# Direct measurements of clock-protein dynamics using quasielastic neutron scattering

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## 1. Introduction

Circadian (approximately 24 h) clocks are endogenous timing systems enabling organisms to fit daily changes in environments. These time-measuring systems share three properties. First, the clock systems reveal an oscillation with a circadian period even without any external cues (self-sustained oscillation). Second, the period of the clock systems is little dependent on the temperature (temperature compensation). Third, the phase of the oscillator can be shifted forward/backward in response to the changes of light/temperature to effect the fitness to the diurnal fluctuation of the environment (synchronization).

It has become widely accepted, especially in eukaryotes, that clock genes and their translational products (clock proteins) constitute a negative-feedback loop as a core oscillator to realize transcriptional-translational oscillation (TTO). On the other hand, both the transcription and translation proceed in the order of minutes, and thus it remains unclear how and why the circadian systems composed of fast-moving macromolecules can be so slow and stable.

To address the issue, we have used cyanobacterium *Synechococcus elongatus* PCC 7942 as the model system and looked for the minimal-and-slow reaction correlated to the frequency of the clock oscillator (Abe *et al*. Science 2015). The rate-limiting reaction we discovered was the steady-state ATPase activity (11 $d^{-1}$) in the N-terminal half of the clock protein KaiC (KaiC1). The ATPase activity in KaiC1 behaves as the pacemaker of *in vivo* frequency of TTO rhythm; i.e. the higher ATPase *in vitro* resulted in higher frequency of TTO *in vivo*. Furthermore, we recorded the time-response curves of KaiC ATPase, and our system-control analyses uncovered a natural frequency of the KaiC ATPase. Astonishingly, the natural frequency of KaiC ATPase was 0.91 $d^{-1}$, in good agreement with the frequency of the *in vivo* cyanobacterial clock (1 $d^{-1}$). This is the first experimental demonstration that *in vivo* frequency of TTO is related on one-to-one correspondence to the rate of minimal biochemical reaction within the particular clock protein. This correspondence implies that the rotation period of the Earth is inherently "encoded" behind the regulatory mechanism of slow KaiC ATPase.

In this research proposal, we conducted a series of quasi-elastic neutron scattering (QENS) experiments to address following two points;

1. Correlation between KaiC dynamics and ATPase activity (circadian clock frequency)
2. Mechanism behind temperature compensation of KaiC ATPase.

## 2. Experiment

Wild-type KaiC (KaiC-WT), a short-period KaiC mutant (KaiC-SP), and a long-period KaiC mutant (KaiC-LP) were expressed in *E. coli*. and purified as described previously (*EMBO J*. **26**, 4029-4037, 2007). After a buffer exchange into a $D_2O$ buffer, the KaiC samples were concentrated up to approximately 10 mg/ml using ultrafiltration. QENS measurements were conducted for the KaiC samples and the $D_2O$ buffer at five different temperatures between 278 and 313 K using the near-backscattering spectrometer, BL02 (DNA), at J-PARC MLF. The obtained QENS spectra, $S(Q,\omega)$, were corrected for contributions from an empty cell and for detector efficiency, and then normalized relative to that of a vanadium standard.

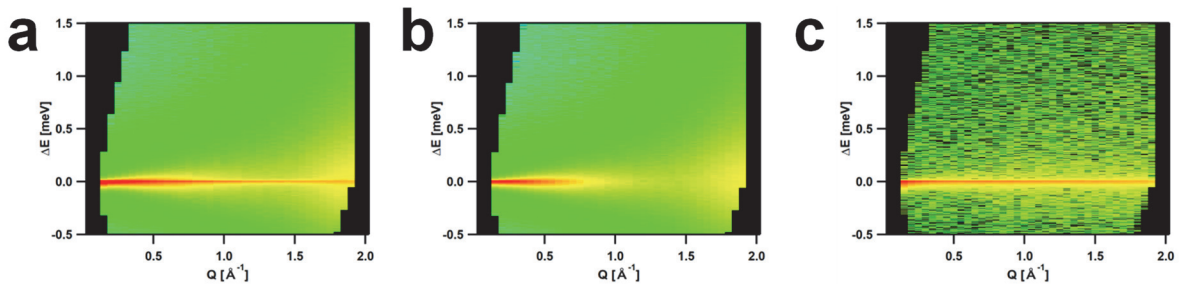


**Figure 1**. QENS profiles at 313 K for a sample solution of KaiC-WT (**a**), a $D_2O$ buffer (**b**), and KaiC-WT after subtracting contributions of $D_2O$ (**c**).

