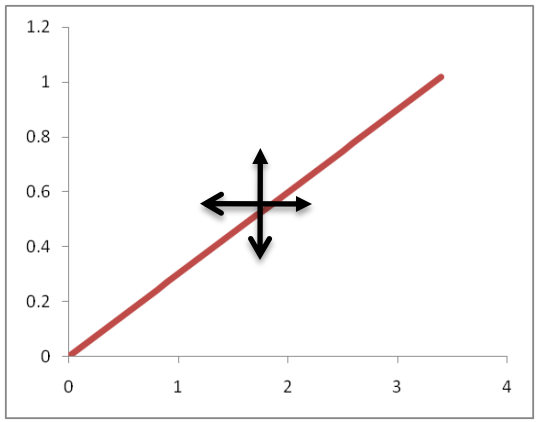
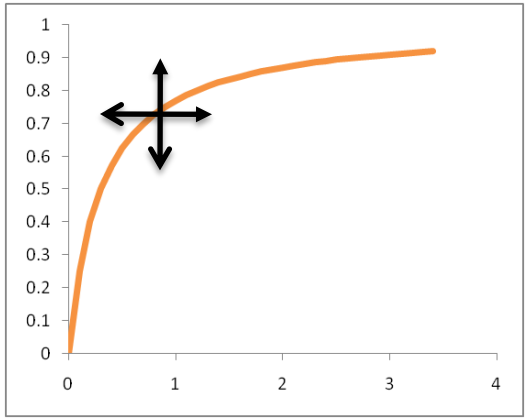
More Complex Kinetics

Unit Responses in Biochemical Networks

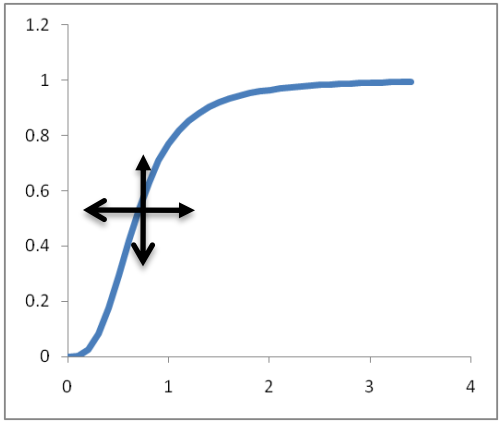
Linear



Hyperbolic



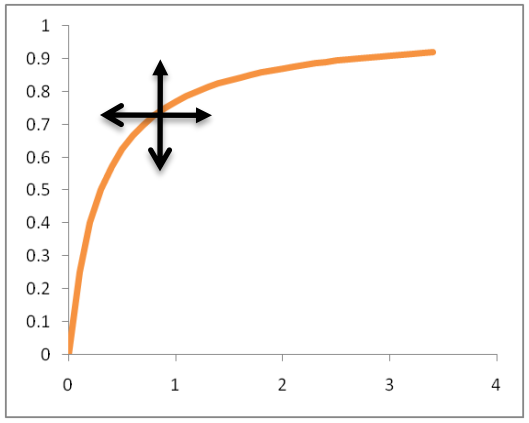
Sigmoidal



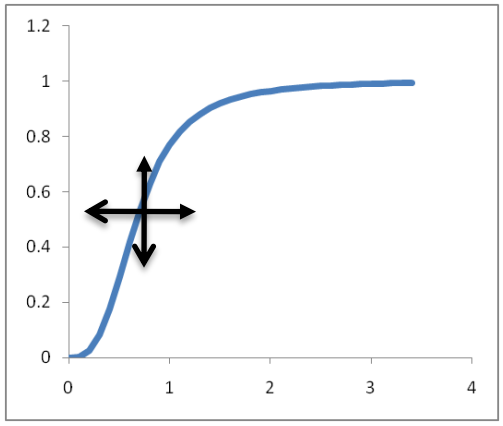
Where does the nonlinearity come from?

- Conservation Laws
- Bimolecular Binding

Hyperbolic



Sigmoidal



Association or Binding Constant

$$H + A \rightleftharpoons HA$$

$$K_a = \frac{HA}{H.\ A}$$

Ka the equilibrium constant for the binding of one molecule to another.

Dissociation Constant

$$HA \rightleftharpoons H + A$$

$$K_d = \frac{H.\ A}{HA}$$

Kd the equilibrium constant for dissociation.

Enzyme Catalysis

The set of reactions shown below is the classic view for enzyme catalysis. Enzyme binds to substrate to form enzyme-substrate complex. Enzyme-substrate complex degrades to free enzyme and product.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

Enzyme Catalysis

Unfortunately the kinetic constants that describe enzyme catalysis are very difficult to measure and as a result researchers do not tend to use the explicit mechanism, instead they use certain approximations.

The two most popular approximations are:

1. Rapid Equilibrium

2. Steady State

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

Rapid Equilibrium Approximation

The rapid equilibrium approximation assumes that the binding and unbinding of substrate to enzyme to much faster than the release of product. As a result, one can assume that the binding of substrate to enzyme is in equilibrium.

That is, the following relation is true at all times (Kd = dissociation constant):

$$K_d = \frac{E.\ S}{ES}$$

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

Rapid Equilibrium Approximation

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

Let Kd be the dissociation constant:

$$K_d = \frac{E.\ S}{ES}$$

$$E_t = E + ES$$

Rapid Equilibrium Approximation

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

The equilibrium concentration of ES can be found:

$$ES = \frac{E_t . \ S}{K_d + S}$$

$$v = k_2 \ ES$$

Rapid Equilibrium Approximation

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

The rate of reaction is then:

$$v = \frac{E_t .\ k_2 .\ S}{K_d + S}$$

Fractional Saturation

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

Rearrange the equation:

$$v = \frac{E_t.\ k_2.\ S}{K_d + S}$$

$$\frac{v}{E_t\ k_2} = \frac{S}{K_d + S}$$

Substitute Kd

$$K_d = \frac{E.\ S}{ES}$$

Fractional Saturation

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

Yields:

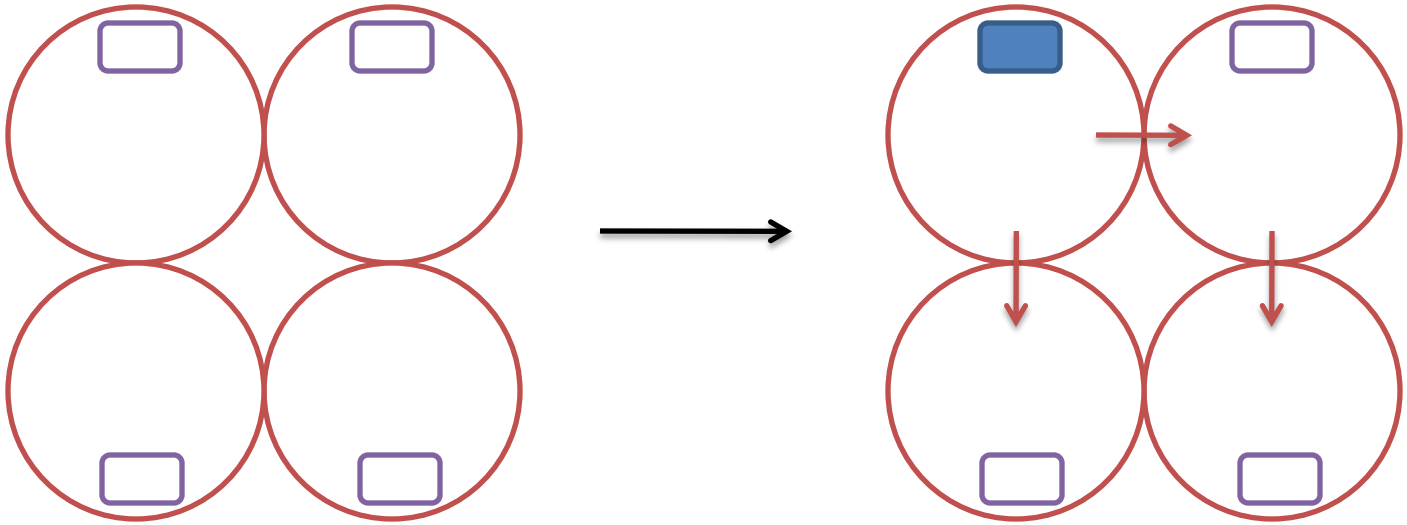
$$\frac{v}{E_t\ k_2} = \frac{ES}{ES + E}$$

This shows that the rate is proportional to the fraction of total enzyme that is bound to substrate.

This expression gives us the fractional saturation.

Sigmoid responses arise from cooperative interactions

Binding at one site results in changes in the binding affinities at the remaining sites.



Many proteins are multimeric

The Ecocyc database reports 774 multimeric protein complexes out of 4316 proteins.

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doi:10.1093/nar/gkm740

SURVEY AND SUMMARY

Multidimensional annotation of the *Escherichia coli* K-12 genome

Peter D. Karp[1,*], Ingrid M. Keseler[1], Alexander Shearer[1], Mario Latendresse[1], Markus Krummenacker[1], Suzanne M. Paley[1], Ian Paulsen[2,3], Julio Collado-Vides[4], Socorro Gama-Castro[4], Martin Peralta-Gil[4], Alberto Santos-Zavaleta[4], Mónica I. Peñaloza-Spínola[4], César Bonavides-Martinez[4] and John Ingraham[5]

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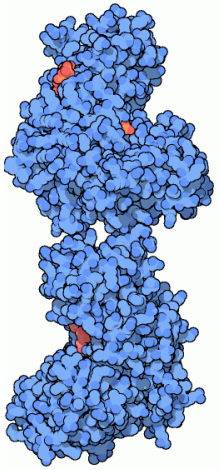
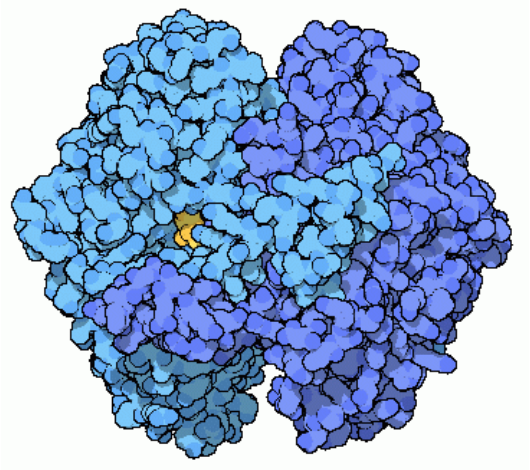
Many Proteins are Multimeric

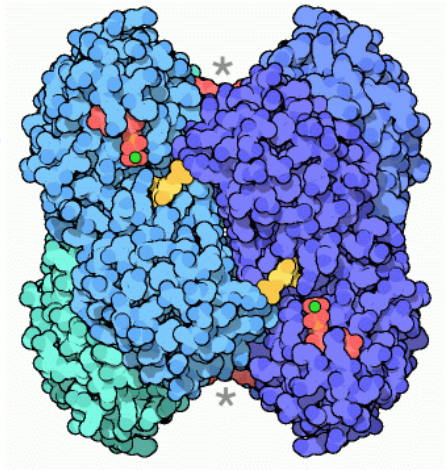
The Ecocyc database reports 774 multimeric protein complexes out of 4316 proteins.

