Engineering Genetic Circuits

Chris J. Myers

Lecture 5: Reaction-Based Abstraction
The grand aim of all science is to cover the greatest number of empirical facts by logical deduction from the smallest number of hypotheses or axioms.
There is no abstract art. You must always start with something. Afterward you can remove all traces of reality.
Several techniques exist to accelerate stochastic simulation, but they are still limited in the size of the models that they can analyze.

To reduce the cost of simulation, this lecture describes *reaction-based abstractions* to simplify the original model.

These abstractions remove irrelevant or rapid reactions.

Each abstraction examines the structure of the reaction-based model and, whenever possible, it applies transformations to simplify the model.

Simulation time is improved by reducing the model size but also eliminating many fast reactions that slow down simulation.

The reduced model is also easier to visualize.
Overview

- Irrelevant node elimination
- Enzymatic approximations
- Operator site reduction
- Statistical thermodynamical model
- Dimerization reduction
- Application to the phage λ model
- Stoichiometry amplification
Some species may not have influence on species of interest, $S_i$.

Even when all species coupled, after applying abstractions, a species may no longer influence the species in $S_i$.

Useful to remove such irrelevant species and reactions.

*Irrelevant node elimination* performs reachability analysis of the model to detect nodes that do not influence species in $S_i$. 
Irrelevant Node Elimination Example
Irrelevant Node Elimination Example

\[
\begin{array}{c}
P_{RE} \\
\downarrow r \\
r_3 \\
\downarrow p \\
S_1 \\
\downarrow m \\
r_5 \\
\downarrow np,p \\
CI \\
\downarrow r \\
r_1 \\
P_R \\
\downarrow r \\
r_4 \\
\downarrow p \\
S_2 \\
\downarrow m \\
r_6 \\
\downarrow np,p \\
CII \\
\downarrow r \\
r_2 \\
\end{array}
\]
A common motif in biochemical systems are enzymatic reactions such as:

\[ E + S \xrightarrow{k_1} C \xleftarrow{k_{-1}} E + P \]

where enzyme, \( E \), used to change substrate, \( S \), into product, \( P \).

Using the law of mass action:

\[
\begin{align*}
\frac{d[C]}{dt} &= k_1 [E][S] - k_{-1} [C] - k_2 [C] \\
\frac{d[P]}{dt} &= k_2 [C]
\end{align*}
\]
Enzymatic Reactions

\[ E + S \stackrel{k_1}{\rightleftharpoons} C \stackrel{k_2}{\rightarrow} E + P. \]

- Transformation of substrate into product, catalyzed by enzyme.
- 4 species and 3 reactions.
- Unproductive when \( k_{-1} \gg k_2 \).
Production-Passage-Time Approximation

\[ E + S \xrightarrow{k_1'} C \xrightarrow{k_2} E + P. \]

- Removes unproductive reaction.
- Approximates passage time of C formation leading to P production.
- 4 species and 2 reactions.
When $[E_t] \ll [S] + K_M$ where $K_M = (k_{-1} + k_2)/k_1$, the network can be reduced using a *steady-state assumption*.

Assumes $[C]$ reaches final concentration quickly (i.e., $d[C]/dt \approx 0$).  

Using this assumption, can derive the following:

$$\frac{d[C]}{dt} = k_1 [E][S] - k_{-1} [C] - k_2 [C]$$
When \([E_t] \ll [S] + K_M\) where \(K_M = (k_{-1} + k_2)/k_1\), the network can be reduced using a **steady-state assumption**.

Assumes \([C]\) reaches final concentration quickly (i.e., \(d[C]/dt \approx 0\)).

Using this assumption, can derive the following:

\[
0 = k_1[E][S] - k_{-1}[C] - k_2[C]
\]
Michaelis-Menten Equation

- When $[E_t] \ll [S] + K_M$ where $K_M = (k_{-1} + k_2)/k_1$, the network can be reduced using a *steady-state assumption*.

- Assumes $[C]$ reaches final concentration quickly (i.e., $d[C]/dt \approx 0$).

- Using this assumption, can derive the following:

$$[C] = \frac{k_1 [E][S]}{k_{-1} + k_2}$$
Using total concentration of enzyme, $[E_t]$:

$$[E] = [E_t] - [C]$$

Substituting this equation into the previous one results in the following:

$$[C] = \frac{k_1 [E] [S]}{k_{-1} + k_2}$$
Michaelis-Menten Equation

Using total concentration of enzyme, $[E_t]$:

$$[E] = [E_t] - [C]$$

Substituting this equation into the previous one results in the following:

$$[C] = \frac{[E_t][S]}{[S] + \frac{k_1}{k_1 + k_2}}$$
Using total concentration of enzyme, \([E_t]\):

\[
[E] = [E_t] - [C]
\]

Substituting this equation into the previous one results in the following:

\[
[C] = \frac{[E_t][S]}{[S] + \frac{k_{-1}+k_2}{k_1}}
\]

After substitution:

\[
\frac{d[P]}{dt} = k_2[C]
\]
Michaelis-Menten Equation

- Using total concentration of enzyme, $[E_t]$:

\[
[E] = [E_t] - [C]
\]

- Substituting this equation into the previous one results in the following:

\[
[C] = \frac{[E_t][S]}{[S] + \frac{k_{-1}+k_2}{k_1}}
\]

