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Supporting Information

DNA Modulates Solvent Isotope Effects in a Nanopore

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Patch Clamp Experiments

Nanopore experiments were performed in a custom built cell, composed of two teflon blocks each with a machine-drilled well ~1 mL in volume which were bolted together (Figure S1). Each well contained a side opening such that when the blocks are clamped together the wells are connected through their side openings. A 25 μm thick Teflon sheet (Goodfellow) was clamped between the two blocks, separating the side openings and fixed in place with silicone glue (3140 RTV coating, Dow Corning). A Teflon sheet containing an aperture of ~100 μm diameter (produced with a 30 kV spark gap generator) was positioned such that the aperture was in the centre of the lower half of the inter-well channel. The cell was placed on a Nano 20/30 anti-vibration platform (Halcyonics) within a custom built faraday cage with acoustic damping to isolate the experiment from external electrical and mechanical noise. A small hanging drop (~5 μL) of 10% solution of hexadecane in *n*-pentane was touched on each side of the Teflon sheet. In the experiments included in the main text 600 μL of a solution containing KCl (1 M) and Tris –HCl or DCl (25 mM) buffered to pH 8.0 or pD 7.6 in H_2O or D_2O respectively was added to the well on each side of the Teflon sheet. Further supporting experiments were performed using 'HDO' buffer prepared with 50:50 v:v mixture of the H_2O and D_2O buffers, this buffer gave a reading on the pH meter of 7.8. Next, 6 μL of 10 mg/mL solution of 1,2-diphytanoyl-*sn*-glycero-3-phosphocholine (Avanti Polar Lipids) in *n*-pentane was dispensed using a syringe on each side of the Teflon sheet. The buffer solution was then aspirated and dispensed into each well multiple times using a Hamilton syringe to paint a phospholipid bilyer across the aperture. Ag/AgCl electrodes (Warner) connected to a patch clamp amplifier (Axopatch 200B, Molecular Devices) were placed on either side of the Teflon sheet and a ±1 mV pulse applied at 1333 Hz to determine when a bilayer was obtained (capacitance of 60-80 pF). A gel loading tip fitted to a 20 μL Gilson type pipettor was introduced into an aqueous solution of α-haemolysin (Sigma Aldrich, ~250 μM), without aspirating, such that a tiny amount << 1 μL of the solution remained on the tip. The pipette tip was then submerged ~5 mm from the aperture in the Teflon sheet and repeatedly aspirated. The process was repeated until a small current corresponding to a single channel arose (Figure S2).

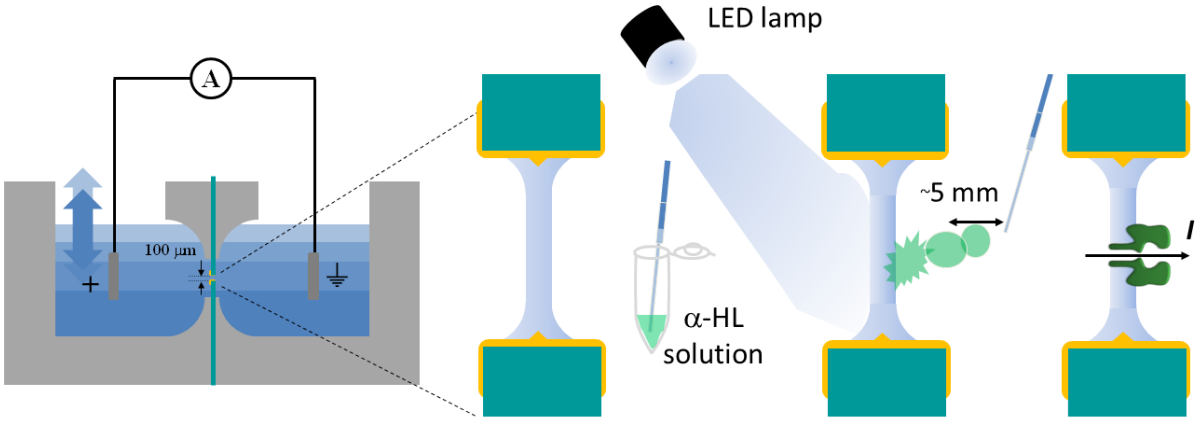

Figure S1. Experimental set-up used for single-channel recordings.

