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Supplementary Materials for

Structural basis of the day-night transition in a bacterial circadian clock

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Materials and Methods

Cloning of constructs

All genes were cloned into pET-28b vector using the Nde I/Hind III sites by PCR. Details of the cloning protocol have been described previously *(27)*. Please see table S1 for full list of constructs.

Protein expression, purification and fluorescence labeling

Proteins were expressed in BL21(DE3) *Escherichia coli* (Novagen) and purified by Ni-NTA affinity chromatography and size-exclusion chromatography as described previously *(27-29)*, except that the CII peptides of KaiC were purified by C4 reverse-phase chromatography column (Vydac, Hesperia, CA) with sample loading buffer consisting of H_2O+0.1% trifluoroacetic acid (TFA) and elution buffer consisting of 100% acetonitrile+0.1% TFA. Gradient elution was set to reach 100% elution buffer in 100 minutes at 1.0 mL/min flow rate. Fluorescent labeling of the CII peptides was described previously *(28)*.

Analytical size exclusion chromatography

All experiments were performed with a Superdex 200 or a Superdex S75 10/300 GL column (GE Healthcare) as described previously *(29)*, except that the sample injection volume was 250 µL. Desired protein sample(s) and buffer were mixed to a total of 250 µL, with or without incubation time at room temperature depending on experimental needs. The reaction sample(s) was then applied with a flow rate of 0.5 mL/min, at room temperature. The volume of the sample loop was 100 µL. Details of experimental conditions are provided in table S1. Molecular weight markers were: albumin (67 kDa), ovalbumin (43 kDa), chymotrypsinogen A (25 kDa), and ribonuclease A (13.7 kDa) (GE Healthcare).

Fluorescence spectroscopy

All data were collected with an ISS PC1 spectrofluorometer. The thiol-reactive fluorophore used was 6-iodoacetamidofluorescein (6-IAF) (Invitrogen). Instrument setup was as previously described *(29)*, except that a three-cuvette sample holder was used to measure triplicates simultaneously. Anisotropy measurements started immediately following a 5-minute incubation. One measurement was taken every minute for 5 minutes they were averaged to determine the starting anisotropy value. For experimental conditions, please see table S1.

Light scattering

Absolute molecular weight calculations were obtained by static light scattering in-line with size exclusion chromatography using a Wyatt Optilab T-rEX refractometer and miniDAWN Treos multiangle light scattering system at 4 ºC. Proteins were incubated overnight in a 1:3 ratio of $\text{KaiC}_{\text{S431E}}$:$\text{KaiB}_{\text{fs-cryst*}}$ (with 30 µM $\text{KaiC}_{\text{S431E}}$) in a binding buffer of 20 mM Tris pH 7.0, 150 mM NaCl, 5 mM DTT, 1 mM $MgCl_2$, 1 mM ATP. Protein samples (injection volume of 100 µL) were run at a 0.5 mL/min flow rate on a Superose 6 10/300 analytical gel filtration column (GE Healthcare) in a running buffer of 20 mM Tris pH 7.0, 150 mM NaCl, 5 mM DTT, 0.5 mM $MgCl_2$, 0.5 mM ATP. Protein concentrations were monitored by a refractometer and light scattering directly after the gel filtration column. Absolute molecular weights were determined using ASTRA version 6.0 (Wyatt Technologies).

Crystallization and X-ray data collection

Crystallization of the KaiB$_{\text{fs-cryst*}}$-KaiC$_{\text{S431E}}$ complex was carried out using the hanging-drop diffusion method at 22 ºC by mixing a 2:1 ratio of protein (12.5 mg/mL) with a reservoir solution containing 0.1 M sodium citrate pH 5.75 and 22% PEG3000. Before being flash frozen in liquid nitrogen, crystals were soaked in reservoir solution with increasing concentrations of glycerol, up to a final concentration of 25% (v/v), as the cryoprotectant. Crystals of the KaiB$_{\text{fs-cryst}}$-CI$_{\text{cryst}}$ complex were grown at 22 ºC in a 1:2 ratio of protein (20 mg/mL) with a reservoir solution containing 0.2 M potassium acetate, 10% (w/v) PEG3350, and 2.5% glycerol. Crystals of the KaiA$_{\text{cryst}}$-KaiB$_{\text{fs-cryst}}$-CI$_{\text{cryst}}$ complex grew using 25 mg/mL protein mixed in a 1:2 ratio with a reservoir solution containing 0.15 M ammonium sulfate, 0.1 M Tris pH 7.0, and 10% PEG4000. Crystals of both KaiB$_{\text{fs-cryst}}$-CI$_{\text{cryst}}$ and KaiA$_{\text{cryst}}$-KaiB$_{\text{fs-cryst}}$-CI$_{\text{cryst}}$ were flash frozen after soaking in reservoir solution with 12.5% glycerol. For the KaiB$_{\text{fs-cryst*}}$-KaiC$_{\text{S431E}}$ complex, diffraction data were collected at the Advanced Photon Source at the Argonne National Laboratory 23-ID-B beamline at 100K and processed using the HKL2000 program. Diffraction data for KaiB$_{\text{fs-cryst}}$-CI$_{\text{cryst}}$ and KaiA$_{\text{cryst}}$-KaiB$_{\text{fs-cryst}}$-CI$_{\text{cryst}}$ crystals were collected at the Advanced Light Source at Lawrence Berkeley National Laboratory at the 8.3.1 beamline at 100K. KaiB$_{\text{fs-cryst}}$-CI$_{\text{cryst}}$ data were processed using Mosflm and Scala, while KaiA$_{\text{cryst}}$-KaiB$_{\text{fs-cryst}}$-CI$_{\text{cryst}}$ data were processed with HKL2000.

Structure determination and refinement

The structures of KaiB$_{\text{fs-cryst}}$-CI$_{\text{cryst}}$ and KaiA$_{\text{cryst}}$-KaiB$_{\text{fs-cryst}}$-CI$_{\text{cryst}}$ complexes were solved by molecular replacement with Phaser, using the KaiB$_{\text{fs-nmr}}$ solution structure (5JYT) as well as modified domains of KaiA and KaiC from coordinates 4O0M and 1V2Z as search models. Further manual model building was done using Coot *(62)*, combined with structure refinement in Phenix *(63)*. The structure of the KaiB$_{\text{fs-cryst*}}$-KaiC$_{\text{S431E}}$ complex was solved by molecular replacement using the isolated hexameric CII ring from coordinate 4O0M and the binary KaiB$_{\text{fs-cryst}}$-CI$_{\text{cryst}}$ complex as a starting model, and further refined in a similar way. Refinement statistics and crystallographic data can be found in table S2. Protein-protein interfaces were analyzed with PDBePISA *(64)* (http://www.ebi.ac.uk/pdbe/prot_int/pistart.html). Structural figures were made with UCSF Chimera software *(65)*.

Cyanobacterial strains, culture conditions and DNA manipulations

Plasmids and *S. elongatus* PCC 7942 strains are described in tables S12 and S13. All *S. elongatus* strains were grown as previously described *(66, 67)*. Plasmids were constructed using the GeneArt Seamless Cloning and Assembly Kit (Life Technologies) and propagated in *Escherichia coli* DH5α. Mutant alleles were made using the QuikChange (Stratagene) protocol and clones were verified by DNA sequencing.

In vivo bioluminescence monitoring

Bioluminescence from luciferase reporter cyanobacterial strains was monitored at 30 °C under constant light (LL) conditions after two cycles of 12 h light:12 h dark to synchronize the population as described previously *(66)*. Data were analyzed with Microsoft Excel and the Biological Rhythms Analysis Software System (http://millar.bio.ed.ac.uk/pebrown/brass/brasspage.htm).

Live-cell microscopy

Cells were placed on a pad of 1.2% agarose in BG-11 media and covered with a coverslip. Microscopy was performed with a DeltaVision Core system (Applied Precision) with a WeatherStation attached to an Olympus IX71 inverted microscope and an Olympus Plan Apochromat 100× objective at 30 °C. To image autofluorescence emitted from photosynthetic pigments, a tetramethyl rhodamine isocyanate (TRITC) filter set was used (EX555/EM617) with an exposure time of 0.1 s. Images were captured using a CoolSnap HQ CCD camera (Photometrics) and deconvolved using the SoftWorx imaging program (Applied Precision). Cell length measurements were determined using SoftWorx imaging software. Statistics of cell lengths were analyzed with Graphpad Prism.

Hydrogen deuterium exchange mass spectroscopy (HDX-MS)

The protocols for proteolysis, hydrogen/deuterium exchange and LC/MS analysis were similar to previously described *(39)*. Various protein ratios were used for $\text{KaiA}_{\Delta\text{N}}$-$\text{KaiB}_{\text{fs-cryst}}$-$\text{CI}_{\text{cryst}}$ samples to probe the relative hydrogen/deuterium exchange of $\text{KaiA}_{\Delta\text{N}}$ free and mixed with $\text{KaiB}_{\text{fs-cryst}}$-$\text{CI}_{\text{cryst}}$ (1.0:1.2:1.44 ratio for $\text{KaiA}_{\Delta\text{N}}$:$\text{KaiB}_{\text{fs-cryst}}$:$\text{CI}_{\text{cryst}}$), or $\text{KaiB}_{\text{fs-cryst}}$-$\text{CI}_{\text{cryst}}$ in the absence or the presence of $\text{KaiA}_{\Delta\text{N}}$ (1.2:1.0:1.2 for $\text{KaiA}_{\Delta\text{N}}$:$\text{KaiB}_{\text{fs-cryst}}$:$\text{CI}_{\text{cryst}}$). For the binary sample, CikA_{PsR}:$\text{KaiB}_{\text{fs-nmr}}$ was prepared with 1.0:1.2 ratio to detect exchange for CikA_{PsR} and 1.2:1.0 ratio to detect exchange for $\text{KaiB}_{\text{fs-nmr}}$. Labeling time points before quenching were 10, 100 and 1000 seconds. The optimized quench condition was 3.2 M GuHCl for the $\text{KaiA}_{\Delta\text{N}}$-$\text{KaiB}_{\text{fs-cryst}}$-$\text{CI}_{\text{cryst}}$ complex or 1.6 M GuHCl for the CikA_{PsR}-$\text{KaiB}_{\text{fs-nmr}}$ complex. Sub-localized exchange level was analyzed as described previously *(68)* and datasets are listed in tables S4 to S11. The 100 s difference profiles of significantly protected regions from tables S5, S7, S9 and S11 were mapped to the structures of $\text{KaiA}_{\text{cryst}}$-$\text{KaiB}_{\text{fs-cryst}}$-$\text{CI}_{\text{cryst}}$ and CikA_{PsR}-$\text{KaiB}_{\text{fs-nmr}}$ in figs. S7 and S10, respectively.

NMR spectroscopy

A Bruker 600 MHz AVANCE III spectrometer equipped with a TCI cryoprobe was used for all of the NMR experiments described here. Chemical shifts were referenced to internal 2,2-dimethyl-2-silapentane-5-sulfonate. Data were processed using NMRPipe and visualized using NMRDraw *(69)*. Assignments were analyzed using PIPP *(70)* and XIPP *(71)*. For the single-entity protein structures, all experiments were performed at 25 °C. For the binary CikA_{PsR}-$\text{KaiB}_{\text{fs-nmr}}$ complex, all experiments were performed at 50 °C. 3D NMR experiments used to assign backbone, sidechain and intramolecular proton nuclear Overhauser effects (NOEs) and residual dipolar couplings (RDCs) were: HNCACB, HN(CO)CACB, HN(CA)CO, HNCO, HBHA(CO)NH, HCC(CO)NH, ^{15}N-NOESY-HSQC, ^{13}C-NOESY-HSQC, HCCH-COSY, HCAN, HCA(CO)N, IPAP-J-HNCO(CA) for ^{1}J(CA-HA), IPAP-J-HNCO for ^{1}J(N-H), and IPAP-J-HNCO for ^{1}J(CA-CO). For the binary complex, additional experiments for assigning side chain chemical shifts, intramolecular NOESY cross peaks, and RDCs were: 3D HCCH-TOCSY, ^{13}C-edited 3D NOESY-HSQC (in a sample containing ^{13}C-enriched CikA$_{\mathrm{PsR}}$ with unlabeled KaiB$_{\mathrm{fs\text{-}nmr}}$ or ^{13}C-enriched KaiB$_{\mathrm{fs\text{-}nmr}}$ with unlabeled CikA$_{\mathrm{PsR}}$), ^{15}N-edited 3D NOESY-HSQC (in a sample containing ^{15}N-enriched CikA$_{\mathrm{PsR}}$ with unlabeled KaiB$_{\mathrm{fs\text{-}nmr}}$ or ^{15}N-enriched KaiB$_{\mathrm{fs\text{-}nmr}}$ with unlabeled CikA$_{\mathrm{PsR}}$), and 2D ^{15}N HSQC-IPAP (in a sample containing ^{15}N-enriched CikA$_{\mathrm{PsR}}$ with unlabeled KaiB$_{\mathrm{fs\text{-}nmr}}$ or ^{15}N-enriched KaiB$_{\mathrm{fs\text{-}nmr}}$ with unlabeled CikA$_{\mathrm{PsR}}$). All NMR samples included 1x complete Mini Protease Inhibitor (Roche). Intermolecular NOE distance restraints for the binary complex were collected by: ^{15}N,^{13}C-edited 4D NOESY-HSQC (in a sample containing ^{15}N-enriched CikA$_{\mathrm{PsR}}$ with ^{13}C-enriched KaiB$_{\mathrm{fs\text{-}nmr}}$ or ^{15}N-enriched KaiB$_{\mathrm{fs\text{-}nmr}}$ with ^{13}C-enriched CikA$_{\mathrm{PsR}}$), and ^{13}C-edited, ^{12}C-filtered 3D NOESY-HSQC (in a sample containing ^{13}C-enriched CikA$_{\mathrm{PsR}}$ with unlabeled KaiB$_{\mathrm{fs\text{-}nmr}}$ or ^{13}C enriched KaiB$_{\mathrm{fs\text{-}nmr}}$ with unlabeled CikA$_{\mathrm{PsR}}$). Methyl-TROSY NMR experiments *(72, 73)* were performed as previously described *(27)*. Please see table S1 for full details.

The equations used to calculate Δδ is given below *(74)*:

$$\Delta\delta = \sqrt{\frac{1}{2}\left[\Delta \mathrm{H}^2 + (\alpha \times \Delta \mathrm{C}^2)\right]} \quad (1)$$

$$\alpha = \frac{\text{Range of H}}{\text{Range of C}} = 0.231165207 \quad (2)$$

where H and C are chemical shifts of proton and ^{13}C carbon, respectively.

Residual dipolar coupling

Samples of free CikA$_{\mathrm{PsR}}$ and KaiB$_{\mathrm{fs\text{-}nmr}}$ were separately soaked in 5.16% stretched polyacrylamide gels made with 6 mm diameter chamber (New Era NMR), and samples of the CikA$_{\mathrm{PsR}}$-KaiB$_{\mathrm{fs\text{-}nmr}}$ complex were soaked in 5.5% stretched polyacrylamide gels made with 5 mm diameter chamber (New Era NMR). The protocol for making an alignment gel has been described previously *(75)*. Please see table S1 for full details.

NMR structure determination

Dihedral angles were predicted from backbone chemical shifts using TALOS-N (http://spin.niddk.nih.gov/bax/software/TALOS-N). Cis-prolines were predicted using Promega (http://spin.niddk.nih.gov/bax/software/PROMEGA). Modified from Cornilescu et al. *(76)*, the range of intramolecular distance restraints were converted from a continuous distribution of assigned NOESY peak intensities, with a uniform 40% distance error for free CikA$_{\mathrm{PsR}}$ and KaiB$_{\mathrm{fs\text{-}nmr}}$ and 50% distance error for the CikA$_{\mathrm{PsR}}$-KaiB$_{\mathrm{fs\text{-}nmr}}$ complex, to account for spin diffusion. Intermolecular NOE distance restraints were classified as strong, medium and weak *(77)*. Distance restraints involving methyl groups were given an additional 0.5 Å for the upper bound. Structures were calculated with XPLOR-NIH *(78)* following protocols from Tian et al. *(79)*. RDC refinements followed Chou et al. *(80)*, except for the last stage, when the force constant for the dihedral potential was set to 200 instead of 50 kcal mol^{-1} rad^{-2}. Structure validation statistics were generated by Molprobity *(81)* and DC (Restrained Fit of Dipolar Couplings to a PDB Structure Using Non-Linear Least-Squares) (http://spin.niddk.nih.gov/bax/nmrserver/dc/fit.html). Structure refinement statistics are in table S3. Protein-protein interfaces were analyzed with PDBePISA *(64)* (http://www.ebi.ac.uk/pdbe/prot_int/pistart.html). Structural figures were made with UCSF Chimera software *(65)*.

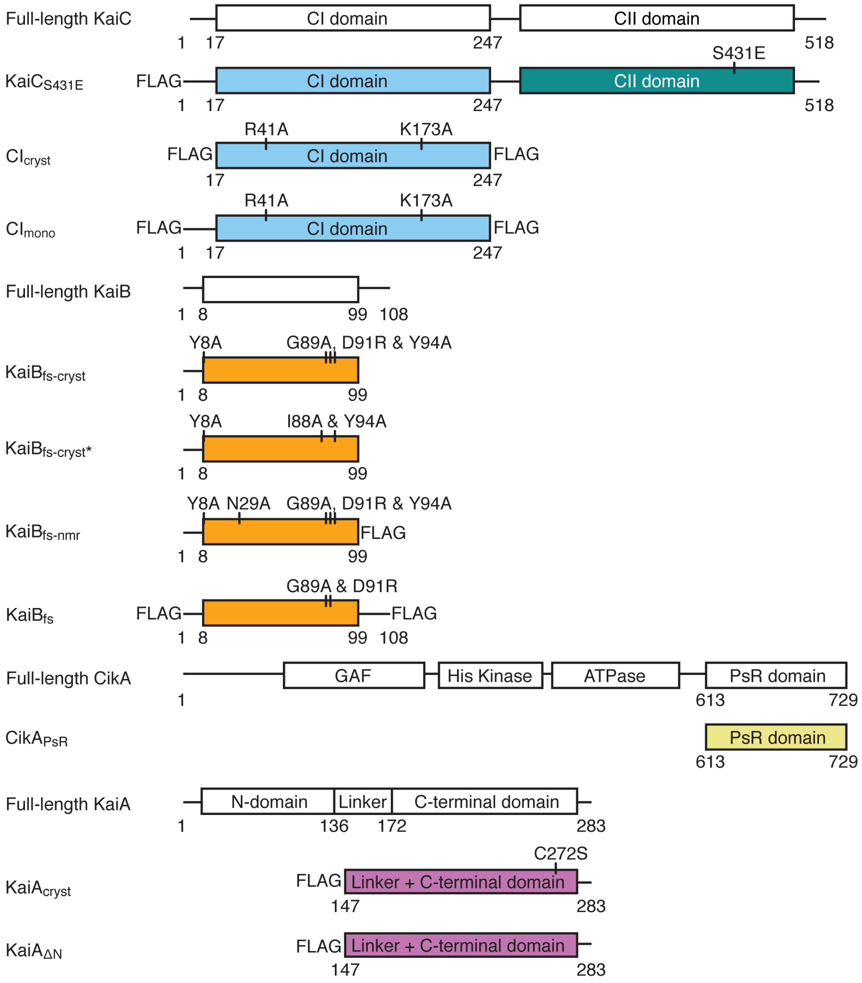
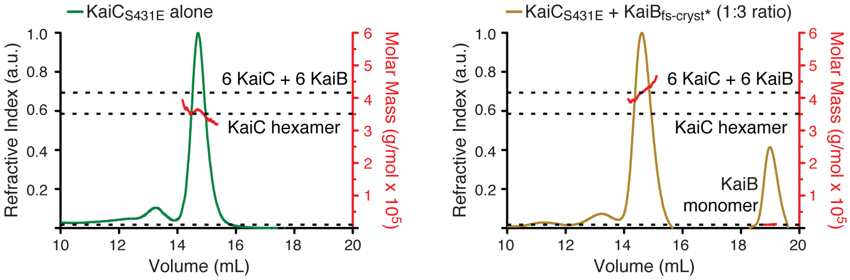


Fig. S1. Minimal constructs of *Thermosynechococcus elongatus* proteins.

Clock protein constructs from *Thermosynechococcus elongatus* BP-1 used in this study (not drawn to scale). Please see table S1 for additional construct details.



	MW (kDa)		
protein	calculated	experimental	polydispersity (Mw/Mn)
KaiC$_{S431E}$ hexamer	351	361.2 ± 0.5%	1.003 ± 0.75%
KaiB$_{fs\text{-}cryst*}$	11	11.3 ± 14.7%	1.003 ± 20.93%
KaiB$_{fs\text{-}cryst*}$-KaiC$_{S431E}$ complex	416 (6:6)	423.8 ± 0.3%	1.006 ± 0.48%

Fig. S2. Stoichiometry of the KaiB$_{fs\text{-}cryst*}$-KaiC$_{S431E}$ complex.

Size-exclusion chromatography and multi-angle light scattering (SEC-MALS) analyses of molecular masses of complexes containing KaiC$_{S431E}$ alone (green) or KaiC$_{S431E}$+KaiB$_{fs\text{-}cryst*}$ (brown). Calculated and experimental values for molecular weights are given below. Please see fig. S1 and table S1 for construct details.

