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Question & Answer

Q&A: Cooperativity

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What is cooperativity?

Cooperativity is a type of behavior where a number of seemingly independent components of a system act collectively, in unison or near-unison. Think of a school of fish, a flock of birds, or a pack of lemmings. Cooperativity implies some sort of communication among the system's seemingly independent components.

In biochemistry the term cooperativity is almost always used in one particular context: binding-dissociation reactions at equilibrium. The classic example is the binding of oxygen to hemoglobin (Figure 1). But cooperativity is also important in cell-cell signaling, transcriptional regulation and more complex processes governing the behavior of cells.

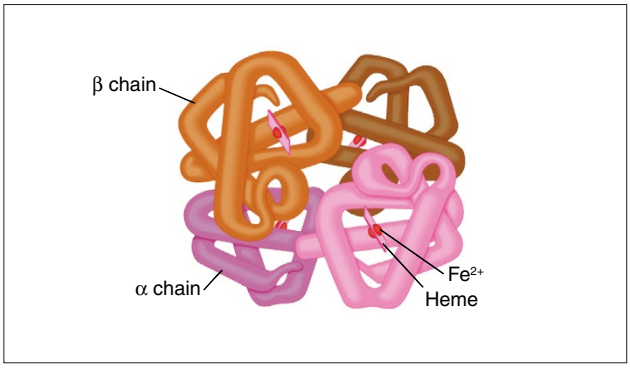

Figure 1
The oxygen-transporting protein hemoglobin, a tetrameric protein consisting of four globin subunits with four oxygen-binding hemes. Reproduced with permission from Michael W King.

What is the importance of cooperativity?

That depends on the system, but let's take hemoglobin. Hemoglobin's mission is to pick up a large amount of oxygen in the lungs, where the oxygen concentration (or partial pressure) is about 100 torr, and then drop off a good fraction of it in the peripheral tissues where the oxygen concentration is about 20 torr. Cooperativity helps make this transport efficient.

To see why, first suppose that hemoglobin were a monomeric oxygen binding protein (Figure 2).

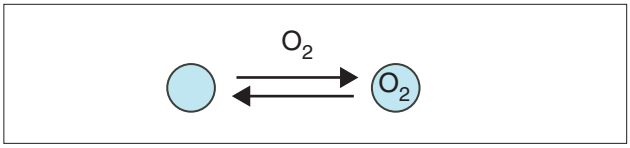

Figure 2
Monomeric oxygen-transporting protein.

If the binding and dissociation reactions are described by simple mass action kinetics, then hemoglobin's oxygen saturation at equilibrium would be given by the Langmuir equation:

$$y = \frac{x}{K + x}$$

where y is the oxygen saturation, x is the partial pressure of oxygen, and K is the dissociation constant. Because this equation is identical in form to the familiar Michaelis-Menten equation, the relationship between x and y is sometimes called Michaelian. A Langmuir (or Michaelian) binding curve is hyperbolic, shaped like the green curve shown in Figure 3, and at most 38% of the hemoglobin molecules could deliver an oxygen to the peripheral tissues.

You would like to do better. Ideally, the binding curve should be higher than the green curve at 100 torr, so hemoglobin would pick up more oxygen in the lungs, and lower at 20 torr, so that hemoglobin would unload more completely. A sigmoidal curve would fit the bill, and the experimentally determined binding curve is in fact steeply sigmoidal, like the red curve shown in Figure 3. The sigmoidal shape of the oxygen binding curve helps hemoglobin to achieve a high oxygen-delivery throughput. Sigmoidal binding curves occur if binding is cooperative.

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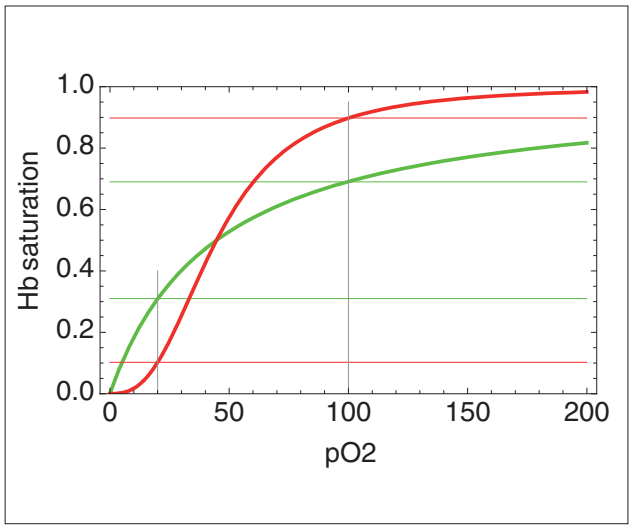

Figure 3
Michaelian (green) and sigmoidal (red) oxygen-binding curves.

