## Supporting Information

# Direct Probing of Solvent Accessibility and Mobility at the Binding Interface of Polymerase (Dpo4)-DNA Complex

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### Data Analysis

All the fluorescence transients can be fitted by a multi-exponential decay function. The apo protein transients can be fitted by three solvation components and two lifetime components. The DNA transients can be fitted by a triple-exponential decay function. The transients of the complex consist of contributions from both protein and DNA. Therefore, we build a general fitting model for the complex signal:

$$S_{complex}=\left(\sum_{i=1}^{3} a_i e^{-\frac{t}{\tau_i}}\right)_{solvation} + \left(\sum_{i=4}^{5} a_i e^{-\frac{t}{\tau_i}}\right)_{lifetime} + \varepsilon \times \left(\sum_{j=1}^{3} a_j e^{-\frac{t}{\tau_j}}\right)_{DNA} \quad [S1]$$

The lifetime $\tau_4$ and $\tau_5$ can be directly determined by the time correlated single photon counting (TCSPC) measurements. $\varepsilon$ is a parameter to account for the DNA contribution to the overall complex signal. The accurate DNA signal in the complex can be obtained from our control mutant of Y312W or the wild-type complex (no tryptophan) and thus $a_j$ and $\tau_j$ are precisely determined.

Y312W, a control mutant, contains a tryptophan which does not interact with the DNA in the complex and hence, the solvation does not change from the apo to the complex state. Therefore, we measured the fluorescence transients of Y312W in the apo and complex states, then subtracted the apo-protein contribution from the complex signal and thus obtained the DNA signal in the complex form. Meanwhile, we also measured the fluorescence transients of DNA in a buffer solution. We observed that the DNA signal does not change from the buffer condition to the complex environment (see Figure S1), indicating no changes of DNA base-pair structures and related hydration. For Dpo4, we can also determine the DNA signal in the complex directly from the wild-type Dpo4-DNA complex because the wild-type Dpo4 contains no single tryptophan. All these methods to determine the DNA signal in the complex state also meet the requirement that the integration ratio of protein and DNA decay transients should match the ratio of their steady-state emissions at a given wavelength.

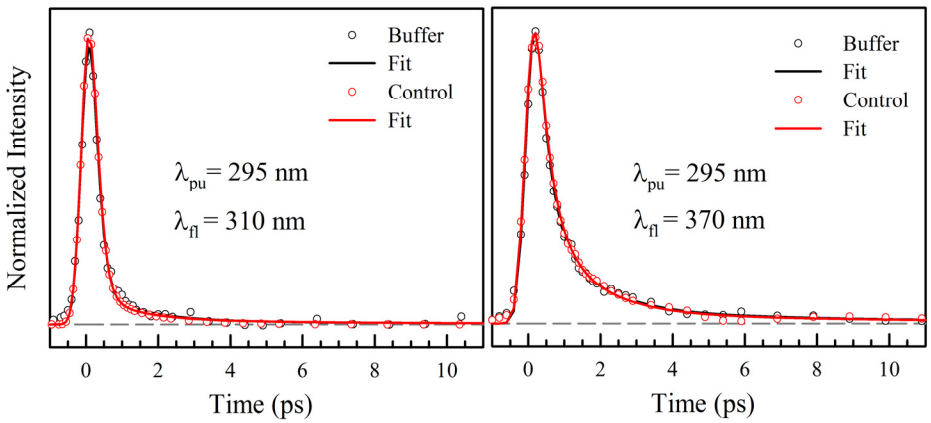


**Figure S1.** Fluorescence transients of DNA at two typical wavelengths of 310 nm (left) and 370 nm (right). The transients measured from DNA in buffer solution (black) and reproduced from the control of Y312W (red) overlap very well. This clearly indicates that DNA in buffer solution has the similar dynamics as in the Dpo4 complex. The scatter points and lines are the experimental data and best exponential fit, respectively.

