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Protein−Protein Interactions in the Cyanobacterial Circadian Clock: Structure of KaiA Dimer in Complex with C-Terminal KaiC Peptides at 2.8 Å Resolution

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Supporting Information

ABSTRACT: In the cyanobacterial circadian clock, the KaiA, -B, and -C proteins with ATP constitute a post-translational oscillator. KaiA stimulates the KaiC autokinase, and KaiB antagonizes KaiA action. KaiA contacts the intrinsically disordered C-terminal regions of KaiC hexamer to promote phosphorylation across subunit interfaces. The crystal structure of KaiA dimer from *Synechococcus elongatus* with two KaiC C-terminal 20mer peptides bound reveals that the latter adopt an α-helical conformation and contact KaiA α-helical bundles via mostly hydrophobic interactions. This complex and the crystal structure of KaiC hexamer with truncated C-terminal tails can be fit into the electron microscopy (EM) density of the KaiA:KaiC complex. The hybrid model helps rationalize clock phenotypes of KaiA and KaiC mutants.

The discoveries that the circadian rhythm of KaiC phosphorylation in the cyanobacterium *Synechococcus elongatus* proceeds in the absence of transcription−translation feedback[1] and that the clock can be reconstituted *in vitro* from the KaiA, KaiB, and KaiC proteins in the presence of ATP[2] allowed detailed biochemical and biophysical investigations of this molecular timer.[3−6] Post-transcriptional and -translational regulatory mechanisms have also been identified in clocks of mammals and other eukaryotes (ref 7 and references cited therein).

KaiC undergoes phosphorylation and dephosphorylation at two sites, Thr-432 and Ser-431,[8,9] in strict order[10,11] and with a period of ∼24 h. In addition to phosphorylation [autokinase in the C-terminal hexameric CII ring (Figure S1)] and autodephosphorylation (CII ATP synthase[12,13]), KaiC also displays ATPase activity in the CI and CII rings.[14] The KaiC phosphorylation level is a marker for clock phase and regulates its activities and nanocomplex formation with KaiA and KaiB.[6] Three-dimensional (3D) structures for all three proteins have been determined and revealed a KaiC hexamer, a domain-swapped KaiA dimer, and a dimer of dimers for KaiB.[15]

Kai hexamer displays the shape of a double doughnut with N-terminal CI and C-terminal CII rings formed by gene-duplicated domains, whereby the N-terminal peptides of CII subunits link domains on the outer surface of the barrel (Figure S1). Six ATP molecules are bound at subunit interfaces in both CI and CII, and C-terminal peptides protruding from the dome-shaped CII surface constitute an intrinsically disordered region (IDR). These peptides are contacted by KaiA dimer,[16,17] and this interaction is key to stimulation of KaiC phosphorylation by KaiA.[18,19] However, the lack of high-resolution structures of full-length KaiA:KaiC[17] and KaiB:KaiC[20] complexes has precluded a full understanding of the mechanisms of KaiC kinase promotion by KaiA[21,22] and KaiB's antagonistic role,[23] respectively.[24]

To visualize the interaction between C-terminal KaiC peptide and KaiA, we determined the crystal structure of full-length *S. elongatus* KaiA homodimer (amino acids V1−T284/His_6) in complex with two KaiC 20mer peptides (D500−S519) NH_3^+-DEKSELSRIVRGVQEKGPES-[K-5-FITC]-COO^- (Figure 1).

Figure 1. Sequence alignment of C-terminal portions of KaiC proteins from selected cyanobacterial strains. The sequence alignment was generated with CLUSTAL Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/)[25] and modified manually in UCSF Chimera.[26]

The KaiC peptide carried a C-terminal lysine that was modified with fluorescein 5-isothiocyanate (5-FITC), i.e., the primary amino group of the lysine side chain reacts with the isothiocyanate group, N═C═S, of 5-FITC under formation of a thiourea moiety. The labeled peptide has a yellow tint, which allows for facile monitoring of the formation of complex crystals with the KaiA protein (Figure S2). Crystals of the complex were obtained

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by soaking the peptide into KaiA crystals or by cocrystallization of KaiA with KaiC peptide. The latter crystals were of better quality and were used for the determination of the structure described here. The structure was phased by molecular replacement using the full-length KaiA dimer as the search model and refined to 2.8 Å resolution (see the Supporting Information for details and Table 1 for crystal and refinement parameters).

Table 1. KaiA:KaiC Complex Crystal Structure Statistics

Crystal Data	
space group	$P4_32_12$
unit cell constants ($a = b$, c) (Å)	97.43, 124.52
resolution (Å) (last shell)	46.2−2.82 (2.89−2.82)
no. of unique reflections (last shell)	15075 (1082)
completeness (%) (last shell)	100 (100)
R_{merge} [a] (last shell)	0.110 (0.805)
$I/\sigma(I)$ (last shell)	37.1 (4.7)
Refinement	
no. of working set reflections	15075
no. of test set reflections	732 (4.9%)
no. of protein non-H atoms	5139
no. of water molecules	108
R_{work}, [b] R_{free} [c]	0.239, 0.298
average B factor (Å^2)	
protein (all residues)	57.3
solvent	54.3
root-mean-square deviation	
bond lengths (Å)	0.01
bond angles (deg)	1.2
Ramachandran analysis (%)	
favored	91
allowed	6
outliers	2

[a] $R_{merge} = \sum_{hkl}\sum_{j=1,N}|\langle I_{hkl}\rangle - I_{hkl,j}|/\sum_{hkl}\sum_{j=1,N}|I_{hkl,j}|$, where the outer sum ($hkl$) is taken over the unique reflections. [b] $R_{work} = \sum_{hkl}||F_{ohkl}| - k|F_{chkl}||/\sum_{hkl}|F_{ohkl}|$, where $|F_{ohkl}|$ and $|F_{chkl}|$ are the observed and calculated structure factor amplitudes, respectively. [c] $R_{free} = R_{work}$ for the set of reflections omitted from the refinement process.

The electron density permits visualization of amino acids D500−K515 from one KaiC peptide (G chain) and amino acids D500−S519 from the other (H chain) (Figure 2; KaiA subunit chains are termed A and B). The five C-terminal residues of peptide G, including the added lysine with 5-FITC, are disordered. For peptide H, only that C-terminal lysine is invisible.

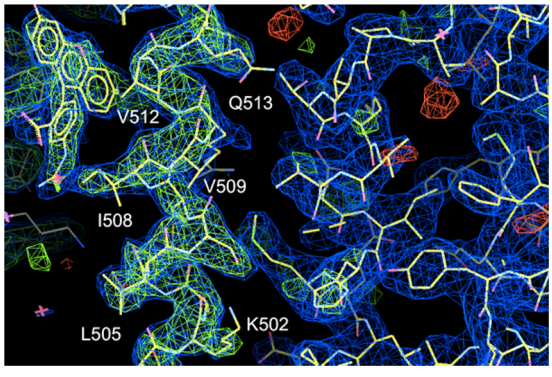

Figure 2. Omit ($2F_o - F_c$) Fourier electron density drawn at the 1σ level around the KaiC peptide (H chain; K502 is at the bottom and Q513 at the top) and one of the 5-FITC molecules (top left).

