

Replacing radiolabeling techniques with the SURFE²R N1

Dr. Randy Stockbridge
featured by Nanion Technologies



Dr. Randy Stockbridge is Assistant Professor at the University of Michigan, Ann Arbor, MI, USA. She holds a Bachelor of Arts from Princeton University 2005, and received her Ph. D. from the University of North Carolina in 2010. After that she went to Brandeis University for postdoctoral work before coming to Michigan.

contended with vast and unusual chemical threats throughout evolutionary time and retain a catalog of idiosyncratic export proteins to deal with hostile elements. Delving deeply into bacterial export systems reveals novel physiologies and structural surprises. By understanding bacteria's intrinsic vulnerabilities, we can generate new leads on antimicrobials.

We have discovered exporters of two different toxic ions: fluoride (F⁻) and guanidinium (Gdm⁺). Fluoride channels are interesting because they protect bacteria against toxic environmental fluoride ion, and guanidinium ion exporters, which have an interesting evolutionary relationship to multidrug exporters involved in antibiotic resistance. We have four big questions:

- what do they look like?
- how do they work?
- how did they evolve?
- what is their biological role?

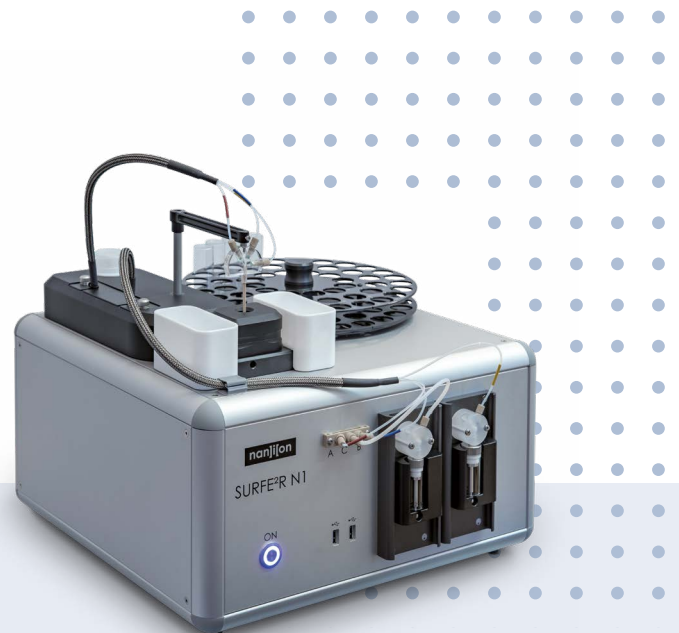
New ways to explore "old" targets

In 2018 Nanion Technologies ran a contest where researchers from all over the world had the chance to win a SURFE²R N1 platform for a 6-month period to perform transporter research in their own laboratories and with their own scientific scope. Randy Stockbridge and her research group convinced the Nanion team with their application. Shortly after, a system was installed, the assays optimized, and Dr. Randy Stockbridge and her team started with their research project at once.

NT: Randy, tell us about your research!

RS: My lab focuses on discovering and understanding membrane transport proteins.

Membrane transport proteins – channels and energy-coupled pumps – are the molecular gatekeepers of the cell. For microbes afloat in a hostile environment, these proteins import vital nutrients and export dangerous toxins. Bacteria have



Nanion's SURFE²R N1 platform

allows for the investigation of electrogenic membrane transport proteins with high sensitivity. So far over 100 targets have been studied including symporters, exchangers, uniporters, ion pumps and ion channels.

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NT: What techniques are you currently using in your scientific work?

RS: Our lab uses a breadth of biochemical and biophysical techniques to study these proteins, including electrophysiology, membrane protein biochemistry, x-ray crystallography, and macromolecular NMR, and since 2018 also the SURFE²R N1 from Nanion Technologies.

NT: What prompted you to enter the competition to win a SURFE²R N1?