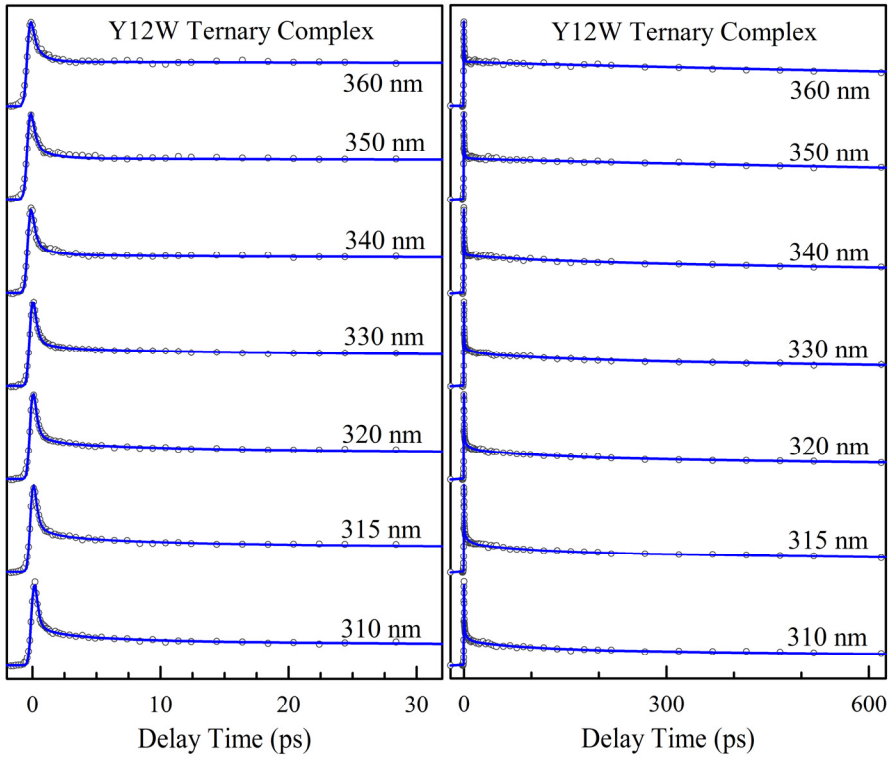


**Figure S2.** Normalized fluorescence transients of the Y12W ternary complex at several typical gated wavelengths in the short (left) and long (right) time ranges. The circles are the experimental data and the solid lines are the best exponential fits.

