Supplementary information for:

Rapid and Selective DNA-Based Detection of Melamine Using

α-Hemolysin Nanopore

Yingying Sheng,^{a,b} Yi You,^a Zhong Cao*^a, Lei Liu*^b and Hai-Chen Wu^b

^aCollaborative Innovation Center of Micro/nano Bio-sensing and Food Safety Inspection, Hunan Provincial Key Laboratory of Materials Protection for Electric Power and Transportation, School of Chemistry and Biological Engineering, Changsha University of Science and Technology, Changsha 410114, China. E-mail: <u>zhongcao2004@163.com</u>

^bKey Laboratory for Biomedical Effects of Nanomaterials & Nanosafety, Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100049, China. Fax: +86 10 88235745; Tel: +86 10 88235745; E-mail: <u>leiliu@ihep.ac.cn</u> **Materials.** All the DNA samples were purified by HPLC and purchased from Sangon Biotechnology Co., Ltd. (Shanghai). 1,2-Diphytanoyl-*sn*-glycero-3-phosphochline (DPhPc) was purchased from Avanti Polar Lipids (Alabaster, AL). Melamine and other chemicals were purchased from J&K, Sigma-Aldrich and Alfa Aesar, and used without further purification. All the samples and buffers were prepared in deionized water (Millipore, MA). DNA concentration was measured on ND-2000c Spectrophotometer. Wild type α HL-D8H6 was prepared as described previously (ref. 26 in the main text).

Buffer preparation. A total of 29.827 g of KCl (99.999%, Sigma-Aldrich) and 0.486 g of Tris·HCl (99.0%, Sigma-Aldrich) were dissolved in 370 mL of deionized water (Millipore, MA). 1 M NaOH was used to adjust the pH to 8.0. The solution was diluted with deionized water to 400 mL. The final buffer solution consists of 1 M KCl and 10 mM Tris at pH 8.0.

Melamine detection experiments. DNA samples (final concentration 1 μ M) were incubated in binding buffer (0.10 M PB, 0.30 M NaCl, pH 7.4, total volume 40 μ L) with various concentrations of melamine (final concentrations: 200, 100, 50, 10, 1, 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , $10^{-5} \mu$ M) or other interfering substances (100 μ M) for 2 h at room temperature. The resulting solution was subject to single channel recording experiments.

Pretreatment of milk samples. Melamine aqueous solution was prepared and was added to the liquid milk to obtain different concentration of samples (10, 1.0, 0.1, 0.01 mM). In order to get the clear signals of melamine, the spiked milk must be pretreated. First, the spiked milk was transferred into centrifuge tubes and centrifuged for 20 min at 14,000 rpm, and the supernatant was collected from the centrifuge tubes to discard most of the big molecules such as protein and fat. Second, 1.0 mL HCl (1.0 M) was added into the collected liquid. Then the mixture was shaken for 10 s until it became turbid, and then centrifuged again to discard residual big molecules. Finally, the transparent solution was collected and filtered with 0.22 µm disposable filters. The sample was stored at 4°C for the further experiments.

Single-channel current recording. 1,2-Diphytanoyl-*sn*-glycero-3-phosphocholine was used to form a synthetic lipid bilayer across an aperture 100-150 μ m in diameter in a 25- μ m-thick polytetrafluoroethylene film (Goodfellow, Malvern, PA) that divided a planar bilayer chamber into two compartments, *cis* and *trans*. Both compartments contained 1 mL of buffer solution. DNA samples were added to the *cis* compartment, which was connected to ground. The *trans* compartment was connected to the head-stage of the amplifier. All experiments were carried out in 1 M KCl, 10 mM Tris, pH 8.0, at 25 ± 2 °C, unless otherwise stated. Ionic currents were measured by using Ag/AgCl electrodes with a patch-clamp amplifier (Axopatch 200B; Axon instruments, Foster City, CA), filtered with a low-pass Bessel filter with a corner frequency of 5 kHz and then digitized with a Digidata 1440A A/D converter (Axon Instruments) at a sampling frequency of 100 kHz. The potential was held at +120 mV unless otherwise stated.

Data analysis. Current traces were analyzed with Clampfit 10.2 software (Axon Instruments). Events were detected using the Event Detection feature, and used to construct amplitude and dwell time histograms. Origin (Microcal, Northampton, MA) and Clampfit were used for histogram construction, curve fitting and graph presentation. Adobe Illustrator was used for making figures. Current signature events were manually selected for statistical analysis.

