

Structural analysis of protein complex in solution with AUC- iCM-SANS

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In a typical SANS measurement for biomacromolecule in a solution, the sample should be monodispersed to obtain the scattering profile purely from the target molecule. However, a protein complex under fast association-dissociation equilibrium cannot be purified in all cases; The complex could coexist with the unassociated proteins in the solution. In such case, the scattering profile given as an ensemble average in the solution.

To overcome above issues, the integrated method of analytical-ultracentrifugation (AUC) and small-angle X-ray/neutron scattering (SAXS and SANS; collectively called SAS), namely “AUC-SAS”, is promising^[1]. AUC-SAS decomposes the SAS-profile of target component from the ensemble averaged SAS-profile of the multi-component solution. On the other hand, inversed-contrast matching (iCM) method is useful to investigate the partial structure of protein complex^[2]. In this study, we demonstrated that the integrative approach of AUC-SAS and iCM-SANS (=AUC-iCM-SANS) gives the partial structure of protein complex in multi-component solution. In this experiment, we analyzed the partial structure of clock protein complex KaiA-KaiC (AC). Especially, we selectively observed the partial structure of KaiA in the AC complex consisting of hydrogenated KaiA (h-KaiA) and 75%-deuterated KaiC (75d-KaiC) with AUC-iCM-SANS.

SANS measurement was conducted with SANS-U located at JRR-3 (Proposal#: 22914). A neutron beam at wavelength = 6.0 Å with 10 % of resolution was irradiated to the samples. The sample-to-detector-distances (SDD) were set to be 4000 and 1030 mm which cover the q -range of 0.01 – 0.3 Å⁻¹. AUC measurement was carried out with ProteomeLab XL-I (Beckman Coulter) at 60,000 rpm.

Figure 1 shows the scattering profile of h-KaiA solo (magenta circles) and h-KaiA in AC

complex (blue circles) which is derived with AUC-iCM-SANS. The gyration radii (R_g) of h-KaiA in the AC complex was larger than that of h-KaiA solo (h-KaiA in AC complex: $R_g = 31.4 \pm 0.7$ Å, and h-KaiA solo: $R_g = 25.7 \pm 0.4$ Å). Thus, it is indicated that the structure of KaiA in the AC complex is different from free KaiA.

[1] K. Morishima, *et al. Commn. Biol.* **3**, 294 (2020).

[2] Y. Yunoki, *et al. Commn. Biol.* **5**, 184, (2022).

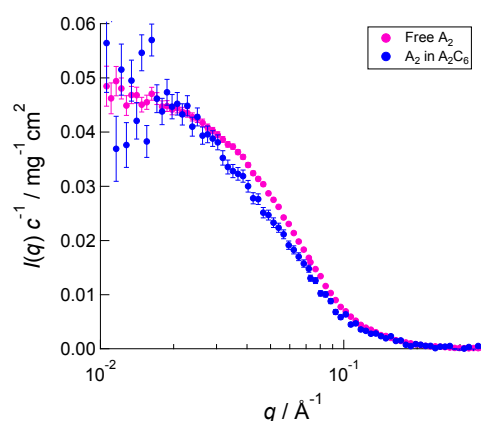


Figure 1. Magenta and blue circles represent the scattering profile of h-KaiA solo and h-KaiA in AC complex which is derived with AUC-iCM-SANS.