**Feedback in the model**

Given the nonlinearities associated with dimerisation reactions, the distribution of protein between the four states is expected to vary depending on total protein amounts. This is particularly interesting in the case of PAR-2, as partitioning between the cytoplasm and membrane doesn’t follow simple linear kinetics, but varies depends on dosage (section x). I therefore investigated whether our simple thermodynamic behaviour can capture similar behaviour, by varying phi across a range of concentrations and solving for phi\_m and phi\_c.

Figure x shows that, in certain parameter regimes, clear nonlinearity can be observed. For a given value of wm, changing wd impacts both the shape and the amplitude of the cytoplasm vs membrane relationship (fig xA). By stabilising the high affinity dimeric state, increases to wd increase overall membrane affinity for a constant wm, leading to a steeper relationship.

As shown in fig xB, effective exponent of the cytoplasm vs membrane relationship (beta), obtained by fitting <>, varies across parameter space between 1 (linear relationship) and 2 (quadratic relationship). Peak nonlinearity occurs in regions of high membrane energy and intermediate dimerisation energy. As shown in fig x, this corresponds to regions of parameter space in which membrane protein is largely dimeric and cytoplasmic protein largely monomeric, across the relevant range of dosages. Where wd is low, protein is unable to dimerise, even at enriched membrane concentrations, and thus follows a linear relationship like that described in section x. If, on the other hand, wd is too high, protein is constitutively dimeric in both the membrane and cytoplasm, and will behave similarly to a monomer (albeit with a higher membrane affinity). If wd is intermediate, then an asymmetry is observed whereby protein is dimeric on the membrane but not in the cytoplasm, provided that wm is sufficiently large to set up a substantial concentration asymmetry between the two compartments.

**Two species model**

To simplify further analysis, we can convert the four species model to a two species model, in which we only track overall membrane and cytoplasmic concentrations, phi\_m and phi\_c, irrespective of dimeric state.

If we assume that dimerisation reations in the membrane and cytoplasm are fast relative to membrane binding, we can define instantaneous monomer and dimer concentrations as a function of overall concentration. For example, for cytoplasmic protein

<equations>

which is identical to the description in section x. As a result, and because we can describe the chemical potential of each of these species individually (equations xxx), we can combine expression <> and <> to define the overall chemical potential of cytoplasmic protein (mu\_c) as a function of overall cytoplasmic concentrations (phi\_c):

<equation>

And analogously for membrane bound protein:

<equation>

These two equations can be solved analytically at equilibrium (mu\_m = mu\_c) to find equilibrium membrane and cytoplasmic concentrations.

**Assessment of PAR-2 membrane binding kinetics**

Overall, this analysis shows that dimerisation can have a strong influence on the membrane binding kinetics of a protein. With the assumption that the membrane binding energy of a dimer is twice that of a monomer, dimerisation is expected to strongly enhance membrane affinity and overall membrane localisation. I have also shown that nonlinear membrane binding kinetics can arise if dimerisation and membrane binding energies are sufficient to so that dimerisation is supported on the membrane but not in the cytoplasm.

A few behaviours of the model make it highly relevant for consideration of PAR-2.

Firstly, the model suggests that a reduction in dimerisation energy ($w\_d$), as would be expected for a RING-disrupting mutant, can substantially decrease membrane association

Secondly, the requirements that membrane protein be dimeric and cytoplasmic protein monomeric both appear to be the case for PAR-2 based on in vivo observations.