- After substitution:

\[
\frac{d[P]}{dt} = \frac{k_2[E_t][S]}{[S] + \frac{k_{-1}+k_2}{k_1}}
\]
Michaelis-Menten Equation

- Using total concentration of enzyme, $[E_t]$:
  
  $$[E] = [E_t] - [C]$$

- Substituting this equation into the previous one results in the following:

  $$[C] = \frac{[E_t][S]}{[S] + \frac{k_{-1} + k_2}{k_1}}$$

- After substitution:

  $$\frac{d[P]}{dt} = V_{max} \frac{[S]}{[S] + K_M}$$

  where $V_{max} = k_2[E_t]$ and $K_M = (k_{-1} + k_2)/k_1$. 
Michaelis-Menten Equation

- Using total concentration of enzyme, $[E_t]$:
  
  $$[E] = [E_t] - [C]$$

- Substituting this equation into the previous one results in the following:
  
  $$[C] = \frac{[E_t][S]}{[S] + \frac{k_{-1} + k_2}{k_1}}$$

- After substitution:
  
  $$\frac{d[P]}{dt} = V_{max} \frac{[S]}{[S] + K_M}$$

  where $V_{max} = k_2[E_t]$ and $K_M = (k_{-1} + k_2)/k_1$.

- This is the Michaelis-Menten equation.
Using total concentration of enzyme, \([E_t]\):

\[
[E] = [E_t] - [C]
\]

Substituting this equation into the previous one results in the following:

\[
[C] = \frac{[E_t][S]}{[S] + \frac{k_{-1}+k_2}{k_1}}
\]

After substitution:

\[
\frac{d[P]}{dt} = V_{max} \frac{[S]}{[S] + K_M}
\]

where \(V_{max} = k_2[E_t]\) and \(K_M = (k_{-1} + k_2)/k_1\).

This is the Michaelis-Menten equation.

This is also known as the quasi-steady state assumption.
Original Enzymatic Reaction Model

\[
S \xrightarrow{r} E \xrightarrow{r} E \\
\quad k_1[S][E] - k_{-1}[C] \\
C \xrightarrow{p} P \\
\quad k_2[C] \\
P \xrightarrow{p} P \\
\]

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After Quasi-Steady-State Approximation

\[
S \xrightarrow{r} p \quad \frac{k_2 [E_t] [S]}{[S] + \frac{k_{-1} + k_2}{k_1}}
\]
After Rapid Equilibrium Approximation

Assuming $k_{-1} >> k_2$. 

\[
\frac{k_2 [E_t] [S]}{[S] + \frac{k_{-1}}{k_1}}
\]
Species $E \not\in S_i$, and it must be a reactant in at least one reaction $r_1$.

Reaction $r_1$ must be reversible, have two reactants, a kinetic law of the form: $k_f[E][S] - k_r[C]$, and species $C$ must not be in $S_i$.

The initial concentration of the complex species $C$ must be zero.

Species $C$ must be a product of only one reaction, $r_1$, reactant in only one reaction, $r_2$, and modifier in no reactions.

Reaction $r_2$ must not be reversible and have only one reactant and no modifiers.

Reaction $r_2$ must have only one or two products including species $E$, and it must have a kinetic law of the form: $k_2[C]$. 

$$E + S \xrightleftharpoons[k_f]{k_r} C \xrightarrow{k_2} E + P.$$
Enzymatic Approximation Transformation

- Model is updated using one of the enzymatic approximations.
- Note that enzyme E may be a reactant in multiple reactions known as competitive enzymatic reactions.
- For each reaction, a configuration is formed that includes the substrate S, complex C, equilibrium constant $K_1 = k_f / k_r$, production rate $k_2$, complex forming reaction $r_1$, and product forming reaction $r_2$.
- Procedure loops through the set of configurations to form an expression that is used in the denominator in each new rate law as well as forming a list of all the substrates that bind to the enzyme E.
- For each configuration $(S, C, K_1, k_2, r_1, r_2)$, it makes the substrate S a reactant for $r_2$, makes all other substrates modifiers for $r_2$, creates a new rate law for $r_2$, and removes species C, enzyme E, and reaction $r_1$.
- The application of enzymatic approximations not only reduces the size of the model, but also improves simulation time by removing fast reactions.
Competitive Enzymatic Reaction Example: Original

\[
k_8 \cdot [\text{CII}] \cdot [\text{P1}] - k_9 \cdot [\text{P1} \cdot \text{CII}] \\
\]

\[
k_{11} \cdot [\text{CIII}] \cdot [\text{P1}] - k_{12} \cdot [\text{P1} \cdot \text{CIII}] \\
\]

\[
k_{10} \cdot [\text{P1} \cdot \text{CII}] \\
\]

\[
k_{13} \cdot [\text{P1} \cdot \text{CIII}] \\
\]
Competitive Enzymatic Reaction Example: Abstracted

\[
\frac{k_{10} \cdot P_1 \cdot \text{tot} \cdot k_8}{1 + k_8 / k_9 \cdot [\text{CII}] + k_{11} / k_{12} \cdot [\text{CIII}]}\]

\[
\frac{k_{13} \cdot P_1 \cdot \text{tot} \cdot k_{11}}{1 + k_8 / k_9 \cdot [\text{CII}] + k_{11} / k_{12} \cdot [\text{CIII}]}\]

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Models of genetic circuits include many operator sites to which transcription factors bind.

Rates of transcription factor binding and unbinding often rapid compared to rate of open complex formation.

