Some very hard problems in nature (biology-biochemistry) “solved” using physical algorithms that reduce the hardness

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add your favorite problem

PENN HUNT PROJECT
September 18, 2008

Harvey Rubin MD, PhD
University of Pennsylvania
Cooperativity at the monomolecular level binding of B or C to the common partner A affects binding of the other

\[ a \quad \text{A} + \text{B} \xrightleftharpoons{K_m} \xrightleftharpoons{k_m} \text{A} \text{B} \quad \Delta G^{\text{r}} = -RT \ln K_1 \quad K_1 = \frac{[\text{A}][\text{B}]}{[\text{AB}]} = \frac{k_{ri}}{k_m} \]

\[ \Delta G^{\text{r}} = -RT \ln K_2 \quad K_2 = \frac{[\text{A}][\text{C}]}{[\text{AC}]} \]

\[ b \quad \text{A} + \text{B} \xrightleftharpoons{K_1} \text{AB} \quad \text{C} \xrightleftharpoons{K_2} \text{AC} \quad \text{B} \xrightleftharpoons{K_3} \text{AB} \quad \text{C} \xrightleftharpoons{K_4} \text{AC} \quad K_1 K_2 = K_3 K_4 \]

\[ \Delta G^{\text{r}}_1 + \Delta G^{\text{r}}_3 = \Delta G^{\text{r}}_2 + \Delta G^{\text{r}}_4 \]

\[ \Delta \Delta G = \Delta G^{\text{r}}_1 - \Delta G^{\text{r}}_3 = \Delta G^{\text{r}}_2 - \Delta G^{\text{r}}_4 \]

\[ c \quad \text{A} + \text{B} \xrightleftharpoons{\Delta G^{\text{r}}_4} \text{AB} \quad \text{C} \xrightleftharpoons{\Delta G^{\text{r}}_2} \text{AC} \quad \text{B} \xrightleftharpoons{\Delta G^{\text{r}}_1} \text{AB} \quad \text{C} \xrightleftharpoons{\Delta G^{\text{r}}_3} \text{AC} \]

**Figure 1** Thermodynamic cycles and cooperativity. (a) Hypothetical set of bimolecular complexes between component A and two other components (B and C), with the rate constants, equilibrium constants and free energies for complex formation. (b) A thermodynamic cycle for formation of the ternary complex ABC by two different possible routes: either B binds first, or C binds first. There are four equilibrium constants that describe the formation of the various complexes. Because they converge on the common product ABC, the thermodynamics must be independent of the pathway chosen around the cycle, and constraints are placed on the relative values of the equilibrium constants and hence the free energies. The thermodynamic coupling free energy (\(\Delta \Delta G\)) gives the difference between binding of one component in the presence of the other. (c) Definition of cooperativity in terms of binding of B in the presence or absence of C. The two vertical binding reactions are gray to emphasize the comparison of \(\Delta G^{\text{r}}_1\) and \(\Delta G^{\text{r}}_4\). If B binds better in the presence of C, the binding is cooperative. If B binds worse in the presence of C, the binding is anticooperative. In the third case, binding of B is independent of C, and there is no cooperativity.

Cooperativity in macromolecular assembly
James R Williamson
volume 4 number 8 August 2008 nature chemical biology
Cooperativity/heterogeneity

1. complex interactions among identical ligands binding to multiple sites on an oligomeric protein--oxygen binding to hemoglobin.
   Homotropic *allosteric* regulators—e.g. O2
   Heterotropic *allosteric* regulators—e.g. 2,3 BPG

2. the thermodynamics of macromolecular conformational transitions--protein folding or nucleic acid helix-coil transitions.

3. the thermodynamics of forming multicomponent complexes—multimeric complexes, surface interactions, cellular communication, organism organization, multicellular dynamics, social structures

Cooperativity and biological complexity
Adrian Whitty Nature Chemical Biology Volume 4 Number 8 August 2008
Interaction of Hemoglobin with Three Ligands: Organic Phosphates and the Bohr Effect

Ruth E. Hensch and Harvey Rubin

Department of Biochemistry, Columbia University, New York, N.Y.

