10

Signalling and metabolic networks

10.1 Signalling across networks

10.1.1 *Calculate (plot as two-dimensional surfaces) promoter activities for AND, OR, NAND and XOR logic as functions of A and B concentrations between 0.001 and 100. Set all binding constants equal to 1, A and B binding with co-operativity equal to 0.01 when they bind to neighboring operators. Disregard any leaking, and assume perfect recruitment of A or B or AB, when needed.*

Answer The equations in the four cases read:

$$\text{Activity} = \frac{(A \cdot B/0.01)}{1 + A + B + (A \cdot B/0.01)} \quad \text{AND Gate}$$

$$\text{Activity} = \frac{A}{1+A} + \frac{B}{1+B} \quad \text{OR Gate}$$

$$\text{Activity} = \frac{1}{1 + A + B + AB/0.01} \quad \text{NAND Gate}$$

$$\text{Activity} = \left(\frac{A}{1+A} + \frac{B}{1+B}\right) \cdot \frac{1}{1 + A + B + (A \cdot B/0.01)} \quad \text{XOR Gate}$$

where the geometry is two operators, where the AB complex is only used in the NAND gate and where it could also have been the result of binding that involved strong co-operativity between neighboring sites. The results shown in the left-hand panel of Fig. 10.1.

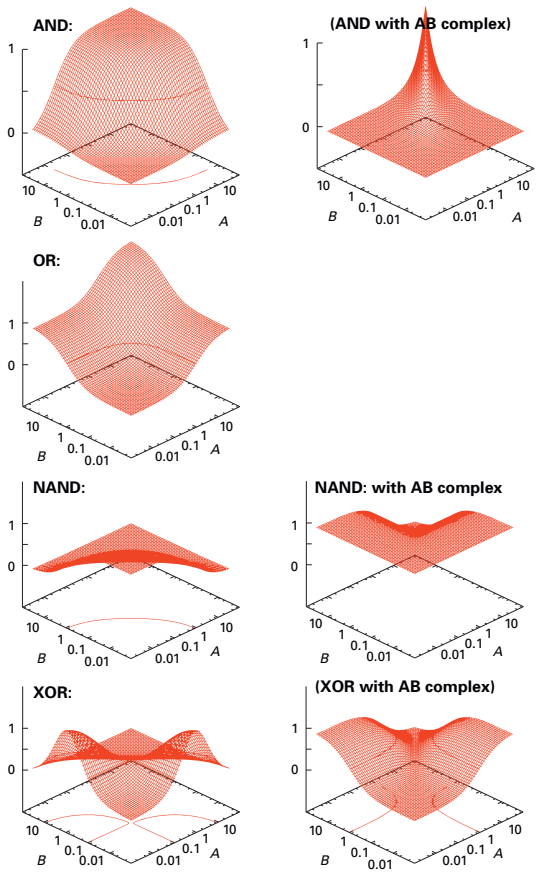

Figure 10.1 Logic gates for genetic circuits. In the right-hand column we use a complex AB in solution that both regulates and sequesters A and B from the solution.

10.1.2 *Repeat the AND, NAND and XOR gates above, using an AB complex in solution, with binding constant 0.01.*

Answer Using the AB complex more extensively, one may make AND, NAND and XOR gates using:

$$\text{Activity} = \frac{AB}{AB + (A - AB) + (B - AB) + 1} \quad \text{AND Gate}$$

$$\text{Activity} = \frac{1}{1 + AB} \quad \text{NAND Gate}$$

$$\text{Activity} = \left(\frac{A - AB}{(1 + A - AB)} \frac{B - AB}{(1 + B - AB)} \right) \cdot \frac{1}{1 + AB} \quad \text{XOR Gate}$$

of which, in particular, the NAND gate appears to be better than the one that does not use complex formation. Results shown in the right-hand panel of Fig. 10.1.

10.1.3 *Consider the network of three proteins on a line* 0−1−2*, with total initial concentrations* $C_0 = C_1 = C_2 = 1$*. Calculate the free concentration of all proteins for* $K = 1$*. Investigate the steady-state change* $\Delta F/F$ *for all proteins, as the total concentration of the first protein is increased to 10 (*F *denotes free protein concentration).*

Answers The iterative equations are:

$$F_1 = \frac{C_1}{1 + F_2}$$
$$F_2 = \frac{C_2}{1 + F_1 + F_3}$$
$$F_3 = \frac{C_3}{1 + F_2}$$

with results for $C_1 = C_2 = C_3 = 1$, and $C_1 = 10$, $C_2 = C_3 = 1$ shown in Fig. 10.2.

