The following questions consider binding of a transcription factor protein, denoted X, to a gene promoter with N binding sites. The number of transcription factors bound is denoted n, and in the general case

$$n \in \{0, 1, 2, 3, \ldots, N\}.$$

The number of copies of the gene with n sites bound is denoted G_n. The total number of copies of the gene is the constant

$$G_T = \sum_{n=0}^{N} G_n,$$

and the fraction of copies in state n is G_n/G_T. The transcription rate for a copy of the gene with n transcription factors bound is denoted ν_n, and the total transcriptional rate is

$$\beta = \sum_{n=0}^{N} \nu_n G_n.$$

We will assume that $\nu_0 = 0$ and that increased binding gives increased transcription, which implies that the maximum possible transcription rate occurs when G_N is the only occupied state. Thus, the maximum transcription rate is $\beta_{\max} = \nu_N G_T$. The concentration of transcription factor is sufficiently large that changes in the value of unbound X need not be considered. All questions assume that binding has reached equilibrium.

Cooperativity at a particular value of X is defined as $(d/d\ln X)\ln[\beta/(\beta_{\max} - \beta)]$, usually evaluated at the value of X giving half-maximal transcription, $\beta = \beta_{\max}/2$. Note that $d/d\ln X$ is $X d/dX$ by the chain rule.

1. Fully cooperative binding. The standard model for fully cooperative binding is that only the two states G_0 and G_N exist, $G_0 + NX \rightleftharpoons G_N$, with forward rate constant k_f and backward rate constant k_b.

   (a) Show that β as a function of X is proportional to $(X/K)^N/[1 + (X/K)^N]$. Provide K as a function of model parameters.
   At equilibrium, $G_0 X^N k_f = G_N k_b$. Since only two states exist, $G_T = G_N + G_0$, we have:

$$(G_T - G_N) X^N k_f = G_N k_b$$

$$G_N = \frac{k_f X^N}{k_b + k_f X^N} G_T = \frac{(X/K)^N}{1 + (X/K)^N} G_T$$

   assuming $K^N = \frac{k_b}{k_f}$. Then we have

$$\beta = \sum_{n=0}^{N} \nu_n G_n = \nu_N G_N = \frac{(X/K)^N}{1 + (X/K)^N} \nu_N G_T$$

(b) Provide $\beta/(\beta_{\max}-\beta)$.
Simply plug in everything, we get

$$\frac{\beta}{\beta_{\max}-\beta}=\frac{\frac{(X/K)^N}{1+(X/K)^N}G_T}{\nu_N G_T-\frac{(X/K)^N}{1+(X/K)^N}G_T}=(\frac{X}{K})^N$$

(c) Show that the cooperativity is N regardless of the value of X.
$\frac{d}{d\ln X}\ln\frac{\beta}{\beta_{\max}-\beta}=X\frac{d}{dX}\ln(\frac{X}{K})^N=NX\frac{d}{dx}[\ln X-\ln K]=NX\frac{1}{X}=N$

2. Fully independent and additive binding. Suppose each site is bound independently. Each site has two states, u (unbound) and b (bound), with $u+X\rightleftharpoons b$. The forward rate constant is k'_f, and the backward rate constant is k'_b. Because this expression in in terms of a single site, $u+b=1$. When n sites are bound, the transcription rate is additive, $\nu_n=n\Delta\nu$.

(a) What is the probability θ that a particular site is bound?
At equilibrium

$$uXk'_f=bk'_b$$
$$(1-b)Xk'_f=bk'_b$$

which gives us $b=\frac{Xk'_f}{Xk'_f+k'_b}$. Also, $\theta=\frac{b}{u+b}=b$.

(b) In terms of θ, what is the probability G_n/G_T that n of the N sites are bound for a particular copy of the gene?
G_n/G_T follows a binomial distribution, so $G_n/G_T=C(N,n)\theta^n(1-\theta)^{N-n}$

(c) Show that the transcription rate is $\beta=G_T\Delta\nu\sum_{n=0}^{N}nC(N,n)\theta^n(1-\theta)^{N-n}$, where $C(N,n)$ is the combinatorial factor $N!/n!(N-n)!$.

$$\begin{aligned}\beta&=\sum_{n=0}^{N}\nu_n G_n\\&=\sum_{n=0}^{N}n\Delta\nu\frac{G_n}{G_T}G_T\\&=G_T\Delta\nu\sum_{n=0}^{N}nG_n/G_T\\&=G_T\Delta\nu\sum_{n=0}^{N}nC(N,n)\theta^n(1-\theta)^{N-n}\end{aligned}$$

