel pairs if a loop length is shorter than 12 residues long (Loop), ii) All residues on β -strands have at least one pairing partner(s) (Mask). By the pairing score and the "Loop" and "Mask" restrictions, accuracies of order and direction prediction were up to 57, 36, 21 and 8 % for 3-, 4-, 5- and 6-stranded sheets, respectively, and accuracies of order, direction and register were 42, 16 and 8 and 5 % for 3-, 4-, 5- and 6-stranded sheets, respectively. In the latter case, H-bond formation is completely predicted. When maximum number of possible H-bonds is considered as one of sheet formation restrictions, prediction accuracies tend to increase for 3-stranded sheets, but to decrease for 5- and 6-stranded sheets. This tendency is related to the ratio of anti-parallel to parallel sheets.

3G1136 Molecular Dynamics Simulation of Protein Using Robot Dynamics Algorithm

Yu Yamamori, Kazuhiro Takemura, Akio Kitao (*Laboratory of computational Protein Science, Center for Structural Biology of Challenging Proteins, Tokyo University*)

In the simulation study of biophysical phenomena, a major bottleneck of Cartesian Molecular Dynamics (CMD) is the time step gap between the simulation and that of the target process. High frequency motions in proteins prohibit CMD from enlarging the simulation time step beyond 1.0-2.0 fs. To

overcome this limitation, we adopted a strategy to consider the torsion angles as the only degrees of freedom (DOF) which enables us to employ the methods developed originally in robotics. The Articulated-Body Algorithm (ABA) can solve the equations of motion of internal coordinates with the small computational cost that linearly depends on the number of DOF. Our strategy has three advantages, i.e. reduction of DOF, longer time step and the efficient linear algorithm. The program of Molecular Dynamics in Torsion Angle space (TAMD) is equipped with ABA. TAMD can treat amber and ECEPP force field and can generate NVE and NVT ensemble. To evaluate our program, we compared the spectra of 20 distinct dipeptides calculated by TAMD, CMD with bond constraints normal mode analysis (NMA) in torsion angle space. Comparing the spectrum of TAMD to those of CMD, found some peak differences originated from the different treatment of angles and improper torsion angles. Comparison of the spectrum of TAMD with those of NMA showed good agreement. We confirmed that this program can give reasonable results. We also searched the maximum time step length with reasonable energy conservation and we concluded that time step 6.0 fs can be employed.

3G1148 溶媒と自由エネルギーを用いた蛋白質-蛋白質ドッキング複合体のP15 評価

Solvation Free Energy of Complex: a potential tool for improving scoring function in protein-protein docking

Kazuhiro Takemura¹, Hao Guo^{1,2}, Shun Sakuraba³, Nobuyuki Matubayasi^{2,4}, Akio Kitao^{1,2} (¹IMCB, Univ. of Tokyo, ²JST,CREST, ³Institute for Molecular Science, National Institutes of Natural Sciences, ⁴Institute for Chemical Research, Kyoto University)

In protein-protein docking study, several decoys of candidate are generated and scored. The scoring of decoys is one of the key factors to select near native complex. For the purpose of developing the method to predict complex accurately, we evaluated docking decoys using solvation free energy. Two protein complexes are simulated: bovine trypsin and CMTI-1 squash inhibitor (1PPE), RNase SA and barstar (1AY7). Unbound protein structures were used as initial structures of rigid body dockings using ZDOCK. Several decoys which vary in RMSD from the crystal structure were picked up. Solvation free energies of decoys were calculated by molecular dynamics simulations and energy representation method by Matubayasi et. al. Decoys are evaluated by total energy of the complex structural energy and solvation free energy. The structures with low RMSD from the crystal structure tend to have lower total energy. Thus we concluded our method is useful to predict complex.

13:10~16:10 G会場 / Room G

蛋白質_構造 4

Protein: Structure 4

3G1322 Docking study to investigate the interaction of p53 domains with Mdm2 and Azurin

Ragunadha Reddy Burri, Akio Kitao (*Laboratory of Computational Protein Science, Institute of Molecular and Cellular Biosciences, University of Tokyo*)

The tumour suppressor p53 plays a crucial role in preventing cancer cell growth and maintaining genomic stability. Its anticancer activity is mainly down-regulated by Mdm2 that, upon binding to p53, blocks its transcriptional activity and promotes its ubiquitin-dependent degradation. It is a new and promising strategy for anticancer drug design to block the Mdm2-p53 interaction using a peptide or non-peptide inhibitor. It has been reported that the copper containing redox protein azurin, plays an anticancer role, as observed in different cell lines: it can enter cancer cells and induce their apoptotic death. This apparent antagonist action with respect to the Mdm2-p53 functional interaction suggests that binding of Azurin to p53 might interfere with the Mdm2-p53 association and, thus, preventing p53 from degradation. Recent AFM studies have suggested the possible formation of a ternary interaction among Azurin, Mdm2 and p53. To gain further insight about the ternary complex interactions (p53-Mdm2-Azurin), it is necessary to deeply characterize the interface between three proteins. By a protein docking study, combined with MD simulations and free energy calculations, we have predicted a possible structure for this ternary complex between p53 domain, Mdm2 and azurin, evidencing some details about their binding sites. The understanding of the molecular mechanisms on the basis of this phenomenon can open the way to new anti-cancer strategies.