10.1.4 *Repeat the above question with the stronger binding* $K = 0.1$*.*

Answers The iterative equations are:

$$F_1 = \frac{C_1}{1 + F_2/0.1}$$
$$F_2 = \frac{C_2}{1 + F_1/0.1 + F_3/0.1}$$
$$F_3 = \frac{C_3}{1 + F_2/0.1}$$

with results for $C_1 = C_2 = C_3 = 1$, and $C_1 = 10$, $C_2 = C_3 = 1$ shown in the lower panels of Fig. 10.2.

10.1.5 *Repeat Question 10.1.4, but assume that total concentrations are* $C_0 = 10$*,* $C_1 = 3$ *and* $C_2 = 1$*, changing to* $C_0 = 100$*,* $C_1 = 3$ *and* $C_2 = 1$*. Finally, repeat 10.1.3, but with* $C_0 = 1$*,* $C_1 = 2$ *and* $C_2 = 10$ *changing to* $C_0 = 10$*,* $C_1 = 3$ *and* $C_2 = 10$*.*

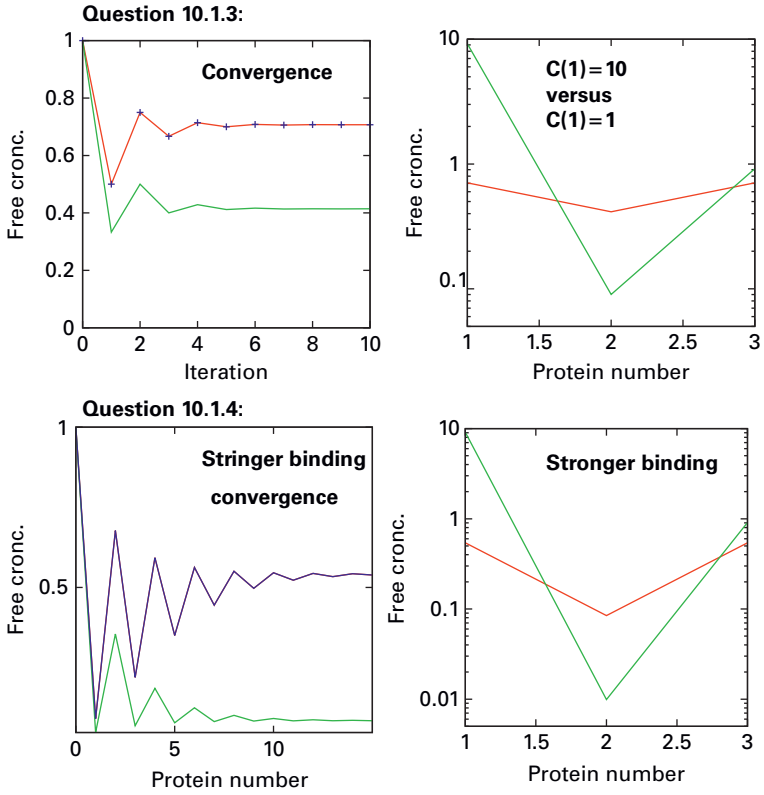

Figure 10.2 Three proteins 1,2 and 3 bind sequentially. In the left-hand panels we investigate convergence of our method for the case of equal total concentrations, in the right-hand and panels we investigate the difference in free concentrations when all proteins have equal concentrations versus the case where the first protein has 10 times the total concentration.

Answer The iterative equations are:

$$F_1 = \frac{C_1}{1 + F_2/0.1}$$
$$F_2 = \frac{C_2}{1 + F_1/0.1 + F_3/0.1}$$
$$F_3 = \frac{C_3}{1 + F_2/0.1}$$

with C_i given in the question formulation. Results are shown in Fig. 10.3.