Typically number of operator sites is much smaller than number of RNAP molecules and transcription factors.

Operator site reduction merges reactions and removes operator sites and their complexes from reaction models.
Rate of production of a protein may be inhibited by a repressor molecule. As amount of a repressor increases, rate of protein production decreases. A repressor typically binds to an operator site to prevent RNAP from binding to a promoter to start transcription. However, other mechanisms exist with similar dynamical behavior. It may take multiple repressor molecules to inhibit production. In phage λ, it takes 4 molecules of CI (two dimers) to repress Cro.
Model for Repression

- $n$ molecules of repressor $R$ bind to the operator $O$.

\[
\begin{align*}
\text{d}[R]\text{dt} & = n(k_{-1}[R_n] - k_1[R]^n) \\
\text{d}[O]\text{dt} & = k_{-2}[R_{nO}] - k_2[R_n][O]
\end{align*}
\]
Assuming reactions are rapid (i.e. \( \frac{d[R]}{dt} = \frac{d[O]}{dt} \approx 0 \)):

\[
[R_n] = K_1[R]^n \quad (1)
\]
\[
[R_nO] = K_2[R_n][O] \quad (2)
\]

where \( K_1 = \frac{k_1}{k_{-1}} \) and \( K_2 = \frac{k_2}{k_{-2}} \).

- Assume concentrations of \( R_1, \ldots, R_{n-1} \) are negligible.
- Assume \( [O] \ll [R_t] \) (total concentration of repressor).

Using assumptions and Equations 1 and 2:

\[
[O_t] = [O] + [R_nO] = [O](1 + K_1 K_2 [R]^n)
\]

\[
f([R]) = \frac{[O]}{[O_t]} = \frac{1}{1 + K_1 K_2 [R]^n}
\]

This is a sigmoid function known as a Hill function.

\( f([R]) = 1/2 \) when \( [R] = \frac{1}{\sqrt[n]{K_1 K_2}} \).
The Fraction of Operator Sites Free of Repressor \( f([R]) \)
Model for Activation

- $n$ molecules of activator $A$ bind to the operator $O$.

$$
\begin{align*}
nA & \overset{k_1}{\underset{k_{-1}}{\rightleftharpoons}} A_n \\
A_n + O & \overset{k_2}{\underset{k_{-2}}{\rightleftharpoons}} A_n O
\end{align*}
$$

- Using the Law of Mass Action:

$$
\begin{align*}
\frac{d[A]}{dt} &= k_{-1}[A_n] - k_1[A]^n \\
\frac{d[O]}{dt} &= k_{-2}[A_n O] - k_2[A_n][O]
\end{align*}
$$
Assuming reactions are rapid (i.e. \( \frac{d[A]}{dt} = \frac{d[O]}{dt} = 0 \)):

\[
\begin{align*}
[A_n] &= K_1[A]^n \\
[A_nO] &= K_2[A_n][O]
\end{align*}
\]

where \( K_1 = k_1/k_{-1} \) and \( K_2 = k_2/k_{-2} \).

Assume concentrations of \( A_1, \ldots, A_{n-1} \) are negligible.

Assume \( [O] << [A_t] \) (total concentration of activator).

Using assumptions and Equations 3 and 4:

\[
\begin{align*}
[A_nO] &= K_1 K_2[A]^n ([O_t] - [A_nO]) \\
f([A]) &= \frac{[A_nO]}{[O_t]} = \frac{K_1 K_2[A]^n}{1 + K_1 K_2[A]^n}
\end{align*}
\]
The Fraction of Operator Sites Bound to Activator ($f([A])$)
Consider an operator site, \(O\), which can be repressed by \(R\), preventing production of protein \(P\).

Assume that production is at a low basal rate, \(k_b\), until enhanced by \(A\), to a higher activated rate, \(k_a\).

\[
\begin{align*}
O + R & \overset{k_1}{\rightleftharpoons} O \cdot R \\
& \overset{k_{-1}}{\longleftarrow} \\
O + \text{RNAP} & \overset{k_2}{\rightleftharpoons} O \cdot \text{RNAP} \\
& \overset{k_{-2}}{\longleftarrow} \\
O + \text{RNAP} + \text{A} & \overset{k_3}{\rightleftharpoons} O \cdot \text{RNAP} \cdot \text{A} \\
& \overset{k_{-3}}{\longleftarrow} \\
O + \text{RNAP} & \overset{k_a}{\rightarrow} O \cdot \text{RNAP} \cdot \text{A}
\end{align*}
\]
Using the law of mass action, some of the differential equations are:

\[
\begin{align*}
\frac{d[O \cdot R]}{dt} &= k_1 [O][R] - k_{-1} [O \cdot R] \\
\frac{d[O \cdot RNAP]}{dt} &= k_2 [O][RNAP] - k_{-2} [O \cdot RNAP] \\
\frac{d[O \cdot RNAP \cdot A]}{dt} &= k_3 [O][RNAP][A] - k_{-3} [O \cdot RNAP \cdot A] \\
\frac{d[P]}{dt} &= np k_b [O \cdot RNAP] + np k_a [O \cdot RNAP \cdot A]
\end{align*}
\]
Using the law of mass action, some of the differential equations are:

\[
\begin{align*}
0 &= k_1 [O][R] - k_{-1} [O \cdot R] \\
0 &= k_2 [O][RNAP] - k_{-2} [O \cdot RNAP] \\
0 &= k_3 [O][RNAP][A] - k_{-3} [O \cdot RNAP \cdot A] \\
\frac{d[P]}{dt} &= np k_b [O \cdot RNAP] + np k_a [O \cdot RNAP \cdot A]
\end{align*}
\]

