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Single-molecule comparison of DNA Pol I activity with native and analog nucleotides OSMAN GUL, TIVOLI OLSEN, YONGKI CHOI, BRAD CORSO, GREGORY WEISS, PHILIP COLLINS, Univ of California - Irvine — DNA polymerases are critical enzymes for DNA replication, and because of their complex catalytic cycle they are excellent targets for investigation by single-molecule experimental techniques. Recently, we studied the Klenow fragment (KF) of DNA polymerase I using a label-free, electronic technique involving single KF molecules attached to carbon nanotube transistors [1]. The electronic technique allowed long-duration monitoring of a single KF molecule while processing thousands of template strands. Processivity of up to 42 nucleotide bases was directly observed, and statistical analysis of the recordings determined key kinetic parameters for the enzyme's open and closed conformations. Subsequently, we have used the same technique to compare the incorporation of canonical nucleotides like dATP to analogs like 1-thio-2'-dATP. The analog had almost no affect on duration of the closed conformation, during which the nucleotide is incorporated. On the other hand, the analog increased the rate-limiting duration of the open conformation by almost 40%. We propose that the thiolated analog interferes with KF's recognition and binding, two key steps that determine its ensemble turnover rate. [1] T. J. Olsen, et. al., JACS 135, 7855 (2013).

Osman Gul
Univ of California - Irvine

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