Presentation: "Cryo-EM structures of human neuronal glutamate transporter" by Olga Boudker, Professor of Physiology and Biophysics at Weill Cornell Medical College

Q: You reconstituted a protein for the SSM measurements, do you expect a mixed orientation of the protein and do you think this has an influence on the current properties?

A: This is a very good question. Yes, we expect mixed orientation and I think it is interesting something that we've been struggling with is, that, the KMs that we see in cell membranes for example, are lower, so they apparent affinity is higher than what we see in the constituting systems, and what we see on the grids appear to be show even lower affinity (at least for the inward facing state). So, this kind of discrepancies of affinities made us think a lot about what is it that we're actually seeing in different conditions, and I don't really have answer, but I think if we had mixed orientation, then we would see contributions from both what we believe is a high affinity and low affinity parity.

Q: Beautiful work, as always, curious if you have been able to obtain SSM currents with potassium?

A: We actually haven't tried it.

Q: Have you had a closer look at the chloride conductance? Do the observations in the SSM experiments fit to the expected properties?

A: No, we have not. I am very excited to look at that. I think it could be a really powerful technique for us to look at the conductance properties of isolated protein.

Q: Can you go over the part where you switch from sodium chloride to sodium phosphate?

A: What we saw is that in sodium chloride the currents were decaying very fast and had a negative overshoot. So, they didn't look like a multiple turnover transport currents. We're quite new to this technique so it wasn't obvious to us until that was pointed out by the reviewers. However, when we switched to phosphate buffer and phosphate is not permanent and iron, we now saw a pretty normal transport currents. So, that made us speculate and we think that the neuro-currents that we saw were due to the fact that as the transporter was transporting that there was a compensating ion current that basically decayed the capacitive currents faster than they normally would.

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

Presentation: "SSM-based electrophysiological characterization of a metal transporter" by Lars Jeuken, Professor of Molecular Biophysics at Faculty of Biological Sciences, University of Leeds

Q: Did you try using different lipids to see if the background can be improved that way? Meaning, do different lipids interact more or less with the metals?

A: No, actually, that's a very good suggestion. We've been struggling with this protein for a while due to the low currents and that's probably one of the things we could have used. I think for most I would have to check, as most for of the work I presented today we used total ecoli poly lipid extract to create a mixture of lipids that is relevant for bacteria, obviously the e coli lipid extract id not the same as the enterococcus lipid extract. But this is a very good question, something that we haven't done.

Q: Why are the peak currents not going linear with lipid to protein ratio? Do you have a way of determining the effective LPR after reconstitution? It looks like you got constant LPRS for all reconstitutions.

A: So, we haven't independently determined whether the phosphate sort of the phospholipid concentration is correct so there we just assumed that we recovered most of the phospholipids where we use normal protein assays to make sure that our lipid to protein ratios were in the order of what we would expect depending on what we put in the reconstitution protocol. So, we've fairly sure that those lipid protein ratios are, if not, too many significant digits but are roughly correct, so why don't we see the different activities? And that's exactly what I indicated on the slide but didn't explain enough. That already suggests that what we see is not a total activity of the enzyme, as you would expect with multiple turnovers where you would expect especially with the decay rate to be different between the different LPRS. So, one hypothesis, and I am just saying this is really speculation, is that what we may be seeing with this protein and that is also suggested by the low currents is maybe one turnover after which the system is in kind of thermodynamic equilibrium or otherwise locked, so the amount of protein might not be sort of limited, but it might be limited by something else, not by the number of proteins in the liposomes.

Q: You mentioned that buffers are important to lower artifacts. What buffers do you recommend?

A: The metal has a problem because I would actually recommend phosphate, in general. The problem is that with some metals you cannot use phosphates because metal phosphates will crash out because it doesn't solidify us very well. So, in that case, you're

bound to use sort of your other buffers that are really well known like HEPES and MOBS for instance. I would always be careful with Tris being an amine. Again, tris and metals are not a good combination because the amines start to coordinate to your metals, so always be careful. The transition methods come with a couple of additional rules.

Q: I had a question about the respiratory complex, when you said it could be activated and deactivated sate. Could you elaborate what triggers the transition in deactivated or activated state?

A: So in the complex one, this is exactly what we would like to know. Cryo-EM structures are now available not by us, but by our collaborator who I should have mentioned, Judy Hurst, in Cambridge. So, what really governs that transition is currently under investigation. But, if you don't incubate it with the substrate and the H for a long time at 37 degrees it just shuts down so electron transfer doesn't happen anymore. That also means that the reverse electron transfer doesn't happen, and that's quite important, because if the electrons normally go from NADH to the quinone pool but in reverse electron transfer you might go from the quinone pool to oxygen creating radical oxygen species. So, it's not at the NADH converting site, there's something around the quinone binding site that switches off and there're some structures available that show how this might occur.

Q: So, if you take the enzyme and you add NADH to it for a while it's not going to be active and then it will gradually activate?

A: right yeah and interestingly if you do a normal assay in a cuvette you can do that and you can just see that after a bit of a lag phase your energy H concentration goes down so you can see the whole thing it's sort of the lag phase and then the activity but of course the supported membrane has this one second time frame that we look at, right, so it's a completely different time frame than most enzyme assays and that just means you have to do things differently. In other words, you do one measurement after adding NADH and then you incubate the end of the H and then you repeat the system where you first briefly get energy H out and then you reintroduce it; and then it also takes some time before the session to deactivate if that would have gone too quickly of course then you would be in real trouble so you you're really playing with the kinetics of the deactivation and activation and the actual sort of normal turnover. With the hydrogenate there's also this deactive state which has nothing to do with the quinone side. There is a very complicated system to do with one particular iron sofa cluster which actually has kept the community busy for what 20 years of really loads of fun experiments trying to see how that happens. It's going to be difficult to explain exactly how that happens but it is called a super oxidized state if there's no hydrogen available one of the iron sulfur clusters goes into a superoxide state after which it's sort of the electron transfer to that site is then very very

slow. in other words, it doesn't transport electrons anymore on the normal time scale and the only way to reactivate it is to to slowly re-reduce that into the normal reduced and oxidized state so nature seems to have different sort of ways of deactivating enzymes when it needs to. It's not a one-size-fits-all type solution of evolution and this is really why I do this work.

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