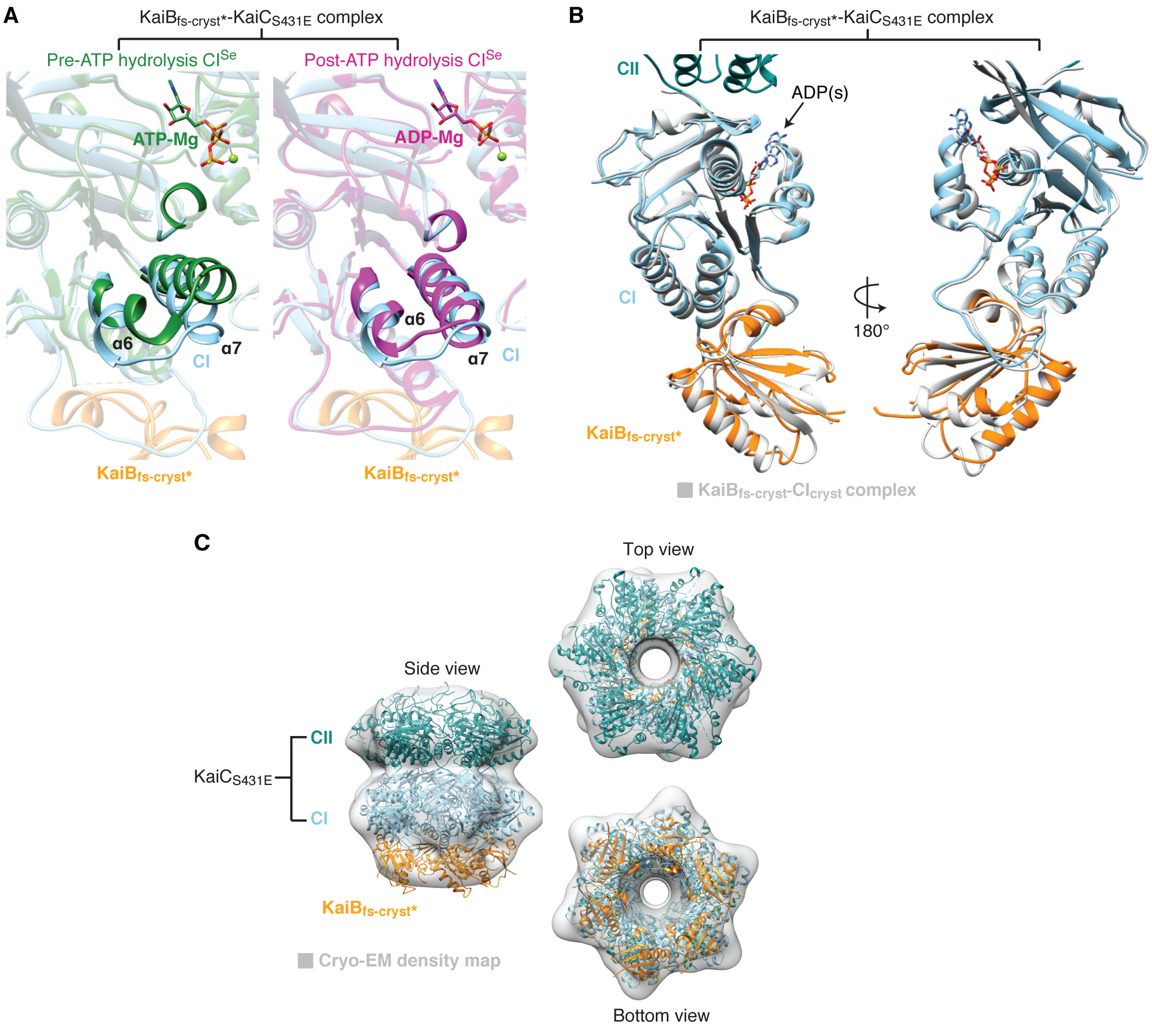


Fig. S3. Analysis of the KaiB$_{\text{fs-cryst*}}$-KaiC$_{\text{S431E}}$ complex.

(**A**) Superposition of the KaiB$_{\text{fs-cryst*}}$-KaiC$_{\text{S431E}}$ complex to the pre-ATP hydrolysis state of CI$^{\text{Se}}$ (dark green) (PDB 4TLC, subunit C) and post-ATP hydrolysis state of CI$^{\text{Se}}$ (magenta) (PDB 4TLA, subunit E). Orange, KaiB$_{\text{fs-cryst*}}$; sky-blue, KaiC$_{\text{S431E}}$ CI domain. (**B**) Superposition of the KaiB$_{\text{fs-cryst}}$-CI$_{\text{cryst}}$ complex to the KaiB$_{\text{fs-cryst*}}$-KaiC$_{\text{S431E}}$ structure. Orange, KaiB$_{\text{fs-cryst*}}$; sky-blue, KaiC$_{\text{S431E}}$ CI domain; dark cyan, KaiC$_{\text{S431E}}$ CII domain; gray, KaiB$_{\text{fs-cryst}}$-CI$_{\text{cryst}}$ complex. (**C**) Fitting the hexameric KaiB$_{\text{fs-cryst*}}$-KaiC$_{\text{S431E}}$ complex to the 16 Å resolution cryo-EM density map of a KaiB-KaiC complex made with native KaiB *(51)* (EMD-5672). Please see fig. S1 and table S1 for construct details.

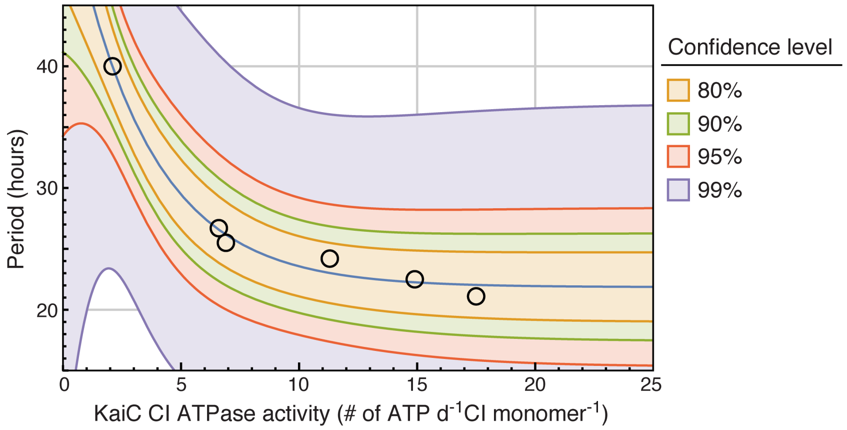


Fig. S4. Analysis of CI ATPase activity versus clock period data in Figure 1G of the paper by Abe et al. *Science* (2015) 349: 312-316.

Exponential fit (blue line) of CI ATPase activity versus clock period data in Figure 1G of the paper by Abe et al. *Science* (2015) 349: 312-316. Predicted confidence levels of the fit by Mathematica (Wolfram) are 80% (yellow), 90% (green), 95% (red), and 99% (purple). The fit predicts a lower limit of ~21.9 hours for clock period, irrespective of CI ATPase activity, assuming wild-type CII activity.

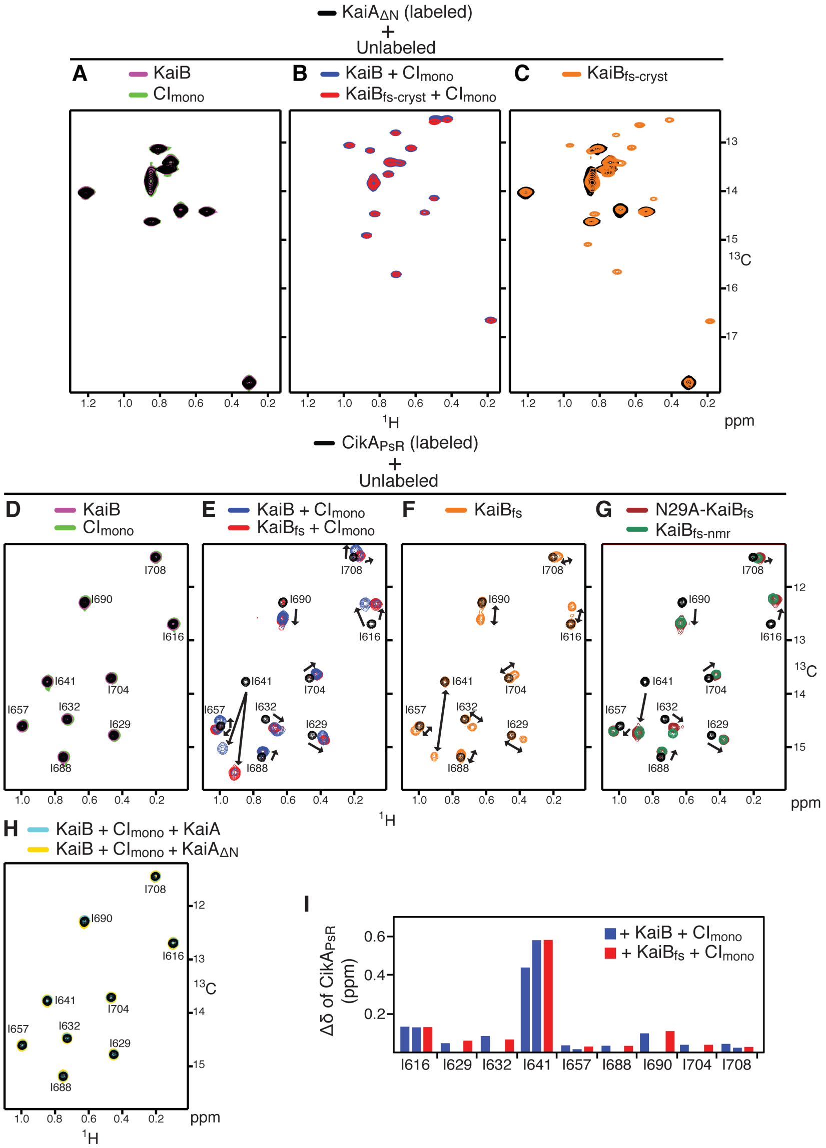


Fig. S5. NMR of KaiA$_{\Delta N}$-KaiB$_{fs\text{-}cryst}$-CI$_{mono}$ and CikA$_{PsR}$-KaiB$_{fs}$-CI$_{mono}$ interactions. (**A**) Methyl-TROSY spectra of U-[^{15}N, ^{2}H]-Ile-δ1-[^{13}C, ^{1}H]-labeled KaiA$_{\Delta N}$ alone (black), or in the presence of wild-type KaiB (magenta), or CI$_{mono}$ (green); or **(B)** in the presence of KaiB+CI$_{mono}$ (blue) or KaiB$_{fs\text{-}cryst}$+CI$_{mono}$ (red); or (**C**) in the presence of KaiB$_{fs\text{-}cryst}$ (orange). (**D**) Assigned methyl-TROSY spectra of U-[^{15}N, ^{2}H]-Ile-δ1-[^{13}C, ^{1}H]–labeled CikA$_{PsR}$ alone (black), or in the presence of wild-type KaiB (magenta) or CI$_{mono}$ (green); or (**E**) in the presence of KaiB+CI$_{mono}$ (blue), or KaiB$_{fs}$+CI$_{mono}$ (red); or (**F**) in the presence of KaiB$_{fs}$ (orange); or (**G**) in the presence of N29A-KaiB$_{fs}$ (brown), or KaiB$_{fs\text{-}nmr}$ (dark green); or (**H**) in the presence of KaiB+CI$_{mono}$+KaiA (cyan), or KaiB+CI$_{mono}$+KaiA$_{\Delta N}$ (yellow). (**I**) Perturbation of CikA$_{PsR}$ ^{1}H, ^{13}C chemical shifts by KaiB+CI$_{mono}$ (blue) or KaiB$_{fs}$+CI$_{mono}$ (red). Two blue bars for I616, I641, I657, and I708 represent two different conformations. Please see fig. S1 and table S1 for construct details.

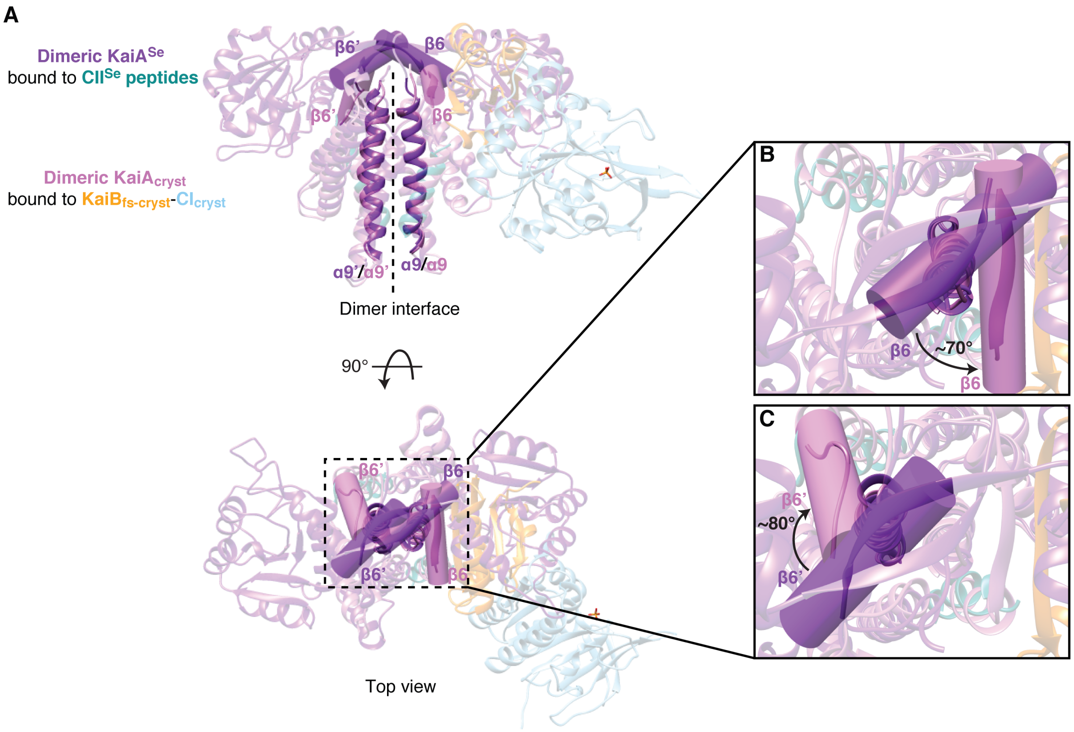


Fig. S6. Rotations of KaiA$_{cryst}$ β6 strands in the KaiA$_{cryst}$-KaiB$_{fs\text{-}cryst}$-CI$_{cryst}$ complex. (**A**) Superposition of KaiA^{Se} (purple) crystal structure (PDB 5C5E) and KaiA$_{cryst}$ (orchid) in complex with KaiB$_{fs\text{-}cryst}$ and CI$_{cryst}$. Prime symbols denote the other protomer within the KaiA dimer. Top view is shown below. Ribbons are shown in transparent mode, except for strand β6 and helix α9. (**B**) Zoomed-in view of the boxed region in (A). Only one set of β6 strands is shown. (**C**) Zoomed-in view of the boxed region in (A). The other set of β6 strands is shown. Angles are calculated from cylindral axes using residues 167-172 in KaiA^{Se} and residues 166-171 in KaiA$_{cryst}$. Please see fig. S1 and table S1 for construct details.

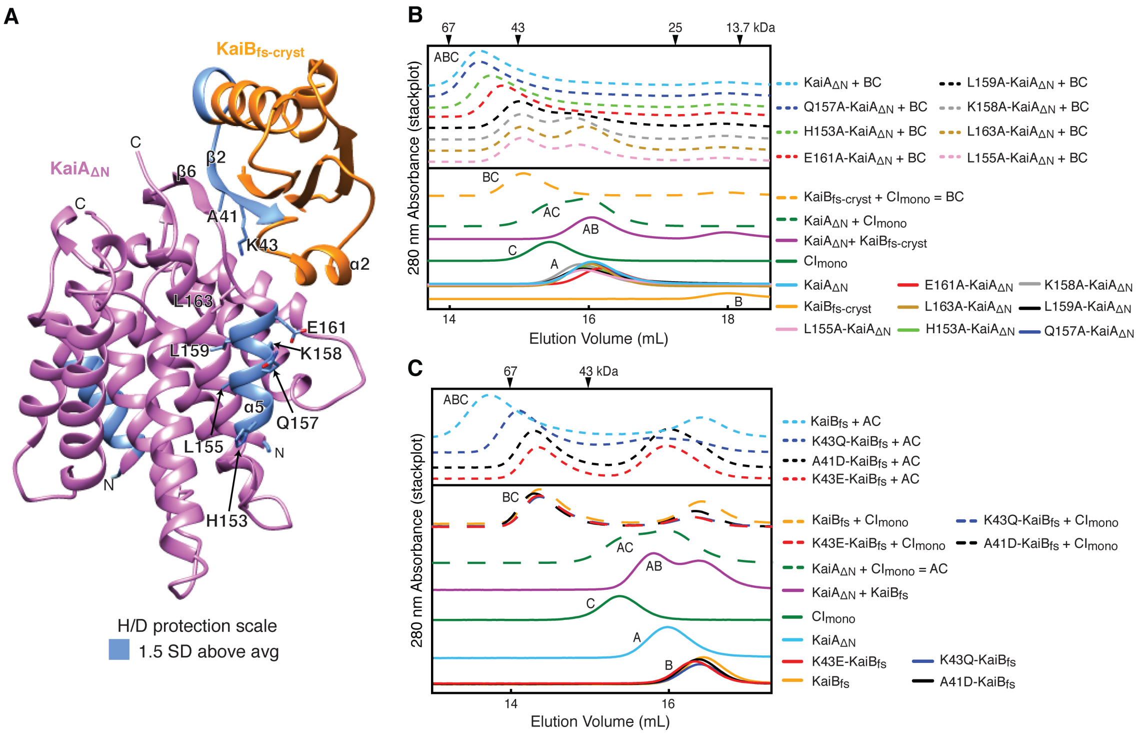


Fig. S7. HDX-MS and mutagenesis of KaiA$_{\Delta N}$-KaiB$_{fs\text{-}cryst}$ interface.

(**A**) Protection against hydrogen-deuterium exchange upon formation of the KaiA$_{\Delta N}$-KaiB$_{fs\text{-}cryst}$-CI$_{cryst}$ complex, as measured by HDX-MS. Blue, protection 1.5 SD above average protection. (**B**) Size-exclusion chromatography of Kai protein complex formation. Bottom panel, KaiA$_{\Delta N}$ mutants and control samples: KaiA$_{\Delta N}$ (sky blue), H153A-KaiA$_{\Delta N}$ (green), L155A-KaiA$_{\Delta N}$ (pink), Q157A-KaiA$_{\Delta N}$ (blue), K158A-KaiA$_{\Delta N}$ (gray), L159A-KaiA$_{\Delta N}$ (black), E161A-KaiA$_{\Delta N}$ (red), L163A-KaiA$_{\Delta N}$ (coffee), KaiB$_{fs\text{-}cryst}$ (orange), CI$_{mono}$ (dark green), KaiA$_{\Delta N}$+KaiB$_{fs\text{-}cryst}$ (purple), KaiA$_{\Delta N}$+CI$_{mono}$ (dark green, long dash), and KaiB$_{fs\text{-}cryst}$+CI$_{mono}$ (orange, long dash). The label BC represents KaiB$_{fs\text{-}cryst}$+CI$_{mono}$ (top panel). Top panel, ternary mixtures: KaiA$_{\Delta N}$+BC (sky blue, short dash), H153A-KaiA$_{\Delta N}$+BC (green, short dash), L155A-KaiA$_{\Delta N}$+BC (pink, short dash), Q157A-KaiA$_{\Delta N}$+BC (blue, short dash), K158A-KaiA$_{\Delta N}$+BC (gray, short dash), L159A-KaiA$_{\Delta N}$+BC (black, short dash), E161A-KaiA$_{\Delta N}$+BC (red, short dash), and L163A-KaiA$_{\Delta N}$+BC (coffee, short dash). (**C**) Size-exclusion chromatography of Kai protein complex formation. Bottom panel, KaiB$_{fs}$ mutants and control samples: KaiA$_{\Delta N}$ (sky blue), KaiB$_{fs}$ (orange), A41D-KaiB$_{fs}$ (black), K43E-KaiB$_{fs}$ (red), K43Q-KaiB$_{fs}$ (blue), CI$_{mono}$ (dark green), KaiA$_{\Delta N}$+KaiB$_{fs}$ (purple), KaiB$_{fs}$+CI$_{mono}$ (orange, long dash), A41D-KaiB$_{fs}$+CI$_{mono}$ (black, long dash), K43E-KaiB$_{fs}$+CI$_{mono}$ (red, long dash), K43Q-KaiB$_{fs}$+CI$_{mono}$ (blue, long dash), and KaiA$_{\Delta N}$+CI$_{mono}$ (dark green, long dash). The label AC represents KaiA$_{\Delta N}$+CI$_{mono}$ (top panel). Ternary mixtures (top panel): KaiB$_{fs}$+AC (sky blue, short dash), A41D-KaiB$_{fs}$+AC (black, short dash), K43E-KaiB$_{fs}$+AC (red, short dash), and K43Q-KaiB$_{fs}$+AC (blue, short dash). Molecular weight markers (inverted triangles) are indicated along the top of the chromatograms. Please see fig. S1 and table S1 for construct details.

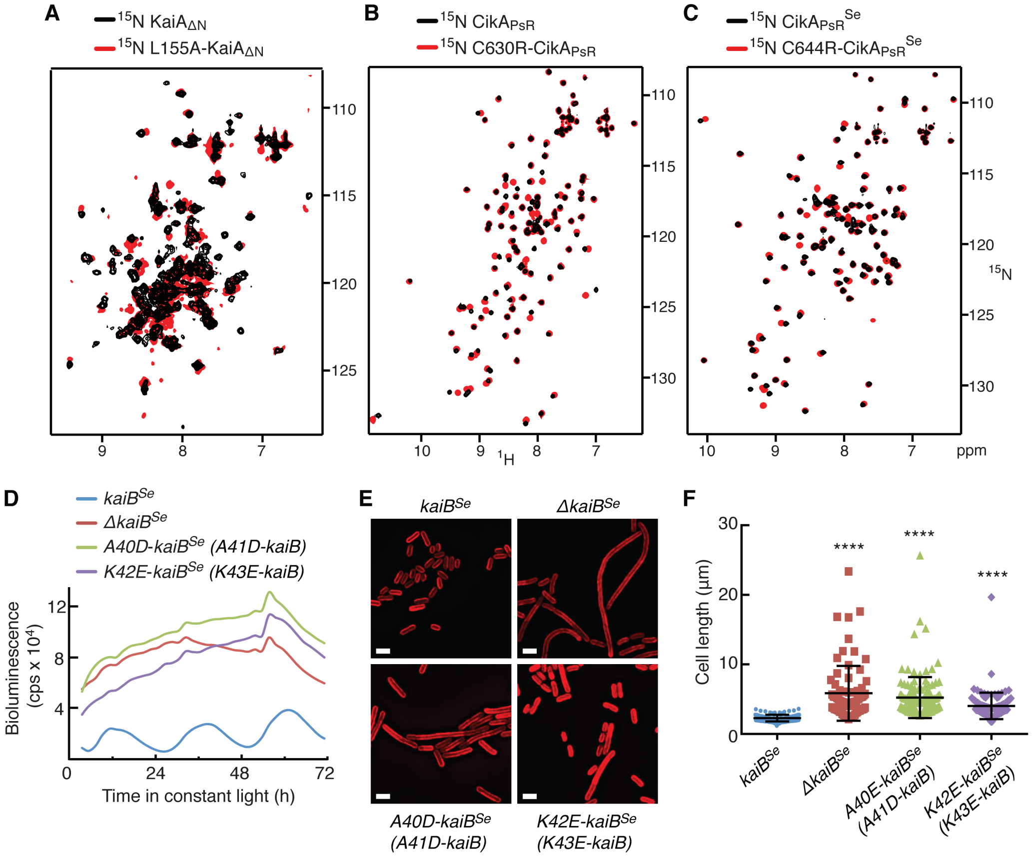


Fig. S8. NMR and *in vivo* experiments of KaiA, KaiB, and CikA$_{PsR}$ mutants.