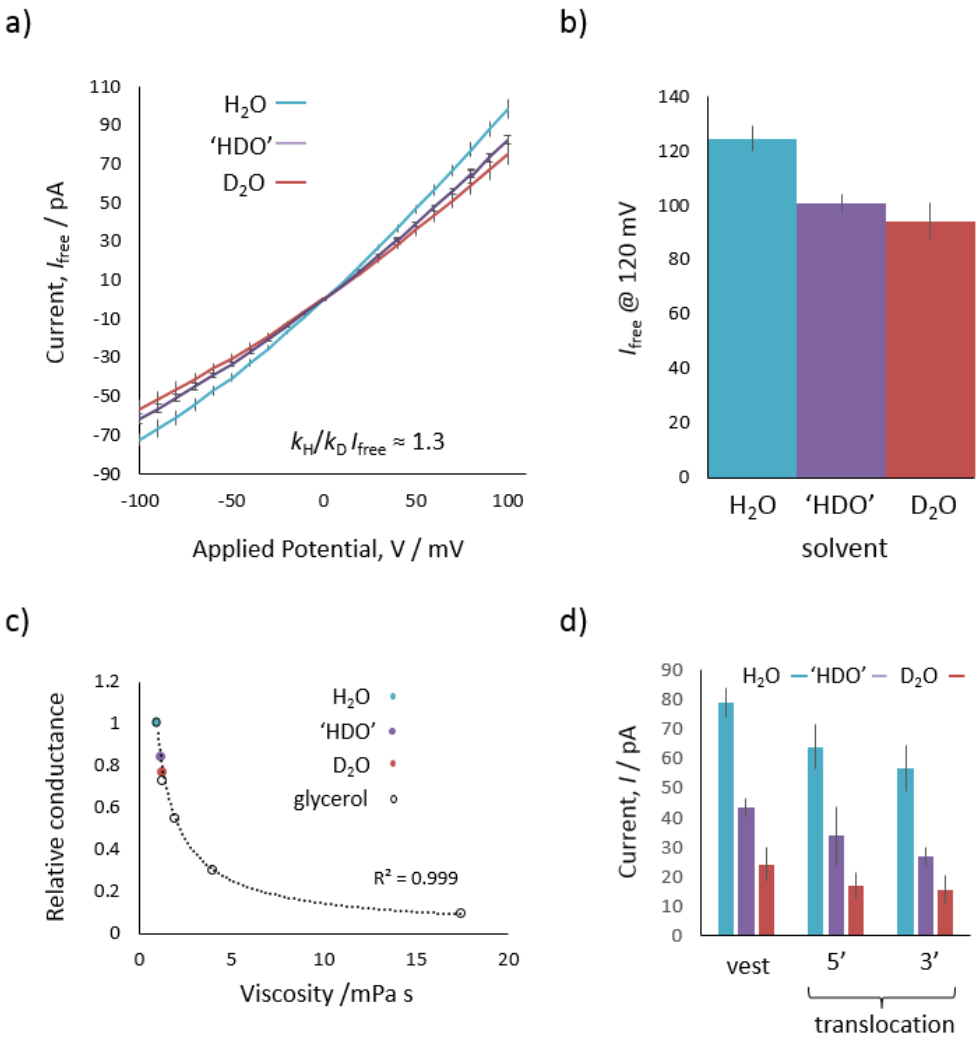

Figure S2. Nanopore ion current in D_2O, H_2O and 50:50 H_2O:D_2O buffers. a) Average current response calculated from 1.5 seconds of real-time current recordings measured at 10 mV intervals across an applied potential range of 100 to –100 mV for 10 individual α-haemolysin channels in H_2O or D_2O buffer (8 individual channels in HDO). Error bars represent the standard deviation. b) Average free channel current response at 120 mV for the 38 channels used in the main study (n = 16 in H_2O and n = 22 in D_2O) as well as n = 11 in HDO, error bars represent the standard deviations in each sample. c) relative conductance versus viscosity for a single alpha-hemolysin nanopore using buffer prepared with H_2O, 'HDO' and D_2O used in the present work and H_2O, H_2O/glycerol mixtures used in reference[1]. d) most probable average currents through a single alpha-hemolysin nanopore during DNA translocation (5' or 3') or vestibule (vest) events in H_2O, 'HDO' or D_2O used in the present work. The procedure by which these data were acquired is outlined below.

DNA Experiments

DNA experiments were performed at 21 ± 2 ^{o}C. DNA (5 μL, 300 μM) was added to the ground well of the experimental cell shown in Figure S1 (and described above). A 120 mV transmembrane voltage was applied, and data were recorded using an Axon Axopatch 200B (Molecular Devices) equipped with a CV203BU headstage (Molecular Devices), and digitized using an Axon Instruments Digidata 1332A at a sample rate of 500 kHz. Single-channel ion current recordings were processed