The electron density also showed two 5-FITC molecules, and their locations [adjacent to V512.G/H and thus at some distance from the C-termini (Figure 2)] suggest that the fluorescent probe had undergone partial hydrolysis (during crystallization, as matrix-assisted laser desorption ionization time of flight showed the stored peptide to be intact). However, FITCs do not mediate interactions between KaiC peptides and KaiA dimer and can be ignored in terms of the analysis of the KaiC peptide conformation and the KaiA:KaiC binding interface.

KaiC peptides bind on opposite sides of the KaiA dimer, and adjacent to the dimer interface formed by two helices from C-terminal α-helical bundle domains of KaiA subunits (Figure 3).

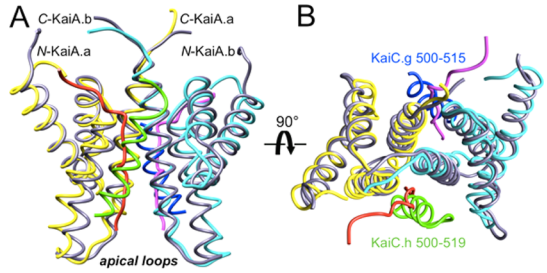

Figure 3. KaiA dimer (C-terminal domains of subunits in the crystal structure colored yellow and cyan) in complex with KaiC peptides (green, H chain; blue, G chain), viewed (A) perpendicular to the noncrystallographic dyad and (B) rotated by 90° around the horizontal. The NMR solution structure of C-KaiA dimer (gray) in complex with the corresponding portions of KaiC peptides (red and magenta; the complex exhibits precise 2-fold rotational symmetry)[16] is superimposed.

The stretch comprising D500−K515 in both peptides adopts a regular α-helical conformation, and the four C-terminal residues of the H chain end in a curl (Figure 3A). The distance between Cα atoms of D500 and S519 is just above 28 Å, and the length of the KaiC peptide thus matches the height of the KaiA C-terminal α-helical bundles minus the apical loop. Thus, C-terminal KaiC peptides contact almost exclusively C-terminal domains of KaiA dimer. The closest contacts between KaiC peptide (H chain) and a residue from the KaiA N-terminal domain (R127.A) are formed Oγ of S519 (4.4 Å) and the C═O group of P517 [3.2 Å; the primary contact of R127 is to E124 (Figure S3)].

The ordered α-helical KaiC peptides in the complex differ drastically from the dynamic and partially disordered random coil conformations of C-terminal tentacles in the crystal structure of free KaiC hexamer (Figure S1).[17] The isolated KaiC peptide forms a random coil (Figure S4). The switch to the α-helix seems to be induced by binding to KaiA and allows for a facile binding mode with KaiA α-helical bundle domains via a coiled coil motif. Indeed, each peptide forms a parallel, right-handed coiled coil with a portion of the long KaiA helix comprising residues E255−S279 from one subunit (Figure 3B, KaiC.H:KaiA.B). Further interactions are established with residues V230−R249 of the antiparallel helix from the other KaiA subunit (KaiC.H:KaiA.A).

Perhaps not surprisingly, the KaiC peptide does not feature the heptad repeat sequence motif hpphcpc (a-f; h = hydrophobic, p = polar, c = charged amino acid) characteristic of coiled coils,[27,28] as the same stretch in free KaiC forms an IDR. KaiC C-terminal peptides evidently fluctuate through a range of conformations, but docking to KaiA may organize the region from at least D500 to K515 into an α-helix. The KaiC sequence in this region displays a fairly high degree of conservation across proteins from different cyanobacterial strains (Figure 1). No fewer than 13 of the 20 C-

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terminal residues are charged or polar in the protein from *S. elongatus*, and just four are hydrophobic: L505, I508, V509, and V512. Beyond residue 515, the sequence variations increase, and KaiC proteins exhibit a range of lengths. This is consistent with the KaiA:KaiC peptide interface in the crystal structure of the complex: among amino acids 515−519, only P517 (via C═O) interacts with KaiA [R127.A NH2, 3.2 Å (Figure S3)]. The relatively low content of bulky hydrophobic amino acids is not untypical for a disordered region, especially considering the solvent-exposed C-terminal location.[29−31]

In the α-helical segment encompassing residues 500−515, the only charged amino acid to engage in an interaction with KaiA is R510 [to E235 (Figures 4 and 5)]. However, R510 is exposed to

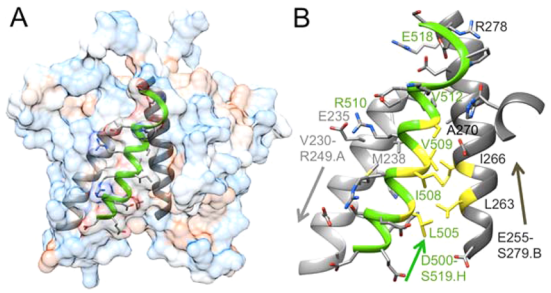

Figure 4. Coiled coil interaction between KaiC peptide (H chain, green) and KaiA dimer (only C-terminal domains are shown). (A) Surface model of $KaiA_2$ colored according to hydrophobicity (pink, most hydrophobic, light blue, least hydrophobic). (B) Three-stranded coiled coil between KaiA (gray) and KaiC α-helices (green).

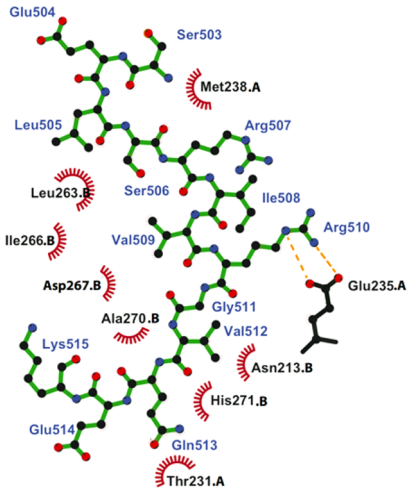

Figure 5. Ligplot[32] analysis of interactions of KaiC peptide (H chain, residues S503−E514) with $KaiA_2$. Peptide bonds are colored green and KaiA bonds black. H-Bonds are shown as dashed orange lines, and hydrophobic contacts are indicated with spoked red arcs.

