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Most mitochondrial proteins are synthesized in the cytosol and imported into mitochondria. The N-terminal presequences of mitochondrial-precursor proteins contain a diverse amino acid sequence, which is recognized by Tom20 on the mitochondrial surface. Saitoh et al. previously found a consensus motif in the precursor proteins and determined the X-ray crystal structures of the Tom20-presequence complex [1]. They proposed the existence of a dynamic equilibrium among multiple bound states, which may facilitate the broad selective specificity of the Tom20 receptor toward diverse mitochondrial presequences. In the current study, we performed all-atom molecular dynamics (MD) simulations of the Tom20-presequence in solution, starting from two different crystal structures (A and Y-state). We simulated 500-ns dynamics for four different models using the NAMD software and observed only one transition from the Y to A states in one of the four MD simulations. We next performed umbrella sampling MD simulations in order to examine the energy barrier between these two states and search other bound states. As a result we found a new stable bound state along the structural transition pathway between the A and Y states. The structure of the new bound state obtained in the simulation we found was in good agreement with a new X-ray crystal structure of Tom20-presequence complex recently determined [2].

[1] T. Saitoh, D. Kohda, et al. *The EMBO Journal* 26, 4777-4787 (2007).

[2] T. Saitoh, D. Kohda, et al. *Biochemistry* (2011)

3G1458 配列順序の入れ替えを考慮したスレッディングによる構造予測法の開発

Development of a threading-based structure prediction technique utilizing sequentially shuffled templates

Kengo Sawada, Shintaro Minami, George Chikenji (*Dept. of eng., Nagoya univ.*)

Recently, many researches have reported interesting examples of non-sequential alignments such as circular permutations and domain or region swaps. For example, Alexj et al. conducted a comprehensive large-scale study of non-sequential alignments between available protein structures in Protein Data Bank (PDB) and found that 35.2% of all significant alignments are non-sequential[1]. Furthermore, Aysam et al. identified significant non-sequential structural similarity between some new folds and existing structures in PDB[2]. These observation suggest that appropriately utilizing non-sequential template enables us to predict new fold targets.

In this study, we propose a new threading-based structure prediction technique in which secondary structure ordering of template structures are shuffled. The method evaluates fitness between target sequences and sequentially shuffled template by several score function such as empirical potential function. In this talk, we will present the detailed algorithm of the method and results of prediction benchmark test.

[1]Alexej Abyzov and Valentin A Ilyin. 2007. *BMC Structural Biology* 7:78

[2]Aysam Guerler and Ernst-walter Knapp. 2008. *Protein Science*. 17:1374-1382

3G1522 修飾糖を含む全長 PrP モデリングによる既存プリオン繊維モデルの比較

A comparison of mouse prion protein PrP fibril models contained full-length residues(24-231) and its N-Glycan

Mihoko Saito, Tuiyoshi Shirai (*Nagahama. Inst. BioSci.Tech*)

The prion hypothesis declares that harmless cellular prion protein (PrP^c) is converted into cytotoxic conformational isomer (PrP^{sc}) in the prion diseases, and the protein in the latter form is the sole pathogenic entity that transmits disease without a mediation of nucleic acids. Although this hypothesis has been supported by many experimental evidences, the structural detail of PrP^{sc}, the most critical point of the hypothesis, is still elusive. Several 3D atomic models of fibril PrP^{sc} have been proposed, which are not consistent to each other. These PrP^{sc} models were constructed by referring to a wide variety of experimental data, but their coordinates are not publicly available, which prevent a comprehensive comparison of PrP^{sc} models. Therefore we have constructed the proposed PrP^{sc} models, which contain 231 amino acid residues and typical N-glycan at Asn180 (1660-2340 Da) and Asn196 (2000-3020 Da). MOE and COOT/REFMAC5 applications were used for the manual constructions and refinement of the models. The models were checked by using Verify3D and

PROCHECK. These PrP^{sc} models were used to evaluate the adequacy of the models in terms of steric hindrance of the disordered N terminal region (residues 24-90), two N-glycans, and C-terminal GPI-anchor moieties. Also, the reported mutations, which promote PrP^{sc} conversion, were tested on these models with the 3D1d method.

3G1534 A multi-dimensional umbrella sampling approach for structure prediction of transmembrane helical dimer

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Transmembrane (TM) proteins function as receptors, transporters, or enzymes. Integrin, for example, transmits the signal across the cell membrane inside-out and vice versa. Enormous efforts have been made experimentally and theoretically on understanding the relationships between TM proteins' functions and structures. Even though one third of proteins encoded in the human genome are estimated to be TM proteins, there are only ca. 1000 structures (ca. 200 unique structures) deposited in the protein data bank. It is extremely difficult to obtain their structures by employing the techniques of the X-ray crystallography and the NMR spectroscopy. Theoretical approach plays an important role in the structure prediction and in the interpretation of the experimental data. We have applied replica-exchange molecular dynamics (REMD) method to predict amyloid precursor protein and its homodimer structure in biological membrane [1]. Herein, we present a different approach, a multi-dimensional umbrella sampling method combined with the recent developed helix-helix restraint potentials [2], for the prediction of the transmembrane protein complex. We have applied this umbrella sampling method on the study of the association of the Glycophorin A transmembrane helical dimer and the results are compared with that obtained by the REMD simulation.

[1] N. Miyashita, J. E. Straub, D. Thirumalai, and Y. Sugita. *J. Am. Chem. Soc.* Vol. 131, pp. 3438, 2009.

[2] W. Im, J. Lee, T. Kim, and H. Rui. *J Comp Chem*. Vol. 30, No.11, pp.1622, 2009.

9:00~12:00 H会場 / Room H

蛋白質_物性 4

Protein: Property 4

3H0912 シャペロニンが BFP のフォールディングにおける熱容量と経路を変化させる

Chaperonin changes the heat capacity and pathway of BFP folding

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We studied the folding of blue fluorescent protein (BFP) in real-time to elucidate how chaperonin changes protein folding. Recently, we found that two reactions in the chaperonin-assisted folding, the in-cage folding in which protein folds in the cage, and the out-of-cage folding in which denatured protein escapes out of the cage and subsequently folds spontaneously, compete with each other. Thus, the in-cage folding consists of a slow step competing with escape and the subsequent rapid finishing step, although spontaneous folding has a single step. We analyzed the temperature dependency of the in-cage folding and spontaneous folding. The thermodynamic parameters were estimated from Eyring plot. The ΔC_p of spontaneous folding was more negative than that of the in-cage folding. In case of the chaperonin mutant that contains Y203C and five methionine residues replaced with phenylalanine at C-terminal peptide, ΔC_p is increased. Thus, hydrophobic surface is important to increase ΔC_p . These results also indicated that chaperonin-assisted folding is not always faster than spontaneous folding.

3H0924 マルチスケールアンサンブルモデリングの p53 全体構造への適用 On application of multi-scale ensemble modeling method to tumor suppressor p53 quaternary structure

Tsuyoshi Terakawa, Shoji Takada (*Kyoto university*)

The representative tumor suppressor p53 forms tetramer, and each chain consists of folded DNA binding domain (DBD), tetramerization domain (TET), intrinsically disordered N and C terminal domain (NTD and CTD), and flexible linker between DBD and TET. These flexible regions cause heterogeneity of