In situ monitoring of a slight intermediate during DNA phosphorylation by T4 polynucleotide kinase for transient kinetic studies

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Supplementary Information

Sauerbrey's Equation (S1) was used for the AT-cut shear mode QCM in the air phase,

$$\Delta F_{\mathrm{air}} = -\frac{2F_0^2}{A\sqrt{\rho_q \mu_q}}\Delta m \qquad \text{(S1)}$$

where ΔF_{air} is the measured frequency change [Hz] in the air phase, F_0 is the fundamental frequency of the quartz crystal prior to a mass change [27×10^6 Hz], Δm is the mass change [g], A is the electrode area [0.033 cm^2], ρ_q is the density of quartz [2.65 $g \cdot cm^{-3}$], and μ_q is the shear modulus of quartz [2.95×10^{11} dyn cm^{-2}]. In the air phase, 0.62 ng cm^{-2} of mass increase per 1 Hz of frequency decrease was obtained in previous experiments. This agrees well with the value of 0.61 ng cm^{-2} Hz^{-1}, calculated numerically from Eq. S1. While the frequency change measured in the water phase can be described by Eq. S2,

$$\Delta F_{\mathrm{water}} = -\frac{\Delta F_{\mathrm{water}}}{\Delta F_{\mathrm{air}}} \cdot \frac{2F_0^2}{A\sqrt{\rho_q \mu_q}}\Delta m \qquad \text{(S2)}$$

where $\Delta F_{\mathrm{water}}$ is the measured frequency change in the water phase (Hz). A converting factor $\Delta F_{\mathrm{water}} / \Delta F_{\mathrm{air}}$ is introduced into Eq. S1 to assess the effects of the hydrodynamic water and/or viscoelasticity of the biomolecules on a QCM.[20] When $\Delta F_{\mathrm{water}}$ and ΔF_{air} values were measured for dsDNAs immobilized onto a QCM plate, a good linear correlation between $\Delta F_{\mathrm{water}}$ and ΔF_{air} with a slope ($=\Delta F_{\mathrm{water}} / \Delta F_{\mathrm{air}}$) of 6.3 was observed. Thus, frequency decreases ($\Delta F_{\mathrm{water}}$) owing to binding of a 30 bp dsDNA were 6.3 times larger than those in the air phase (ΔF_{air}), because hydrating water vibrates with dsDNA on a oscillating QCM plate. Therefore, the

$\Delta F_{water} / \Delta F_{air}$ value for the 30 bp dsDNA was determined to be 6.3 ± 0.2 and the converting factor of Sauerbrey's Equation [Eq. (S2)] for 30 bp dsDNA in aqueous solutions was 0.62/6.3 = 0.10 ± 0.02 ng·cm^{-2} Hz^{-1}. We also applied this converting factor ($\Delta F_{water} / \Delta F_{air}$ = 6.3) to the enzyme bound to the dsDNA, because when the enzyme is bound to the DNA it should vibrate with the DNA. In fact, we have observed that the binding of DNA-binding peptides and polymerase or DNase to the dsDNA (20 – 60 bp) shows a 1:1 molar binding ratio from ΔF_{water} in aqueous solutions. This indicates that peptides and proteins bound to the dsDNA may vibrate with DNA in a similar manner.[15,18,19]

