Abstract Submitted
for the MAR16 Meeting of
The American Physical Society

Single molecule fluorescence studies of transition paths in DNA hairpin folding KATHERINE TRUEX, HOI SUNG CHUNG, JOHN LOUIS, WILLIAM EATON, National Institutes of Health — DNA hairpins are the simplest structures for investigating fundamental aspects of nucleic acid folding mechanisms. For two-state hairpins, all of the mechanistic information on how the hairpin folds is contained in the transition path (TP), the rare event in single molecule trajectories when the free energy barrier between folded and unfolded states is actually crossed. The only previous experimental study of TPs in nucleic acids used optical tweezer measurements and Szabo's analytical theory for diffusive barrier crossing to reconstruct the free energy surface for an indirect determination of average TP times (Neupane *et al.* *PRL* 2012). We used confocal single molecule FRET and maximum likelihood analysis of photon trajectories to determine an upper bound of 2.5 μs for the average TP time of a DNA hairpin (Truex *et al.*, *PRL* 2015), compared to the value of 4 μs predicted by Neupane *et al.*, providing an important test of energy landscape theory. Current experiments are aimed at eventually characterizing structural changes during TPs, which will provide a very demanding test of mechanisms predicted by both theoretical models and simulations.

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Date submitted: 06 Nov 2015 Electronic form version 1.4