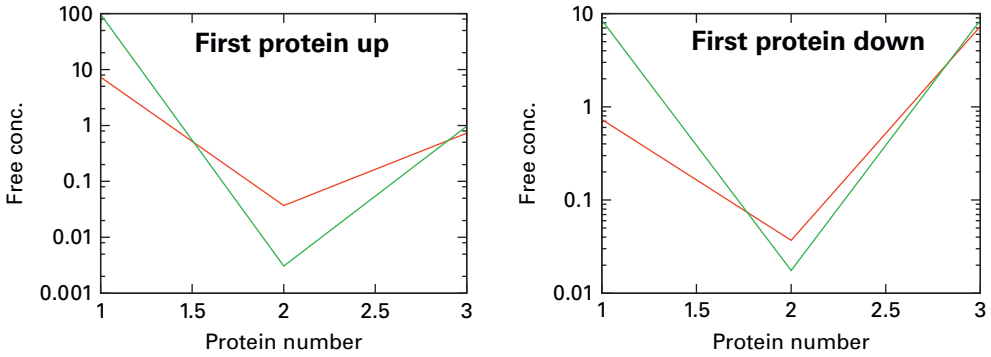

Figure 10.3 Three proteins 1,2 and 3 bind sequentially. In the left-hand panel we shown what happens when $C_1 = 10$ and $C_1 = 100$, whereas the right-hand panels investigates what happens for $C1 = 1$ and $C_1 = 10$. In all cases $C_2 = 3$ and $C_3 = 10$.

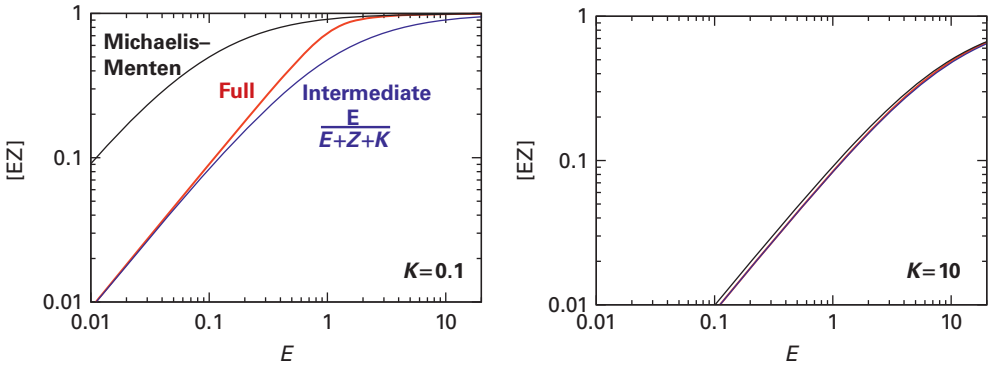

Figure 10.4 Comparison of correct expression with two approximations, of which Michaelis–Menten is the worst.

10.2 Goldbeter-Koshland...

10.2.1 *Compare the exact expression for [EZ] with [EZ] = [E], with [EZ]* $= \frac{[K][Z]}{K+[z]}$ *and with [EZ]* $= \frac{[E][Z]}{K+[E]+[Z]}$ *by plotting the expressions as a function of [E]* $\in [0:10]$ *for fixed [Z] = 1. Use* $K = 1$. *See what happens when* $K = 10$ *and* $K = 0.1$.

Answer Set $Z = 1$ and plot the corresponding curves as in Fig. 10.4.

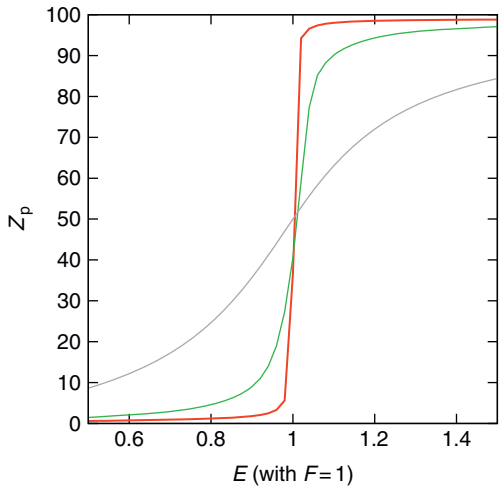

Figure 10.5 Push–pull reactions in steady state, as function of pull, for $K = 0.1, K = 1$ and $K = 10$ (same K in both directions).

