To test whether PAR-2 is constitutively dimeric in vivo, I devised an in vivo pull-down assay based on dual labelling of PAR-2 with two different fluorophores. Expressing PAR-2 with a mix of green and red fluorophores means that, were PAR-2 to be forming stable dimers, a mixed population of green/green, green/red and red/red dimers would be expected to be present within the cytoplasm. Thus, forced relocalisation of one of these pools to a detectable exogenous location in the cell should result in a detectable signal of the other fluorophore.

A similar assay was used previously by Reich, who demonstrated a stable interaction between PAR-6 and PKC-3, through forced localisation of GFP::PKC-3 to the plasma membrane with a membrane-tethered GFP nanobody. (In this case the plasma membrane was a suitable target because, under the conditions of the assay, PAR-6/PKC-3 do not normally bind to the PM)

Here I propose a similar approach, relying instead on recruitment to mitochondrial membranes within the cell. Mitochondrial membranes are dense within the cell, and can be probed easily with a localisation signal from the TOMM-20 protein.

To first test the potential utility of this method, I built a TOMM-20::GBP probe tagged with mKate, and introduced this into the worm by CRISPR. Crossing this line to GFP::PAR-2, and imaging the F1s (heterozygous for both GFP::PAR-2 and the probe), shows that the probe is well expressed, displays the expected mitochondrial localisation pattern, and is able to recruit GFP::PAR-2 to the mitochondria.

To perform the proposed dimerisation assay, I next attempted to build an untagged version of the probe (lacking mKate). Whilst I was successfully able to build and introduce the construct into the genome of the worm, I found that this untagged probe failed to recruit any GFP::PAR-2 to the mitochondria, likely indicating that the probe failed to express. This suggests that the mKate tag may be stabilising expression of the full construct, possibly due to the presence of introns within the mKate sequence. As an alternative method to free up the red channel, I introduced a point mutation into mKate by CRISPR designed to disrupt the chromophore region. This construct, by contrast, was well expressed and, like the tagged parent construct, able to pull GFP::PAR-2 to the mitochondria.

Next, I crossed this line to a line expressing PAR-2::mCherry at the endogenous locus, homozygosing both constructs. I then crossed this line to a line homozygous for endogenous GFP::PAR-2, giving F1s heterozygous for GFP/mCherry PAR-2, and heterozygous for the probe. Imaging embryos from these F1s showed that, whereas GFP::PAR-2 is fully pulled to the mitochondria, there is no detectable colocalisation with mCherry::PAR-2.

Overall, this strongly argues against constitutive dimerisation of PAR-2.