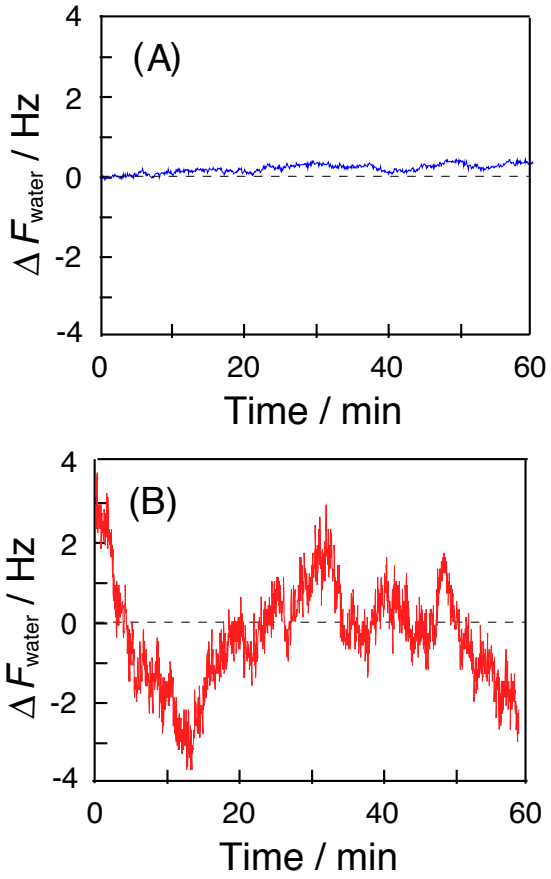

Fig. S1. (A) Typical time courses of a frequency change (ΔF_{water}) of the flow cell-type QCM, flowing ultrapure water (Milli-Q water) at a flow rate of 10 μL/min at 25 °C. (B) Typical time courses of a frequency change (ΔF_{water}) of the batch cell-type QCM in ultrapure water (Milli-Q water) at 25 °C.