Supplementary Figures



Fig. S1 The control experiments. Either (**a**) 100 μ M melamine or (**b**) 1 μ M DNA does not cause any long current blockage. All data were acquired in the buffer of 1 M KCl, 10 mM Tris, pH 8.0, with the transmembrane potential held at +120 mV.



Fig. S2 The current signals produced by melamine with different DNA probes. (a-g) Typical current traces generated by hybrids of melamine with DNA1-7 respectively. All data were acquired in the buffer of 1 M KCl, 10 mM Tris, pH 8.0, with the transmembrane potential held at +120 mV.





Fig. S3 Histograms of the current blockage induced by DNA-melamine duplexes. (**a-j**) Corresponding histograms of the normalized current blockage I/I_0 under different melamine concentrations (200, 100, 50, 10, 1, 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} µM), respectively. All data were acquired in the buffer of 1 M KCl, 10 mM Tris, pH 8.0, with the transmembrane potential held at +120 mV. The final concentration of DNA is 1.0 µM. Number of individual experiments n = 3.











Fig. S4 Histograms of the event intervals caused by DNA-melamine duplexes. (**a-j**) Corresponding histograms of the event intervals under different melamine concentrations (200, 100, 50, 10, 1, 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} µM), respectively. All data were acquired in the buffer of 1 M KCl, 10 mM Tris, pH 8.0, with the transmembrane potential held at +120 mV. The final concentration of DNA is 1.0 µM. Number of individual experiments n = 3.





Fig. S5 Histograms of the event intervals caused by DNA-melamine duplexes in spiked milk samples. (a-j) Corresponding histograms of the event intervals under different melamine concentrations (200, 100, 50, 10, 1, 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , $10^{-5} \mu$ M), respectively. All data were acquired in the buffer of 1 M KCl, 10 mM Tris, pH 8.0, with the transmembrane potential held at +120 mV. The final concentration of DNA is 1.0 μ M. Number of individual experiments n = 3.



Fig. S6 Selectivity of DNA6 for the detection of melamine in α HL. Experimental conditions are the same as in Fig. 3. The final concentration of DNA is 1 μ M; the concentration of melamine is 10 nM while other small molecules or metal ions are 100 μ M. Number of individual experiments n = 3.

Supplementary Table

Detection methods	Linear range (µM)	Detection limit (µM)	Reference
HPLC	7.93~634.32	0.79	[1]
GC/MS	Not given	0.05 ng	[2]
SERS	2.45~39.64	1.34	[3]
Fluorescence with a truncated aptamer	Not given	0.12	[4]
FRET/carbon dots and Au nanoparticles	0.05~0.50	0.036	[5]
Bare gold nanoparticles	3.96×10 ⁻² ~1.58	1.58×10 ⁻³	[6]
β-CD-functionalized silver nanoparticles	50~1000	4.98	[7]
DNA-based aHL nanopore	1.0×10 ⁻⁵ ~0.1	1.0×10 ⁻⁵	This work

Table S1. Comparison of the method developed in this work with literature reports.

Supplementary References:

- 1 G. Venkatasami and J. R. Sowa, Jr., Anal. Chim. Acta, 2010, 665, 227-230.
- 2 R. A. Yokley, L. C. Mayer, R. Rezaaiyan, M. E. Manuli and M. W. Cheung, J. Agric. Food Chem., 2000, 48, 3352-3358.
- 3 A. M. Giovannozzi, F. Rolle, M. Sega, M. C. Abete, D. Marchis and A. M. Rossi, *Food Chem.*, 2014, **159**, 250-256.
- 4 C. Gu, Y. Xiang, H. Guo and H. Shi, Analyst, 2016, 141, 4511-4517.
- 5 H. Dai, Y. Shi, Y. Wang, Y. Sun, J. Hu, P. Ni and Z. Li, *Sens. Actuators, B*, 2014, **202**, 201-208.
- W. Chen, H. H. Deng, L. Hong, Z. Q. Wu, S. Wang, A. L. Liu, X. H. Lin and X. H. Xia, *Analyst*, 2012, 137, 5382-5386.
- 7 S. S. John Xavier, C. Karthikeyan, G. Gnana kumar, A. R. Kim and D. J. Yoo, *Anal. Methods*, 2014, 6, 8165-8172.