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Supplemental Information

Single-Molecule DNA Polymerase Dynamics at a Bacterial Replisome in Live Cells

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Single-molecule DNA polymerase dynamics at a bacterial replisome in live cells

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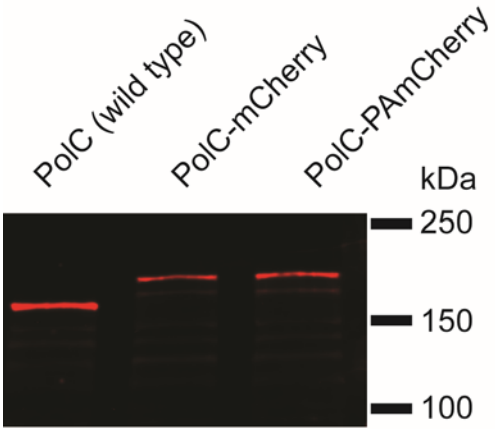

Figure S1. Western blot of PolC and PolC variants in *B. subtilis*. Shown are Western blots of PolC, PolC-mCherry and PolC-PAmCherry from whole cell lysates of *B. subtilis* cells. We used anti-serum directed against purified PolC at a 1:1000 dilution (lot #1266) as described previously (Liao et al., *PNAS* 2015; main text ref. 35). Cells were harvested in exponential phase at OD_{600} 0.5 – 0.7 prior to analysis. The Western blot was developed using LI-COR imaging with a goat anti rabbit conjugated IR dye secondary.

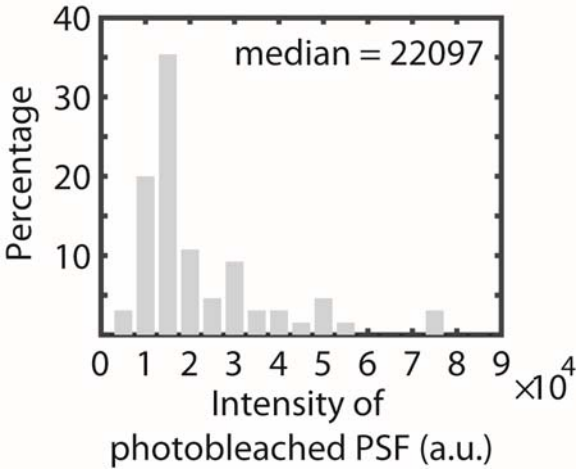

Figure S2. Distribution of single PolC-mCherry molecule intensities from photobleaching measurements. The smallest jump between two states (i.e., the step corresponding to ΔI_{min} in each intensity time trace in Fig. 1c) identifies a single PolC-mCherry molecule undergoing photobleaching within that PolC-mCherry focus. To obtain the intensity of the single PolC-mCherry molecule that was photobleached in this step, we compute the difference between the average image over 10 frames before the change point and the average image over 10 frames after the change point. This difference is a background-subtracted image of the photobleached PolC-mCherry molecule; this point spread function (PSF) was then fit to a 2D Gaussian function to obtain the intensity.

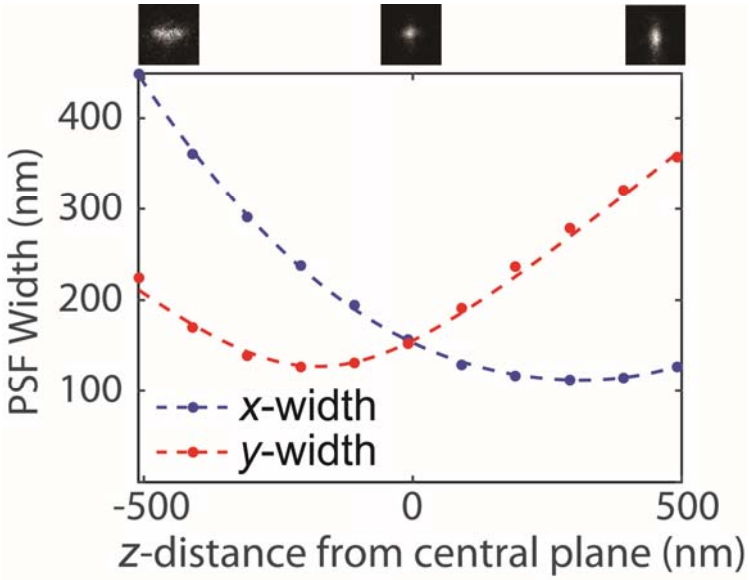

Figure S3. Calibration curve for 3D localization microscopy, with sample point spread functions (PSFs) of single PolC-PAmCherry molecules at various *z*-positions shown on top. For a given signal, the *z*-position is determined from the PSF widths in the *x*- and *y*-directions.