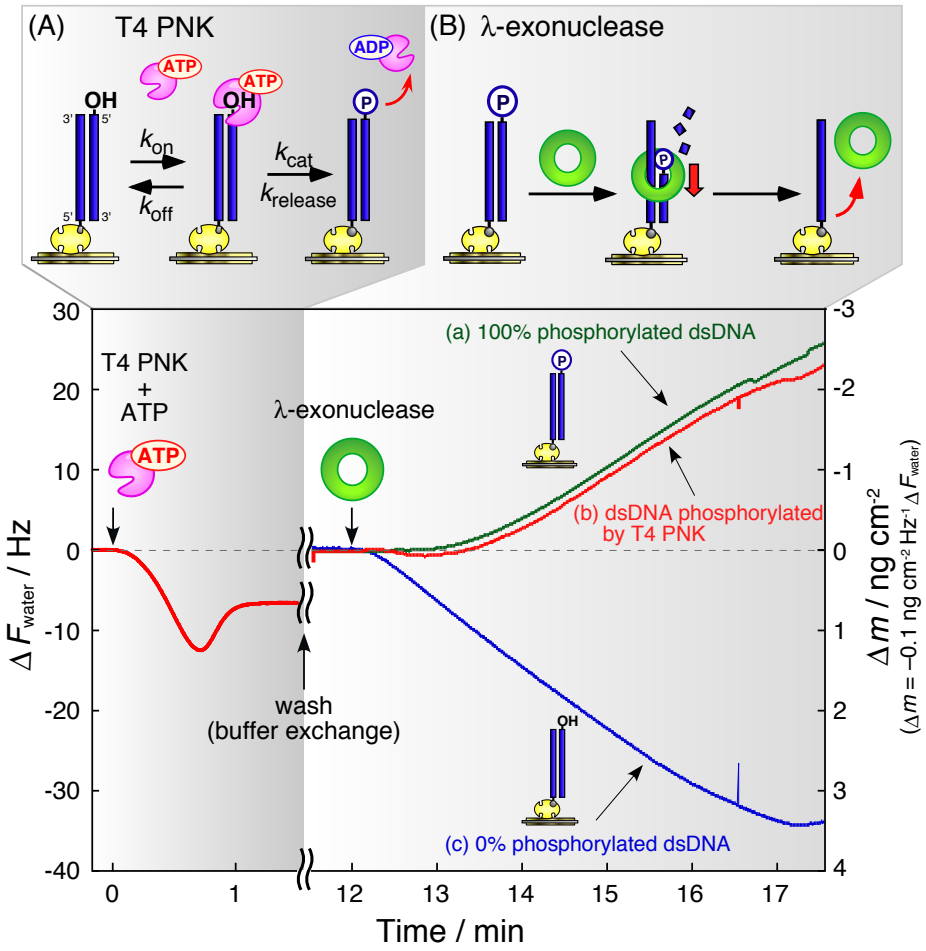

Fig. S2. Observation of (A) phosphorylation of 5′-OH dsDNA catalyzed by T4 PNK ([T4 PNK]=7.1 nM, 50 mM Tris-HCl, pH 8.0, 10 mM $MgCl_2$, 200 mM NaCl at 25 °C), and (B) degradation of 5′-phosphorylated dsDNA to 3′-ssDNA catalyzed by λ-exonuclease at a flow rate of 100 μL/min for 5 min ([λ-exonuclease]=10 nM, 50 mM Tris-HCl, pH 9.0, 2.5 mM $MgCl_2$ at 25 °C). The frequency increase (mass decrease) of the red curve (b) responding to the addition of λ-exonuclease indicates that T4 PNK phosphorylates the 5′-OH dsDNA. λ-Exonuclease then degrades the 5′-phosphorylated dsDNA to ssDNA on the QCM. The green curve (a) and the blue curve (c) indicate degradation of synthetic 5′-phosphorylated dsDNA and binding to 5′-OH dsDNA by λ-exonuclease, respectively. From a comparison of the red curve (b) with these model experiments, the phosphorylation rate of dsDNA by T4 PNK determined using the QCM plate is estimated to be 98%.

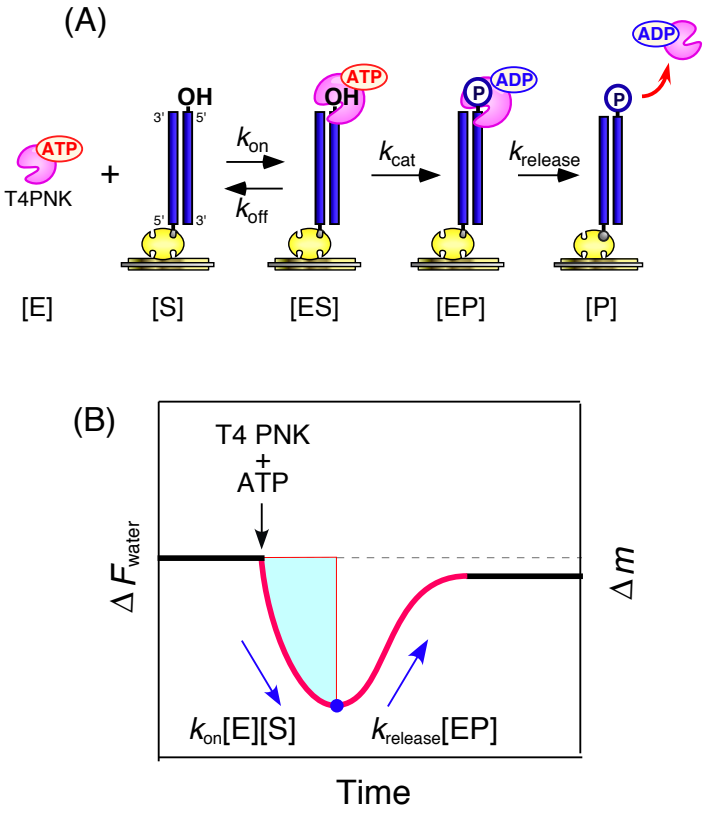

Fig. S3. (A) Reaction schemes describing phosphorylation of the dsDNA-immobilized QCM by T4 PNK with ATP and (B) schematic illustrations of the frequency change (ΔF_{water}) during the phosphorylation process. Rates of the frequency decrease (mass increase) and the frequency increase (mass decrease) correspond to k_{on}[E][S] and $k_{release}$[EP], respectively. These have equality at the time of minimum ΔF_{water} (a graph vertex).