10.2.2 *Solve the Goldbeter–Koshland system from lower panel of Fig. 10.7 using* $[Z] + [Z_p] = 100$*, and plotting [Z] as function of E between 0.5 and 1.5 when* $F = 1$ *is fixed Hint: simulate* $\frac{dz_P}{dt}$ *unit steady state for each [E] value. First consider the case of strong binding* $K_E = K_F = 0.1$*. Subsequentles consider* $K_{\mathrm{E}} = K_{\mathrm{F}} = 10$*.*

Answer For each value of $E = 0.5, 0.6, ...1.5$, iterate the equations:

$$\frac{Z(t+\mathrm{d}t) - Z(t)}{\mathrm{d}t} = -\frac{Z+E+K}{2} + \sqrt{\frac{(Z+E+K)^2}{4} - Z\cdot E} + \frac{(100-Z)+F+K}{2} - \sqrt{\frac{((100-Z)+F+K)^2}{4} - (100-Z)\cdot F}$$

with $\mathrm{d}t = 0.05$ for $t = 1000$ time units (convergence is slow). Results are shown in Fig. 10.5.

10.2.3 *Consider again the Gold beter–Koshland system for Fig. 10.27 but now for* $E = 1$ *and* $E = 0.9$ *and* $K_{\mathrm{E}} = K_{\mathrm{F}} = 0.1$ *and simulate dynamics using the Gillespie algorithm (that is, one has to simulate, say, at least 1000 events, each being one of the two competing reactions* $\mathrm{Z_p} \to \mathrm{Z_p} + 1$ *and* $\mathrm{Z} \to \mathrm{Z} + 1$*.*

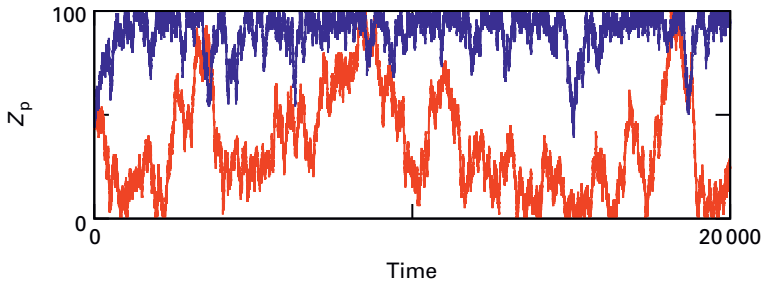

Figure 10.6 Trajectory for the $E = 1$ and the $E = 0.9$ cases using a Gillespie simulation of a push–pull reaction.

Each event is selected according to rates $= k_\mathrm{p} \cdot [\mathrm{ZE}]$, *respectively* $= k \cdot [\mathrm{Z_pF}]$ *where concentrations of complexes can be expressed from the equations in Fig. 10.7. Set* $k_\mathrm{p} = k = 1\,\mathrm{s}^{-1}$.

Answer There are two processes with rates given by:

$$r(Z \to Z-1) = \frac{Z+E+K}{2} - \sqrt{\frac{(Z+E+K)^2}{4} - Z \cdot E}$$
$$r(Z \to Z+1) = \frac{100-Z+F+K}{2} - \sqrt{\frac{((100-Z)+F+K)^2}{4} - (100-Z) \cdot F}$$

At each step select the next event as the first of the two times:

$$t(Z \to Z-1) = -\ln(\mathrm{random})/r(Z \to Z-1))$$
$$t(Z \to Z+1) = -\ln(\mathrm{random})/r(Z \to Z+1))$$

and update time t accordingly. Results are shown in Fig. 10.6.

10.2.4 *Construct logical gates based on phosphorylation processes, with the output being counted in terms of the phosphorylation status of one protein. Hint: allow for complexes of enzymes, with the possibility that these complexes may be active, or passive, respectively.*

Answer This in principle opens up many answers. Most simply, consider an AND gate between two enzymes E and G, which is only active as a complex EG. In a push-pull reaction, where this complex attempts a conversion of $Z \to Z_p$ that is opposed by a pull from an enzyme F with strength $F = 1$, the concentration of [EF] $> F$ (if all rates are assumed equal and binding

strong ($K \sim 1$). Therefore one will have a sharp AND gate that will only respond if both E and G are above 1. At the same time Z is a NAND gate.

And OR gate would NOT be obtained if the two enzymes both catalyze the reaction independently, because half of each in total would do the job. However an OR gate could be obtained if they both pass through a push–pull reaction that secures that they are both either on or off, and then subsequently have both outputs as inputs in catalyzing a third push–pull reaction.

