

Abstract Submitted
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Single-Molecule Measurements of Synthesis by DNA Polymerase with Base-Pair Resolution¹ THOMAS CHRISTIAN, LOUIS ROMANO, DAVID RUEDA, Wayne State University — The catalytic mechanism of DNA polymerases involves multiple steps that precede and follow the transfer of a nucleotide to the 3'-hydroxyl of the growing DNA chain. Here we report a single-molecule approach to monitor the movement of *E. coli* DNA polymerase I (Klenow fragment) on a DNA template during DNA synthesis with single base-pair resolution. As each nucleotide is incorporated, the single-molecule Förster resonance energy transfer intensity drops in discrete steps to values consistent with single nucleotide incorporations. Purines and pyrimidines are incorporated with comparable rates. A mismatched primer-template junction exhibits dynamics consistent with the primer moving into the exonuclease domain, which was used to determine the fraction of primer-termini bound to the exonuclease and polymerase sites. Most interestingly, we observe a structural change following the incorporation of a correctly paired nucleotide, consistent with transient movement of the polymerase past the pre-insertion site or a conformational change in the polymerase. This may represent a previously unobserved step in the mechanism of DNA synthesis that could be part of the proofreading process.

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