What sort of physical mechanism can give you cooperative binding and thus a sigmoidal binding curve?

One answer goes back to AV Hill in 1910. He assumed that hemoglobin is a polymeric complex capable of binding n molecules of oxygen per molecule of complex. So far so good - we now know that hemoglobin is a tetramer. He then assumed that oxygen binding only occurs when n oxygen molecules simultaneously collide with hemoglobin. The binding reaction is therefore nth order in the oxygen concentration, and in a few lines of algebra one can show that the equilibrium oxygen saturation is:

$$y = \frac{x^n}{EC50^n + x^n}$$

where $EC50$ is the partial pressure of oxygen at which the binding is 50% of maximal, and n, the polynomial order of the binding reaction, is commonly referred to as the Hill coefficient.

This is the famous Hill equation, and it has many virtues. It is simple, not much more complicated than the Langmuir equation (which is equivalent to a Hill equation with n =1). Its parameters, n and $EC50$, are easy to understand, empirically determinable quantities. And, perhaps most importantly, the equation fits the experimental data on hemoglobin's oxygen binding very well. Not perfectly, but very well. Moreover, whenever one encounters a sigmoidal response in biochemistry, chances are good that the Hill equation will fit the experimental data adequately.

What's the rub?

One problem is that hemoglobin's oxygen binding is not fit by a Hill equation with a Hill coefficient of 4, but rather with a Hill coefficient of approximately 2.7 (and the red curve plotted above is, in fact, a Hill equation curve with n = 2.7). But the bigger problem, of course, is that the model's assumption that n molecules of ligand or stimulus simultaneously collide with the multimeric protein is patently unrealistic. And without this assumption, you do not get a Hill equation.

So suppose you assume the oxygens bind sequentially rather than simultaneously. What sort of curve can you come up with?

The first such model was published by Adair in 1925, in the sixth of six back-to-back papers in the *Journal of Biological Chemistry* on hemoglobin's oxygen binding. If you assume the binding proceeds as in Figure 4 then it follows, after a lot of algebra, that the oxygen saturation is given by:

$$y = \frac{\frac{1}{4}a_1x + \frac{1}{2}a_2x^2 + \frac{3}{4}a_3x^3 + a_4x^4}{1 + a_1x + a_2x^2 + a_3x^3 + a_4x^4}$$

where the coefficients a_1 through a_4 are functions of the four equilibrium constants. This is the Adair equation, and you can indeed fit this equation quite well to experimental oxygen binding data, if you choose the right values for the

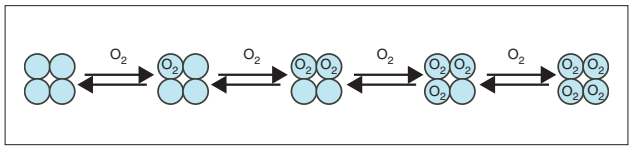

Figure 4
Sequential binding of oxygen to the subunits of tetrameric hemoglobin.

a coefficients. Invariably, to get the right values, you need to assume that the binding of the last couple of oxygens is much more favorable than the binding of the first.

What physical mechanism could account for that?

In 1966, Dan Koshland, George Némethy and David Filmer provided a simple rationalization, known as the Koshland-Némethy-Filmer (KNF) model, for these puzzling equilibrium constants. Koshland, Némethy and Filmer assumed that when the first oxygen bound to one of the hemes (with relatively poor affinity), the binding allosterically induced the globin subunits that had not yet bound oxygens to increase their affinity for oxygen. This mechanism requires a fairly complicated chain of events; the 'information' that the

first oxygen has bound needs to be transmitted from one heme group out to the surface of that globin subunit; the information is relayed from that globin's surface to a neighboring globin; and then it is relayed from there to the neighboring globin's heme. Complicated or not, the conceptual framework fitted very well with Koshland's idea of induced fit as the basis of enzymatic catalysis, and it provided a credible, tangible physical picture to show why the oxygen binding curve of hemoglobin is sigmoidal.

