CRIB

Module 4G7

CONTROL & COMPUTATION IN LIVING SYSTEMS

*Answer **both** questions in section A. Answer **one** question in section B*

All questions carry the same number of marks.

*The **approximate** percentage of marks allocated to each part of a question is indicated in the right margin.*

Manuscripts referenced in part B are attached.

STATIONERY REQUIREMENTS
Single-sided script paper

SPECIAL REQUIREMENTS TO BE SUPPLIED FOR THIS EXAM
CUED approved calculator allowed
Attachment 1: Briat et al (2016) paper for Question 3 (11 pages)
Attachment 2: Gregor et al (2007) paper for Question 4 (11 pages)
Engineering Data Book

10 minutes reading time is allowed for this paper at the start of the exam.

You may not start to read the questions printed on the subsequent pages of this question paper until instructed to do so.

You may not remove any stationery from the Examination Room.

SECTION A

Answer **both** questions.

1 Consider a model of gene expression in which mRNA concentration (m) evolves as:

$$\dot{m} = \alpha - \beta m$$

where α and β are positive constants.

(a) Outline the assumptions underlying this model. Does it violate the Law of Mass-Action? [10%]

Assumptions: mRNA can be modelled as a concentration (continuous variable); system is well mixed and at thermal equilibrium; nucleotides are not rate limiting; RNase enzymes are not saturated; transcriptional activation is on and/or modelled at a quasi steady state over a long time period. Strictly speaking the equation violates mass action because matter enters the system, but this is because reactants (nucleotides) are not explicitly accounted for.

(b) Suppose we replace α with $f(m)$, where f is a monotonically decreasing function such that the system retains the same equilibrium.

(i) What effect does this have on the settling rate of the system? [10%]

Increases settling rate because difference between birth and death rate is higher near the equilibrium if f is monotonically decreasing (can illustrate with rate plot).

(ii) How does this impact the sensitivity of the system to fluctuations in β? [10%]

Reduces sensitivity because locus of equilibrium moves less as β is varied (diagram allowed)

(iii) Biologically, why might one expect β to fluctuate? [10%]

Biomolecules do not spontaneously degrade, they are actively degraded by enzymes whose abundance and occupancy may vary from cell to cell due to variations in copy number and physiological state

(c) Returning to the original form of the model with constant production rate, α, consider an alternative scenario in which the mRNA copy number, m, is a discrete variable and reactions are probabilistic.

(i) Write down the master equation for the resulting system. [10%]

$$\frac{dP(m,t)}{dt} = \text{flux into state } (m,t) - \text{flux out of state } (m,t)$$
$$= \alpha P(m-1,t) + \beta(m+1)P(m+1,t) - \alpha P(m,t) - \beta m P(m,t)$$

(ii) Derive an expression for the probability, $P(m)$, of there being m molecules present at steady state in terms of $P(0)$. [20%]

For steady-state, set $\frac{dP}{dt} = 0$. Then

$$\beta P(1) - \alpha P(0) = 0 \implies P(1) = \frac{\alpha}{\beta} P(0)$$

$$\alpha P(0) + 2\beta P(2) - \alpha P(1) - \beta P(1) \implies P(2) = \frac{1}{2}\frac{\alpha^2}{\beta^2} P(0)$$

and so in. In general, theis gives the following expression:

$$P(m) = \frac{1}{m!}\left(\frac{\alpha}{\beta}\right)^m P(0)$$

as required.

(iii) Show that the steady state distribution of m is Poisson and find an expression for the mean and variance. [20%]

The laws of probability imply that $P(m)$ sums to one:

$$1 = \sum_{m=0}^{\infty} P(m) = P(0) \sum_{m=0}^{\infty} \frac{1}{m!}\left(\frac{\alpha}{\beta}\right)^m \implies P(0) = \exp(-\alpha/\beta).$$

Therefore:

$$P(m) = \frac{1}{m!}\left(\frac{\alpha}{\beta}\right)^m e^{-\alpha/\beta}$$

which is the Poisson probability density function with mean = variance = α/β.