solvent, as are D500, E501, S503, E504, R507, E514, and K515. In addition, the side chains of K502 and S506 establish a somewhat long H-bond (3.5 Å) in the core but appear not to form any electrostatic interactions with KaiA. This leaves only hydrophobic KaiC amino acids L505, I508, V509, V512, and Q513, the Cβ and Cγ methylene groups of the latter, as mediators of stabilizing interactions with KaiA residues. Indeed, all of these take part in the KaiA:KaiC interface (Figure 4, side chains colored yellow, and Figure 5), consistent with the nature of stabilizing contributions in coiled coils and basic principles of protein−protein interactions (i.e., the hydrophobic effect dominates).[33,34] The experimentally determined K_D for the KaiA:KaiC complex (full-length proteins) was as low as 0.2 μM (native PAGE, AMPPnP) and as high as 1.3 μM (IAsys biosensor, ATP).[35] There is thus no need to expect an extensive buried surface, and the structure of the complex between KaiC peptides and the KaiA dimer is in line with the transient nature of the interaction in the clock cycle.[4,5,15]

No high-resolution structure of the complex between the full-length KaiA and KaiC proteins is currently available. However, the crystal structures of KaiC hexamer[15,17] and the KaiA dimer in complex with KaiC C-terminal peptides can be fused readily (Figure 6A). The distances between Cα atoms from correspond-

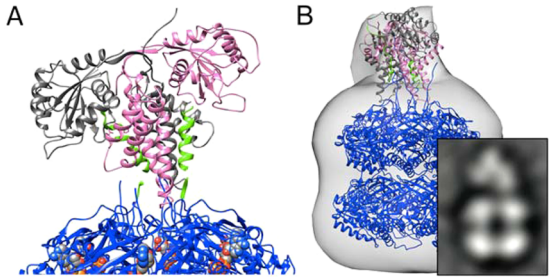

Figure 6. Model of the 3D structure of the KaiA:KaiC complex. (A) Crystal structure of the complex between *S. elongatus* KaiA dimer (subunits colored gray and pink) and KaiC C-terminal peptides (green), described here, combined with the crystal structure of KaiC hexamer[15,17] (Protein Data Bank entry 3DVL, blue ribbon). C-Termini of KaiC in the model were truncated at V499, and a slight gap was left between valines from A and C subunits and D500 residues from G and H peptide chains in the KaiA:KaiC-peptide complex. (B) Combined complex from panel A, fit into the electron density based on the negative-stain EM structure of the *S. elongatus* KaiA:KaiC complex.[17] The inset shows an example of an EM class sum image of the $KaiA_2$:$KaiC_6$ complex.

ing residues in KaiC subunits at the locations where C-terminal tails protrude from the hexameric barrel (I497) range from ~10 to 22 Å (adjacent, i.e., A/B, B/C, etc., and opposing, i.e., A/D, B/E, etc., subunits, respectively). The distance between Cα atoms of D500 residues in the G and H chains of the KaiA:KaiC-peptide complex is 28 Å. Because KaiC peptides contact opposing sides of the KaiA dimer (Figure 2), it is unlikely that the peptides capturing KaiA are from adjacent KaiC subunits. We joined the two structures by roughly aligning the N-termini (D500) of the G and H chains in the peptide complex and the C-termini (V499) of the A and C chains of a truncated KaiC hexamer (Figure 6A). Although some adjustments are necessary to fuse the chains, these appear to be rather minor and no large-scale conformational changes need to be envisioned. The combined model of the complex fits nicely into the low-resolution density (<20 Å) derived from negative stain electron microscopy (EM)[17] (Figure 6B). The 3D model of the KaiA:KaiC complex based on the two fused crystal structures is consistent with the appearance of the complex particle in EM class sum images (Figure 6). Further, the 1:1 $KaiA_2$:$KaiC_6$ complex is supported by biochemical experiments, and a single KaiA dimer was found to be sufficient for converting KaiC to the hyperphosphorylated form.[35]

The conformation of KaiC peptides in the crystal structure of the complex with full-length KaiA dimer described here is completely different from the conformation of the corresponding portion in a longer C-terminal peptide that also contains the adjacent A-loop residues E487−V497 (Figure 1), in complex with the C-terminal domain of KaiA from *Thermosynechococcus elongatus* BP-1[16] (Figure 2). The structure was derived by solution NMR, and KaiC peptides adopt a random coil

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conformation with a sharp turn between the stretched E487−I497 region and the portion corresponding to D500−E518 [Figure S5; KaiC from *T. elongatus* is one residue shorter than the protein from *S. elongatus* (see Figure 1)]. In fact, the conformation of the *T. elongatus* I497−E518 peptide in the solution structure of the complex closely resembles that of the corresponding region in subunit A in the crystal structure of free KaiC hexamer that was tracked to full length[17] (Figure S6). It is unlikely that both models of the KaiA:KaiC-peptide complex are correct. On one hand, there are differences in the constructs used and the methods applied: NMR, solution, *T. elongatus* C-terminal KaiA domain, and KaiC 35mer peptide versus X-ray, crystal, *S. elongatus* full-length KaiA, and KaiC 20mer peptide. On the other hand, the KaiABC clocks from *S. elongatus*[2] and *T. elongatus*[36] can both be reconstituted *in vitro*, and they form a PTO. One would expect matching structures to underlie the same function in closely related strains. However, deviating KaiC conformations result in different interactions with KaiA dimer (Figure 5, Figure S7, and Table S1). Further, the NMR KaiA:KaiC peptide complex cannot be fused with a single KaiC hexamer as N-termini of KaiC peptides point in opposite directions (Figure S5).

In summary, the crystal structure of the KaiA:KaiC peptide complex reveals that C-terminal IDRs of KaiC bound to KaiA dimer adopt an α-helical conformation. The interaction is predominantly stabilized by hydrophobic contributions, and this complex and the structure of KaiC hexamer can be almost seamlessly combined to generate a model of the full-length KaiA:KaiC complex. Docking of KaiA to KaiC peptides will likely destabilize the underlying A-loops (E487−I497 region), but not necessarily require a complete unravelling of the loops,[16,18] to boost KaiC phosphorylation via a concerted allosteric mechanism.[19] The model of the KaiA:KaiC complex derived here is fully consistent with the consequences of site-directed mutagenesis. Thus, deletion of the KaiC C-terminal tails (Δ25 mutant) abolishes clock function,[17] and mutations in the KaiA apical loop region (located close to the KaiCII dome surface) result in severely distorted clock periods (Figure S8).[17]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.biochem.5b00694.

Methods and materials, Figures S1−S8, Table S1 (PDF).

Accession Codes

The Protein Data Bank entry for the complex is 5C5E.

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Notes

The authors declare no competing financial interest.

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Supporting Information

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Table of Contents:

Protein expression and purification

The *S. elongatus* KaiA protein with a C-terminal His_6-tag was overexpressed in *Escherichia coli* (BL21 cell line, Novagen) and purified following previously described protocols.[1] The quality of the protein was assessed by SDS-PAGE (4-20%, BioRad). The modified C-terminal KaiC peptide NH_3^+-DEKSE LSRIV RGVQE KGPES-[K-5-FITC]-COO^- was purchased in purified form from CPC Scientific (Sunnyvale, CA).

