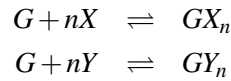


1. A gene exists in an unbound state, G , and also binds to two activating transcription factors, X and Y . ODE models for binding are



with forward and reverse rate constants k_f and k_r and fixed total gene concentration $G_T = G + GX_n + GY_n$. Assume that steady state has been reached and that the concentrations of X and Y are sufficiently large to be unaffected by binding.

- (a) What is the concentration ratio GX_n/G ?

$$\frac{k_f}{k_r} X^n$$

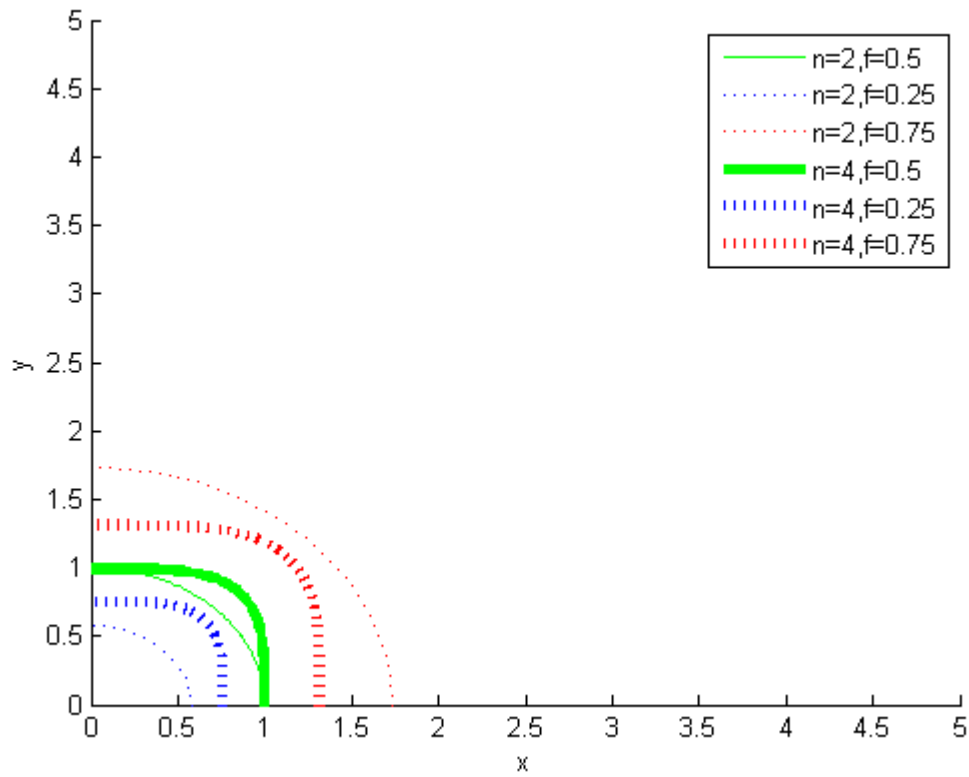
- (b) What is the concentration ratio GY_n/G ?

$$\frac{k_f}{k_r} Y^n$$

- (c) What is the fraction f of active DNA, $f = (GX_n + GY_n)/G_T$?

$$\begin{aligned} f &= \frac{\frac{k_f}{k_r} X^n + \frac{k_f}{k_r} Y^n}{1 + \frac{k_f}{k_r} X^n + \frac{k_f}{k_r} Y^n} = \frac{(X/K)^n + (Y/K)^n}{1 + (X/K)^n + (Y/K)^n} \\ K &= \left(\frac{k_r}{k_f}\right)^{\frac{1}{n}} \end{aligned}$$

- (d) Your answer to the previous question should have the form $(x^n + y^n)/(1 + x^n + y^n)$, where x and y are related to concentrations X and Y and other model parameters. In the xy -plane for the range $x \in 0 \dots 5$, $y \in 0 \dots 5$, plot the lines corresponding to $f = 1/2$ (solid), $f = 0.01$ (dotted), and $f = 0.99$ (dotted) for $n = 2$ (thin lines) and $n = 4$ (thick lines).



