Exercise sheet 10
Systems Biology class 2014

June 12, 2014

Print and return to during classes, tutorials, office hours to Jean Hausser or in the enveloppe outside room 612 in the Wolfson building until June 15th 2014.

1 Trade-offs in kinetic proofreading

Kinetic proofreading is a mechanism that makes it possible for molecules to recognize binding partners with high accuracy. In this exercise, we explore the trade-offs faced by kinetic proofreading. Contrary to the derivation we did in class, we will *not* assume that the rates v and m are slow compared to other rates.

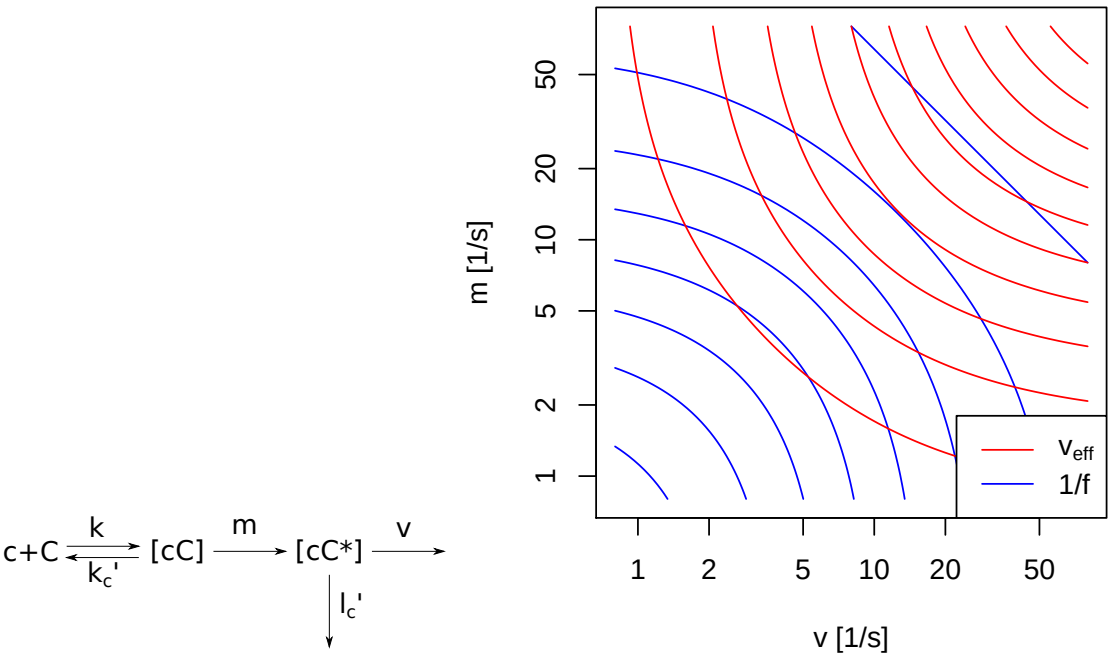

Figure 1: Left: Reactions rates in kinetic proofreading. Right: Contours of the v_{eff} and $1/f$ performance functions.

1. Find an expression for the error rate $f = \frac{v[dC*]}{v[cC*]}$ as a function of the reaction rates in the model. As we did in class, assume that the concentrations of the complexes $[cC]$ and $[cC*]$ are at steady-state.

2. We will now check if we obtained the correct expression for f by making the same assumptions as we made in class and comparing the error rate f to the one we obtained in class.
   For this question only, assume that the modification rate is small compared to the dissociation rate of $[cC]$ ($m \ll k'_c$) and that the elongation rate is small compared to the dissociation rate of $[cC*]$ ($v \ll l'_c$). Under these assumptions, verify that $f = \frac{k'_c}{k'_d}\frac{l'_c}{l'_d}$, which is the value we found in class.

3. Write an expression for the rate of elongation $v_{eff} = v[cC*] + v[dC*]$ as a function of the reaction rates.

4. Figure 1 shows a contour-plot of the translation speed v_{eff} (red) as a function of the elongation rate v and tRNA modification rate m. On the same figure, we represent contours of the translation accuracy $1/f$

(blue). For simplicity, we assumed that the correct and incorrect tRNAs are present in equal concentrations $c = d$.

Represent the archetypes and sketch the possible Pareto front. What are the tasks of the archetypes? In a couple of sentences, propose a biological justification for the shape of the Pareto front.
Reminder: the Pareto front is the set of points at which the performance contours for different tasks are tangent.

2 Reversibility of tRNA modification in kinetic proofreading

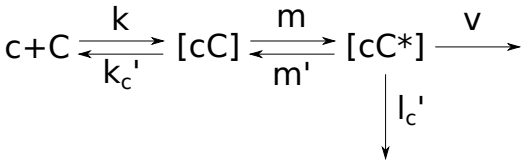

Figure 2: A kinetic-proofreading model in which tRNA modification is reversible.