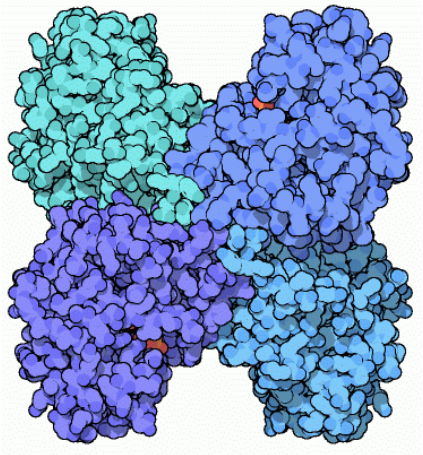
Phosphoglucose Isomerase

Fructose 1,6-bisphosphate Aldolase









Hexokinase

Dimer

Dimer

Phosphofructokinase

Tetramer

Tetramer

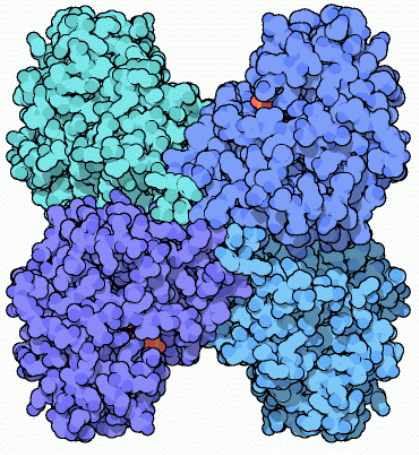
http://www.rcsb.org/pdb/static.do?p=education_discussion/molecule_of_the_month/pdb50_1.html

Hill Equation – Simplest Model

We assume that the ligands bind simultaneously (unrealistic!):

$$E + n\ S \longleftrightarrow ES$$

Assuming Rapid Equilibrium

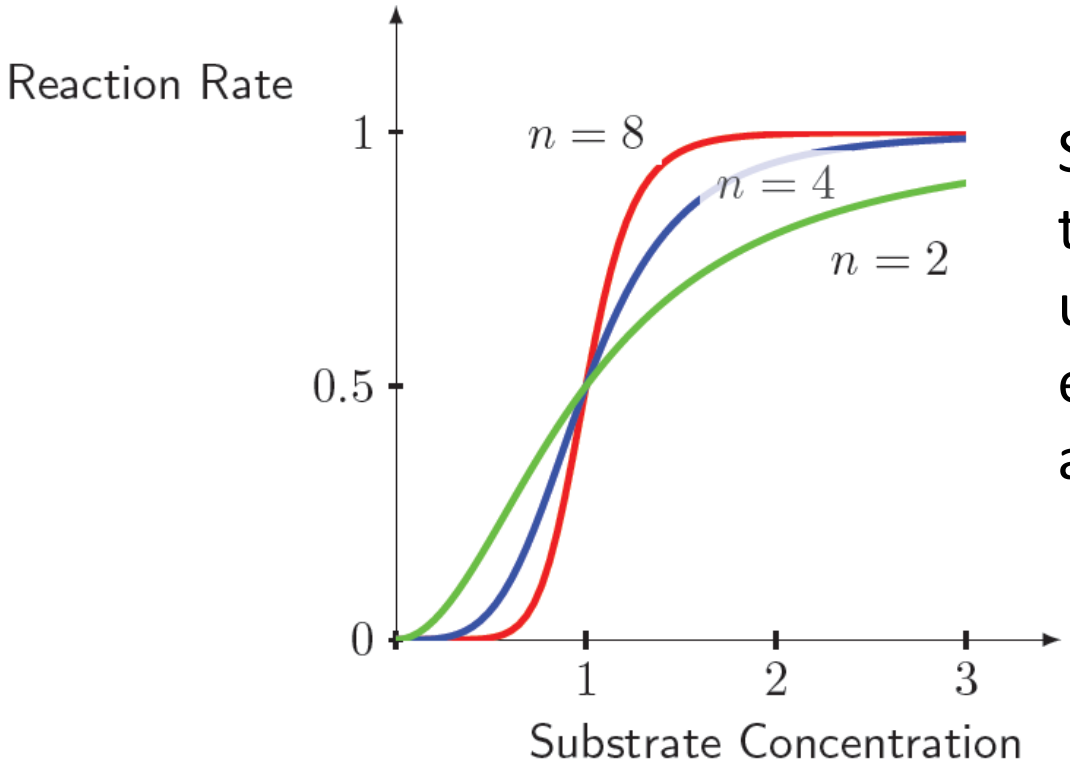


$$K = \frac{ES}{E\ .\ S^n}$$

$$E_t = E + ES$$

$$\frac{ES}{E_t} = \frac{S^n}{1/K + S^n} \quad \Rightarrow \quad v = \frac{Vmax\ S^h}{K_H + S^h}$$

Hill Equation

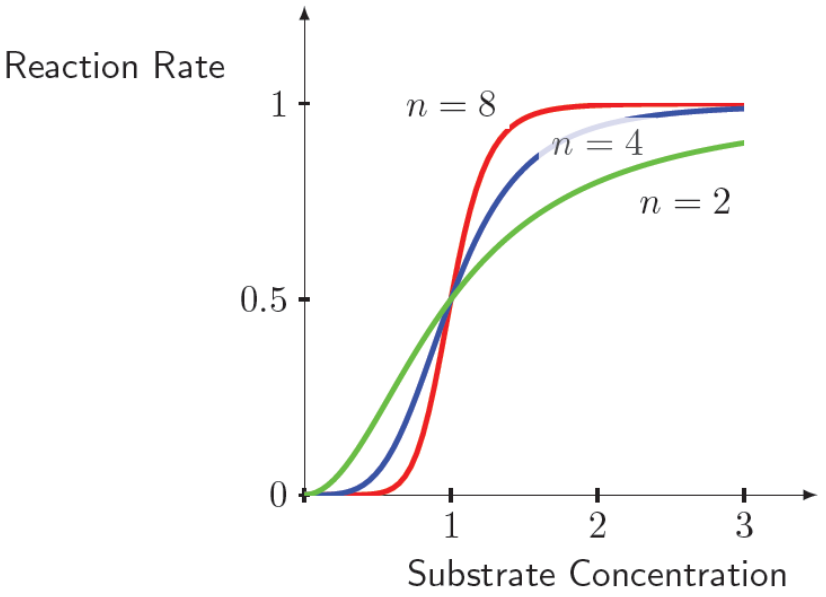


Some researchers feel that the underlying model is so unrealistic that the Hill equation should be considered an empirical result.

$$v = \frac{\text{Vmax } S^{n}}{K + S^{n}}$$

← Hill Coefficient

Hill Coefficient



The Hill Coefficient, n, describes the degree of cooperativity.

If n = 1, the equation reverts to a simple hyperbolic response.

n > 1 : Positive Cooperativity
n = 1 : No Cooperativity
n < 1 : Negative Cooperativity

$$v = \frac{\text{Vmax } S^{n}}{K + S^{n}}$$ ← Hill Coefficient

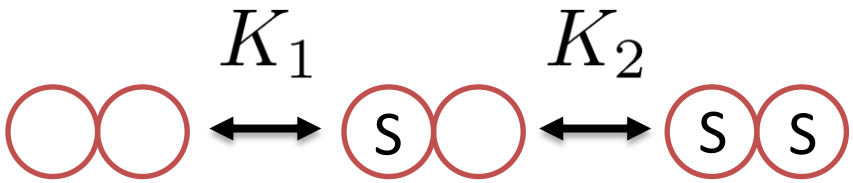
Hill Equation

What is wrong with the Hill equation?

1. The underlying model is unrealistic (assuming this is important)

2. It's a dead-end, no flexibility, one can't add additional effectors such as inhibitors or activators.

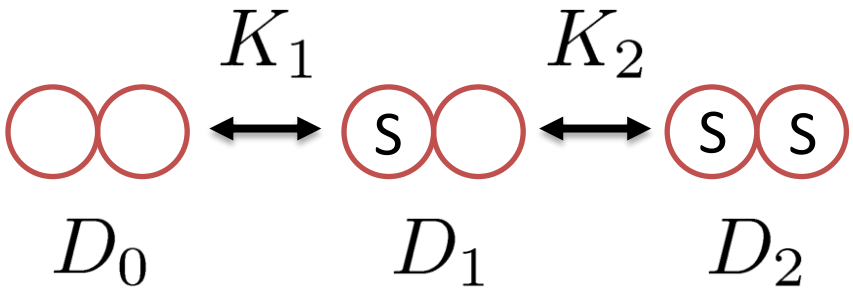
Alternative Models: Sequential Binding



$$f = \frac{\text{S}\bigcirc + 2\,\text{S}\,\text{S}}{2\left(\bigcirc\bigcirc + \text{S}\bigcirc + \text{S}\,\text{S}\right)}$$

$$f = \frac{\text{Total Bound Sites}}{\text{Total UnBound Sites}}$$

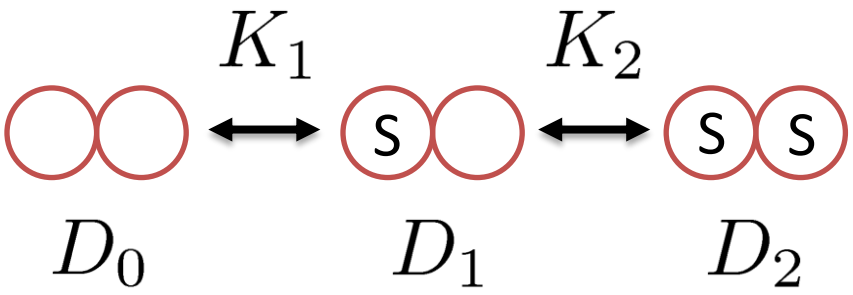
Alternative Models: Sequential Binding



$$f = \frac{D_1 + 2D_2}{2(D_0 + D_1 + D_2)}$$

$$f = \frac{\text{S}\bigcirc + 2\,\text{S}\,\text{S}}{2(\bigcirc\bigcirc + \text{S}\bigcirc + \text{S}\,\text{S})}$$

Alternative Models: Sequential Binding



$$f = \frac{D_1 + 2D_2}{2(D_0 + D_1 + D_2)}$$

Association Constants:

$$K_1 = \frac{D_1}{D_0.\ S}$$

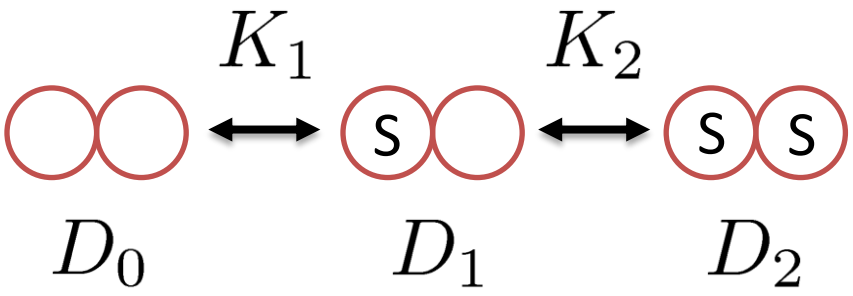
$$K_2 = \frac{D_2}{D_1.\ S}$$

$$D_1 = K_1.\ D_0.\ S$$

$$D_2 = K_2.\ D_1.\ S =$$

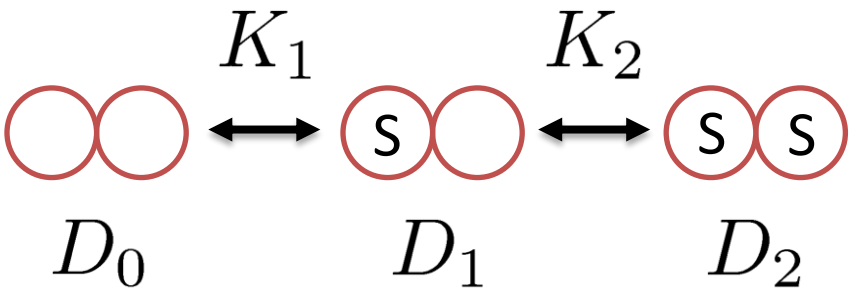
$$K_1.\ K_2.\ D_0.\ S^2$$

Alternative Models: Sequential Binding



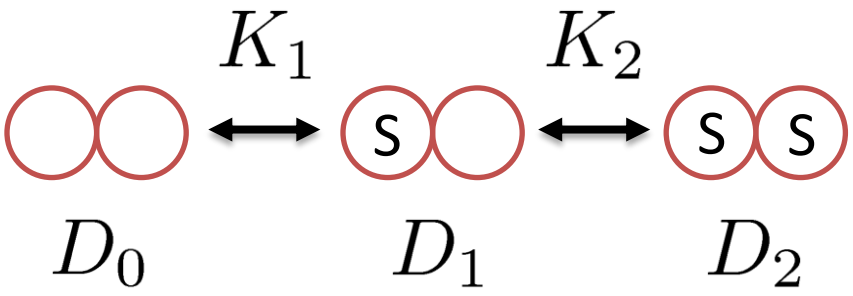
$$f = \frac{K_1.\ D_0.\ S + 2K_1.\ K_2.\ D_0.\ S^2}{2(D_0 + K_1.\ D_0.\ S + 2K_1.\ K_2.\ D_0.\ S^2)}$$

Alternative Models
Sequential Binding



$$f = \frac{K_1.\ \cancel{D_0}.\ S + 2K_1.\ K_2.\ \cancel{D_0}.\ S^2}{2(\cancel{D_0} + K_1.\ \cancel{D_0}.\ S + 2K_1.\ K_2.\ \cancel{D_0}.\ S^2)}$$