with Clampex 10.2 and Clampfit 10.2 software. Examples of characteristic DNA events are shown in Figure S3. Ion current traces were digitally filtered at 10 kHz. Events were extracted using the threshold search function in Clampfit 10.2 and then processed in Origin 9 Pro and Microsoft Excel 2010. Events longer than 1.5 ms were excluded from the analysis as these types of events are attributed to gating events. All raw data is available for download in an open access repository: http://dx.doi.org/10.7488/ds/277

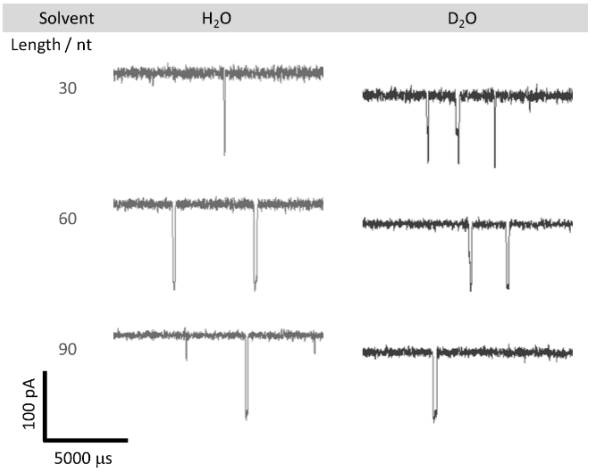

Figure S3. Representative real-time DNA event data in D_2O and H_2O buffers.

DNA Synthesis, Purification and Storage

Poly dT 30, 60 and 90mers were synthesized on a MerMade 4 solid-phase DNA synthesizer (Bioautomation) using phosphoramidites and coupling reagents (Link Technologies) for standard 3’ to 5’ DNA synthesis according to the manufacturer’s instructions and purified by two-stage

(DMT-on and DMT-off) reverse-phase HPLC using a C-18 semi-preparative column (Supelco) with an acetonitrile / 100 mM triethylammonium acetate in 5 : 95, acetonitrile : water pH 7 gradient buffer system at a flow rate of 7 mL/min. A column temperature of 65 °C was used to minimize secondary structure (which could complicate retention behaviour) and elution of DNA fractions was monitored by an integrated UV/vis spectrometer at 260 nm on a Dionex ultimate 3000 series HPLC running Chromeleon software. The DNA was concentrated at reduced pressure to ~300 µM and stored in aliquots of 5 µL in PCR tubes in press-sealed plastic bags at –20 °C.

