First, we have a binding of the solution A to the membrane bound P, with rates and , where . This is thus a 3D reaction, so we can report the *K*D in units of M, (or, e.g. um-3). The lipids P can be converted from units of um-2 concentration by multiplying by Surface Area (SA) divided by solution volume V: SA/V. We typically assume about 1% PIP2, (2x104 copies/um2) but I think Honing uses 10%, (which is 2x105 copies/um2).

(1)

(2)

Next we have a binding of membrane bound AP complex, to the membrane bound receptor, T. We assume based on the experiment, that the solution A cannot directly bind to the receptor T. This is now a 2D reaction, so concentrations are in units of um-2, and *K*D is in units of um-2, as the on rate has units of um2/s. However, at equilibrium we can convert everything into 3D units, by multiplying by A/V, so the equation is indeed the same if you report concentrations of all species (AP, T, and APT) in volume units, and the KD in volume units, but it now has a dimensionality factor that is introduced into the equation! DF=V/(Ah), or the inverse of that. It has no units, and is necessary to convert copies from /A to /V relative to a 3D KD.

(3)

(4)

(4b)

(4c)

Where I used Eq 2 to get the second expression on the RHS.

So we will use volume units (copyNumbers/um3, or mol/L).

**OBSERVED KD BETWEEN SOLUTION AND MEMBRANE**

Then we have the observed equilibrium where now we have more than one choice. Here we have to make assumptions, and it is not entirely clear what the authors used as the other unbound specie (T or P?) when defining the KD between solution and membrane. I think it really only makes sense to use P, but that means P must be >>T (like a factor of 10 is probably sufficient).

First, Let’s assume [P]>>[T], which means that the free or unbound component of the protein partner is the lipid concentration. and thus use

(5)

If we use Eq. 4, the denominator becomes,

(6)

Then we can use Eq 2, to recover:

(7)

And now I will multiply the top and the bottom by to get:

(8)

Alternatively, instead of Eq (5), I will instead assume that the [T]>>[P].

In this case, we have that

(9).

So now we will have the same denominator, and using Eq (4)

(10)

For Eq. 10, unlike Eq. 8, you also need to include the total amount of [APT] on the membrane, or alternatively, a measure of the unbound A, P, and T.

So the relation between and is distinct depending on whether you are in the transferrin, or PIP2 dominated regime.

I think it only really makes sense to compare when [P]>>[T], because the [P] is what controls the localization to the surface.

If you use [T]=50uM, that may be higher or comparable to [P], which may explain the discrepancy in your data.

PLOTS:

I will rearrange Eq (8) to solve for . I recover:

(11)

So this does predict that is linear in , but is not the same as , unless .

So a discrepancy between theory and simulation can exist, if you do not fulfill the assumptions about what is in excess of what else, or whether equilibrium populations are equivalent to starting or total populations of unbound molecules.

Generate some simulation results to test these formula. Write down some formulas that would predict how the residence time of proteins on the membrane is altered by cargo binding, based on rates of cargo binding.