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Unraveling the motion of single-stranded DNA binding proteins on DNA using force and fluorescence spectroscopy

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Single-stranded DNA binding (SSB) proteins bind to and control the accessibility of single stranded (ss) DNA generated as a transient intermediate during a variety of cellular processes. For subsequent DNA processing, however, such a tightly wrapped, high-affinity protein–DNA complex still needs to be removed or repositioned quickly for unhindered action of other proteins. Here we show, using single-molecule two- and three-colour fluorescence resonance energy transfer, that SSB can spontaneously migrate along ssDNA. Diffusional migration of SSB helps in the local displacement of SSB by an elongating RecA filament. SSB diffusion also melts short DNA hairpins transiently and stimulates RecA filament elongation on DNA with secondary structure. This observation of diffusional movement of a protein on ssDNA introduces a new model for how an SSB protein can be redistributed, while remaining tightly bound to ssDNA during recombination and repair processes. In addition, using an optomechanical tool combining single-molecule fluorescence and force methods, we probed how proteins with such a large binding site size ($\sim$ 65 nucleotides) can migrate rapidly on DNA and how protein-protein interactions and tension may modulate the motion. We observed force-induced progressive unravelling of ssDNA from the SSB surface between 1 and 6 pN, followed by SSB dissociation at $\sim$10 pN, and obtained experimental evidence of a reptation mechanism for protein movement along DNA wherein a protein slides via DNA bulge formation and propagation. SSB diffusion persists even when bound with RecO, and at forces under which the fully wrapped state is perturbed, suggesting that even in crowded cellular conditions SSB can act as a sliding platform to recruit and carry its interacting proteins for use in DNA replication, recombination and repair.