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Single-stranded DNA scanning and deamination with Single molecule resolution

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Over the past decade, single-molecule fluorescence resonance energy transfer spectroscopy (smFRET) has become an increasingly popular tool to study the structural dynamics of biopolymers, such as DNA, RNA and proteins. The most attractive aspect of single-molecule experiments is that, unlike ensemble-averaged techniques, they directly reveal the structural dynamics of individual molecules, which would otherwise be hidden in ensemble-averaged experiments. Here, we will present a novel single molecule assay to study, for the first time, scanning of an enzyme (APOBEC3G, involved in the defense against HIV) on single stranded DNA (ssDNA). We have investigated the ssDNA scanning and activity of Apo3G with smFRET. Our data show that Apo3G scans ssDNA randomly and bidirectionally with average excursion lengths of $\sim$ 10 $\mathring{A}$ and $\sim$1 s-1 scanning rates. Apo3G quasi-localization is observed on highly reactive motifs located near the one end of the ssDNA. Motif-dependent ssDNA bending is also observed, where the bending is maximal for highly reactive targets located near the DNA end. Interestingly, both the Apo3G scanning and Apo3G-induced ssDNA bending is reduced with lowered ionic strength, indicating that Apo3G motion on ssDNA is facilitated by salt by reducing 'electrostatic friction'. Although scanning is random, asymmetric catalytic orientation may be the reason for Apo3G directional activity.