Assume binding to operator sites is rapid.
Using the law of mass action, some of the differential equations are:

\[
\begin{align*}
[O \cdot R] &= K_1 [O][R] \\
[O \cdot \text{RNAP}] &= K_2 [O][\text{RNAP}] \\
[O \cdot \text{RNAP} \cdot A] &= K_3 [O][\text{RNAP}][A] \\
\frac{d[P]}{dt} &= np \, k_b [O \cdot \text{RNAP}] + np \, k_a [O \cdot \text{RNAP} \cdot A]
\end{align*}
\]

Rewrite using \( K_1 = k_1/k_{-1} \), \( K_2 = k_2/k_{-2} \), and \( K_3 = k_3/k_{-3} \).
Using the law of mass action, some of the differential equations are:

\[
\begin{align*}
[O \cdot R] &= K_1 [O][R] \\
[O \cdot \text{RNAP}] &= K_2 [O][\text{RNAP}] \\
[O \cdot \text{RNAP} \cdot A] &= K_3 [O][\text{RNAP}][A] \\
\frac{d[P]}{dt} &= np \, k_b [O \cdot \text{RNAP}] + np \, k_a [O \cdot \text{RNAP} \cdot A] \\
[O_t] &= [O] + [O \cdot R] + [O \cdot \text{RNAP}] + [O \cdot \text{RNAP} \cdot A]
\end{align*}
\]
Using the law of mass action, some of the differential equations are:

\[
\begin{align*}
[O \cdot R] &= K_1 [O][R] \\
[O \cdot \text{RNAP}] &= K_2 [O][\text{RNAP}] \\
[O \cdot \text{RNAP} \cdot A] &= K_3 [O][\text{RNAP}][A] \\
\frac{d[P]}{dt} &= np k_b [O \cdot \text{RNAP}] + np k_a [O \cdot \text{RNAP} \cdot A] \\
[O_t] &= [O] (1 + K_1 [R] + K_2 [\text{RNAP}] + K_3 [\text{RNAP}][A])
\end{align*}
\]
Putting It All Together

Using the law of mass action, some of the differential equations are:

\[
\begin{align*}
[O \cdot R] &= K_1 [O][R] \\
[O \cdot \text{RNAP}] &= K_2 [O][\text{RNAP}] \\
[O \cdot \text{RNAP} \cdot A] &= K_3 [O][\text{RNAP}][A] \\
\frac{d[P]}{dt} &= np k_b [O \cdot \text{RNAP}] + np k_a [O \cdot \text{RNAP} \cdot A] \\
[O] &= \frac{[O_t]}{1 + K_1[R] + K_2[\text{RNAP}] + K_3[\text{RNAP}][A]}
\end{align*}
\]
Using the law of mass action, some of the differential equations are:

\[
\begin{align*}
[O \cdot R] &= K_1 [O][R] \\
[O \cdot \text{RNAP}] &= K_2 [O][\text{RNAP}] \\
[O \cdot \text{RNAP} \cdot A] &= K_3 [O][\text{RNAP}][A] \\
\frac{d[P]}{dt} &= \frac{np (k_b K_2[\text{RNAP}] + k_a K_3[\text{RNAP}][A]) [O_t]}{1 + K_1[R] + K_2[\text{RNAP}] + K_3[\text{RNAP}][A]} \\
[O] &= \frac{[O_t]}{1 + K_1[R] + K_2[\text{RNAP}] + K_3[\text{RNAP}][A]}
\end{align*}
\]
First step is to identify operators $O$ assuming that it is a species small in number that is neither produced nor degraded.

Check that $O$’s initial concentration is not greater than a threshold.

$O$ must not be in $S_i$ and a reactant in at least one reaction $r_1$.

Reaction $r_1$ must be reversible, have two or more reactants, exactly one product, and a kinetic law of the form: $k_f \cdot f([s_1], \ldots, [s_n]) - k_r [C]$.

The operator complex $C$ must not be in $S_i$, and it must be the product of one reaction and reactant of no reactions.

In each $r_2$ that $C$ appears as a modifier, there must be no reactants, no other modifiers, one product, and a kinetic law of the form: $k_o [C]$.

For each $r_1$, create a configuration $K_i X_i$ where $K_i = k_{fi}/k_{ri}$, and $X_i$ is the product of concentrations for the reactants of $r_1$ excluding $O$. 
Create sum: $Z = 1 + \sum_{j=1}^{N} K_j X_j$.

For each configuration $i$, create a new reaction which has as modifiers the reactants of the corresponding complex formation reaction $r_1$.