(**A**) Overlay of ^{15}N, ^{1}H-HSQC spectra of U-[^{15}N, ^{1}H]-labeled KaiA$_{\Delta N}$ (black) and L155A-KaiA$_{\Delta N}$ (red). (**B**) Overlay of ^{15}N, ^{1}H-HSQC spectra of U-[^{15}N, ^{1}H]-labeled CikA$_{PsR}$ (black) and C630R-CikA$_{PsR}$ (red). (**C**) Overlay of ^{15}N, ^{1}H-HSQC spectra of U-[^{15}N, ^{1}H]-labeled CikA$_{PsR}{}^{Se}$ (black) and C644R-CikA$_{PsR}{}^{Se}$ (red). C630R and C644R are analogous mutations of CikA$_{PsR}$ and CikA$_{PsR}{}^{Se}$, respectively. (**D**) Bioluminescence rhythms from *kaiB*^{Se}-knockout *S. elongatus* strains transformed with *kaiB*^{Se} (blue), *A40D-kaiB*^{Se} (green), *k42E-kaiB*^{Se} (purple), or nothing (red). In (D to F), A40D and K42E in *kaiB*^{Se} are analogous to A41D and K43E in *kaiB*, respectively. (**E**) Representative micrographs of cells harboring *kaiB*^{Se}, *A40D-kaiB*^{Se}, *K42E-kaiB*^{Se}, or lacking any *kaiB*^{Se} gene. Red: membrane autofluorescence. Scale bars: 2.5 µm. (**F**) Scatterplots of cell lengths from strains expressing complemented *kaiB*^{Se} (blue, n=150), *A40D-kaiB*^{Se} (green, n=125), *K42E-kaiB*^{Se} (purple, n=112), or lacking *kaiB*^{Se} (red, n=76). One-way ANOVA of log$_{10}$-transformed cell length data produced $P < 0.0001$. **** denotes Bonferroni-corrected p values (< 0.0001) for pairwise comparisons to *kaiB*^{Se} (α=0.05). Please see fig. S1 and table S1 for construct details.

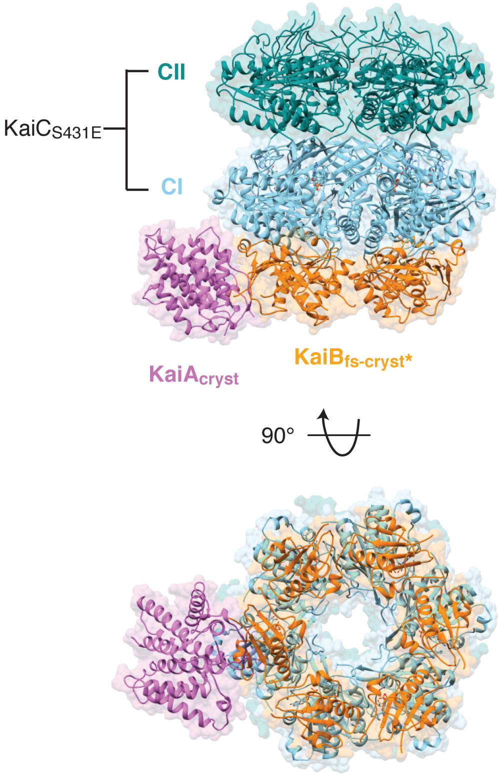


Fig. S9. Superposition of the KaiA$_{cryst}$-KaiB$_{fs\text{-}cryst}$-CI$_{cryst}$ complex onto the KaiB$_{fs\text{-}cryst*}$-KaiC$_{S431E}$ hexamer.

Superposition of the KaiA$_{cryst}$-KaiB$_{fs\text{-}cryst}$-CI$_{cryst}$ complex from Fig. 4A with that of the KaiB$_{fs\text{-}cryst*}$-KaiC$_{S431E}$ hexamer from Fig. 3A.

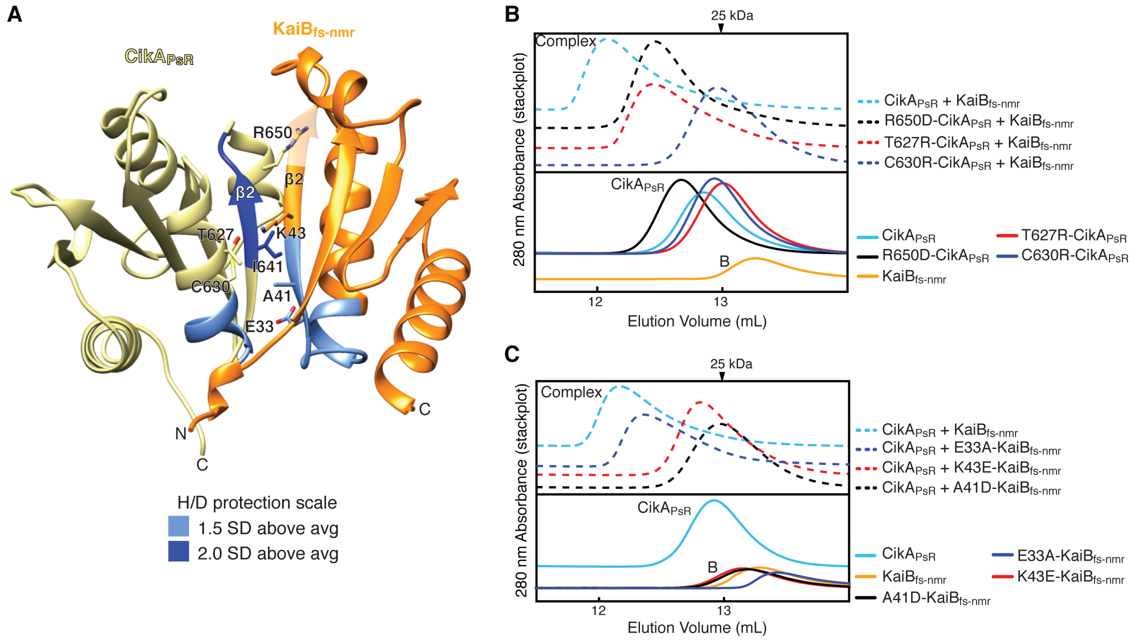


Fig. S10. HDX-MS and mutagenesis of the CikA$_{\mathrm{PsR}}$-KaiB$_{\mathrm{fs\text{-}nmr}}$ complex.

(**A**) Protection against hydrogen-deuterium exchange upon formation of the CikA$_{\mathrm{PsR}}$-KaiB$_{\mathrm{fs\text{-}nmr}}$ complex, as measured by HDX-MS. blue and dark blue, protection 1.5 and 2.0 SD, respectively, above average protection. (**B**) Size-exclusion chromatography of CikA$_{\mathrm{PsR}}$-KaiB$_{\mathrm{fs\text{-}nmr}}$ complex formation. Bottom panel, CikA$_{\mathrm{PsR}}$ mutants and control samples: CikA$_{\mathrm{PsR}}$ (sky blue), T627R-CikA$_{\mathrm{PsR}}$ (red), C630R-CikA$_{\mathrm{PsR}}$ (blue), R650D-CikA$_{\mathrm{PsR}}$ (black) and KaiB$_{\mathrm{fs\text{-}nmr}}$ (orange). Top panel, binary mixtures: CikA$_{\mathrm{PsR}}$+KaiB$_{\mathrm{fs\text{-}nmr}}$ (sky-blue, dash), T627R-CikA$_{\mathrm{PsR}}$+KaiB$_{\mathrm{fs\text{-}nmr}}$ (red, dash), C630R-CikA$_{\mathrm{PsR}}$+KaiB$_{\mathrm{fs\text{-}nmr}}$ (blue, dash), and R650D-CikA$_{\mathrm{PsR}}$+KaiB$_{\mathrm{fs\text{-}nmr}}$ (black, dash). (**C**) Size-exclusion chromatography of CikA$_{\mathrm{PsR}}$-KaiB$_{\mathrm{fs\text{-}nmr}}$ complex formation. Bottom panel, KaiB mutants and control samples: CikA$_{\mathrm{PsR}}$ (sky blue), KaiB$_{\mathrm{fs\text{-}nmr}}$ (orange), E33A-KaiB$_{\mathrm{fs\text{-}nmr}}$ (blue), A41D-KaiB$_{\mathrm{fs\text{-}nmr}}$ (black), and K43E-KaiB$_{\mathrm{fs\text{-}nmr}}$ (red). Top panel, binary mixtures: CikA$_{\mathrm{PsR}}$+KaiB$_{\mathrm{fs\text{-}nmr}}$ (sky-blue, dash), CikA$_{\mathrm{PsR}}$+E33A-KaiB$_{\mathrm{fs\text{-}nmr}}$ (blue, dash), CikA$_{\mathrm{PsR}}$+A41D-KaiB$_{\mathrm{fs\text{-}nmr}}$ (black, long dash), and CikA$_{\mathrm{PsR}}$+K43E-KaiB$_{\mathrm{fs\text{-}nmr}}$ (red, dash). Molecular weight markers (inverted triangles) are indicated along the top of the chromatograms. Please see fig. S1 and table S1 for construct details.

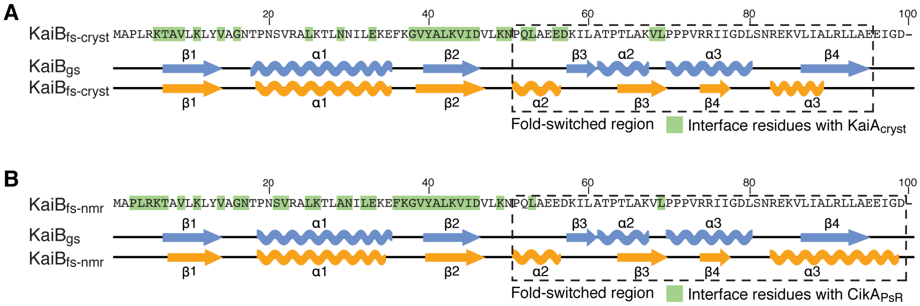


Fig. S11. Interacting residues of KaiB to KaiA$_{cryst}$ and CikA$_{PsR}$.

(**A**) Secondary structures of KaiB$_{gs}$ (PDB 2QKE, subunit A) and KaiB$_{fs\text{-}cryst}$ bound to CI$_{cryst}$ and KaiA$_{cryst}$. Residues of KaiB$_{fs\text{-}cryst}$ that interact with KaiA$_{cryst}$ are highlighted in green. (**B**) Secondary structures of KaiB$_{gs}$ (PDB 2QKE, subunit A) and KaiB$_{fs\text{-}nmr}$ bound to CikA$_{PsR}$. Residues of KaiB$_{fs\text{-}nmr}$ that interact with CikA$_{PsR}$ are highlighted in green.

Table S1. Abbreviation and full name of proteins used in each experimental condition.

Experiment Type	[Abbreviation]-Protein Full Name (N-terminal Tag_Protein_Mutation_Length of Protein_ C-terminal Tag) / Final Concentration (μM if not indicated)	Experimental Condition
Crystallization for KaiB$_{\text{fs-cryst}}$-CI$_{\text{cryst}}$ complex (Fig. 2)	1) [CI$_{\text{cryst}}$]-FLAG_KaiC_R41A-K173A_17-247_FLAG / ~20 mg/mL 2) [KaiB$_{\text{fs-cryst}}$]-KaiB_Y8A_Y94A_G89A_D91R_1-99 / ~20 mg/mL	• Temperature: 22 °C • Crystallization buffer: 0.2 M potassium acetate, 10% (w/v) PEG3350, and 2.5% glycerol • Proteins stored in 20 mM Tris, 50 mM NaCl, 1 mM MgCl$_2$, 1 mM ADP, pH 7.0
Crystallization for KaiB$_{\text{fs-cryst*}}$-KaiC$_{\text{S431E}}$ complex (Fig. 3)	1) [KaiC$_{\text{S431E}}$]- FLAG_KaiC_S431E_1-518 / ~12.5 mg/mL 2) [KaiB$_{\text{fs-cryst*}}$]-KaiB_Y8A_Y94A_I88A_1-99 / ~12.5 mg/mL	• Temperature: 22 ºC • Crystallization buffer: 0.1 M sodium citrate pH 5.75 and 22% PEG3000 • KaiC$_{\text{S431E}}$ stored in 20mM Tris, 150mM NaCl, 5mM DTT, 1mM ATP, 1mM MgCl$_2$, 5% glycerol pH 7.0 • KaiB$_{\text{fs-cryst*}}$ stored in 20mM Tris, 150mM NaCl, 5mM DTT, 5% glycerol pH 7.0
Crystallization for KaiA$_{\text{cryst}}$-KaiB$_{\text{fs-cryst}}$-CI$_{\text{cryst}}$ Complex (Fig. 4)	1) [CI$_{\text{cryst}}$]-FLAG_KaiC_R41A-K173A_17-247_FLAG / ~25 mg/mL 2) [KaiB$_{\text{fs-cryst}}$]-KaiB_Y8A-Y94A-G89A-D91R_1-99 / ~25 mg/mL 3) [KaiA$_{\text{cryst}}$]-FLAG_KaiA_C272S_147-283 / ~25 mg/mL	• Temperature: 22 °C • Crystallization buffer: 0.15 M ammonium sulfate, 0.1 M Tris pH 7.0, and 10% PEG 4000 • Proteins stored in 20 mM Tris, 50 mM NaCl, 1 mM MgCl$_2$, 1 mM ADP, pH 7.0
SEC-MALS of KaiB$_{\text{fs-cryst*}}$-KaiC$_{\text{S431E}}$ complex (fig. S2)	1) [KaiC$_{\text{S431E}}$]-FLAG_KaiC_S431E_1-518 / 30 2) [KaiB$_{\text{fs-cryst*}}$]-KaiB_Y8A_Y94A_I88A_1-99 / 90	• Volume: 200 μL • Pre-experiment incubation time of mixture: overnight • Buffer: 20 mM Tris, 150 mM NaCl, 5 mM DTT, 0.5 mM MgCl$_2$, 0.5 mM ATP, pH 7.0 • Temperature: 4 ºC
Fluorescence Spectroscopy for CII peptides (Fig. 4D)	1) [CII peptides]-Cystine_KaiC_488-518 (6-IAF-labeled) / 0.05 2) [KaiA]-KaiA_1-283 / 10 3) [CI$_{\text{cryst}}$]-FLAG_KaiC_R41A-K173A_17-247_FLAG / 5, 10, 15, 20, 30 4) [KaiB$_{\text{fs-cryst}}$]-KaiB_Y8A-Y94A-G89A-D91R_1-99 / 5, 10 , 15, 20, 30	• Volume: 350 μL • Pre-experiment incubation time of mixture: 5 mins • Temperature: 25 °C • Buffer: 20 mM Tris, 50 mM NaCl, 0.2% NaN$_3$, pH 7.0
HDX-MS for KaiA$_{\Delta\text{N}}$-KaiB$_{\text{fs-cryst}}$-CI$_{\text{cryst}}$ complex (fig. S7A) Mixture ratio of KaiA$_{\Delta\text{N}}$: KaiB$_{\text{fs-cryst}}$:CI$_{\text{cryst}}$ for probing KaiA$_{\Delta\text{N}}$ free and bound is 1.0:1.2:1.44 Mixture ratio of KaiA$_{\Delta\text{N}}$: KaiB$_{\text{fs-cryst}}$:CI$_{\text{cryst}}$ for probing KaiB$_{\text{fs-cryst}}$-CI$_{\text{cryst}}$ with and without KaiA$_{\Delta\text{N}}$ is 1.2:1.0:1.2	1) [KaiA$_{\Delta\text{N}}$]-FLAG_KaiA_147-283 / 49.3, 69.3 2) [KaiB$_{\text{fs-cryst}}$-FLAG]-KaiB _Y8A_Y94A_G89A_D91R_1-99_FLAG / 60, 56.6 3) [CI$_{\text{cryst}}$]-FLAG_KaiC_R41A-K173A_17-247_FLAG / 72, 68	• Volume: 60 μL • Buffer: 10 mM Tris, 100 mM NaCl, pH 7.2 • Quenching buffer: 3.2M GuHCl • Exchange time points: 10s, 100s and 1000s

HDX-MS for CikA$_{\text{PsR}}$-KaiB$_{\text{fs-nmr}}$ complex (fig. S10A) Mixture ratio of CikA$_{\text{PsR}}$: KaiB$_{\text{fs-nmr}}$ for probing CikA$_{\text{PsR}}$ free and bound is 1.0:1.2 Mixture ratio of CikA$_{\text{PsR}}$: KaiB$_{\text{fs-nmr}}$ for probing KaiB$_{\text{fs-nmr}}$ free and bound is 1.2:1.0	1) [CikA$_{\text{PsR}}$]-CikA_613-729 / 78, 104.6 2) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG / 93.3, 84.6	• Volume: 60 µL • Buffer: 10 mM Tris, 100 mM NaCl, pH 7.2 • Quenching buffer: 1.6M GuHCl • Exchange time points: 10s, 100s and 1000s
KaiA-KaiB-CI$_{\text{cryst}}$ complex mutagenesis study by S200 analytical gel filtration chromatography (Fig. 4E)	1) [KaiA]-KaiA_1-283 / 30 2) [KaiB]-KaiB_1-108 / 30 3) [CI$_{\text{cryst}}$]-FLAG_KaiC_R41A-K173A_17-247_FLAG / 30 4) [L155A-KaiA]-KaiA_L155A_1-283 / 30 5) [K158A-KaiA]-KaiA_L158A_1-283 / 30 6) [N212A-KaiA]-KaiA_N212A_1-283 / 30 7) [D266A-KaiA]-KaiA_D266A_1-283 / 30 8) [N212A-D266A-KaiA]-KaiA_N212A_D266A_1-283 / 30	• Volume: 250 µL • Temperature: room temperature (~22 °C) • Buffer: 20 mM Tris, 100 mM NaCl, pH 7.0 • Pre-experiment incubation time for mixtures: 24 hrs.
KaiA$_{\Delta\text{N}}$-KaiB$_{\text{fs-cryst}}$-CI$_{\text{mono}}$ complex mutagenesis study by S200 analytical gel filtration chromatography (fig. S7B)	1) [KaiB$_{\text{fs-cryst}}$]-KaiB_Y8A_Y94A_G89A_D91R_1-99 / 30 2) [CI$_{\text{mono}}$]-FLAG_KaiC_R41A-K173A_1-247_FLAG / 15 3) [KaiA$_{\Delta\text{N}}$]-FLAG_KaiA_147-283 / 30 4) [H153A-KaiA$_{\Delta\text{N}}$]-FLAG_KaiA_H153A_147-283 / 30 5) [L155A-KaiA$_{\Delta\text{N}}$]-FLAG_KaiA_L155A_147-283 / 30 6) [Q157A-KaiA$_{\Delta\text{N}}$]-FLAG_KaiA_Q157A_147-283 / 30 7) [K158A-KaiA$_{\Delta\text{N}}$]-FLAG_KaiA_K158A_147-283 / 30 8) [L159A-KaiA$_{\Delta\text{N}}$]-FLAG_KaiA_L159A_147-283 / 30 9) [E161A-KaiA$_{\Delta\text{N}}$]-FLAG_KaiA_E161A_147-283 / 30 10) [L163A-KaiA$_{\Delta\text{N}}$]-FLAG_KaiA_L163A_147-283 / 30	• Volume: 250 µL • Temperature: room temperature (~22 °C) • Buffer: 20 mM Tris, 100 mM NaCl, pH 7.0
KaiA$_{\Delta\text{N}}$-KaiB$_{\text{fs}}$-CI$_{\text{mono}}$ complex mutagenesis study by S200 analytical gel filtration chromatography (fig. S7C)	1) [KaiA$_{\Delta\text{N}}$]-FLAG_KaiA_147-283 / 30 2) [CI$_{\text{mono}}$]-FLAG_KaiC_R41A-K173A_1-247_FLAG / 16 3) [KaiB$_{\text{fs}}$]-FLAG_KaiB_G89A-D91R_1-108_FLAG / 30 4) [A41D-KaiB$_{\text{fs}}$]-FLAG_KaiB_ G89A-D91R_A41D_1-108_FLAG / 30 5) [K43E-KaiB$_{\text{fs}}$]-FLAG_KaiB_ G89A-D91R_K43E_1-108_FLAG / 30 6) [K43Q-KaiB$_{\text{fs}}$]-FLAG_KaiB_ G89A-D91R_K43Q_1-108_FLAG / 30	• Volume: 250 µL • Temperature: room temperature (~22 °C) • Buffer: 20 mM Tris, 100 mM NaCl, pH 7.0

