Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

Solid-state nanopore analysis of alcohol-soluble molecules

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Materials and Methods

Zein preparation: For reconstitution of α-zein from commercially extracted zein isolate (Amazein™, Prairie Gold, Inc.), raw material was mixed in a 90% ethanol solution in a 1.5 ml eppendorf tube. The resultant mixture was heated to 65 °C for 1 hour with intermittent vortexing, followed by a quick sonication (1 min) in a low power bath sonicator (FS20D, Thermo Fisher). The sample was subsequently centrifuged at 10,000 rpm for 5 minutes, after which the soluble supernatant was carefully removed and put through a syringe filter (200 nm pore size, Sartorius). Protein concentration was verified with absorption spectroscopy (Nanodrop 2000, Thermo Fisher). 60% ethanol material was initially resuspended in 90% ethanol as indicated above and then diluted to its final concentration to avoid additional soluble zein proteins (Supplementary Fig. S5). Other material shown in Fig. 3b in the main text were initially resuspended in a 1% SDS solution or in pure water instead of 90% ethanol, but all other treatments were identical.

Gel analysis: Protein content and purity was evaluated with SDS-PAGE, using NuPAGE® Novex® 4-12% Bis-Tris polyacrylamide gels (Invitrogen) in 1X MES buffer and stained with Coomassie blue. Gel images were captured using a Gel Doc system (BioRad, Hercules). All samples, regardless of solution content, were denatured in aqueous buffer before running on gel, following the manufacturer's protocol.

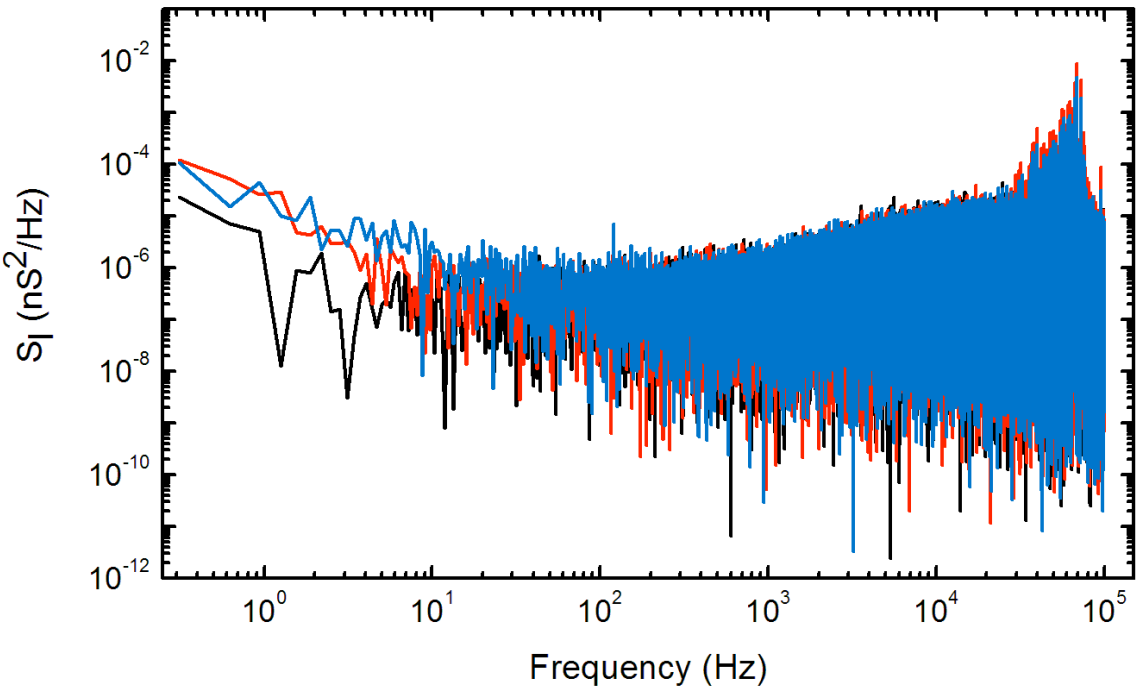
Nanopore Fabrication, Detection, and Analysis: Silicon chips, each featuring a single 10 μm window of 30 nm thick, free-standing silicon nitride were purchased commercially (Norcada, Inc.) and used as-received. Individual nanopores were fabricated with a scanning helium ion microscope (Carl Zeiss

Orion Plus) using an approach described elsewhere[1] and stored in 50% ethanol solution immediately after fabrication. Prior to an experiment, a chip was rinsed with deionized water and ethanol, dried under filtered air flow, and treated with air plasma (30 W) for 2 min on each side. Next, the chip was mounted in a custom Ultem 1000 flow cell and introduced with measurement buffer on both *cis*- and *trans*- reservoirs. All electrical characterization and measurements were performed with a patch clamp amplifier (Axopatch 200B, Axon Instruments) using Ag/AgCl electrodes, and each nanopore was verified to exhibit steady baseline and linear IV characteristics. Current traces were collected at 200 kHz with a 100 kHz four-pole Bessel filter and a 20 kHz low-pass filter was applied to all data prior to analysis. Data was saved in discrete increments of 3.2 s. An event was defined as having amplitude greater than 4.5 times the RMS noise and duration between 10 to 500 μs and rate was determined by analyzing uninterrupted current traces (150 s for Fig. 3d in the main text and 450 s for Supplementary Fig. S6a). The measurement error was defined as the standard deviation of the rates measured from all discrete trace increments.