Crystallization experiments

The KaiA protein buffer (50 mM Tris pH 7.8 and 150 mM NaCl) was exchanged with 20 mM Hepes pH 7.5 and 20 mM NaCl for crystallization. The protein concentration was ca. 14 mg/mL and the peptide concentration was 1 mg/mL. Protein (35 μL) and peptide (3 μL) solutions were mixed and the mixture incubated at 4 °C for five hours. Crystals of the complex were grown using the hanging drop vapor diffusion technique. The reservoir solution was 250 mM ammonium acetate, 100 mM sodium citrate dihydrate, pH 5.6, and 30% (w/v) PEG 4000. Equal volumes (1.5 μL) of the complex and reservoir solutions were mixed and the droplets equilibrated against 1 mL of reservoir solution. Crystals grew within a week at a temperature of 18 °C and were subsequently soaked in cryo solution (reservoir solution with 25% glycerol added) prior to mounting in nylon loops (**Figure S2**) and storage in liquid nitrogen before data collection.

X-ray data collection, structure determination and refinement

X-ray diffraction data were collected on the 21-ID-F beam line of the Life Sciences Collaborative Access Team (LS-CAT) at the Advanced Photon Source (APS), located at Argonne National Laboratory (Argonne, IL), using a MARCCD 300 detector at a wavelength of 0.978 Å. Crystals were kept at 110 K during data collection. Diffraction data were integrated, scaled and merged with HKL2000.[4] Selected data collection and refinement statistics are listed in **Table 1**. The structure was determined by Molecular Replacement with the program Phaser,[5] using the crystal structure of the KaiA dimer from *S. elongatus* (PDB ID 4G86) as the search model.[3] Initial rigid body refinement and all subsequent refinement was performed with PHENIX,[6] setting aside 4.8% of reflections to calculate the R-free. Manual rebuilding of the model was done in Coot.[7] KaiC peptides were identified in Fourier $2F_o$-F_c difference electron

density maps. An example of the quality of the final omit electron density around a KaiC peptide is depicted in **Figure 2** and refinement statistics are summarized in **Table 1**.

Data deposition

Atomic coordinates and structure factor data for the KaiA:KaiC-peptide complex have been deposited in the Protein Data Bank (http://www.rcsb.org, entry code 5C5E).

Circular dichroism spectroscopy

The CD spectrum was recorded at room temperature on a JASCO J-710 spectropolarimeter (JASCO Inc., Easton, MD), using a cell with 1 cm path length, a response time of 1 sec, a scan rate of 5 nm/min, and a step resolution of 1 nm across a wavelength range from 190 to 250 nm. Two scans were signal-averaged. The peptide concentration was 2.5 mg/mL in 20 mM Hepes, pH 7.5, and 20 mM NaCl.

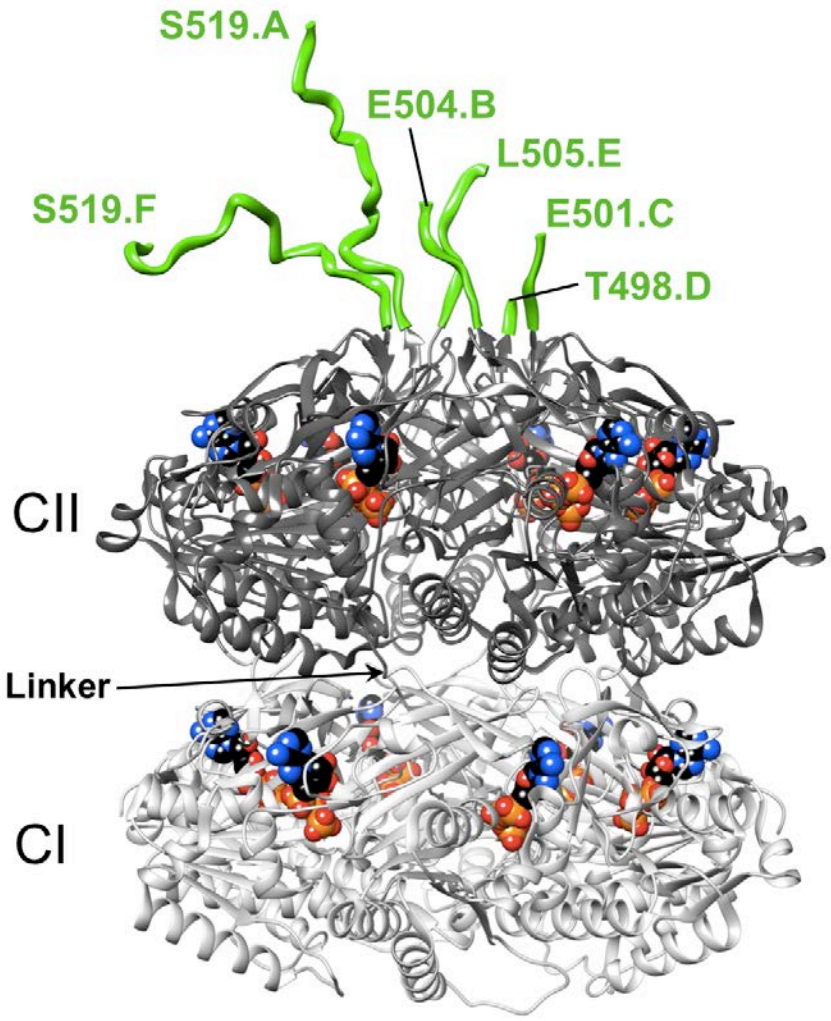

Figure S1. Architecture of the *S. elongatus* KaiC protein.[8,9] KaiC features two domains - N-terminal CI and C-terminal CII - that are the result of a gene duplication,[10] and forms a homo-hexamer (PDB ID code 3DVL). Twelve ATP molecules are lodged between subunits (labeled A to F), six each in the CI and CII rings. The C-terminal intrinsically disordered region (IDR) comprising residues 498 to 519 and traced to various lengths in the crystal structure at 2.8 Å is highlighted in green. Individual C-terminal tails exhibit various degrees of disorder as a result of differing lattice contacts with nearest neighbors in the KaiC crystal.[9] The image was generated with the program UCSF Chimera.[11]

