Commentary and Perspective

Protein large-scale motions revealed by quantum beams: A new era in understanding protein dynamics

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Biological systems function by constantly changing their hierarchical or inter-hierarchical interactions among molecules, cells, and individuals. To visualize these dynamics, it is effective to approach them using penetrating quantum beams such as X-rays and neutron beams. Furthermore, the interpretation of the experimental results can be deepened by theoretical and computational techniques such as molecular dynamics simulations, which also complement phenomena hard to trace by experiments.

For these issues, we have a symposium at the 60th Annual Meeting of the Biophysical Society of Japan held in September 2022 inviting 7 speakers, which is organized by Sekiguchi and Yamamoto. At the symposium, we will review recent advances in quantum beam techniques for biophysical research, mainly focusing on protein large conformational changes using X-ray (Sekiguchi, Yamamoto), neutron scattering (Matsuo), or a combination of both techniques (Inoue). We will also show theoretical studies that interpret or support experimental results (Kurisaki, Zhao) and a combination of experimental and theoretical studies (Hishikawa).

Allosteric Transition Dynamics in Hemoglobin Reconsidered by Diffracted X-ray Tracking (Sekiguchi)

Biological functions are usually governed by allosteric conformational transitions. To unveil the underlying mechanisms, it is requisite to understand how the allostery occurs. Hemoglobin, which is known to effectively carry oxygen in red blood cells, is the best-studied among allosteric proteins. Despite a large number of experiments conducted to understand the secret of its allostery, a complete understanding has not been achieved yet. Recently, a traditional model to describe the allostery has been reconsidered by experimental breakthroughs using crystallography and spectroscopy [1]. To support the revised model from the viewpoint of dynamics, diffracted X-ray tracking (DXT) has been performed to monitor single-molecule allosteric transitions at a sub-millisecond scale [2]. We succeeded in observing structural perturbations upon oxygen detachment from hemoglobin. Detail will be discussed in the Symposium with recent DXT results.

Internal Dynamics of Intrinsically Disordered Protein as Studied by Neutron Scattering (Inoue)

Intrinsically disordered proteins (IDP) are known to play significant roles to achieve biological activities, especially in...
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Eukaryotes [3]. Both high contents of hydrophilic and charged residues in its constituent amino acid sequence render the destabilization of ordered structures, contributing to highly flexible structures. The change in solution environment such as temperature could influence the hydrophilicity and charge distribution in IDP, rendering the change of its solution structure. As an example of such environmental changes, we especially focus on temperature change. Interestingly, IDPs tend to possess compact structures with increasing temperature and this is a totally different behavior observed for globular proteins [4]. It is expected that the anomalous sensitivity of structure to temperature change could be originated from temperature-dependent internal dynamics in IDP. To investigate internal dynamics directly, we applied quasielastic neutron scattering, of which time scale is fitted to detect the internal dynamics of IDP [5]. In this symposium, the significance of the internal dynamics of temperature-dependent structure of IDP will be discussed.

**Thermodynamic and Molecular Dynamic Analysis of Aromatic Interaction Networks in Protein Cages (Hishikawa)**

Aromatic residues in proteins assemble to form highly ordered structures, aromatic clusters, which play essential roles in biological systems such as folding and self-assembly of proteins [6]. It is essential to evaluate the thermodynamic properties and molecular dynamic behaviors of the aromatic interactions to establish their correlations with atomic-level structures. To address this issue, we have constructed aromatic clusters using protein cages based on our previous design principle [7]. Our protein cage system enabled us to evaluate the thermodynamic and dynamical properties of aromatic clusters using differential scanning calorimetry and circular dichroism spectroscopy combined with molecular dynamics simulations. In the Symposium, we will discuss the molecular mechanism of how the aromatic clusters stabilize the protein cages.

