SUPPORTING INFORMATION

EXPERIMENTAL

All experiments were conducted with λ-phage DNA (48.5 kbp, New England Biolabs). The DNA was stained with the fluorescent dye YOYO®-1 (Invitrogen) at a binding ratio of one dye molecule per ten base pairs. The DNA was dissolved in a 0.5x TBE buffer (44.5mM tris(hydroxymethyl)aminomethane (TRIS), 44.5mM sodium borate, 1mM ethylenediaminetetraacetic acid (EDTA)) or a 1x TBE buffer. 3% β-mercaptoethanol was added to suppress photobleaching of YOYO®-1.

Unpolarized DNA imaging was performed using an epifluorescence video microscopy system consisting of a Nikon Eclipse TE2000-U inverted microscope, 60x water immersion (NA 1.0, Nikon) objective (with a 1.5x additional magnification lens for a total magnification of 90x) coupled to a cooled back-illuminated EMCCD camera (Andor Technology, iXon DV *887-ECS* BV). The polarization-sensitive imaging of DNA was carried out on another microscope setup consisting of the same model of microscope and objective but with a back-illuminated EMCCD camera from another manufacturer (Photometrics, Cascade II 512). Polarization-sensitive imaging was achieved by placing a Dual-View™ (DV-2) unit (from Photometrics) on the collecting side. This splits the light into two paths, which enables us to visualize the two polarization directions simultaneously.

In each frame the molecule is automatically located and fitted by a rectangle where all sides are set to the position where the intensity is 50% of the maximum intensity within the molecule after background has been subtracted. The DEI value is then the sum of all pixel values within this rectangle.

The chip consists of 180 nm deep funnels with a width that varies continuously from 50 nm to 650 nm (see Figure S1). The total length of each funnel is 600 μm. The rate of change in the width along the channel is 1 nm/μm, so that locally the DNA is exposed to a channel with more or less straight walls. The DNA is manipulated in the micro and nanochannels using pressure driven flow. The channels were defined by electron beam and UV lithography followed by CF4/CHF3 based reactive ion etching. Finally a thin layer (50 nm) of dry thermal oxide was grown to render the surfaces hydrophilic. The devices were sealed with a 550 μm thick borosilicate glass lid. The depth of the channel was chosen to minimize the aspect ratios when changing the width of the funnel in the optically interesting regime 50 – 650 nm. With 180 nm deep channels, the aspect ratio varies from 1:3.6 to 3.6:1.

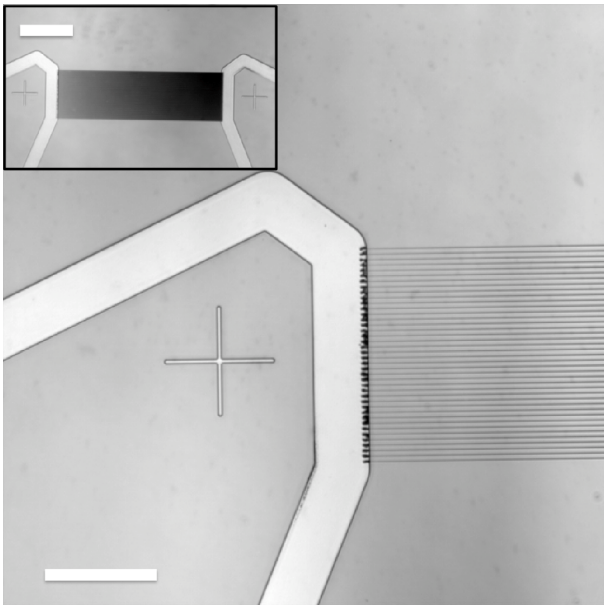

Figure S1. *Optical bright field image showing the narrow end of the nanofunnels and the connecting microchannels used for sample transport. Scale bar corresponds to 100 μm. Inset: Image of the whole nanofunnel region with the narrow entrance to the left. Scale bar corresponds to 200 μm.*

For more details on data analysis and chip fabrication, see F. Persson and J. O. Tegenfeldt, *Chem. Soc. Rev.*, 2010, **39**, 985–999.

