Abstract Submitted
for the MAR16 Meeting of
The American Physical Society

Single-Molecule Electronic Monitoring of DNA Polymerase Activity DENYS O. MARUSHCHAK, KAITLIN M. PUGLIESE, MACKENZIE W. TURVEY, YONGKI CHOI, O. TOLGA GUL, TIVOLI J. OLSEN, ARITH J. RAJAPAKSE, GREGORY A. WEISS, PHILIP G. COLLINS, Univ of California - Irvine
— Single-molecule techniques can reveal new spatial and kinetic details of the conformational changes occurring during enzymatic catalysis. Here, we investigate the activity of DNA polymerases using an electronic single-molecule technique based on carbon nanotube transistors. Single molecules of the Klenow fragment (KF) of polymerase I were conjugated to the transistors and then monitored via fluctuations in electrical conductance. Continuous, long-term monitoring recorded single KF molecules incorporating up to 10,000 new bases into single-stranded DNA templates. The duration of individual incorporation events was invariant across all analog and native nucleotides, indicating that the precise structure of different base pairs has no impact on the timing of incorporation. Despite similar timings, however, the signal magnitudes generated by certain analogs reveal alternate conformational states that do not occur with native nucleotides. The differences induced by these analogs suggest that the electronic technique is sensing KF's O-helix as it tests the stability of nascent base pairs [1]. [1] K.M. Pugliese, et. al., "Processive Incorporation of Deoxynucleoside Triphosphate Analogs by Single-Molecule DNA Polymerase I (Klenow Fragment) Nanocircuits." JACS 137, 9587 (2015).

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Date submitted: 06 Nov 2015 Electronic form version 1.4