(d) Define $\tilde{\theta}(s) = \sum_{n=0}^{N} e^{-sn} C(N,n)\theta^n(1-\theta)^{N-n}$. Show that $\tilde{\theta}(s) = (1-\theta+e^{-s}\theta)^N$. Use this result to calculate $\langle n \rangle$ as $-(d/ds)\ln\tilde{\theta}(s)|_{s=0}$.

$$\begin{aligned}\tilde{\theta}(s) &= \sum_{n=0}^{N} e^{-sn} C(N,n)\theta^n(1-\theta)^{N-n} \\ &= \sum_{n=0}^{N} C(N,n)(e^{-s}\theta)^n(1-\theta)^{N-n} \\ &= (1-\theta+e^{-s}\theta)^N\end{aligned}$$

$$\begin{aligned}\langle n \rangle &= -\frac{d}{ds}\ln\tilde{\theta}(s)|_{s=0} \\ &= -\frac{d}{ds}(1-\theta+e^{-s}\theta)^N|_{s=0} \\ &= N(1-\theta+e^{-s}\theta)^{N-1}\theta e^{-s}|_{s=0} \\ &= N\theta\end{aligned}$$

(e) Show that β for a particular value of X is $\beta_{\max}\theta$.

$\beta = \langle n \rangle \Delta\nu G_T = N\theta\Delta\nu G_T = \beta_{\max}\theta$

(f) What is the cooperativity for independent binding and additive transcription? How does the cooperativity depend on the transcription factor concentration?

$$\begin{aligned}\frac{d}{d\ln X}\ln\frac{\beta}{\beta_{\max}-\beta} &= X\frac{d}{dX}\ln\frac{\theta}{1-\theta} \\ &= X\frac{d}{dX}\ln X + X\frac{d}{dX}\ln k'_f - X\frac{d}{dX}\ln k'_b \\ &= 1\end{aligned}$$

So cooperativity does not depend on TF concentration.

3. Sequential binding. Here we model a system that is intermediate between independent binding and cooperative binding. In this model, the transcription factors bind in spatial order with transitions possible only between neighboring states:

$$G_n + X \rightleftharpoons G_{n+1}.$$

The forward rate constant is k''_f and the backward rate constant is k''_b.

(a) Define $\lambda = G_1/G_0$. Provide λ in terms of model parameters k''_f, k''_b, and X.

$$\begin{aligned}G_1 k''_b &= G_0 k''_f X \\ \frac{G_1}{G_0} &= \frac{k''_f X}{k''_b} = \lambda\end{aligned}$$

(b) Provide the general term G_n/G_0 in terms of λ.

$$G_{n+1}k_b'' = G_n k_f'' X$$
$$\frac{G_{n+1}}{G_n} = \frac{k_f'' X}{k_b''} = \lambda$$

$$\frac{G_n}{G_0} = \frac{G_n}{G_{n-1}} \times \frac{G_{n-1}}{G_{n-2}} \times \dots \frac{G_1}{G_0} = \lambda^n$$

(c) Use the normalization $\sum_{n=0}^{N} G_n = G_T$ to provide G_n/G_T in terms of λ without G_0.

$$\sum_{n=0}^{N} G_n = G_0 \times (1 + \lambda^1 + \dots + \lambda^N) = G_0 \frac{1-\lambda^{N+1}}{1-\lambda} = G_T$$
$$\frac{G_n}{G_T} = \frac{(1-\lambda)\lambda^n}{1-\lambda^{N+1}}$$

(d) At what value of λ are all the states equally occupied, $G_0 = G_1 = \ldots = G_N = G_T/(N+1)$? $\lambda = 1$

(e) Suppose that the transcriptional activation is additive, $\nu_n = n\Delta\nu$. Show that $\beta = G_T\langle n \rangle \Delta\nu$, where

$$\langle n \rangle = \frac{\sum_{n=0}^{N} n\lambda^n}{(\lambda^{N+1}-1)/(\lambda-1)}.$$

$$\beta = \sum_{n=0}^{N} \nu_n G_n$$
$$= \sum_{n=0}^{N} n\Delta\nu \frac{G_n}{G_T} G_T$$
$$= G_t \Delta\nu \sum_{n=0}^{N} n \frac{(1-\lambda)\lambda^n}{1-\lambda^{N+1}}$$