(e) Defining logical inputs as $a = \Theta(x > 1)$ and $b = \Theta(y > 1)$, with $\Theta(u) = 1$ if u is true and 0 if false, what logical function of a and b does the active region resemble?

a OR b

2. Now consider the case that X and Y bind together. The ODE model for binding is



(a) What is the concentration ratio GX_nY_n/G ?

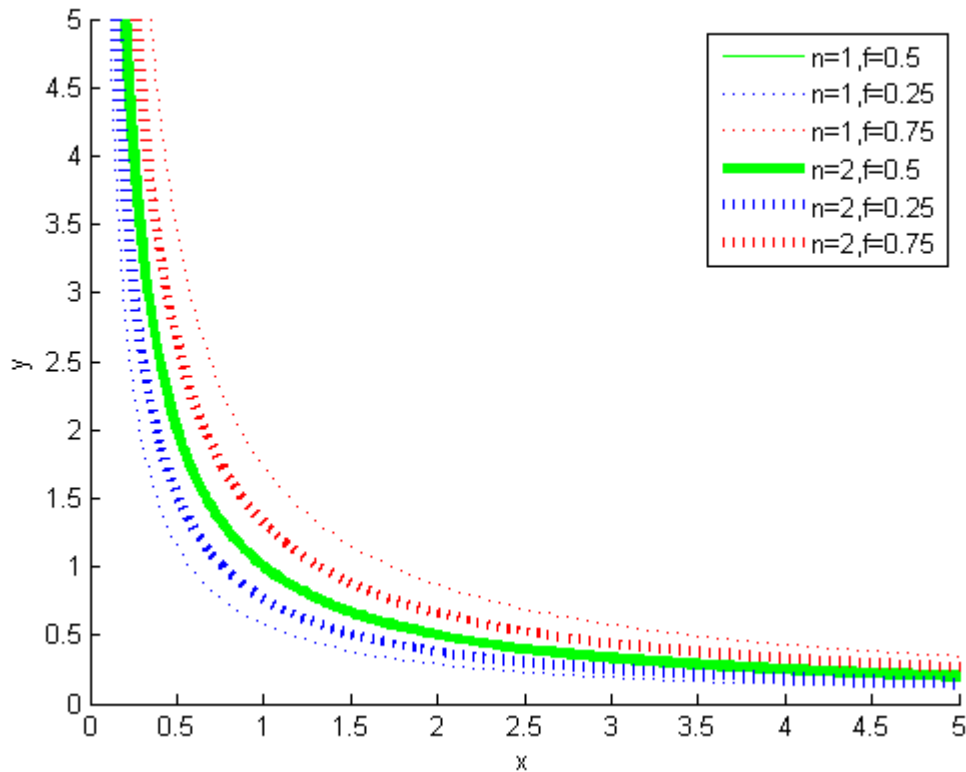
$$\frac{k_f}{k_r} X^n Y^n$$

(b) What is the fraction f of active DNA, $f = (GX_nY_n)/G_T$?

$$f = \frac{\frac{k_f}{k_r} X^n Y^n}{1 + \frac{k_f}{k_r} X^n Y^n} = \frac{(X/K)^n (Y/K)^n}{1 + (X/K)^n (Y/K)^n}$$

$$K = \left(\frac{k_r}{k_f}\right)^{\frac{1}{2n}}$$

(c) Your answer to the previous question should have the form $(x^n y^n)/(1 + x^n y^n)$, where x and y are related to concentrations X and Y and other model parameters. In the xy -plane for the range $x \in 0 \dots 5, y \in 0 \dots 5$, plot the lines corresponding to $f = 1/2$ (solid), $f = 0.01$ (dotted), and $f = 0.99$ (dotted) for $n = 1$ (thin lines) and $n = 2$ (thick lines).



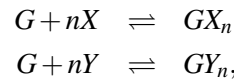
(d) What logical function does the active region resemble in terms of $a = \Theta(x > 1)$ and $b = \Theta(y > 1)$?

a AND b

- (e) How does the match to the logical function differ for $n = 2$ and $n = 4$? Is one response sharper than the other (smaller distance between contour lines for $f = 0.01$ and $f = 0.99$, or closer to the logical function)?

