Visualization of domain motion of tri-ubiquitin through segment deuteration and small-angle neutron scattering

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It is well recognized that domain motions in multi-domain proteins play crucial roles in essential processes such as cellular signaling and gene regulation. Therefore, identification of their domain motions must be significant for revealing the mechanism to develop functions.

Ubiquitin (Ub) is a small protein comprised of 76 amino acid residues and is deeply related to regulatory roles in various cellular events such as cell cycle progression, DNA repair, transcriptional regulation, apoptosis and so on. The C-terminal group of Ub can be linked to another Ub through seven lysine (K) residues as well as the N-terminal amino groups, producing various types of poly-Ubs. We especially focused on linear K48 poly-Ub, which are expected to have high degree of freedom of constituting domains. As the first step, we started to study the structure and dynamics of linear K48-tri-ubiqutin (linear K48-tri-Ub). Solution NMR studies supposed that linear K48-tri-Ub could have four possible different states. Additional experimental approaches are indispensable for validating the expectation from NMR studies. Elucidation of relative spatial arrangements of two domains in linear K48-tri-Ub is one of the candidates for above-mentioned experimental requirements.Through the usage of ubiquitin-conjugating enzymes and deuteration of a domain, selective deuteration of concerned domain in linear K48-tri-Ubis is technically possible. We then prepared K48-tri-Ub consisted of two hydrogenated domains and 75% deuterated domain at different positions (H-H-75D). And we performed performed small-angle neutron scattering (SANS) measurement on H-H-75D in 100% D2O at 42 C, using Quokka installed at ANSTO. Figure 5 shows the SANS profiles from H-H-75D (red circle) and H-H-H (black circle). Clear difference of scattering profiles was observed between them, supporting the successful introduction of 75% deuterated domain into linear K48-tri-Ub. Aiming at the detailed structural analysis, we are on the progress of performing long time all- atom MD simulation.

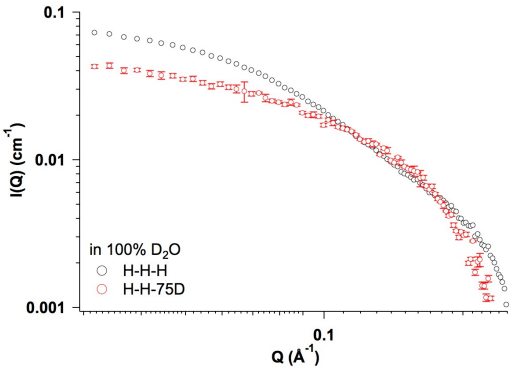

Fig. 1. SANS profiles from H-H-H and H-H-75D in 100% D2O at 42 C.