(iv) Provide a quantitative argument to explain why stochastic fluctuations around the steady-state mean value of m become negligible as m becomes large. [10%]

Let μ_m be mean mRNA count. Then given the previous answer, the fluctuations in m around μ_m scale as $\sqrt{\mu_m}$. The relative magnitude of fluctuations is therefore:

$$\frac{\sqrt{\mu_m}}{\mu_m} = \frac{1}{\sqrt{\mu_m}} \to 0 \text{ as } \mu_m \to \infty$$

2 A population of cells in a bioreactor grows according to:

$$\dot{c} = rc(K - c) + u + d$$

where c is the density of cells, r and K are positive parameters, u is the rate at which cells are harvested and d represents external disturbances.

(a) (i) Provide a biological interpretation of the model parameters r and K. [10%]

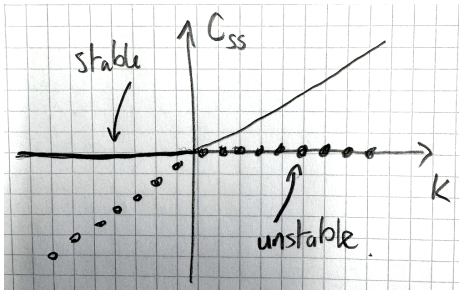
r = fecundity or growth rate, K = carrying capacity of system (nutrient richness, etc)

(ii) Characterise the solutions of this system without solving the above ODE explicitly. [10%]

Unstable fixed point at origin, stable fixed point at $c = K$ as can be illustrated in a sketch of flow field.

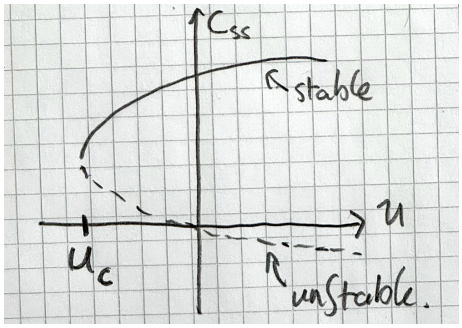
(iii) For $u = 0, d = 0$, sketch the bifurcation diagram of this system as the parameter K changes from positive to negative values, identifying any bifurcations that occur. [15%]

Transcritical bifurcation at K=0:



(iv) For a fixed $K > 0$, and $d = 0$, sketch the bifurcation diagram with u as a bifurcation parameter, identifying the bifurcation that occurs as well as its critical value, u_c. [15%]

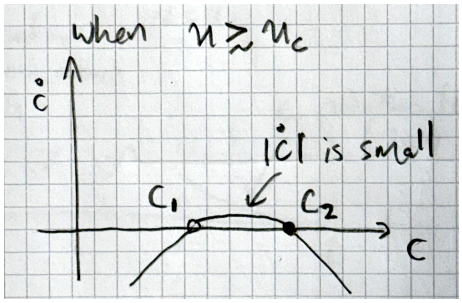
Saddle node (or fold) bifurcation at $u_c = -\frac{rK^2}{4}$:



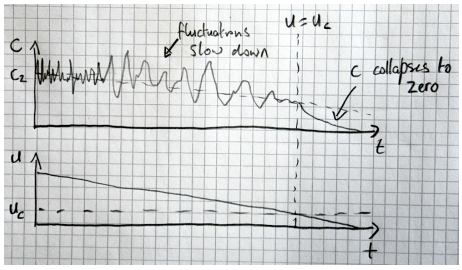
(cont.

