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## **Supplementary information**

Evidence for hydrophobic catalysis of DNA strand exchange

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## **Experimental details**

The FAM (carboxyfluorescein) and TAMRA (carboxytetramethylrhodamine) labeled strands are presented in Figure S1. An unlabeled strand (sequence identical to the TAMRA-strand) is added five times in excess. Upon strand exchange, the unlabeled strand displaces the TAMRA strand, which dissociates the quenched FRET pair. The exchange yield is proportional to the FAM fluorescence intensity increase and can be normalized between the intensity before adding unlabeled strand (0 % yield) and intensity after heating the reaction mixture to 80 °C and then cooling (100 % yield). All experiments were performed at 37 °C, and unless otherwise noted, in sodium phosphate buffer (50 mM Na+, pH 7.5). Mixtures of co-solutes and buffer were equilibrated for one week; concentrations of co-solutes are given as weight percentage.

3'-GCA GTT GTA TGT ATA GTG GT-5'-FAM 5'-CGT CAA CAT ACA TAT CAC CA-3'-TAMRA



Figure S1. Above: FAM and TAMRA labeled DNA strands used for strand exchange. The CD spectra and the melting curves are obtained using unlabeled strands of the same sequences. Below: Principle of FRETbased DNA strand exchange assay. FAM is initially quenched by TAMRA, but fluorescence is recovered after strand exchange.



Figure S2. Strand exchange yields for Dextran (50% w/w, average molecular weight 6000) and Ficoll (50% w/w, average molecular weight 70 000).



Figure S3. UV (260 nm absorbance) melting curves for DNA in the presence of 25% DME, 30% PEG-200, 45% PEG-1000, 50% PEG-6000, and pure buffer (solid lines). Dotted lines indicate that additional salt was added to increase  $T_m$  in each solution to match  $T_m$  in pure buffer.



Figure S4. CD spectra for DNA at different temperatures in the presence of 25% DME, 30% PEG-200, 45% PEG-1000, 50% PEG-6000, and pure buffer. B-DNA is preserved for all samples at 37 °C, and the thermally induced distortions compared to DNA at 20 °C are small.