Translocation and capture kinetics of structurally defined DNA in solid-state nanopores

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The ability to fabricate solid-state nanopores in-situ by controlled-breakdown (CBD) has the potential to democratize the field of nanopore sensing. We present the new features of our small benchtop instrument that leverages this method, which allows users to fabricate solid-state nanopores of virtually any size in a fully automated fashion. Once fabricated, these nanopores are immediately ready to perform single-molecule sensing experiments. In addition, we investigate translocations of short, structured DNA molecules using solid-state nanopores. Using site-specific modifications with non-natural nucleotides along the backbone of DNA fragments, we are able to graft side-branches at fixed positions by “click” chemistry. The resulting structure is a T-shaped DNA molecule with a backbone of 51bp double-stranded DNA and a 25bp double-stranded DNA branch. Nanopores are fabricated by controlled breakdown (CBD) in ultra-thin 10-nm SiN membranes. The DNA is electrophoretically translocated through pores ranging from 3 to 10nm in size. By analyzing the ionic current blockades produced by these structured molecules and comparing them to regular, linear DNA, we are able to distinguish the different topologies. Such structurally-defined branched DNA molecules can be used for the development of multiplexed nanopore-based assays and as position-controlled building blocks of much larger DNA polymers to further our understanding of the fundamentals of molecular transport through nanopores by precisely measuring intra molecular velocity fluctuations.