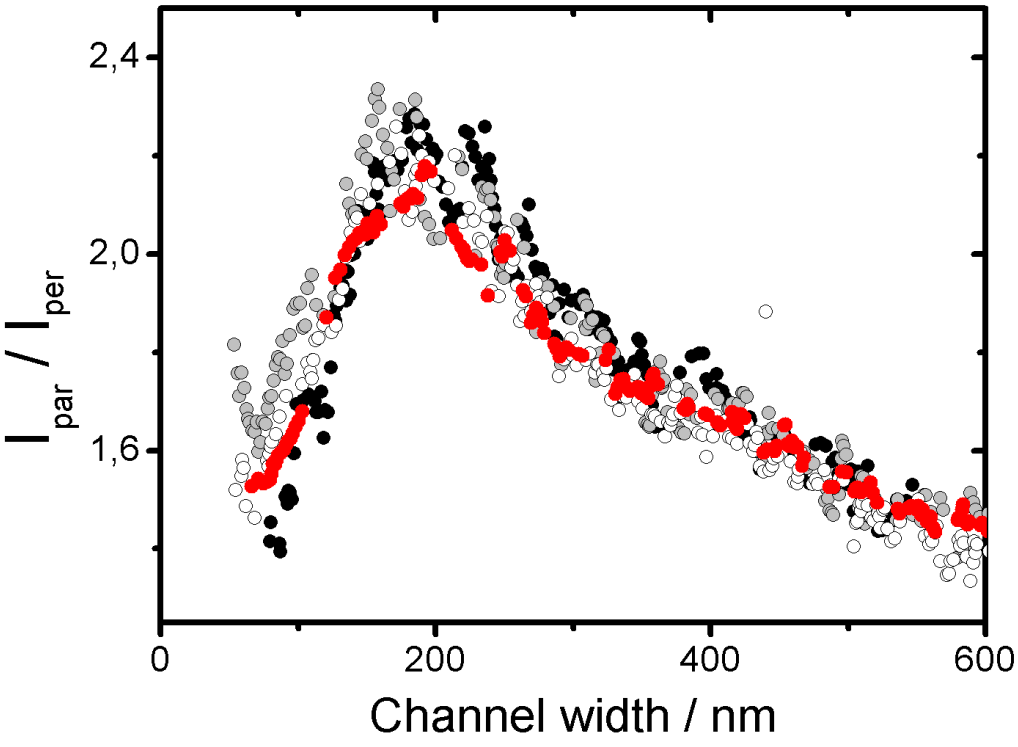

Figure S2. *Ratio of the detected emission intensity polarized parallel (I_{par}) and perpendicular (I_{perp}) to the 180 nm deep nanofunnel for YOYO-labeled λ-DNA in 0.05x TBE (black), 0.5x TBE (gray) and 1x TBE (white) buffer*

as well as T4GT7 DNA (166 kbp, red). The ratio is plotted to allow for direct comparison between the four cases.

The polarization ratio trace (I_{par}/I_{perp}) in Fig. S2 has a shape that is independent of both the DNA length and the ionic strength of the buffer. At varying ionic strengths the DNA will have a different extension at a given confinement (see C. Zhang, F. Zhang, J. A. van Kan and J. R. C. van der Maarel, *J. Chem. Phys.*, 2008, **128**, 225109). The identical traces at the three ionic strengths thus strongly support that the shape of the trace is exclusively governed by the nanostructure.

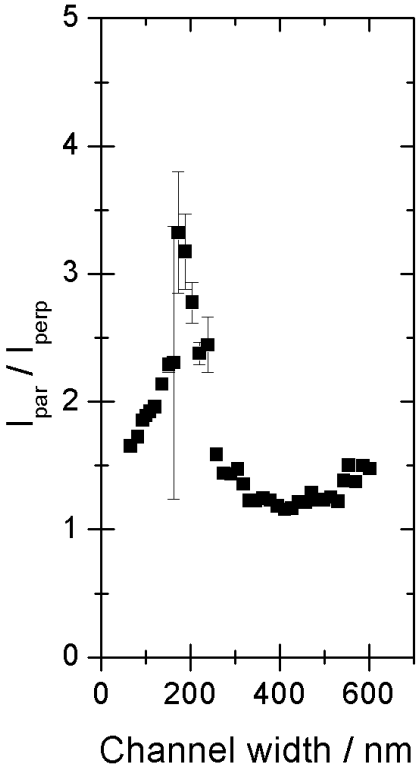

Figure S3. *Detected intensity polarized parallel to the nanochannels (I_{par}) divided by the detected intensity polarized perpendicular to the nanochannels (I_{perp}) for fluorescent beads (D=22 nm, Thermo Scientific) at different positions along a 180 nm deep funnel. Each datapoint represents the average of 100 measurements. The error bars correspond to one standard deviation.*

Using fluorescent polymer beads (D = 22 nm, Thermo Scientific) we see that, just as for the DNA studied in the main text, the detected emission intensity polarized parallel to the channel is significantly higher than that polarized perpendicular to the channel, especially when the channel width is between 150 and 300 nm (Figure S3). This supports the conclusion in the main text that the effect we see is not due to the polymer physics of the DNA but rather resonance effects in the channel structure.

One important source of uncertainty in this measurement is any non-uniform distribution of the fluorescent nanospheres along the funnel structure. However, since that would affect the two polarizations equally, we can compensate for this effect by looking at the ratio rather than the absolute values of the detected emission intensities in the two polarization directions. Without any optical effects due to the channel walls, we would see a constant ratio, whereas we in fact observe a ratio that peaks at a channel width of approximately 180 nm.