

## ELECTRONIC SUPPLEMENTARY INFORMATION

### Additional experimental details

Fluorescence detection system characterization: The background counts detected on the photodetectors when the chip was filled with water was 180 counts/sec for both detection channels (ch1: bandpass filter 513BP17 and ch2: bandpass filter 670DF40). For the bead experiment depicted in Fig. 2a, we extract average signal levels of 1942 counts/sec for the 100nm yellow-green beads and 1052 counts/sec for the 40nm dark-red beads. This corresponds to signal-to-noise-ratios (SNR) in excess of 10 (100nm beads) and 5 (40nm beads), respectively – much higher than previously reported SNRs of 2 in microfluidic FCCS detection.<sup>13</sup> A typical time trace displaying signals from both channels is shown in Fig. S1.

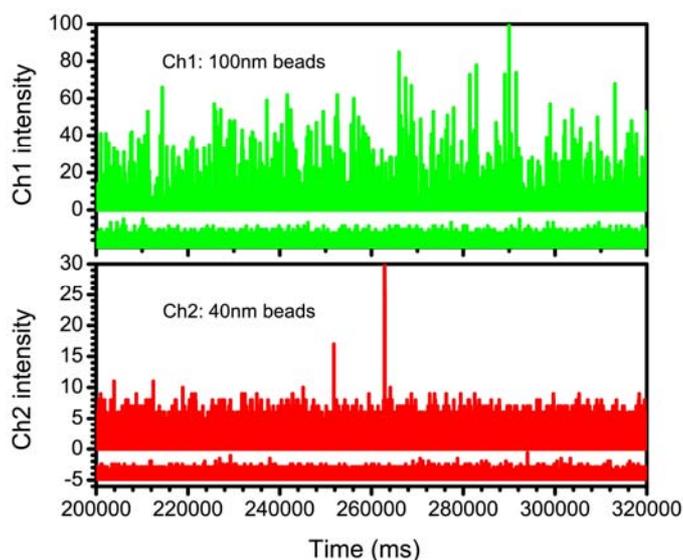


Fig. S1: Time trace of fluorescent bead signals in both detection channels (ch1: bandpass filter 513BP17 and ch2: bandpass filter 670DF40). The background counts (lower traces in each panel) were shifted for clarity. All data are plotted in 5 ms bin time.

FCS and FCCS studies of oligonucleotides: Diffusion coefficients and flow velocities for the oligonucleotides were extracted from two-parameter fits to the donor fluorescence curves. These fits yielded values of  $D=2.3 \times 10^{-6} \text{ cm}^2/\text{s}$  and  $v_z=3.4 \mu\text{m}/\text{s}$  (FRET pair in Fig. 3a),  $D=2.3 \times 10^{-6} \text{ cm}^2/\text{s}$  and  $v_z=0.024 \text{ m}/\text{s}$  (FRET pair in Fig. 3b at  $T=30^\circ\text{C}$ ), and  $D=2.7 \times 10^{-6} \text{ cm}^2/\text{s}$  and  $v_z=0.029 \text{ m}/\text{s}$  (FRET pair in Fig. 3b at  $T=50^\circ\text{C}$ ), respectively. The higher flow velocity for the FCCS measurements are due to the fact that the experiment was carried out with an in-house vacuum line connected to the waste reservoir. The vacuum was applied to increase the flow rate and minimize channel clogging due to salt buildup near the reservoirs. The donor fluorescence signals corresponding to Fig. 3b are shown in the figure below. All values were found in agreement with a diffusion coefficient directly proportional to temperature.

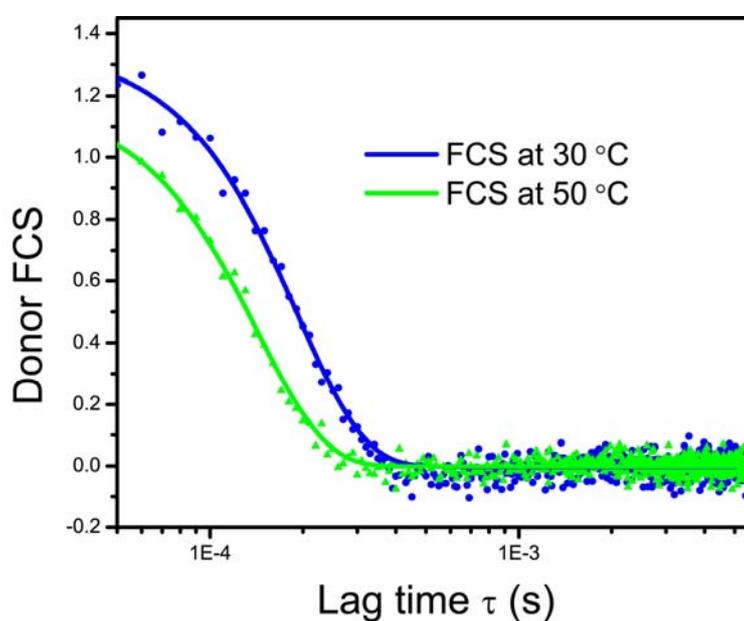


Fig. S2: Donor FCS signal for double-stranded oligonucleotide experiment (FCCS curves shown in Fig. 3b in main text).