WEBINAR Q&A

Did you consider the effect of salt on the assay. Did you keep the osmolarity constant?

Christine Ziegler: Yes we considered this. I have to say, that's why we went for this non-physiological stimuli. It's a good question. It's something we need to try, also now using different ways in how we apply the driving force. We thought that we are on the safe side as we are using that ionized sugar, but if we can now really avoid osmolarity changes, I cannot tell. This is an excellent question, especially for an osmolyte transporter this must be considered.

Could you use other organic ions to match out the osmolarity, to have the same concentration on both sides?

CZ: No, not yet. As I said, we are kind of at the beginning of this study. First of all, we wanted to understand what we see and it took us some time to understand at which parameters we have to look at but it's definitely on the plan now to go into that.

Did you validate the inhibition data which you got from your SURFE2R measurements, maybe with traditional liposome assays using radioactivity, just to see whether the numbers are in the same range?

Camilo Perez: We haven't done it, it's a very fair point. I will tell Natalie to go back and do this measurement, thanks for the suggestion!

Can you observe a difference in the inhibition characteristics of nanobodies compared to small molecule inhibitors? And secondly: is the recovery time of the signal different comparing the nanobodies?

CP: For this transporter, we just have one other small molecular inhibitor which is a very well-known molecule, that inhibits cholin transport, and unfortunately that inhibitor is not reversible, it was not possible to get rid of it after washing extensively. We don't really know why, so we can't compare with inhibitors that are reversible inhibitors. Among different nanobodies, we didn't see major differences except for the height of the peak currents.

How can you distinguish between transport and binding in the case of BetP?

CZ: I have to say, for a long time I was not an SSM believer. sorry to say that, this was exactly my point, how can I do that? That is exactly why we have these mutants that I presented to see where there is definitely no transport. At the moment I would say, you will always have a mixture as I said; pre-steady state and then a real steady-state where you can see transport but as long as we do not have an inhibitor it will be very difficult to answer that question to the very end. We compare it for sure, to uptake measurements - actually most of these were done by Camilo, during his PhD. But at the moment I cannot

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tell you that"this part" is exactly the transport component. Let's see, perhaps with a different mutant or with an inhibitor we can narrow It down a little bit better. It is definitely something which has to be solved. But I am absolutely convinced we see transport in the wildtype because the transient looks very different and the charged translocation pattern looks very different for the mutant I presented.

If the k(on) and k(off) rates for the nanobodies to the choline transporter are relatively low, could you use the SURFE²R to measure or confirm these rates and if so, how do they compare to the SPR data?

CP: I guess that can be done. We haven't analyzed the data in looking in that, but I will say that is definitely feasible, we will need to do it because we have both sets of data, also the SPR Data. We will give it a try but we haven't done it yet.

Would the higher time resolution of 1mm sensors help distinguish binding and transport better? Did you try?

CZ: Yes we tried this, but unfortunately this mutant was so fast that even with a 1mm sensor it was not possible. But it is definitely the first thing to check, to go from a 3 mm to a 1 mm sensor. You saw differences but even with that sensor we were at a resolution limit.

In the case of unspecific nanobody binding, how could this be prevented during the SSM experiment?

CP: We will recommend - and actually this was suggested by Randy Stockbridge when she was here presenting in the last seminar- she recommended to use BSA in your buffers. So include BSA before applying nanobodies so that these can't bind to unspecific sites.On that I agree with her - this can be a good way to deal with this.

Did all the nanobodies you screened bind to the same epitope? Can you choose the epitope?

CP: We have the different Cyro-Em Structures with two different nanobodies, those two binds to different epitopes. I cannot tell about the rest and I don't think we will attempt to solve them…