CikA$_{\mathrm{PsR}}$-KaiB$_{\text{fs-nmr}}$ complex mutagenesis study by S75 analytical gel filtration chromatography (fig. S10B)	1) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG / 30 2) [CikA$_{\mathrm{PsR}}$]-CikA_613-729 / 30 3) [T627R-CikA$_{\mathrm{PsR}}$]-CikA_T627R_613-729 / 30 4) [C630R-CikA$_{\mathrm{PsR}}$]-CikA_C630R_613-729 / 30 5) [R650D-CikA$_{\mathrm{PsR}}$]-CikA_R650D_613-729 / 30	• Volume: 250 µL • Temp: room temp (~22 °C) • Buffer: 20 mM Tris, 100 mM NaCl, pH 7.0
CikA$_{\mathrm{PsR}}$-KaiB$_{\text{fs-nmr}}$ complex mutagenesis study by S75 analytical gel filtration chromatography (fig. S10C)	1) [CikA$_{\mathrm{PsR}}$]-CikA_613-729 / 30 2) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG / 30 3) [E33A-KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_E33A_1-99_FLAG / 30 4) [A41D-KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_A41D_1-99_FLAG / 30 5) [K43E-KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_K43E_1-99_FLAG / 30	• Volume: 250 µL • Temperature: room temperature (~22 °C) • Buffer: 20 mM Tris, 100 mM NaCl, pH 7.0
Methyl-TROSY NMR of KaiA$_{\Delta\mathrm{N}}$-KaiB-CI$_{\mathrm{mono}}$ interactions (fig. S5, A to C)	1) [KaiA$_{\Delta\mathrm{N}}$]-FLAG_KaiA_147-283 (U-[^{15}N, ^{2}H]-Ile-δ-[^{13}C, ^{1}H] labeled) / 20 2) [KaiB]-KaiB_1-108 / 50 3) [CI$_{\mathrm{mono}}$]-FLAG_KaiC_R41A-K173A_1-247_FLAG / 50 4) [KaiB$_{\text{fs-cryst}}$]-KaiB_Y8A_Y94A_G89A_D91R_1-99 / 50	• Volume: 330 µL • Pre-experiment incubation time for mixtures: 24 hrs. • ^{13}C / ^{1}H sweep width (Hz): 1209.247 / 3894.081 • ^{13}C / ^{1}H carrier (Hz): 1626.00 / -379.00 • ^{13}C / ^{1}H acquisition time (ms): 79.3 / 63.9 • Temp: 25 °C • Buffer in 99.96 % D$_2$O: 20 mM Tris, 100 mM NaCl, 5 mM DTT, 1 mM ATP, 1 mM MgCl$_2$, 10 µM DSS, 0.02% NaN$_3$, pH 7 • Shaped tube
Methyl-TROSY NMR of CikA$_{\mathrm{PsR}}$-KaiB-CI$_{\mathrm{mono}}$ interactions (fig. S5, D to I)	1) [CikA$_{\mathrm{PsR}}$]-CikA_613-729 (U-[^{15}N, ^{2}H]-Ile-δ-[^{13}C, ^{1}H] labeled) / 20 2) [CI$_{\mathrm{mono}}$]-FLAG_KaiC_R41A-K173A_1-247_FLAG / 50 3) [KaiB]-KaiB_1-108 / 50 4) [KaiB$_{\mathrm{fs}}$]-FLAG_KaiB_ G89A-D91R_1-108_FLAG / 50 5) [N29A-KaiB$_{\mathrm{fs}}$]-FLAG_KaiB_G89A-D91R_N29A_1-108_FLAG / 50 6) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG / 50 7) [KaiA$_{\Delta\mathrm{N}}$]-FLAG_KaiA_147-283 / 110 8) [KaiA]-KaiA_1-283 / 110	• Volume: 330 µL • Pre-experiment incubation time for mixtures: 24 hrs. • ^{13}C / ^{1}H sweep width (Hz): 2418.494 / 3894.081 • ^{13}C / ^{1}H carrier (Hz): 1626.00 / -379.00 • ^{13}C / ^{1}H acquisition time (ms): 41.3 / 63.9 • Temp: 25 °C • Buffer in 99.96 % D$_2$O: 20 mM Tris, 100 mM NaCl, 5 mM DTT, 1 mM ATP, 1 mM MgCl$_2$, 10 µM DSS, 0.02% NaN$_3$, pH 7 • Shaped tube

^{15}N-HSQC NMR for KaiA$_{\Delta N}$ and L155A-KaiA$_{\Delta N}$ (fig. S8A)	1) [KaiA$_{\Delta N}$]- FLAG_KaiA_147-283 (U-[^{15}N] labeled) / 40 2) [L155A-KaiA$_{\Delta N}$]-FLAG_KaiA_L155A_147-283 (U-[^{15}N] labeled) / 40	• Volume: 350 µL • ^{15}N / ^{1}HN sweep width (Hz): 1583.901 / 9615.385 • ^{15}N / ^{1}HN carrier (Hz): 7278.86 / 2824.00 • ^{15}N / ^{1}HN acquisition time (ms): 63.1 / 68.8 • Temp: 25 °C • Buffer in 5% D_2O: 20 mM Tris, 100 mM NaCl, 0.02% NaN_3, 10 µM DSS, pH 7 • Shaped tube
^{15}N-HSQC NMR for CikA$_{PsR}$ and C630R-CikA$_{PsR}$ (fig. S8B)	1) [CikA$_{PsR}$]-CikA_613-729 (U-[^{15}N] labeled) / 100 2) [C630R-CikA$_{PsR}$]-CikA_C630R_613-729 (U-[^{15}N] labeled) / 100	• Volume: 350 µL • ^{15}N / ^{1}HN sweep width (Hz): 1583.901 / 9615.385 • ^{15}N / ^{1}HN carrier (Hz): 7278.86 / 2824.00 • ^{15}N / ^{1}HN acquisition time (ms): 63.1 / 68.8 • Temp: 25 °C • Buffer in 5% D_2O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7 • Shaped tube
^{15}N-HSQC NMR for *Synechococcus elongatus* PCC 7942 proteins: CikA$_{PsR}^{Se}$ and C644R-CikA$_{PsR}^{Se}$ (fig. S8C)	1) [CikA$_{PsR}^{Se}$]-CikA^{Se}_627-745 (U-[^{15}N] labeled) / 100 2) [C644R-CikA$_{PsR}^{Se}$]-CikA^{Se}_C644R_627-745 (U-[^{15}N] labeled) / 100	• Volume: 350 µL • ^{15}N / ^{1}HN sweep width (Hz): 1583.901 / 9615.385 • ^{15}N / ^{1}HN carrier (Hz): 7278.86 / 2824.00 • ^{15}N / ^{1}HN acquisition time (ms): 63.1 / 68.8 • Temp: 25 °C • Buffer in 5% D_2O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7 • Shaped tube
3D HNCACB and HN(CO)CACB NMR for free KaiB$_{fs\text{-}nmr}$	1) [KaiB$_{fs\text{-}nmr}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG (U-[^{15}N, ^{13}C] labeled) / 800	• Volume: 350 µL • ^{13}C / ^{15}N / ^{1}HN sweep width (Hz): 9500.000 / 1583.899 / 7211.539 • ^{13}C / ^{15}N / ^{1}HN carrier (Hz): 6575.41 / 7278.86 / 2828.00 • ^{13}C / ^{15}N / ^{1}HN acquisition time (ms): 6.9 / 23.9 / 67.9 • Temp: 25 °C • Buffer in 5% D_2O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor • Shaped tube
3D HN(CA)CO and HNCO NMR for free KaiB$_{fs\text{-}nmr}$	1) [KaiB$_{fs\text{-}nmr}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG (U-[^{15}N, ^{13}C] labeled) / 800	• Volume: 350 µL • ^{13}C / ^{15}N / ^{1}HN sweep width (Hz): 1500.000 / 1583.899 / 7211.539 • ^{13}C / ^{15}N / ^{1}HN carrier (Hz): 26225.41 / 7278.86 / 2828.00 • ^{13}C / ^{15}N / ^{1}HN acquisition time (ms): 30.0 / 23.3 / 67.9 • Temp: 25 °C • Buffer in 5% D_2O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor • Shaped tube

3D HBHA(CO)NH NMR for free KaiB$_{\text{fs-nmr}}$	1) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG (U-[^{15}N, ^{13}C] labeled) / 800	• Volume: 350 µL • ^{1}H / ^{15}N / ^{1}HN sweep width (Hz): 4000.000 / 1583.899 / 7211.539 • ^{1}H / ^{15}N / ^{1}HN carrier (Hz): 2828.00 / 7278.86 / 2828.00 • ^{1}H / ^{15}N / ^{1}HN acquisition time (ms): 13.0 / 23.9 / 67.9 • Temp: 25 °C • Buffer in 5% D_2O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor • Shaped tube
3D HCC(CO)NH NMR for free KaiB$_{\text{fs-nmr}}$ Proton version	1) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG (U-[^{15}N, ^{13}C] labeled) / 800	• Volume: 350 µL • ^{1}H / ^{15}N / ^{1}HN sweep width (Hz): 4000.000 / 1583.899 / 7211.539 • ^{1}H / ^{15}N / ^{1}HN carrier (Hz): 2828.00 / 7278.86 / 2828.00 • ^{1}H / ^{15}N / ^{1}HN acquisition time (ms): 13.0 / 23.9 / 67.9 • Mixing time (ms): 120 • Temp: 25 °C • Buffer in 5% D_2O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor • Shaped tube
3D HCC(CO)NH NMR for free KaiB$_{\text{fs-nmr}}$ Carbon version	1) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG (U-[^{15}N, ^{13}C] labeled) / 800	• Volume: 350 µL • ^{13}C / ^{15}N / ^{1}HN sweep width (Hz): 9674.291 / 1583.899 / 7211.539 • ^{13}C / ^{15}N / ^{1}HN carrier (Hz): 6537.93 / 7278.86 / 2828.00 • ^{13}C / ^{15}N / ^{1}HN acquisition time (ms): 8.0 / 23.9 / 67.9 • Mixing time (ms): 120 • Temp: 25 °C • Buffer in 5% D_2O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor • Shaped tube
3D ^{15}N-NOESY-HSQC NMR for free KaiB$_{\text{fs-nmr}}$	1) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG (U-[^{15}N, ^{13}C] labeled) / 800	• Volume: 350 µL • ^{1}H / ^{15}N / ^{1}HN sweep width (Hz): 6011.328 / 1583.899 / 7211.539 • ^{1}H / ^{15}N / ^{1}HN carrier (Hz): 2828.00 / 7278.86 / 2828.00 • ^{1}H / ^{15}N / ^{1}HN acquisition time (ms): 24.9 / 21.4 / 67.9 • Mixing time (ms): 180 • Temp: 25 °C • Buffer in 5% D_2O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor • Shaped tube

3D ^{13}C-NOESY-HSQC NMR for free KaiB$_{\text{fs-nmr}}$	1) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG (U-[^{15}N, ^{13}C] labeled) / 800	• Volume: 350 μL • ^{1}H / ^{13}C / ^{1}H sweep width (Hz): 4027.582 / 5215.029 / 6009.615 • ^{1}H / ^{13}C / ^{1}H carrier (Hz): 1643.56 / 6011.05 / 1643.56 • ^{1}H / ^{13}C / ^{1}H acquisition time (ms): 32.2 / 9.9 / 67.8 • Mixing time (ms): 150 • Temp: 25 °C • Buffer in 99.96% D$_2$O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN$_3$, 10 μM DSS, pH 7, 1x protease inhibitor • Shaped tube
3D ^{13}C-HCCH-COSY NMR for free KaiB$_{\text{fs-nmr}}$ Proton version	1) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG (U-[^{15}N, ^{13}C] labeled) / 800	• Volume: 350 μL • ^{1}H / ^{13}C / ^{1}H sweep width (Hz): 4027.584 / 5215.027 / 6009.615 • ^{1}H / ^{13}C / ^{1}H carrier (Hz): 1643.56 / 6011.05 / 1643.56 • ^{1}H / ^{13}C / ^{1}H acquisition time (ms): 32.2 / 9.9 / 67.8 • Temp: 25 °C • Buffer in 99.96% D$_2$O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN$_3$, 10 μM DSS, pH 7, 1x protease inhibitor • Shaped tube
3D ^{13}C-HCCH-COSY NMR for free KaiB$_{\text{fs-nmr}}$ Carbon version	1) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG (U-[^{15}N, ^{13}C] labeled) / 800	• Volume: 350 μL • ^{13}C / ^{13}C / ^{1}H sweep width (Hz): 5215.027 / 5215.027 / 6009.615 • ^{13}C / ^{13}C / ^{1}H carrier (Hz): 6011.05 / 6011.05 / 1643.56 • ^{13}C / ^{13}C / ^{1}H acquisition time (ms): 9.9 / 9.9 / 67.8 • Temp: 25 °C • Buffer in 99.96% D$_2$O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN$_3$, 10 μM DSS, pH 7, 1x protease inhibitor • Shaped tube
3D HCAN and HCA(CO)N NMR for free KaiB$_{\text{fs-nmr}}$	1) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG (U-[^{15}N, ^{13}C] labeled) / 800	• Volume: 350 μL • ^{15}N / ^{13}C / ^{1}H sweep width (Hz): 1583.901 / 4534.871 / 6009.615 • ^{15}N / ^{13}C / ^{1}H carrier (Hz): 7278.98 / 8086.90 / 2850.40 • ^{15}N / ^{13}C / ^{1}H acquisition time (ms): 23.9 / 9.9 / 63.8 • Temp: 25 °C • Buffer in 99.96% D$_2$O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN$_3$, 10 μM DSS, pH 7, 1x protease inhibitor • Shaped tube

3D IPAP-J-HNCO(CA) NMR with 5.16% stretched polyacrylamide gel RDC of free KaiB$_{\text{fs-nmr}}$ measures: ^{1}J(CA-HA)	1) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG (U-[^{15}N, ^{13}C] labeled) / 356	• ^{13}C / ^{15}N / ^{1}HN sweep width (Hz): 1500.000 / 1583.899 / 7211.539 • ^{13}C / ^{15}N / ^{1}HN carrier (Hz): 26187.80 / 7278.86 / 2823.20 • ^{13}C / ^{15}N / ^{1}HN acquisition time (ms): 50.6 / 23.3 / 67.9 • Temp: 25 °C • Buffer in 10% D_2O: 10 mM Tris, 50 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor • Aligned sample: 4.2mm new era NMR tubes (NE-UP5-GT-7) • Isotropic sample: shaped tube
3D IPAP-J-HNCO NMR for 5.16% stretched polyacrylamide gel RDC of free KaiB$_{\text{fs-nmr}}$ measures: ^{1}J(N-H)	1) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG (U-[^{15}N, ^{13}C] labeled) / 356	• ^{13}C / ^{15}N / ^{1}HN sweep width (Hz): 1500.004 / 1583.899 / 7211.539 • ^{13}C / ^{15}N / ^{1}HN carrier (Hz): 26187.80 / 7278.86 / 2823.20 • ^{13}C / ^{15}N / ^{1}HN acquisition time (ms): 19.9 / 46.7 / 67.9 • Temp: 25 °C • Buffer in 10% D_2O: 10 mM Tris, 50 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor • Aligned sample: 4.2mm new era NMR tubes (NE-UP5-GT-7) • Isotropic sample: shaped tube
3D IPAP-J-HNCO NMR for 5.16% stretched polyacrylamide gel RDC of free KaiB$_{\text{fs-nmr}}$ measures: ^{1}J(CA-CO)	1) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG (U-[^{15}N, ^{13}C] labeled) / 356	• ^{13}C / ^{15}N / ^{1}HN sweep width (Hz): 1500.000 / 1583.899 / 7211.539 • ^{13}C / ^{15}N / ^{1}HN carrier (Hz): 26187.80 / 7278.86 / 2823.20 • ^{13}C / ^{15}N / ^{1}HN acquisition time (ms): 50.6 / 23.3 / 67.9 • Temp: 25 °C • Buffer in 10% D_2O: 10 mM Tris, 50 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor • Aligned sample: 4.2mm new era NMR tubes (NE-UP5-GT-7) • Isotropic sample: shaped tube
3D HNCACB and HN(CO)CACB NMR for free CikA$_{\text{PsR}}$	1) [CikA$_{\text{PsR}}$]-CikA_613-729 (U-[^{15}N, ^{13}C] labeled) / 1500	• Volume: 350 µL • ^{13}C / ^{15}N / ^{1}HN sweep width (Hz): 11337.012 / 1583.899 / 9615.385 • ^{13}C / ^{15}N / ^{1}HN carrier (Hz): 6537.93 / 7278.86 / 2823.00 • ^{13}C / ^{15}N / ^{1}HN acquisition time (ms): 6.3 / 23.3 / 68.8 • Temp: 25 °C • Buffer in 5% D_2O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor • Shaped tube

3D HN(CA)CO and HNCO NMR for free CikA$_{\mathrm{PsR}}$	1) [CikA$_{\mathrm{PsR}}$]-CikA_613-729 (U-[^{15}N, ^{13}C] labeled) / 1500	• Volume: 350 µL • ^{13}C / ^{15}N / ^{1}HN sweep width (Hz): 1500.004 / 1583.899 / 9615.385 • ^{13}C / ^{15}N / ^{1}HN carrier (Hz): 26225.41 / 7278.86 / 2823.00 • ^{13}C / ^{15}N / ^{1}HN acquisition time (ms): 29.9 / 23.9 / 68.8 • Temp: 25 °C • Buffer in 5% D$_2$O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN$_3$, 10 µM DSS, pH 7, 1x protease inhibitor • Shaped tube
3D HBHA(CO)NH NMR for free CikA$_{\mathrm{PsR}}$	1) [CikA$_{\mathrm{PsR}}$]-CikA_613-729 (U-[^{15}N, ^{13}C] labeled) / 1500	• Volume: 350 µL • ^{1}H / ^{15}N / ^{1}HN sweep width (Hz): 4000.000 / 1583.899 / 9615.385 • ^{1}H / ^{15}N / ^{1}HN carrier (Hz): 2823.00 / 7278.86 / 2823.00 • ^{1}H / ^{15}N / ^{1}HN acquisition time (ms): 13.0 / 23.9 / 68.8 • Temp: 25 °C • Buffer in 5% D$_2$O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN$_3$, 10 µM DSS, pH 7, 1x protease inhibitor • Shaped tube
3D HCC(CO)NH NMR for free CikA$_{\mathrm{PsR}}$ Proton version	1) [CikA$_{\mathrm{PsR}}$]-CikA_613-729 (U-[^{15}N, ^{13}C] labeled) / 1500	• Volume: 350 µL • ^{1}H / ^{15}N / ^{1}HN sweep width (Hz): 4000.000 / 1583.899 / 9615.385 • ^{1}H / ^{15}N / ^{1}HN carrier (Hz): 2823.00 / 7278.86 / 2823.00 • ^{1}H / ^{15}N / ^{1}HN acquisition time (ms): 13.0 / 23.9 / 68.8 • Mixing time (ms): 120 • Temp: 25 °C • Buffer in 5% D$_2$O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN$_3$, 10 µM DSS, pH 7, 1x protease inhibitor • Shaped tube
3D HCC(CO)NH NMR for free CikA$_{\mathrm{PsR}}$ Carbon version	1) [CikA$_{\mathrm{PsR}}$]-CikA_613-729 (U-[^{15}N, ^{13}C] labeled) / 1500	• Volume: 350 µL • ^{13}C / ^{15}N / ^{1}HN sweep width (Hz): 11337.012 / 1583.899 / 9615.385 • ^{13}C / ^{15}N / ^{1}HN carrier (Hz): 6537.93 / 7278.86 / 2828.00 • ^{13}C / ^{15}N / ^{1}HN acquisition time (ms): 6.2 / 23.9 / 68.8 • Mixing time (ms): 120 • Temp: 25 °C • Buffer in 5% D$_2$O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN$_3$, 10 µM DSS, pH 7, 1x protease inhibitor • Shaped tube

3D ^{15}N-NOESY-HSQC NMR for free CikA$_{PsR}$	1) [CikA$_{PsR}$]-CikA_613-729 (U-[^{15}N, ^{13}C] labeled) / 1500	• Volume: 350 µL • ^{1}H / ^{15}N / ^{1}HN sweep width (Hz): 5530.422 / 1583.899 / 9615.385 • ^{1}H / ^{15}N / ^{1}HN carrier (Hz): 2823.00 / 7278.86 / 2823.00 • ^{1}H / ^{15}N / ^{1}HN acquisition time (ms): 23.1 / 21.4 / 68.8 • Mixing time (ms): 180 • Temp: 25 °C • Buffer in 5% D$_2$O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN$_3$, 10 µM DSS, pH 7, 1x protease inhibitor • Shaped tube
3D ^{13}C-NOESY-HSQC NMR for free CikA$_{PsR}$	1) [CikA$_{PsR}$]-CikA_613-729 (U-[^{15}N, ^{13}C] labeled) / 1500	• Volume: 350 µL • ^{1}H / ^{13}C / ^{1}H sweep width (Hz): 4027.582 / 4534.808 / 6009.615 • ^{1}H / ^{13}C / ^{1}H carrier (Hz): 1643.56 / 6011.05 / 1643.56 • ^{1}H / ^{13}C / ^{1}H acquisition time (ms): 32.2 / 10.1 / 63.9 • Mixing time (ms): 150 • Temp: 25 °C • Buffer in 99.96% D$_2$O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN$_3$, 10 µM DSS, pH 7, 1x protease inhibitor • Shaped tube
3D ^{13}C-HCCH-COSY NMR for free CikA$_{PsR}$ Proton version	1) [CikA$_{PsR}$]-CikA_613-729 (U-[^{15}N, ^{13}C] labeled) / 1500	• Volume: 350 µL • ^{1}H / ^{13}C / ^{1}H sweep width (Hz): 4027.582 / 4534.808 / 6009.615 • ^{1}H / ^{13}C / ^{1}H carrier (Hz): 1643.56 / 6011.05 / 1643.56 • ^{1}H / ^{13}C / ^{1}H acquisition time (ms): 32.2 / 10.1 / 63.9 • Temp: 25 °C • Buffer in 99.96% D$_2$O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN$_3$, 10 µM DSS, pH 7, 1x protease inhibitor • Shaped tube
3D ^{13}C-HCCH-COSY NMR for free CikA$_{PsR}$ Carbon version	1) [CikA$_{PsR}$]-CikA_613-729 (U-[^{15}N, ^{13}C] labeled) / 1500	• Volume: 350 µL • ^{13}C / ^{13}C / ^{1}H sweep width (Hz): 4534.808 / 4534.808 / 6009.615 • ^{13}C / ^{13}C / ^{1}H carrier (Hz): 6011.05 / 6011.05 / 1643.56 • ^{13}C / ^{13}C / ^{1}H acquisition time (ms): 10.1 / 10.1 / 63.9 • Temp: 25 °C • Buffer in 99.96% D$_2$O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN$_3$, 10 µM DSS, pH 7, 1x protease inhibitor • Shaped tube

3D IPAP-J-HNCO(CA) NMR for 5.16% stretched polyacrylamide gel RDC of free CikA$_{PsR}$ measures: ^{1}J(CA-HA)	1) [CikA$_{PsR}$]-CikA_613-729 (U-[^{15}N, ^{13}C] labeled) / 450	- ^{13}C / ^{15}N / ^{1}HN sweep width (Hz): 1500.004 / 1583.899 / 9615.385 - ^{13}C / ^{15}N / ^{1}HN carrier (Hz): 26187.80 / 7278.86 / 2823.20 - ^{13}C / ^{15}N / ^{1}HN acquisition time (ms): 50.6 / 23.3 / 68.9 - Temp: 25 °C - Buffer in 10% D_2O: 10 mM Tris, 50 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor - Aligned sample: 4.2mm new era NMR tubes (NE-UP5-GT-7) - Isotropic sample: shaped tube
3D IPAP-J-HNCO NMR for 5.16% stretched polyacrylamide gel RDC of free CikA$_{PsR}$ measures: ^{1}J(N-H)	1) [CikA$_{PsR}$]-CikA_613-729 (U-[^{15}N, ^{13}C] labeled) / 450	- ^{13}C / ^{15}N / ^{1}HN sweep width (Hz): 1500.004 / 1583.899 / 9615.385 - ^{13}C / ^{15}N / ^{1}HN carrier (Hz): 26187.80 / 7278.86 / 2823.20 - ^{13}C / ^{15}N / ^{1}HN acquisition time (ms): 19.9 / 46.7 / 68.8 - Temp: 25 °C - Buffer in 10% D_2O: 10 mM Tris, 50 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor - Aligned sample: 4.2mm new era NMR tubes (NE-UP5-GT-7) - Isotropic sample: shaped tube
3D IPAP-J-HNCO NMR for 5.16%, stretched polyacrylamide gel RDC of free CikA$_{PsR}$ measures: ^{1}J(CA-CO)	1) [CikA$_{PsR}$]-CikA_613-729 (U-[^{15}N, ^{13}C] labeled) / 450	- ^{13}C / ^{15}N / ^{1}HN sweep width (Hz): 1500.004 / 1583.899 / 9615.385 - ^{13}C / ^{15}N / ^{1}HN carrier (Hz): 26187.80 / 7278.86 / 2823.20 - ^{13}C / ^{15}N / ^{1}HN acquisition time (ms): 50.6 / 23.3 / 68.8 - Temp: 25 °C - Buffer in 10% D_2O: 10 mM Tris, 50 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor - Aligned sample: 4.2mm new era NMR tubes (NE-UP5-GT-7) - Isotropic sample: shaped tube
3D HNCACB and HN(CO)CACB NMR for labeled KaiB$_{fs\text{-}nmr}$ + 1.1x unlabeled CikA$_{PsR}$	1) [KaiB$_{fs\text{-}nmr}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG (U-[^{15}N, ^{13}C] labeled) / 800 2) [CikA$_{PsR}$]-CikA_613-729 / 880	- Volume: 380 µL - ^{13}C / ^{15}N / ^{1}HN sweep width (Hz): 9500.000 / 1583.899 / 7211.539 - ^{13}C / ^{15}N / ^{1}HN carrier (Hz): 6159.72 / 7278.86 / 2828.00 - ^{13}C / ^{15}N / ^{1}HN acquisition time (ms): 7.0 / 23.9 / 62.8 - Temp: 50 °C - Buffer in 5% D_2O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor - Shaped tube