In what sense does the KNF model invoke the concept of cooperativity?

In the KNF model, once one heme binds oxygen it becomes progressively easier for the other hemes to bind it. One heme leads and the others follow. Allosteric communication between the hemoglobin subunits allows the whole protein to behave collectively in a way that non-cooperative, truly independent subunits would not.

Could you get cooperativity without allosteric communication between the subunits of the binding protein?

Yes - you could have a multivalent ligand binding to a multi-subunit protein. The classic example is an antigen with repeated structural features, or epitopes (for example, the surface of a bacterium or a virus) interacting with the two arms of an antibody. The binding of one antigen epitope to the antibody makes the second binding event much more favorable by forcing the antigen's second epitope into close proximity of the antibody's remaining free antigen binding site. This type of cooperative interaction is often referred to as enforced proximity, or the avidity effect, and it is common in protein-protein interactions.

While the avidity effect is similar to KNF cooperativity in that one binding event promotes the next, it is different in terms of the consequences for the shape of the saturation curve. Because the first binding event promotes a zero-order second binding event (that is, it occurs within the complex rather than between the complex and a second ligand), the result here is a Langmuir/Michaelian-type binding curve, not a sigmoidal one.

Do all cooperative interactions increase binding?

No. You can have anti-cooperativity, or negative cooperativity, in which binding the first molecule makes it harder, not easier, for the second one to bind. The result is usually a binding curve that looks sort of like a Langmuir curve, but approaches maximal binding even more slowly than the Langmuir curve does.

And if the binding of one molecule of ligand has absolutely no effect on the binding of any of the others, the complicated Adair/KNF equation can be reduced to a simple Langmuir equation, and the binding is said to be non-cooperative.

Note that the KNF concepts of cooperativity (or positive cooperativity) and anti-cooperativity (or negative cooperativity) are most cleanly defined for a dimeric protein with two binding sites. If the first binding event increases the affinity of the second site, there is positive cooperativity. If the first binding event decreases the affinity of the second site, there is negative cooperativity. With a four subunit protein like hemoglobin the distinction can be a bit murkier. For example, what would you call it if the first binding event makes the second one weaker (as with negative cooperativity), which makes the third one stronger (as with positive cooperativity), and then the fourth one weaker? This sort of behavior has actually been inferred from fits of the Adair/KNF equation to (some) hemoglobin oxygen-binding datasets, and so technically you might consider the whole process to exhibit mixed positive and negative cooperativity. However, since the net effect is a sigmoidal binding curve, as with simple positive cooperativity, that is what it might as well be called.

Is negative cooperativity important?

Well, negative cooperativity is fairly common. For example, most G-protein coupled receptors probably function as dimers. For some, the binding curves are sigmoidal, indicating positive cooperativity. But for about as many, the binding curves are even more graded than Langmuir curves, indicating negative cooperativity. So negative cooperativity is common and therefore probably important.

One thought is that negative cooperativity occurs in cells when it is worth sacrificing the ability of a system to respond decisively to one particular range of ligand concentrations in favor of the ability to respond at least a little to a very wide range of concentrations. Positive cooperativity gives you a response that is decisive, but only over the limited range of ligand concentrations that correspond to the steep upslope of the binding curve. Negative cooperativity gives you a response that is less decisive but also less restricted with respect to the range of ligand concentrations.

Is allosteric cooperativity the only way to get a sigmoidal curve?

No. The famous team of Jacques Monod, Jeffries Wyman and Jean-Pierre Changeux proposed a different model for

oxygen binding by hemoglobin. They broke the binding of oxygen to hemoglobin down into four sequential steps, just as Adair and Koshland, Némethy and Filmer did. However, they assumed that the binding of the first oxygen had no effect on the affinities of the other globins. At this point there was nothing in the model that would make the binding curve different from a Langmuir curve.

