Binding in a Ternary mixture 2

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Three lipids: α, β, γ

The probability that lipid α, β, γ binds to conformational state A are:

$pA_\alpha(x_\alpha, x_\beta), \mathrm{p}A_\beta(x_\alpha, x_\beta), \mathrm{p}A_\gamma(x_\alpha, x_\beta)$

Where $x_\alpha, x_\beta, x_\gamma$ are mol fractions of each lipid and $x_\gamma = 1 - (x_\alpha + x_\beta)$

There is also the probability that no lipid binds:
$pA_0(x_\alpha, x_\beta)$

The total probability of state A is

$$pA = pA_\alpha + pA_\beta + pA_\gamma + pA_0$$

Where $pA = 1$ if state A is the only state available. Let's start by assuming that situation. Then

$$pA_\alpha = \frac{pA_\alpha}{pA_\alpha + pA_\beta + pA_\gamma + pA_0} = \frac{1}{1 + \frac{pA_\beta}{pA_\alpha} + \frac{pA_\gamma}{pA_\alpha} + \frac{pA_0}{pA_\alpha}}$$

So $\frac{pA_\beta}{pA_\alpha}$ and $\frac{pA_\gamma}{pA_\alpha}$ are both determined by relative calculations. But we technically **do not have access to $\frac{pA_0}{pA_\alpha}$,** which would properly be determined through an absolute calculation or a TI calculation on lipid α.

However, suppose we assume that the site is nearly always occupied, i.e. $\frac{\mathrm{p}A_0}{pA_\alpha} \ll 1$, in which case

$$pA_\alpha \sim \frac{1}{1+\frac{pA_\beta}{pA_\alpha}+\frac{pA_\gamma}{pA_\alpha}}$$

So we can actually get an absolute probability from relative probabilities. That's great!!

But .... what if there is a second state (B) available, where

$$pB = pB_\alpha + pB_\beta + pB_\gamma + pB_0$$

Our goal is to determine if lipid α stabilizes one state -- where stabilization could be defined by a few different things. I'll get to those options. But for now, I need to do a little more to get my *mise en place* together because all the equations where I assumed $pA = 1$ no longer hold.

But as long as there are only two conformations,

$$pA + pB = 1$$

$$p_A = \frac{p_A}{p_A + p_B} = \frac{1}{1+\frac{p_B}{p_A}} = \frac{1}{1+Y}$$

Where I defined the probability ratio Y as:

$$Y = \frac{p_B}{p_A} = \frac{1-p_A}{p_A} = \frac{1-pA_\alpha - pA_\beta - pA_\gamma - pA_0}{pA_\alpha + pA_\beta + pA_\gamma + pA_0}$$
$$= \frac{\frac{1}{pA_\alpha} - \left(1+\frac{pA_\beta}{pA_\alpha}+\frac{pA_\gamma}{pA_\alpha}+\frac{pA_0}{pA_\alpha}\right)}{1+\frac{pA_\beta}{pA_\alpha}+\frac{pA_\gamma}{pA_\alpha}+\frac{pA_0}{pA_\alpha}}$$

Alternatively, we can divide the numerator and denominator by A_0 instead:

$$Y = \frac{\frac{1}{pA_0} - \left(1 + \frac{pA_\alpha}{pA_0} + \frac{pA_\beta}{pA_0} + \frac{pA_\gamma}{pA_0}\right)}{1 + \frac{pA_\alpha}{pA_0} + \frac{pA_\beta}{pA_0} + \frac{pA_\gamma}{pA_0}}$$

To make life easier for now, define the following fractions:

$f_{A\beta} = \frac{pA_\beta}{pA_\alpha + pA_\beta + pA_\gamma + pA_0}$ *or*

$f_{X\beta} = \frac{1}{\sum_i \frac{pX_i}{pX_\beta}}$ is the fraction of binding sites on conformation X proteins that are occupied by lipid β

Similarly the fraction of conformation A proteins that have nothing bound is

$$f_{A0} = \frac{pA_0}{pA_\alpha + pA_\beta + pA_\gamma + pA_0} = \frac{1}{1 + \frac{pA_\alpha}{pA_0} + \frac{pA_\beta}{pA_0} + \frac{pA_\gamma}{pA_0}}$$

Where

$$Y = \frac{f_{A\alpha}}{pA_\alpha} - 1 = \frac{f_{A0}}{pA_0} - 1$$

***Important*: f is a sum we can calculate as long as we have all relative calculations and one absolute calculation (or estimate that it is negligible).**

Now, let's get to considering stabilization.