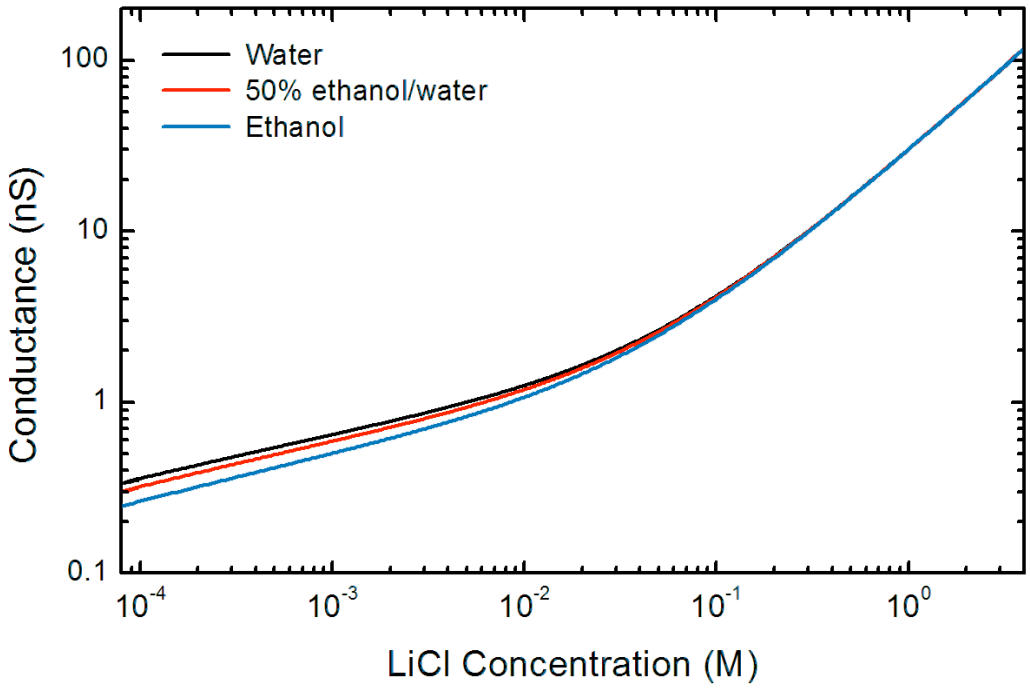
To minimize evaporation-mediated perturbations of the salt concentration, the flow cell was covered with parafilm during measurement. Nonetheless, the analytes and buffers in both chambers were regularly replenished. We display typical results from a single nanopore for consistency and to avoid effects of pore-to-pore variation. Additional data is presented in Supplementary Figure S6 to demonstrate the reproducibility of the measurement.

References:

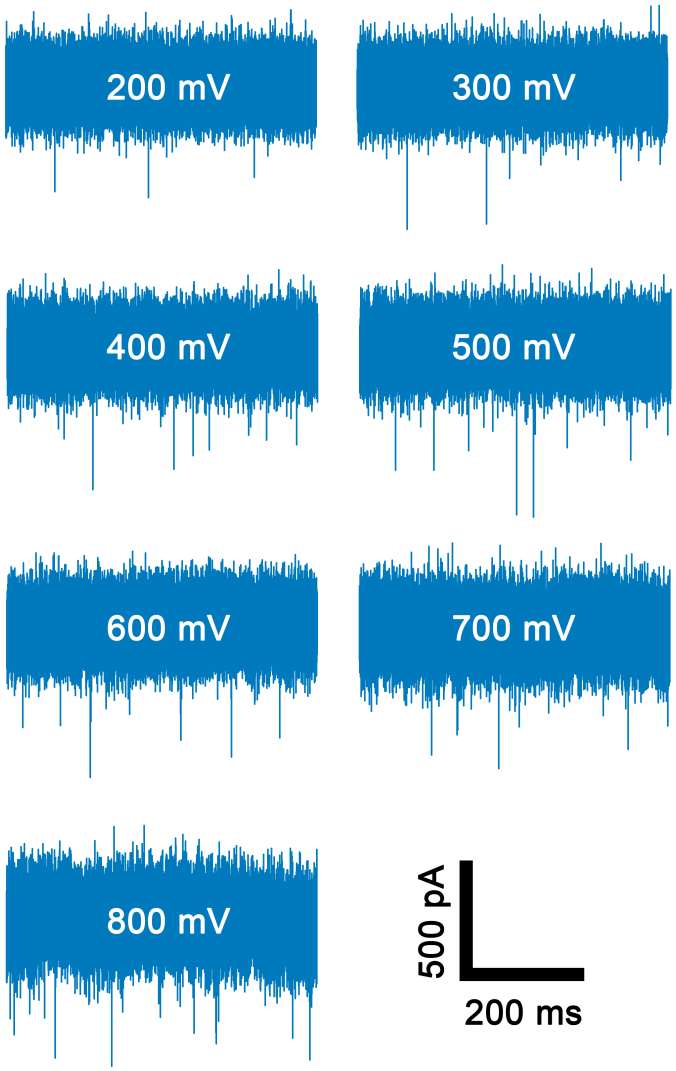
(1) Yang, J.; Ferranti, D. C.; Stern, L. A.; Sanford, C. A.; Huang, J.; Ren, Z.; Qin, L.-C.; Hall, A. R. *Nanotechnology* **2011**, *22* (28), 285310.