## 3. Results

Figs. 1a and 1b show QENS profiles for the KaiC sample and the $D_2O$ buffer, respectively, recorded at 313 K. Contributions from the $D_2O$ buffer (Fig. 1b) were subtracted from the sample dataset (Fig. 1a) using scaling factors calculated from theoretical scattering cross section, and then a resultant QENS spectrum (Fig. 1c) of wild-type KaiC (KaiC-WT) was fitted to the following equation,

$$S(Q,\omega) = [A_0(Q)\delta(\omega) + A_1(Q)L_{local}(Q,\omega)] \otimes L_{global}(Q,\omega) \otimes RF(Q,\omega) + BG(Q)$$

where $A_0(Q)$ and $A_1(Q)$ are amplitudes of global and local motions, respectively; $\delta(\omega)$ is Dirac delta-function; $L_{local}(Q,\omega)$ and $L_{global}(Q,\omega)$ are Lorentzian functions describing local and global atomic motions, respectively; $R(Q,\omega)$ is an instrumental resolution function; $BG(Q)$ is a background function. Fig.2 is a typical result of fitting to the experimental QENS spectrum of KaiC-WT at $Q$ = 1.225 $Å^{-1}$.

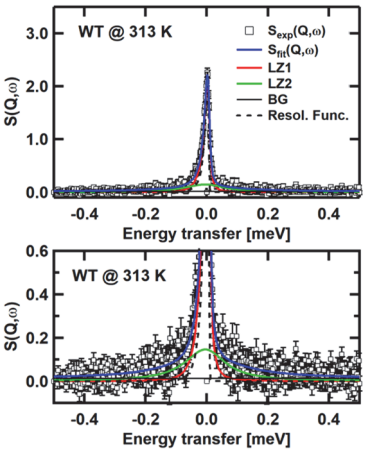


**Figure 2**. QENS spectrum of KaiC-WT at $Q$ = 1.225 $Å^{-1}$ at 313 K.

A half-width at half-maximum (HWHM) of $L_{global}(Q,\omega)$, $\Gamma_{global}(Q)$, provides information on diffusive motions of proteins. The $\Gamma_{global}(Q)$ values for KaiC-WT increased linearly with $Q^2$. Apparent diffusion coefficients, $D_{app}$, were estimated from the linear slope of a plot of $\Gamma_{global}(Q)$ against $Q^2$. The $D_{app}$ values of KaiC-SP ($4.42 \pm 0.22 \times 10^{-7}$ $cm^2$ $s^{-1}$) and KaiC-LP ($4.30 \pm 0.17 \times 10^{-7}$ $cm^2$ $s^{-1}$) were indistinguishable from that of KaiC-WT ($4.42 \pm 0.14 \times 10^{-7}$ $cm^2$ $s^{-1}$) at 313 K, indicating that all KaiC samples adopted the functional hexamer throughout the QENS measurements.

A HWHM of $L_{local}(Q,\omega)$, $\Gamma_{local}$ $(Q)$, provides information on local motions of proteins. $Q^2$-dependency of $\Gamma_{local}(Q)$ was analyzed under the assumption of jump-diffusion model to estimate local residence time ($\tau$). Interestingly, the $\tau$ value for KaiC-WT revealed a unique temperature dependence. A slope of an Arrhenius plot of the $\tau$ value for KaiC-WT is seemingly different from each of other two KaiC mutants, suggesting a potential correlation between the speed of the clock oscillator (ATPase activity) and the local motions in KaiC.

## 4. Conclusion

Although great care must be taken on interpreting the present data with a limited S/N ratio, the local motions in KaiC are seemingly more sensitive to the clock property than we expected. We believe that our first QENS experiment conducted under the approval of the trial use is successful and should be a good starting point to further experiments using a variety of the KaiC mutants.