Case study

Real-Time Monitoring of Pore-Forming Toxin Activity in Live Cells

Tools:
AtlaZ

Dr. Tobias Ensslen & Anna-Sophia Kittel
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Dr. Tobias Ensslen, Group Leader of Nanopore Biochemistry at Hahn-Schickard, Freiburg, focuses his research on biological nanopores and nanotechnology. His work aims to deepen the understanding of the molecular mechanisms behind pore-forming toxins and their effects on cells, ultimately leveraging these systems to develop novel biosensors and bio-tools. The research presented in this case study is a joint effort, co-led in equal parts by Dr. Tobias Ensslen and Anna-Sophia Kittel from the research group of Prof. Dr. Winfried Römer, in close collaboration with Prof. Dr. Jan C. Behrends (both University of Freiburg).

Pore-forming toxins (PFTs) are among the most potent virulence factors produced by a wide range of microbial and eukaryotic organisms. By disrupting cellular membrane integrity, PFTs compromise essential barrier functions and ultimately induce cell death. While this cytotoxic activity underlies their role in pathogenesis, it has also sparked significant interest in their potential applications beyond infectious disease. In particular, PFTs have emerged as valuable tools in single-molecule sensing, where they function as biological nanopores, and as promising candidates for targeted therapeutic strategies, including the selective elimination of cancer cells. Despite decades of study, however, the molecular mechanisms governing pore formation, membrane insertion, and cell specificity remain incompletely understood.

Pore formation by many PFTs typically involves the assembly of soluble monomers into oligomeric, membrane-spanning β-barrel structures. This process is not uniform across all cell types but often depends on the presence of specific membrane components, such as glycosphingolipids (e.g. Gb3 (globotriaosylceramide)) or other carbohydrate-containing molecules. Indeed, several PFTs exhibit lectin-like binding properties, enabling selective recognition of membrane-associated glycoconjugates. These interactions can play a decisive role in determining the efficiency and specificity of pore formation. Importantly, such membrane components are sometimes upregulated in tumor cells, providing a potential basis for selective targeting in cancer therapy. Nevertheless, the complexity of membrane composition and the dynamic nature of toxin-membrane interactions make these systems difficult to study and control using conventional experimental approaches.

A major limitation in the field has been the lack of experimental platforms capable of generating rapid,



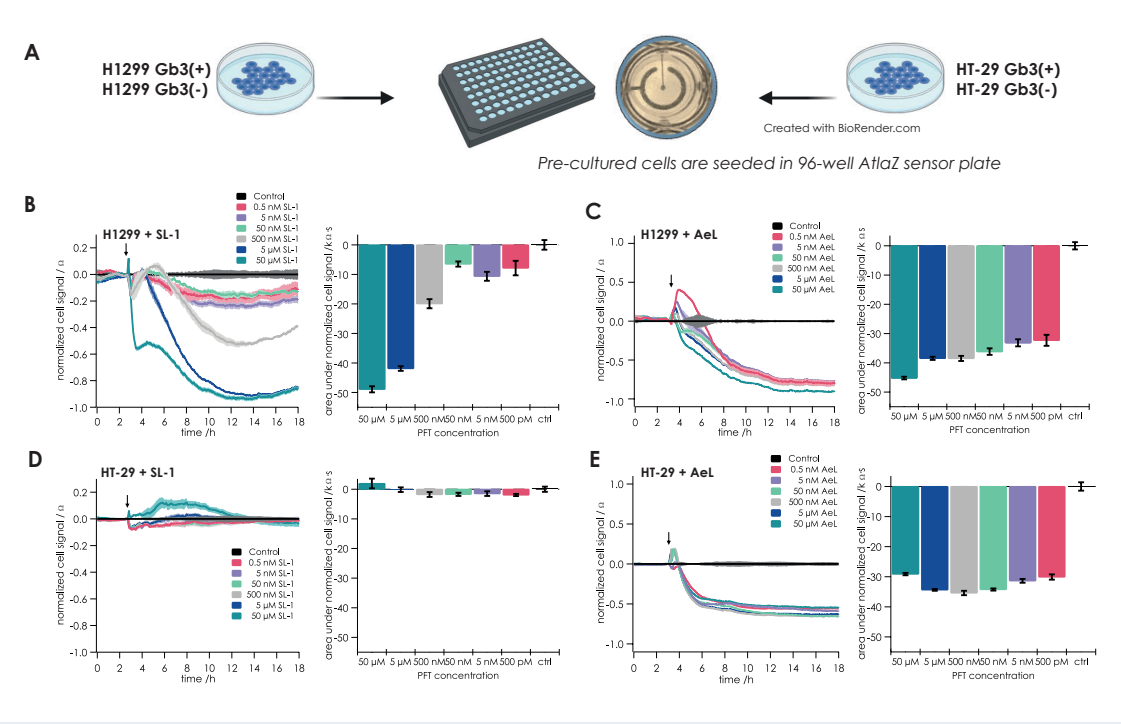
Nanion's AtlaZ platform
AtlaZ enables label-free and real-time cellular research on cell adhesion and proliferation, cytotoxicity, GPCR signaling, morphology change or barrier function. Up to six 96-well plates can be assessed simultaneously or independently.