And Exclusive OR gate is in fact also relatively simple to obtain, assuming that each enzyme E and G catalyze the $Z \to Z_p$ reaction, whereas the complex EG then should catalyze the pull reaction $Z \to Z_p$. A small F also independently catalyzes the pull reaction, but with a lower value $F \sim 0.5$.

10.3 Adaptation

10.3.1 *A pedagogical example of adaptation could be our intestines, occupied in part by bacteria B, which consumes the available food F. Argue for and identify variables in the following model:*

$$\begin{aligned} \frac{\mathrm{d}B}{\mathrm{d}t} &= F \cdot B - B \\ \frac{\mathrm{d}F}{\mathrm{d}t} &= E - F \cdot B - C \cdot F \end{aligned} \tag{10.1}$$

Assume that host consumption $C = 0.5$ and simulate the system for a switch from steady state at $E = 1$ to a steady state where the host eats twice as much, i.e. $E = 2$. What could make this adaptation less perfect?

Answer B is bacterial density, F is food density and E the supply of food per time unit. C is the consumption of the intestines, which will grow with the surface area of the intestine.

The equations are simulated in discrete time steps, with $\mathrm{d}t = 0.01$, with results shown in Fig. 10.7.

The equations are idealized in the sense that B in practice would be limited by the size of intestine:

$$\begin{aligned} \frac{\mathrm{d}B}{\mathrm{d}t} &= F \cdot B \cdot \left(1 - \frac{B}{B_{\max}}\right) - B \\ \frac{\mathrm{d}F}{\mathrm{d}t} &= E - F \cdot B - C \cdot F \end{aligned} \tag{10.2}$$

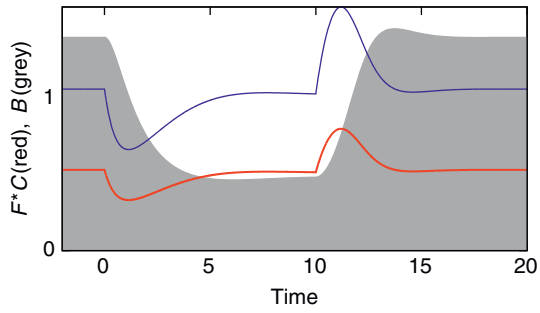

Figure 10.7 Simulation of Question 10.3.1 with $C = 0.5$. The simulation is started with B = 1 and F = 0, whereas E changes between 1 and 2 every 10th time unit.

an equation which makes adaptation less perfect, in the sense that a small $B_{\max}$ will make it impossible to obtain enough bacteria to maintain a steady-state food level.

10.3.2 *Consider the simulation in the right-panel of Fig. 10.1, but replacing the assumed total concentration of* $B_{\mathrm{T}} = 1$ *with a* $B_{\mathrm{T}} = 10$. *Examine the equations as* $B_{\mathrm{T}} \to \infty$ *(i.e. that the buffer is in huge excess). Demonstrate that the output* C *obtains perfect adaptation* ($\Delta C \sim 0$). *Hint: set* $L = 0.02$ *and solve the equations by simulating a trajectory with small time increments* ($\mathrm{d}t = 0.01$) *until steady, then change* L *abruptly to* $L = 0.2$ *and plot the response of output* C.

Answer Shown in Fig. 10.8. Notice that, a larger B_{tot} gives a better adaptation. The equations are:

$$\frac{\mathrm{d}B}{\mathrm{d}t} = \frac{C \cdot (B_T - B)}{(B_T - B) + C + 0.001} - \frac{0.25 \cdot B}{B + 0.001} \tag{10.3}$$

$$\frac{\mathrm{d}C}{\mathrm{d}t} = \frac{L \cdot (1 - C)}{(1 - C) + L + 0.1} - \frac{B \cdot C}{B + C + 0.1} \tag{10.4}$$

with $B_{\mathrm{tot}} = 1$ respectively $B_{\mathrm{tot}} = 2$. Also notice that we explore a two-step increase in input L, and that the second step challenges the capacity of the buffer more.