The response is sharper when $n = 4$.

3. Now consider the case that X is an activator and Y is a repressor that competes for the same binding site. The ODE models for binding are the same as independent activators,



but now the active fraction is $f = GX_n/G_T$.

- (a) What is the fraction f of active DNA in terms of model parameters?

We can use the chemical kinetics equations above to write a system of ODEs:

$$\begin{aligned} \frac{d}{dt} [GX_n] &= k_f [G] \cdot [X]^n - k_r [GX_n] \\ \frac{d}{dt} [GY_n] &= k_f [G] \cdot [Y]^n - k_r [GY_n] \end{aligned}$$

From here on out we will write all concentration without the brackets (ie we will write $[G]$, $[GX_n]$ as G , GX_n . We can now solve for the steady state values of GX_n and GY_n by setting the derivatives in the above ODEs to zero. Doing some algebra we conclude:

$$GX_n = \frac{k_f}{k_r} G \cdot X^n \tag{1}$$

Now we solve for G_T :

$$\begin{aligned} G_T &= G + GX_n + GY_n \\ &= G \left(1 + \frac{k_f}{k_r} X^n + \frac{k_f}{k_r} Y^n \right) \end{aligned}$$

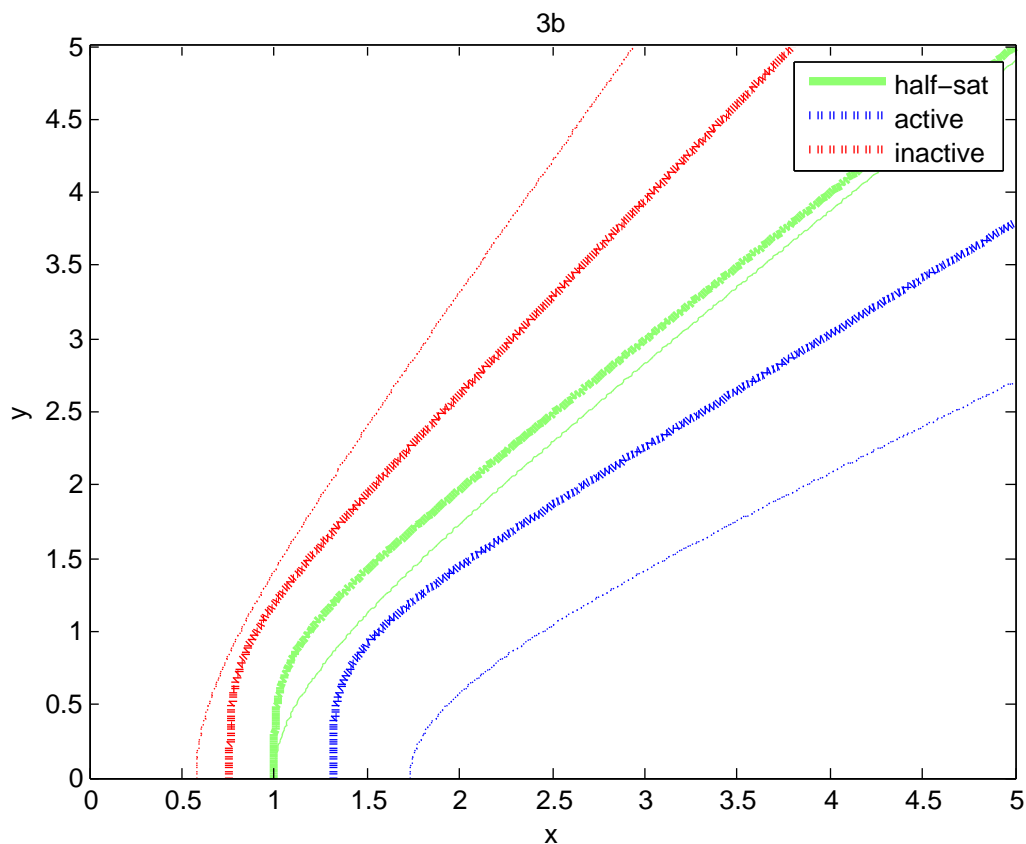
We are told in the problem statement that the active fraction is:

$$f = \frac{GX_n}{G_T}$$

Plugging in we get:

$$\begin{aligned}
 f &= \frac{GX_n}{G_T} \\
 &= \frac{\frac{k_f}{k_r}GX^n}{G\left(1 + \frac{k_f}{k_r}X^n + \frac{k_f}{k_r}Y^n\right)} \\
 &= \frac{\frac{k_f}{k_r}X^n}{\left(1 + \frac{k_f}{k_r}X^n + \frac{k_f}{k_r}Y^n\right)}
 \end{aligned}$$

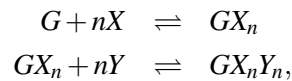
- (b) Your answer should have the form $x^n/(1+x^n+y^n)$, where x and y are related to concentrations and model parameters. In the xy -plane for the range $x \in 0 \dots 5$, $y \in 0 \dots 5$, plot the lines corresponding to $f = 1/2$ (solid), $f = 0.01$ (dotted), and $f = 0.99$ (dotted) for $n = 2$ (thin lines) and $n = 4$ (thick lines).



- (c) What logical function does the active region resemble in terms of $a = \Theta(x > 1)$ and $b = \Theta(y > 1)$?

Answers may vary. Acceptable answers include:

- i. a AND (NOT b)
 - ii. a
 - iii. a AND $x > y$
 - iv. a AND (a XOR b) (Which reduces to a AND (NOT b))
 - v. There is no logic function.
4. Consider the case that X is an activator and Y is a repressor that binds to X rather than to DNA. The ODE models for binding are the same as independent activators,



with active fraction is $f = GX_n/G_T$.

- (a) What is the fraction f of active DNA in terms of model parameters?

Just like problem 3 we start by solving for the steady state of the system of ODEs:

$$\begin{aligned} \frac{d}{dt}[GX_n] &= k_f[G] \cdot [X]^n - k_r[GX_n] \\ \frac{d}{dt}[GX_nY_n] &= k_f[GX_n] \cdot [Y]^n - k_r[GX_nY_n] \end{aligned}$$

Dropping the brackets and evaluating when the derivatives are zero we can compute:

$$\begin{aligned} GX_n &= \frac{k_f}{k_r} G \cdot X^n \\ GX_nY_n &= \left(\frac{k_f}{k_r}\right)^2 G \cdot X^n \cdot Y^n \end{aligned}$$

Now we solve for G_T :