The Bohr effect (a decrease in oxygen affinity with increasing bicarbonate concentration) is a well-known phenomenon in hemoglobin. The Bohr effect is thought to be mediated by the interaction of the iron with a bicarbonate molecule, leading to a conformational change in the hemoglobin molecule.

RESULTS AND DISCUSSION

The results of our experiments support the hypothesis that bicarbonate interacts with the hemoglobin molecule, causing a conformational change.

Figure 1: A timeline showing the discovery and progression of the concept of allosteric regulation in protein.
Logarithmic scale of $k_{\text{cat}}$ and $k_{\text{non}}$ values for representative reactions at 25 °C. The length of each vertical bar represents the rate enhancement by each enzyme.

ADC ) arginine decarboxylase; ODC ) orotidine 5’-phosphate decarboxylase; STN ) staphylococcal nuclease; GLU ) sweet potato $\alpha$-amylase; FUM ) fumarase; MAN ) mandelate racemase; PEP ) carboxypeptidase B; CDA ) E. coli cytidine deaminase; KSI ) ketosteroid isomerase; CMU ) chorismate mutase; CAN ) carbonic anhydrase.
How does “Biology” cope?

“After total war can come total living”

Mutually Assured Destruction: Cold War exhibit at the Smithsonian
Stringent response and growth control

- Triggered by adverse conditions, e.g. starvation

Transcription control (p)ppGpp:
- Lack of nutrients
- Stalled ribosomes
- ppGpp synthesis
- Reprogramming of transcription

Translation shutdown:
- Proteases
- (p)ppGpp involved
- Activation of toxin-antitoxin modules
- Toxin reversibly disables ribosomes
Stringent response and growth control

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  - Toxin reversibly disables ribosomes
The Stringent Response is mediated by two opposing $\text{Rel}_{\text{Mt}}$ activities which must be tightly regulated.

1) pppGpp synthesis:

\[
\begin{align*}
\text{p-p-p-G} + \text{p-p-p-A} & \leftrightarrow \text{p-p-p-G-p-p} + \text{p-A} \\
\text{GTP} + \text{ATP} & \leftrightarrow \text{G5} + \text{AMP}
\end{align*}
\]

2) pppGpp hydrolysis:

\[
\begin{align*}
\text{p-p-p-G-p-p} & \leftrightarrow \text{PPi} + \text{p-p-p-p-G}
\end{align*}
\]

pppGpp alters RNAP kinetics and mediates the transcriptional response to environmental conditions to which Mtb is exposed.
The RAC Allosterically Activates Transferase Activity

<table>
<thead>
<tr>
<th></th>
<th>$K_{\text{ATP}}$ (mM)</th>
<th>$K_{\text{GTP}}$ (mM)</th>
<th>$k_{\text{cat}}$ (s$^{-1}$)</th>
<th>$k_{\text{cat}}/K_{\text{ATP}}$ (mM$^{-1}$ s$^{-1}$)</th>
<th>$k_{\text{cat}}/K_{\text{GTP}}$ (mM$^{-1}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rel$_{\text{Mt}}$(Basal Level)</td>
<td>2.0</td>
<td>1.4</td>
<td>1.2</td>
<td>0.6</td>
<td>0.9</td>
</tr>
<tr>
<td>Rel$_{\text{Mt}}$ + Ribosome•UtRNA•mRNA</td>
<td>0.5</td>
<td>0.3</td>
<td>24.7</td>
<td>54.8</td>
<td>79.6</td>
</tr>
</tbody>
</table>

RAC = Rel$_{\text{Mt}}$ Activating Complex
Ribosome•Uncharged tRNA•mRNA

5'..AUGCCGACGUACAGUUUGUUGUCGGGC...3'
Figure 1: Summary of Rel$_{Mtb}$ truncated. Full-length Rel$_{Mtb}$ protein is at the top followed by the different truncated proteins. Amino acid numbers are at the beginning and end of each fragment and corresponding activity is listed below. 87-187 overlapping site is noted in the full-length Rel$_{Mtb}$. 