3D HN(CA)CO and HNCO NMR for labeled KaiB$_{\text{fs-nmr}}$ + 1.1x unlabeled CikA$_{\text{PsR}}$	1) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG (U-[^{15}N, ^{13}C] labeled) / 800 2) [CikA$_{\text{PsR}}$]-CikA_613-729 / 880	- Volume: 380 µL - ^{13}C / ^{15}N / ^{1}HN sweep width (Hz): 1500.000 / 1583.899 / 7211.539 - ^{13}C / ^{15}N / ^{1}HN carrier (Hz): 25809.72 / 7278.86 / 2828.00 - ^{13}C / ^{15}N / ^{1}HN acquisition time (ms): 30.0 / 23.3 / 62.8 - Temp: 50 °C - Buffer in 5% D_2O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor - Shaped tube
3D HBHA(CO)NH NMR for labeled KaiB$_{\text{fs-nmr}}$ + 1.1x unlabeled CikA$_{\text{PsR}}$	1) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG (U-[^{15}N, ^{13}C] labeled) / 800 2) [CikA$_{\text{PsR}}$]-CikA_613-729 / 880	- Volume: 380 µL - ^{1}H / ^{15}N / ^{1}HN sweep width (Hz): 4000.000 / 1583.899 / 7211.539 - ^{1}H / ^{15}N / ^{1}HN carrier (Hz): 2828.00 / 7278.86 / 2828.00 - ^{1}H / ^{15}N / ^{1}HN acquisition time (ms): 13.0 / 23.9 / 62.8 - Temp: 50 °C - Buffer in 5% D_2O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor - Shaped tube
3D ^{15}N-edited-NOESY-HSQC NMR for labeled KaiB$_{\text{fs-nmr}}$ + 1.1x unlabeled CikA$_{\text{PsR}}$ For intramolecular NOE of labeled KaiB$_{\text{fs-nmr}}$ in complex	1) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG (U-[^{15}N, ^{13}C] labeled) / 800 2) [CikA$_{\text{PsR}}$]-CikA_613-729 / 880	- Volume: 380 µL - ^{1}H / ^{15}N / ^{1}HN sweep width (Hz): 5590.535 / 1583.899 / 9615.385 - ^{1}H / ^{15}N / ^{1}HN carrier (Hz): 2828.00 / 7278.86 / 2828.00 - ^{1}H / ^{15}N / ^{1}HN acquisition time (ms): 20.0 / 21.4 / 67.8 - Mixing time (ms): 120 - Temp: 50 °C - Buffer in 5% D_2O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor - Shaped tube
3D ^{13}C-edited-NOESY-HSQC NMR for labeled KaiB$_{\text{fs-nmr}}$ + 1.1x unlabeled CikA$_{\text{PsR}}$ For intramolecular NOE of labeled KaiB$_{\text{fs-nmr}}$ in complex	1) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG (U-[^{15}N, ^{13}C] labeled) / 800 2) [CikA$_{\text{PsR}}$]-CikA_613-729 / 880	- Volume: 380 µL - ^{1}H / ^{13}C / ^{1}H sweep width (Hz): 3727.018 / 3023.204 / 7211.539 - ^{1}H / ^{13}C / ^{1}H carrier (Hz): 1918.60 / 5944.17 / 1918.60 - ^{1}H / ^{13}C / ^{1}H acquisition time (ms): 19.0 / 10.6 / 59.9 - Mixing time (ms): 120 - Temp: 50 °C - Buffer in 99.96% D_2O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor - Shaped tube

3D ^{13}C-HCCH-COSY NMR for labeled KaiB$_{\text{fs-nmr}}$ + 1.1x unlabeled CikA$_{\text{PsR}}$ Proton version	1) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG (U-[^{15}N, ^{13}C] labeled) / 800 2) [CikA$_{\text{PsR}}$]-CikA_613-729 / 880	• Volume: 380 µL • ^{1}H / ^{13}C / ^{1}H sweep width (Hz): 4809.062 / 3023.204 / 7211.539 • ^{1}H / ^{13}C / ^{1}H carrier (Hz): 1918.60 / 5944.17 / 1918.60 • ^{1}H / ^{13}C / ^{1}H acquisition time (ms): 12.9 / 10.6 / 59.9 • Temp: 50 °C • Buffer in 99.96% D$_2$O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN$_3$, 10 µM DSS, pH 7, 1x protease inhibitor • Shaped tube
3D ^{13}C-HCCH-COSY NMR for labeled KaiB$_{\text{fs-nmr}}$ + 1.1x unlabeled CikA$_{\text{PsR}}$ Carbon version	1) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG (U-[^{15}N, ^{13}C] labeled) / 800 2) [CikA$_{\text{PsR}}$]-CikA_613-729 / 880	• Volume: 380 µL • ^{13}C / ^{13}C / ^{1}H sweep width (Hz): 3023.204 / 3023.204 / 7211.539 • ^{13}C / ^{13}C / ^{1}H carrier (Hz): 5944.17 / 5944.17 / 1918.60 • ^{13}C / ^{13}C / ^{1}H acquisition time (ms): 10.6 / 10.6 / 59.9 • Temp: 50 °C • Buffer in 99.96% D$_2$O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN$_3$, 10 µM DSS, pH 7, 1x protease inhibitor • Shaped tube
3D ^{13}C-HCCH-TOCSY NMR for labeled KaiB$_{\text{fs-nmr}}$ + 1.1x unlabeled CikA$_{\text{PsR}}$ Carbon version	1) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG (U-[^{15}N, ^{13}C] labeled) / 800 2) [CikA$_{\text{PsR}}$]-CikA_613-729 / 880	• Volume: 380 µL • ^{13}C / ^{13}C / ^{1}H sweep width (Hz): 3023.204 / 3023.204 / 7211.539 • ^{13}C / ^{13}C / ^{1}H carrier (Hz): 5944.17 / 5944.17 / 1918.60 • ^{13}C / ^{13}C / ^{1}H acquisition time (ms): 10.6 / 10.6 / 59.9 • Temp: 50 °C • Buffer in 99.96% D$_2$O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN$_3$, 10 µM DSS, pH 7, 1x protease inhibitor • Shaped tube
3D HCAN and HCA(CO)N NMR for labeled KaiB$_{\text{fs-nmr}}$ + 1.1x unlabeled CikA$_{\text{PsR}}$	1) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG (U-[^{15}N, ^{13}C] labeled) / 800 2) [CikA$_{\text{PsR}}$]-CikA_613-729 / 880	• Volume: 380 µL • ^{15}N / ^{13}C / ^{1}H sweep width (Hz): 1583.899 / 3023.247 / 7211.539 • ^{15}N / ^{13}C / ^{1}H carrier (Hz): 7278.98 / 8086.90 / 2850.40 • ^{15}N / ^{13}C / ^{1}H acquisition time (ms): 23.9 / 10.6 / 59.9 • Temp: 50 °C • Buffer in 99.96% D$_2$O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN$_3$, 10 µM DSS, pH 7, 1x protease inhibitor • Shaped tube

3D IPAP-J-HNCO(CA) NMR for 5.5% stretched polyacrylamide gel RDC of labeled KaiB$_{\text{fs-nmr}}$ + 1.1x unlabeled CikA$_{\text{PsR}}$ measures: ^{1}J(CA-HA)	1) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG (U-[^{15}N, ^{13}C] labeled) / 700 2) [CikA$_{\text{PsR}}$]-CikA_613-729 / 770	• ^{13}C / ^{15}N / ^{1}HN sweep width (Hz): 1500.000 / 1583.899 / 7211.539 • ^{13}C / ^{15}N / ^{1}HN carrier (Hz): 26187.80 / 7278.86 / 2823.20 • ^{13}C / ^{15}N / ^{1}HN acquisition time (ms): 50.6 / 23.3 / 67.9 • Temp: 50 °C • Buffer in 10% D_2O: 10 mM Tris, 50 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor • Aligned sample: 4.2mm new era NMR tubes (NE-UP5-GT-7) • Isotropic sample: shaped tube
3D IPAP-J-HNCO NMR for 5.5% stretched polyacrylamide gel RDC of labeled KaiB$_{\text{fs-nmr}}$ + 1.1x unlabeled CikA$_{\text{PsR}}$ measures: ^{1}J(N-H)	1) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG (U-[^{15}N, ^{13}C] labeled) / 700 2) [CikA$_{\text{PsR}}$]-CikA_613-729 / 770	• ^{13}C / ^{15}N / ^{1}HN sweep width (Hz): 1500.004 / 1583.899 / 7211.539 • ^{13}C / ^{15}N / ^{1}HN carrier (Hz): 26187.80 / 7278.86 / 2823.20 • ^{13}C / ^{15}N / ^{1}HN acquisition time (ms): 19.9 / 46.7 / 67.9 • Temp: 50 °C • Buffer in 10% D_2O: 10 mM Tris, 50 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor • Aligned sample: 4.2mm new era NMR tubes (NE-UP5-GT-7) • Isotropic sample: shaped tube
3D IPAP-J-HNCO NMR for 5.5% stretched polyacrylamide gel RDC of labeled KaiB$_{\text{fs-nmr}}$ + 1.1x unlabeled CikA$_{\text{PsR}}$ measures: ^{1}J(CA-CO)	1) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG (U-[^{15}N, ^{13}C] labeled) / 700 2) [CikA$_{\text{PsR}}$]-CikA_613-729 / 770	• ^{13}C / ^{15}N / ^{1}HN sweep width (Hz): 1500.000 / 1583.899 / 7211.539 • ^{13}C / ^{15}N / ^{1}HN carrier (Hz): 26187.80 / 7278.86 / 2823.20 • ^{13}C / ^{15}N / ^{1}HN acquisition time (ms): 50.6 / 23.3 / 67.9 • Temp: 50 °C • Buffer in 10% D_2O: 10 mM Tris, 50 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor • Aligned sample: 4.2mm new era NMR tubes (NE-UP5-GT-7) • Isotropic sample: shaped tube
4D ^{15}N, ^{13}C-edited-NOESY-HSQC NMR for labeled KaiB$_{\text{fs-nmr}}$ + 1.1x unlabeled CikA$_{\text{PsR}}$ For intermolecular NOE	1) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG (U-[^{15}N] labeled) / 800 2) [CikA$_{\text{PsR}}$]-CikA_613-729 (U-[^{13}C] labeled) / 880	• Volume: 380 µL ^{13}C / ^{1}H / ^{15}N / ^{1}HN • ^{13}C / ^{1}H / ^{15}N / ^{1}HN sweep width (Hz): 3023.208 / 4809.062 / 1583.899 / 7211.539 • ^{13}C / ^{1}H / ^{15}N / ^{1}HN carrier (Hz): 6121.94 / 2828.00 / 7278.86 / 2828.00 • ^{13}C / ^{1}H / ^{15}N / ^{1}HN acquisition time (ms): 5.2 / 11.8 / 10.1 / 62.8 • Mixing time (ms): 180 • Temp: 50 °C • Buffer in 5% D_2O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor • Shaped tube

3D ^{13}C-edited, ^{12}C-filtered NOESY-HSQC NMR for labeled KaiB$_{\text{fs-nmr}}$ + 1.1x unlabeled CikA$_{\text{PsR}}$ For intermolecular NOE	1) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG (U-[^{15}N, ^{13}C] labeled) / 800 2) [CikA$_{\text{PsR}}$]-CikA_613-729 / 880	• Volume: 380 µL • ^{1}H / ^{13}C / ^{1}H sweep width (Hz): 3606.792 / 3023.204 / 7211.539 • ^{1}H / ^{13}C / ^{1}H carrier (Hz): 1918.60 / 5944.17 / 1918.60 • ^{1}H / ^{13}C / ^{1}H acquisition time (ms): 19.9 / 11.2 / 59.9 • Mixing time (ms): 150 • Temp: 50 °C • Buffer in 99.96% D_2O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor • Shaped tube
2D IPAP-J-HSQC NMR for 5.5% stretched polyacrylamide gel RDC of labeled KaiB$_{\text{fs-nmr}}$ + 1.1x unlabeled CikA$_{\text{PsR}}$ measures: ^{1}J(N-H)	1) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG (U-[^{15}N] labeled) / 400 2) [CikA$_{\text{PsR}}$]-CikA_613-729 / 440	• ^{15}N / ^{1}HN sweep width (Hz): 1583.899 / 7211.539 • ^{15}N / ^{1}HN carrier (Hz): 7278.86 / 2835.00 • ^{15}N / ^{1}HN acquisition time (ms): 202 / 62.6 • Temp: 50 °C • Buffer in 10% D_2O: 10 mM Tris, 50 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor • Aligned sample: 4.2mm new era NMR tubes (NE-UP5-GT-7) • Isotropic sample: shaped tube
3D HNCACB and HN(CO)CACB NMR for labeled CikA$_{\text{PsR}}$ + 1.1x unlabeled KaiB$_{\text{fs-nmr}}$	1) [CikA$_{\text{PsR}}$]-CikA_613-729 (U-[^{15}N, ^{13}C] labeled) / 800 2) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG / 880	• Volume: 380 µL • ^{13}C / ^{15}N / ^{1}HN sweep width (Hz): 9500.000 / 7211.539 / 1583.899 / • ^{13}C / ^{15}N / ^{1}HN carrier (Hz): 6575.41/ 2828.00 / 7278.86 / • ^{13}C / ^{15}N / ^{1}HN acquisition time (ms): 7.0 / 62.8 / 23.9 • Temp: 50 °C • Buffer in 5% D_2O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor • Shaped tube
3D HN(CA)CO and HNCO NMR for labeled CikA$_{\text{PsR}}$ + 1.1x unlabeled KaiB$_{\text{fs-nmr}}$	1) [CikA$_{\text{PsR}}$]-CikA_613-729 (U-[^{15}N, ^{13}C] labeled) / 800 2) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG / 880	• Volume: 380 µL • ^{13}C / ^{15}N / ^{1}HN sweep width (Hz): 1500.004 / 1583.899 / 7211.539 • ^{13}C / ^{15}N / ^{1}HN carrier (Hz): 26225.41 / 7278.86 / 2828.00 • ^{13}C / ^{15}N / ^{1}HN acquisition time (ms): 30.0 / 23.3 / 62.8 • Temp: 50 °C • Buffer in 5% D_2O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor • Shaped tube

3D HBHA(CO)NH NMR for labeled CikA$_{\text{PsR}}$ + 1.1x unlabeled KaiB$_{\text{fs-nmr}}$	1) [CikA$_{\text{PsR}}$]-CikA_613-729 (U-[^{15}N, ^{13}C] labeled) / 800 2) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG / 880	• Volume: 380 µL • ^{1}H / ^{15}N / ^{1}HN sweep width (Hz): 4000.000 / 1583.899 / 7211.539 • ^{1}H / ^{15}N / ^{1}HN carrier (Hz): 2828.00 / 7278.86 / 2828.00 • ^{1}H / ^{15}N / ^{1}HN acquisition time (ms): 13.0 / 23.9 / 62.8 • Temp: 50 °C • Buffer in 5% D_2O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor • Shaped tube
3D ^{15}N-edited-NOESY-HSQC NMR for labeled CikA$_{\text{PsR}}$ + 1.1x unlabeled KaiB$_{\text{fs-nmr}}$ For intramolecular NOE of labeled CikA$_{\text{PsR}}$ in complex	1) [CikA$_{\text{PsR}}$]-CikA_613-729 (U-[^{15}N, ^{13}C] labeled) / 800 2) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG / 880	• Volume: 380 µL • ^{1}H / ^{15}N / ^{1}HN sweep width (Hz): 5590.535 / 1583.899 / 9615.385 • ^{1}H / ^{15}N / ^{1}HN carrier (Hz): 2828.00 / 7278.86 / 2828.00 • ^{1}H / ^{15}N / ^{1}HN acquisition time (ms): 20.0 / 21.4 / 67.8 • Mixing time (ms): 120 • Temp: 50 °C • Buffer in 5% D_2O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor • Shaped tube
3D ^{13}C-edited-NOESY-HSQC NMR for labeled CikA$_{\text{PsR}}$ + 1.1x unlabeled KaiB$_{\text{fs-nmr}}$ For intramolecular NOE of labeled CikA$_{\text{PsR}}$ in complex	1) [CikA$_{\text{PsR}}$]-CikA_613-729 (U-[^{15}N, ^{13}C] labeled) / 800 2) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG / 880	• Volume: 380 µL • ^{1}H / ^{13}C / ^{1}H sweep width (Hz): 4027.584 / 3023.204 / 7211.539 • ^{1}H / ^{13}C / ^{1}H carrier (Hz): 1918.60 / 5944.17 / 1918.60 • ^{1}H / ^{13}C / ^{1}H acquisition time (ms): 19.1 / 10.6 / 59.9 • Mixing time (ms): 120 • Temp: 50 °C • Buffer in 99.96% D_2O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor • Shaped tube
3D ^{13}C-HCCH-COSY NMR for labeled CikA$_{\text{PsR}}$ + 1.1x unlabeled KaiB$_{\text{fs-nmr}}$ Proton version	1) [CikA$_{\text{PsR}}$]-CikA_613-729 (U-[^{15}N, ^{13}C] labeled) / 800 2) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG / 880	• Volume: 380 µL • ^{1}H / ^{13}C / ^{1}H sweep width (Hz): 4809.062 / 3023.204 / 7211.539 • ^{1}H / ^{13}C / ^{1}H carrier (Hz): 1918.60 / 5944.17 / 1918.60 • ^{1}H / ^{13}C / ^{1}H acquisition time (ms): 12.9 / 10.6 / 59.9 • Temp: 50 °C • Buffer in 99.96% D_2O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor • Shaped tube

3D ^{13}C-HCCH-COSY NMR for labeled CikA$_{\text{PsR}}$ + 1.1x unlabeled KaiB$_{\text{fs-nmr}}$ Carbon version	1) [CikA$_{\text{PsR}}$]-CikA_613-729 (U-[^{15}N, ^{13}C] labeled) / 800 2) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG / 880	• Volume: 380 µL • ^{13}C / ^{13}C / ^{1}H sweep width (Hz): 3023.204 / 3023.204 / 7211.539 • ^{13}C / ^{13}C / ^{1}H carrier (Hz): 5944.17 / 5944.17 / 1918.60 • ^{13}C / ^{13}C / ^{1}H acquisition time (ms): 10.6 / 10.6 / 59.9 • Temp: 50 °C • Buffer in 99.96% D$_2$O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN$_3$, 10 µM DSS, pH 7, 1x protease inhibitor • Shaped tube
3D ^{13}C-HCCH-TOCSY NMR for labeled CikA$_{\text{PsR}}$ + 1.1x unlabeled KaiB$_{\text{fs-nmr}}$ Carbon version	1) [CikA$_{\text{PsR}}$]-CikA_613-729 (U-[^{15}N, ^{13}C] labeled) / 800 2) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG / 880	• Volume: 380 µL • ^{13}C / ^{13}C / ^{1}H sweep width (Hz): 3023.204 / 3023.204 / 7211.539 • ^{13}C / ^{13}C / ^{1}H carrier (Hz): 5944.17 / 5944.17 / 1918.60 • ^{13}C / ^{13}C / ^{1}H acquisition time (ms): 10.6 / 10.6 / 59.9 • Temp: 50 °C • Buffer in 99.96% D$_2$O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN$_3$, 10 µM DSS, pH 7, 1x protease inhibitor • Shaped tube
3D IPAP-J-HNCO(CA) NMR for 5.5% stretched polyacrylamide gel RDC of labeled CikA$_{\text{PsR}}$ + 1.1x unlabeled KaiB$_{\text{fs-nmr}}$ measures:^{1}J(CA-HA)	1) [CikA$_{\text{PsR}}$]-CikA_613-729 (U-[^{15}N, ^{13}C] labeled) / 800 2) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG / 880	• ^{13}C / ^{15}N / ^{1}HN sweep width (Hz): 1500.000 / 1583.899 / 7211.539 • ^{13}C / ^{15}N / ^{1}HN carrier (Hz): 26187.80 / 7278.86 / 2823.20 • ^{13}C / ^{15}N / ^{1}HN acquisition time (ms): 50.6 / 23.3 / 67.9 • Temp: 50 °C • Buffer in 10% D$_2$O: 10 mM Tris, 50 mM NaCl, 5 mM TCEP, 0.02% NaN$_3$, 10 µM DSS, pH 7, 1x protease inhibitor • Aligned sample: 4.2mm new era NMR tubes (NE-UP5-GT-7) • Isotropic sample: shaped tube
3D IPAP-J-HNCO NMR for 5.5% stretched polyacrylamide gel RDC of labeled CikA$_{\text{PsR}}$ + 1.1x unlabeled KaiB$_{\text{fs-nmr}}$ measures:^{1}J(N-H)	1) [CikA$_{\text{PsR}}$]-CikA_613-729 (U-[^{15}N, ^{13}C] labeled) / 800 2) [KaiB$_{\text{fs-nmr}}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG / 880	• ^{13}C / ^{15}N / ^{1}HN sweep width (Hz): 1500.004 / 1583.899 / 7211.539 • ^{13}C / ^{15}N / ^{1}HN carrier (Hz): 26187.80 / 7278.86 / 2823.20 • ^{13}C / ^{15}N / ^{1}HN acquisition time (ms): 19.9 / 46.7 / 67.9 • Temp: 50 °C • Buffer in 10% D$_2$O: 10 mM Tris, 50 mM NaCl, 5 mM TCEP, 0.02% NaN$_3$, 10 µM DSS, pH 7, 1x protease inhibitor • Aligned sample: 4.2mm new era NMR tubes (NE-UP5-GT-7) • Isotropic sample: shaped tube