$$k_{on}[E][S] = k_{release}[EP]$$

When the minimum ΔF_{water} is –20 Hz, [EP] is calculated to be 2 ng cm^{-2} (0.016 pmol cm^{-2}) using the calibration of 0.1 ng cm^{-2} Hz^{-1} (see the calibration of the 27-MHz QCM) and the value of MW for T4 PNK tetramer (132 kDa). Thus, [S] is calculated to be 0.019 pmol cm^{-2} using values of k_{on} =1.2 × 10^{6} M^{-1} s^{-1}, $k_{release}$ = 0.02 s^{-1}, and [E] = 14 nM. Because the amount of immobilized dsDNAs, $[S]_0$ is 1.0 pmol cm^{-2}, the amount of the phosphorylated dsDNA ([P]) is estimated to be 0.98 pmol·cm^{-2}. This suggests that 98% of the immobilized dsDNAs should be phosphorylated at time of the minimum ΔF_{water} (a graph vertex).

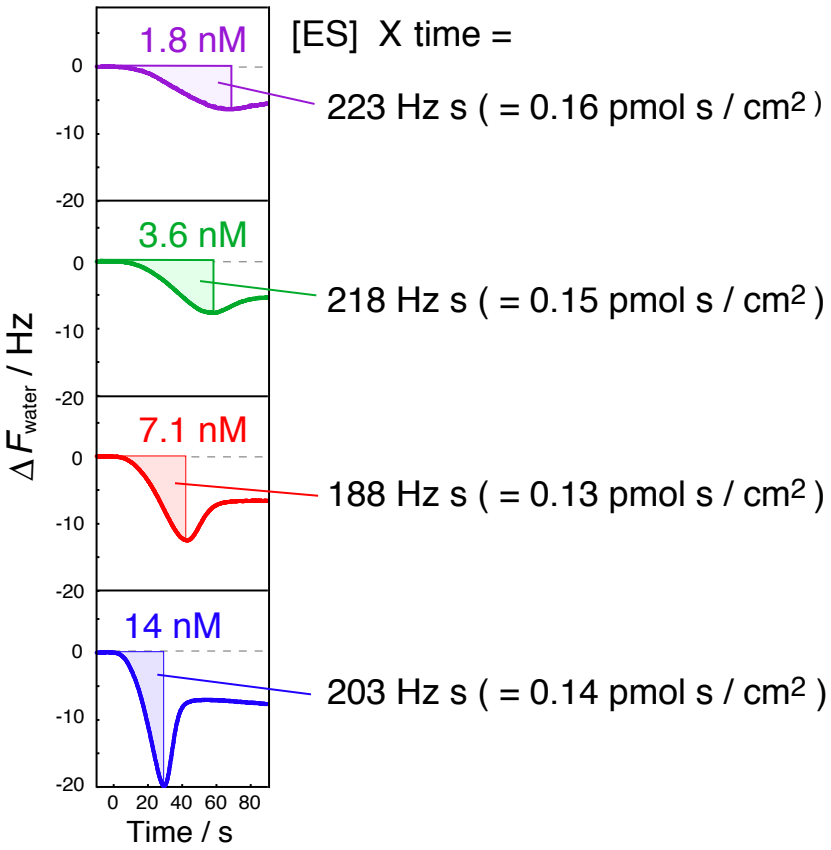

Fig. S4. Integration values of [ES] against time *t* obtained from the area between the time-axis and the curve of ΔF_{water} corresponding to the amount of the ES complex in Fig. 2A. These values indicate around 200 Hz/s even in different enzyme concentrations. The values are calculated to be 0.145 ± 0.006 pmol cm^{-2} using the calibration of 0.1 ng cm^{-2} Hz^{-1} and a value of MW for the T4 PNK tetramer (132 kDa). The k_{cat} value was calculated to be 7.1 ± 0.28 s^{-1} from the following equation, using the value of the amount of the phosphorylated dsDNA [P-DNA] (1.0 pmol cm^{-2}).

$$k_{\mathrm{cat}} = \frac{[P-DNA]}{[ES]\times \mathrm{Time}}$$