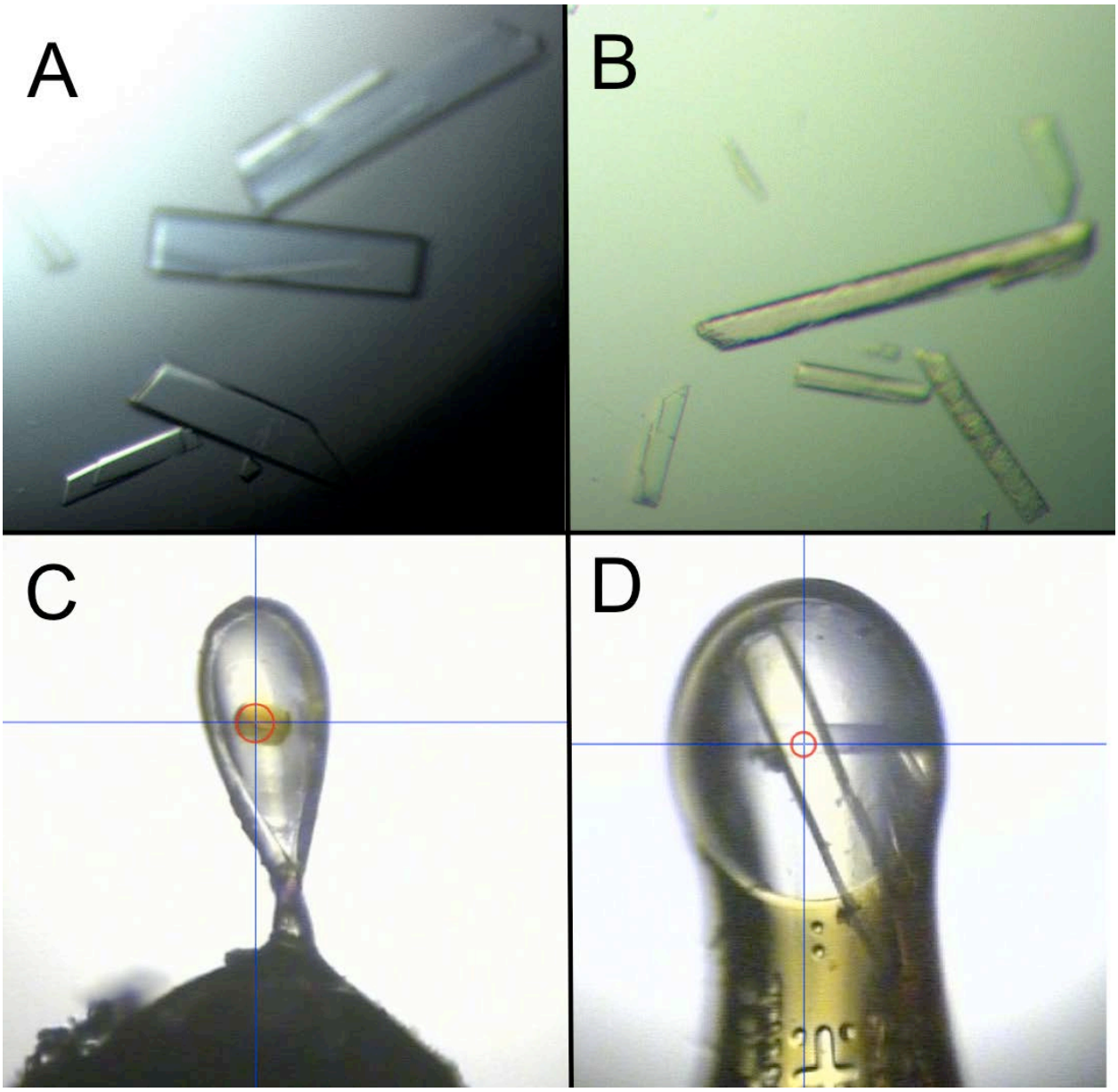

Figure S2. (A) Crystals of full-length *S. elongatus* KaiA dimer. (B) Crystals of the complex between KaiA dimer and color-labeled C-terminal KaiC peptide NH_3^+-DEKSE LSRIV RGVQE KGPES-[K-5-FITC]-COO^- obtained by co-crystallization; 5-FITC is fluorescein-5-isothiocyanate. (C and D) Frozen crystals of the KaiA:KaiC-peptide complex mounted in nylon loops.

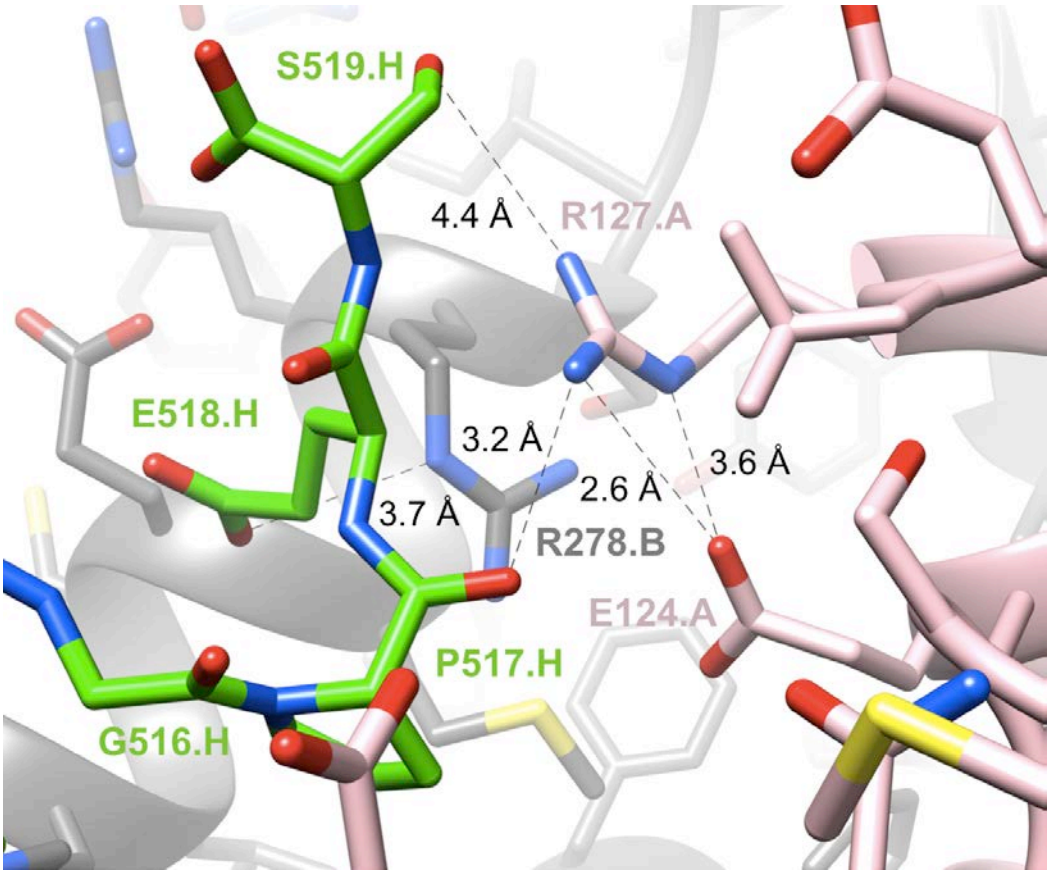

Figure S3. Interactions at the C-terminal end of the KaiC peptide (H chain; carbon atoms colored in light green). The peptide interacts almost exclusively with the C-terminal domain of KaiA dimer (carbon atoms colored in grey) and the closest distances between an amino acid from the N-terminal domain of one KaiA subunit (colored in pink; R127) and KaiC residues are 4.4 Å (Oγ, S519) and 3.2 Å (C=O, P517). The image was generated with the program UCSF Chimera.[11]

