Functional studies on membrane transportes using the SURFE²R N1– Introduction and Remote Demonstration Webinar

WEBINAR Q&A

Q: How do you distinguish between an inhibitor and a substrate based on inhibition experiments?

A: When performing an inhibition experiment by applying one test compound (inhibitor or substrate) to all measurement solutions, an inhibitor will behave similar to a substrate: the transport signal will decrease due to competition. The difference between substrate and inhibitor becomes visible in an activation assay: Only the test compound is added without a standard substrate. Here, only a substrate induces a transport current. You can easily perform both experiments on the same sensor sequentially.

Q: Is it possible to test voltage dependence of transport

A: The key behind the SURFE²R technology is capacitive coupling, meaning we detect changes in membrane potential due to electrogenic transport. Clamping the voltage would annihilate our read-out. We wouldn't be able to measure. But we have established some assays to apply a membrane potential by using the potassium ionophor valinomycine and application of a solution exchange providing or removing potassium. These experiments can provide information about the impact of voltage on transport rates, but are by far not as accurate as voltage clamp experiments.

Q: What was the activating solution for cardiomyocytes? How were all the other transporters excluded from the assay?

A: Typically we measure transporters which are overexpressed and therefore dominate the signal. But when assays are developed with whole cells or membrane preparations, the contribution of other transporters to the signal has to be ruled out, e.g. by specific inhibitors. In the case of the NCX assay using cardiomyocytes we tested different NCX inhibitors which yielded >90% inhibition of the signal. Our HEPES buffered activating solution contained 300 µM $CaCl_2$ and 140 mM KCl.

Q: Can you use intact cells and measure transport?

A: In general, any kind of sample containing stable membranes can be used. Intact cells, too. So far we have tested HEK and CHO cells, besides cardiomyocytes. But in contrast to membrane preparations or proteoliposomes they have some disadvantages: lower signal-to-noise values and sensor stability as well as a more tricky sensor preparation workflow. That's why we always recommend to start with membranes or vesicles, especially when the assay still needs to be optimized.

Q: But if your inhibitor is a substrate and generating the same charge as your substrate you will see no change?

A: In the inhibition assay you will see the same change, no matter if inhibitor or substrate. A typical inhibition experiment consists of two sequential measurements on the same sensor: First you need to measure the transport current of the standard substrate (full activation).

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Afterwards you apply your inhibitor or a second substrate into activating and non-activating solutions and repeat the full activation of your transporter. The signal will be decreased, since inhibitor or second substrate block binding sites of the transporter. Then % of inhibition can be calculated. You can distinguish between inhibitor and substrate by using the test compound only in the activating solution – without another standard substrate added. Inhibitors will not induce transport, substrates will. A sequence how you could test these things in one run on the same sensor would be: First use only the standard substrate for full activation, then use only the test compound (if it's a substrate you see a signal), then use the standard substrate again plus the test compound in all buffers (in the background) and you can see potential inhibition effects. Then use again the standard substrate to test for reversibility.

Q:
1. Is it possible to add solutions to the external solution manually?
2. Can measurements be done automatically with multiple sensors on the surfer?
3. How well does the membrane system on the sensor behave over time?"

A1: You can always use a pipette and add solution into the sensor (the sensor well has a max volume of about 250 µl). However, this is not suitable for an activation (recording) and could only be used to e.g. incubate with a compound (but there are also automatic workflows for that).

A2: The SURFE2R N1 only allows for one sensor to be measured at a time and sensors need to be exchanged manually after one experiment is finished. But there is the SURFE2R 96SE which is able to measure 96 sensors simultaneously using 96 sensor well plates.

A3: Sensor stability over time mainly depends on the transporter stability. We observed perfectly stable signals for more than 24 hours or 100 measurements on a single sensor at room temperature. But there are also transporters which tend to loose signal over a period of a few hours.

Q: can pH be used for activation and if so, is it tricky?

A: pH can be used as an activator. But pH jumps should be small, since large pH jumps also induce pH artifacts due to H^+/membrane interactions. When the task is to only investigate the pH dependence of the transport of another substrate, we would recommend to use solutions with different pH and activate transport via substrate concentration jumps instead of pH jumps.

Q: "Can the instrument be used to measure binding of a charged ligand to membrane protein, without transmembrane transport?"
A: This only depends on the binding process itself. Prerequisite for successful detection is that this process is electrogenic, e.g. binding may trigger a movement of charged amino acids within the protein or binding may close a cavity with a charge which was formerly accessible by water. In Na+ and H+ coupled sugar transporters we observe such sugar induced

pre steady-state currents when no transport occurs. Success of such experiments depends also on the protein density – binding currents can be small and amplification via high protein density may be required. Best chances are using liposomes with low LPR.

Q: Can the sensor be cleaned and reused? Thank you
A: Yes, it can be re-used. Typically, each sensor can be re-used 5 to 10 times, depending how well it is treated during the cleaning process. Keeping in mind that a single sensor can be used for multiple sequential measurements during one day, this pulls down the costs per measurement a lot.

Q: How was the capacitance at the beginning measured, without voltage step?
A: The SURFE2R has a built-in voltage generator. But this is only used to apply voltage during the capacitance and conductance measurements during sensor quality tests. It cannot be used during transporter measurements.

Q: "Can you change the internal solution of proteoliposomes on the fly, or do you need to prepare sensors with a variety of different internal solutions?"

A: This depends on the membrane sample and the type of compound you want to apply a gradient for. The membrane needs to be leaky for the compound on a second to minute time scale and stable for at least 1 s to apply a gradient and measure under the influence of such gradients. This is true for pH gradients and is often true for compounds which are transported by some transporters within the sample membrane. Whenever the membrane is not leaky for a compound, it has to be added during sensor preparation. To do so we usually dilute the membrane sample in a buffer, containing the compound following sonication. During sonication the vesicles are loaded with the compound. Membrane fragments from cells or tissue are generally much more leaky than liposomes.