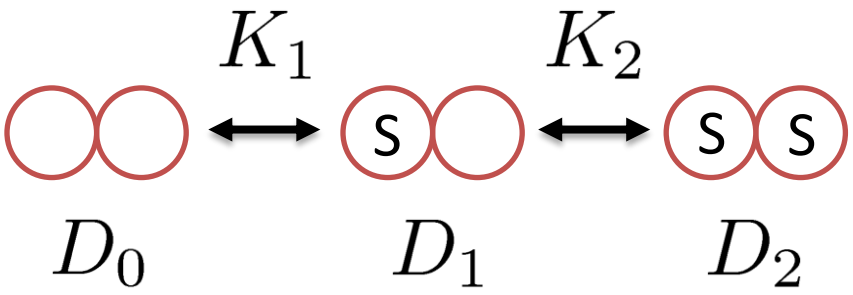
Alternative Models
Sequential Binding



$$f = \frac{K_1.\ S + 2K_1.\ K_2.\ S^2}{2(1 + K_1.\ S + 2K_1.\ K_2.\ S^2)}$$

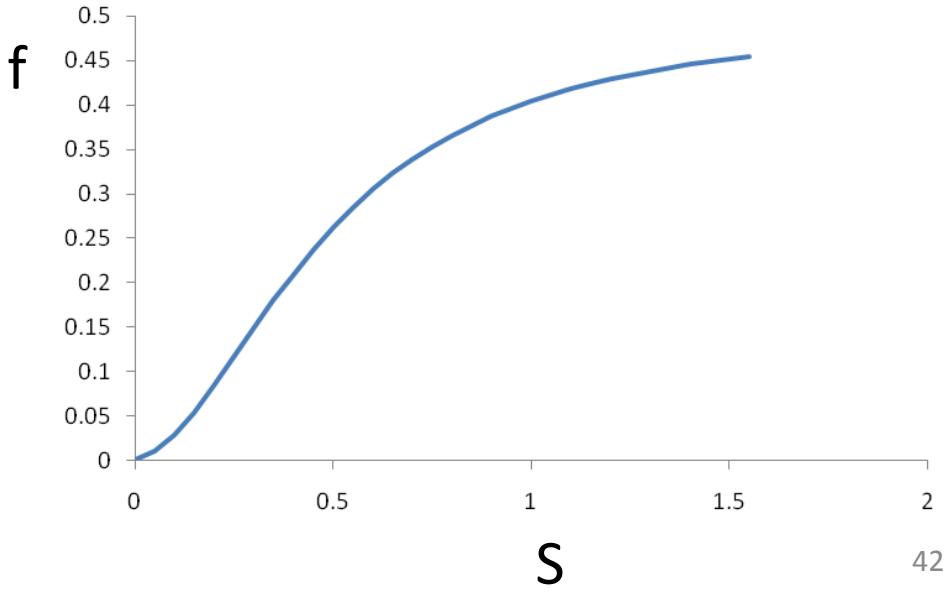
Adair Model

Alternative Models
Sequential Binding



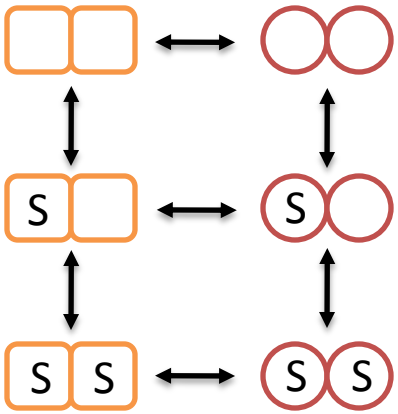
$$f = \frac{K_1.\ S + 2K_1.\ K_2.\ S^2}{2(1 + K_1.\ S + 2K_1.\ K_2.\ S^2)}$$

K1 = 0.2; K2 = 10



Other Models – MWC Model

MWC Model or concerted model (Monod, Wyman, Changeux)



Is disallowed

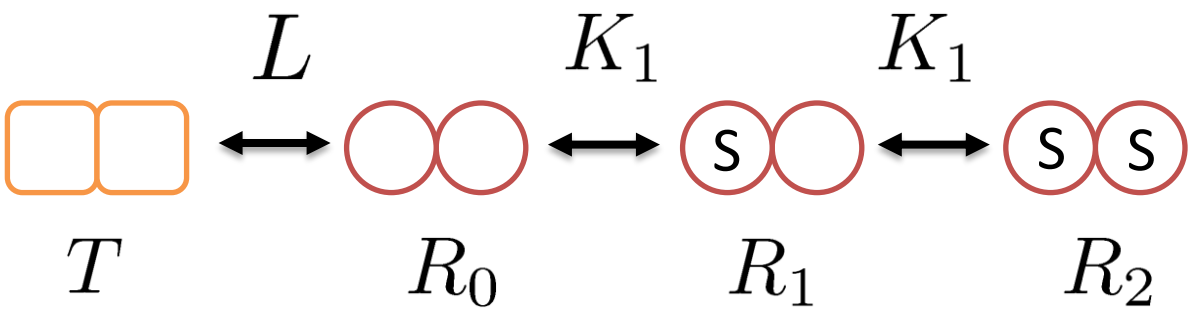
1. Subunits exist in two conformations, relaxed (R) and taut (T)
2. One conformation has a higher binding affinity than the other (R)
3. Conformations within a multimer are the same
4. Conformations are shifted by binding of ligand

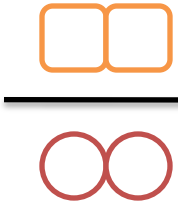
Taut (T) – less active

Relaxed (R) – more active

Other Models – MWC Model

MWC Model or concerted model (Monod, Wyman, Changeux)

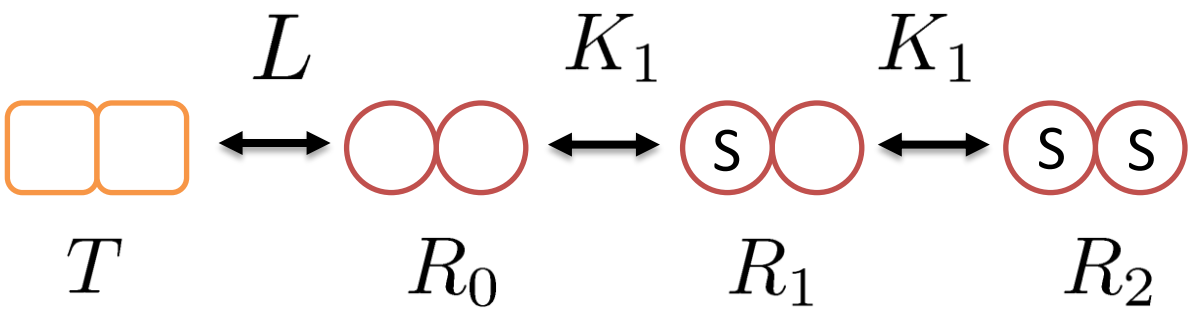


Where L = 

$$f = \frac{R_1 + 2R_2}{2(T + R_0 + R_1 + R_2)}$$

Other Models – MWC Model

MWC Model or concerted model (Monod, Wyman, Changeux)

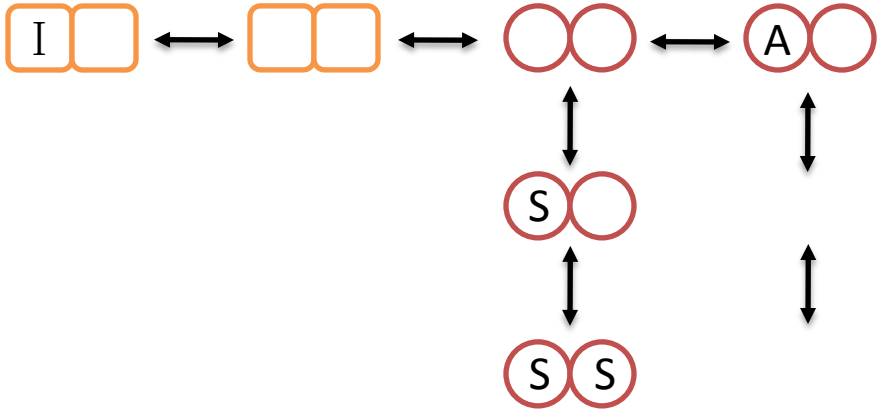


$$f = \frac{S/K_1(1+S/K_1)}{(1+S/K_1)^2 + L}$$

Modifiers in Sigmoid Kinetics

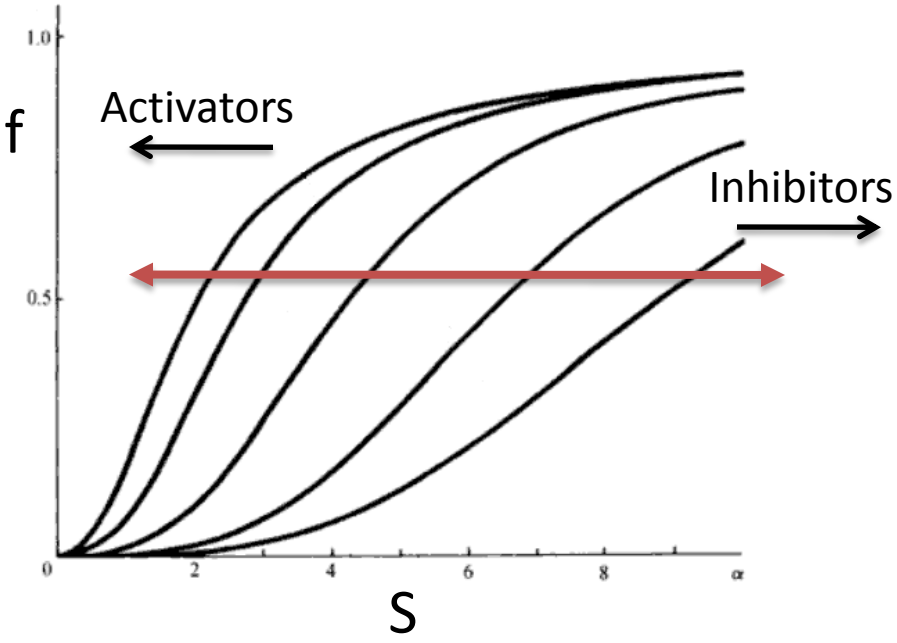
Modifiers can be incorporated into the models by assuming that the modifier molecule can bind either exclusively to the relaxed or taut forms.

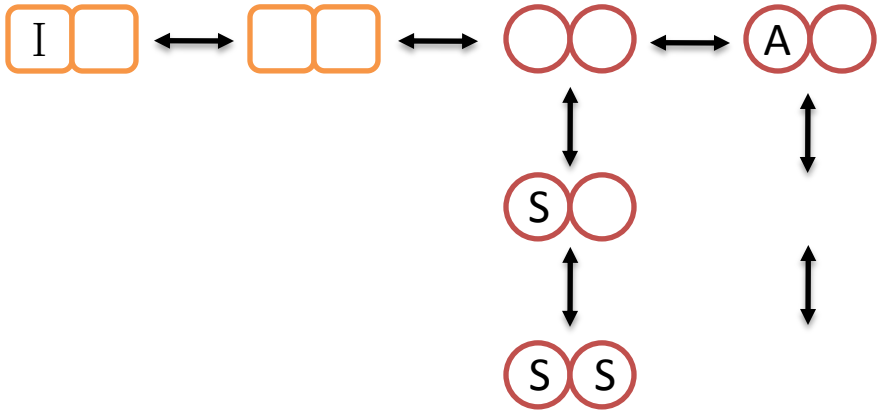
Thus inhibitors will bind to the taut form while activators can bind to the relaxed form. They effectively change the L constant.



$$f = \frac{S/K_1(1+S/K_1)}{(1+S/K_1)^2 + L\left(\frac{1+I/K_I}{1+A/K_A}\right)}$$

Modifiers in Sigmoid Kinetics





$$f = \frac{S/K_1(1 + S/K_1)}{(1 + S/K_1)^2 + L\left(\frac{1+I/K_I}{1+A/K_A}\right)}$$