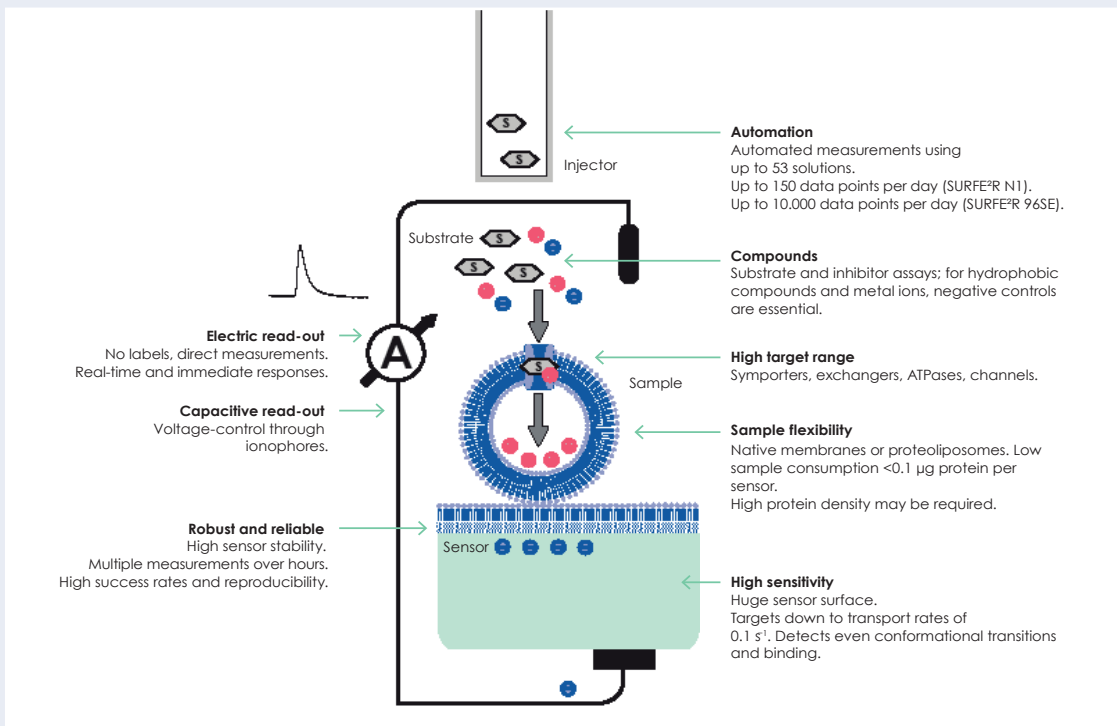
RS: We had done the initial characterization of our new family of transporters using radiolabeled substrate uptake, but we needed a more general assay in order to expand beyond

commercially available radiolabeled substrates. Since many of the substrates aren't fluorescent, fluorescence-based methods would not work, and the currents generated by these transporters were not large enough to see with planar lipid bilayer electrophysiology.

Because of the sensitivity of the SURFE²R N1 where small electrogenic currents can be measured, it seemed like the ideal device for us to measure this protein.

NT: What was the project you had in mind and did the SURFE²R N1 fulfill your expectations?

RS: We wanted to characterize the electrophysiological characteristics of our new family of transporters to find out properties such as K_m and V_{max} of the substrate, and ideally



Solid Supported Membrane (SSM) Electrophysiology Measurements on the SURFE²R Technology

The figure shows the SURFE²R technology with its benefits and limitations explained.