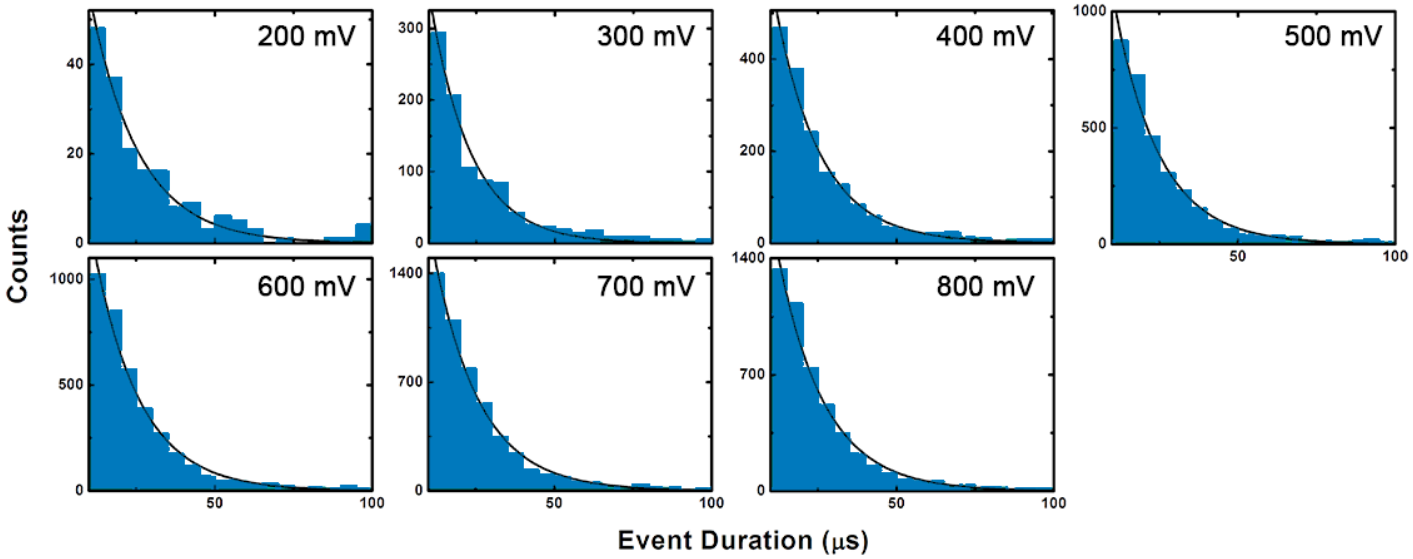
Supplementary Figure S1. Conductance noise comparison for a single SS-nanopore (diameter 12 nm) at 200 mV applied voltage between 50 mM LiCl in pure water (black), 300 mM LiCl in 50% ethanol/water (red), and 3 M LiCl in pure ethanol (blue). Concentrations were chosen to yield approximately equal, and therefore comparable, nanopore conductances (see Fig. 2 from the main text). A slight increase in low-frequency noise is observed in ethanol solutions.