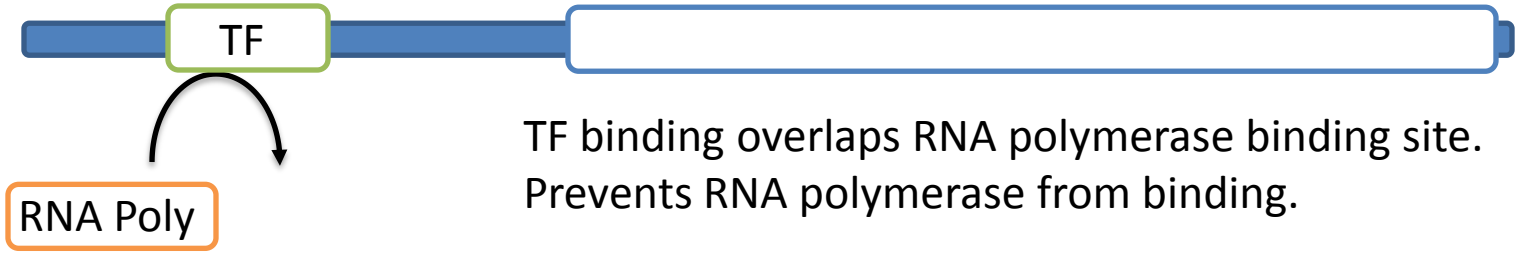
Gene Expression

TF = Transcription Factor

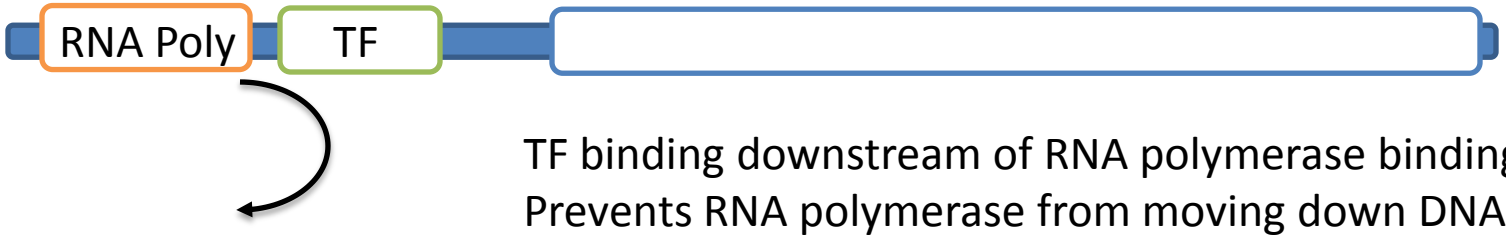
Molecular Details - Activation

Binding of a TF can increase the apparent strength of the Promoter

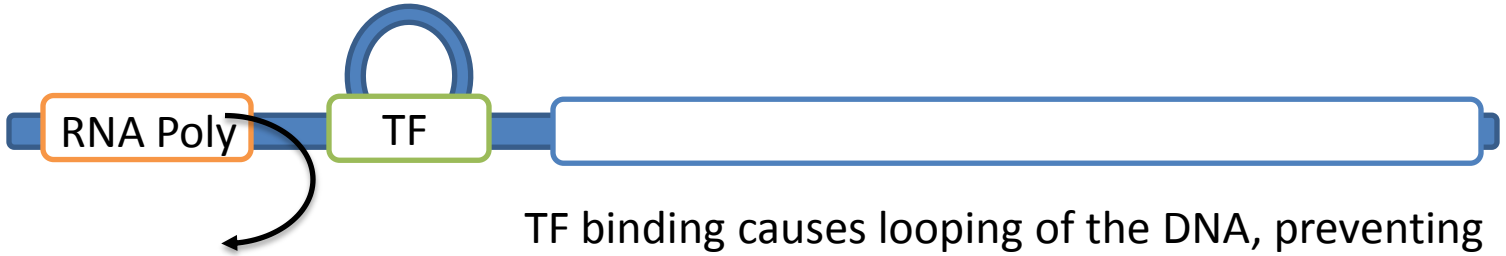
Molecular Details - Inhibition



TF binding overlaps RNA polymerase binding site.
Prevents RNA polymerase from binding.



TF binding downstream of RNA polymerase binding site.
Prevents RNA polymerase from moving down DNA strand.

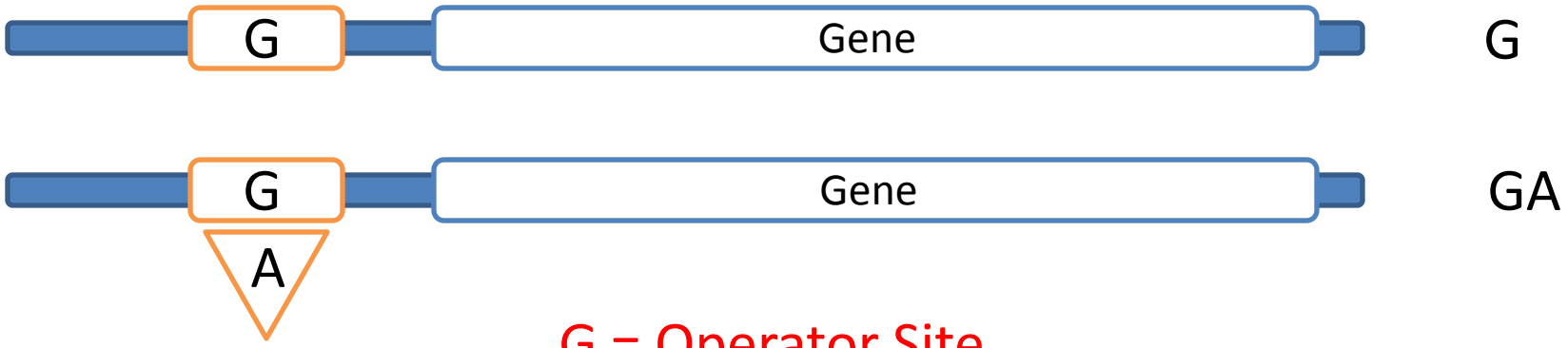


TF binding causes looping of the DNA, preventing
RNA polymerase from moving down DNA strand.

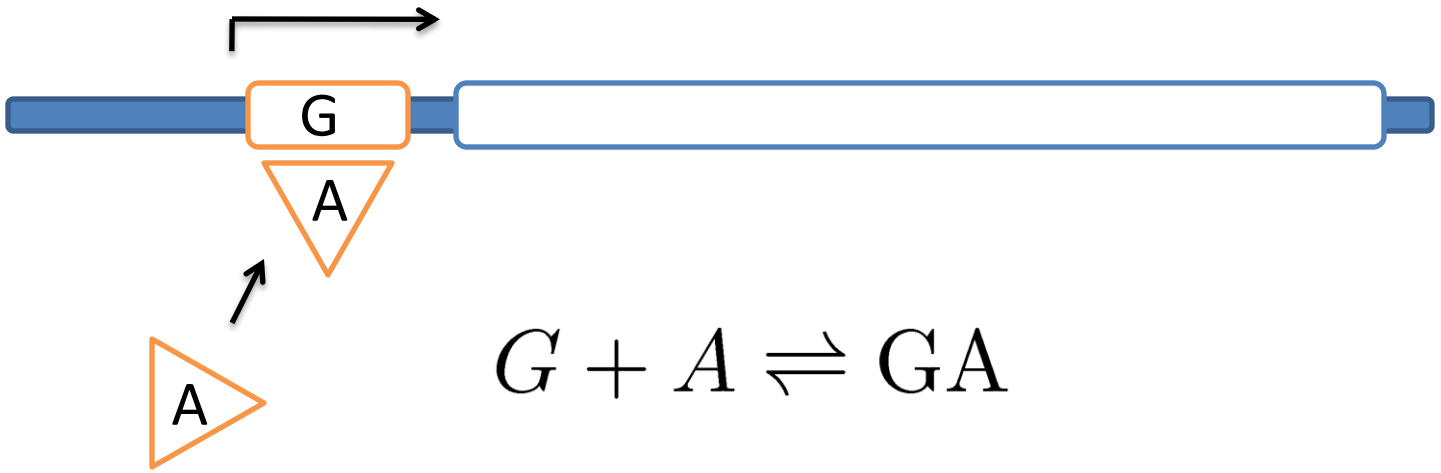
The Central Equation – Fractional Saturation

$$f = \frac{\text{Total Active States that Lead to Expression}}{\text{All States}}$$

A state refers to the state of the operator site, eg is it free or bound to a TF.



Single TF Binds and Activates Expression

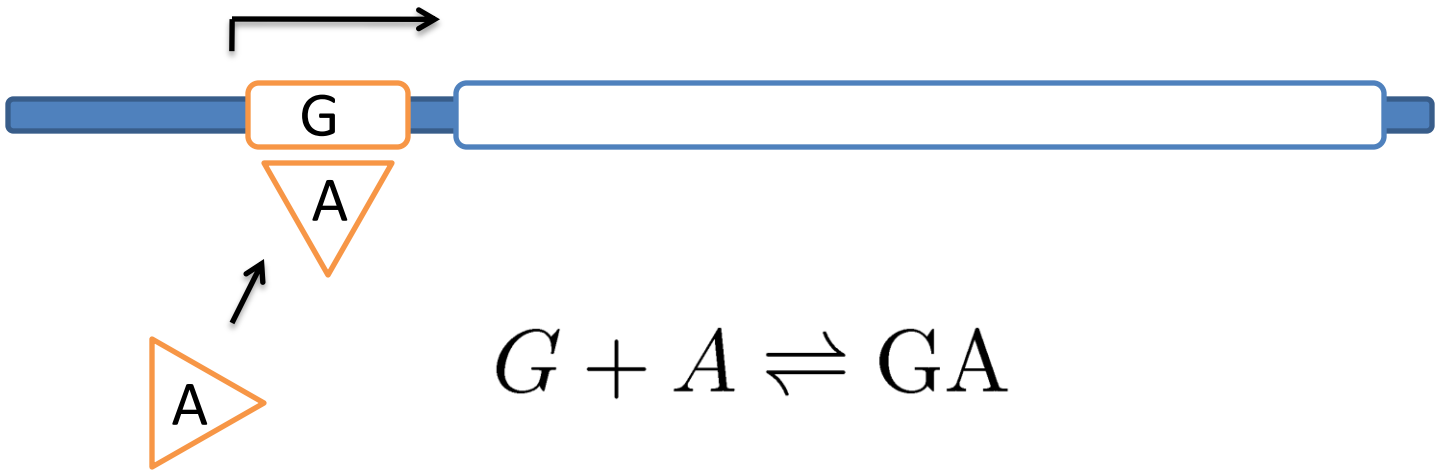


$$G + A \rightleftharpoons \mathrm{GA}$$

$$K = \frac{GA}{G.\ A}$$

Equilibrium Condition

Single TF Binds and Activates Expression



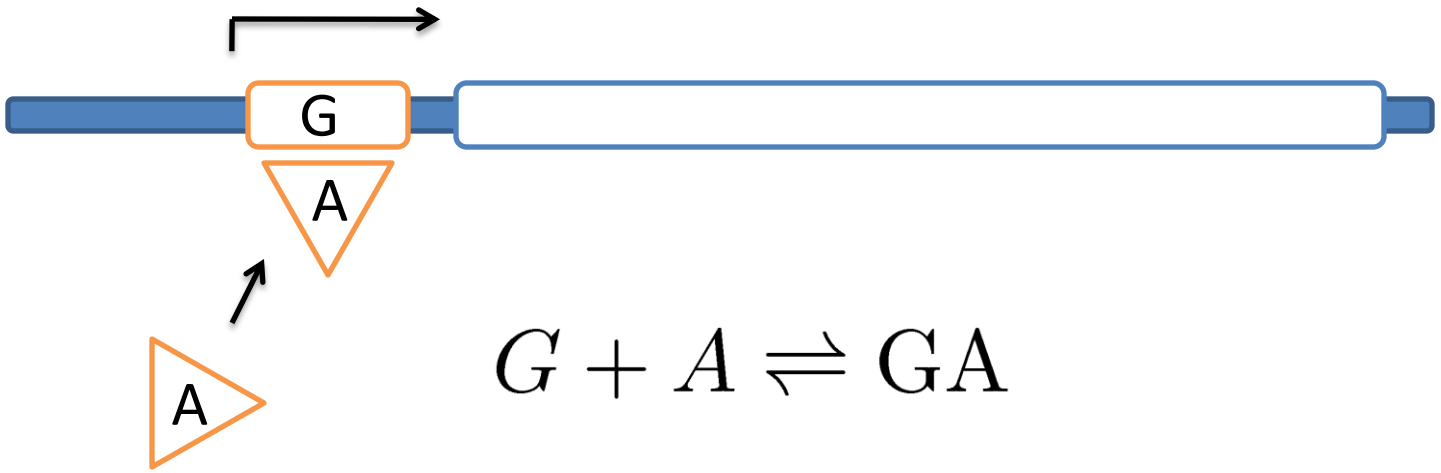
$$G + A \rightleftharpoons \mathrm{GA}$$

