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Free Energy Analysis on the Tom20-Presequence Complex in Solution for Understanding a Dynamic-Equilibrium Model

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Tom20 locates at outer membrane of mitochondria and recognizes the N-terminal presequence of mitochondrial-precursor proteins. Recently, three atomic structures of the Tom20-presequence complex were determined using X-ray crystallography and classified into A-pose, M-pose, and Y-pose in terms of the presequence-binding forms. From the experimental data, a dynamic-equilibrium model has been proposed for this system, although its functional role has been still elusive. To understand this in detail, we performed all-atom molecular dynamics (MD) simulations of the Tom20-presequence complex in explicit water. We used the replica-exchange molecular dynamics (REMD) method as an enhanced sampling technique to obtain a reliable free-energy landscape. One major distribution and another minor one were observed at 300 K in the landscape. using k-means clustering algorithm, we found structures similar to A-pose and M-pose in the major distribution, and those similar to Y-pose in the minor one. A new structure (S-pose), which has double salt bridges between Arg14 in presequence and Glu78 or Glu79 in Tom20 can interpret previous pull-down assay experiment on binding affinity of the Tom20-presequence complex. Correlations between binding pose of presequence and domain motion of Tom20 were consistent with X-ray structures. Hydration analysis of the REMD trajectory shows that a hydrophobic residue in presequence that does not directly attach to Tom20 has a role to prevent water from penetrating the interface between the presequence and Tom20. In summary, solution structures of the Tom20-presequence complex observed in the REMD simulations are consistent with the dynamic-equilibrium model based on the X-ray crystal structures.

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Different Scale Conformational Changes of SERCA Analyzed with Motion Tree Method

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Proteins have flexibility, and undergo relevant conformational change upon ligand binding or an alteration of environment. In many cases, these conformational change affect reaction activity of the proteins, i.e., the conformational changes of proteins are important to the functions. Many biologically-significant proteins exhibit multiple reaction steps to complete their functions. The proteins have more than two reaction sites and allosteric effects are observed frequently. To analyze the conformational changes at each of multiple steps and clarify their relationships to the biological functions, new methodology to describe the conformational change is required. We apply the motion tree algorithm originally developed by Koike et al. to a representative P-type ATPase, Sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA), as an example of large proteins composed of multiple domains. SERCA is the best studied member of P-type ATPases. Atomic structures of SERCA in different physiological states have been determined with X-ray crystallography. To uptake Ca^{2+} from the cytoplasm to sarcoplasmic reticulum lumen against a large concentration gradient, SERCA undertakes rearrangements of the transmembrane helices coupled with large-scale cytoplasmic domain movements. Thus, SERCA is an appropriate multi-domain protein to investigate the interplay between conformational change and biological function. We detect characteristic conformational changes in the reaction steps. We also perform 100ns molecular dynamics simulations starting from either of E2 and E2•Pi states and analyze the conformational change seen in the trajectories. The method is effective to detect essential conformational changes within the trajectories including large fluctuations in more detail.

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Multiple Conformers and their Spectroscopic Properties of N-Glycan Predicted using Replica-Exchange Simulations

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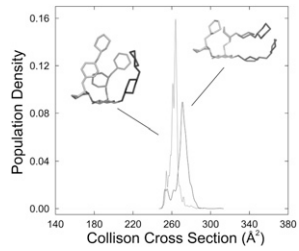
N-glycans play essential roles in many biological functions such as protein quality control and cell-to-cell communications. Nuclear Magnetic Resonance (NMR) and Mass Spectroscopy (MS) are powerful experimental means to analyze glycan structures, but a precise characterization of various glycan conformers are still challenging. We performed replica-exchange MD (REMD) simulations of N-glycans to identify a family of conformers to interpret experimental data. The results show that N-glycans have distinct multiple conformers and their population sizes change upon the chemical modifications. The experimental NMR data (NOEs, J-couplings) and MS data (collision cross sections) are well reproduced taking the multiple conformers into account. For example, the difference in the major conformer between two isomeric N-glycans leads to the different distribution of collision cross sections observed in the experiment. Further details of the analyses as well as future perspective will be discussed.



3416-Pos Board B571

Modelling TSH and its Receptor Complex for Binding Affinity

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Thyroid stimulating hormone (TSH), a glycoprotein hormone consisting of two distinct subunits, binds to its receptor (TSHR) and regulates the production and secretion of thyroid hormones via a complex signaling cascade. Two recent works have reported the crystal structure of stimulating and blocking type antibody-Fab fragments binding to the ectodomain of the TSHR . However, as of today no crystal structure of the TSH-TSHR complex has been reported. In this work, homology modeling of TSH has been carried out with Modeller (Sali and Blundell 1993) using the crystal structure (PDB ID: 1FL7) of the related follicle stimulating hormone (FSH) as the template (Fox, Dias et al. 2001). A fast molecular dynamics minimization of the structure has been performed as part of the Modeller routine. Docking of TSH on the TSHR was carried out on the HEX server (http://hexserver.loria.fr/) and residual contacts of the TSH-TSHR complex have been identified. Conformational studies and the free energy of binding will be reported based on Molecular Dynamics simulations using the AMBER force field. The calculations will be complemented by experimental determination of the binding affinity using Surface Plasmon Resonance (SPR). This study provides useful insight into the kinetics and dynamics of TSH binding to its receptor.

Fox, K. M., J. A. Dias, et al. (2001). Molecular Endocrinology 15(3): 378-389.
Sali, A. and T. L. Blundell (1993). Journal of Molecular Biology 234(3): 779-815

3417-Pos Board B572

Mitochondrial Proteins and Molecular Interaction with Cardiolipin at Atomistic Level

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Functional coupling between mitochondrial creatine kinase (MtCK) and adenine nucleotide translocase (ANT) can play an important role in determining energy transfer pathways in the cell. MtCK, which is an octameric enzyme, catalyzes the conversion of creatine (Cr) and consumes adenosine triphosphate (ATP) to create phosphocreatine (PCr) and adenosine diphosphate (ADP). Mitochondrial CK is located partly in contact sites of outer and inner mitochondrial membranes and partly in the cristae region, but always firmly attached to the inner membrane and in the vicinity of transmembrane ANTs. It is shown that MtCK binds electrostatically to the negatively charged cardiolipins (CL) of the inner membrane. CL is a kind of diphosphatidylglycerol lipid and is found almost exclusively in the inner mitochondrial membrane where it is essential for keeping spatially close and functional CK, ANT and other respiratory complexes. The formation of contact sites of CK-ANT by CL patches is a dynamic process and important to understand at atomistic level, which is the main goal of this work.

The molecular dynamics simulation technique is used to study the atomistic details of the functional role of CL close to mitochondrial CK and ANT complexes. The thermodynamics-based coarse-grained force-field, called MARTINI (version 2.0), has been used together with the GROMACS molecular dynamics package (version 4.5.4) for a model of a patch of the mitochondrial inner membrane containing several transmembrane ANTs, and a single CK above the membrane. The membrane model consists of three major type of lipids - phosphatidylcholine, phosphatidylethanolamine and cardiolipin in roughly 2:1:1 molar ratio.

3418-Pos Board B573

A Proposed Solution Structural Model for Human Coagulation Tenase Complex between Factor VIIIa and IXa: A Protein-Protein Docking and MD Refinement Study

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Activation of blood coagulation factor X to Xa plays central role in the clotting cascade. The protein-protein complex between the co-factor VIIIa and the