

Microfluidic Integration of Interfacial Nanopores for Single-Molecule Sensing under FPGA-Based Closed-Loop Control

Lorenzo Petrella¹, Lena Dysli¹, Jonah Imfeld¹, Justin N. Cronk¹, Morteza Aramesh¹

¹ Laboratory of Single-Molecule and Single-Cell Biophysics, Department of Information Technology and Electrical Engineering, ETH Zürich, Zürich, Switzerland
lpetrella@ethz.ch

Summary:

We present interfacial nanopores integrated into microfluidic devices as a versatile platform for single-molecule sensing in small-volume samples. On-chip integration combined with FPGA-based closed-loop control enables leak-free, mechanically stable nanopore formation with tunable resistance up to the hundred megaohm range. Initial tests on double-stranded DNA demonstrate the working principle, with single-cell secretomics envisioned as the target application.

Keywords: interfacial nanopore, microfluidics, single-molecule sensing, FPGA closed-loop control, secretomics

Background, Motivation and Objective

Achieving real-time, single-molecule sensitivity in highly diluted samples is a major challenge toward unlocking access to the wealth of information encoded in the secretions of single cells. Interfacial nanopores have emerged as a promising technique to address this challenge, owing to their ability to detect single molecules in diluted samples or in situ proximal to single cells [1]. Microfluidic platforms are well-established tools for the handling and analysis of small-volume samples in a cost-efficient fashion [2]. Building on the growing body of evidence supporting pipette–elastomer interfacial nanopores (PEINs) as controllable-size, clog-free single-molecule sensors with high spatiotemporal resolution, we aim to embed interfacial nanopores directly into microfluidic chips to leverage their versatile, high-throughput capabilities and expand the range of applications.

Description of the New Method or System

Interfacial nanopores were formed at the interface of a flexible surface and a submicron aperture. The geometry, assembly, and operation were optimized to enable stable ionic current measurements across the highly deformable channels. Nanomanipulators with single-nanometre resolution, patch-clamp amplifiers, and high-speed digitisers orchestrated by FPGA-based logic provide real-time, automated feedback control of the interfacial nanopores.

Results

We achieved on-chip integration of interfacial nanopores by establishing leak-free and

mechanically stable nanopore formation with tunable resistance up to the 100 M Ω range in 2 M KCl solution. The power spectral density of an actuated interfacial nanopore measured in buffer shows rejection of electromagnetic interference at the power line and servo loop frequencies, and limited vibrational noise in the expected range despite the mechanical actuation of the system (Figure 1).

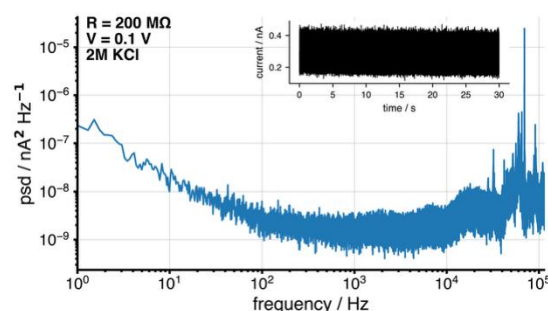


Fig. 1. Power spectral density of a 200 M Ω interfacial nanopore in 2 M KCl when a 100 mV bias is applied. The inset shows the 30 s current trace used to compute the PSD.

Combined with mechanical and electromagnetic noise rejection, the setup enabled the sensing of biomolecules such as double-stranded DNA (Figure 2), exhibiting voltage dependence of capture rate, dwell time and amplitude. Ultimately, we aim to establish a platform for single-cell secretomics that combines the single-molecule sensitivity of interfacial nanopores with the small-volume handling capabilities of microfluidics. Future work will focus on leveraging the low-latency FPGA-based control to influence the

translocation dynamics of individual analyte molecules, and on integrating multiple interfacial nanopores on a single device.

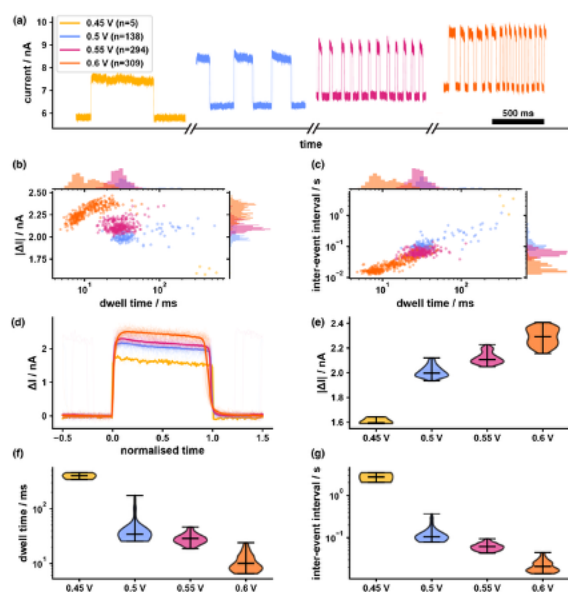


Fig. 2. Sensing of λ -DNA at varying pore bias in 2 M KCl with an 80 M Ω pore. (a) Representative traces of nanopore current at increasing pore bias; scatter plots of (b) event amplitude and (c) inter-event interval versus dwell time, with marginal distributions; (d) time-warped average event traces per pore bias condition; violin plots of (e) event amplitude (f) dwell time and (g) inter-event interval versus pore bias, with distributions shown for the 5th-95th percentile range.

References

- [1] T. Schlotter, S. Weaver, C. Forró, D. Momotenko, J. Vörös, T. Zambelli, M. Aramesh, Force-Controlled Formation of Dynamic Nanopores for Single-Biomolecule Sensing and Single-Cell Secretomics. *ACS nano* 14(10), 12993–13003 (2020); doi:10.1021/acsnano.0c04281
- [2] A.-G. Niculescu, C. Chircov, A. C. Bîrcă, A. M. Grumezescu, Fabrication and Applications of Microfluidic Devices: A Review. *International Journal of Molecular Sciences* 22(4) (2011); doi: 10.3390/ijms22042011