$$K = \frac{GA}{G.\ A}$$

$$f = \frac{GA}{G + GA}$$

Fractional Saturation

Single TF Binds and Activates Expression

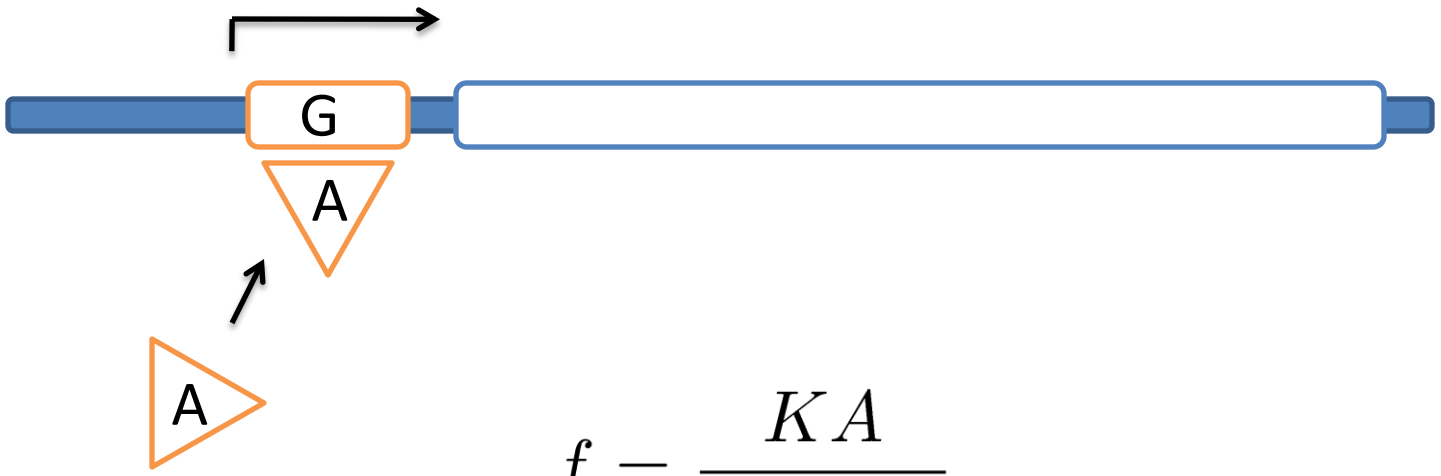


$$G + A \rightleftharpoons \mathrm{GA}$$

$$K = \frac{GA}{G.\ A}$$

$$f = \frac{K.\cancel{G}.\ A}{\cancel{G} + K.\cancel{G}.\ A} = \frac{KA}{1 + KA}$$

Single TF Binds and Activates Expression



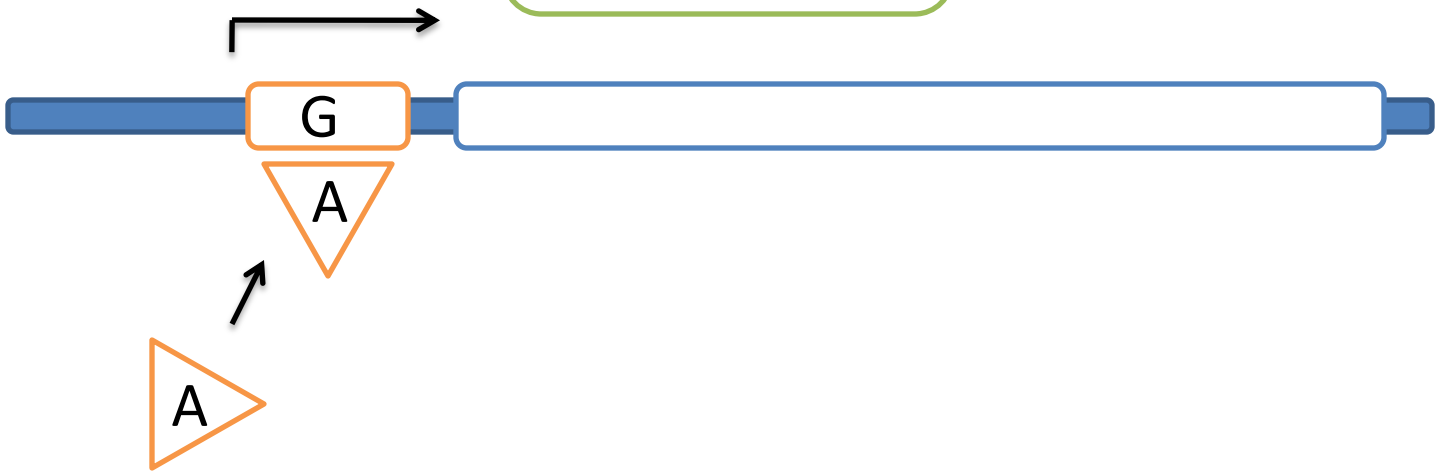
$$f = \frac{KA}{1 + KA}$$

$$\frac{v}{Vmax} = \frac{KA}{1 + KA}$$

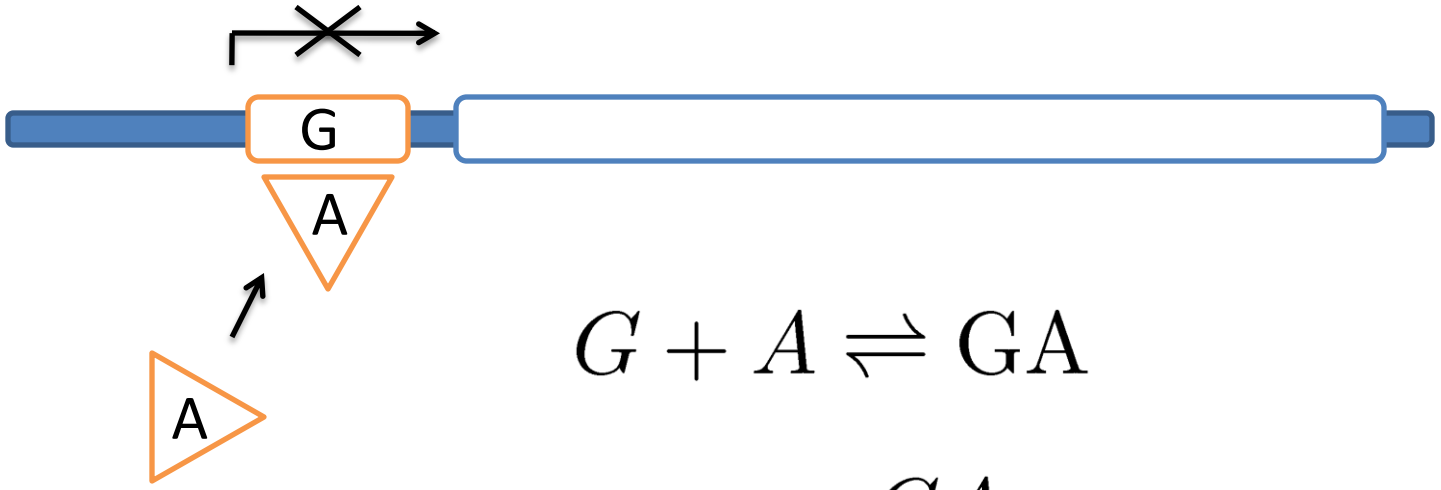
Probability Interpretation

Inside a cell there are of course only a few operator binding sites. Therefore we should strictly interpret the fractional saturation as a probability that a TF will be bound to the operator site. The rate of gene expression is then proportional to the probability of the TF being bound.

$$p = \frac{KA}{1 + KA}$$



Single TF Binds and Inhibits Expression



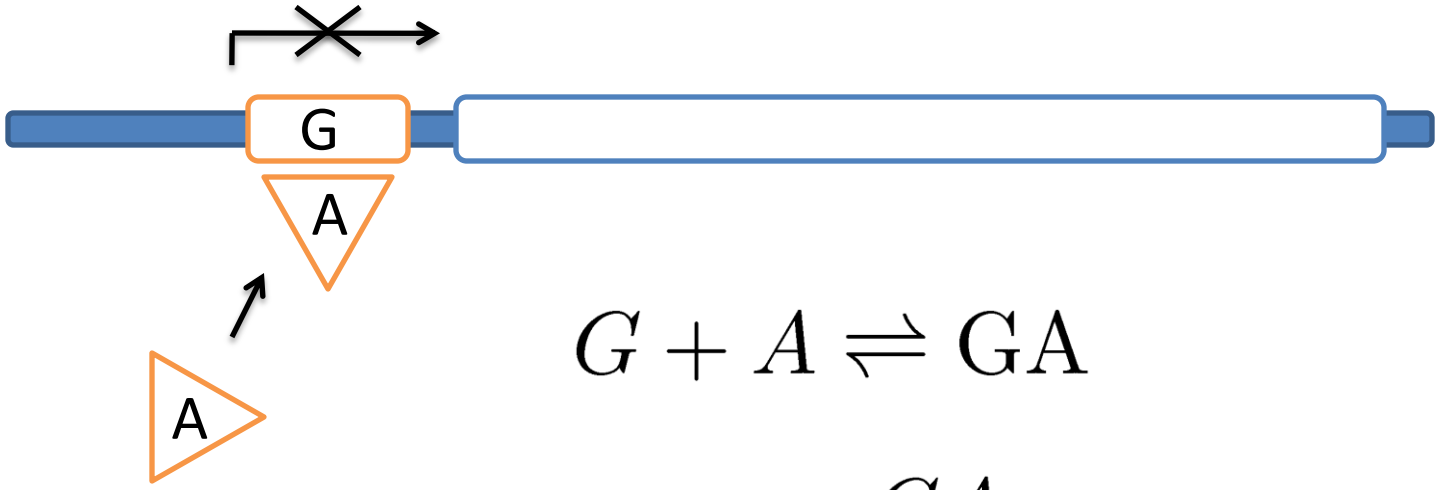
$$G + A \rightleftharpoons \mathrm{GA}$$

$$K = \frac{GA}{G.\ A}$$

$$f = \frac{G}{G + GA}$$

The active state is when the TF is not bound.

Single TF Binds and Inhibits Expression



$$G + A \rightleftharpoons \mathrm{GA}$$

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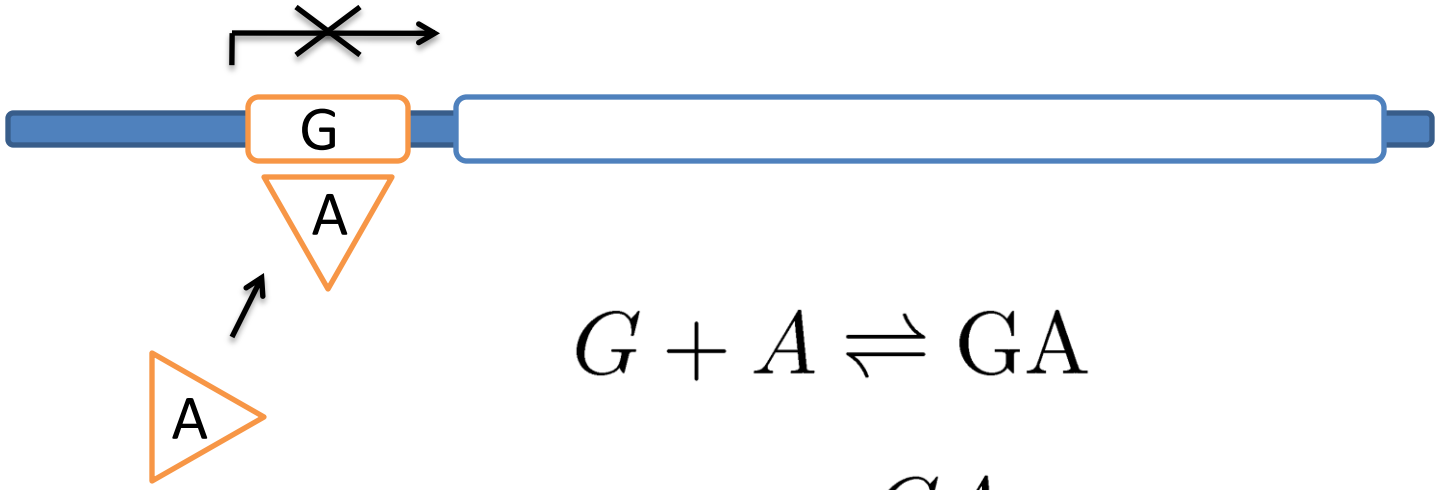
Single TF Binds and Inhibits Expression