Kinetic law for this reaction is:

$$\frac{k_2 O_0 K_i X_i}{Z}$$

Assuming $O_0$ is the total amount of operator, then $K_i X_i / Z$ is the proportion of $O_0$ in the $i$-th configuration.
Operator Site Reduction: Original

\[
\begin{align*}
\text{RNAP} & \quad \text{PRE} \quad \text{RNAP} - [S_1] \\
K_{o1}[\text{PRE}][\text{RNAP}] & \quad r \quad K_{a}[\text{PRE}][\text{CII}]^{na}[\text{RNAP}] - [S_4] \\
S_1 & \quad p \quad S_4 \\
k_b[S_1] & \quad m \quad k_a[S_4] \\
\end{align*}
\]
Operator Site Reduction: Abstracted

\[
k_b K_{o1} [\text{RNAP}] \frac{PRE_0}{1 + K_{o1} [\text{RNAP}] + K_a [\text{CII}]^{na} [\text{RNAP}]}
\]

\[
k_a K_a [\text{CII}]^{na} [\text{RNAP}] \frac{PRE_0}{1 + K_{o1} [\text{RNAP}] + K_a [\text{CII}]^{na} [\text{RNAP}]}
\]
After Similar Reaction Combiner

\[
\frac{(k_b K_{o1} + k_a K_a [CII]^{na})[RNAP] PRE_0}{1 + K_{o1}[RNAP] + K_a[CII]^{na}[RNAP]}
\]
After Modifier Constant Propagation

\[
\frac{(k_b K_{o1} + k_a K_a [CII]^{na}) \text{RNAP}_0 \text{PRE}_0}{1 + K_{o1} \text{RNAP}_0 + K_a [CII]^{na} \text{RNAP}_0}
\]

CII

\[m\]

\[np, p\]

CI

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• Alternative approach to operator site reduction.
• Assumes occupancy of operator sites can be determined by equilibrium statistical thermodynamic probabilities.
• Probability of each potential configuration of transcription factors and RNAP bound to operator sites can be determined.
• Does not include reactions for operator site binding, but determines configuration during each simulation cycle.
Interactions of Repressor Molecules

2

\[ P_{RM} \quad \leftrightarrow \quad P_R \]

\[ cl \quad O_{R3} \quad O_{R2} \quad O_{R1} \quad cro \]

\[ P_{RM} \quad \leftrightarrow \quad P_R \]

\[ cl \quad O_{R3} \quad O_{R2} \quad O_{R1} \quad cro \]

\[ P_{RM} \quad \leftrightarrow \quad P_R \]

\[ cl \quad O_{R3} \quad O_{R2} \quad O_{R1} \quad cro \]
Assumptions

- Occupancy of the operator sites are determined by equilibrium statistical thermodynamic probabilities.
- Repressors bound to adjacent operator sites interact.
- Cooperative interaction b/w $O_R^2$ and $O_R^3$ only when $O_R^1$ is vacant.
- $P_R$, cro gene, is off when $O_R^1$ or $O_R^2$ are occupied.
- $P_{RM}$, cl gene, is off when $O_R^3$ is occupied.
- In mutants with one operator damaged, others work the same.
## Configurations

<table>
<thead>
<tr>
<th>s</th>
<th>$O_{R3}$</th>
<th>$O_{R2}$</th>
<th>$O_{R1}$</th>
<th>Free energy contributions</th>
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<tbody>
<tr>
<td>1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Reference</td>
</tr>
<tr>
<td>2</td>
<td>—</td>
<td>—</td>
<td>$\text{Cl}_2$</td>
<td>$\Delta G_1$</td>
</tr>
<tr>
<td>3</td>
<td>—</td>
<td>$\text{Cl}_2^*$</td>
<td>—</td>
<td>$\Delta G_2$</td>
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<tr>
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<td>—</td>
<td>$\Delta G_3$</td>
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<tr>
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<td>—</td>
<td>$\text{Cl}_2$</td>
<td>$\text{Cl}_2$</td>
<td>$\Delta G_1 + \Delta G_2 + \Delta G_{12}$</td>
</tr>
<tr>
<td>6</td>
<td>$\text{Cl}_2$</td>
<td>—</td>
<td>$\text{Cl}_2$</td>
<td>$\Delta G_1 + \Delta G_3$</td>
</tr>
<tr>
<td>7</td>
<td>$\text{Cl}_2^*$</td>
<td>$\text{Cl}_2$</td>
<td>—</td>
<td>$\Delta G_2 + \Delta G_3 + \Delta G_{23}$</td>
</tr>
<tr>
<td>8</td>
<td>$\text{Cl}_2$</td>
<td>$\text{Cl}_2^*$</td>
<td>$\text{Cl}_2$</td>
<td>$\Delta G_1 + \Delta G_2 + \Delta G_3 + \Delta G_{12}$</td>
</tr>
</tbody>
</table>

*Indicates that adjacent $\text{Cl}_2$ molecules bind cooperatively.

\[
\Delta G_i = -RT \ln K_i.
\]
Mathematical Relationships of the Model

\[ f_s = \frac{\exp(-\Delta G_s/RT) [\text{Cl}_2]^{i(s)}}{\sum_s \exp(-\Delta G_s/RT) [\text{Cl}_2]^{i(s)}} \]

\[ f_{OR1} = f_1 + f_4 + f_5 + f_7 \]

\[ f_{OR2} = f_2 + f_4 + f_6 + f_7 \]

\[ f_{OR3} = f_3 + f_5 + f_6 + f_7 = f_{PRM} \]

\[ f_{PR} = f_1 + f_2 + f_4 + f_5 + f_6 + f_7 \]
Values for $[\text{Cl}_2]$ for Half Occupation (units of 3nM)

