* PAGE NUMBERS!
* I know we discussed this at B&B but you should be extra sure that there are no other examples of 100 mutant enzymes generated with kinetic data. Somebody’s feelings could get hurt if this data exists and they’re reviewing the paper…
* I don’t think I explicitly read it anywhere regarding your yields but it seems that you are getting 1 mg/ ml culture? If so, incredible (but make it clear that it \*is\* per ml culture)!
* I’m not sure why you have a mixture of hyperbolic and linear plots in supplemental Fig 3? You should pretty much always derive kcat & Km from hyperbolas. In the M&M section, you say that you fit to the M-M equation.
* I think that the effects of mutating Gln19 are pretty well expected since you are pretty much completely destroying binding interactions. If I am interpreting Fig 3A correctly, it looks like the only other binding interactions are H-bonds with ether oxygens. Those are pretty weak. High affinity sugar binding sites have specific interactions with all of their hydroxyls (like Gln19).
* Fig. 2 – I don’t like the log 1/Km column – why not make it log Km so it is easier to understand for most folks (particularly since you talk about the Km range in the figure legend? Fig. 4, Table 1 too.
* Speaking of Fig. 4 – I’ll admit I’m an idiot. What does SRC stand for? I suspect any bio-guy like me might make a similar remark. OK, I see it defined in the text now.
* Towards the end, you talk about the most important influence on 1/Km is “protein packing”. Does this mean minimization of internal voids arising from the mutation? Or maybe have something to do with stability? If so, too bad no data for stability then!
* It would be useful to add an extra step to the mechanism in Fig. 1B (with catalytic residues) to illustrate the description that you give in the text of the introduction. This will help make Figure 3A make more sense, too.
* In Fig. 4, the axes labels should be measured or predicted LOG kcat/Km or LOG kcat, etc., correct? Same for the insets. If so, it looks to me by eye that you’ve got a bunch of Kms around 1 M – both greater & less than? In Fig 2, you say that the range is .6-85 mM. Am I missing something? Also, your biggest kcat/Km is 560K which seems like it should give >5 for the log value. AHHH – I see these are \*relative\* values. I think you should emphasize this – the axes are deceptive since it looks like you are comparing measured values versus predicted values…
* In Table 1, negative numbers indicate that these hinder catalysis? The descriptions aren’t very thorough (I guess they can’t be…) but if I understand correctly then hydrogen bonding between pNPG and the protein is one of the worst things for kcat/Km? That’s sorta hard to picture. Other lines in this table are also difficult to either rationalize or understand.