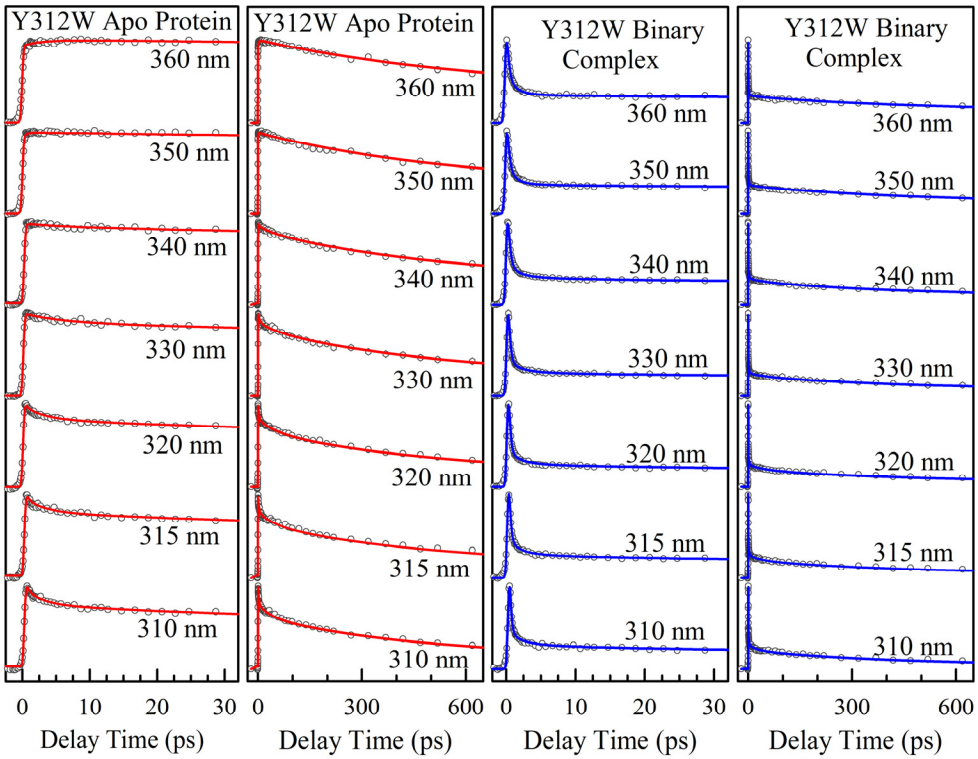


**Figure S3.** Normalized fluorescence transients of the mutant Y312W in the apo state (left two) and the binary complex (right two) at several typical gated wavelengths on the short and long time scales. The circles and lines are the experimental data and best exponential fits, respectively. The transients of the Y312W ternary complex are extremely similar to those of the binary complex and thus are not shown here.

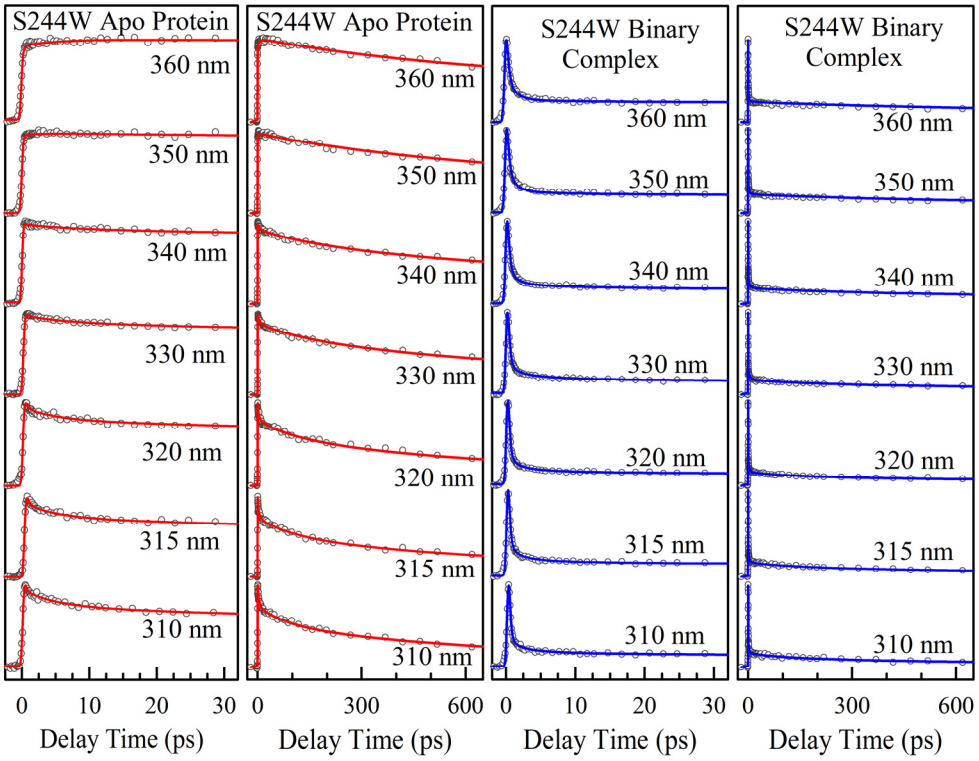


**Figure S4.** Normalized fluorescence transients of the mutant S244W in the apo state (left two) and the binary complex (right two) at several typical gated wavelengths in the short and long time ranges. The circles and lines are the experimental data and best exponential fits, respectively. The transients of the ternary complex are extremely similar to those of the binary complex and therefore are not shown here.