In exercise 1, we studied a model of kinetic-proofreading in which the tRNA modification step is irreversible. In this exercise, we will study the consequence of relaxing this assumption. As illustrated by the model sketched on Figure 2, the modification of the tRNA can be reversed at a rate m'.

We assume that the elongation rate v is small compared to the dissociation rate of $[cC*]$ ($v \ll l'_c$) and to the tRNA unmodification rate ($v \ll m'_c$). We also assume that the tRNA modification rate m_c is small compared to the dissociation rate of $[cC]$ ($m_c \ll k'_c$).

1. Write an expression for the amount of $[cC*]$ at steady-state as a function $c.C$, the product of the tRNA and codon concentrations.

2. For a wrong tRNA of concentration d, only the dissociation rates k'_c and l'_c of the $[cC]$ and $[cC*]$ complexes differ from case of the c tRNA.
Write an expression for the error rate $f = \frac{v[dC*]}{v[cC*]}$.

3. What value of m' minimizes the error rate? Discuss the biological implication of this result in 2-3 sentences.

3 A two-state model of conformational proofreading

In this exercise, we study conformational proofreading using a two-state thermodynamic model of tRNA – codon complex formation.

1. The binding free energy of a correct tRNA c to its codon $\Delta G_{b,c}$ is -47.0 kcal/mol. A wrong but similar tRNA d binds the same codon with a free energy that is higher by 6.0 kcal/mol. What is the binding free energy $\Delta G_{b,d}$ of the tRNA d to the codon?

   In the two-state model of conformational proofreading, the tRNA – codon system has only two states: bound (b) or unbound (u). In class and in the tutorial, we saw that the probability of finding the correct tRNA bound to the codon is
   $$P(b|c) = \frac{1}{1 + e^{\Delta G_c / RT}}$$
   where $\Delta G_c = \Delta G_{b,c} + \Delta G_{def}$ is the combined free energy of tRNA – codon binding ($\Delta G_{b,c}$) and of tRNA deformation (ΔG_{def}). $\Delta G_{b,c}$ is negative because the tRNA has electrostatic interactions that favor binding to the codon. ΔG_{def} is typically positive because energy is required to stretch the tRNA so that it can bind the codon. $T = 300$K is the temperature and $R = 1.99 \times 10^{-3}$ kcal/K.mol is Boltzmann's constant.

2. Assuming there is no conformational proofreading ($\Delta G_{def} = 0$), what is the error rate *i.e.* the ratio $\frac{P(b|d)}{P(b|c)}$ between the probability of binding d to the probability of binding c? Is this error rate in translation sufficiently low for cells to grow?
Reminder: cell growth is significantly hampered if the error rate exceeds 10^{-3}.

3. Plot the probability $P(b|c)$ of c binding the codon as a function of ΔG_{def}, for ΔG_{def} in the $0-60$ kcal/mol range. In the same figure, plot the probability $P(b|d)$ of d binding the codon as a function of ΔG_{def}.

4. Plot the error rate $\frac{P(b|d)}{P(b|c)}$ as a function of ΔG_{def}, for ΔG_{def} in the $0-60$ kcal/mol range. Use a log-scale on the y-axis (error rate). Graphically, what value of ΔG_{def} minimizes the error rate while keeping the probability of binding the right tRNA over 0.5? What is the associated error rate in this model?

4 Optimal genetic code for minimizing errors (optional)

In this exercise we consider an additional mechanism for reducing translation errors, based on the structure of the genetic code.

1. First consider a code based on an alphabet of two letters (0 and 1), and where codons have two letters each. Thus, there are four possible codons ([00], [01], [10], and [11]). This genetic code encodes two amino acids, A and B (and no stop codons). Each amino acid is assigned two of the four codons.
   What are the different possible genetic codes?

2. Assume that misreading errors occur, such that a codon can be misread as a codon that differs by one letter (e.g., [00] can be misread as [01] or [10], but not as [11]). Which of the possible codes make the fewest translation errors?

3. Assume that the first letter in the codon is misread at a higher probability than the second letter (e.g., [00] is misread as [10] more often than as [01]). Which of the codes have the lowest translation errors?

4. Study the real genetic code in Figure 3. Compare the grouping of codons that correspond to the same amino acid. How can this ordering help reduce translation errors? Based on the structure of the genetic code, can you guess which positions in the codon are most prone to misreading errors? Can you see in the code a reflection of the fact that U and C in the third letter of the codon cannot be distinguished by the translation machinery (a phenomenon called "third-base wobble")?

First letter	Second letter: U	Second letter: C	Second letter: A	Second letter: G	Third letter
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Figure 3: The genetic code. Each 3-letter codon maps to an amino acid or a stop signal that ends translation. For example, CUU codes for the amino acid leucine (Leu). Polar amino acids are shaded, non-polar amino acids in white.

5. In the real genetic code, chemically similar amino acids tend to be encoded by similar codons (Figure 3). Discuss how this might reduce the impact of translation errors on the fitness of the organism.