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WEBINAR Q&A

Q: Is it possible to measure electroneutral antiport with SURFER?

A: Sometimes it is possible to measure electrogenic binding reactions in otherwise electroneutral antiporters. E.g. Cation binding in the electroneutral Na^+/H^+ Antiporter NhaP was measured with SSM-based electrophysiology (Calinescu, 2016, JBC).

Q: Empty liposomes give a good background but what about HEK293 cells or other mamallian cells?

A: Since the activation procedure of an assay is very specific for a transporter or pump (only certain ions and substrates present, no membrane potential steps) potential endogenously expressed proteins are usually silent and the background is good. But transporters with very similar function could be activated. Therefore, control measurements in parental cells are recommended.

Q: How precisely does the signal follow actual kinetics of charge transfer? Can one extract kinetic parameters for one turnover of the transport reaction from a Nanion trace?

A: The charge transfer in the vesicles on the sensor and the current readout have no measurable time delay. The limiting factor is the speed of addition of the substrate. The perfusion system is optimized for a fast exchange and enables the resolution of events starting from about 10 ms duration. This means it is possible to extract some kinetic information. Extracting kinetic parameters for one single turnover is possible in case of binding reactions, meaning only one specific reaction takes place in each protein once.

Q: How fast is the perfusion system?

A: Standard exchange speed is 200 µl/s, enabling the resolution of 10 ms duration events.

Q: How much protein do you need per sensor and how long does the sensor preparation take?

A: Using membrane fragments usually the total protein concentration is measured. For one sensor 0.1-1 µg total protein is used. In case of liposomes the amount of protein depends on the LPR used for reconstitution. Amounts from low nanogram range to 1 µg have been reported. Which protein concentration works for a successful measurement depends on the turnover of the protein.

The sensor preparation takes in total about 1.5h. However, 60 min are incubation or centrifugation steps and do not require active input.

Q: How many measurements can you do per sensor

Nanion Technologies GmbH
Ganghoferstraße 70a
80339 Munich
Germany
www.nanion.de

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A: Typically, 10-50 or even more activation cycles are possible with a stable amplitude. The range depends on the individual protein stability.

Q: Is it possible to use the method for deorphanization?

A: Yes. Deorphanization is challenging, but SSM-based Electrophysiology is a useful tool for it. The most interesting feature is that it enables testing of different substances as potential substrates in a direct assay. It can also easily differentiate between inhibitors and substrates.

Q: Have you tested common inhibitors that target choline transporters?

A: We are doing so. Some highly hydrophobic compounds do not give a clean background noise.

Q: How much sybody have you use for the inhibitory assay?

A: We use a 10x fold molar excess compared to that of transporter in the chip

Q: Are you using this Sybodies for structural studies?

A: Yes, inhibitory sybodies are being used for structures determination

Q: Can this method be used for transporters that use other coupling ions?

A: There are no limitation in this regard. The coupling ions are typically present in the basic buffer or the liposomes are even loaded with certain ions. The ionic composition of the basic buffer is not restricted. E.g., K^+, Na^+, H^+, Ca^{2+}, Mg^{2+} and Cl^- have been used as coupling ions.

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80339 Munich
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