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Binding Study of T7 Gene 2.5 Protein to Single- and Double–Stranded DNA from Single Molecule Stretching LEILA SHOKRI, Department of Physics, Northeastern University, BORIANA MARINTCHEVA, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, CHARLES C. RICHARDSON, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, MARK C. WILLIAMS, Department of Physics, Northeastern University — Bacteriophage T7 gene 2.5 protein binds preferentially to single-stranded DNA. This property is essential for its role in DNA replication, recombination, and repair. We present the first quantitative study of the thermodynamics and kinetics of equilibrium and non-equilibrium DNA helix destabilization in the presence of gp2.5 and a deletion mutant lacking 26 C-terminal amino acids that binds with higher affinity to ssDNA (gp2.5-delta26C). Our measured force-extension curves of lambda-DNA in the presence of these proteins suggest strong cooperative binding. By measuring the DNA melting force as a function of time and pulling rate, we obtained binding site size and the association constants of these proteins to ssDNA and dsDNA, over a range of salt and protein concentrations. The results are used to characterize the electrostatic interactions that determine the DNA-protein binding in each case.

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