Supplemental information

Structural characterization of the circadian clock protein complex composed of KaiB and KaiC by inverse contrast-matching small-angle neutron scattering

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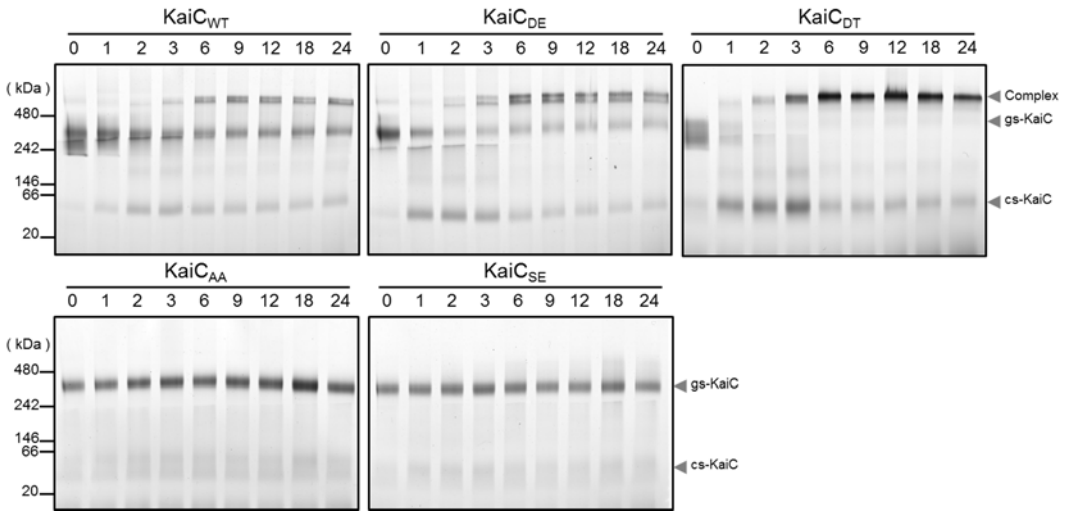
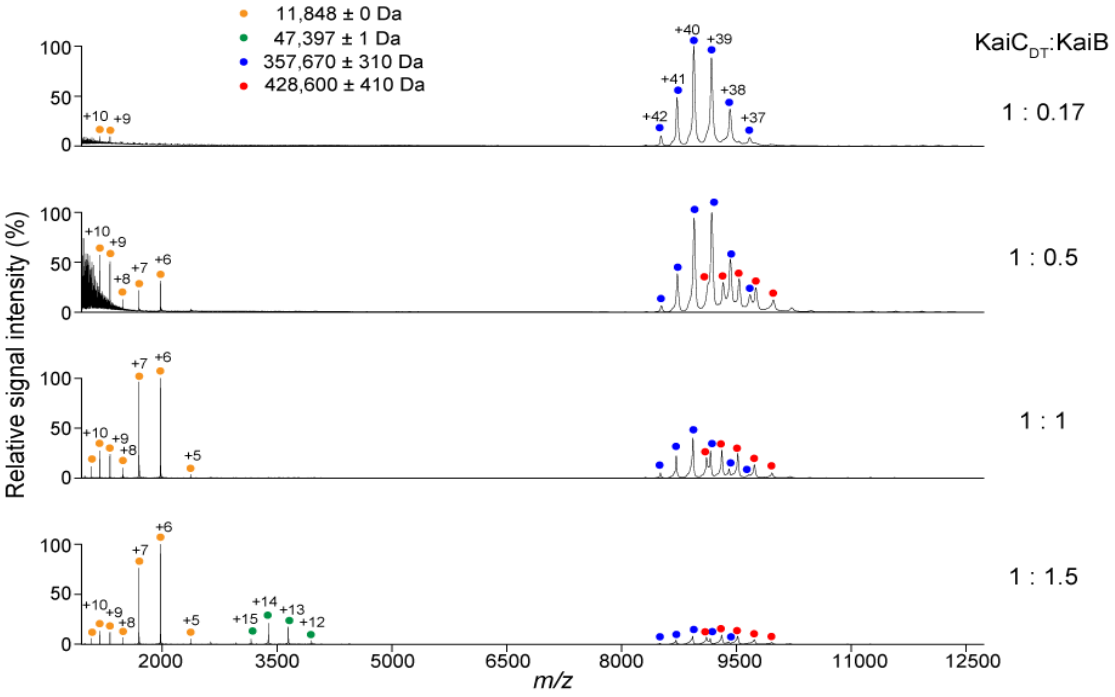
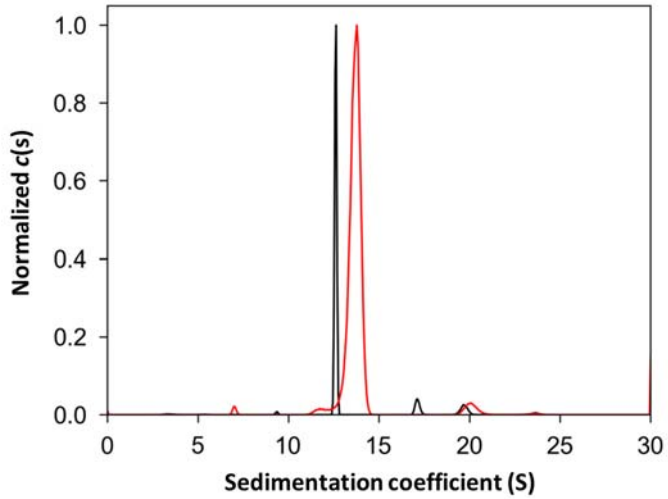