(v) Suppose cells are being harvested at a constant rate, $u(t) = u < 0$, and that $d(t)$ consists of small, time-varying random fluctuations. Using appropriate sketches and clear reasoning, describe the effect these fluctuations have on the cell density, $c(t)$, as u is varied in a range approaching the value u_c calculated in part (iv). [20%]

As u approaches u_c, magnitude of flow field decreases:

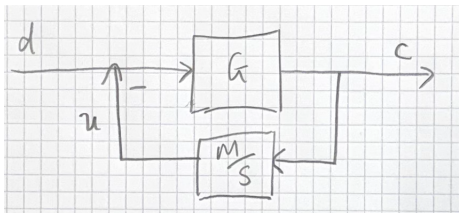


this increases the variance and autocorrelation time of c:



(b) An integral feedback controller with gain M is implemented in a linear model, G, of the bioreactor to counteract the disturbances, d, and maintain the system at the nonzero equilibrium point corresponding to $u = d = 0$.

(i) Draw a block diagram of the system. [10%]



(ii) Linearise the system at its equilibrium to obtain G. [10%]

Full system is:

$$\dot{c} = f(c) = rc(K - c) + u + d$$

Equilibrium is at $c = K$.
Linearising:

$$\frac{\partial f}{\partial c} = rK - 2rc = -Kr$$

at equilibrium. So linear system is given by:

$$\dot{c} = -Krc + u + d$$

(iii) Calculate the closed loop transfer function from d to c. [10%]

Take Laplace transform of linearised system:

$$s\bar{c} = -Kr\bar{c} + \bar{u} + \bar{d}$$

putting in $\bar{u} = M\frac{\bar{c}}{s}$ this becomes:

$$s\bar{c} = -Kr\bar{c} + M\frac{\bar{c}}{s} + \bar{d}$$

Rearranging gives the required transfer function, $T_{d\to c}$:

$$T_{d\to c} = \frac{1}{s + Kr + M/s} = \frac{s}{s^2 + Krs + M}$$

SECTION B

Answer **one** question.

3 Refer to the attached paper by Briat et al (2016).

(a) Summarise the aim, approach, assumptions and findings of the paper in no more than 600 words. You may use diagrams if you wish. [60%]

Aim

The primary aim of this paper is to develop a theory and a specific regulatory motif—termed antithetic integral feedback—for biological regulation that accounts for the stochastic nature of biochemical reactions. The authors sought to create a controller that ensures robust perfect adaptation, meaning the system can maintain a desired set point for a regulated species despite environmental disturbances and biochemical noise.

Motivation

The study was motivated by the observation that while integral feedback is a well-known engineering strategy for achieving homeostasis in deterministic systems, its application and theoretical underpinnings in the noisy, stochastic environment of the cell were poorly understood. Existing synthetic circuits often relied on simpler feedback strategies that failed to achieve perfect adaptation or required cumbersome parameter tuning. Furthermore, integral action is challenging to achieve in a purely biochemical system due to mass-action relations and degradation/dilution.

Approach

- **Controller Design:** They proposed a "stochastic antithetic integral controller" consisting of two species (Z_1 and Z_2) that annihilate each other. Z_1 acts as the "actuation" species (increasing the production of the target species X_1), while Z_2 acts as the "sensing" species (produced at a rate proportional to X_1).
- **Theoretical Framework:** Instead of using deterministic models or moment-closure approximations (which require infinite equations), they worked directly with the stochastic chemical reaction network.
- **Mathematical Proof:** They proved that this specific motif guarantees ergodicity (convergence to a stationary distribution), stability, and robust set-point tracking for a large class of networks.

Findings

- **Robust Perfect Adaptation:** The proposed antithetic integral feedback motif successfully steers the population average of a regulated species to a desired set point.
- **Parameter Independence:** The steady-state average of the regulated species depends *only* on the ratio of two controller parameters (μ and θ) and is independent of the network's internal parameters. This proves the adaptation is robust to parameter variations.
- **Noise Utilization:** The study found that stochastic noise can be beneficial; the controller can stabilise systems that would be unstable in a purely deterministic setting.
- **Biological Relevance:** The authors suggest this motif may already exist in nature, citing the regulation of *E. coli* sigma factor σ^{70} by its anti-sigma factor Rsd as a plausible endogenous example.