> **"The AtlaZ proved to be highly effective for resolving the kinetics of pore-forming toxins on both short and long time scales, and across multiple conditions simultaneously."**
>
> **Dr. Tobias Ensslen**, Group leader, Hahn Schickard, Freiburg

quantitative, and statistically robust data on pore formation across diverse cellular environments. To address this challenge, we employed AtlaZ, a high-throughput impedance spectroscopy platform featuring a 6 × 96-well format, to investigate the kinetics and cellular effects of two structurally related PFTs: aerolysin (AeL) from *Aeromonas hydrophila* and SaroL-1 (SL-1) from *Salpingoeca rosetta*[1,2]. The AtlaZ label-free approach enables real-time monitoring of electrical impedance changes in living cell layers, providing a sensitive and non-invasive readout of membrane integrity, cell adhesion, barrier function, and overall cellular fitness[3].

Using human lung carcinoma (H1299) and colorectal carcinoma (HT-29) cell lines, we systematically characterized toxin-induced responses. Both AeL and SL-1 triggered pronounced impedance changes in H1299 cells, indicating significant disruption of cellular homeostasis[1,4]. In contrast, only AeL exhibited detectable activity in HT-29 cells, despite Gb3 abundance in these cells, highlighting cell-type-specific differences in susceptibility. Importantly, impedance-based measurements further allowed us to distinguish between rapid and delayed toxin activities, thereby generating detailed kinetic profiles of pore formation and downstream cellular effects.

To probe the molecular basis of these differences, we examined the role of the glycosphingolipid Gb3 as a potential receptor mediating toxin activity[1]. In general, impedance-based measurements acquired over a series of increasing compound concentrations allow the construction of a dose-dependent response relationship, since changes in morphology translate into concentration-dependent shifts in the impedance signal. In this context, the AtlaZ measurement readout can also be used as a typical endpoint parameter,



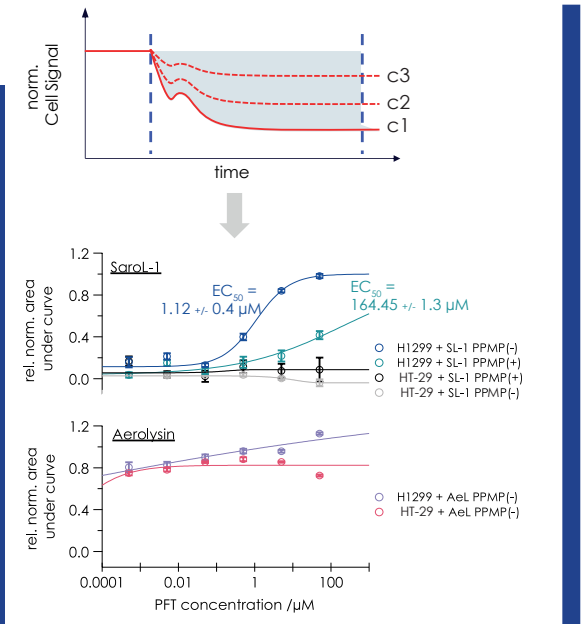
***H1299 and HT-29 cells in the presence of SaroL-1 or Aerolysin. A**, Schematic of the workflow and AtlaZ 96-well plate, alongside a detailed photograph of a single well containing a central working electrode and a C-shaped reference electrode. **B - E**, Normalized timetraces and corresponding area under the curve bar plots for H1299 and HT-29 cells. The observed reduction in Cell Signal is caused by addition of varying concentrations of SL-1 or AeL. Instantaneous peak right after compound addition (arrow) is hinting to cell swelling.*