<table>
<thead>
<tr>
<th>DNA Template</th>
<th>$O_R^+$ (wild type)</th>
<th>$O_R^- 1$</th>
<th>$O_R^- 2$</th>
<th>$O_R^- 3$</th>
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<tr>
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<td>-</td>
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<tr>
<td>$O_R^- 1$, $O_R^- 2$</td>
<td>25</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>$O_R^- 1$, $O_R^- 3$</td>
<td>-</td>
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<td>-</td>
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</tr>
<tr>
<td>$O_R^- 3$</td>
<td>-</td>
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<td>1</td>
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</table>

(Data courtesy of Johnson et al., 1979)
Resolved Interaction Free Energies for $O_R$

<table>
<thead>
<tr>
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<th>Energy, kcal</th>
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<tbody>
<tr>
<td>Individual site binding</td>
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<tr>
<td>$\Delta G_1$</td>
<td>$-11.69 \pm 0.03$</td>
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<tr>
<td>$\Delta G_2$</td>
<td>$-10.10 \pm 0.05$</td>
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<tr>
<td>$\Delta G_3$</td>
<td>$-10.09 \pm 0.02$</td>
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<tr>
<td>Cooperative interaction</td>
<td></td>
</tr>
<tr>
<td>$\Delta G_{12}$</td>
<td>$-1.99 \pm 0.06$</td>
</tr>
<tr>
<td>$\Delta G_{23}$</td>
<td>$-1.94 \pm 0.06$</td>
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</table>

(Results courtesy of Ackers et al., 1982)
### Free Energies for Configurations

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<thead>
<tr>
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<th>$O_{R2}$</th>
<th>$O_{R1}$</th>
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<tr>
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<td>—</td>
<td>Cl₂</td>
<td>Cl₂</td>
<td>$\Delta G_1 + \Delta G_2 + \Delta G_{12}$</td>
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<tr>
<td>6</td>
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<td>Cl₂</td>
<td>$\Delta G_1 + \Delta G_3$</td>
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<tr>
<td>7</td>
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<td>$\Delta G_2 + \Delta G_3 + \Delta G_{23}$</td>
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<tr>
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<td>Cl₂</td>
<td>$\Delta G_1 + \Delta G_2 + \Delta G_3 + \Delta G_{12}$</td>
<td>-33.9</td>
</tr>
</tbody>
</table>

*Indicates that adjacent Cl₂ molecules bind cooperatively.

(Result courtesy of Ackers et al., 1982)
Predicted Behavior of the System

![Graph showing the predicted behavior of the system with respect to Log[Cl2]. The graph plots Repression against Log[Cl2] and includes three lines representing different conditions: PR (no cooperativity), PR, and PRM.](image-url)

Log[Cl2] range: -10 to -5
Repression range: 0 to 1

- **PR** (no cooperativity)
- **PR**
- **PRM**

Chris J. Myers (Lecture 5: Abstraction)  Engineering Genetic Circuits
Discussion

- 25-fold more repressor is needed to half repress $P_{RM}$ than $P_R$.
- Cooperativity makes $P_R$ behavior more switch-like than $P_{RM}$.
- Cooperativity maintains stable lysogen, yet allows induction.
## Free Energies for the $O_R$ Operator Configurations

<table>
<thead>
<tr>
<th>s</th>
<th>$O_R3$</th>
<th>$O_R2$</th>
<th>$O_R1$</th>
<th>$\Delta G_s$ (kcal mol$^{-1}$)</th>
<th>$k_{PR}(s)$ (sec$^{-1}$)</th>
<th>$k_{PRM}(s)$ (sec$^{-1}$)</th>
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</thead>
<tbody>
<tr>
<td><strong>Non-liganded species</strong></td>
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</tr>
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<tr>
<td><strong>Singly liganded species</strong></td>
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<tr>
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<td>0.0</td>
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<tr>
<td>4</td>
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<td>—</td>
<td>-10.1</td>
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<td>0.0</td>
</tr>
<tr>
<td>5</td>
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<td>—</td>
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<td>0.0</td>
</tr>
<tr>
<td>6</td>
<td>—</td>
<td>Cro$_2$</td>
<td>—</td>
<td>-10.8</td>
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<td>0.0</td>
</tr>
<tr>
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<td>0.0</td>
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</table>

(Courtesy of Shea and Ackers (1985))
### Free Energies for the $O_R$ Operator Configurations

<table>
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<tr>
<th>$s$</th>
<th>$O_R3$</th>
<th>$O_R2$</th>
<th>$O_R1$</th>
<th>$\Delta G_s$ (kcal mol$^{-1}$)</th>
<th>$k_{PR}(s)$ (sec$^{-1}$)</th>
<th>$k_{PRM}(s)$ (sec$^{-1}$)</th>
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<tbody>
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<tr>
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<tr>
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</tbody>
</table>

*Doubly liganded species*

*Indicates that adjacent Cl$_2$ molecules bind cooperatively.*
Free Energies for the $O_R$ Operator Configurations

<table>
<thead>
<tr>
<th>State</th>
<th>$O_{R3}$</th>
<th>$O_{R2}$</th>
<th>$O_{R1}$</th>
<th>$\Delta G_s$ (kcal mol$^{-1}$)</th>
<th>$k_{PR}(s)$ (sec$^{-1}$)</th>
<th>$k_{PRM}(s)$ (sec$^{-1}$)</th>
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<tbody>
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<td>20</td>
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<td>—</td>
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</table>
## Free Energies for the $O_R$ Operator Configurations

<table>
<thead>
<tr>
<th>s</th>
<th>$O_R3$</th>
<th>$O_R2$</th>
<th>$O_R1$</th>
<th>$\Delta G_s$ (kcal mol$^{-1}$)</th>
<th>$k_{PR}(s)$ (sec$^{-1}$)</th>
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</tbody>
</table>

*Indicates that adjacent Cl$_2$ molecules bind cooperatively.