10.3.3 *Investigate Barkai–Leibler chemotaxis adaptation in Fig. 10.11 numerically, in particular with respect to increasing the rates between* Z *and* Z_{p} *by a factor of 10. Normally these reactions are assumed to be fast, whereas methylation reactions are assumed to be slower for this system.*

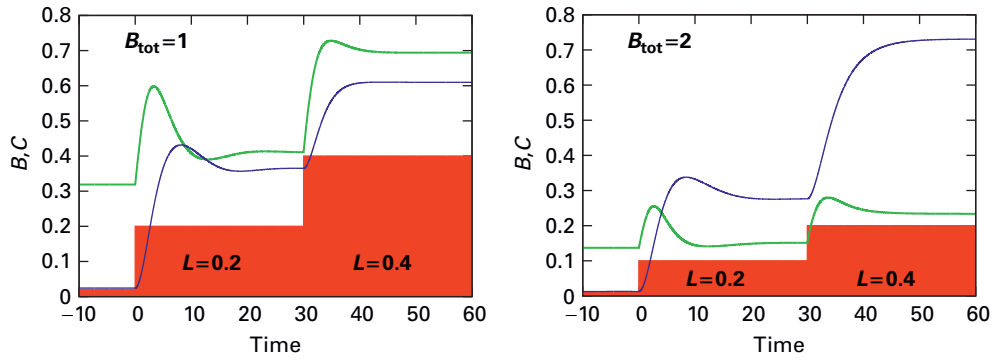

Figure 10.8 Response for different values of buffer, as input is increased from 0.02, to 0.2, to 0.4.

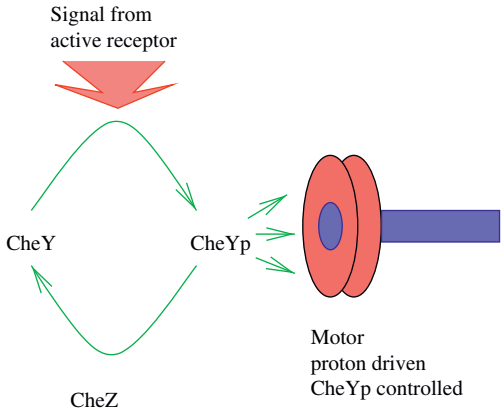

Figure 10.9 The downstream signaling in chemotaxis of *E. coli*. Z in our notation is the molecule CheY, whereas unfortunately the CheZ in our notation is taken by the enzyme F.

Answer The appropriate equations with the factor $f = 10$ are:

$$\frac{\mathrm{d}Z_p}{\mathrm{d}t} = f \cdot \frac{L \cdot Z}{Z+1} - f \cdot \frac{Z_p}{Z_p+1} - \frac{Z_p}{Z_p+1} \tag{10.5}$$

$$\frac{\mathrm{d}Z}{\mathrm{d}t} = -f \cdot \frac{L \cdot Z}{Z+1} + f \cdot \frac{Z_p}{Z_p+1} + \frac{0.2 \cdot (1-Z-Z_p)}{(1-Z-Z_p)+0.001} \tag{10.6}$$

which is simulated to steady state starting with $Z = Z_p = 0.1$ at time $t = -40$ and using $L = 1$. At time $t > 0$ $L = 10$. See Fig. 10.10, left-hand panel with $f = 1$, right-hand with $f = 10$.

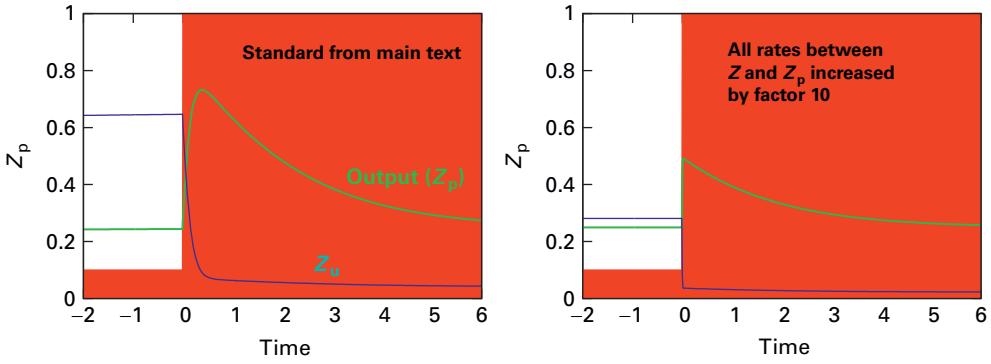

Figure 10.10 Response for variations of the Barkai–Leibler system.

