Abstract Submitted
for the MAR14 Meeting of
The American Physical Society

Direct observation of transcription activator-like effector (TALE) protein dynamics LUKE CUCULIS, Department of Chemistry, University of Illinois at Urbana-Champaign, ZHANAR ABIL, Department of Biochemistry, University of Illinois at Urbana-Champaign, HUIMIN ZHAO, CHARLES M. SCHROEDER, Department of Chemical and Biomolecular Engineering, University of Illinois at Urbana-Champaign — In this work, we describe a single molecule assay to probe the site-search dynamics of transcription activator-like effector (TALE) proteins along DNA. In modern genetics, the ability to selectively edit the human genome is an unprecedented development, driven by recent advances in targeted nuclease proteins. Specific gene editing can be accomplished using TALE proteins, which are programmable DNA-binding proteins that can be fused to a nuclease domain. In this way, TALENs are a leading technology that has shown great success in the genomic editing of pluripotent stem cells. A major hurdle facing clinical implementation, however, is the potential for deleterious off-target binding events. For these reasons, a molecular-level understanding of TALE binding and target sequence search on DNA is essential. To this end, we developed a single-molecule fluorescence imaging assay that provides a first-of-its-kind view of the 1-D diffusion of TALE proteins along stretched DNA. Taken together with co-crystal structures of DNA-bound TALEs, our results suggest a rotationally-coupled, major groove tracking model for diffusion. We further report diffusion constants for TALE proteins as a function of salt concentration, consistent with previously described models of 1-D protein diffusion.

Luke Cuculis
Department of Chemistry, University of Illinois at Urbana-Champaign

Date submitted: 13 Nov 2013 Electronic form version 1.4