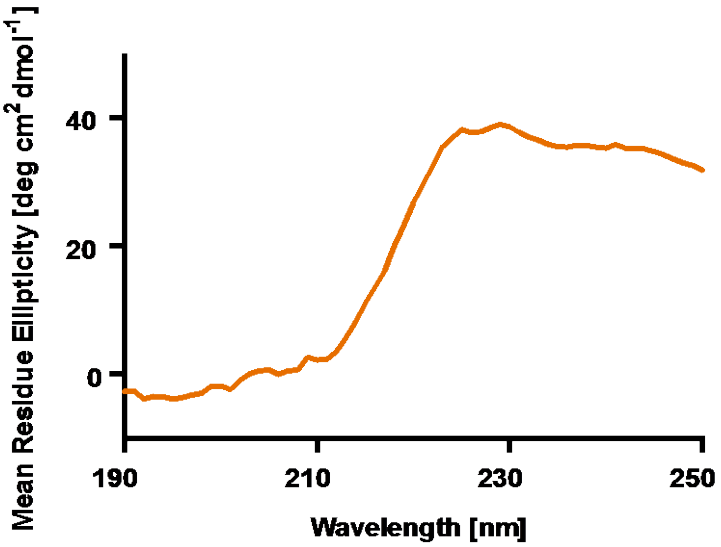

Figure S4. Circular dichroism (CD) spectrum of the K-FITC-labeled, C-terminal *S. elongatus* KaiC 21mer peptide *N*-DEKSE LSRIV RGVQE KGPES K(FITC)-*C*. The spectrum is consistent with a random coil conformation of the peptide, i.e. a minimum at ca. 195 nm and a maximum at ca. 220 nm.

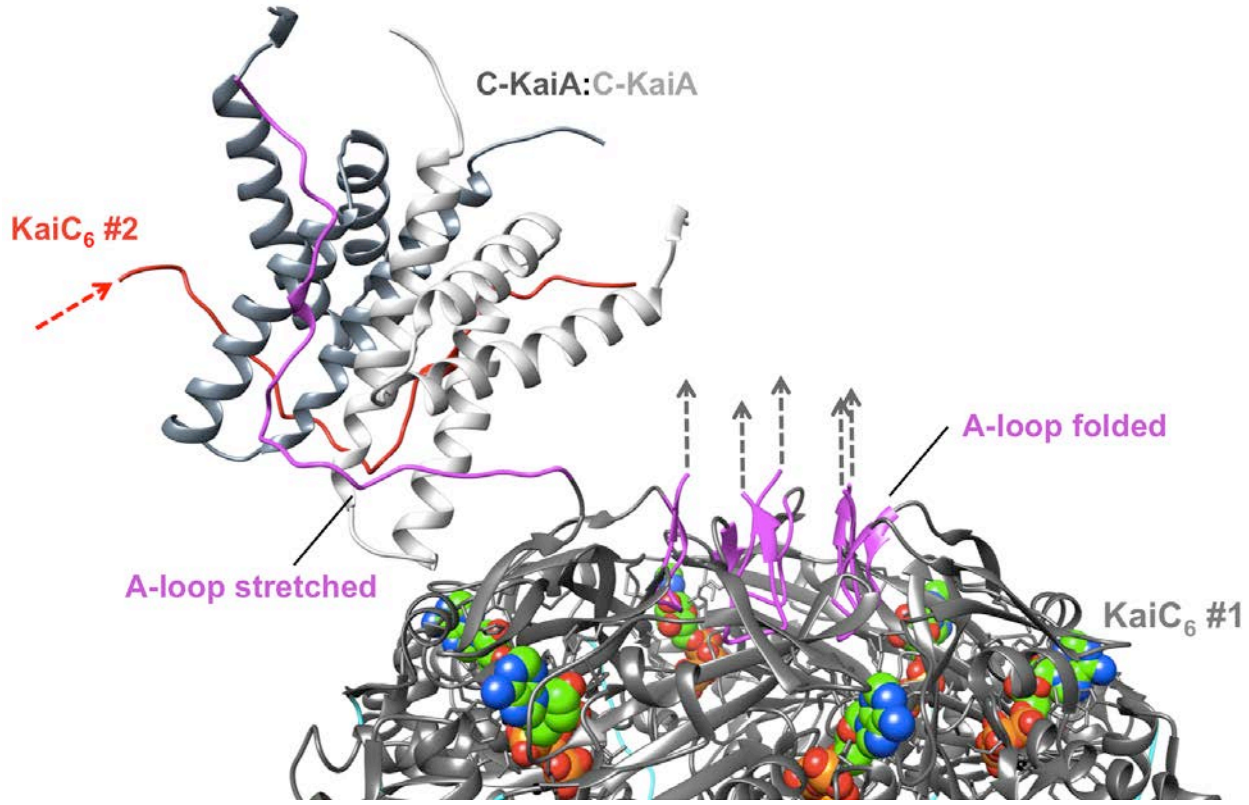

Figure S5. KaiA dimer (only C-terminal domains, C-KaiA, are depicted and subunits are colored in light gray and dark grey) interacting with KaiC C-terminal peptides (red and magenta) based on the NMR structure of the protein/peptide from *T. elongatus*[12] (PDB ID code 1SUY). In the NMR structure the region of the peptide that corresponds to the A-loop (residues 487-497) adopts a stretched conformation. In the crystal structures of the KaiC hexamers from *S. elongatus*[8,9] (PDB ID code 3DVL; shown here) and *T. elongatus*[13] (PDB ID code 4O0M), A-loops (highlighted in magenta) are folded into the C-terminal ring and the last 20 residues constitute an intrinsically disordered region (IDR, grey dashed arrows; see **Figure S1**). The model of the complex shown is a fusion of the KaiC crystal structure and the NMR structure of the KaiA:KaiC-peptide complex. The model demonstrates that a KaiA dimer interacting with KaiC peptides that adopt a kinked, random coil conformation with unraveled A-loops as observed by NMR cannot bind to a single KaiC hexamer ($KaiC_6$ #1). Instead the second C-terminal tail would have to be contributed by another KaiC hexamer ($KaiC_6$ #2; highlighted in red). The image was generated with the program UCSF Chimera.[11]