Next they assumed that hemoglobin exists in two alternative conformations. They termed these conformations 'tense' (the blue states below) and 'relaxed' (the pink states). Furthermore, they assumed that if one hemoglobin monomer was relaxed, all of the hemoglobins in that complex would be, and that if one was tense, they all would be. Essentially, they replaced Hill's assumption of the simultaneous binding of four oxygens to one hemoglobin with the assumption of concerted conformation changes among the four hemoglobin monomers. This assumption seems much more reasonable. Think of four kittens sleeping cuddled up in a pile. For one kitten to shift position, perhaps all four will need to.

Finally, they assumed that the relaxed (pink) globins bind oxygen more avidly than the tense (blue) globins. This means that as the hemoglobin picks up more oxygens, the equilibrium between tense and relaxed shifts progressively in favor of relaxed. The Monod, Wyman and Changeux (MWC) mechanism is shown schematically in Figure 5. This

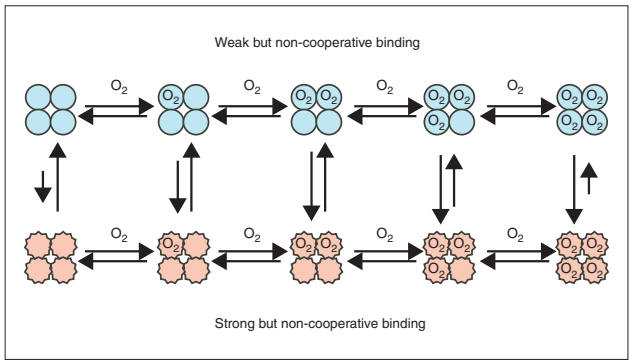

Figure 5
Concerted flipping of hemoglobin subunits between two states with different affinities for oxygen.

scheme yields a relatively simple equilibrium binding expression containing just three thermodynamic parameters: the equilibrium constant for the binding of oxygen to the tense globins (K_1), the equilibrium constant for the binding of oxygen to the relaxed globins (K_2), and the equilibrium constant for the concerted flipping of the unliganded hemoglobin species between the relaxed and tense conformations (K_3):

$$y = \frac{K_2 \frac{x}{K_1}\left(1+\frac{x}{K_1}\right)^3 + \frac{x}{K_3}\left(1+\frac{x}{K_3}\right)^3}{K_2\left(1+\frac{x}{K_1}\right)^4 + \left(1+\frac{x}{K_3}\right)^4}$$

Like the Adair/KNF equation, the MWC equation is a ratio of two complicated *n*th order polynomials. And as with the Adair/KNF equation, it is possible to choose *K* values that yield sigmoidal curves and reproduce experimental oxygen binding data extremely well.

In what sense does the MWC model invoke the concept of cooperativity?

In the MWC model, the oxygen binding seems, at first glance, to be totally noncooperative; the binding of an oxygen to a globin within a tense hemoglobin complex is explicitly assumed to have no effect on the affinities of other globins for oxygen. And the same is true of the binding of an oxygen to a globin within a relaxed complex. Instead, the cooperativity here is embodied in the notion that the whole hemoglobin complex flips between the tense and relaxed states as a unit.

This concerted conformation change has the effect of allowing the binding of the first oxygen to indirectly promote the binding of the second, and the second to promote the binding of the third, and the third to promote the binding of the fourth. This is because the binding of each oxygen makes the flip to the tight-binding state more favorable, and the flip to the tight-binding state makes the binding of the next oxygen more favorable.

Which is more realistic? The KNF model or the MWC model?

For most cooperative systems it is nearly impossible to choose between the two models simply on the basis of the shape of the binding curve - either model can usually be fitted to experimental binding data quite well. For that matter, even the Hill equation, based on a patently unrealistic physical scenario, usually fits experimental data satisfactorily. What is really needed is some other type of evidence that gets at the nature of the intermediates that are formed when the cooperative protein is partially saturated.

What sort of evidence?

The most fruitful approaches in this regard have been studies on single molecules of cooperative, multimeric ion

channels. For example, the nicotinic cholinergic receptor consists of five homologous subunits with two to five acetylcholine binding sites. When the receptor binds acetylcholine, it opens, allowing cations to flow through its central pore from one side of the plasma membrane to the other. In patch clamp experiments, in which ion flow through single nicotinic receptors can be monitored, one observes the channel flipping between two conductance states, consistent with an MWC-style symmetrical, concerted transition of all of the subunits between a closed and an open conformation, rather than the three or more conductance states that might be expected in a KNF-style mechanism. Moreover, crystal structures of open and closed nicotinic receptors show that the whole complex does seem to change conformation in concert.