$$G + A \rightleftharpoons \mathrm{GA}$$

$$K = \frac{GA}{G.\ A}$$

$$f = \frac{1}{1 + KA}$$

Summary

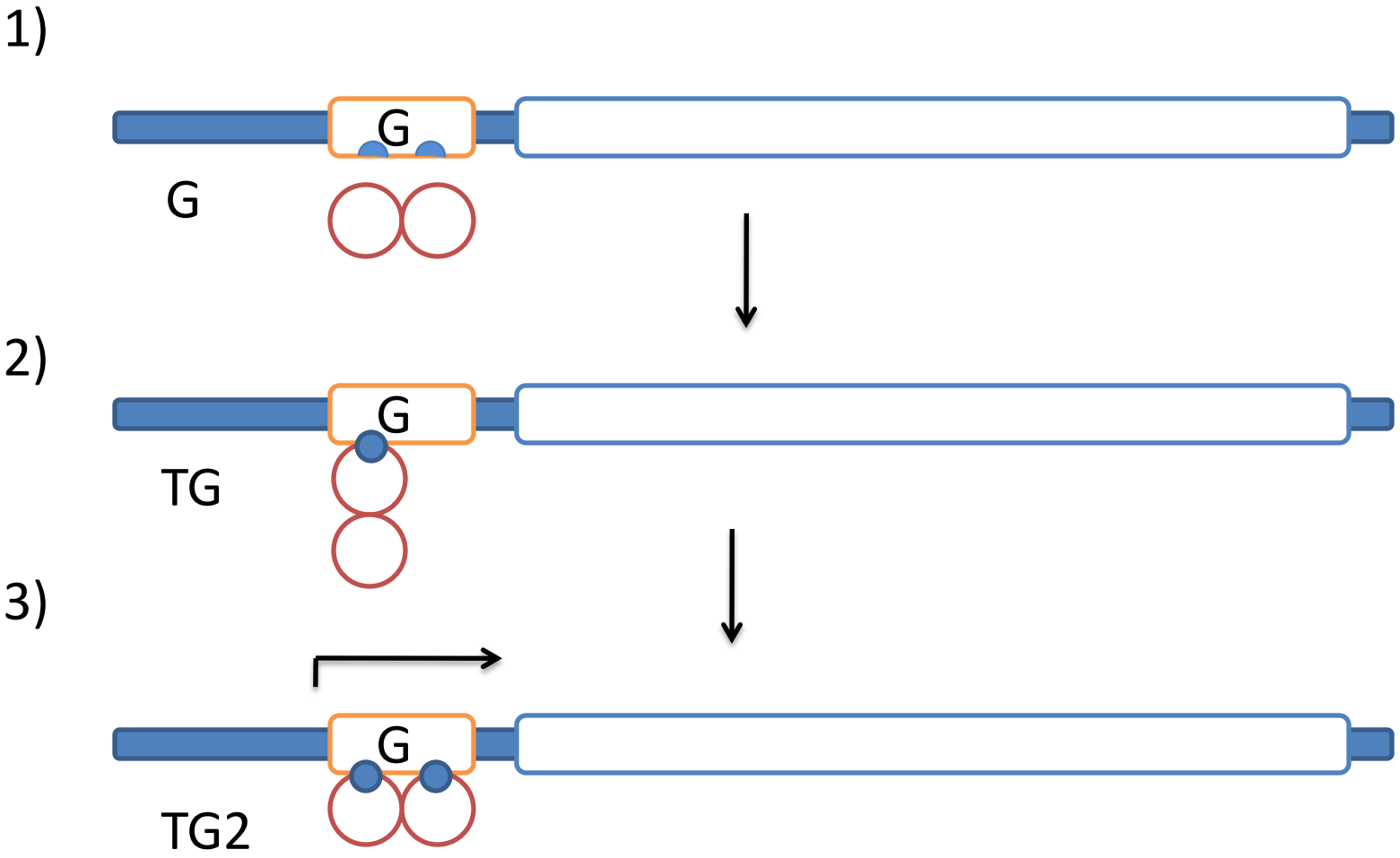
Activation

$$\frac{v}{\mathrm{Vmax}} = \frac{KA}{1+KA}$$

Repression

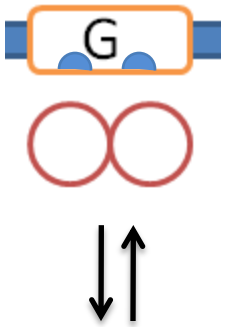
$$\frac{v}{\mathrm{Vmax}} = \frac{1}{1+KA}$$

Single TF Binds, Activates with Cooperativity



Single TF Binds, Activates with Cooperativity

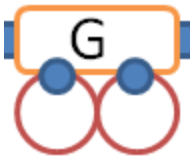
$$K_1 = \frac{TG}{T.\ G}$$



G

TG

$$K_2 = \frac{TG_2}{TG.\ T}$$



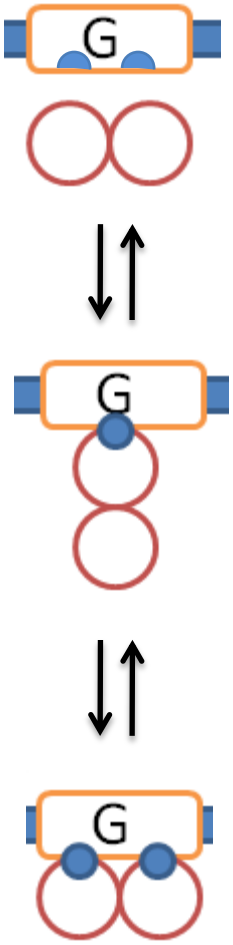
TG2

$$f = \frac{TG_2}{G + TG + TG_2}$$

$$TG = K_1.\ T.\ G$$

$$TG_2 = K_1.\ K_2.\ G.\ T^2$$

Single TF Binds, Inhibits with Cooperativity



G

$$f = \frac{K_1.\ K_2.\ \cancel{G}\ T^2}{\cancel{G} + K_1.\ T.\ \cancel{G} + K_1.\ K_2.\ \cancel{G}\ T^2}$$

TG

$$f = \frac{K_1.\ K_2.\ T^2}{1 + K_1.\ T.\ + K_1.\ K_2.\ T^2}$$

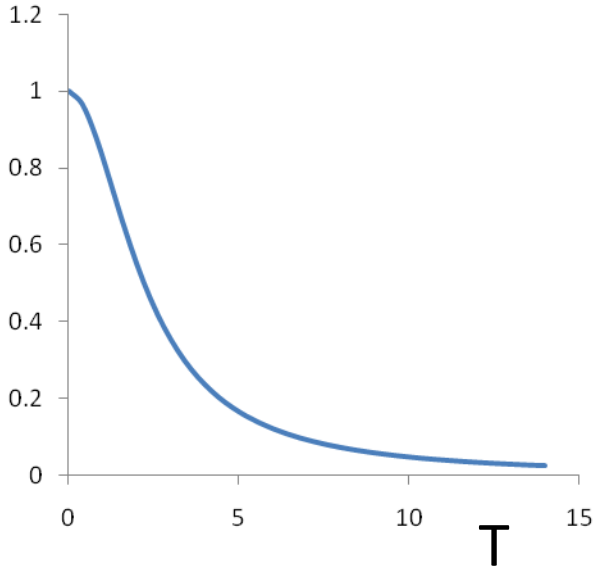
TG2

$$\frac{v}{Vmax} = \frac{K_1.\ K_2.\ T^2}{1 + K_1.\ T.\ + K_1.\ K_2.\ T^2}$$

Single TF Binds, Inhibits with Cooperativity

$$f = \frac{G}{G + TG + TG_2}$$

v/Vmax

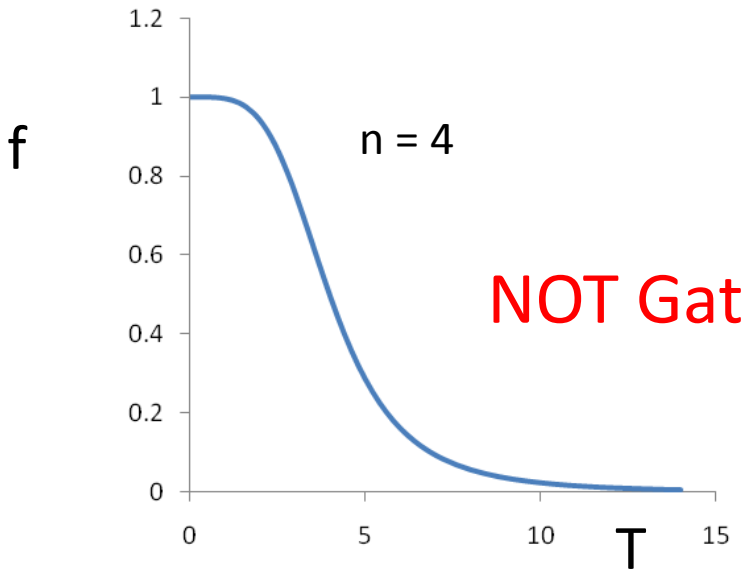


$$\frac{v}{Vmax} = \frac{1}{1 + K_1.\,T.\, + K_1.\,K_2.\,T^2}$$

Repression with n binding sites:

$$f = \frac{G}{G + TG + TG_2 + TG_3 + \ldots}$$

$$\frac{v}{Vmax} = \frac{1}{1 + K_1.\,T.\, + K_1.\,K_2.\,T^2 + K_1.\,K_2.\,K_3.\,T^3 + \ldots}$$



NOT Gate

In the literature you'll often find then following variants:

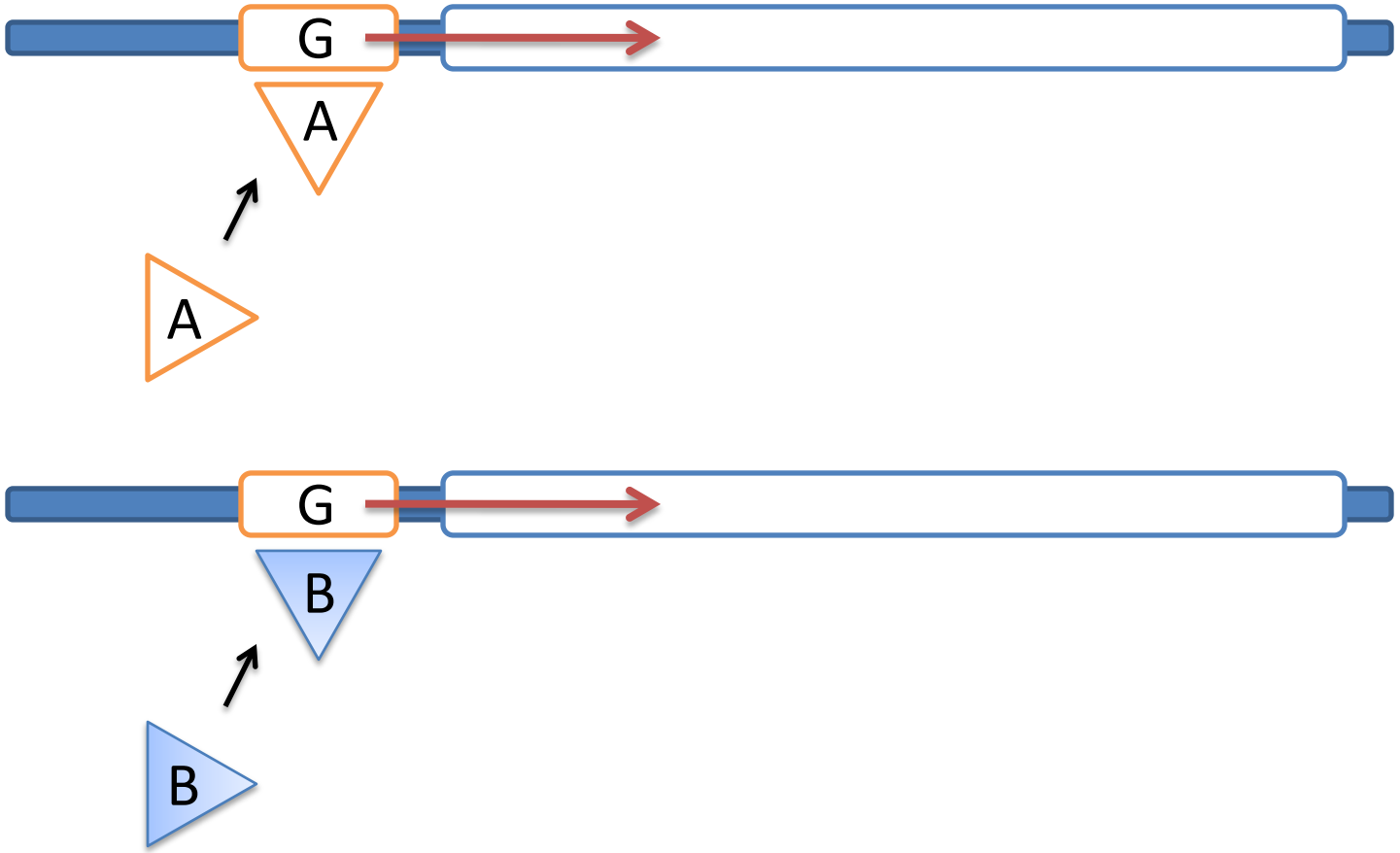
Activation

$$\frac{v}{\mathrm{Vmax}} = \frac{KA^n}{1 + KA^n}$$

Repression

$$\frac{v}{\mathrm{Vmax}} = \frac{1}{1 + KA^n}$$

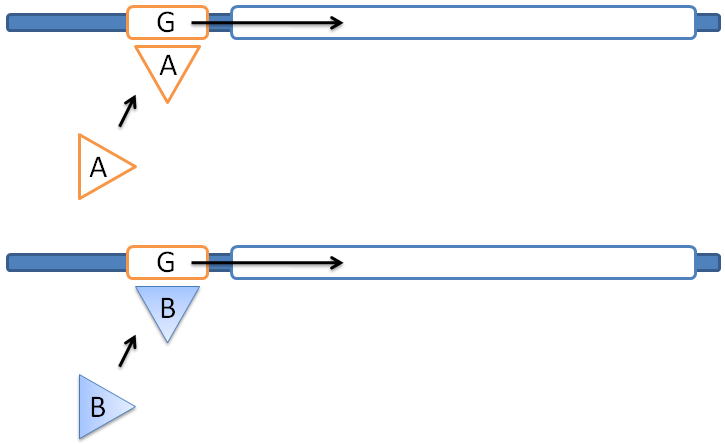
Two Activating TFs Compete for the Same Site