3D IPAP-J-HNCO NMR for 5.5% stretched polyacrylamide gel RDC of labeled CikA$_{PsR}$ + 1.1x unlabeled KaiB$_{fs\text{-}nmr}$ measures: ^{1}J(CA-CO)	1) [CikA$_{PsR}$]-CikA_613-729 (U-[^{15}N, ^{13}C] labeled) / 800 2) [KaiB$_{fs\text{-}nmr}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG / 880	• ^{13}C / ^{15}N / ^{1}HN sweep width (Hz): 1500.000 / 1583.899 / 7211.539 • ^{13}C / ^{15}N / ^{1}HN carrier (Hz): 26187.80 / 7278.86 / 2823.20 • ^{13}C / ^{15}N / ^{1}HN acquisition time (ms): 50.6 / 23.3 / 67.9 • Temp: 50 °C • Buffer in 10% D_2O: 10 mM Tris, 50 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor • Aligned sample: 4.2mm new era NMR tubes (NE-UP5-GT-7) • Isotropic sample: shaped tube
4D ^{15}N, ^{13}C-edited-NOESY-HSQC NMR for labeled CikA$_{PsR}$ + 1.1x unlabeled KaiB$_{fs\text{-}nmr}$ For intermolecular NOE	1) [CikA$_{PsR}$]-CikA_613-729 (U-[^{15}N] labeled) / 800 2) [KaiB$_{fs\text{-}nmr}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG (U-[^{13}C] labeled) / 880	• Volume: 380 µL • ^{13}C / ^{1}H / ^{15}N / ^{1}HN sweep width (Hz): 3023.208 / 4809.062 / 1583.899 / 7211.539 • ^{13}C / ^{1}H / ^{15}N / ^{1}HN carrier (Hz): 6121.94 / 2828.00 / 7278.86 / 2828.00 • ^{13}C / ^{1}H / ^{15}N / ^{1}HN acquisition time (ms): 5.2 / 11.8 / 10.1 / 62.8 • Mixing time (ms): 150 • Temp: 50 °C • Buffer in 5% D_2O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor • Shaped tube
3D ^{13}C-edited, ^{12}C-filtered, NOESY-HSQC NMR for labeled CikA$_{PsR}$ + 1.1x unlabeled KaiB$_{fs\text{-}nmr}$ For intermolecular NOE	1) [CikA$_{PsR}$]-CikA_613-729 (U-[^{15}N, ^{13}C] labeled) / 800 2) [KaiB$_{fs\text{-}nmr}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG / 880	• Volume: 380 µL • ^{1}H / ^{13}C / ^{1}H sweep width (Hz): 3606.792 / 3023.204 / 7211.539 • ^{1}H / ^{13}C / ^{1}H carrier (Hz): 1918.60 / 5944.17 / 1918.60 • ^{1}H / ^{13}C / ^{1}H acquisition time (ms): 19.9 / 11.2 / 59.9 • Mixing time (ms): 150 • Temp: 50 °C • Buffer in 99.96% D_2O: 20 mM Tris, 100 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor • Shaped tube
2D IPAP-J-HSQC NMR for 5.5% stretched polyacrylamide gel RDC of labeled CikA$_{PsR}$ + 1.1x unlabeled KaiB$_{fs\text{-}nmr}$ measures: ^{1}J(N-H)	1) [CikA$_{PsR}$]-CikA_613-729 (U-[^{15}N] labeled) / 400 2) [KaiB$_{fs\text{-}nmr}$]-KaiB_Y8A_Y94A_G89A_D91R_N29A_1-99_FLAG / 440	• ^{15}N / ^{1}HN sweep width (Hz): 1583.901 / 7211.539 • ^{15}N / ^{1}HN carrier (Hz): 7278.86 / 2835.00 • ^{15}N / ^{1}HN acquisition time (ms): 202 / 62.6 • Temp: 50 °C • Buffer in 10% D_2O: 10 mM Tris, 50 mM NaCl, 5 mM TCEP, 0.02% NaN_3, 10 µM DSS, pH 7, 1x protease inhibitor • Aligned sample: 4.2mm new era NMR tubes (NE-UP5-GT-7) • Isotropic sample: shaped tube

Table S2. Crystal structures and refinement statistics (molecular replacement).

	$\text{KaiB}_{\text{fs-cryst}}$:$\text{CI}_{\text{cryst}}$	**$\text{KaiA}_{\text{cryst}}$:$\text{KaiB}_{\text{fs-cryst}}$:$\text{CI}_{\text{cryst}}$**	**$\text{KaiB}_{\text{fs-cryst*}}$:$\text{KaiC}_{\text{S431E}}$**
Data collection			
Space group	$P22_12_1$	P1	$P2_13$
Cell dimensions			
a, *b*, *c* (Å)	42.5, 56.6, 146.1	74.6, 79.1, 80.8	155.9, 155.9, 155.9
α, β, γ (°)	90.0, 90.0, 90.0	107.3, 90.7, 111.2	90.0, 90.0, 90.0
Resolution (Å)^a	73.07-1.9 (1.9-1.80)	50.0-2.60 (2.64-2.60)	90.02-3.87 (4.08-3.87)
No. total reflections	109956	95617	100649
No. unique reflections	32797	47380	11991
R_{merge}	10.3 (44.7)	6.2 (58.1)	27.2 (105.9)
R_{pim}	6.5 (26.8)	5.9 (54.8)	9.6 (38.1)
I/σI	6.5 (3.1)	21.3 (2.5)	7.2 (2.5)
Completeness (%)	97.8 (99.3)	96.4 (97.4)	99.7 (99.9)
$CC_{1/2}$	0.987 (0.701)	0.997 (0.557)	0.987 (0.479)
Wilson B-factor	15.9	48.5	96.1
Redundancy	3.4 (3.6)	2.0 (2.1)	8.4 (8.3)
Refinement			
Resolution (Å)	1.80	2.60	3.87
$R_{\text{work}}/R_{\text{free}}$	21.9/25.7	18.5/24.2	32.7/34.5
No. atoms			
Protein	2382	9412	7303
Ligand/ion	27	10	54
Water	262	106	N/A
B-factors			
Protein	24	68	96
Ligand/ion	43	59	87
Water	34	53	N/A
RMS deviations			
Bond lengths (Å)	0.014	0.004	0.006
Bond angles (°)	1.54	0.92	1.49
Ramachandran^b			
Favored region (%)	97	98	84
Outliers (%)	0.3	0.2	4.6

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[a] Highest resolution shell is shown in parenthesis, [b] Values obtained from MolProbity *(81)*.

One crystal was used for data collection. Data were collected at a wavelength of 1 Å
Please see fig. S1 and table S1 for more details.

Table S3. NMR structures and refinement statistics.

	KaiB$_{\text{fs-nmr}}$	**CikA$_{\text{PsR}}$**	**CikA$_{\text{PsR}}$: KaiB$_{\text{fs-nmr}}$**
Total residue number	106	117	117:106
NMR constraints			
Distance restraints			
Total NOE	2298	2766	2671
Intra-residue	711	961	1111
Inter-residue			
Sequential (|i-j| = 1)	530	628	531
Medium-range (|i-j| ≤ 4)	466	494	346
Long-range (|i-j ≥ 5)	591	683	508
Intermolecular	N/A	N/A	175
Total dihedral angle restraints			
phi	90	98	188
psi	90	98	188
Total RDCs	145	165	188
HN−N	48	57	109
CO−CA	51	58	68
CA−HA	46	50	11
Q (%)[¶]	30.4	41.9	32.5
Structure statistics			
Violations (mean ± SD)			
Distance constraints (Å)	0.120 ± 0.094	0.099 ± 0.088	0.103 ± 0.088
Dihedral angle constraints (°)	1.205 ± 1.064	1.061 ± 0.865	1.176 ± 0.918
Max. dihedral angle violation (°)	4.193	3.877	4.407
Max. distance restrant violation (Å)	0.450	0.451	0.480
Deviations from idealized geometry			
Bond lengths (Å)	0.003	0.003	0.003
Bond angles (°)	0.528 ± 0.005	0.441 ± 0.008	0.445 ± 0.008
Impropers (°)	0.467 ± 0.011	0.440 ± 0.015	0.430 ± 0.013
Average pairwise r.m.s.d. [§]* (Å)			
Heavy atoms	1.06 ± 0.25	1.30 ± 0.32	1.64 ± 0.16
Backbone atoms	0.21 ± 0.07	0.49 ± 0.15	0.81 ± 0.18
Ramachandran Plot Summary from Richardson Lab's Molprobity*			
Most favored regions (%)	98.5 ± 0.8	96.7 ± 0.8	97.7 ± 0.8
Allowed regions (%)	1.2 ± 0.8	2.6 ± 0.9	2.2 ± 0.7
Disallowed regions (%)	0.1 ± 0.2	0.7 ± 0.6	0.1 ± 0.3
Favored rotamers (%)	88.7 ± 1.9	92.4 ± 1.8	90.8 ± 1.7
Poor rotamers (%)	6.6 ± 1.5	4.3 ± 1.5	4.8 ± 1.3

[¶] Q (%) is calculated using the average structure as input to "DC: Servers for Dipolar Coupling Calculations." (http://spin.niddk.nih.gov/bax/nmrserver/dc/fit.html)

[§] Non-flexible regions are used for r.m.s.d. calculation. KaiB$_{\text{fs-nmr}}$ (residues 9-98), CikA$_{\text{PsR}}$ (residues 615-692 and 702-724), CikA$_{\text{PsR}}$:KaiB$_{\text{fs-nmr}}$ complex (residues 615-692 and 702-724:9-98).

*Pairwise r.m.s.d. and Ramachandran statistics were calculated among 20 lowest energy structures. Please see fig. S1 and table S1 for more details.

Table S4. HDX-MS deuterium uptake profile of KaiA$_{\Delta N}$ free and bound to KaiB$_{fs\text{-}cryst}$-CI$_{cryst}$ complex.

Peptide residues[♯]		Deuterium uptake					
		Free KaiA$_{\Delta N}$			KaiA$_{\Delta N}$ bound to KaiB$_{fs\text{-}cryst}$-KaiC CI$_{cryst}$		
Start	End	10 sec	100 sec	1000 sec	10 sec	100 sec	1000 sec
139	159	13.81789826	17.26475761	17.52098749	10.07688224	13.49029432	15.76590238
139	159	13.72123783	17.33740953	17.55168455	9.768155727	13.3996007	16.07466933
139	161	14.46877912	18.89943235	19.16631365	9.525967974	14.14513259	17.85478268
169	176	3.583107249	4.374315294	5.316333044	2.434258241	2.971906259	4.448038687
169	176	3.560830754	4.378620832	5.327276714	2.541486362	3.314250173	4.59356447
170	176	2.527111016	3.384804972	4.375910844	1.871624518	2.504393485	3.795756537
170	176	2.495332819	3.407050239	4.320319925	1.921679843	2.533827084	3.882738617
177	193	1.18859629	2.250459413	3.841352442	0.815642146	1.490605433	2.311517741
177	193	1.358769705	2.256433321	3.876451723	0.840732463	1.666519402	2.458983776
177	195	1.210955638	2.308848535	3.910844761	0.768696062	1.456431279	2.742425378
177	199	0.952649027	2.026021142	3.637017577	0.654907112	1.35516357	2.61368612
178	193	0.879760032	1.684146029	3.282693785	0.559242053	1.080678202	2.023210301
178	193	0.927714741	1.607021929	3.236949336	0.59916912	1.076856286	1.972317535
178	199	0.653544812	1.508599274	3.097681381	0.28138735	0.999076374	2.09618447
200	203	0.026178261	0.064849558	0.228282583	0.021444807	0.033204862	0.134418935
200	204	0.071906909	0.128000265	0.277682005	0.038191221	0.095378597	0.255602705
200	213	8.062206349	9.477543161	9.743145523	7.389108591	9.246800976	9.46712695
202	213	8.247799402	9.384034564	9.448235484	7.572680287	9.235645643	9.381813777
203	209	4.266043241	5.22335405	5.226640485	3.592314015	4.935225754	5.167491488
203	213	8.754071336	9.671695474	9.700919173	8.118793086	9.759141772	9.909756219
203	217	9.288707549	11.28996709	11.93246839	8.454756612	11.09912345	11.88703629
204	209	3.968989509	4.28708	4.255622193	3.66884202	4.084756287	4.117741505
204	213	8.0191361	8.255935389	8.359103108	7.334408029	8.230884241	8.125740565
204	213	8.102489565	8.415070884	8.457484967	7.444998527	8.296461418	8.25157506
205	213	6.779704621	6.84186355	6.917860191	6.582768849	6.724794736	6.828307894
205	213	7.032709173	7.119182719	7.167977337	6.661600253	7.040856275	7.054748831
205	217	8.058015029	8.833450142	9.410797025	7.881984192	8.834520889	9.411867772
206	217	6.817715763	7.698377318	8.346558488	6.736804565	7.650677603	8.355920114
208	217	4.645576536	5.494450533	6.426245816	4.511013482	5.393458354	6.295066287
209	217	3.58480328	4.502641267	5.225919419	3.445186415	4.395492103	5.213043954
218	223	0.06865422	0.066046158	0.059849	0.035798016	0.036699729	0.043372408
218	224	0.117647059	0.106592973	0.1091591	0.014607185	0.051125148	0.035333597
223	233	1.175263343	2.307354431	4.27058547	1.096543802	1.803900843	3.621974311
224	233	1.393951613	2.709879032	4.840725806	1.227721774	1.739112903	3.804737903
224	233	1.323468418	2.600398867	4.869440278	1.11104705	1.803872904	3.804318407
224	235	1.405256284	2.619512496	4.902343373	1.278732922	1.881205553	3.822845746
225	231	1.090777852	2.110291538	4.067263622	0.965791905	1.546623165	3.204907817
225	233	1.158705701	2.295497346	4.417907892	1.071819894	1.654939223	3.551189865

225	233	1.163073435	2.264581485	4.405859302	0.994880033	1.601118276	3.39212305
233	239	0.097222222	0.087896825	0.099404762	0.001785714	0.021428571	0.007539683
234	239	0.050078379	0.051221984	0.047322007	0.085384144	0.091900281	0.123623529
240	249	2.990044482	6.958695192	7.860622749	2.094895149	6.165431053	7.267951705
240	255	7.383823229	12.93016436	13.83601582	6.089852008	12.3310373	13.46937871
240	255	7.437679671	12.95107803	13.8835729	6.215554415	12.49075975	13.50734086
240	258	8.487508125	15.5687219	17.34810483	7.077154836	14.76018663	16.71470227
243	249	2.154279838	4.998532289	5.130563895	1.288166102	4.614516145	4.992964281
243	251	3.628518972	7.538963688	7.632190942	2.607915137	7.050999592	7.461852305
243	253	5.251436846	9.232969481	9.375116953	4.456293161	9.144553353	9.351459122
243	255	6.665271705	10.9338122	11.01053508	5.626420987	10.72998126	10.99816781
243	255	6.65070922	10.91206296	10.93772552	5.70461615	10.44192485	10.79401518
243	257	7.684557191	13.35370667	13.53418051	6.461160216	12.91344212	13.43406461
243	257	7.711561971	13.35716551	13.55944439	6.360974471	13.06103839	13.58205099
243	258	7.452642284	14.1811551	14.45755164	5.978609251	13.67107339	14.81397061
244	255	6.105401679	9.695241676	9.843965409	5.006893094	9.480511342	9.74641768
244	255	5.901445155	9.368633025	9.58747927	4.633084577	9.111288794	9.511075575
247	255	5.330779376	6.86459838	6.930980343	4.922156629	6.81909816	6.903724173
256	264	0.618307198	2.279949258	4.526552969	0.573841981	2.302194032	4.512009921
258	264	0.190712076	1.12368248	2.93415286	0.216418758	1.163199611	2.938741805
258	264	0.191640001	1.02242832	2.812269723	0.218737484	1.053686764	2.780585026
263	271	0.028570806	0.145985574	0.228103294	0.121838573	0.103392392	0.18528711
265	271	0.003019423	0.09737495	0.231894181	0.07678363	0.065775925	0.101565402
265	273	0.02005695	0.022922228	0.036252002	0.042231714	0.000249155	0.022299342
265	273	0.007790063	0.005025847	0.00427197	0.009046525	0.014574957	0.056540781
265	274	-0.002407633	-0.002407633	-0.000723625	-0.002407633	0.003598734	-0.001927124
268	273	0.067465487	0.099605883	0.126520313	0.002303055	0.039648316	0.088608095
275	283	2.13264115	3.531455428	5.162775696	1.495300117	2.679762856	3.939792345

[♯] Residues 139-146 is FLAG tag insertion on KaiA$_{\Delta N}$.

Table S5. HDX-MS difference profile between KaiA$_{\Delta N}$ free and bound to KaiB$_{fs\text{-}cryst}$-CI$_{cryst}$ complex.

	Difference in number of deuteron (bound − free)[¶]		
Residue number[#]	10 sec	100 sec	1000 sec
141	-0.234	-0.238	-0.066
142	-0.234	-0.238	-0.066
143	-0.234	-0.238	-0.066
144	-0.234	-0.238	-0.066
145	-0.234	-0.238	-0.066
146	-0.234	-0.238	-0.066
147	-0.234	-0.238	-0.066
148	-0.234	-0.238	-0.066
149	-0.234	-0.238	-0.066
150	-0.234	-0.238	-0.066
152	-0.234	-0.238	-0.066
153	-0.234	-0.238	-0.066
154	-0.234	-0.238	-0.066
155	-0.234	-0.238	-0.066
156	-0.234	-0.238	-0.066
157	-0.234	-0.238	-0.066
158	-0.234	-0.238	-0.066
159	-0.234	-0.238	-0.066
160	-0.234	-0.238	-0.066
161	-0.234	-0.238	-0.066
171	-0.175	-0.208	-0.137
172	-0.175	-0.208	-0.137
173	-0.175	-0.208	-0.137
174	-0.175	-0.208	-0.137
175	-0.175	-0.208	-0.137
176	-0.175	-0.208	-0.137
179	-0.029	-0.051	-0.100
180	-0.029	-0.051	-0.100
181	-0.029	-0.051	-0.100
182	-0.029	-0.051	-0.100
184	-0.029	-0.051	-0.100
185	-0.029	-0.051	-0.100
186	-0.029	-0.051	-0.100
187	-0.029	-0.051	-0.100
188	-0.029	-0.051	-0.100
189	-0.029	-0.051	-0.100
190	-0.029	-0.051	-0.100
191	-0.029	-0.051	-0.100

192	-0.029	-0.051	-0.100
193	-0.029	-0.051	-0.100
194	0.000	0.000	0.037
195	0.000	0.000	0.037
196	0.000	0.000	0.037
197	0.000	0.000	0.037
198	0.000	0.000	0.037
199	0.000	0.000	0.037
202	-0.104	-0.028	-0.030
203	-0.104	-0.028	-0.030
204	-0.104	-0.028	-0.030
205	-0.104	-0.028	-0.030
206	-0.104	-0.028	-0.030
207	-0.015	-0.008	-0.004
208	-0.015	-0.008	-0.004
209	-0.015	-0.008	-0.004
210	-0.015	-0.008	-0.004
211	-0.015	-0.008	-0.004
212	-0.015	-0.008	-0.004
213	-0.015	-0.008	-0.004
214	-0.015	-0.008	-0.004
215	-0.015	-0.008	-0.004
216	-0.015	-0.008	-0.004
217	-0.015	-0.008	-0.004
220	-0.018	-0.012	-0.012
221	-0.018	-0.012	-0.012
222	-0.018	-0.012	-0.012
223	-0.018	-0.012	-0.012
224	-0.018	-0.012	-0.012
225	-0.020	-0.091	-0.117
226	-0.020	-0.091	-0.117
227	-0.020	-0.091	-0.117
228	-0.020	-0.091	-0.117
229	-0.020	-0.091	-0.117
230	-0.020	-0.091	-0.117
231	-0.020	-0.091	-0.117
232	0.001	-0.008	-0.021
233	0.001	-0.008	-0.021
234	0.001	-0.008	-0.021
235	0.001	-0.008	-0.021
236	0.001	-0.008	-0.021
237	0.001	-0.008	-0.021
238	0.001	-0.008	-0.021

239	0.001	-0.008	-0.021
242	-0.101	-0.074	-0.070
243	-0.101	-0.074	-0.070
244	-0.101	-0.074	-0.070
245	-0.101	-0.074	-0.070
246	-0.101	-0.074	-0.070
247	-0.101	-0.074	-0.070
248	-0.101	-0.074	-0.070
249	-0.042	-0.002	0.000
250	-0.042	-0.002	0.000
251	-0.042	-0.002	0.000
252	-0.042	-0.002	0.000
253	-0.042	-0.002	0.000
254	-0.108	-0.058	0.000
255	-0.108	-0.058	0.000
256	-0.108	-0.058	0.000
257	-0.108	-0.058	0.000
258	-0.108	-0.058	0.000
259	-0.007	0.013	-0.002
260	-0.007	0.013	-0.002
261	-0.007	0.013	-0.002
262	-0.007	0.013	-0.002
263	-0.007	0.013	-0.002
264	-0.007	0.013	-0.002
265	0.018	0.000	-0.002
266	0.018	0.000	-0.002
267	0.018	0.000	-0.002
268	0.018	0.000	-0.002
269	0.018	0.000	-0.002
270	-0.004	-0.010	-0.010
271	-0.004	-0.010	-0.010
272	-0.004	-0.010	-0.010
273	-0.004	-0.010	-0.010
274	-0.004	-0.010	-0.010
277	-0.107	-0.142	-0.203
278	-0.107	-0.142	-0.203
279	-0.107	-0.142	-0.203
281	-0.107	-0.142	-0.203
282	-0.107	-0.142	-0.203
283	-0.107	-0.142	-0.203

[♯] Residues 139-146 is FLAG tag insertion on KaiA$_{\Delta N}$.

[¶] Sub-localized exchange level was analyzed as described previously *(68)*.

Table S6. HDX-MS deuterium uptake profile of KaiB$_{\text{fs-cryst}}$-CI$_{\text{cryst}}$ complex with and without KaiA$_{\Delta N}$.