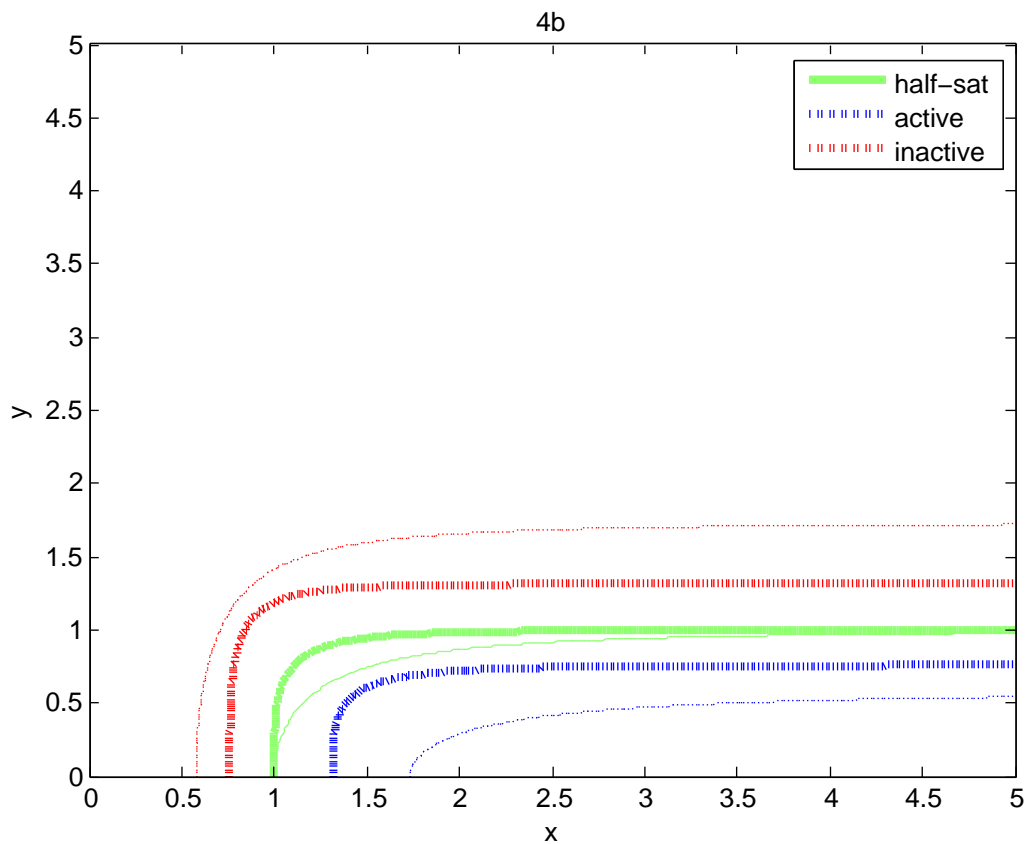
$$\begin{aligned} G_T &= G + GX_n + GX_nY_n \\ &= G \left[1 + \frac{k_f}{k_r} X^n + \left(\frac{k_f}{k_r}\right)^2 X^n \cdot Y^n \right] \end{aligned}$$

Solving for the active fraction:

$$f = \frac{GX_n}{G_T}$$

$$= \frac{\frac{k_f}{k_r} \cdot X^n}{1 + \frac{k_f}{k_r} X^n + \left(\frac{k_f}{k_r}\right)^2 X^n \cdot Y^n}$$

- (b) Your answer should have the form $x^n / (1 + x^n + x^n y^n)$, where x and y are related to concentrations and model parameters. In the xy -plane for the range $x \in 0 \dots 5$, $y \in 0 \dots 5$, plot the lines corresponding to $f = 1/2$ (solid), $f = 0.01$ (dotted), and $f = 0.99$ (dotted) for $n = 2$.



- (c) What logical function does the active region resemble in terms of $a = \Theta(x > 1)$ and $b = \Theta(y > 1)$?
a AND (NOT b)
- (d) Compare this example to the previous repressor model with competitive binding. How do the active regions differ? Is one response sharper than the other (smaller distance between contour lines for $f = 0.01$ and $f = 0.99$, or closer to the logical function)?
System 3 does not converge to a logic function of a and b as n goes to ∞ whereas system 4 converges to a AND (NOT b). System 4 is sharper away from the origin but they have approximately the same sharpness around $x = 1$.
5. Suppose that three transcription factors X , Y , and Z bind to the same promoter, and the fraction of active DNA is $f = (x^2 + y^2 + x^2y^2)/(1 + x^2 + y^2 + x^2y^2 + z^2 + x^2y^2z^2)$.
- (a) What are the transcriptional complexes that bind to the promoter?
 $X_2, Y_2, X_2Y_2, Z_2, X_2Y_2Z_2$
- (b) Which of these are transcriptionally active?
 X_2, Y_2, X_2Y_2
- (c) Suppose that the transcriptional rate is $(\beta_1x^2 + \beta_2y^2 + \beta_3x^2y^2)/(1 + x^2 + y^2 + x^2y^2 + z^2 + x^2y^2z^2)$. How would you interpret the coefficients β_1 , β_2 , and β_3 ?
 $\beta_1 \rightarrow$ Transcription rate (transcripts/second) when all genes have X bound (ie $G_T = GX_2$).
 $\beta_2 \rightarrow$ Transcription rate (transcripts/second) when all genes have Y bound (ie $G_T = GY_2$).
 $\beta_3 \rightarrow$ Transcription rate (transcripts/second) when all genes have both X and Y bound (ie $G_T = GX_2Y_2$).