### Triply liganded species

- **29 CI$_2$ Cl$_2$ Cl$_2$**
  - $\Delta G_s$: -33.9 kcal mol$^{-1}$
  - $k_{PR}(s)$: 0.0 sec$^{-1}$
  - $k_{PRM}(s)$: 0.0 sec$^{-1}$
- **30 Cro$_2$ Cro$_2$ Cro$_2$**
  - $\Delta G_s$: -33.7 kcal mol$^{-1}$
  - $k_{PR}(s)$: 0.0 sec$^{-1}$
  - $k_{PRM}(s)$: 0.0 sec$^{-1}$
- **31 Cro$_2$ Cl$_2^*$ Cl$_2$**
  - $\Delta G_s$: -35.8 kcal mol$^{-1}$
  - $k_{PR}(s)$: 0.0 sec$^{-1}$
  - $k_{PRM}(s)$: 0.0 sec$^{-1}$
- **32 Cl$_2$ Cro$_2$ Cl$_2$**
  - $\Delta G_s$: -32.6 kcal mol$^{-1}$
  - $k_{PR}(s)$: 0.0 sec$^{-1}$
  - $k_{PRM}(s)$: 0.0 sec$^{-1}$
- **33 Cl$_2^*$ Cl$_2$ Cro$_2$**
  - $\Delta G_s$: -33.0 kcal mol$^{-1}$
  - $k_{PR}(s)$: 0.0 sec$^{-1}$
  - $k_{PRM}(s)$: 0.0 sec$^{-1}$
- **34 Cl$_2$ Cro$_2$ Cro$_2$**
  - $\Delta G_s$: -33.7 kcal mol$^{-1}$
  - $k_{PR}(s)$: 0.0 sec$^{-1}$
  - $k_{PRM}(s)$: 0.0 sec$^{-1}$
- **35 Cro$_2$ Cl$_2$ Cro$_2$**
  - $\Delta G_s$: -33.0 kcal mol$^{-1}$
  - $k_{PR}(s)$: 0.0 sec$^{-1}$
  - $k_{PRM}(s)$: 0.0 sec$^{-1}$
- **36 Cro$_2$ Cro$_2$ Cl$_2$**
  - $\Delta G_s$: -34.6 kcal mol$^{-1}$
  - $k_{PR}(s)$: 0.0 sec$^{-1}$
  - $k_{PRM}(s)$: 0.0 sec$^{-1}$
- **37 RNAP Cl$_2^*$ Cl$_2$**
  - $\Delta G_s$: -35.2 kcal mol$^{-1}$
  - $k_{PR}(s)$: 0.0 sec$^{-1}$
  - $k_{PRM}(s)$: 0.011 sec$^{-1}$
- **38 RNAP Cro$_2$ Cro$_2$**
  - $\Delta G_s$: -33.1 kcal mol$^{-1}$
  - $k_{PR}(s)$: 0.0 sec$^{-1}$
  - $k_{PRM}(s)$: 0.001 sec$^{-1}$
- **39 RNAP Cro$_2$ Cl$_2$**
  - $\Delta G_s$: -34.0 kcal mol$^{-1}$
  - $k_{PR}(s)$: 0.0 sec$^{-1}$
  - $k_{PRM}(s)$: 0.001 sec$^{-1}$
- **40 RNAP Cl$_2$ Cro$_2$**
  - $\Delta G_s$: -32.4 kcal mol$^{-1}$
  - $k_{PR}(s)$: 0.0 sec$^{-1}$
  - $k_{PRM}(s)$: 0.011 sec$^{-1}$
Computing Average Open Complex Rates

\[
f_s = \frac{\exp\left(-\frac{\Delta G_s}{RT}\right)[\text{Cl}_2]^{i(s)}[\text{Cro}_2]^{j(s)}[\text{RNAP}]^{k(s)}}{\sum_s \exp\left(-\frac{\Delta G_s}{RT}\right)[\text{Cl}_2]^{i(s)}[\text{Cro}_2]^{j(s)}[\text{RNAP}]^{k(s)}}
\]

\[
k_{PR} = \sum_s k_{PR}(s) f_s
\]

\[
k_{PRM} = \sum_s k_{PRM}(s) f_s
\]
Open Complex Rates for $P_R$ and $P_{RM}$

![Graph of $P_R$](image1)

![Graph of $P_{RM}$](image2)

$P_R$

$P_{RM}$
Dimerization is another rapid reaction that would be useful to remove.

\[
2s_m \leftrightarrow \frac{k_+}{k_-} s_d
\]

Assuming \(s_m\) and \(s_d\) are in equilibrium:

\[
[s_d] = K_d [s_m]^2.
\]

where \(K_d\) is the equilibrium constant (\(K_d = k_+/k_-\)).

