Supporting Information

**Title:** Rapid determination of sample purity and composition by nanopore stochastic sensing

**Authors:** D.M. Milan Krishantha, Zachary S. Breitbach, Nilusha L. T. Padivitage, Daniel. W. Armstrong, and Xiyun Guan*

**Author affiliation:**
Department of Chemistry and Biochemistry, The University of Texas at Arlington, Arlington, Texas 76019-0065, USA

**Corresponding author:**
Department of Chemistry and Biochemistry, The University of Texas at Arlington, 700 Planetarium Place, Arlington, Texas 76019-0065, USA. Tel: 817-272-6086; Fax: 817-272-3808; E-mail: xguan@uta.edu
Materials and Reagents. Lipid 1,2-diphytanoylphosphatidylcholine was obtained from Avanti Polar Lipids (Alabaster, AL). Teflon film was purchased from Goodfellow (Malvern, PA). Samples of cyclofructans (CFs) were generously provided by AZYP (Arlington, Texas). The purities of CF6 and CF7 standards were greater than 99%, as verified by High Performance Liquid Chromatography (HPLC). All the CFs samples were dissolved in HPLC-grade water (ChromAR, Mallinckrodt Baker). Except the HPLC grade acetonitrile (ACN) which was purchased from EMD (Gibbstown, NJ), all of the other reagents were purchased from Sigma Aldrich.

Preparation of the wild-type and mutant α-hemolysin (αHL) proteins. The production of the wild-type and mutant αHL protein pores has been described elsewhere. Briefly, the mutant αHL M113F genes were constructed by site-directed mutagenesis. Then, the wild-type and mutant monomers were first synthesized by coupled in vitro transcription and translation (IVTT) using the E. coli T7 S30 Extract System for Circular DNA from Promega (Madison, WI). Subsequently, they were assembled into homoheptamers by adding rabbit red cell membranes and incubating for 1 h. The heptamers were purified by SDS-polyacrylamide gel electrophoresis and stored in aliquots at -80 °C.

Single channel recording. A bilayer of 1,2-diphytanoylphosphatidylcholine was formed on an aperture (150 μm) in a Teflon septum (25 μm thick) that divided a planar bilayer chamber into two compartments, cis and trans. The formation of the bilayer was achieved by using the Montal-Mueller method. The experiments were carried out at 22 ± 1 °C under symmetrical buffer conditions with a 2.0 mL of 1 M NaCl solution buffered with 10 mM NaH₂PO₄/H₃PO₄ (pH 3.0). Unless otherwise noted, the αHL protein (with the final concentration of 0.2-2.0 ng·mL⁻¹) was added to the cis compartment which is connected to “ground”, while CFs samples were added to the trans compartment. The applied potential was -120 mV unless otherwise noted. Currents were recorded with a patch clamp amplifier (Axopatch 200B, Axon...
instruments, Foster City, CA). They were low-pass filtered with built-in four pole Bessel filter at 10 kHz and sampled at 50 kHz by a computer equipped with a Digidata 1322 A/D converter (Molecular Devices). In all the cases, the final concentrations of CF standards were 40 μM each. At least three separate experiments were carried out for each sample of CFs. All the results were reported as mean values ± standard deviation.

**Data Analysis.** Data were analyzed with pClamp 10.1 (Molecular devices), QuB (www.qub.buffalo.edu), and Origin 6.0 (Microcal, Northampton, MA) software. Conductance values were obtained from the amplitude histograms after the peaks were fit to Gaussian functions. Mean residence time (τ_{off}) values of the events were obtained from the dwell time histograms by fitting the distributions to single exponential functions by the Levenberg-Marquardt procedure.

**HPLC Separation.** The HPLC system used was a Shimadzu (Kyoto, Japan) LC-6A, equipped with a Shimadzu SPD-6A detector. All samples were dissolved in water and injected using a 6-port injector and a 10 μL sample loop. The separations were performed using a FRULIC-N™ column (4.6 x 150 mm) obtained from AZYP, LLC (Arlington, TX). The mobile phase was 70% ACN / 30% water. A flow rate of 1 mL/min and UV detection at 190 nm was used. The separations were performed in triplicate and the percentages of CF6 and CF7 were directly calculated by using the peak area ratios.
**Fig. S1** Structure of cyclofructan
Fig. S2 Event residence time histograms of (a) CF6 (0.50 ± 0.03 ms) and (b) CF7 (4.64 ± 0.10 ms). The experiments were performed with the mutant αHL (M113F)γ pore at -120 mV in a 1M NaCl solution buffered with 10 mM NaH$_2$PO$_4$/H$_3$PO$_4$ (pH 3.0) in the presence of 40 µM CF6 and CF7, respectively.
**Fig. S3** Effect of the concentration of (■) CF6, or (●) CF7 on their interactions with the mutant αHL protein (M113F)7 pore: (a) event frequency; (b) mean residence time; and (c) amplitude. The experiments were performed at -120 mV in 1M NaCl buffered with 10 mM NaH$_2$PO$_4$/H$_3$PO$_4$ (pH 3.0) in the presence of various concentrations of CF6 and CF7.
Fig. S4 Determination of the composition of a cyclofructan sample, which was consisted of 20% CF6 and 80% CF7. (a) Typical single channel current recording trace; (b) the corresponding scatter plot of event residence time vs. amplitude; and (c) the corresponding current blockage amplitude histogram. $I/I_o$ in Figs. S4b and S4c is normalized blockage residual current, which was obtained by dividing the average blockage residual current of an event by the average open channel current. The experiment was performed with a mutant $\alpha$HL (M113F)$_7$ pore at -120 mV in a 1 M NaCl solution buffered with 10 mM NaH$_2$PO$_4$/H$_3$PO$_4$ (pH 3.0).
Fig. S5 HPLC separation of CF6 and CF7 samples: (a) CF6 (>99% pure); (b) CF7 (>99% pure); (c) CF6 (96.4% ± 0.5%); and (d) CF7 (85.9% ± 0.4%). The separations were performed using a FRULIC-N™ column (4.6 x 150 mm) obtained from AZYP, LLC (Arlington, TX). The mobile phase was 70% ACN / 30% water. A flow rate of 1 mL/min and UV detection at 190 nm was used.
**Fig. S6** Effect of the concentration of (■) αCD, (●) βCD, or (▲) γCD on their interactions with the mutant αHL protein (M113F)7 pore: (a) event frequency; (b) mean residence time; and (c) amplitude. The experiments were performed with the wild-type (WT) αHL protein at -120 mV in a 3M NaCl solution buffered with 10 mM Tris-HCl (pH 7.5) in the presence of various concentrations of αCD, βCD, or γCD.
Supporting References