Interpretation:
They demonstrate that precise, robust control is possible in biological systems without complex parameter fine-tuning, provided the right topology (antithetic feedback) is used. The finding that the set point is determined solely by the ratio of production rates (μ/θ) offers a powerful "dial" for synthetic biologists to program cellular behaviour predictably.

Limitations:

- **Implementation Constraints:** While the theory is robust, the physical implementation requires specific "annihilation" reactions (e.g., stoichiometric binding) which might be difficult to engineer perfectly in vivo without side reactions. If there is degradation in the interacting species this can undermine perfect adaptation, for example, by causing leaking integration.
- **Metabolic Load:** Although the paper claims the motif works with low copy numbers, introducing new species and reactions still imposes some metabolic burden on the cell, which the theoretical model might not fully capture.
- **Dilution Effects:** The model assumes cell volume is constant or that dilution due to cell division is negligible for the relevant timescales, which is a common simplification that may not hold in rapidly dividing cells. The "annihilation" must be faster than dilution for the integral action to hold effectively.

(b) Show that the model system in Fig 4A of Briat et al has a unique fixed point and find

conditions for the stability of the fixed point for $\eta = k = 1$ and $\mu = 2$. You may make use of (a special case of) the Routh-Hurwitz criterion, stated below. [40%]

Fixed point is at $x_1 = x_2 = \mu$; $z_1 = \mu/k$; $z_2 = k/\eta$. Stability of this fixed point is determined by eigenvalues of the matrix:

$$A = \begin{pmatrix} -1 & 0 & k & 0 \\ 1 & -1 & 0 & 0 \\ 0 & 0 & -\eta z_2 & -\eta z_1 \\ 0 & 1 & -\eta z_2 & -\eta z_1 \end{pmatrix} = \begin{pmatrix} -1 & 0 & k & 0 \\ 1 & -1 & 0 & 0 \\ 0 & 0 & -k & -\frac{\mu\eta}{k} \\ 0 & 1 & -k & -\frac{\mu\eta}{k} \end{pmatrix} = \begin{pmatrix} -1 & 0 & 1 & 0 \\ 1 & -1 & 0 & 0 \\ 0 & 0 & -1 & -2 \\ 0 & 1 & -1 & -2 \end{pmatrix}$$

which has characteristic equation,

$$|sI - A| = s^4 + 5\,s^3 + 7\,s^2 + 3\,s + 2$$

The fixed point is stable iff all roots are in the left half plane. All coefficients are positive, so applying Routh-Hurwitz criterion tells the fixed point is stable iff:

$$105 > 59$$

which is the case. Direct calculation of eigenvalues by other means is acceptable.

The Routh-Hurwitz criterion for the polynomial:

$$p(s) = a_4 s^4 + a_3 s^3 + a_2 s^2 + a_1 s^1 + a_0.$$

Suppose $a_0, \ldots, a_4 > 0$. Then the roots of $p(s)$ lie in the left half plane if and only if

$$a_1 a_2 a_3 > a_1^2 a_4 + a_3^2 a_0.$$

4 Refer to the attached paper by Gregor et al (2007).

(a) Summarise the aim, approach, assumptions and findings of the paper in no more than 600 words. You may use diagrams if you wish. [60%]

Aim

The study aims to quantify the precision and reproducibility of the Bicoid (Bcd) morphogen gradient and its readout by the Hunchback (Hb) gene in *Drosophila* embryos. Specifically, the authors sought to determine whether the embryo achieves patterning through robustness to noisy signals or by exerting precise control over absolute concentrations down to physical

limits.

Motivation:
The work was motivated by the "readout problem": how organisms generate precise multicellular patterns given that chemical signals are inherently stochastic. Prior theories suggested development must rely on robustness to "sloppy" initial data. The authors challenged this by investigating if the biological hardware instead operates at the edge of theoretical physical performance.

Approach
The authors defined four distinct measures of precision to investigate: (1) the concentration difference required to distinguish neighbouring cells, (2) the physical limits imposed by random molecular arrival (noise), (3) the actual noise in the Hb readout, and (4) the reproducibility of Bcd profiles across embryos.