A simple dimerization reduction is replace \([s_d]\) with \(K_d [s_m]^2\).
The total concentration of monomer molecules, \([s_t]\), is:

\[
[s_t] = [s_m] + 2[s_d].
\]

Combining equations, we can derive the following:

\[
K_d[s_t]^2 - (4K_d[s_t] + 1)[s_d] + 4K_d[s_d]^2 = 0.
\]

Solving this equation, we can express \([s_m]\) and \([s_d]\) in terms of \([s_t]\):

\[
\begin{align*}
[s_d] &= \frac{[s_t]}{2} - \frac{1}{8K_d} \left( \sqrt{8K_d[s_t] + 1} - 1 \right) \\
[s_m] &= \frac{1}{4K_d} \left( \sqrt{8K_d[s_t] + 1} - 1 \right)
\end{align*}
\]
Consider $r$ as a potential dimerization reaction.

Reaction $r$ must have one reactant, one product, no modifiers, and a kinetic law of the form: $k_+ [s_m]^2 - k_- [s_d]$.

The monomer form, $s_m$, must not appear as a modifier in any reaction, and the dimer form, $s_d$, must not appear as a product in any reaction other than the dimerization reaction.
Create species $s_t$ with $[s_t]_0 = [s_m]_0 + 2[s_d]_0$.

In all reactions with $s_m$ as a reactant, replace $[s_m]$ with
\[
\frac{1}{4K_d} \left( \sqrt{8K_d[s_t]} + 1 - 1 \right)
\]
in the kinetic law.

In all reactions with $s_m$ as a product, replace with $s_t$.

In all reactions with $s_d$ as a reactant or modifier, replace $[s_d]$ with
\[
\frac{[s_t]}{2} - \frac{1}{8K_d} \left( \sqrt{8K_d[s_t]} + 1 - 1 \right)
\]
Dimerization Reduction Example: Original
Dimerization Reduction Example: Abstracted

\[ k_d \cdot [CI] \quad f_1(K_d[CI]^2) \]
Dimerization Reduction Example: Abstracted

\[ f_2 \left( \frac{1}{4K_d} \left( \sqrt{8K_d[C_l]+1-1} \right) \right) \]

\[ C_l \]

\[ f_1 \left( \frac{[C_l]}{2} - \frac{1}{8K_d} \left( \sqrt{8K_d[C_l]+1-1} \right) \right) \]
Abstracted Reaction Model for Part of Phage $\lambda$

\[ f_2 \left( \frac{1}{4K_d} \left( \sqrt{8K_d[C_{lt}] + 1} - 1 \right) \right) \]

\[ f_1 \left( \frac{[C_{lt}]}{2} - \frac{1}{8K_d} \left( \sqrt{8K_d[C_{lt}] + 1} - 1 \right) \right) \]

\[ f_4 ([CII]) \]

\[ f_3 ([CII]) \]
Comparison of Results for 10,000 SSA Runs

Original simulates in 20 minutes while abstracted in only 40 seconds.
Abstracted Reaction Model for Phage $\lambda$

Reduced from 61 species and 75 reactions to 5 species and 11 reactions. (Courtesy of Kuwahara et al. (2006))
Probability of Lysogeny Versus Multiplicity of Infection

(Courtesy of Kuwahara et al. (2006))
Probability of Lysogeny Versus Multiplicity of Infection

(Courtesy of Kuwahara et al. (2006))
Probability of Lysogeny Versus Multiplicity of Infection

Original simulates in 56.5 hours while abstracted in only 9.8 hours.
(Courtesy of Kuwahara et al. (2006))
Amplification with factor \( n \).
Can advance the system and the time faster.
Lower the cost of stochastic simulation.
Amplification with factor $n$.
- Can advance the system and the time faster.
- Lower the cost of stochastic simulation.
Stoichiometry Amplification Example

\[ f_2 \left( \frac{1}{4K_d} \left( \sqrt{8K_d [Cl_t] + 1} - 1 \right) \right) \]

\[ f_1 \left( \frac{[Cl_t]}{2} - \frac{1}{8K_d} \left( \sqrt{8K_d [Cl_t] + 1} - 1 \right) \right) \]

\[ f_3 ([CII]) \]

\[ f_4 ([CII]) \]
Stoichiometry Amplification Example

\[ \frac{1}{10} f_2 \left( \frac{1}{4K_d} \left( \sqrt{8K_d [C_{lt}] + 1} - 1 \right) \right) \]

\[ f_1 \left( \frac{[C_{lt}]}{2} - \frac{1}{8K_d} \left( \sqrt{8K_d [C_{lt}] + 1} - 1 \right) \right) \]

\[ \frac{1}{10} f_4 ([C_{ll}]) \]

\[ f_3 ([C_{ll}]) \]
Sources

- **Enzymatic approximations:**
  - Original enzymatic formulation - Henri (1903)
  - Michaelis-Menten approximation - Michaelis and Menten (1913)
  - Theoretical basis - Briggs and Haldane (1925)
  - Justified with perturbation theory - Segel and Slemrod (1989)
  - Production-passage time approximation - Kuwahara and Myers (2008)

- **Operator side reduction:**
  - Theoretical basis - Tyson and Othmer (1978)
  - Algorithmic approach - Kuwahara et al. (2006)

- **Statistical thermodynamical model:**
  - Proposed - Ackers et al. (1982) and Shea and Ackers (1985)

- **Dimerization reduction:**
  - Theoretical basis - Santillán and Mackey (2004)
  - Algorithmic approach - Kuwahara et al. (2006)

- **Abstraction of phage λ** - Kuwahara et al. (2006) and Kuwahara (2007).