

Probing Transcription Factor Dynamics at the Single Molecule Level in a Living Cell

Johan Elf¹†, Gene-Wei Li²†, X. Sunney Xie¹*

¹Department of Chemistry and Chemical Biology, ²Department of Physics,
Harvard University, Cambridge, MA 02138

† These authors contributed equally to this work.

Transcription factors regulate gene expression through their binding to DNA. In a living *E. coli* cell, we directly observe specific binding of a lac repressor, labeled with a fluorescent protein, to a chromosomal lac operator. Using single-molecule detection techniques, we measure the kinetics of binding and unbinding of the repressor in response to metabolic signals. Furthermore, we characterize the nonspecific binding to DNA, 1D diffusion along DNA segments, and 3D translocation among segments through cytoplasm at the single molecule level. In searching for the operator, a lac repressor spends ~90% of time non-specifically bound to and diffusing along DNA with a residence time <5ms. The methods and findings can be generalized to other nucleic acid binding proteins.