**3D Structural Determination of Protein from Fluctuation X-ray Scattering Data (Zhao)**

Cellular functions, such as intracellular transport/mitosis/movement with microtubules, transmembrane ion/molecule transport with ribosomes, are mostly realized by polymerization, depolymerization, and conformational change of proteins and biological complexes. In general, “seeing” their structures is the prerequisite to understanding their large-scale dynamics. While well-established techniques such as X-ray crystallography and cryo-electron microscopy investigate their structures in crystallized or frozen conditions, fluctuation X-ray scattering (FXS) [8] can measure non-crystallized biological particles dispersed in solutions in near-physiological conditions, which is promising for future dynamics study. This emerging technique is not only experimentally challenging but also has a theoretical difficulty in interpreting experimental data. In the experiment, thousands or millions of 2D X-ray coherent diffraction images are collected, while each image averages the information of many randomly oriented biological particles. Reconstruction to 3D structures requires not only an explicit mathematical theory but also high-performance computation. In the Symposium, our recent progress in theoretical work and results with simulated experimental data will be shown.

**Microscopic Mechanisms of Stable Amyloid β (1-42) Oligomer Formation (Kurisaki)**

Dysregulation of interaction between proteins often brings about undesirable aggregate formation such as amyloid fibrils, which causes serious neurodegenerative diseases. Amyloid β (1-42) (Aβ42), a paradigmatic protein of fibril formation protein, has been widely examined as a pathogenic cause of Alzheimer’s disease. A stable formation and accumulation of Aβ42 oligomers are supposed as a critical step leading to the amyloid fibril formation, although the microscopic mechanism has not been elucidated sufficiently. To give further insights into Aβ42 oligomer formation, we theoretically examined the thermal stability of dimer formation of Aβ42 oligomers (Aβ42(d)) with atomistic molecular dynamics simulation [9]. We found that the configurational fluctuation of the Aβ42(d) is reduced by an increase of Aβ42 oligomer size and the reduction correlates with the thermodynamics stability of Aβ42(d). At the formation of Aβ42 pentamer dimer, the dimer dissociation can be remarkably suppressed, suggesting a turning point in Aβ42 fibril formation. In the Symposium, further details of this study will be shown.

**Sub-nanosecond Dynamics of Amyloid Polymorphs and Phospholipid Molecules Observed by Incoherent Neutron Scattering (Matsuo)**

Amyloid fibrils are known to possess cytotoxicity [10]. However, the molecular mechanism is yet to be understood. Dynamical properties of amyloid fibrils might be related to their cytotoxicity because dynamic interactions between fibrils and bio-membranes are considered to cause cytotoxicity. It has been known that two lysozyme amyloid polymorphs, LP27 and LP60, exhibit different levels of cytotoxicity. As a first step to elucidate the mechanism of the phenomenon, we
performed incoherent neutron scattering (iNS) measurements on isolated LP27 and LP60 to monitor dynamics in a sub-nanosecond timescale [11]. We found that LP60 possessed enhanced molecular dynamics compared with those of LP27, indicating that this difference in dynamics could explain the difference in their cytotoxicity. Recently, we also developed a new model called Matryoshka-model [12-14] describing the dynamics of phospholipids using iNS, and found that iNS data of phospholipids in various forms are successfully reproduced with this model. In the Symposium, how our research can contribute to understanding the molecular mechanism of amyloid cytotoxicity will be discussed.

Structural Development of Amyloid Prefibrillar Intermediates and its Inhibition (Yamamoto)

Before amyloid fibril formation, accumulation of intermediate species has usually been observed [15]. These prefibrillar intermediates are thought to play roles in efficiently inducing nucleation requisite for amyloid fibril formation. However, detailed molecular mechanisms were not established yet. In this study, using an insulin-derived peptide, B chain, we investigated how its prefibrillar intermediates converted to amyloid fibrils. Furthermore, the molecular mechanism of the inhibition of conversion by a blood-clotting protein, fibrinogen, was also investigated [16]. Transmission electron microscope images obtained in a time-lapse manner showed that prefibrillar intermediates, which possessed wavy rod-like structures, bundled together to form protofilaments of amyloid fibrils. Furthermore, time-resolved small-angle X-ray scattering experiments revealed that the prefibrillar intermediates became thicker and longer as a function of time. Interestingly, fibrinogen inhibited this structural development of the prefibrillar intermediates by restricting the elongation of the prefibrillar intermediates. In the Symposium, we will discuss molecular mechanisms of amyloid fibril formation via prefibrillar intermediates and its inhibition by the inhibitor.

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