Peptide residues of KaiB$_{\text{fs-cryst}}$ ♯		Deuterium uptake					
		KaiB$_{\text{fs-cryst}}$-KaiC CI$_{\text{cryst}}$ complex without KaiA$_{\Delta N}$			KaiB$_{\text{fs-cryst}}$-KaiC CI$_{\text{cryst}}$ complex with KaiA$_{\Delta N}$		
Start	**End**	**10 sec**	**100 sec**	**1000 sec**	**10 sec**	**100 sec**	**1000 sec**
1	9	4.325397337	5.210385551	5.850849827	4.127490775	4.715578739	5.470383164
1	9	4.315906858	5.336473748	5.992348864	4.172364496	4.786205141	5.554580146
1	12	4.021731375	4.852208871	5.57342795	3.778567655	4.418840532	5.09834258
1	12	3.976619147	4.800348059	5.58152822	3.668023173	4.374665107	5.156315649
11	32	1.218769137	3.013112039	6.790166051	1.245495017	2.92446732	6.725582199
11	40	3.622703378	8.124843953	14.41411185	3.397601676	6.692547297	13.08064192
13	24	2.194494806	3.775504897	6.894335089	2.168163593	3.762289849	6.842225639
13	32	1.259595727	3.177667545	7.081369805	1.179674025	3.09511419	7.001209832
13	32	1.202115104	3.079073552	7.115730387	1.160309821	2.982391039	7.094305058
13	33	1.299486393	3.296227997	8.051488282	1.20939079	3.209364658	7.979965914
13	40	3.562467414	8.138233352	14.36855845	3.104191122	6.561724406	12.95944617
13	41	3.766935373	8.742659438	15.40213253	3.552277669	6.911543809	13.60818975
13	42	4.194025996	9.692712114	16.40971517	3.80682251	7.624396319	14.44001243
33	40	2.17271224	3.423084145	5.313278077	1.998974482	3.050576824	4.80185532
41	46	1.05182995	1.723569133	1.998383618	0.705296392	1.09796282	1.642578134
41	53	1.548357807	3.501372579	5.557524117	1.019646136	2.580750385	4.663251005
41	53	1.625177659	3.656565987	5.780554119	0.983690732	2.602830063	4.761466353
41	55	1.685795908	4.18036763	7.292804183	0.964360897	2.802083973	6.078417857
42	53	1.470554607	3.396480163	5.501071358	0.900117534	2.241587951	4.145398947
43	53	1.269923058	2.56901724	4.164912061	0.698168792	1.563515657	3.295933373
45	53	0.849346644	2.11891552	3.650032555	0.596661795	1.327482163	2.883253623
47	53	0.762464908	2.074394871	3.907221737	0.434891263	1.257615481	2.927046891
47	53	0.825676272	2.156632893	3.979840909	0.567833403	1.518190353	3.051833884
54	65	1.166066304	3.441041753	5.185862302	0.809828353	2.370005344	3.895661059
57	65	0.864889683	2.475817239	3.276551461	0.528427012	1.833196834	2.542154479
63	89	3.910343071	8.500145141	12.29239714	3.837291788	8.358981448	12.23366589
66	87	3.326820218	7.930183561	12.00097658	3.298457793	7.759850408	11.92587791
66	87	3.484642953	7.982871732	11.68500932	3.36148486	7.825926922	11.55993817
66	89	3.74553562	8.265382959	11.84920116	3.676654847	8.060648525	11.76812784
93	106	9.96511625	11.6189459	12.70363391	9.939350697	11.51599241	12.1443236
93	106	10.16271963	11.58150805	12.76614184	10.19606201	11.73015225	12.54420464
95	106	7.930833791	9.29493882	10.07574206	8.015446383	9.30687506	10.19069604
96	106	7.83856633	8.719861455	9.146675513	7.900298625	8.828250017	9.190754989
97	106	6.509807142	7.310494631	8.162434245	6.362084598	7.323511085	8.198399797
99	106	4.27058273	5.413327037	6.134663652	4.265191043	5.371220063	6.080559559
100	106	2.694160142	3.929688831	4.700635552	2.698624157	3.955052357	4.708672533

♯Residues 99-106 is FLAG tag insertion on KaiB$_{\text{fs-cryst.}}$

Table S7. HDX-MS difference profile between KaiB$_{\text{fs-cryst}}$-CI$_{\text{cryst}}$ complex with and without KaiA$_{\Delta\text{N}}$.

Residue number of KaiB$_{\text{fs-cryst}}$ ♯	Difference in number of deuteron (with KaiA$_{\Delta\text{N}}$ − without KaiA$_{\Delta\text{N}}$) ¶		
	10 sec	100 sec	1000 sec
4	-0.037	-0.080	-0.072
5	-0.037	-0.080	-0.072
6	-0.037	-0.080	-0.072
7	-0.037	-0.080	-0.072
8	-0.037	-0.080	-0.072
9	-0.037	-0.080	-0.072
10	0.000	0.000	0.000
11	0.000	0.000	0.000
12	0.000	0.000	0.000
13	0.000	0.000	0.000
14	0.000	0.000	0.000
15	-0.007	-0.008	-0.006
16	-0.007	-0.008	-0.006
17	-0.007	-0.008	-0.006
18	-0.007	-0.008	-0.006
20	-0.007	-0.008	-0.006
21	-0.007	-0.008	-0.006
22	-0.007	-0.008	-0.006
23	-0.007	-0.008	-0.006
24	-0.007	-0.008	-0.006
25	0.000	0.000	-0.004
26	0.000	0.000	-0.004
27	0.000	0.000	-0.004
28	0.000	0.000	-0.004
29	0.000	0.000	-0.004
30	0.000	0.000	-0.004
31	0.000	0.000	-0.004
32	0.000	0.000	-0.004
33	-0.031	-0.169	-0.156
34	-0.031	-0.169	-0.156
35	-0.031	-0.169	-0.156
36	-0.031	-0.169	-0.156
37	-0.031	-0.169	-0.156
38	-0.031	-0.169	-0.156
39	-0.031	-0.169	-0.156
40	-0.031	-0.169	-0.156
41	-0.070	-0.177	-0.135
42	-0.070	-0.177	-0.135

43	-0.070	-0.177	-0.135
44	-0.070	-0.177	-0.135
45	-0.070	-0.177	-0.135
46	-0.070	-0.177	-0.135
47	-0.047	-0.115	-0.140
48	-0.047	-0.115	-0.140
49	-0.047	-0.115	-0.140
50	-0.047	-0.115	-0.140
52	-0.047	-0.115	-0.140
53	-0.047	-0.115	-0.140
54	-0.008	-0.142	-0.148
55	-0.008	-0.142	-0.148
56	-0.008	-0.142	-0.148
57	-0.008	-0.142	-0.148
58	-0.008	-0.142	-0.148
59	-0.058	-0.128	-0.146
60	-0.058	-0.128	-0.146
61	-0.058	-0.128	-0.146
62	-0.058	-0.128	-0.146
64	-0.058	-0.128	-0.146
65	-0.003	-0.006	-0.003
66	-0.003	-0.006	-0.003
67	-0.003	-0.006	-0.003
68	-0.003	-0.006	-0.003
69	-0.003	-0.006	-0.003
73	-0.003	-0.006	-0.003
74	-0.003	-0.006	-0.003
75	-0.003	-0.006	-0.003
76	-0.003	-0.006	-0.003
77	-0.003	-0.006	-0.003
78	-0.003	-0.006	-0.003
79	-0.003	-0.006	-0.003
80	-0.003	-0.006	-0.003
81	-0.003	-0.006	-0.003
82	-0.003	-0.006	-0.003
83	-0.003	-0.006	-0.003
84	-0.003	-0.006	-0.003
85	-0.003	-0.006	-0.003
86	-0.003	-0.006	-0.003
87	-0.003	-0.006	-0.003
88	-0.003	-0.006	-0.003
89	-0.003	-0.006	-0.003
95	0.008	0.000	0.000

96	0.008	0.000	0.000
97	0.008	0.000	0.000
98	0.008	0.000	0.000
99	0.008	0.000	0.000
100	0.008	0.000	0.000
101	-0.003	0.003	0.000
102	-0.003	0.003	0.000
103	-0.003	0.003	0.000
104	-0.003	0.003	0.000
105	-0.003	0.003	0.000
106	-0.003	0.003	0.000

[♯] Residues 99-106 is FLAG tag insertion on $\text{KaiB}_{\text{fs-cryst}}$.

[¶] Sub-localized exchange level was analyzed as described previously *(68)*.

Table S8. HDX-MS deuterium uptake profile of KaiB$_{fs\text{-}nmr}$ free and bound to CikA$_{PsR}$.

Peptide residues$^{\sharp}$		Deuterium uptake					
		Free KaiB$_{fs\text{-}nmr}$			KaiB$_{fs\text{-}nmr}$ bound to CikA$_{PsR}$		
Start	End	10 sec	100 sec	1000 sec	10 sec	100 sec	1000 sec
7	12	0.041427678	0.094911817	0.46612337	0.363030184	0.999787486	1.068320095
7	12	0.035104947	0.080706823	0.444075163	0.398847126	0.990550949	1.068564499
7	32	3.013152048	6.364816689	10.33551495	2.73564996	5.711523115	9.227485531
8	12	0.038257007	0.099714531	0.386848477	0.169545115	0.596000977	0.71839449
11	24	3.658285902	6.412843395	8.614620076	3.604798941	6.365156108	8.577406943
11	27	3.550830244	6.404541963	9.062164694	3.214218284	5.956265211	8.453419955
11	29	3.25242483	5.870889796	8.847956899	2.791936421	5.373874055	7.858569972
11	32	3.036576242	6.359246515	10.27665627	2.326521839	5.13326905	8.500642493
11	32	2.944387679	6.306325105	10.22808155	2.294786834	5.09418736	8.349726107
11	33	3.131476434	6.693915757	11.3069616	2.417703319	5.076156347	8.865451175
11	40	6.305585047	12.45097052	17.82433597	3.17868338	8.358595424	14.34047313
11	40	6.325219824	12.43119546	17.80540885	3.154074227	8.314518715	14.33217383
11	40	6.325871371	12.4653491	17.78982326	3.139949482	8.309858064	14.29410126
11	41	6.486073155	12.54186932	18.06775252	3.262850381	8.445569052	14.83801197
11	42	6.765527593	13.46455156	19.3370279	3.498279105	8.758732513	15.41399996
11	46	7.165163504	14.91749879	21.13142047	3.378149619	9.131994353	15.96049872
11	46	7.232421551	15.00910611	21.15275733	3.347314271	9.026881288	16.3271977
11	46	7.251613227	14.7375019	21.39158493	3.186763817	8.869263007	16.33074189
11	46	7.198071065	14.84640525	20.97501651	3.258060496	9.031509873	16.15716664
11	53	9.309007063	18.63191771	24.87188569	4.633959053	11.89227338	20.08384386
11	53	9.302428265	18.66780099	24.84624763	4.536695062	11.86485779	20.14718201
11	53	9.402419943	18.64894722	24.86081379	4.541124067	11.88306128	20.13210439
12	43	6.771435482	13.53160941	19.07680399	3.48443018	8.723969974	15.43561213
13	21	3.590479855	5.310674149	5.93315181	3.579401451	5.30530999	5.897021972
13	24	3.987329995	6.58751952	8.633504028	3.888645239	6.483269294	8.632291619
13	27	3.665203802	6.579516307	9.216293394	3.237617954	5.892365743	8.720079707
13	30	3.522017679	7.028843375	9.930149201	3.000633097	5.889220023	8.87377338
13	31	3.246229786	6.795414443	10.50594234	2.536286444	5.255975834	8.533804769
13	32	3.003446387	6.438074211	10.3670571	2.423920501	5.121661853	8.471158354
13	33	3.041932753	6.862299815	11.20001042	2.416280613	5.184299654	8.777390401
13	33	3.045427728	6.873249774	11.30301618	2.448823588	5.157289552	8.823348825
13	35	4.101607396	8.630468068	13.06041177	2.603233152	6.333694195	10.46599631
13	40	6.411200822	12.56634182	17.75661773	3.214917641	8.307248555	14.05851333
13	40	6.269118518	12.43857063	17.78973717	3.073298273	8.270377242	14.22846421
13	41	6.47928756	12.7377699	18.3799646	3.229479998	8.484492009	14.50750764
13	41	6.406796379	12.64748369	18.33097733	3.219634911	8.36711477	14.3890799
13	42	6.645861199	13.45611166	19.28728011	3.178385719	8.508438165	15.08198947

13	44	6.828065498	14.32008392	20.47773511	3.227900821	8.608704692	15.63119469
13	44	6.853591032	14.25926238	20.33845582	3.274109121	8.542883152	15.66511376
13	46	7.238659731	14.92662993	21.175948	3.312360469	8.931535953	16.36561104
13	46	7.221342093	14.86791689	21.17790556	3.319273599	8.942420201	16.30904039
13	46	7.240292069	14.82309715	21.23545495	3.336904817	8.937682136	16.38178339
13	53	9.328870663	18.57034226	24.8539857	4.583295775	11.84088713	20.02263784
13	53	9.317521664	18.55213311	24.85136078	4.644704324	11.76121401	20.05682509
13	53	9.297142139	18.53188582	24.80902191	4.638583644	11.78401287	19.97240487
22	32	0.480062725	1.486652369	3.257081133	0.045786835	0.599765165	1.444158635
23	32	0.517001593	1.182203158	2.574914047	0.076794737	0.263436014	0.951978736
23	40	4.242381106	7.872913053	10.69831552	1.417639866	4.222848103	7.445718089
25	32	0.31358323	1.085278703	2.277792892	0.018345674	0.222719729	0.92111116
25	32	0.437517438	1.097223621	2.259462366	0.002383435	0.226856672	1.028193628
25	40	4.287916309	8.021851073	10.3945062	1.402984555	4.187651511	7.396487166
25	40	4.167822377	7.577792066	10.32729862	1.37434035	4.266520624	7.475412172
25	40	4.366253979	7.968918104	10.55992209	1.068254023	4.038238991	7.130614498
28	40	4.021293717	7.303699866	9.171530509	1.262753323	3.949266713	6.876113564
30	40	3.708304486	6.337461447	7.745806207	1.313528523	3.537861963	5.638247489
30	42	4.052434603	7.358270778	9.241385615	1.300402666	3.947881669	6.902354079
30	46	4.88244266	9.087859997	11.22804269	1.4363987	4.551708266	8.452043647
30	53	6.917565422	12.58443334	14.77916152	2.980369305	7.605303746	12.21460381
31	40	3.962748075	6.434757738	7.845325198	1.503443734	3.832978473	6.19096698
32	40	3.84713248	5.699545924	6.727934197	1.105974433	3.211977921	5.495274877
33	40	2.998688821	4.842287228	5.845978491	1.152689083	2.92711627	4.720410717
33	40	3.011212687	4.755688179	5.776998437	1.296948139	3.009875077	4.77552394
33	41	3.055117115	4.82867103	6.211315602	1.356347366	3.034958031	4.794429557
33	42	3.322800001	5.724880328	7.239690615	1.152350192	2.885068652	5.522536561
33	42	3.177215052	5.730441922	7.131328503	1.214418471	2.954315876	5.549881212
33	44	3.169502462	6.185713126	7.944094901	0.522107801	2.601361351	5.433288304
33	44	3.041867315	6.128202669	7.875233753	0.657956038	2.622454602	5.25132613
33	46	3.662834864	6.859591504	8.679535008	1.400785712	3.373201999	6.287639962
33	47	3.518501629	6.640911666	8.662588536	1.10405857	2.871903033	6.188068736
33	47	3.4480874	6.811863881	8.540931702	1.090847009	2.762799335	6.0125405
33	53	5.591622651	10.34607616	12.1709178	2.549738621	5.999325671	9.968957361
33	53	5.549873785	10.29140145	12.10549802	2.437868263	5.982264363	9.81898563
33	53	5.518227832	10.25364014	12.09693881	2.460495641	5.9113114	9.89305075
34	40	2.365094079	3.871874253	4.813515292	0.831617607	2.190167219	3.782773046
34	40	2.345414984	3.934486688	4.792951591	0.967841524	2.328456802	3.916044876
34	42	2.725360945	4.839830225	6.319573849	1.080937708	2.469976632	4.688339619
34	46	3.331796383	6.242293724	7.928252281	1.07914796	2.826703169	5.717921586
34	46	3.233891068	6.358257483	7.944843035	1.092855301	2.78324034	5.672332268
36	46	2.267738203	4.617517102	6.147201754	0.658640278	1.827300901	4.075382938
41	46	0.990374644	1.76767243	2.061557742	0.112742173	0.641739223	1.418098843

41	46	0.976877829	1.985353005	2.264833872	0.238284921	0.835450877	1.470414447
41	47	0.890841807	1.627240366	1.948287515	0.131928932	0.665297221	1.352334192
41	47	0.899185956	1.606458784	1.988375123	0.060391418	0.736968832	1.189654084
41	53	2.993260942	5.235772105	5.71510459	1.608318038	3.541580635	4.895592557
41	53	3.089195782	5.277945316	5.715552378	1.593198079	3.518907454	4.873633949
41	55	4.017303698	7.283239324	7.708969091	2.037391432	5.248134253	6.928952527
41	55	4.010371295	7.311834198	7.707110386	2.042212459	5.319545812	6.896293097
41	56	4.652448847	8.263501251	8.613330249	2.37091058	5.974672209	7.683233263
41	57	5.011541028	9.098717271	9.300069398	2.645767822	6.7117177	8.732446681
41	65	8.709714178	14.27247929	15.90629937	5.657858929	11.17117287	15.06272708
42	46	0.6730649	1.216702336	1.509504938	-0.040633536	0.554572087	1.076701661
42	52	2.775199893	4.300564327	4.527492738	1.632605115	3.416566076	4.326991822
42	53	2.949111099	5.096537483	5.553108428	1.580642584	3.484248515	4.831861344
42	53	3.044318894	5.086378554	5.528484255	1.529834382	3.614472506	4.907941391
42	55	3.811909206	7.005028624	7.368722625	2.01072688	4.86563431	6.708208444
43	53	2.681692067	4.106009781	4.567076183	1.448929033	3.478357845	4.323311593
43	53	2.775199893	4.300564327	4.548383389	1.632605115	3.416566076	4.384149327
43	53	2.796264806	4.326146569	4.607376112	1.758550988	3.50390674	4.339546362
43	55	3.821467362	6.372380011	6.641858401	2.219635479	5.247435089	6.432883627
43	56	4.50102112	7.374683583	7.674014234	2.339805595	6.009632466	7.272380774
43	65	8.3163072	13.164038	14.97335363	5.393095664	10.1690059	14.18183738
43	65	8.522043291	13.25668398	14.93675625	5.544120135	10.72223669	13.98371449
45	53	2.189949958	3.635503578	3.814091937	1.605970737	2.920843817	3.764971693
45	53	2.133253252	3.61932508	3.849894631	1.490305529	2.927259697	3.722672121
45	55	3.363110275	5.722618243	5.977989949	2.094607211	4.735755513	5.685708861
45	65	7.882275826	12.64390102	14.02039643	5.441775175	10.29920048	13.40962784
45	65	8.101358033	12.67434551	14.17826521	5.296805379	10.26082822	13.3684927
47	53	2.225589759	3.983764648	4.022433606	1.422292864	3.13767955	3.964032163
47	55	3.305096155	5.970900146	5.97842373	1.93598054	4.962752354	6.025627113
47	56	3.927312135	6.957992439	7.061107375	2.355814674	5.891415277	7.139380002
47	65	7.914159367	12.5812282	13.84263549	5.509918865	10.48227248	13.83120065
47	65	7.855010097	12.59260857	13.97303244	5.283438382	10.20812198	13.77653683
48	53	1.708532417	2.822847999	2.952186714	1.098281437	2.436339107	2.983178296
49	53	1.159009263	1.919344566	1.951705867	0.752810221	1.593607107	1.96912031
54	62	4.4039254	5.992527483	6.853668875	3.658036669	5.111275771	6.847369814
54	62	4.588050114	6.083823274	6.985628004	3.620670261	5.473435218	6.949454898
54	65	4.378862363	6.176891471	7.547112646	3.523558176	5.277945295	7.322277099
54	65	4.434382156	6.132983491	7.585190543	3.656907898	5.331348819	7.513051025
54	87	10.68933213	15.34230345	18.75826385	11.40688281	16.09086849	20.37098366
54	87	10.66262448	15.27310356	18.65319913	11.54969888	16.12754303	20.48105259
54	89	10.31928657	14.89805251	18.60061117	11.03550925	15.63283622	19.8028108
54	89	10.58251502	15.26636649	18.41982149	11.24815608	16.02784473	20.26807648
54	89	10.50092618	15.01195453	18.4723006	11.20666957	15.8362318	20.31827265

54	92	10.39892043	14.71160839	18.43056301	11.23226364	15.94942631	20.53885504
56	65	3.55854058	4.466978671	5.668311075	2.688421999	3.616085803	5.376556242
57	62	2.844487725	3.329925404	3.940474643	2.825501064	3.305637377	3.943799937
57	65	3.126788416	3.770547424	4.747284205	2.824913946	3.401509117	4.651378002
57	65	3.180818921	3.772683338	4.714045531	2.839654825	3.449479754	4.509555554
57	80	6.938573224	9.241882912	11.26256835	7.403538418	9.753253675	12.38297085
57	80	7.223370215	9.681605619	11.7538868	7.550199179	10.36554559	12.98790826
57	87	9.525279765	13.44575456	16.36410264	10.53731098	14.41759411	18.05500717
58	65	2.947557688	3.135772684	3.654906184	2.76509038	2.93220852	3.494012325
58	87	9.661634679	12.8722554	15.22759945	10.6906714	14.08266447	17.1965711
63	89	6.828534979	10.16442539	12.31912371	7.937056837	11.41980309	13.84904863
63	89	6.782001817	9.970096177	12.23583387	7.803608695	11.44748915	14.06402849
66	82	5.92568515	8.394713556	9.23576632	6.910995063	9.14553456	10.32864624
66	84	7.285860557	10.05493997	11.03090332	8.001334302	11.05688438	12.47403386
66	87	6.839443153	9.970832154	11.75626728	8.017573673	11.28857166	13.44028268
66	87	7.048124993	10.04300097	11.75352375	8.324048866	11.5124073	13.25526374
66	89	7.45016404	10.40249499	11.93041198	8.312143331	11.99888365	13.50326564
67	87	6.988009015	9.582421597	11.32143568	7.683596552	10.52293537	12.32735286
81	87	0.779385385	1.524421541	2.435626001	0.777037097	1.53938568	2.456005996
81	89	0.774865908	1.431243668	2.186170504	0.733606579	1.487028793	2.230818824
81	89	0.782747934	1.573113221	2.226910143	0.792016629	1.59091222	2.256396314
83	89	0.034163973	0.088275708	0.446987011	0.002181712	0.113620141	0.510248767
85	89	0.054243008	0.066757776	0.076341098	0.018121413	0.050115581	0.10412056
88	92	0.089851712	0.216551718	0.600901248	0.091336313	0.230291032	0.721571168
88	94	0.141242607	0.433127086	1.458032172	0.234257708	0.91425511	2.316717701
88	94	0.291367284	0.68430383	1.554138362	0.417608291	1.22966127	2.448027948
90	96	0.724512076	2.272142841	3.61131647	1.557544169	3.177964002	4.60746324
90	96	1.079373805	2.371457931	3.6961364	1.594048968	3.141195188	4.612957722
90	106	6.698807232	10.81604274	12.77564288	8.586297183	12.20596363	14.07300781
90	106	6.591233986	10.67827336	12.76003946	8.299080952	11.98466271	14.13312054
91	96	0.777290636	2.143153634	3.19341438	1.427590581	2.930166334	3.914733024
95	106	7.769273915	9.163955287	10.12423524	7.806766485	9.205381418	9.922148768
96	106	7.389955766	8.205081301	8.896397974	7.459875655	8.196172723	8.905999885
97	100	2.621088683	2.63056205	2.571603754	2.459672622	2.524704981	2.554903427
97	106	6.383450746	7.225477754	7.975450184	6.374565416	7.15184653	7.874848906

♯Residues 99-106 is FLAG tag insertion on KaiB$_{\text{fs-nmr}}$.