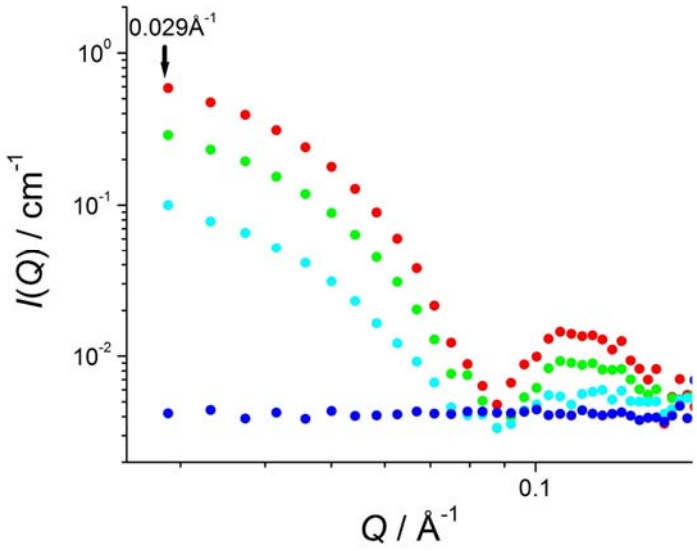
Supplemental Fig. S1. Full-length BN-PAGE images of Fig.1. KaiC$_{WT}$, KaiC$_{DE}$, KaiC$_{DT}$, KaiC$_{AA}$, and KaiC$_{SE}$ were incubated with KaiB at 30ºC and were subjected to BN-PAGE at individual time points. The 12hr-lanes of individual BN-PAGE images are highlighted in Fig. 1.



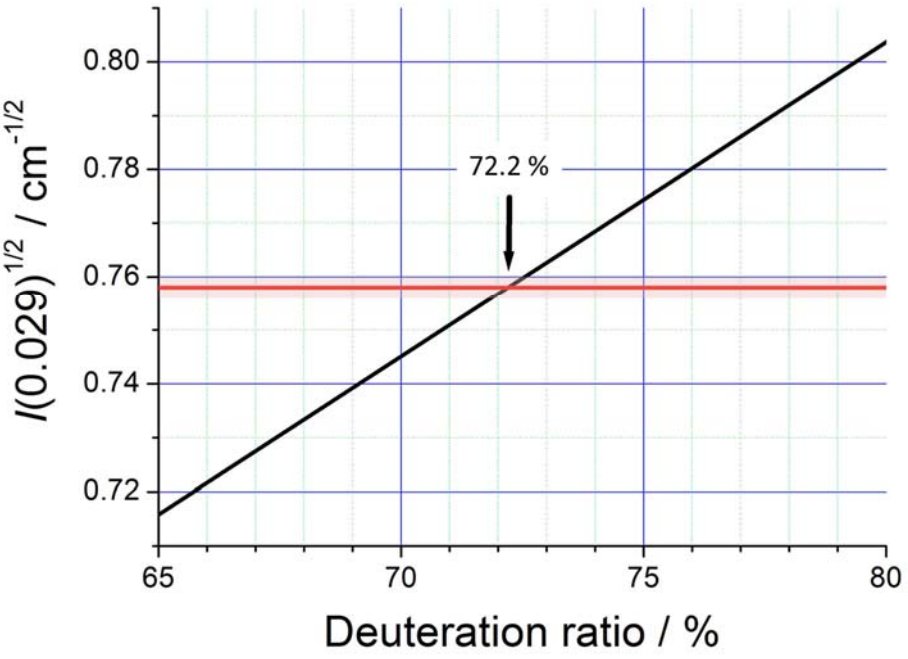
Supplemental Fig. S2. Mass spectra of mixtures of KaiC$_{DT}$ and KaiB at 1:0.17, 1:0.5, 1:1, and 1:1.5 molar ratios (KaiC$_{DT}$ to KaiB). The mass spectra in the range from *m/z* 1,000 to *m/z* 12,700 in Figure 2 are shown. Orange, green, blue, and red circles show the ion series of the monomeric KaiB, the homo-tetrameric KaiB, the homo-hekameric KaiC$_{DT}$, and the 6:6 hetero-dodecamer complexes of KaiC$_{DT}$ and KaiB, respectively.



