Preparation and behavior of ssDNA in porous roof nanochannels

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While there is a large body of research describing and analyzing double-stranded (ds) DNA behavior in nanochannels, surprisingly little has been reported on long, single-stranded (ss) DNA molecules. Here, we present experiments demonstrating several approaches to introducing long ssDNA molecules into nanochannels monitoring of ssDNA behavior in nanochannels with a porous roof. The nanochannels were fabricated on quartz or silicon substrates using interferometric lithography to create a parallel array of 100- to 500-nm wide photoresist (PR) lines on a 1 μm period. The remaining bottom antireflection coating (BARC) in the channels was etched by reactive ion etching, and then ~ 50 nm diameter silica nanoparticles were self-assembled in ~ monolayer increments by spin coating of a colloidal nanoparticle suspension. The final fabrication step was an 800°C bake to burn out the PR/BARC and sinter the nanoparticles (Fig. 1). This chip design allows us to introduce DNA into the nanochannels either through wells etched to access the ends of the nanochannels or directly through the roof, since the nanopores formed by sintering the nanoparticles are large enough to allow snake-like movement of long DNA molecules through the pores.

Double-stranded lambda-phage DNA (48,502 base pairs and approximately 20 μm contour length) was used. The DNA was converted into single-stranded form using either heating at 95ºC for 10 min and rapid transfer to an ice bath at 0ºC ("snap-cooling"), or using digestion by lambda DNA exonuclease, an enzyme that selectively digests both strands of double-stranded DNA starting from their respective 5' ends. Both strands are digested to half-length, at which point the enzyme stops because ssDNA is not a substrate for lambda exonuclease. Both of these processes can be conducted in a tube ("bulk") or *in situ* inside the nanochannels. Thus, we varied the following parameters: 1) ssDNA generation by exonuclease digestion (Fig.2) or snap-cooling (Fig.3) either in bulk or *in situ;* 2) Introduction of both ds- and ss-DNA into nanochannels through the roof pores or through the wells and 3) staining with a ssDNA-specific OliGreen dye or a dsDNA-specific YOYO dye. The results were observed using a microscope an emCCD camera with single DNA molecule sensitivity. We demonstrate that the initial shape of ssDNA molecules depends on the method of preparation and introduction into nanochannels. However, at the low buffer concentrations we employed, ssDNA spontaneously stretches out with time (minutes) to its full length (20 μm for snap cooling and ~10 μm for exonuclease digestion), likely due to the influence of negative surface charges on the nanochannel walls that force the DNA to the center of the channel.

ssDNA in nanochannels is potentially important for many biological investigations including optical mapping and sequencing. The demonstration that ssDNA linearizes in the channels and is transported through the pores in the roof is highly relevant to sequencing applications.

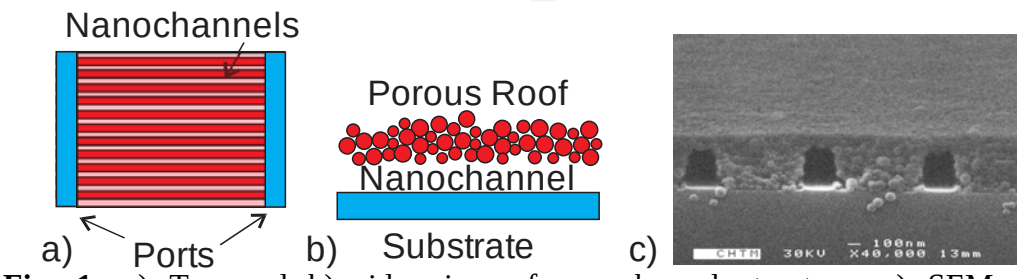

Fig. 1: a) Top and b) side view of nanochannel structure; c) SEM of nanochannels with porous silica particle roof and sidewalls.

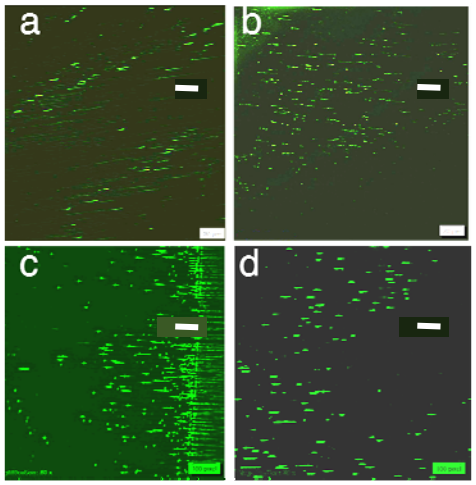

Fig. 2: ssDNA generated from Exonuclease digestion of Lambda ds-DNA. a) Oligreen labelled ss-DNA formed by exonuclease digestion of ds-DNA in the chip. B) ssDNA (exonuclease digested in bulk) intro-duced in to the nano-channels through tortuous nanopore. This indicates that ssDNA transits through the pores. c) Exonuclease digested ssDNA (bulk) introduced into the nanochannels through ports on the side of the chip. Note the number of bright small dots of balled-up ssDNA at the entry port. Those dots gradually stretch out in nanochannels after ~20 min to ~10μm as seen in panel d (scale bars are 20-μm long).

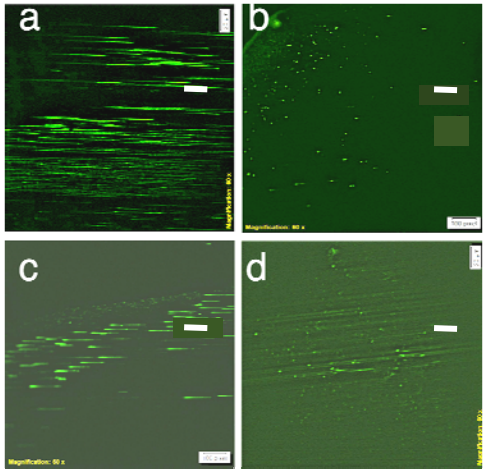

Fig. 3: ssDNA generated from heating and snap cooling of dsDNA. a) ds-DNA heated in the chip and snap cooled on ice water to form ssDNA strands. Strands are much longer than with the exonuclease approach. b) Heated and snap cooled ssDNA introduced through the roof. ssDNA appears as shorter balled-up segments. c) Heated and snap cooled ssDNA introduced into channels through ports on the side of the chip. Marker corresponds to 20 μm which is consistent with fully extended ssDNA. d) Same as a) but YOYO labelled to show majority of dsDNA has converted to ssDNA since YOYO fluoresces only when intercalated into dsDNA.