Table S9. HDX-MS difference profile between KaiB$_{\text{fs-nmr}}$ free and bound to CikA$_{\text{PsR}}$.

	Difference in number of deuteron (bound − free)[¶]		
Residue number[♯]	10 sec	100 sec	1000 sec
9	0.066	0.158	0.128
10	0.066	0.158	0.128
11	0.066	0.158	0.128
12	0.066	0.158	0.128
13	0.066	0.158	0.128
14	-0.054	-0.027	-0.006
15	-0.054	-0.027	-0.006
16	-0.054	-0.027	-0.006
17	-0.054	-0.027	-0.006
18	-0.054	-0.027	-0.006
20	-0.054	-0.027	-0.006
21	-0.054	-0.027	-0.006
22	0.000	-0.030	-0.060
23	0.000	-0.030	-0.060
24	0.000	-0.030	-0.060
25	0.000	-0.030	-0.060
26	0.000	-0.030	-0.060
27	-0.040	-0.170	-0.286
28	-0.040	-0.170	-0.286
29	-0.040	-0.170	-0.286
30	-0.040	-0.170	-0.286
31	-0.040	-0.170	-0.286
32	-0.283	-0.303	-0.183
33	-0.283	-0.303	-0.183
34	-0.283	-0.303	-0.183
35	-0.283	-0.303	-0.183
36	-0.283	-0.303	-0.183
37	-0.283	-0.303	-0.183
38	-0.192	-0.342	-0.234
39	-0.192	-0.342	-0.234
40	-0.192	-0.342	-0.234
41	-0.192	-0.342	-0.234
42	-0.192	-0.342	-0.234
43	-0.116	-0.204	-0.138
44	-0.116	-0.204	-0.138
45	-0.116	-0.204	-0.138
46	-0.116	-0.204	-0.138
47	-0.116	-0.204	-0.138
48	-0.168	-0.100	-0.005
49	-0.168	-0.100	-0.005

50	-0.168	-0.100	-0.005
52	-0.168	-0.100	-0.005
53	-0.192	-0.174	-0.008
54	-0.192	-0.174	-0.008
55	-0.192	-0.174	-0.008
56	-0.192	-0.174	-0.008
57	-0.192	-0.174	-0.008
58	-0.142	-0.094	-0.026
59	-0.142	-0.094	-0.026
60	-0.142	-0.094	-0.026
61	-0.142	-0.094	-0.026
62	-0.142	-0.094	-0.026
64	0.000	-0.073	-0.028
65	0.000	-0.073	-0.028
66	0.000	-0.073	-0.028
67	0.000	-0.073	-0.028
68	0.089	0.127	0.152
69	0.089	0.127	0.152
73	0.089	0.127	0.152
74	0.089	0.127	0.152
75	0.089	0.127	0.152
76	0.089	0.127	0.152
77	0.089	0.127	0.152
78	0.089	0.127	0.152
79	0.089	0.127	0.152
80	0.089	0.127	0.152
81	0.053	0.045	0.035
82	0.053	0.045	0.035
83	0.053	0.045	0.035
84	0.053	0.045	0.035
85	0.053	0.045	0.035
86	0.053	0.045	0.035
87	0.000	0.010	0.006
88	0.000	0.010	0.006
89	0.000	0.010	0.006
90	0.000	0.010	0.006
91	0.000	0.010	0.006
92	0.188	0.184	0.210
93	0.188	0.184	0.210
94	0.188	0.184	0.210
95	0.188	0.184	0.210
96	0.188	0.184	0.210
97	0.046	0.030	0.013

98	0.046	0.030	0.013
99	0.046	0.030	0.013
100	0.046	0.030	0.013
101	0.046	0.030	0.013
102	0.046	0.030	0.013
103	0.046	0.030	0.013
104	0.046	0.030	0.013
105	0.046	0.030	0.013
106	0.046	0.030	0.013

[♯] Residues 99-106 is FLAG tag insertion on $\mathrm{KaiB}_{\text{fs-nmr}}$.

[¶] Sub-localized exchange level was analyzed as described previously *(68)*.

Table S10. HDX-MS deuterium uptake profile of CikA$_{\mathrm{PsR}}$ free and bound to KaiB$_{\mathrm{fs\text{-}nmr}}$.

		Deuterium uptake					
Peptide residues			**Free CikA$_{\mathrm{PsR}}$**			**CikA$_{\mathrm{PsR}}$ bound to KaiB$_{\mathrm{fs\text{-}nmr}}$**	
Start	**End**	**10 sec**	**100 sec**	**1000 sec**	**10 sec**	**100 sec**	**1000 sec**
613	618	0.264219456	0.563218745	1.350385604	0.209768297	0.320782476	0.734109818
613	622	0.715149758	2.880299664	4.477796906	0.399514108	2.337970165	3.617821864
613	628	4.978974448	8.945037292	11.20576012	4.520142797	8.429767097	10.26781958
619	627	3.051098216	5.573155713	6.643972942	3.067056408	5.489052443	6.629201991
619	627	3.036511268	5.665307939	6.620525189	3.069160323	5.622080246	6.601268122
619	628	3.642354916	6.406070608	7.667589042	3.614303792	6.377956789	7.658445295
619	628	3.645776212	6.568742932	7.664001839	3.577912244	6.542993231	7.59494848
622	628	2.14540874	3.879259836	4.619765878	2.111798239	3.860147079	4.601504582
623	628	1.18896958	3.047505448	3.618981882	1.174300582	3.028259699	3.592937919
629	633	0.441694715	2.376758381	2.928331376	0.108691381	1.385526117	2.46479932
632	637	1.597803224	2.555469594	3.460298832	0.72125505	1.954050626	2.821227129
632	638	1.685329035	2.588535059	3.532316922	0.689027239	2.106701548	2.964791325
632	639	1.643016781	2.90610793	4.615330384	0.729611379	2.043358798	3.305649038
632	639	1.630423458	2.900704768	4.290581823	0.726551588	2.028064047	3.358677331
632	643	1.897430329	4.300326624	7.761769879	0.914522692	2.4581909	4.92551996
632	651	3.86051372	9.83005424	15.06839895	3.272867765	7.155784244	11.23528013
632	651	4.24351229	10.00833487	15.2555697	3.233457347	6.733305076	10.95878442
633	639	1.364274923	2.558310711	3.986956367	0.535340859	1.760491583	2.953068101
634	638	0.247698253	1.030126285	1.819963307	0.139885531	0.547533654	1.300272237
634	639	0.315292382	1.445401691	2.854285147	0.15845902	0.660685284	1.798368267
634	643	0.536962452	2.762402239	6.120052004	0.018203741	0.743938707	3.425102081
634	643	0.599795855	2.909518558	6.139905186	0.124728222	1.036780304	3.374968913
634	651	3.049036082	8.495159476	13.36357068	2.653868754	6.081774894	9.839782426
634	651	3.230913904	8.56393698	13.49790193	2.6634851	5.489088551	9.336119292
638	642	0.200610888	1.211300555	2.825332122	0	0.229097478	1.333396573
638	643	0.25450018	1.460157433	3.288818664	0.04745516	0.29742519	1.556681246
638	646	0.598548606	2.810279078	6.150063408	0.45070703	1.419809179	3.3526458
638	651	2.685336575	6.923570347	10.28601454	2.447555466	5.0598628	7.405495438
639	643	0.230682245	1.582851955	2.583748864	0	0.294130598	1.762203874
639	651	2.446495945	6.885170977	9.589415387	2.202308566	5.152884832	7.396088854
644	651	1.780419961	4.324879553	4.987831586	1.820322844	4.317885082	4.92065088
647	651	0.50333164	1.679022145	2.169364788	0.466822149	1.652330363	2.15702142
652	658	0.556678828	1.71015534	2.508111757	0.23291584	0.918124943	1.80707373
652	659	0.631934348	2.038677709	3.178885193	0.22932882	1.02376632	2.204497756
652	660	0.721869501	2.240441106	3.515180874	0.17771495	1.128983422	2.38704472
652	660	0.678860033	2.093225814	3.391039614	0.252769176	1.038277559	2.187384463
659	673	4.577544107	7.302481438	8.088358078	3.812096821	6.768978076	7.8641884

661	666	1.42656876	2.257053087	2.463056334	1.410970046	2.261904172	2.458519182
661	672	4.157334897	6.548310564	7.254041144	3.432741479	6.107158307	6.876615698
661	672	4.049767252	6.491230663	7.156293485	3.436944695	6.152627627	6.864498644
661	673	4.145742475	6.613460626	7.34828134	3.495258237	6.234775436	7.073951174
661	677	4.427986936	7.143473176	8.02951336	3.217689395	6.264920677	7.421479508
661	686	5.861049445	11.2367402	14.53532872	5.07484894	10.57528563	13.83702999
661	688	5.82333462	11.59051019	14.88330575	5.280797614	10.90970869	14.05014731
664	672	2.948453235	5.198370407	5.717655699	2.39509768	4.774159263	5.694960431
669	673	0.114144083	0.210610248	0.849381579	0.083299691	0.192316981	0.810364313
673	677	0.017902135	0.01829996	0.078042532	0.004972815	0.019692348	0.045225047
673	685	2.381729889	5.16137513	7.504779389	2.820275669	5.712042474	7.656324198
673	688	2.509063631	5.677479619	8.320573943	2.890920504	6.182041579	8.394137658
674	686	2.383711581	5.12641076	7.561111604	2.823977919	5.61368486	7.595370566
674	688	2.406872283	5.421776206	7.998947005	2.81928412	5.979755008	8.053392952
678	688	2.042601472	4.364292072	6.516283129	2.272394146	4.650891313	6.675287007
686	701	6.52745671	10.19306706	11.49273032	6.060487918	9.589921238	11.0975231
686	712	7.372153092	12.79394011	15.43291755	6.434235292	11.67885392	14.47917736
686	713	7.358455025	13.36976695	16.95633312	6.68169303	12.26496897	15.81642224
686	714	7.413139986	13.4280289	17.41279052	6.428335605	12.48157466	16.09326942
687	701	6.266231886	10.02698833	10.98083476	5.931709181	9.549547986	10.62287718
687	712	7.495542544	12.49352103	15.00241292	6.837389963	11.33991197	13.76476424
687	713	7.433711091	12.83486722	16.03875745	6.611280253	11.67261284	14.65703741
687	713	7.312240894	12.80574282	16.02686516	6.871336416	11.64022993	14.702655
687	714	7.488511932	13.25318158	16.99363885	7.169275225	12.39623604	16.33647921
687	714	7.359894372	13.37065313	17.04658046	6.683194698	12.26595686	15.99963135
688	713	7.075425736	12.82007219	16.53181622	6.386985171	11.80937743	15.17208852
689	701	5.788951892	9.132874323	9.86978492	5.486958173	8.750184496	9.510881862
689	712	7.100879437	11.72841365	13.97405533	6.666620906	11.02262538	12.9805072
689	712	7.219900045	11.93888577	14.21650608	6.699922706	11.22159077	13.20126259
689	713	7.166301387	12.23731501	15.24287801	6.497023265	11.14216495	13.89013193
689	713	7.098836369	12.19120626	15.16164465	6.420684002	11.17873295	13.91802351
689	714	7.173503498	12.82339698	16.40749771	6.549207178	11.7670434	14.97123437
689	714	7.073773386	12.82115029	16.53700711	6.463478942	11.79167431	15.27526578
690	713	7.219900045	11.93888577	14.21650608	6.798416041	11.11883758	13.35574663
702	712	2.853227548	3.983085305	4.142098815	2.838812507	3.936734816	4.074756885
702	712	2.88116394	4.112976154	4.28153232	2.779912094	4.105582171	4.187363294
702	713	2.70814609	4.28145612	5.127882339	2.647973688	4.215618463	5.033089475
702	713	2.803472206	4.315040072	5.294526945	2.768534567	4.248775926	5.245515535
702	714	2.843364936	4.927421997	6.483081298	2.808582897	4.845372486	6.470377136
702	714	2.680779034	4.803594749	6.323428136	2.5996412	4.730053273	6.296307261
702	716	3.278250625	5.982794564	7.916290656	3.197858315	5.912237875	7.908690112
704	713	2.785881466	4.602775951	5.11362702	2.725780004	4.558550406	5.094846594
713	721	1.778742367	3.552220315	4.996554114	1.776935494	3.54636927	4.965277248

713	721	1.679951429	3.43661472	4.965687967	1.699270884	3.452319337	4.91980891
713	724	5.032047597	6.845189733	8.264101351	4.95334738	6.756849076	8.204210437
714	718	0.029717857	0.063042354	0.479308001	0.019166302	0.067016139	0.454413337
714	721	1.289250964	2.678705266	4.083061681	1.257849738	2.645373417	4.077917385
714	721	1.285993638	2.678650282	4.019855746	1.227282866	2.616738874	3.968883402
714	724	4.803702935	6.254057578	7.410329008	4.775066537	6.21055317	7.382204191
714	724	4.680043244	6.150427263	7.407972224	4.661033554	6.100308486	7.374249632
714	729	9.760836639	11.34950138	12.4819737	9.707187619	11.29760296	12.42672862
715	721	1.238844382	2.526867658	3.803804904	1.217728002	2.524278148	3.784337889
715	724	4.469208636	5.907340904	6.941955802	4.4865544	5.914341075	6.902006387
715	724	4.515080008	5.935056944	7.046206032	4.426771999	5.865230951	7.004288034
720	724	2.69935152	3.061781182	3.079462407	2.668099312	2.967421368	3.092850809
721	729	6.728224992	7.044733626	7.112826966	6.718685418	7.141505822	7.123548928
722	729	5.784321938	6.132914677	6.149517638	5.779280212	6.008336955	6.146640295
722	729	5.797680054	5.977911944	6.035576214	5.769529239	5.940453232	6.016020144

Table S11. HDX-MS difference profile between CikA$_{PsR}$ free and bound to KaiB$_{fs\text{-}nmr}$.

	Difference in number of deuteron (bound − free) ¶		
Residue number	10 sec	100 sec	1000 sec
615	-0.060	-0.083	-0.148
616	-0.060	-0.083	-0.148
617	-0.060	-0.083	-0.148
618	-0.060	-0.083	-0.148
619	-0.060	-0.083	-0.148
620	-0.060	-0.083	-0.148
621	-0.006	-0.004	-0.003
622	-0.006	-0.004	-0.003
623	-0.006	-0.004	-0.003
624	-0.006	-0.004	-0.003
625	-0.006	-0.004	-0.003
626	-0.006	-0.004	-0.003
627	-0.006	-0.004	-0.003
628	-0.006	-0.004	-0.003
631	-0.178	-0.216	-0.118
632	-0.178	-0.216	-0.118
633	-0.178	-0.216	-0.118
634	-0.178	-0.216	-0.118
635	-0.178	-0.216	-0.118
636	-0.056	-0.170	-0.352
637	-0.056	-0.170	-0.352
638	-0.056	-0.170	-0.352
639	-0.056	-0.170	-0.352
640	-0.056	-0.170	-0.352
641	-0.026	-0.362	-0.374
642	-0.026	-0.362	-0.374
643	-0.026	-0.362	-0.374
644	-0.026	-0.362	-0.374
645	-0.026	-0.362	-0.374
646	0.002	-0.008	-0.038
647	0.002	-0.008	-0.038
648	0.002	-0.008	-0.038
649	0.002	-0.008	-0.038
650	0.002	-0.008	-0.038
651	0.002	-0.008	-0.038
654	-0.083	-0.198	-0.175
655	-0.083	-0.198	-0.175
657	-0.083	-0.198	-0.175

658	-0.083	-0.198	-0.175
659	-0.060	-0.050	-0.070
660	-0.060	-0.050	-0.070
661	-0.060	-0.050	-0.070
662	-0.060	-0.050	-0.070
663	-0.060	-0.050	-0.070
665	-0.060	-0.050	-0.070
666	-0.106	-0.100	-0.060
667	-0.106	-0.100	-0.060
668	-0.106	-0.100	-0.060
669	-0.106	-0.100	-0.060
670	-0.106	-0.100	-0.060
671	-0.006	-0.004	0.000
672	-0.006	-0.004	0.000
673	-0.006	-0.004	0.000
674	-0.006	-0.004	0.000
675	-0.006	-0.004	0.000
676	0.038	0.033	0.000
677	0.038	0.033	0.000
678	0.038	0.033	0.000
679	0.038	0.033	0.000
680	0.038	0.033	0.000
681	0.038	0.033	0.000
682	0.038	0.033	0.000
684	0.038	0.033	0.000
685	0.038	0.033	0.000
686	-0.012	-0.010	0.004
687	-0.012	-0.010	0.004
688	-0.012	-0.010	0.004
689	-0.012	-0.010	0.004
690	-0.012	-0.010	0.004
691	-0.041	-0.065	-0.079
692	-0.041	-0.065	-0.079
693	-0.041	-0.065	-0.079
694	-0.041	-0.065	-0.079
695	-0.041	-0.065	-0.079
696	-0.041	-0.065	-0.079
697	-0.041	-0.065	-0.079
698	-0.041	-0.065	-0.079
699	-0.041	-0.065	-0.079
700	-0.041	-0.065	-0.079
701	-0.041	-0.065	-0.079
702	-0.015	-0.014	-0.031

703	-0.015	-0.014	-0.031
704	-0.015	-0.014	-0.031
706	-0.015	-0.014	-0.031
708	-0.015	-0.014	-0.031
709	-0.015	-0.014	-0.031
711	-0.015	-0.014	-0.031
712	-0.015	-0.014	-0.031
713	0.003	-0.003	-0.003
714	0.003	-0.003	-0.003
715	0.003	-0.003	-0.003
716	0.003	-0.003	-0.003
717	0.003	-0.003	-0.003
718	0.003	-0.003	-0.003
719	-0.006	-0.008	0.000
720	-0.006	-0.008	0.000
721	-0.006	-0.008	0.000
722	-0.006	-0.008	0.000
723	-0.006	-0.008	0.000
724	-0.002	0.000	0.000
725	-0.002	0.000	0.000
726	-0.002	0.000	0.000
727	-0.002	0.000	0.000
728	-0.002	0.000	0.000
729	-0.002	0.000	0.000

[¶] Sub-localized exchange level was analyzed as described previously *(68)*.

Table S12. Plasmids for making cyanobacterial strains used in this study.

Plasmid	Description	Antibiotic resistance	Source
pAM4731	P_{kaiB}-*kaiB*^{Se} in neutral site 1. Transformed into strain AMC2133 and replaces gentamycin marked *kaiB*^{Se} with nourseothricin marked *kaiB*^{Se} in neutral site 1.	Nourseothricin	Chang et al. *(39)*
pAM4985	Neutral site 1 vector with nourseothricin resistance cassette. Transformed into strain AMC2133 and replaces gentamycin marked *kaiB*^{Se} with nourseothricin marker to create a *ΔkaiB*^{Se} strain.	Nourseothricin	Chang et al. *(39)*
pAM5290	P_{kaiB}-*A40D-kaiB*^{Se} in neutral site 1. Transformed into strain AMC2133 and replaces gentamycin marked *kaiB*^{Se} with *A40D-kaiB*^{Se} marked with nourseothricin in neutral site 1.	Nourseothricin	This work
pAM5291	P_{kaiB}-*K42E-kaiB*^{Se} in neutral site 1.Transformed into strain AMC2133 and replaces gentamycin marked *kaiB*^{Se} with *K42E-kaiB*^{Se} marked with nourseothricin in neutral site 1.	Nourseothricin	This work
pAM5292	P_{kaiA}-*kaiA*^{Se} expressed from neutral site 2. Transformed into strain AMC1898 for *kaiA*^{Se} complementation studies.	Nourseothricin	This work
pAM5293	P_{kaiA}-*L155A-kaiA*^{Se} expressed from neutral site 2. Transformed into strain AMC1898 for *kaiA*^{Se} complementation studies.	Nourseothricin	This work
pAM3389	P_{trc}-*cikA*^{Se} expressed from neutral site 1. Transformed into strain AMC1475 for *cikA*^{Se} complementation studies. *cikA*^{Se} is thioredoxin tagged *cikA*^{Se} published in Zhang et al. *(60)*.	Spectinomycin and Streptomycin	Zhang et al. *(60)*
pAM5294	P_{trc}-*C644R-cikA*^{Se} expressed from neutral site 1. Transformed into strain AMC1475 for *cikA*^{Se} complementation studies. *C644R-cikA*^{Se} has a thioredoxin tag similar to thioredoxin tagged *cikA*^{Se} in Zhang et al. *(60)*.	Spectinomycin and Streptomycin	This work

Table S13. Cyanobacterial strains used in this study.

Strain	Genotype (NS denotes neutral site)	Antibiotic resistance	Source
AMC1825	*S. elongatus* with NS1-P_{kaiB}-*luc*	Spectinomycin and Streptomycin	Chang et al. *(39)*
AMC1898	Insertional mutation of *kaiA*^{Se} in AMC1825 (NS1-P_{kaiB}-*luc*)	Kanamycin, Spectinomycin and Streptomycin	This work
AMC2133	*ΔkaiBC* strain transformed with NS1-Gm-*KaiB*^{Se}, NS2-Km-*KaiC*^{Se}, and NS3-P_{kaiB}-*luc* reporter	Gentamycin, Kanamycin and Chloramphenicol	Chang et al. *(39)*
AMC669	*S. elongatus* transformed with pAM2195 (NS2-P_{psbAI}-*luxAB*, P_{psbAI}-*luxCDE*)	Chloramphenicol	Mackey et al. *(66)*
AMC1475	AMC669 (NS2-P_{psbAI}-*luxAB*, *luxCDE*), transformed with pAM2152 to delete *cikA*^{Se}	Chloramphenicol and Gentamycin	Mackey et al. *(82)*

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