Heterogeneity even within a single molecule
Cooperativity, heterogeneity, stochasticity
Another example: Controllers for nanomachines
Aerobic and anaerobic respiratory chain in Mtb

- Fumarate Reductase: *FrdABCD* (Rv1552-Rv1555)
- Succinate:Menaquinone Oxidoreductase: *sdhABCD* (Rv3316-Rv3319)
- Electron-transferring Flavoproteins
- NADH:Menaquinone Oxidoreductases: *menABCDEFGHJKLMN* (Rv3145-Rv3158), *ndh* (Rv1854c), *ndhA* (Rv0392c)

Menaquinone Pool

- Cytochrome *bd* Oxidase: *cydABCD* (Rv1620c-Rv1623c)
- Cytochrome *c* Oxidase (*aa)*: *ctaBCDE* (Rv1451, Rv2200c, Rv3043c, Rv2193)
- Cytochrome *bc1* complex

Nitrate Reductase: *Fused: narX* (Rv1736c)
Multisubunit: *narGHJI* (Rv1161-Rv1164)

Aerobic Pathway

Anaerobic Pathway
Electrons enter the chain through NADH oxidoreductase

Plot of the NADH-Q2 reductase reaction with varying Q concentrations and fixed concentrations of NADH. Lineweaver-Burk plot (inset), slopes (Vmax/Km) of the lines are not affected by NADH concentration—ping pong mechanism.

\[ K_{m}^{\text{NADH}} = 42 \text{ uM}, \ K_{m}^{Q2} = 12.5 \text{ uM}, \ V_{\text{max}} = 26 \text{ unit mg}^{-1} \]
Ping Pong tetra-uni mechanism

- NDH-2 catalyzes the following two electron transfer reactions:

\[ \text{Ndh(}F_{\text{ox}}\text{)} + \text{NADH} \rightarrow \text{Ndh(}F_{\text{red}}\text{)} + \text{NAD}^+ \]  
(eq1)

\[ \text{Ndh(}F_{\text{red}}\text{)} + \text{Q} \rightarrow \text{Ndh(}F_{\text{ox}}\text{)} + \text{QH}_2 \]  
(eq2)

\[ \text{E is Mtb NDH-2, A is NADH and B is the quinone} \]
Phenothiazine inhibition of Mtb respiration.

(A) TPZ inhibition of NADH-dependent oxygen consumption by Mtb membranes measured with a Clark-type oxygen electrode. Respiration was initiated by the addition of 10 mM NADH and arrested upon the addition of 1mMTPZ. Addition of 10mM ascorbate and 1 mM TMPD produced an immediate resumption of respiration.
A 3D model of *E. coli* Ndh according to Schmid and Gerloff (2004). Putative flavin-, NADH-, and membrane-binding domains are shown in ovals.
## A drug for dormant TB

<table>
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<tr>
<th>Drug</th>
<th>MBC (mg/L)</th>
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<tr>
<td></td>
<td>Log-phase</td>
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<tr>
<td>Rifampin</td>
<td>&lt;0.625</td>
</tr>
<tr>
<td>Trifluoperazine</td>
<td>10~20</td>
</tr>
<tr>
<td>Chorpromazine</td>
<td>10~20</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>&lt;0.625</td>
</tr>
<tr>
<td>Ethionamide</td>
<td>&lt;0.625</td>
</tr>
<tr>
<td>Capreomycin sulfate</td>
<td>0.625</td>
</tr>
<tr>
<td>Amikacin sulfate</td>
<td>&lt;0.625</td>
</tr>
<tr>
<td>Thiacetazone</td>
<td>&lt;0.625</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>0.625</td>
</tr>
<tr>
<td>Streptomycin sulfate</td>
<td>&lt;0.625</td>
</tr>
<tr>
<td>p-aminosalicylic acid</td>
<td>&lt;0.625</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>&lt;0.625</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>10~20</td>
</tr>
<tr>
<td>Cycloserine</td>
<td>10~20</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>40</td>
</tr>
<tr>
<td>Dapsone</td>
<td>&gt;40</td>
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**MBC\textsubscript{99} s of 17 Drugs for Log-phase and 6-week-starved \textit{M.tuberculosis}H37Rv by cfu counts.**
We shall go on to the end, we shall fight in France, **we shall fight** on the seas and oceans, **we shall fight** with growing confidence and growing strength in the air, we shall defend our Island, whatever the cost may be, **we shall fight on the beaches, we shall fight** on the landing grounds, **we shall fight** in the fields and in the streets, **we shall fight** in the hills; **we shall never surrender.**