10.3.4 *Vary the saturation condition for methylation by CheR for the model in Fig. 10.11 (change the constant 0.001 to 1). Investigate what happens if one changes the CheB rate limits* $(Z_\mathrm{p}/(Z_\mathrm{p}+1) \to Z_\mathrm{p}/(Z_\mathrm{p}+0.1)$ *in one of the terms for* $\mathrm{d}Z/\mathrm{d}t)$.

Answer For variations in CheR, the equations are:

$$\frac{\mathrm{d}Z_p}{\mathrm{d}t} = \frac{L\cdot Z}{Z+1} - \frac{Z_p}{Z_p+1} - \frac{Z_p}{Z_p+1} \tag{10.7}$$

$$\frac{\mathrm{d}Z}{\mathrm{d}t} = -\frac{L\cdot Z}{Z+1} + \frac{Z_p}{Z_p+1} + \frac{0.2\cdot(1-Z-Z_p)}{(1-Z-Z_p)+1} \tag{10.8}$$

which is simulated to steady state starting with $Z = Z_p = 0.1$ at time $t = -40$ and using $L = 1$. At time $t > 0$ $L = 10$. See Fig. 10.11, left-hand panel.

For variations in CheB the equations read:

$$\frac{\mathrm{d}Z_p}{\mathrm{d}t} = \frac{L\cdot Z}{Z+1} - \frac{Z_p}{Z_p+1} - \frac{Z_p}{Z_p+0.1} \tag{10.9}$$

$$\frac{\mathrm{d}Z}{\mathrm{d}t} = -\frac{L\cdot Z}{Z+1} + \frac{Z_p}{Z_p+1} + \frac{0.2\cdot(1-Z-Z_p)}{(1-Z-Z_p)+0.001} \tag{10.10}$$

which is simulated to steady state starting with $Z = Z_p = 0.1$ at time $t = -40$ and using $L = 1$. At time $t > 0$ $L = 10$. See Fig. 10.11, right-hand panel.

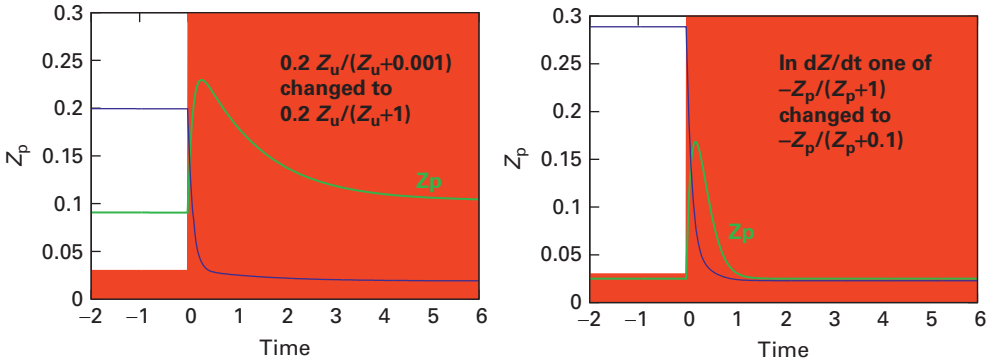

Figure 10.11 Response for variations of the Barkai–Leibler system.

10.4 Metabolic Fluxes

10.4.1 *Examine possible solutions for the glucolysis network in Fig. 10.14, assuming that all v are in the interval [0,1]. Which solutions maximize production of Fluc1-6P2. What are values of all fluxes in that state? Explain the solution in words?. Hint: sample solutions by selecting v_2, v_3 and v_7 randomly in the chosen interval and obtain other vs from the equation for the null space. Investigate the subset of solutions that fulfill all constraints.*

Answer Let v_2, v_3 and v_7 take random values between 0 and 1. For each such selection set:

$$v_1 = v_2 + v_3 \tag{10.11}$$
$$v_4 = v_3 \tag{10.12}$$
$$v_5 = v_3 \tag{10.13}$$
$$v_6 = v_7 + 2v_2 + 2v_3 \tag{10.14}$$
$$v_8 = 0 \tag{10.15}$$

and accept a solution if all $v_i \in [0, 1]$. For each accepted selection calculate production of Fruc1-6P2, given by $V = v_5$. Select the solution with the maximum value of V. For a sample of 1000 attempted solutions we obtain $V = 0.48$, as seen from the figure in main text. At this point the fluxes in the system are: $\mathbf{v} = (0.49, 0.01, 0.48, 0.48, 0.48, 0.99, 0.01, 0.00)$.