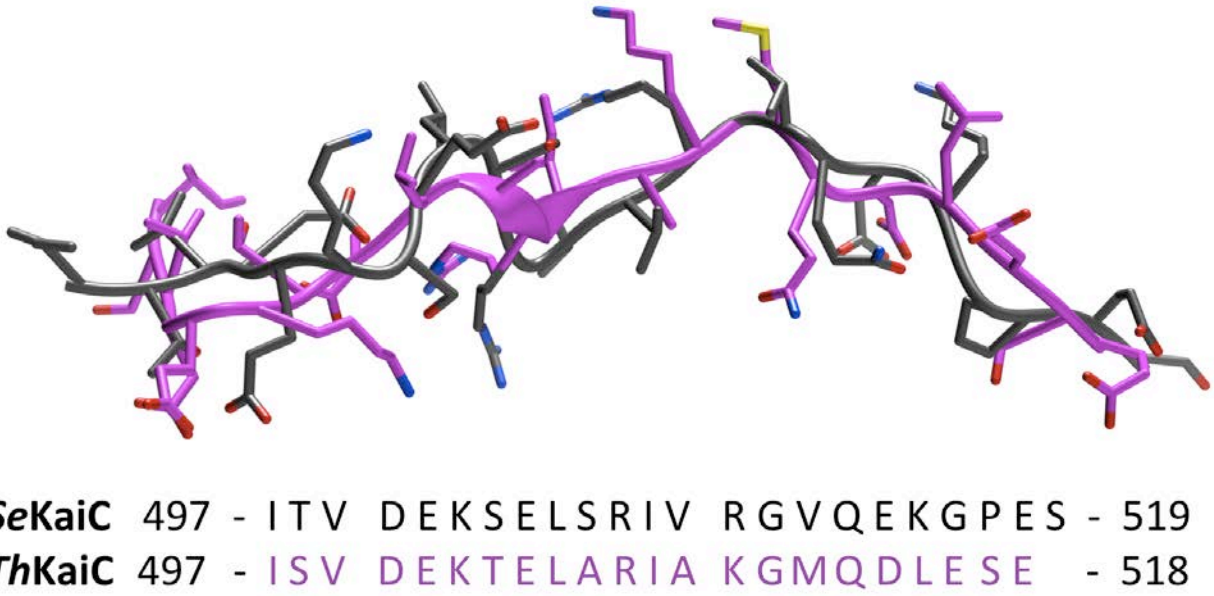

Figure S6. Overlay of the C-terminal KaiC peptides from the crystal structure of *S. elongatus* KaiC hexamer[8,9] (PDB ID code 3DVL; subunit A, carbon atoms colored in grey) and the NMR solution structure of the *T. elongatus* C-KaiA:KaiC-peptide complex[12] (PDB ID code 1SUY; carbon atoms colored in magenta), indicating similar, random coil conformations of the peptide in the free and bound states. Note, however, the drastically different conformation of *S. elongatus* KaiC peptide (residues D500 to S519) bound to full-length KaiA dimer in the crystal structure of the KaiA:KaiC-peptide complex (**Figures 3** and **4**; main paper). The drawing was generated using the MatchMaker option in the program UCSF Chimera.[11]

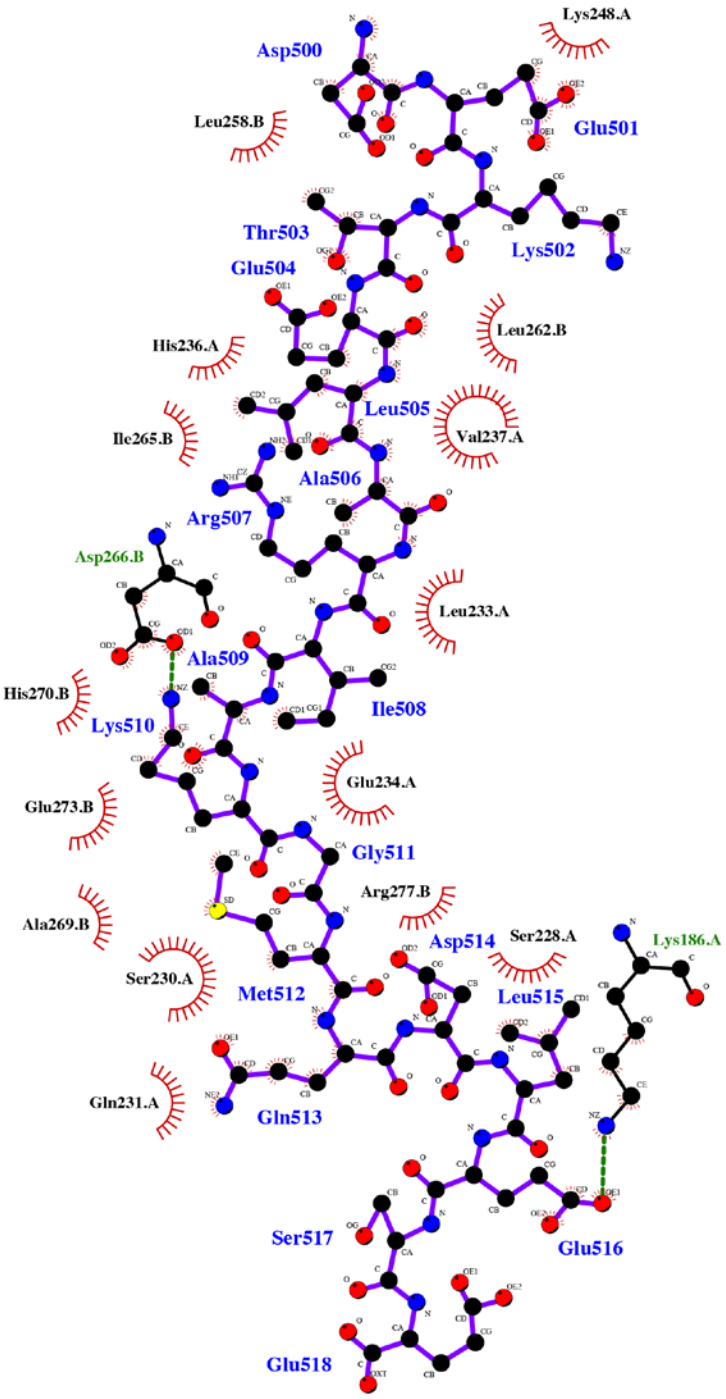

Figure S7. Ligplot analysis of KaiC : KaiA interactions (KaiC C chain, residues D500-E518; C-terminal domain of KaiA) in the NMR solution structure of the $KaiA_2$:KaiC-peptide complex.[12] KaiA and KaiC residues were renumbered to match residue numbers in the crystal structure of the complex with full-length KaiA protein. Peptide bonds are purple, KaiA bonds are black, H-bonds are dashed green lines and hydrophobic contacts are indicated with spoked arcs in red.

