Things I’m confident about:

**PAR-2 can polarise in systems with uniform aPAR.** Data from me, KangBo and Joana showing polarity with S950A in no-flow regimes. We also have P1 data from KangBo and C1B data from Florent’s paper to back this up.

RING domain mutants have a lower membrane affinity than wild type, even in the absence of PKC

**LGL-1 (and CHIN-1) can polarise in a similar manner.** I think I have reasonably good data for LGL-1. At this point I can’t say for sure whether this is PAR-2 dependent. I’ve made an nmy-2(ts), PAR-6 mCh, LGL GFP line, PAR-3 (S950A) line, which I can do a PAR-2 RNAi on top of (a lot going on!), but haven’t had time to do the experiment yet. My CHIN-1 data is less good, and I don’t have the right line to do the equivalent experiment, so I’ll probably have to skip this.

**PAR-2 can dimerise via its RING domain**. SEC MALS data using RING domain fragment, and Y2H using full length protein. Both need to be tidied up, but I’m convinced. Dave is going to do a concentration series for SEC MALS. AUC and structure will also help.

**Mutations to the dimerisation interface reduce membrane affinity**. Currently based on structure predictions, but I think these are pretty good and should have a real structure soon to compare to. SEC MALS confirms that dimerisation is distupted in L109R. Difficult to say for certain that we’re not unstructuring the whole domain, but shouldn’t do based on other RING domains. Also the fact that L109R RING was easier to purify than C56S RING is reassuring to me.

**Membrane tethered RING can polarise in a PAR-2 dependent manner**. RING-PH data, with PAR-2/PAR-6 RNAi and C56S. I think that dimerisation with PAR-2 is the most obvious explanation, especially given some of my other data, but it’s not 100% definitive. For example, you would see the same thing if the RING domain was interacting with something else that was enriched in the posterior in a PAR-2 dependent manner. It’s also a relatively large fragment which includes the first microtubule binding site and the phosphatase site. The C56S is a nice control, but we think it’s a pretty disruptive mutation so it could plausibly disrupt other interactions. I need to do this with L109R (in progress).

**Non-linear membrane binding kinetics can be captured with a thermodynamic model**. At its most extreme this gives a quadratic relationship between cyt and mem, but this is parameter dependent.

**Thermodynamic models predict that this should be an off-rate phenomenon**. Not sure I entirely follow David’s reasoning, but it fits with my intuition. May have to work on how we explain this idea.

**PAR-2 displays concentration dependent membrane affinity**. This is obvious just from looking at images from the rundown experiments after af-correction (and by taking cross-cortex profiles). Of course, this depends heavily on the af-correction method, but I think this is all solid. PH rundown is a good control. Quantifying the images to get an accurate cyt vs mem relationship is a separate issue which I’ll come on to.

**Positive feedback + linear antagonism *can* combine to allow polarity**. However, this depends heavily on how the positive feedback is implemented. This is pretty easy if you don’t care about the mechanism, e.g. adding a simple interaction term between membrane and cytoplasmic protein (kpos \* cyt \* mem). In this case the model also seems pretty prone to spontaneous symmetry breaking. <is this bistability or more like a Turing model>

Nonlinear membrane-binding kinetics introduced by dimerisation are insufficient for bistability in systems with linear antagonism. I’ve tried a few approaches for modelling this. The Dawes/Munro model only seems to work when detailed balance is broken.

A kinetic models inspired by David’s thermodynamic descriptions

**Results I’m more uneasy about:**

**Quantifying the PAR-2 cyt vs mem relationship**. Putting accurate numbers on the cyt vs mem relationship is pretty tough, and relies on being able to accurately fit the shape of the cross-cortex profile. This requires accurate descriptions of ‘expected’ profile shapes for cytoplasmic and membrane contributions. Small differences in the shape of these profiles can have quite a big impact on the quantification. I’ve tried to give this my best shot, but it might be a tough sell. Cytoplasmic profiles are easy – currently I’m using my glh::tPT2A::Neon line (entirely cytoplasmic), but there are a few other ways (e.g. opposite pole in a polarised cell, like Jake did). Membrane profiles are much more tricky. I’m not a huge fan of the eggshell staining method. It’s very fiddly, difficult to fully wash out the dye from the buffer meaning I always get a background signal, and the shape of the profile can vary quite a lot from embryo to embryo. The machine-learning-based method I’m using is nice because it extracts profiles direct from polarised embryos, and is very consistent, but it relies on the assumption that the PAR-2 cytoplasm is perfectly uniform (it could well be but it’s hard to know this for sure). It’s reassuring that the thermodynamic model gives something reasonably close to the data, but the data is still a bit more nonlinear and it’s hard to know how much to make of this.

**Cytoplasmic PAR-2 is monomeric**. The mitochondrial GBP experiment tries to address this, but all I have is a negative result without a positive control. It could always be that the GBP is messing up an interaction. PAR-6/PKC-3 could be a good positive control (based on Jakes results using GBP in oocytes this should work), but even still it’s two completely different proteins. PAR-2(GCN4) would be better, but then I’d have to make it in both GFP and mCherry, and I’m not sure this is worth it. If I can get a KD from AUC that’s compatible with being monomeric at cytoplasmic concentrations then we’re probably ok. Also the fact that the RING fragment on its own doesn’t go to the membrane (mentioned in Hao, but I’ve also made the line to confirm this). So overall this is probably ok.

**Forced dimerisation using GCN4 causes PAR-2 to localise to internal membranes**. I think this statement is true, but without an explanation that fits with theory it’s pretty unsatisfying. I think we also need to understand what it’s binding to (e.g. stainings). Repeating with another dimerisation domain would also help to confirm that this is a PAR-2 dimerisation-dependent phenomenon rather than a GCN4 phenomenon (although the GCN4 alone is a good control)

**PAR-2 does not act as a ubiquitin ligase**. My results are in line with this, but it’s just too difficult to rule out. We’re relying on negative results, and I’m also not completely confident with the purified PAR-2 made with Anne/Colin that I used for the assays. There was just lots of weird stuff: two bands, ubiquitination that seemed to persist in C56S, higher expression for C56S. The linchpin mutant isn’t conclusive, as some active E3s don’t rely on this site. If I really wanted to push this I would try some discharge assays with our purified RING fragment. But if I’m expecting a negative result then this still isn’t very useful. We’re also working against a claim from the Hao paper that PAR-2 *does* act a ubiquitin ligase.