providing a quantitative measure of the maximal effect observed at a defined time point that complements the continuous impedance trace. In H1299 cells, pretreatment with the glucosylceramide synthase inhibitor PPMP causing Gb3 depletion led to a dramatic reduction in SL-1 activity, with the EC_{50} shifting from 1.12 µM to 164.45 µM, an approximately 200-fold decrease in potency. This finding confirms a strong dependence of SL-1 on Gb3 for efficient pore formation. In contrast, AeL activity remained unaffected by Gb3 depletion, with an EC_{50} below 5 pM, suggesting a distinct or less restrictive receptor interaction mechanism. These results underscore the importance of membrane composition in modulating PFT function and demonstrate how receptor specificity can dictate cell-type selectivity.

In addition to receptor dependence, we investigated the biophysical consequences of toxin insertion using osmotic protection assays. Upon toxin exposure, both SL-1 and AeL typically induce rapid influx of water and subsequent cell swelling due to the disruption of ionic balance and osmotic equilibrium, as seen in the figure on the previous page. By adding 30 mM dextran as an osmotic protectant, we were able to mitigate this swelling until dextran was flushed out (data not shown). Capturing these dynamic, protective shifts in real time highlights the exceptional sensitivity of the AtlaZ platform in resolving early-stage pore-formation kinetics.

Overall, the AtlaZ platform proved to be a powerful and versatile tool for studying PFT activity. Its high-throughput capabilities enable scalable experiments with strong statistical robustness, making it particularly well suited for screening applications, such as identifying neutralizing agents or characterizing dose-dependent effects. Moreover, the label-free nature of impedance sensing avoids potential artifacts associated with fluorescent or biochemical markers, ensuring an unbiased and direct assessment of native cellular physiology.

In conclusion, our study demonstrates that parallelized impedance spectroscopy offers a comprehensive approach to investigating mechanisms of pore-forming toxins in living cells. By combining kinetic analysis, receptor specificity studies, and functional assays, this methodology provides a deeper and more quantitative understanding of PFT-



Experimental results. *Impedance–time traces, area-under-the-curve analysis at concentrations c1–c3, and derived EC_{50} values for SL-1 or AeL in H1299 cells ± PPMP.*

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"What really excited us about the AtlaZ platform was the level of experimental control and robustness it offered. Watching the omission of the lytic phase until we flushed the protectant out in real time was incredibly satisfying. It perfectly demonstrates the platform's exceptional kinetic resolution."

Anna-Sophia Kittel, University of Freiburg, Freiburg

induced cytotoxicity. Such insights are essential not only for elucidating fundamental aspects of toxin biology but also for advancing the development of PFT-based technologies in diagnostics and targeted therapy.

Innovation-driver: Nanodiag Transfer Lab

This work was the result of a fruitful collaboration between Nanion Technologies, University of Freiburg and Hahn-Schickard, as all partners work towards the development of accurate, rapid and scalable diagnostics, which remain a central challenge in biomedical research and clinical translation. Tobias Ensslen, who is also member of the *nanodiag bw cluster*, addresses this challenge through the development and application of nanopore-based assays. Under his leadership, the *nanodiag BW Transfer Lab* offers dedicated service capabilities for both academic and industrial partners, serving as a translational bridge to accelerate the transition from lab bench to practical application.

Unlike conventional research groups, the *nanodiag BW Transfer Lab* operates as a full-service collaborative unit, providing comprehensive hands-on expertise, assay development, and rapid-turnaround molecular characterization. Within the lab, fabrication processes for both biological and solid-state nanopores are established and continuously refined, enabling reproducible and application-oriented workflows and the provision of specialized nanopore kits. Beyond nanopore technology, the lab offers dedicated pipelines for recombinant protein expression and advanced peptide synthesis, coupled with high-resolution MALDI-MS/MS mass spectrometry for rapid sample validation. Characterization is performed using parallelized electrophysiological, biochemical, and electrochemical methods. Leveraging state-of-the-art measurement stations and a specialized S3** facility, the *nanodiag BW Transfer Lab* covers the complete pipeline from molecular synthesis and component fabrication to functional high-resolution analysis, delivering scalable solutions within a fast service framework.

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