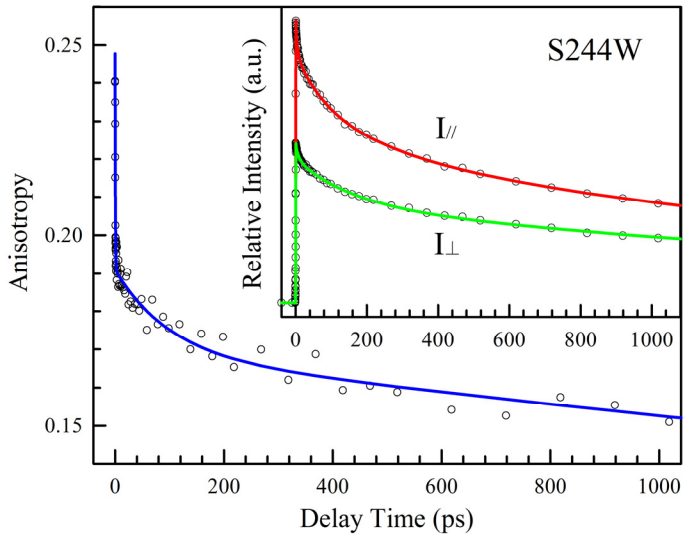


**Figure S5.** Anisotropy dynamics of S244W in the apo state. The inset shows the experimental parallel and perpendicular fluorescence transients. The scatter points are the experiment data and the solid lines are the best exponential fits.

**Table S1**. All the fitting results and the corresponding characteristic quantities.

| | Y312W | | | S244W | | | Y12W | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Apo** | **Binary** | **Ternary** | **Apo** | **Binary** | **Ternary** | **Apo** | **Binary** | **Ternary** |
| $\lambda_{ss}$ (nm) | 339.1 | 339.7 | 339.7 | 342.2 | 342 | 342 | 341.1 | 341.3 | 341.1 |
| $A_1$ | 0.342 | 0.332 | 0.331 | 0.409 | 0.094 | 0.089 | 0.393 | 0.234 | 0.166 |
| $A_2$ | 0.360 | 0.367 | 0.369 | 0.309 | 0.458 | 0.448 | 0.333 | 0.412 | 0.416 |
| $A_3$ | 0.298 | 0.301 | 0.300 | 0.282 | 0.448 | 0.463 | 0.274 | 0.354 | 0.418 |
| $\tau_1$ (ps) | 0.35 | 0.34 | 0.36 | 0.37 | 0.86 | 0.83 | 0.43 | 0.62 | 0.91 |
| $\tau_2$ (ps) | 3.4 | 3.5 | 3.6 | 4.8 | 6.1 | 6.2 | 5.2 | 7.4 | 8.3 |
| $\tau_3$ (ps) | 52 | 54 | 52 | 111 | 136 | 135 | 120 | 140 | 140 |
| $E_1$ ($cm^{-1}$) | 362 | 370 | 374 | 539 | 119 | 112 | 534 | 277 | 196 |
| $E_2$ ($cm^{-1}$) | 381 | 409 | 416 | 407 | 579 | 564 | 453 | 488 | 491 |
| $E_3$ ($cm^{-1}$) | 315 | 336 | 339 | 372 | 567 | 582 | 372 | 419 | 494 |
| E ($cm^{-1}$) | 1058 | 1115 | 1129 | 1318 | 1265 | 1258 | 1359 | 1184 | 1181 |
| $S_1$ ($cm^{-1}$/ps) | 1034 | 1089 | 1038 | 1457 | 138 | 135 | 1242 | 447 | 215 |
| $S_2$ ($cm^{-1}$/ps) | 112 | 117 | 116 | 85 | 95 | 91 | 87 | 66 | 59 |
| $S_3$ ($cm^{-1}$/ps) | 6.1 | 6.2 | 6.5 | 3.3 | 4.2 | 4.3 | 3.1 | 3.0 | 3.5 |
| $\tau_w$ (ps) | 42 | 45 | 43 | 99 | 158 | 165 | 114 | 139 | 133 |
| θ (degree) | 11.2 | 12.0 | 11.4 | 11.5 | 6.3 | 8.6 | 13.1 | 10.2 | 10.3 |
| ω (degree/ps) | 0.267 | 0.267 | 0.265 | 0.116 | 0.040 | 0.052 | 0.115 | 0.073 | 0.077 |