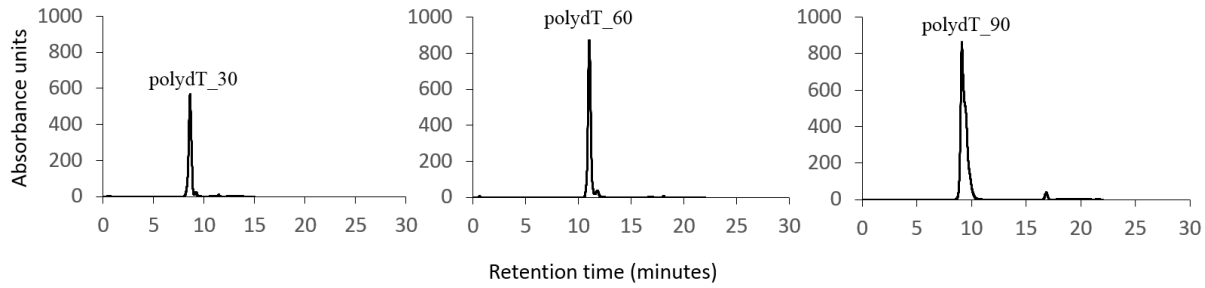

Figure S4. HPLC chromatograms (absorbance at 260 nm) obtained for synthesized and purified DNA.

Relationship between solvent isotope effect and the estimated conductive diameter of the nanopore

It may be possible to assume that electrostatic differences between different DNA events are small,[2] and if such an assumption holds true, then it is possible to estimate the effective conducting diameter of the pore from the recorded ion currents. This assumption can be used to determine the relationship between the effective (conducting) diameter of the pore and the solvent isotope effect, since the cross-sectional area of a conducting channel is proportional to the current flowing through it. Since the diameter of the α-HL pore is known,[3] then I_{free} in each experimental condition can be used as a normalisation factor to convert the measured ionic currents (I_{DNA}) to the effective conducting diameter of the pore during each type of event, as shown in Figure S5. The pattern appears to be well-approximated by a Lennard-Jones potential (black line overlaid), which describes the distance dependence of the repulsive and cohesive components of intermolecular interactions.[4] Thus, this model derived from the Lennard-Jones potential might suggest a suitable basis for rationalising the physical origins of the observed solvent isotope effects resulting from the exclusion/restructuring of water within nanopores that are occluded to different extents by the presence of DNA. However, more sophisticated theoretical models might provide a more complete explanation than this simple, approximate model.

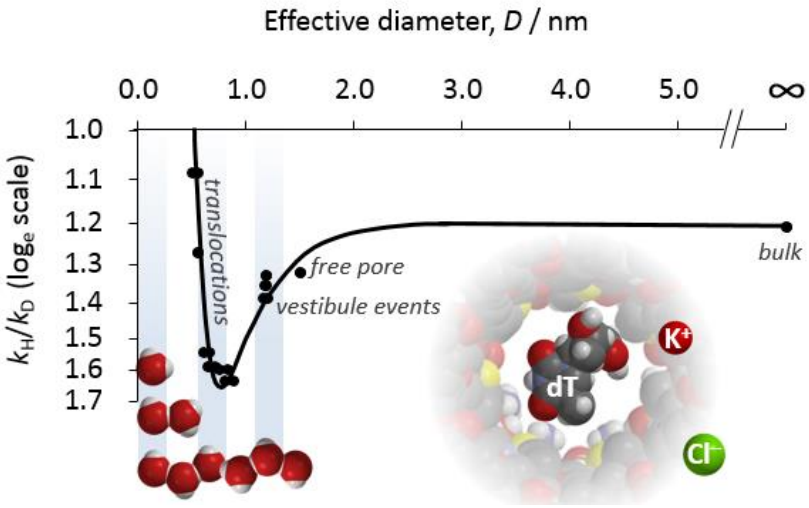

Figure S5. The relationship between the observed solvent isotope effects on the ion current and the derived effective conductive diameter of the pore. The diameters of pore, single-stranded DNA, K^+, Cl^-, and water molecules are indicated in proportion to the effective diameter scale shown.

