

MAR13-2012-008247

Abstract for an Invited Paper  
for the MAR13 Meeting of  
the American Physical Society

**Simultaneous measurement of DNA motor protein conformation and activity with combined optical trap and single-molecule fluorescence<sup>1</sup>**

YANN CHEMLA, University of Illinois, Urbana-Champaign

We present single-molecule measurements of Superfamily 1 UvrD helicase DNA unwinding that reveal directly how helicase stoichiometry and conformation regulate motor activity. Using a new instrument that combines high resolution optical tweezers with single-molecule fluorescence microscopy, we record DNA unwinding activity with base pair-scale resolution (via optical tweezers) simultaneously with helicase stoichiometry and conformation (via fluorescence). Quantifying the fluorescence signal from labeled UvrD, we observe that pairs of UvrD molecules are required for long distance unwinding but that individual molecules exhibit limited, non-processive unwinding activity. UvrD is also known to exhibit two different conformations, ‘closed’ and ‘open’, based on the orientation of its 2B regulatory domain. The function of these conformations has remained elusive. Measuring the fluorescence of FRET labeled proteins, we detect directly the conformation of the 2B domain of individual UvrD molecules during unwinding activity. We observe that UvrD is in the ‘closed’ conformation during DNA unwinding but surprisingly switches to the ‘open’ conformation upon reversal of helicase direction, i.e. when UvrD switches strands and translocates on the opposing strand with the DNA junction re-zipping behind it. We hypothesize that the 2B domain acts as a conformational switch that controls DNA unwinding vs. re-annealing.

<sup>1</sup>Work supported by NSF (PHY-082261, Center for the Physics of Living Cells) and NIH (R21 RR025341A)