Table S1. Comparison of KaiA:KaiC interactions in the crystal structure and NMR solution structure[12] of complexes between the proteins from *S.* and *T. elongatus*, respectively.[a]

Crystal, *S. elongatus* KaiA$_2$:KaiC peptide			**NMR, *T. elongatus C*-KaiA$_2$:KaiC peptide**		
KaiA residue	*Distance [Å]*	*KaiC residue*	*KaiC residue*	*Distance [Å]*	*KaiA residue*
D242.A/*OD1*	(3.78)[b]	D500/*N*	D500/*OD2*	(3.71)[b]	K248.A/*NZ*
—	—	E501	E501/*OE1*	(3.54)	S244.A/*OG*
—	—	K502	K502	—	—
—	—	S503	T503	—	—
—	—	E504	E504	—	—
L263.B	hydrophobic	L505	L505	hydrophobic	I265.B
—	—	S506	A506	hydrophobic	L233.A
—	—	R507	R507	—	—
L263.B	hydrophobic	I508	I508	—	—
D267.B	hydrophobic	V509	A509	—	—
E235.A/*OE2*	3.21	R510/*NH2*	K510/*NZ*	2.14	D266.B/*OD1*
—	—	G511	G511	—	—
N213.B	hydrophobic	V512	M512	hydrophobic	E273.B
H271.B/*ND1*	(3.31)	Q513/*NE2*	Q513/*NE2*	2.21	E234.A/*OE1*
T231.A	hydrophobic	Q513	—	—	—
—	—	E514	D514	—	—
—	—	K515	L515	—	—
—	—	G516	E516/OE1,2	2.95, 3.01	K186.A/*NZ*
R127.A/*NH2*[c]	3.21	P517/*O*	S517	—	—
R278.B/*NE*	(3.73)	E518/*OE1*	E518	—	—
—	—	S519	—	—	—

[a] The *T. elongatus* KaiC peptide in the NMR solution structure is a 35mer. Only the C-terminal 19 residues D500-E518 were included for the comparison, and KaiA and KaiC residues in the published structure[12] were renumbered to match numbers of corresponding residues in the crystal structure of the *S. elongatus* KaiA:KaiC-peptide complex. Unlike in the crystal, the complex in solution adopts twofold rotational symmetry. Only KaiC peptide H from the crystal structure is included in the binding analysis. (interactions by peptide G are similar). Hydrophobic contacts and H-bonds/salt bridges are highlighted in blue and red, resp.

[b] 3.3 Å cut-off for H-bonds/salt-bridges; distances between 3.31 and 4.0 Å are included in parentheses.

[c] Amino acid from the N-terminal KaiA domain. The complex in the crystal structure is based on full-length *S. elongatus* KaiA dimer. The complex in the NMR structure features the C-terminal domain of KaiA (*C*-KaiA).

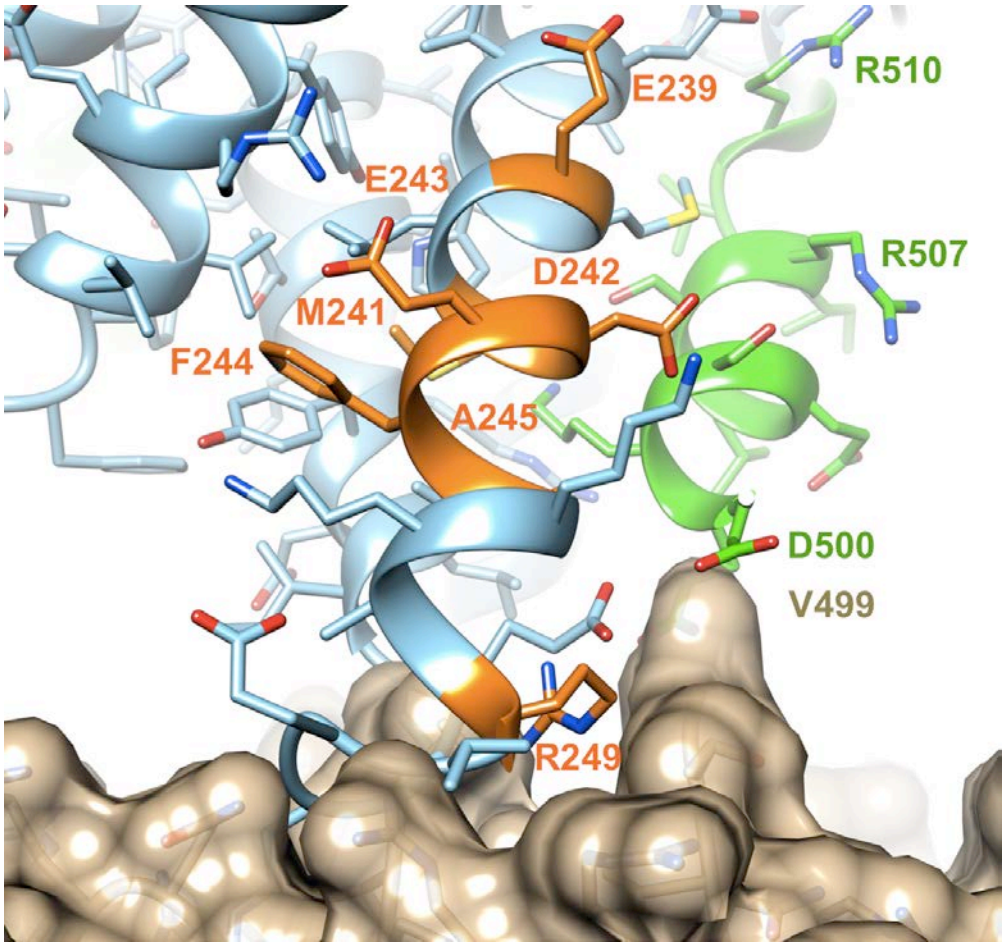

Figure S8. Postulated secondary interaction mode between the KaiA apical loop region and the KaiCII dome surface based on the model of full-length KaiA:KaiC complex depicted in **Figure 6** (main paper). Contacts between C-terminal KaiC peptide, residues D500-S519 (green), and KaiA dimer are considered the primary interaction. Side chains of KaiA amino acids are shown in stick mode and residues that result in periods > 24 h upon mutation are highlighted in orange: E239G, M241T, D242V/G, E243A, F244V, A245D, and R249H. The drawing was generated with the program UCSF Chimera.[11] To avoid a clash between KaiC residues protruding from the dome-shaped KaiCII surface (**Figure S1**) and amino acids from the KaiA apical loop region (R249-D256), the conformation of the KaiC A-loop (E487-I497) seen in the crystal structure of the hexamer alone (**Figure S4**) needs to adapt. However, completely pulling out the A-loops from the KaiC barrel and adoption of a stretched geometry (as shown in **Figure S4**) appear unnecessary.

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