Two TFs Compete for the Same Site

$$G + A \rightleftharpoons \mathrm{GA}$$

$$G + B \rightleftharpoons \mathrm{GB}$$



$$K_1 = \frac{GA}{G.\ A}$$

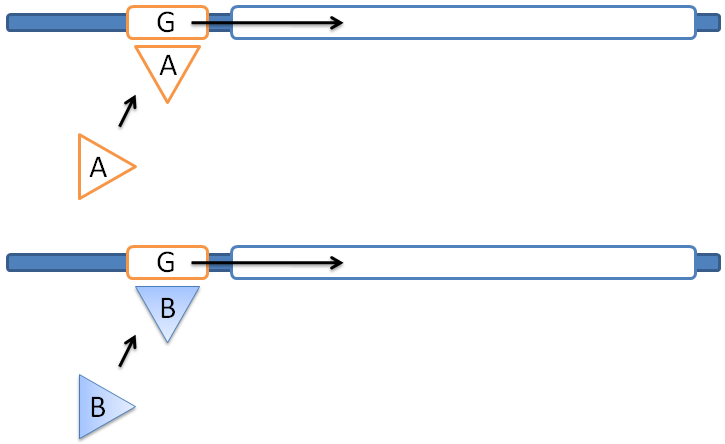
$$f = \frac{\text{Total Number of Active States}}{\text{Total Number of States}}$$

$$K_2 = \frac{GB}{G.\ B}$$

$$f = \frac{GA + GB}{G + GA + GB}$$

Two Activating TFs Compete for the Same Site

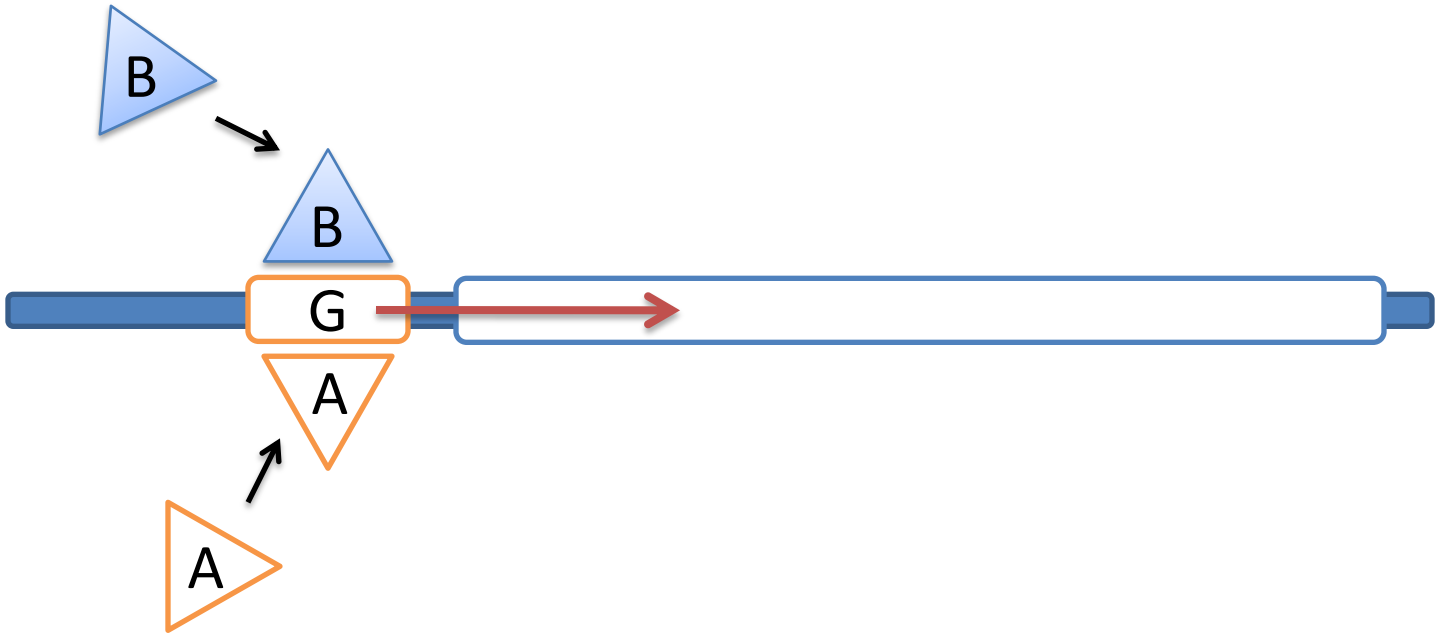
$$f = \frac{GA + GB}{G + GA + GB}$$



$$f = \frac{K_1.\ A + K_2.\ B}{1 + K_1.\ A + K_2.\ B}$$

OR Gate

Two Activating TFs Bind to two different sites



$$f = \frac{GA + GB + GAB}{G + GA + GB + GAB}$$

Two Activating TFs Bind to two different sites

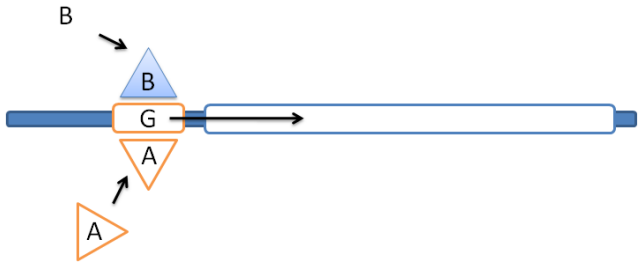
.....but there is a slight complication

$$G + A \rightleftharpoons \mathrm{GA}$$

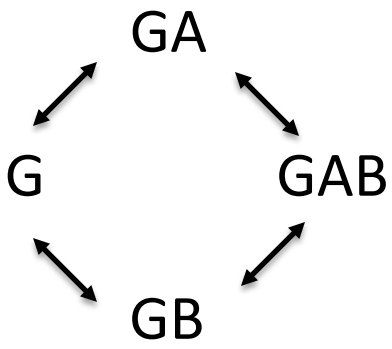
$$G + B \rightleftharpoons \mathrm{GB}$$

$$GA + B \rightleftharpoons \mathrm{GAB}$$

$$GB + A \rightleftharpoons \mathrm{GAB}$$

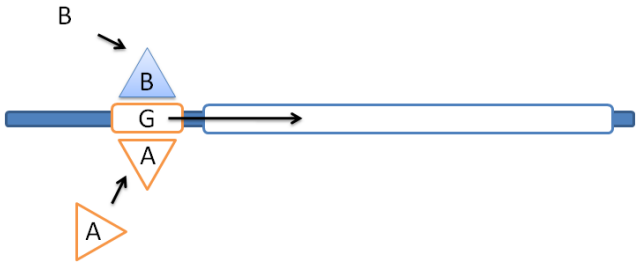


Two different ways to make GAB



Two Activating TFs Bind to two different sites

$$G + A \rightleftharpoons \mathrm{GA} \qquad K_1 = \frac{GA}{G.\ A}$$



$$G + B \rightleftharpoons \mathrm{GB} \qquad K_2 = \frac{GB}{G.\ B}$$

$$GA + B \rightleftharpoons \mathrm{GAB} \qquad K_3 = \frac{GAB}{GA.\ B}$$

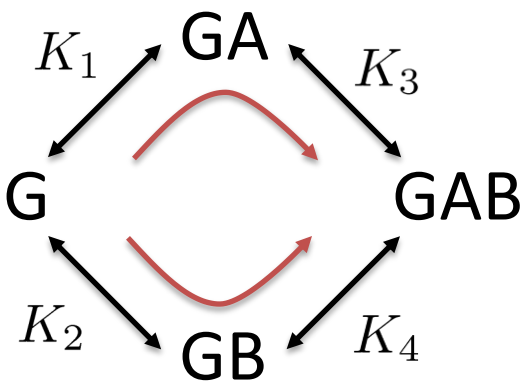
$$GB + A \rightleftharpoons \mathrm{GAB} \qquad K_4 = \frac{GAB}{GB.\ A}$$

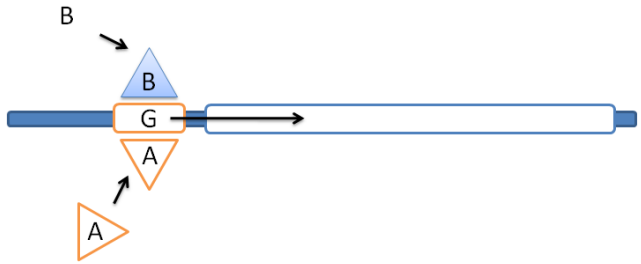
You only need to use one of These relations.

But which one?

Or does it matter?

Two Activating TFs Bind to two different sites

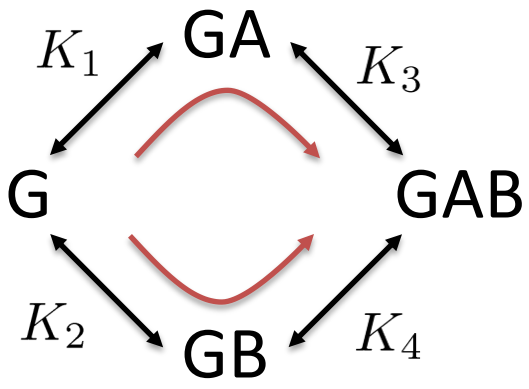


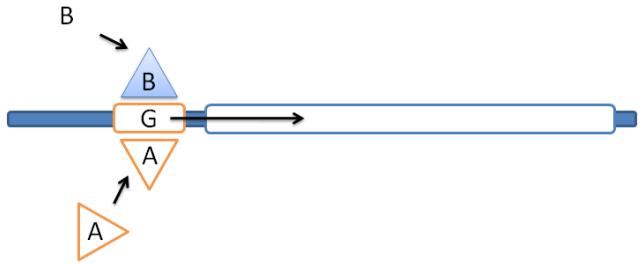


The principle of detailed balance means that the energy change along each path must be the same since the end points (G and GAB) are the same. The overall equilibrium constants for the upper and lower routes must therefore be equal.

$$K_1.\ K_3 = K_2.\ K_4$$

Two Activating TFs Bind to two different sites





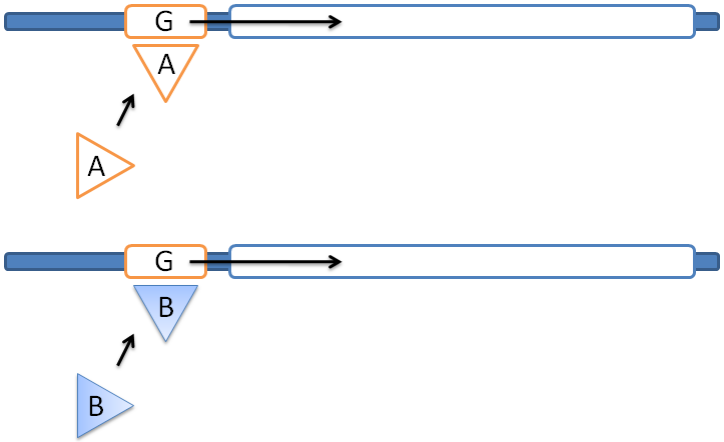
Using: $K_3 = \frac{GAB}{GA.\ B}$ $$f = \frac{K_1.\ A + K_2.\ B + K_1.\ K_3.\ A.\ B}{1 + K_1.\ A + K_2.\ B + K_1.\ K_3.\ A.\ B}$$