WSC June 4, 1940
Can molecular computing say anything

based on irreversible nature of computation

The Fundamental Physical Limits of Computation
What constraints govern the physical process of computing? Is a minimum amount of energy required, for example, per logic step? There seems to be no minimum, but some other questions are open by Charles H. Bennett and Rolf Landauer

A Fredkin Gate: Logically reversible with no energy limit on the computation

CAB is a piece of DNA that we can synthesize
a NAND gate

\[
\begin{array}{ccc|ccc}
A & B & C & \rightarrow & A' & B' & C' \\
1 & 1 & 0 & \rightarrow & 1 & 0 & 1 \\
1 & 0 & 0 & \rightarrow & 1 & 0 & 0 \\
0 & 1 & 0 & \rightarrow & 0 & 1 & 0 \\
0 & 0 & 0 & \rightarrow & 0 & 0 & 0 \\
\end{array}
\]

\[
\begin{array}{cc}
\text{AND} & \text{NOT} \\
A & B & C & \rightarrow & A' & B' & C' \\
1 & 0 & 1 & \rightarrow & 1 & 1 & 0 \\
0 & 0 & 1 & \rightarrow & 0 & 0 & 1 \\
\end{array}
\]

\text{HAND gate}

\[
\begin{array}{c}
\text{AND} \\
A \\
B \\
C \\
\end{array}
\rightarrow
\begin{array}{c}
A' \\
B' \\
C' \\
\end{array}
\text{(A AND B)}
\]

\[
\begin{array}{c}
\text{NOT} \\
A \\
B \\
C \\
\end{array}
\rightarrow
\begin{array}{c}
A' \\
B' \\
C' \rightarrow \text{OUTPUT (NOT A)} \\
\end{array}
\]

\[1 \ A \ 0 -- C \rightarrow \Rightarrow 1 \ A \ 0 -- C' A' B'
\]

\[<-- C' A' B'
\]

\[1 \rightarrow
1 \ A \ 0 -- C' A' B' \Rightarrow 1 \ A \ 0
\]

\[<-- 0
\]

**Figure 2**
Why reversible?

Minimal energy expense

Detection and correction of intrusion

Error checking by reversing computation to recreate inputs

Bidirectional debugging
In principle it can take minimal energy to go through a biochemical gate

\[
\text{DNA}_n + \text{dNTP} \leftrightarrow \text{DNA}_{n+1} + \text{PPi}
\]

\[
\Delta G = kt \ln[\text{dNTP/PPi}]
\]

If dNTPs are just 1% over the equilibrium value:
\[
\Delta G = kt \ln[10.1/10] \quad \text{or about } 0.01kT
\]

a modification of an idea in Bennett and Landaur’s Sci. Am paper—suggested using RNA
We synthesized the oligonucleotides and ran the reactions

The gate works in the lab

Figure 3
How fast could one go through one gate?

$t_{1/2}$ annealing: 3 sec.

DNA polymerization rate: 15 bases/sec

For 60 bases pair input: 10 sec
Some very hard problems in nature (biology–biochemistry) “solved” using physical algorithms that reduce the hardness

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