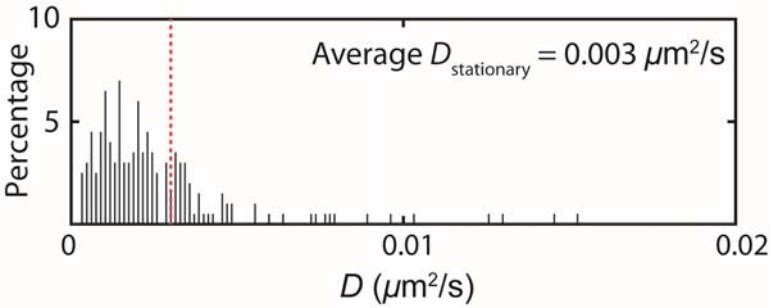

Figure S4. Distribution of apparent diffusion coefficients for PolC-PAmCherry trajectories in fixed cells. The red dashed line represents the average apparent diffusion coefficient of immobile PolC-PAmCherry molecules; this value of 0.003 $\mu m^2/s$ is far slower than even the slowest measured diffusion coefficients (~0.01 – 0.1 $\mu m^2/s$) for PolC-PAmCherry in living cells.

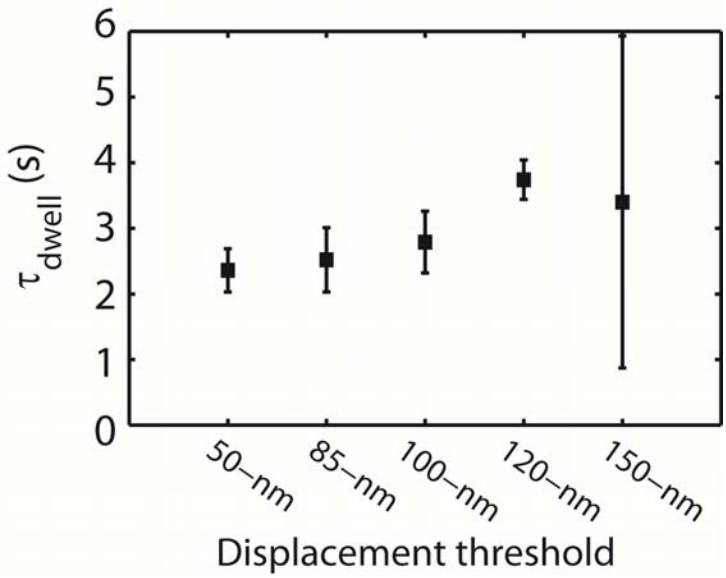

Figure S5. Dwell time analysis performed using different step sizes as the cutoff threshold for defining a dwelling event. Error bars are from bootstrapping. The dwell times obtained were generally not sensitive to the particular step size threshold value until up to ~120 nm. However, when an even larger threshold such as a 150-nm cutoff is chosen, the fit was poor and the dwell time estimation was highly uncertain, indicating that our one-term dwell time model is not sufficient to describe the behavior of PolC when such a large step is taken. These results suggest that considering PolC molecule step sizes above 150 nm includes PolC dynamics characterized by more than one dwelling time scales, likely due to both the fast moving and the slower PolC populations. Results in the text (Figure 3) are reported based on a threshold of 100 nm.

Table S1. Bacterial strains used in this study

Strain Name	Genotype	Source
PY79	Wild type, SPβ^{o}	Youngman P., Perkins, J. B., and Losick, R. 1984. *Plasmid* 12:1-9.
YL001	*polC-mCherry*	This work.
JWS213	*polC*-PAmCherry	This work.