10.4.2 *Repeat question 10.4.1, allowing v_6 and v_7 to take any value, i.e. allowing transitions between ATP and ADP to be very fast.*

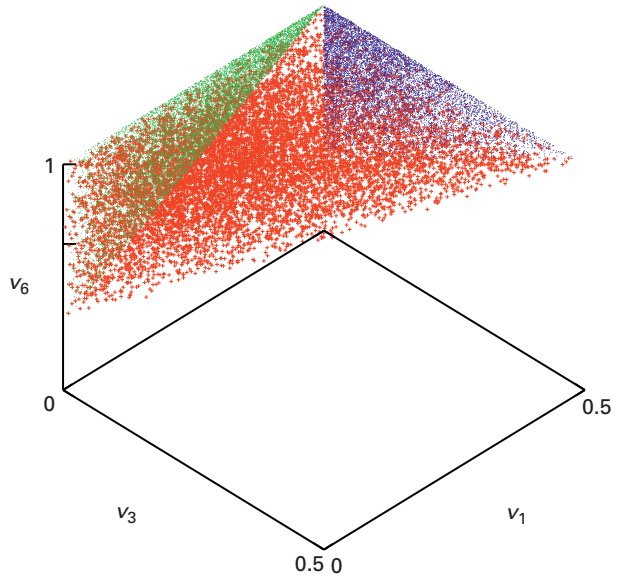

Figure 10.12 Sampling of solutions where all v are between 0 and 1. The blue and green colors are projections of the solution space in various planes.

Answer Let v_2, v_3 take random values between 0 and 1, whereas v_7 can take values between 0 and 1000. Repeat procedure from last questions and again obtain $V = 0.99$ and $\mathbf{v} = (1.00, 0.01, 0.99, 0.99, 0.99, 603, 600.00, 0.00)$.

10.4.3 *Repeat the random sampling in Fig. 10.15, showing instead the allowed values of* v_1, v_3 *and* v_6.

Answer See Fig. 10.12.

10.4.4 *Examine the metabolic model network in Fig. 10.15, and the sample solution space, where we assume that all* $v \in [0; 2]$. *Find solutions where* $v_3 + v_6$ *is maximal.*

Answer Let v_2, v_3 and v_4 takes random values between 0 and 2. For each such selection set:

$$v_1 = v_2 + 2v_4 \tag{10.16}$$
$$v_5 = v_3 - v_2 \tag{10.17}$$
$$v_6 = v_4 - v_5 \tag{10.18}$$

and accept a solution if all $v_i \in [0, 2]$. For each accepted selection calculate $V = v_3 + v_6$ and select the solution with the maximum value of V. For a

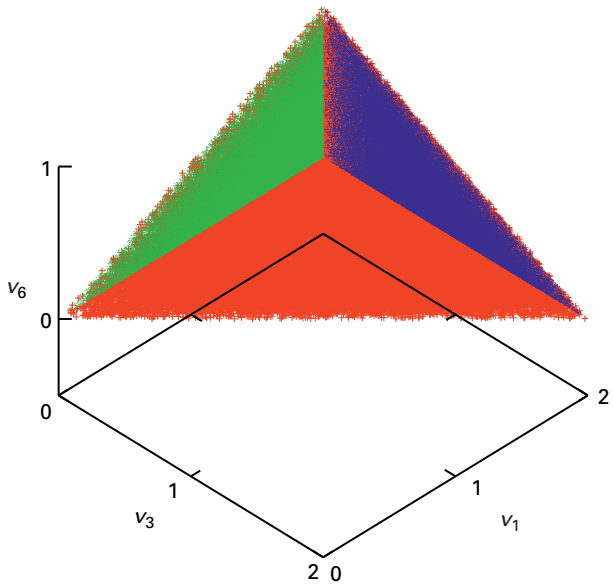

Figure 10.13 Sampling of solutions where all v are between 0 and 2. The blue and green colors are projections of the solution space in various planes.

sample of 1000 attempted solutions we obtain $V = 1.9$. At this point the fluxes in the system are: $\mathbf{v} = (1.97, 1.90, 1.91, 0.03, 0.01, 0.02)$. The solution space is visualized in Fig. 10.13.