# Analysis of flexible structure of multi-domain protein

T. Oda[a], R. Inoue[b], K. Morishima[b], A. Okuda[b], M. Sugiyama[b]

[a]J-PARC Center, Japan Atomic Energy Agency.
[b]Institute for Integrated Radiation and Nuclear Science, Kyoto University.

Multi-domain proteins, which have multiple rigid folded domains and flexible unfolded regions (intrinsically disordered region: IDR), play various biologically important roles. However, due to flexibility, structural analysis of the multi-domain proteins is difficult. In this study, we focused on a DNA repair protein Hef from hyperthermophilic archaeon as a muti-domain protein and attempt to determine the flexible structure as an ensemble using SAXS/SANS data. Hef consists of an N-terminal helicase domain, a C-terminal nuclease domain, and an IDR connecting the two domains. From our preliminary SAXS experiments using only IDR of Hef, we found that the IDR becomes more compact at high temperatures (323~353 K), than at room temperature (298 K). Considering that high temperature (333~353 K) is physiological temperature for hyperthermophilic archaeon, we speculate that this compaction is related to the function of Hef. Therefore, we attempted to analyze the structure of full-length Hef at high temperature (353 K). Since the entire molecule contributes to scattering in SAXS, it is difficult to determine the ensemble based solely on the SAXS data. To obtain additional experimental data that serves as constraints are required, we prepared a full-length Hef protein in which only the helicase domain or nuclease domain was 75% deuterated, and the remaining part was not deuterated. In a 100% heavy water solvent, the 75% deuterated domain is matched out, making it possible to specifically observe scattering from the non-deuterated region (light hydrogen region). By combining this scattering data with the scattering from the entire molecule obtained by SAXS, we can determine the ensemble structure more accurately. The SAXS/SANS data at room temperature has already been acquired. We therefore focused to acquire SANS data at 353K. Since Hef tend to aggregate even at low protein concentration, we performed size exclusion chromatography to remove aggregates from samples before SANS measurement, and protein concentration of the samples were set to low (0.6 mg/mL) to prevent aggregation. SANS measurement was performed with SANS-U installed at JRR-3. To obtain data with sufficient statistical accuracy from low concentration sample, the exposure times were set to 16 and 24 hours. The obtained SANS profiles did not show any sharp rise at the low angle. Thus, at first glance it appears that there are no aggregates in the samples. However, the AUC analysis of the samples after SANS measurement, indicated the presence of aggregates and degradant (Fig. 2). It will be necessary to consider the condition of sample preparation and measurement that prevent aggregation and degradation.

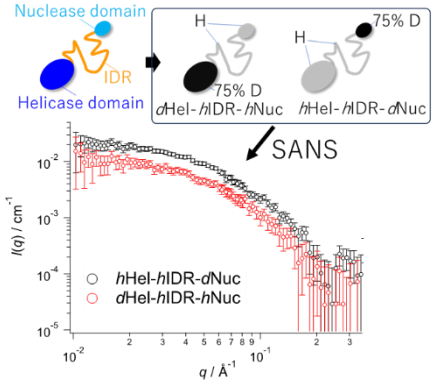


Fig 1. Schematic view of samples used for SANS measurement and SANS profiles.

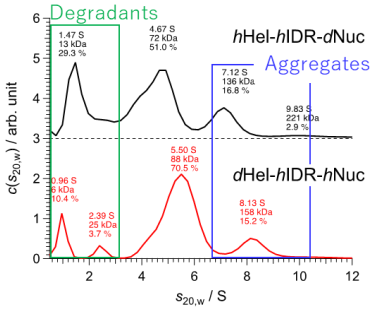


Fig 2. AUC analysis of the samples after SANS measurement.