Stabilization: classic formulation

In a classic interpretation α would "stabilize" state B if

$$\frac{pB_\alpha}{pB_{unocc}} > \frac{pA_\alpha}{pA_{unocc}}$$

The subtlety here is whether "unoccupied" should mean nothing is bound, or "unoccupied" should mean everything that is *not α*. We consider each in turn

Case 1) "Unoccupied" means no bound lipid at all: pB_unocc= pB_0 and pA_unocc= pA_0

Test:

$$\frac{pB_\alpha}{pB_0} >? \frac{pA_\alpha}{pA_0}$$

This is almost good enough on its own, except we don't have any absolute affinities. It is equivalent to

$$\frac{pB_\alpha}{pA_\alpha} >? \frac{pB_0}{pA_0} .$$

And we also know that $\frac{pB_\alpha}{pA_\alpha} = Y \frac{f_{B\alpha}}{f_{A\alpha}}$ and $\frac{pB_0}{pA_0} = Y \frac{f_{B0}}{f_{A0}}$

So if $\frac{pB_\alpha}{pA_\alpha} > \frac{pB_0}{pA_0}$ then $Y \frac{f_{B\alpha}}{f_{A\alpha}} > Y \frac{f_{B0}}{f_{A0}}$, and our test becomes:

$$\frac{f_{B\alpha}}{f_{A\alpha}} >? \frac{f_{B0}}{f_{A0}}$$

If we have a better estimate of these f values than the absolute affinities, we might be in ok shape but overall the reformulation doesn't help that much.

Case 2) "Unoccupied" means only that lipid α is not bound. The other lipids could be bound or not bound.

$$\mathrm{p}B_{unocc} = pB_\beta + pB_\gamma + pB_0$$

Stabilization Test:

$$\frac{pB_\alpha}{pA_\alpha} >? \frac{pB_\beta + pB_\gamma + pB_0}{pA_\beta + pA_\gamma + pA_0} = \frac{pB_0}{pA_0}\frac{1+\frac{pB_\beta}{pB_0}+\frac{pB_\gamma}{pB_0}}{1+\frac{pA_\beta}{pA_0}+\frac{pA_\gamma}{pA_0}} = \frac{pB_\beta}{pA_\beta}\frac{1+\frac{pB_\gamma}{pB_\beta}+\frac{pB_0}{pB_\beta}}{1+\frac{pA_\gamma}{pA_\beta}+\frac{pA_0}{pA_\beta}}$$

Which can also be written in terms of our absolute affinity S values as:

$$\frac{f_{B\alpha}}{f_{A\alpha}} >? \frac{f_{B0}}{f_{A0}}\frac{1+\frac{pB_\beta}{pB_0}+\frac{pB_\gamma}{pB_0}}{1+\frac{pA_\beta}{pA_0}+\frac{pA_\gamma}{pA_0}}$$

Or in terms of relative affinities we can write this test a bunch of different ways:

$$\frac{f_{B\alpha}}{f_{A\alpha}} >? \frac{f_{B\beta}}{f_{A\beta}}\frac{1+\frac{pB_\gamma}{pB_\beta}+\frac{pB_0}{pB_\beta}}{1+\frac{pA_\gamma}{pA_\beta}+\frac{pA_0}{pA_\beta}}$$

In summary, a comparison….

