

A toolbox of ligands to study Acid-sensing ion channels (ASICs)

Dr. Elena Budusan
featured by Nanion Technologies



Elena Budusan completed her PhD at the University of Queensland, Australia studying ASIC channels and modulators. During her PhD, Elena spent 2 months at Nanion's headquarters in Munich using the Port-a-Patch and Patchliner to study ligand-gated ion channels in cell lines. After completing her PhD, Elena started a post doctoral research position at the University of Lausanne, Switzerland and still focuses on ASICs.

Can you give us a brief summary of your PhD?

"My PhD project focused on Acid-sensing ion channels (ASICs), crucial sensors for extracellular protons with implications in various physiological and acidosis-related pathological conditions. This makes ASICs a promising new therapeutic target in health and disease for new drug development. During my PhD, I studied ASIC modulating ligands, particularly peptides derived from venomous animals like snakes, funnel-webs, and tarantula spiders. Using two-electrode voltage clamp (TEVC) and automated patch clamp (APC) techniques, I characterized the pharmacological properties of these peptides. Overall, my work expanded the 'toolbox' of ligands to study these interesting ion channels, laying the groundwork for further exploration into their mechanism of action, evolutionary significance of these peptides, and their potential therapeutic applications."

What brought you to Nanion?

"I was interested in learning more about opportunities beyond academia, especially in the field of

electrophysiology and ion channel research. I was fortunate to have a supportive PhD supervisor who encouraged me to pursue an industry placement. With his connections to Nanion, he arranged a meeting with Ali and me. A couple of months later, I found myself in Munich, working on the Patchliner and the Port-a-Patch."

What was your project at Nanion?

"During my time at Nanion, I focused on validating different cell lines and channel types on both platforms, the Patchliner and the Port-a-Patch. Specifically, I studied the activation and inhibition of $P2X_{2/3}$ and $P2X_3$ receptors stably expressed in CHO cells on the Patchliner. In a second project, I compared the efficiency of the Patchliner to the Port-a-Patch when recording from Neuro2A cells, which endogenously express ASICs, different voltage-gated sodium channels, and $P2X_7$ receptors. I used different activators and inhibitors of these ion channels to show their expression in Neuro2A cells."



Nanion User Meeting 2022

Elena enjoyed meeting users and interacting with colleagues at the 2022 NUM at Nanion's headquarters in Munich.

"There wasn't a single 'best moment'; rather, there were many great moments that I cherished during my time at Nanion."

Dr. Elena Budusan, University of Lausanne

Was your project work at Nanion related to your PhD?

"Although it wasn't initially planned to be part of my PhD project, after observing ASIC currents expressed in Neuro2A cells, I couldn't resist testing some of my ASIC ligands on them. The results obtained on the Patchliner contributed to a research article that we just recently published ([The funnel-web spider venom derived single knot peptide Hc3a modulates acid-sensing ion channel 1a desensitisation](#))."

Did your project at Nanion accelerate your PhD project?

"Absolutely, it did. Using the Patchliner to study ASIC currents in Neuro2A cells, I was able to quickly and effectively validate my results for one of my spider peptides. This approach allowed me to confirm the activity of this particular

peptide in a cell line that endogenously expresses ASICs, complementing my previous findings obtained through TEVC in *Xenopus Laevis* oocytes."

What was your best moment during your time at Nanion?

"There wasn't a single 'best moment', rather, there were many great moments that I cherished during my time at Nanion. These included receiving support and help using the APC instruments, obtaining the first good recordings on the Patchliner and Port-a-Patch, enjoying coffee breaks with colleagues who became friends, and participating in networking events such as the Nanion User meeting. Overall, I thoroughly enjoyed every aspect of my experience there."

The screenshot shows a scientific article from the journal *Biochemical Pharmacology*. The article title is "The funnel-web spider venom derived single knot peptide Hc3a modulates acid-sensing ion channel 1a desensitisation". The authors listed are Elena Budusan^{1,2}, Colton D. Payne¹, Tye I. Gonzalez¹, Alison Obergruber¹, Nadine Becker¹, Richard J. Clark¹, K. Johan Rosengren¹, Lachlan D. Rash^{1,3}, and Ben Cristoforo-Armstrong¹. The article is published in *Biochemical Pharmacology*, Volume 193, 2024, pages 115317. The abstract states: "Acid-sensing ion channel 1a (ASIC1a) is a proton-gated channel involved in synaptic transmission, pain signaling, and several ischemia-associated pathological conditions. The spider venom-derived peptide PVTx1 and Hc3a are two of the most potent ASIC1a inhibitors known and have been instrumental in furthering our understanding of the structure, function, and biological roles of ASIC1a. To date, homologous spider peptides with different pharmacological profiles at ASIC1a have yet to be discovered. Here we report the characteristics of Hc3a, a single inhibitor cationic basic peptide from the Australian funnel-web spider *Phidippus opifex* with unique sensitivity to PVTx1. We show that Hc3a has unique pharmacology and binds different ASIC1a conformational states (closed, open, and desensitized) with different affinities, with the most potent effect on desensitization. We show the pharmacological basis of pronounced ASIC1a currents across multiple applications, and show novel binding of Hc3a to the desensitized conformational protein state. The solution structure of Hc3a was solved, and the peptide-channel interaction examined via mutagenesis studies to highlight key motifs of difference in response between Hc3a and PVTx1 and how to peptide with distinct pharmacology. The discovery of Hc3a expands the pharmacology diversity of spider venom peptides targeting ASIC1a and adds to the toolbox of compounds to study the structure of ASIC1a gating." The article includes an introduction, abstract, and a figure showing current traces.

ASIC 1a recorded from Neuro2A cells and effect of Hc3a.
Elena used some of her time at Nanion to investigate the effect of the spider peptide Hc3a on ASIC1a recorded from Neuro2A cells. Elena gathered enough data for posters presented at the 2022 and 2023 Society for Neuroscience Annual meetings, and contributed to a publication in *Biochemical Pharmacology*, complementing her previous work using *Xenopus laevis* oocytes.