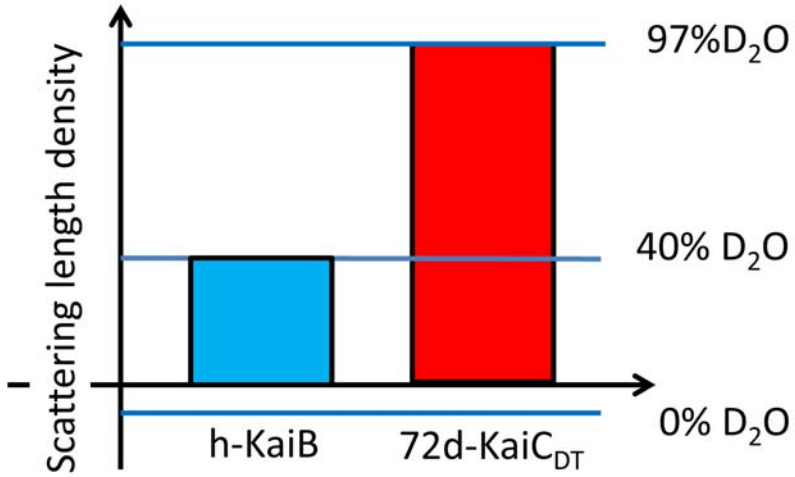
Supplemental Fig. S3. Distribution of sedimentation coefficients of 60 μM KaiC$_{DT}$ (black) and 60 μM KaiB- KaiC$_{DT}$ complex (red). The 13.7 S peak corresponds to the complex at a 6:6 stoichiometry.



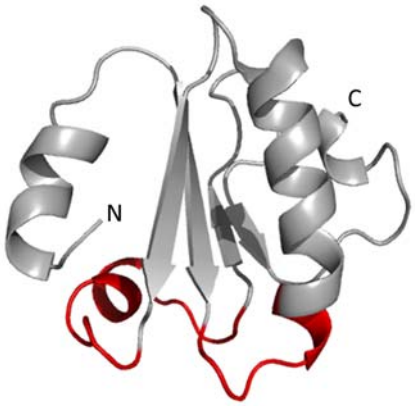
Supplemental Fig. S4. SANS profiles of the partially deuterated KaiC$_{DT}$ preparation in 0% (red), 30% (green), 60% (cyan), and 97% (blue) D_2O solvents. The profiles were measured with 6.0 Å neutron and the sample-to-detector distance of 2.0 m in 300 s by D22 of ILL.



Supplemental Fig. S5. Square root of SANS intensity at 0.029 $Å^{-1}$, $I(0.029)^{1/2}$, in 0% D_2O (H_2O), of partially deuterated $KaiC_{DT}$. The black line indicates the calculated $I_C(0.029)^{1/2}$ as a function of deuteration ratio, while the red line and the orange zone show the observed value and the errors of $I_O(0.029)^{1/2}$ of the partially deuterated $KaiC_{DT}$ preparation, respectively. The cross point of black and red lines indicates that the deuteration ratio of the sample was 72.2 ± 0.3%.



Supplemental Fig. S6. Map of scattering length density. Blue and red bars show the scattering length densities of hydrogenated KaiB (h-KaiB) and 72% deuterated $KaiC_{DT}$ (72d-$KaiC_{DT}$). The scattering length densities of 0%, 40%, and 97% D_2O express with blue cross bars. Because a scattering intensity of substance in a solution is proportional to the square of the difference in scattering length density between solute and solvent, namely "contrast," the scattering of 72d-$KaiC_{DT}$ can be almost ignored compared from that of h-KaiB in 97% D_2O solvent: yellow arrow shows the contrast of h-KaiB in 97% D_2O solvent.



Supplemental Fig. S7. The KaiC-binding site in the structural model of the fold switch KaiB used for calculation of SANS profiles. The KaiC-binding site (red) was mapped on the basis of the previously reported deuterium-exchange MS data[1].

Supplemental Table S1: Samples used for SANS experiments.

Protein	Protein deuteration ratio	Solvent D_2O ratio	Figure of SANS profile
$KaiC_{DT}$	72%	0%	Fig. S3
$KaiC_{DT}$	72%	30%	Fig.S3
$KaiC_{DT}$	72%	60%	Fig.S3
$KaiC_{DT}$	72%	97%	Fig.S3
h-KaiB+ h-$KaiC_{DT}$	0% / 0%	97%	Fig.3
h-KaiB+ d-$KaiC_{DT}$	0% / 72%	97%	Figs. 3 and 5

Reference

1. Chang, Y.G. et al. Circadian rhythms. A protein fold switch joins the circadian oscillator to clock output in cyanobacteria. *Science* **349**, 324-8 (2015).