On the other hand, there are many examples of negative cooperativity - receptors that are saturated by ligand even more gradually than a non-cooperative receptor would be. And although negative cooperativity is easy to account for with a KNF model, it cannot arise for any choice of parameters in an MWC model. Thus, both MWC and KNF types of mechanisms are probably found in nature.

Are sigmoidal responses important outside the context of high-throughput oxygen delivery?

Certainly. We have already mentioned a couple of examples from cell signaling: the cooperative nicotinic cholinergic receptors, and the cooperative or anti-cooperative activation of G-protein coupled receptors. These are both examples of cooperativity in signal reception. We suspect that sigmoidal responses will be at least as important in signal processing. One way of seeing why this might be is to think about how signals would propagate down a signal transduction pathway if none of the components of the pathway exhibited sigmoidal responses to their upstream activators.

Explain please: why do sigmoidal responses help signals propagate down a pathway?

Suppose you have a cascade of three signaling proteins, *A*, *B*, and *C*, in a pathway where an input stimulus x activates *A*, *A* activates *B*, and then *B* activates *C*. Suppose also, for the moment, that the response of each protein to its upstream regulator is described by a Langmuir/Michaelian-type function.

$$y = \frac{x}{EC50 + x}$$

And finally, suppose that the system is asked to respond to a whopping-big change in input stimulus (x), an 81-fold change. You get the largest change in *A* if you use the middle of the response curve, with x ranging from $\frac{1}{9}$ *EC*50 to 9 *EC*50; *A* then goes from 10% to 90% of its maximal activity. Thus, an 81-fold change in input stimulus has yielded a 9-fold change in output response. If you feed this 9-fold change in *A* into the response of *B*, the best you can get is to drive *B* from 25% to 75%. And if you drive this 3-fold change in *B* into the response of *C*, the best you can get is to drive *C* from 37% to 63%, a 1.7-fold change. So, in three steps, this Michaelian cascade has reduced a decisive, 81-fold change in input stimulus to a murky, gray, 1.7-fold change in output. Given that signaling pathways often contain even more than three successive signal relayers, this seemingly ineluctable descent into murkiness is a big problem.

This problem can be circumvented if some of the signaling proteins exhibit sigmoidal response curves; with a sigmoidal curve, the fold change in output can be as big as, or bigger than, the fold change in stimulus. For example, for a system whose response is given by a Hill curve with a Hill coefficient of 3, a 9-fold change in input can give you a 25-fold change in output. So sigmoidal response curves can restore or even amplify the 'contrast' of a signal propagating down a signaling pathway.

Isn't a sigmoidal binding curve inherently cooperative, irrespective of the mechanism that generates it?

In a sense, yes. With a Langmuir binding curve, every increment of ligand concentration gives you a little less binding than the previous increment did. Langmuir binding obeys the law of diminishing returns: every time a binding site is filled, it makes it a little harder for the next ligand molecule to find a binding site. However, with a sigmoidal binding curve, for a while each increment of ligand concentration results in a little more binding than the previous increment did. Regardless of what mechanism makes the curve bend upward, the upward bend itself means that the system is responding in a sort of collective, cooperative, all-or-none fashion. Or at least in a more cooperative fashion than a system with a Langmuir binding curve does.

However, there are a number of well explored mechanisms in cell signaling that can give rise to steeply sigmoidal response curves, but have nothing to do with multisubunit proteins and allosteric communication between binding sites. Probably the best examples are zero-order ultrasensitivity, discovered by Goldbeter and Koshland in the course of theoretical studies of signaling cascades, and inhibitor ultrasensitivity, a simple stoichiometric buffering reaction. These non-cooperative mechanisms for generating sigmoidal response curves are probably at least as important

as cooperativity in the overall scheme of cellular regulation. For more on this interesting topic, see the 1996 *Trends in Biochemical Science* paper referenced below.

Where can I find out more?

Books

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