

AIMS Biophysics, 5(2): 144–154. DOI: 10.3934/biophy.2018.2.144 Received: 14 March 2018 Accepted: 26 April 2018 Published: 10 May 2018

http://www.aimspress.com/journal/biophysics

**Research** article

## Single-molecule FRET reveals proofreading complexes in the large fragment of *Bacillus stearothermophilus* DNA polymerase I

## Thomas D. Christian and William H. Konigsberg\*

Konigsberg Laboratory, Yale University, 333 Cedar Street, New Haven, CT 06520, USA

\* **Correspondence:** Email: william.konigsberg@yale.edu; Tel: +2037854599.

## **Supplementary**



**Figure S1.** Cartoon Representation of Bst pol I LF and the Cy3B-labeled duplex. (A) The sequence of the Biotinylated 20mer primer and the 30 mer-Cy3B labeled template stratd. The location of the C<sub>2</sub>-amine modified thymidine residue is depicted in blue on the template strand. The 5'-end of the primer strand is biotinylated and the 3'-end of the primer is either deoxy or dideoxy terminated. (B) A cartoon representation of Bst pol I LF with the location of modified thymidine residue shown as blue spheres and the location of the cysteine mutation depicted in red spheres on the fingers subdomain. (C) A cartoon diagram depicting the polymerase domain in red and the exonuclease domain in yellow.



**Figure S2.** Polymerization and Exonuclease Crystal Structures of DNA polymerase I. (A) The open binary structure of Bst pol I LF with the DNA depicted in black with the primer-template terminus occupying the polymerase active site. (B) The binary complex of the Klenow fragment of *E. coli* DNA polymerase I where the primer-template terminus has melted and the primer terminus is now located in the exonuclease active site. The location of the donor and acceptor dyes have been depicted as blue and red spheres, respectively.



**Figure S3.** Bulk-solution FRET titrations. (A) Fluorescence spectra of a typical titration in bulk-solution. As the Atto647N-labeled Bst pol I is added to a 10 nM solution of Cy3B-labled duplex the intensity of Cy3B decreases and the intensity of the Atto647N increases due to Forster resonance energy transfer. (B) The decrease in donor intensity can be measured and converted into an apparent FRET efficiency. The increase in FRET efficiency vs polymerase concentration can be fit to a rectangular hyperbola yielding a  $K_{d,app}$  for binary complex formation. Top panel: Titration using the fully complementary DNA duplex yielding a  $K_{d,app}$  of 24 nM. Lower panel: Titration using a DNA duplex with 4 mismatched base pairs at the primer terminus yielded a  $K_{d,app}$  of 46 nM.



**Figure S4.** Example Intensity and FRET trajectory from a typical experiment. (A) The donor intensity is depicted in blue and the acceptor intensity is depicted in red. At this location we see two biding events occurring between 500 and 800 frames. (B) A magnified view of the binding events. Here we observe two distinct anti-correlated binding events. (C) Converting the intensity trajectories to a FRET trajectory we see the polymerase on this particular DNA primer-template spends more time in the lower FRET state than in the higher FRET state. The horizontal lines demarcate the FRET states that we have assigned to the open, ajar, closed and exo states (0.25, 0.35, 0.60 and 0.80 states, respectively).



**Figure S5.** Histogram of Compiled Intensity Trajectories for Deoxy and Dideoxy Terminated Substrates. (A) The histogram compiled from binding events for the Biotinylated-20 mer/30 mer-Cy3B deoxy terminated substrate. Peak 1 corresponds to unbound DNA (0.00848). Peak 2 corresponds to the open, binary complex (0.215). Peak 3 corresponds to the exonuclease binary complex (0.826). (B) The off rate of the polymerase estimated from the lifetimes of the collision complexes observed on the deoxy terminated substrate. (C) The histogram compiled from the binding events recorded on the biotinylated-20 mer/30 mer-Cy3B dideoxy terminated substrate. Peak 1 corresponds to unbound DNA (0.0184). Peak 2 corresponds to the open, binary complex (0.275). In this histogram the high FRET state is dwarfed by the long lived 0.275 FRET state. (D) The off rate of the polymerase estimated from the lifetimes of the collision complexes observed on the dideoxy terminated substrate.



300 250

A.

Figure S6. FRET Histograms of the Ternary Complex of Atto647N-labeled triple mutant. (A) Right panel. The FRET histogram for the binding of Atto647N-labeled triple mutant to a deoxy terminated substrate in the presence of 200  $\mu$ M dCpcpp. Peak 1 represents the unbound DNA FRET state (0.00488). Peak 2 corresponds to the ajar conformation (0.292). Peak 3 corresponds to the closed state (0.638) and Peak 4 corresponds to the exo bound conformation (0.874). (A) Left panel. The FRET histogram for the binding of the labeled mutant to the deoxy terminated substrate in the presence of 1.00 mM dApcpp. Peak 1 corresponds to the unbound DNA FRET state (0.00664). Peak 2 corresponds to the ajar state (0.315). Peak 3 most closely corresponds to the pre-proofreading complex (0.548) observed with mismatched DNA and Peak 4 corresponds to the exo bound conformation (0.851). (B) Right panel. The FRET histogram for the binding of Atto647N-labeled triple mutant to a dideoxy terminated substrate in the presence of 200 µM dCTP. Peak 1 corresponds to the unbound DNA FRET state (0.00571). Peak 2 corresponds to the Ajar state (0.374). Peak 3 represents the closed state (0.637) and Peak 4 represents the Exo state (0.883). (B) Left panel. The FRET histogram of the binding of labeled triple mutant to the dideoxy terminated substrate in the presence of 1.00 mM dATP. The Gaussians fit best to a three state model with Peak 1 representing the unbound DNA FRET state (0.00884). Peak 2 represents the ajar state (0.306) and Peak 3 most closely resembles the closed state (0.698), but most likely contains contributions from both Closed and Exo states. The difference in the FRET peaks of the two ajar states in Figure 6A right panel and Figure 6A left panel is likely due to the two different DNA substrates that were used.



**Figure S7.** Histogram and Lifetime Analysis of the Collision Complexes of Atto647N-labeled triple mutant and the biotinylated-20 mer 4 mismatch/30 mer-Cy3B deoxy terminated substrate. (A) The compilation of individual binding events into a FRET histogram reveals that the polymerase binds in two predominant FRET states; Peaks 0.525 state and a 0.801 state. The 0.525 peak likely represents the pre-proofreading complex observed with mismatched DNA and the 0.801 FRET state represents the exo bound conformation similar to Figure 6A left panel (B) Lifetime analysis of the collision complex yields an off rate (k<sub>-1</sub>) of 1.49 s<sup>-1</sup>; similar to that of the fully complementary deoxy terminated duplex.



© 2018 the Author(s), licensee AIMS Press. This is an open access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0)