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Molecular Dynamics Study of Conserved Water Molecules in PrPc and Pathological Point Mutation T188R

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The secondary conformational change of cellular prion protein (PrPc) into scrapie prion protein (PrPsc) is critical important for prion disease including bovine spongiform encephalopathy and Creutzfeldt-Jakob disease.

This study performed molecular dynamics simulations of both human prion protein (WT-PrPc) and pathogenic mutant prion protein (T188R) and analyze its conserved water molecules in equilibrium state.

Although the secondary structure is not dramatically different, increase of Cα-RMSF occurs not only around mutation point but also in a wide range. This increase of intensity may result from the instability caused by point mutation.

Next, we calculated mean residence time for each protein atoms to find conserved water molecules. Residence time is defined that the time from when the water molecule get into the sphere whose center is a protein atom to the evacuation of water molecule from the sphere. We found four conserved water molecule sites in WT-PrPc. Conserved water molecules also keep the higher rotational correlation than water molecules in bulk. These results shows that conserved water molecules are not only wrapped but also fixed by hydrogen bond between conserved water molecule and amino acids.

On the other hand, every residence time of T188R decrease compared by WT-PrPc and the rotational relaxation is also faster than WT-PrPc although secondary structure is almost not different. These results show that conserved water molecules cannot survive related to the wide range fluctuation caused by point mutation.

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Characterisation of the Prion Protein Oligomerisation by Md Simulations and Small Angle X-Ray Scattering

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Neurodegenerative diseases constitute a large group of affections characterized by brain dysfunction and degeneration, often occurring with advancing age. Among them, prion diseases are fatal transmissible spongiform encephalopathies (TSEs) affecting Humans (Creutzfeldt-Jacob disease) and animals (BSE or the "Mad cow" disease). The causes of prion diseases have long been debated and it is widely accepted by the scientific community that a protein, namely, the prion protein (PrP), plays a critical role in the disease development. The prion protein or PrP is present in two structurally distinct forms: the natural cellular form (PrPc) and an abnormal polymeric form (PrPsc), which is at the origin of fibrillar deposits found in affected brains. PrPc has been shown to convert into small toxic oligomeric species but the mechanisms of oligomerization are not fully understood. In this study, we have combined Molecular Dynamics (MD) simulations and Small Angle X-ray Scattering (SAXS) to investigate the critical unfolding steps of the ovine prion protein (OvPrP) and to determine the low resolution structure of some of the oligomeric species obtained from various truncated forms of the OvPrP.

We show that the domain formed by Helix2 and Helix3 (H2H3) is critical in the unfolding of OvPrP and its conversion of into a beta-rich conformer. The analysis also suggests the incorporation of 9-10 monomer units for all monomeric species studied. We therefore propose that the minimal β-rich core of OvPrP-O3 oligomer is formed by the H2H3 domain while the N-terminal domain either combines with other N-terminal domains to form a second domain or surrounds the oligomeric core.

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Accelerate Lateral Equilibration in Mixed Lipid Bilayers using Replica Exchange with Solute Tempering

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The lateral heterogeneity of cellular membranes may play an important role in many important biological functions such as signaling and regulating membrane proteins. The heterogeneity can result from preferential interactions between membrane components or with membrane proteins. One of the major difficulties with molecular dynamics simulation to study membrane heterogeneity is that lipids diffuse slowly and collectively in bilayers and therefore it is extremely hard to reach equilibrium in lateral organization in bilayer mixtures. Here, we proposed a novel approach to accelerate lateral relaxation in bilayer mixtures by using Replica exchange with Solute Tempering (REST). Since the number of replicas in REST only scales with the number of degrees of freedom in solute, REST enables us to study a much larger bilayer system comparing to traditional replica exchange molecular dynamics simulation (REMD). We applied this method to a cholesterol and DPPC bilayer mixture and found that the lateral distribution of cholesterols in upper and lower monolayers converges much faster than traditional MD simulation. Although REST was initially proposed to study protein folding and despite its efficiency in protein folding is still under debate, we found an unique application of REST to accelerate lateral equilibration in mixed lipid membranes and suggest a promising way to probe membrane lateral heterogeneity through molecular dynamics simulation.

CD and Vibrational Spectroscopy

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Ion-Protein Interaction in Channel and Pump Proteins Studied by FTIR Spectroscopy

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Membrane proteins play crucial roles for receiving external stimuli, transporting and permeating protons and ions to generate biological energy and signal. The molecular mechanisms of membrane proteins have been deepened with the progress in structural biology. Especially, the structural changes connecting the inactive and active states of protein are nowadays investigated by many physicochemical methods. Fourier-transform infrared (FTIR) spectroscopy is one of powerful method for investigating molecular structures in the level of chemical bonds. Perfusion-induced difference infrared spectroscopy has been applied to extract structural changes of protein in response to various stimuli. The selectivity of ion is important for understanding the molecular mechanisms of the ion channels and transporters. I have been studying a sodium ion pump, V-type ATPase [1], and a potassium ion channel, KcsA [2] by using FTIR spectroscopy with attenuated total reflection (ATR) configuration. KcsA is a well-known potassium ion channel whose precise X-ray structures in various conditions have been reported. I have applied ATR-FTIR spectroscopy on KcsA and succeeded to assign the C=O stretching vibrations of the selectivity filter in the amide I region [2]. Recently, I proposed a novel rapid buffer-exchange system for time-resolved ATR-FTIR spectroscopy to monitor ion-binding reaction of protein. The anion-binding reaction of a membrane protein, *Natronomonaspharaonis* halorhodopsin was confirmed by an increase of the retinal absorption band at 1528 cm^{-1} in accordance with the buffer-exchange reaction finished within ~25 ms, which was monitored by the infrared absorption change of a nitrate band at 1350 cm^{-1} [3].

[1] Y. Furutani et al. *J. Am. Chem. Soc.***133** (9), 2860-3, 2011.
[2] Y. Furutani et al. *J. Phys. Chem. Lett.***3**, 3806-10, 2012.
[3] Y. Furutani et al. *BIOPHYSICS***9**, 123-129, 2013.

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Measuring Protein Dynamics and Mechanism using Infrared Spectroscopy

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Infrared spectroscopy has long been used to deduce concentration and structural descriptions of proteins in a variety of static and time resolved experiments. Our focus is on developing new infrared methods to compare the structure, dynamics and function of nearly identical protein samples. We have developed an order parameter describing protein conformation variations around the average molecular values. Here we apply our order parameter to the problem of characterizing membrane protein function and the efficacy of membrane protein reconstitution in different environments. By comparing our order parameter and amide hydrogen/deuterium exchange methods, we explore the relationship between protein stability and the dynamics of the protein conformational distribution. Examples include lysozyme and albumin in solution, and cytochrome c interacting with lipid membranes of varying net-negative surface charge density. We have recently extended our work to the characterization of both native and crystalline forms of bacteriorhodopsin during its photocycle.

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A Novel Microfluidic Mixer Utilizing Infrared Imaging Spectroscopy with a Submillisecond Mixing Time

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We present a novel microfluidic mixer utilizing vibrational spectroscopy with an experimentally determined submillisecond mixing time. The mixer is