Meaning of unoccupied state	**Framed to use….**	**Test for whether lipid α stabilizes conformation B (in terms of probabilities)**	**Test for whether lipid α stabilizes conformation B (in terms of sums over probabilities)**
Apo (no lipid is "bound")	Absolute affinities	$\frac{pB_\alpha}{pB_0} >? \frac{pA_\alpha}{pA_0}$	$\frac{f_{B\alpha}}{f_{A\alpha}} >? \frac{f_{B0}}{f_{A0}}$
lipid α is not bound	Absolute affinities	$\frac{pB_\alpha}{pB_0} >? \frac{pA_\alpha}{pA_0} \frac{1+\frac{pB_\beta}{pB_0}+\frac{pB_\gamma}{pB_0}}{1+\frac{pA_\beta}{pA_0}+\frac{pA_\gamma}{pA_0}}$	$\frac{f_{B\alpha}}{f_{A\alpha}} >? \frac{1-f_{B0}\frac{pB_\alpha}{pB_0}}{1-f_{A0}\frac{pA_\alpha}{pA_0}}$
lipid α is not bound	Relative affinities	$\frac{pB_\alpha}{pB_\beta} > \frac{pA_\alpha}{pA_\beta} \frac{1+\frac{pB_\gamma}{pB_\beta}+\frac{pB_0}{pB_\beta}}{1+\frac{pA_\gamma}{pA_\beta}+\frac{pA_0}{pA_\beta}}$	$\frac{f_{B\alpha}}{f_{A\alpha}} >? \frac{1-f_{B\beta}\frac{pB_\alpha}{pB_\beta}}{1-f_{A\beta}\frac{pA_\alpha}{pA_\beta}};$

These tests suggest a stabilization score:

stabilization score = $\log\left(\frac{f_{B\alpha}}{f_{A\alpha}}\frac{1-f_{A\beta}\frac{pA_\alpha}{pA_\beta}}{1-f_{B\beta}\frac{pB_\alpha}{pB_\beta}}\right)$

Note that for PG,

$\frac{pB_{PG}}{pB_\beta} \approx \frac{1}{f_{B\beta}}$ regardless of β, which means that the correction term $\frac{1-f_{A\beta}\frac{pA_\alpha}{pA_\beta}}{1-f_{B\beta}\frac{pB_\alpha}{pB_\beta}}$ is quite a bit larger than 1.

Chemical Potential Approach

Second approach, discussed by Jerome and Grace Friday April 1 2022:

Calculate $\left(\frac{\partial \ln p_A}{\partial x_\beta}\right)_{x_\gamma} = -\left(\frac{\partial \ln 1+Y}{\partial x_\beta}\right)_{x_\gamma}$; if $\left(\frac{\partial \ln p_A}{\partial x_\beta}\right)_{x_\gamma} > 0$ then lipid β stabilizes state A.

Start by assuming that $pA + pB = 1$ i.e. there are only two states.

$$\left(\frac{\partial \ln p_A}{\partial x_\beta}\right)_{x_\gamma} = -\left(\frac{\partial \ln 1 + Y}{\partial x_\beta}\right)_{x_\gamma} = \frac{1}{pA_\alpha}\frac{\partial pA_\alpha}{\partial x_\beta} + \frac{1}{S_{A\alpha}}\frac{\partial S_{A\alpha}}{\partial x_\beta}$$

The second term is one we can calculate because it only depends on probability ratios. I am not sure how we get the first term.

Perhaps the other unhelpful thing about this formulation is that it doesn't depend on the calculations involving state B. What if we relax the requirement that A and B are the only conformations (i.e. when we don't insist that $p_B = 1 - p_A$):

$$\mathrm{Y} = \frac{p_B}{p_A} = \frac{pB_\alpha + pB_\beta + pB_\gamma + pB_0}{pA_\alpha + pA_\beta + pA_\gamma + pA_0} = \left(\frac{pB_\alpha}{pA_\alpha}\right)\frac{1 + \frac{pB_\beta}{pB_\alpha} + \frac{pB_\gamma}{pB_\alpha} + \frac{pB_0}{pB_\alpha}}{1 + \frac{pA_\beta}{pA_\alpha} + \frac{pA_\gamma}{pA_\alpha} + \frac{pA_0}{pA_\alpha}} = \left(\frac{pB_\alpha}{pA_\alpha}\right)\frac{S_{B\alpha}}{S_{A\alpha}}$$

Or in our alternate reference:

$$Y = \left(\frac{pB_0}{pA_0}\right)\frac{S_{B0}}{S_{A0}}$$

So now we can take the derivative, and it will depend on both states, but we would need data at multiple concentrations to do so.