Label-free, multiplexed, single-molecule analysis of protein-DNA complexes with nanopores

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Protein interactions with specific DNA sequences are crucial in the control of gene expression and the regulation of replication. Single-molecule methods offer unique capabilities to unravel the mechanism and kinetics of these interactions. Here we develop a nanopore approach where a target DNA sequence is contained in a hairpin followed by a ssDNA. This system allows DNA-protein complexes to be distinguished from bare DNA molecules as they are pulled through a single nanopore detector, providing both the equilibrium and kinetic constants of the interactions. We show that this approach can be used to test the inhibitory effect of small molecules on complex formation, and their mechanisms of action. In a proof of concept, we use DNAs with different sequence patterns to probe the ability of the nanopore to distinguish the effects of an inhibitor in a complex mixture of target DNAs and proteins. We anticipate that the use of this technology with arrays of thousands of nanopores will drive the discovery of new inhibitors of transcription factor binding.