Using: $K_4 = \frac{GAB}{GB.\ A}$ $$f = \frac{K_1.\ A + K_2.\ B + K_2.\ K_4.\ A.\ B}{1 + K_1.\ A + K_2.\ B + K_2.\ K_4.\ A.\ B}$$

$$K_1.\ K_3 = K_2.\ K_4$$

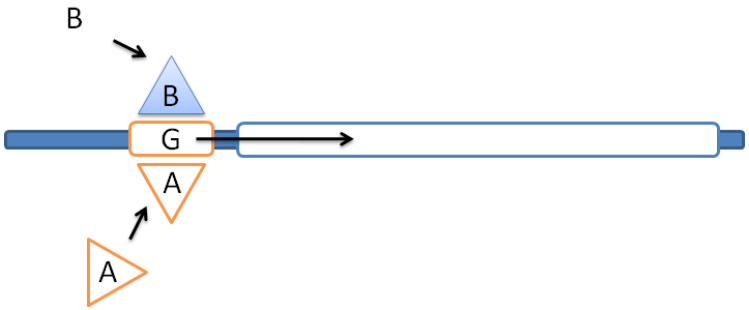
This is: the equations are identical

Comparing the two:



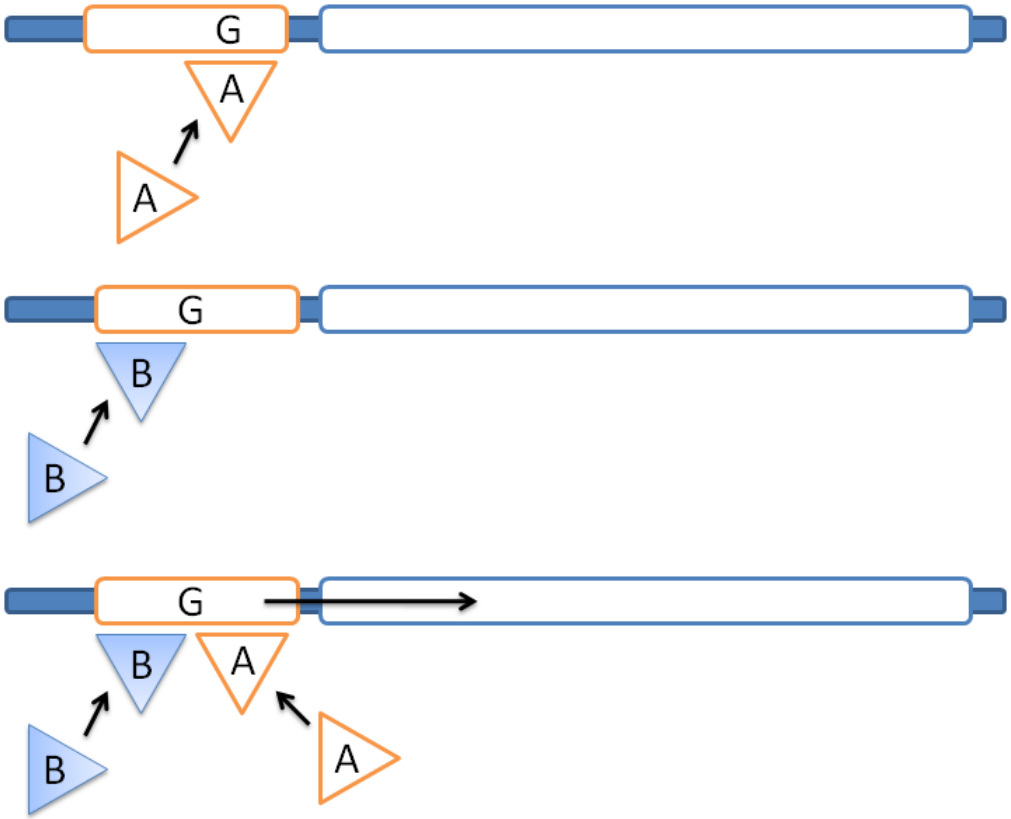
$$f = \frac{K_1.\ A + K_2.\ B}{1 + K_1.\ A + K_2.\ B}$$

OR Gates



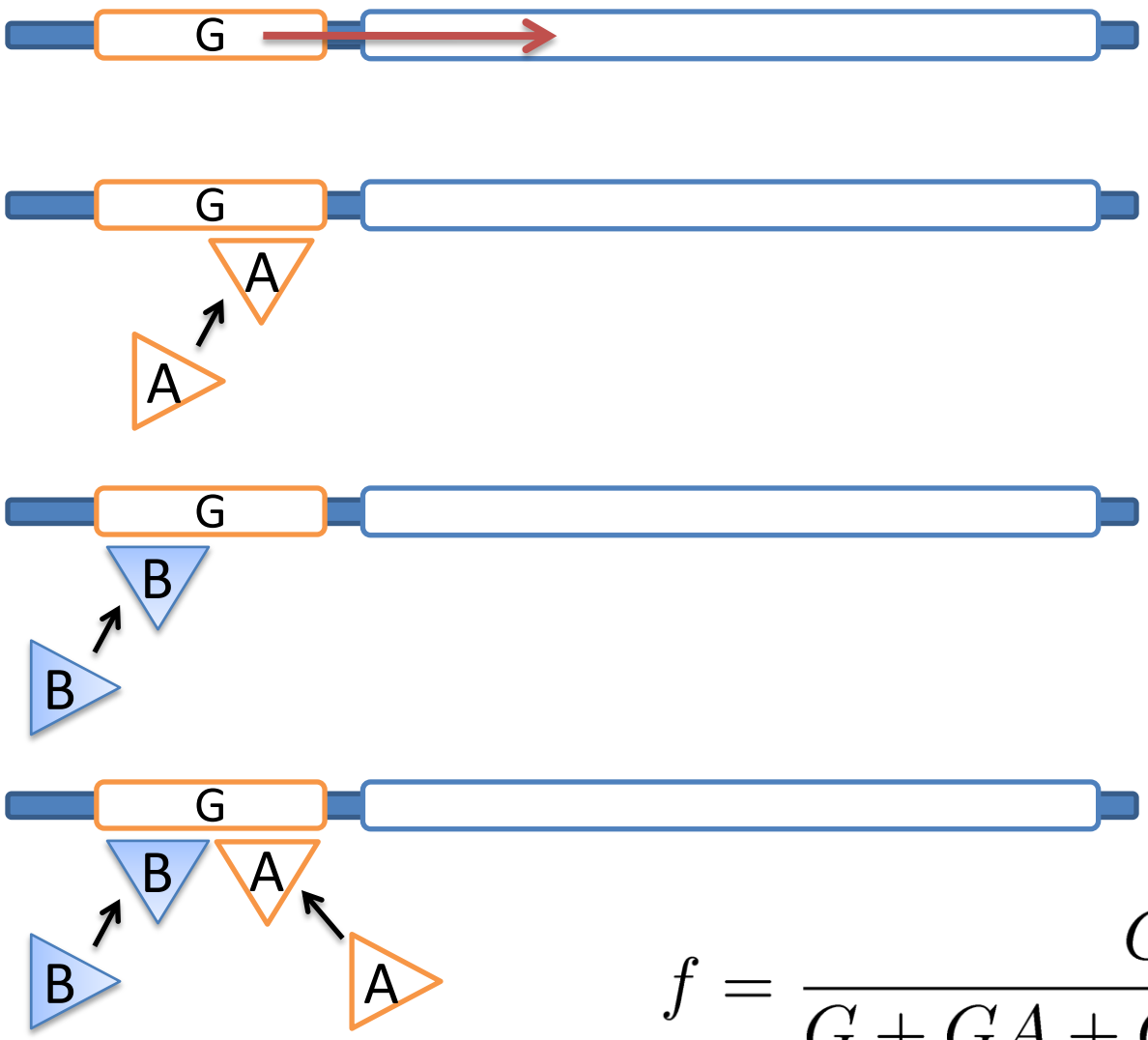
$$f = \frac{K_1.\ A + K_2.\ B + K_1.\ K_3.\ A.\ B}{1 + K_1.\ A + K_2.\ B + K_1.\ K_3.\ A.\ B}$$

AND Gates: Active only when both are bound



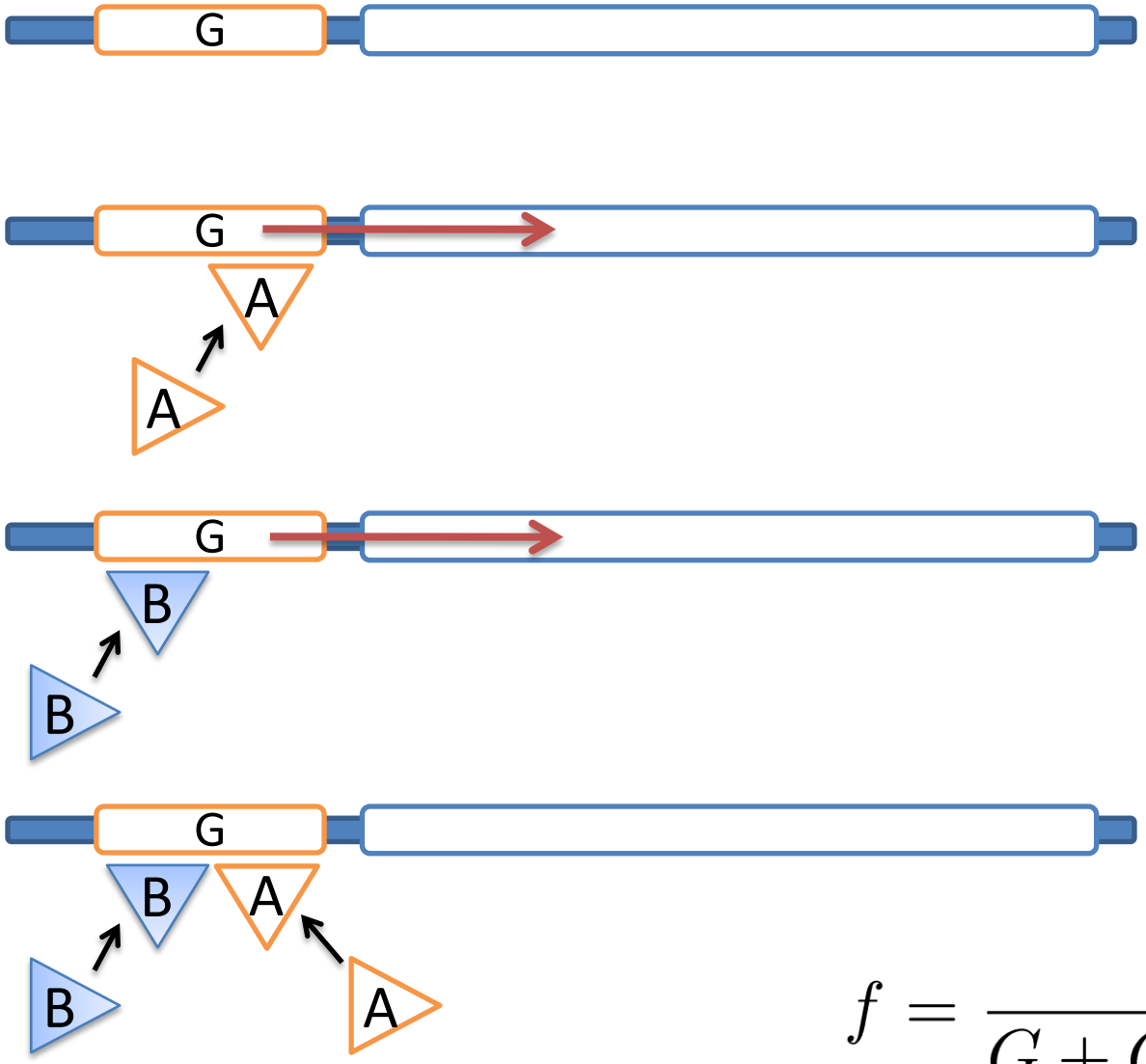
$$f = \frac{GAB}{G + GA + GB + GAB}$$

NAND Gates: Active when neither are bound



$$f = \frac{G}{G + GA + GB + GAB}$$

XOR Gate



Input A	Input B	Output
1	1	0
1	0	1
0	1	1
0	0	0

$$f = \frac{GA + GB}{G + GA + GB + GAB}$$

Other Configurations

When A binds it activates. If B is bound, then A is unable to bind. B therefore acts as an inhibitor of A.

$$f = \frac{GA}{G + GA + GB}$$