**Random thought and ideas about DAB2**

Stopped-flow CO2 hydration

Result: I could not detect any significant activity. However, there are still some uncertainties so I would not rule out that its not active. Raw data and results are attached in separate file.

* Something in the protein sample is messing up the signal. Resulting in a lower total dAbs with the protein sample compared to the control. Not sure how to explain this, but my best guess is that the GFP mess something up.
* DDM makes the measurements messier and causes e.g. random spikes in a lot of traces.
* The protein buffer is 50 mM Tris, 150 mM NaCl (correct me if I’m wrong Jack). I normally have lower buffer conc (and add less protein). This amount might change the pH of rxn and Tris have a different pKa than TAPS, so it will make analysis more complicated but you should still see activity if it’s there. Chloride have shown to be an inhibitor of β-CA, so this could actually be a problem.
* With high protein conc I saw an increase in absorbance after ~100 s. Can this be an indication of that protein is precipitating.

Ideas to try:

* Measure activity with hCAII and CsosCA/carboxysomes in in same amount of DDM to confirm that this is not inhibiting.
* Buffer exchange protein to a no salt or low salt buffer. Chloride have shown to be an inhibitor of β-CA. Currently I had ~50 mM NaCl in the reaction (from DAB2 buffer).
* Remove GFP.
* Purify only the soluble protein/part that is predicted to have CA activity.
* Check so that protein is not precipitating when conc of DDM is lower. If it is add DDM to assay buffer. - did you do this last week Jack?
* Some β-CA have unusual steep pH-profiles. e.g. CAN/ECCA from E. coli have almost no measured activity in pH 7.5 but high activity in pH 8.5. I did the measurement in pH 8. So can try it in a higher pH ~8.5-9.5. Can also try in lower pH – even if it would surprise me.
* If you think it’s a vectoral CA that need something to drive/activate the enzyme. What can this be and any suggestions of what to add to the rxn?

Inhibition of CA

* Which inhibitor did you use and what this in CAfree rescue experiments?
* Acetazolamide (and other sulfonamides) are less potent in microbial β-CA (ref. Frost and McKenna chapter 4 “anions and sulfonamides inhibit plant β-CAs more strongly than for microbial β-CAs )
* There should be other inhibitors to try. Check work from the the Supuran lab. They are one of the leading lab in CA kinetics and inhibition.
* What conc of inhibitor did you use? I think penetration of cell membranes can be a problem with sulfonamides
* Try it in the bicarb uptake assay instead of in CAfree rescue? Does that make sense?

Random thoughts

* Soluble part does not complement CAfree alone. Does it actually express and fold by itself?
* The active site mutations are they tested in CAfree? If we are not be able to measure any activity it might be an idea to 1. Try these in the bicarb uptake assay and then 2. Run a western-blot to confirm they express 3. Purify the complex to show that the mutant still form a complex and behaves the same as wt.
* The protein is huge compared to a “regular” CAs indicating that it’s a multidomain protein with several different functions.
* If the soluble part can’t be expressed by itself can we try to figure out which part is the potential CA and then try expressing only this part?
* There are a lot of examples of human CAs that are membrane bound/associated. Probably a good idea to dig into this literature to get ideas of how it could work and potential experiments to do.
* Also examples of extracellular human CAs that are associated to membranes. Is it a totally crazy idea that it’s on the outside of the cell and not the inside?