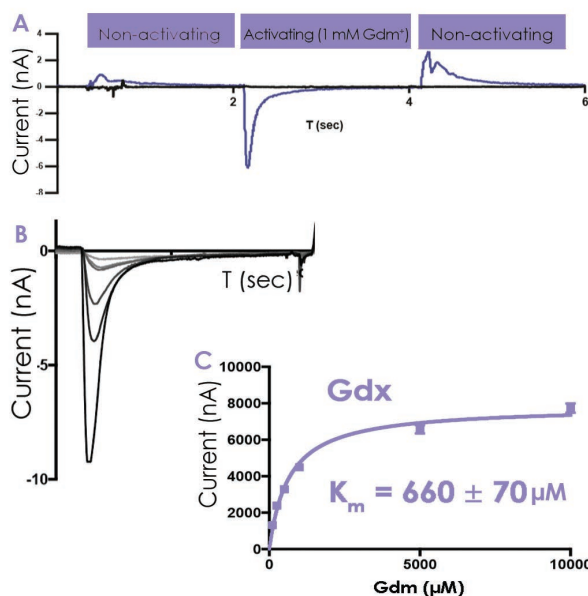
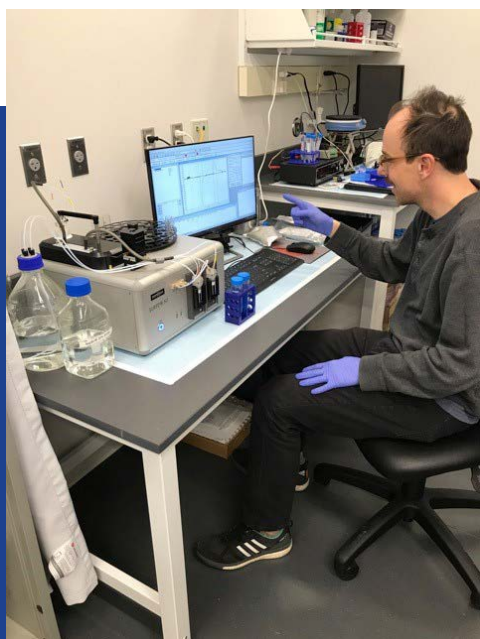
to screen a set of substrates for transporter activity. However, given our difficulties in developing a transport assay, I was pessimistic that we were going to be able to move beyond radiolabeled substrates. Contrary to my expectations, electrophysiology with the SURFE²R N1 platform worked right away. We saw very robust currents in our pilot experiments during the initial training session on the instrument. The data we collected in these first experiments with the SURFE²R N1 agreed with what we already knew about the transporters, and after that we were quickly able to push the project in exciting, unexpected directions by screening a large family of structurally related substrates for transport.

NT: How was the support you received from Nanion?

RS: The support we got from Nanion Technologies was really comprehensive; in addition to an in-depth training session at the start of the SURFE²R N1 demo period, they worked with my graduate student to analyze and interpret the data and develop the proper controls over the 6-month period that we collected data with the instrument.

NT: Are there other techniques you could have used for this project? If so, what are they and what are the benefits of the SURFE²R N1 over these other techniques?

RS: We have been using fluorescence and radiolabeled uptake experiments but these were unsuitable in this case. Now that we've started with solid supported membrane (SSM) electrophysiology, this technique has replaced radiolabeled substrate experiments in my lab, to everyone's benefit. We're not spending as much money on radiolabeled substrates, the experiments are much faster to perform, with fewer regulatory headaches. We were quickly able to push the project in exciting, unexpected directions by screening a large family of structurally related substrates for transport. And my graduate student vastly prefers doing SSM electrophysiology experiments, too!



Experiments on the SURFE²R N1 in Dr. Randy Stockbridge's laboratory. Left, Chris Macdonald is recording the bacterial guanidium transporter. Figure shows SSM (solid supported membrane) electrophysiology measurements of Gdx transporter reactions on the SURFE²R N1. (A) Raw data traces showing activation by application of 1 mM Gdm. In (B) and (C) the concentration dependent current responses and the corresponding Km-plot are shown.

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NT: Do you have any further projects in mind that you would like to use the SURFE²R N1 for? If so, can you tell us a bit more about them?

RS: I also work on a family of microbial fluoride channels. I've primarily characterized these proteins using planar lipid bilayer electrophysiology. However, the SURFE²R N1 is so sensitive that I can monitor currents at much lower substrate concentrations (a few micromolar). We're interested in probing channel function at sub-saturating ion concentrations using the SURFE²R N1.

I've also had several inquiries from colleagues interested in using the SURFE²R N1 for various applications, including measuring transporter or ion channel currents in organelles or membrane vesicles.

NT: Would you recommend the SURFE²R N1 platform to other researchers studying transporters?

RS: The SURFE²R N1 fills a niche that other transport techniques do not. I would definitively recommend it.

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Acknowledgments

We thank Dr. Stockbridge for letting us use images and data, and for the very nice conversation!