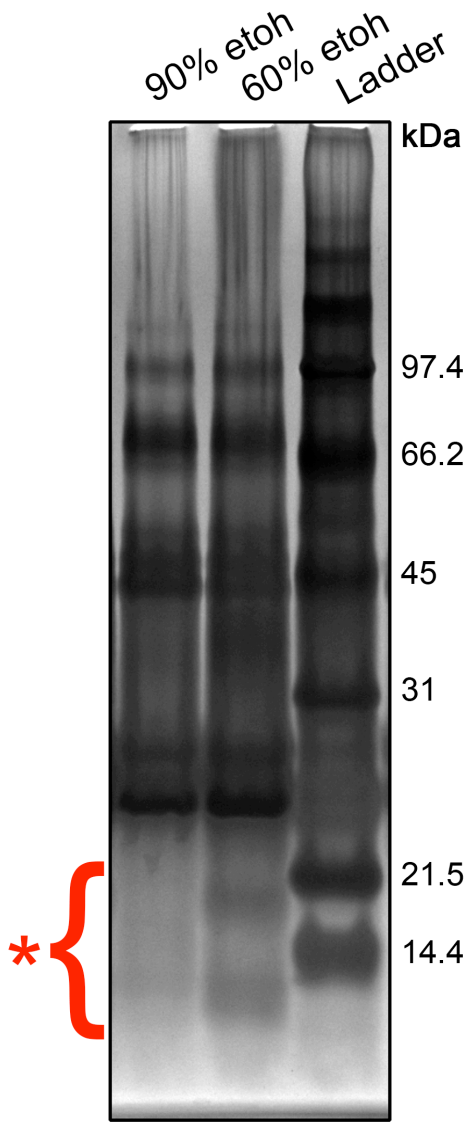
Supplementary Figure S2. Modeled salt dependence of nanopore conductance from Fig. 2 in the main text considering only a change in the electrical permittivity, ε, between pure water (black), a 1:1 mix of water and ethanol (red), and pure ethanol (blue).



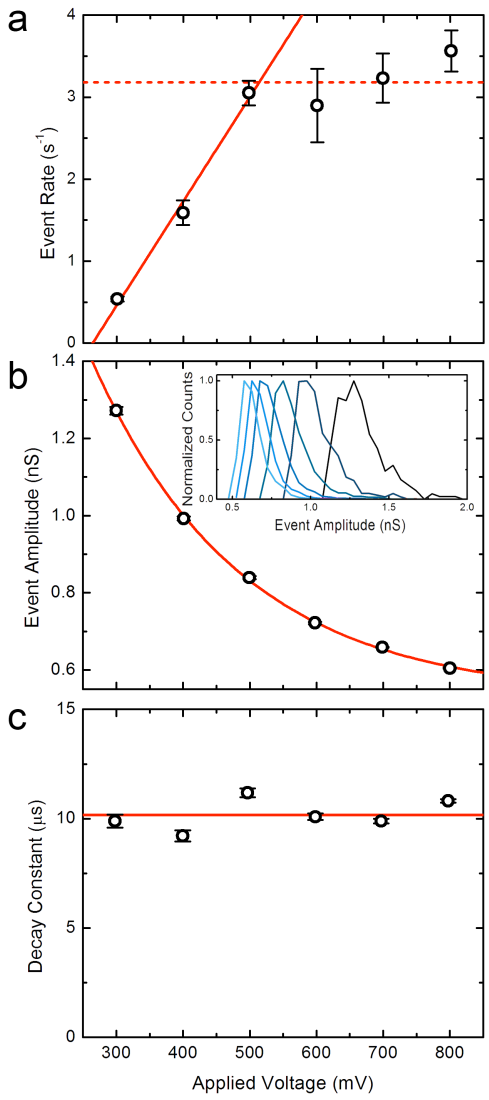
Supplementary Figure S3. Example current traces from Fig. 3d-f (main text) at each of the measured voltages.



Supplementary Figure S4. Dwell time histograms for the 11 nm pore used in the main text, measured across the full voltage range. Black lines are exponential fits to the data, from which the decay constants, t_d, were derived for Fig. 3f in the main text.



Supplementary Figure S5. Silver stained SDS-PAGE gel of raw zein material resuspended directly in 90% and 60% mixtures of ethanol in water. In 60%, two additional faint bands emerge (*, corresponding to β-zein). To avoid this impurity, material for SS-nanopore measurements in the main text were first resuspended in 90% ethanol and then diluted to a final concentration of 60%.



Supplementary Figure S6. Additional α-zein translocation event analyses. (a) Event rate for 10 μM α-zein through a 9 nm pore as a function of applied voltage, showing linear dependence and eventual saturation. Solid line is a linear fit up to 500 mV and dashed line is approximate saturation rate. Each data point represents rate measured from a 450 s uninterrupted trace. (b) Mean event amplitude (depth) as a function of applied voltage. Red line is an exponential fit to the data. Inset: normalized event amplitude histograms from 300-800 mV (right to left). (c) Exponential decay constants derived from fits to event duration histograms as a function of applied voltage. No voltage dependence is observed. For (b) and (c), from 300-800 mV, *n*= 250, 753, 1656, 1322, 1494, and 1729, respectively.