Structural analysis of clock protein complex in solution

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A circadian clock system in *Cyanobacteria* has been investigated as an ideal circadian oscillation model within last decades because of its simplicity. The clock system consists of only three proteins, KaiA, KaiB, and KaiC, and they repeat an association-disassociation in 24h-period. To understand the periodical association-disassociation mechanism, it is necessary to elucidate the structures of the complexes; KaiA-KaiC (AC), KaiB-KaiC (BC), and KaiA-KaiB-KaiC (ABC) complexes. However, the structure of AC complex has not been solved because of following issues: (i) Crystallography is not applicable because of the difficulty of crystallization due to the structural fluctuation. (ii) AC complex cannot be purified because KaiA-KaiC interaction affinity is weak.

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 SAS-profile of the multi-component solution.

So far, we studied the whole structure of AC complex with AUC-SAXS. As the next step, we focused on the partial structure of KaiC in the AC complex. For this purpose, we utilized inverse contrast-matching SANS (iCM-SANS)[2]: When we measure SANS for a protein complex consisting of hydrogenated and 75%-deuterated protein in 100% D_2O buffer, we can selectively observe the structure of hydrogenated protein in the complex. In this study, we selectively observed the partial structure of KaiC in the AC complex consisting of hydrogenated KaiC (h-KaiC) and 75%-deuterated KaiA (75d-KaiA) with AUC-iCM-SANS.

SANS measurement was conducted with SANS-U located at JRR-3 (Proposal#: 21537). A neutron beam at wavelength = 7.0 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 – 0.2 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-KaiC solo (blue circles) and h-KaiC in AC complex (magenta circles) which is derived with AUC-iCM-SANS. Both profiles agree with each other in whole q-region (0.01 – 0.2 Å^{-1}). Furthermore, the gyration radii (R_g) which were given by Guinier analysis are also consistent each other (h-KaiC solo: R_g = 45.2 ± 1.5 Å and h-KaiC in AC complex: R_g = 45.9 ± 1.9 Å). Thus, it is indicated that the structure of KaiC does not change in the AC complex.

[1] K. Morishima, *et al. Commn. Biol.* **3**, 294 (2020).
[2] Y. Yunoki, *et al. Commn. Biol*. **5**, 184, (2022).

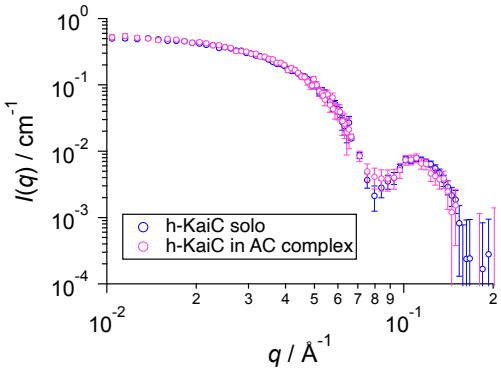

Figure 1. Blue and magenta circles represent the scattering profile of h-KaiC solo and h-KaiC in AC complex which is derived with AUC-iCM-SANS.