- Measurement: They used Bcd-GFP fusion proteins to measure absolute Bcd concentrations in live embryos and immunofluorescence to quantify Bcd and Hb levels in fixed embryos.
- Theoretical Modeling: They applied the Berg-Purcell limit, $\delta c/c \sim 1/\sqrt{DacT}$, to estimate the fundamental physical constraints on concentration sensing based on diffusion (D), receptor size (a), and integration time (T).
- Analysis: They analyzed the input/output relation between Bcd and Hb and calculated spatial correlations in Hb expression noise.

Findings

- Scale of Precision: All four measures of precision converged on a value of approximately 10%. Neighbouring nuclei, separated by $\sim 8\mu m$, experience a Bcd concentration difference of $\sim$ 10%.
- Absolute Concentration: The Bcd concentration at the Hb activation threshold is $\sim 8 \pm 1$ nM, corresponding to ~ 5 molecules/μm^3.
- Readout Noise: The observed noise in Hb readout (input-equivalent noise) is $\sim$ 10% at the threshold.
- Physical Limits: Calculating the time required to achieve 10% precision via temporal averaging alone yielded $T \sim 2$ hours, which is inconsistent with the rapid pace of nuclear cycles ($\sim 3-4$ minutes).
- Spatial Averaging: The study found that Hb output noise is correlated over a

distance of $\sim$ 5 nuclei. This implies that nuclei effectively average signals over a neighbourhood, reducing the required integration time to $\sim$ 3 minutes.

Interpretation: The results demonstrate that the embryo does not operate with noisy inputs; rather, it maintains absolute Bcd concentrations with high reproducibility ($\sim$ 10%) and reads them out with maximal precision. The convergence of experimental data with the Berg-Purcell limit suggests the system performs spatial averaging (likely via diffusion) to overcome the stochastic limitations of counting low-copy molecules within short timeframes.

Limitations: The analysis assumes Hb responds solely to Bcd; if Hb reacts to additional signals correlated with Bcd, the intrinsic precision of the Bcd-Hb transformation would be even higher than observed. Furthermore, a significant portion of the observed variance arises from measurement noise rather than biological fluctuations, necessitating statistical corrections that introduce uncertainty.

(b) Nuclei in the *Drosophila* embryo are not separated by cell membranes and instead exist together in a cytoplasmic gel called a syncytium. Based on the reasoning in the paper, how would the presence of cell membranes affect morphogen sensing in the embryo? [40%]

The system analysed in the paper was a syncitial embryo, so there were no membranes. We thus consider the effect of adding membranes to the calculation of bicoid sensing precision. We make the following assumptions:

- The presence of a membrane will result in a smaller diffusion constant, $D_m = k_m D$, for the bicoid morphogen to the nuclei (where $k_m < 1$ is the attenuation factor due to the membrane).
- Diffusion of bcd in the extracellular space will be unaffected.
- We assume that membranes will not affect the cell/nucleoid spacing.
- We assume that there is no additional signalling mechanism present to counteract the effect of the membrane.

Alternative, but reasonable, assumptions and analyses can be considered. With these assumptions, the single-nucleoid resolution (eq 3 of the paper) in the presence of the membrane, will be altered:

$$\frac{\delta c_m}{c_m} \approx [D_m a c T]^{-1/2}$$

(Berg-Purcell limit). However, the effective averaging radius will be unaffected (eq 7):

$$N = \frac{8\pi D T}{l^2 \sqrt{3}}$$

where N is the number in the absence of the membrane. Combining these in the overall calculation of precision, the effect on the estimated precision will scale as:

$$\frac{\delta c_m}{c_m} = \frac{1}{\sqrt{D_m acTN}} = \frac{1}{\sqrt{k_m}} \frac{\delta c}{c}.$$

Thus, if the membrane reduces diffusion between the extracellular space and the nucleus by 100-fold, the precision will reduce by a factor of 10, which would require an unfeasible integration time.

END OF PAPER