Table of Solvent Isotope Effects

Table S1. Measured and calculated parameters in H_2O and D_2O and calculated solvent isotope effects. Values for the single-molecule measurements are given as the mean value ± the standard deviations in the property distributions.

Condition	Parameter	Units	H_2O	D_2O	SIE
Bulk	1/Viscosity[5]	$mPa^{-1}\ s^{-1}$	0.892	1.095	1.23
	Conductivity[6]	$Ohm^{-1}\ cm^{-1}\ mol^{-1}$	149.94 ± 0.01	124.23 ± 0.01	1.21
Nanopore	I_{free}	$pA = pC\ s^{-1}$	124.4 ± 4.6	94.2 ± 6.8	1.3 ± 0.1
DNA in Nanopore	I_{DNA} 30 nt vest	$pA = pC\ s^{-1}$	79.1 ± 4.9	56.9 ± 5.7	1.4 ± 0.1
	I_{DNA} 60 nt vest	$pA = pC\ s^{-1}$	77.8 ± 6.2	58.7 ± 7.5	1.3 ± 0.2
	I_{DNA} 90 nt vest	$pA = pC\ s^{-1}$	78.2 ± 6.2	57.7 ± 5.7	1.4 ± 0.1
	I_{DNA} 30 nt 5’	$pA = pC\ s^{-1}$	43.7 ± 7.8	26.8 ± 5.0	1.6 ± 0.3
	I_{DNA} 60 nt 5’	$pA = pC\ s^{-1}$	38.0 ± 8.1	23.8 ± 5.2	1.6 ± 0.3
	I_{DNA} 90 nt 5’	$pA = pC\ s^{-1}$	28.0 ± 4.7	17.7 ± 2.8	1.6 ± 0.2
	I_{DNA} 30 nt 3’	$pA = pC\ s^{-1}$	24.2 ± 7.5	15.7 ± 4.5	1.5 ± 0.4
	I_{DNA} 60 nt 3’	$pA = pC\ s^{-1}$	16.9 ± 5.1	13.3 ± 3.1	1.3 ± 0.4
	I_{DNA} 90 nt 3’	$pA = pC\ s^{-1}$	14.0 ± 2.2	12.9 ± 1.4	1.1 ± 0.2
	k_{DNA} 30 nt 5’	$kbase\ s^{-1}$	3.4 ± 0.2	3.0 ± 0.3	1.2 ± 0.1
	k_{DNA} 60 nt 5’	$kbase\ s^{-1}$	2.8 ± 0.4	2.4 ± 0.4	1.2 ± 0.2
	k_{DNA} 90 nt 5’	$kbase\ s^{-1}$	1.5 ± 0.5	1.3 ± 0.5	1.2 ± 0.5
	k_{DNA} 30 nt 3’	$kbase\ s^{-1}$	7.9 ± 0.1	7.0 ± 0.1	1.1 ± <0.1
	k_{DNA} 60 nt 3’	$kbase\ s^{-1}$	5.5 ± 0.1	5.3 ± 0.2	1.0 ± <0.1
	k_{DNA} 90 nt 3’	$kbase\ s^{-1}$	2.9 ± 0.2	2.2 ± 0.2	1.3 ± 0.1

Condition	Parameter	Units	HDO
DNA in Nanopore	I_{DNA} 30 nt vest	$pA = pC\ s^{-1}$	64.0 ± 2.9
	I_{DNA} 30 nt 5’	$pA = pC\ s^{-1}$	33.8 ± 9.9
	I_{DNA} 30 nt 3’	$pA = pC\ s^{-1}$	16.8169 ± 3.1

Supporting References

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