Version SB/3

EGT2
ENGINEERING TRIPOS PART IIA

XXday XX May 2026 xx:xx to yy:yy

Module 3G1

MOLECULAR BIOENGINEERING I

*Answer not more than **three** questions.*

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.*

*Write your candidate number **not** your name on the cover sheet.*

STATIONERY REQUIREMENTS
Single-sided script paper

SPECIAL REQUIREMENTS TO BE SUPPLIED FOR THIS EXAM
CUED approved calculator allowed

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.

1 An *Escherichia coli* strain has been engineered to constitutively express bicyclobutanase, a novel engineered haem-derived enzyme. Bicyclobutanase has a function that is new to nature: it can generate bicyclobutanes, strained ring molecules which are important in medicinal chemistry. This reaction usually require powerful chemical catalysts and replacement with biocatalysis is a step towards more environmentally-friendly production methods but due to the nature of the reaction and the need for bulk synthesis, large amounts of the enzyme will be required per reaction.

The enzyme is being expressed from a high copy number plasmid and during initial characterisation, the strain produces high levels of bicyclobutanase (5mg/L) and grows at an acceptable rate.

(a) After approximately 50 generations of continuous culture, the productivity of the strain declines substantially, despite no change to growth conditions.

(i) Explain why a loss of productivity over time might be expected given the relationship between genotype, phenotype, and selection pressure. [10%]

Crib: Recombinant expression typically imposes a burden (resource use, toxicity, slowed growth). In continuous culture, mutants gain a fitness advantage and outcompete other cells. Selection acts on growth/fitness, not the production goals of the molecular engineer. Marking (2 marks): 1 mark for linking genotype to phenotype to fitness. 1 mark for mentioning selection pressure arising from expression burden.

(ii) List the main components of a protein expression plasmid and briefly describe their role in maintaining expression of the enzyme. [15%]

Crib:

Origin of replication (ori): determines copy number; Selectable marker: enables maintenance of cells carrying the plasmid (e.g. antibiotic resistance); Promoter: initiates transcription, controls when and how strongly gene is expressed; Ribosome binding site (RBS): controls initiation of translation, speed and strength of protein expression; Gene of interest (coding sequence): encodes the protein to be expressed; Transcription terminator: stops transcription at the correct position and prevents read-through. Marking (2 marks): 1 mark for correct list of five components. 1 mark for correct descriptions. Allow 1 mark for partial list of 3 or more components with correct corresponding descriptions.

(iii) Outline an experimental strategy to determine whether the loss of productivity is primarily due to reduced transcription, reduced translation, or loss/inactivation of the enzyme itself. Your answer should reference the appropriate molecular biology

(cont.

techniques and the outcome you would expect in each case. [15%]

Crib:

Transcription: Semi-quantitative PCR or RT-qPCR for RNA quantification. Translation/protein abundance: SDS-PAGE, could also mention Western blot but this was not covered in lectures (low protein with normal mRNA suggests translational issue or degradation); Function: enzyme activity assay (normal protein abundance but low activity suggests misfolding/inactivity). Marking (3 marks): 1 mark for a correct technique for each of reduced transcription, reduced translation, or loss/inactivation of the enzyme itself.

(b) You are asked to redesign the plasmid system to improve long-term stability. Propose two changes you could make that might reduce the rate at which loss-of-function variants emerge or take over. For each change, discuss one trade-off or limitation it introduces. [20%]

Crib: Strategies can include: inducible/conditional expression to reduce burden except when needed; use of lower copy number plasmid to reduce overall burden; use of weaker promoters and RBS to reduce overall burden; coupling expression to growth advantage e.g. auxotrophy; negative autoregulation to reduce noise/burden; toxin–antitoxin/addiction systems. Trade-offs: lower max yield, increased complexity, reduced flexibility, added burden elsewhere. Marking (4 marks): 1 mark each for up to two valid changes proposed (subtotal: 2 marks), 1 mark each for a valid trade-off or limitation with sufficient discussion to demonstrate an understanding of how it links to the proposed change (subtotal: 2 marks).

(c) If it is not possible to design expression systems that produce sufficient bicyclobutanase to be economically viable as a catalyst, another approach is to improve the catalytic activity or the stability of the enzyme. This reduces the amount of enzyme needed to obtain the same yield of product. Often this involves changes to the active site pocket or catalytic residues to improve activity, or to the enzyme surface and the inter-domain interactions to improve stability.

(i) Name and briefly define the four levels of protein structure and the type of bond which stabilises them. [10%]

Crib: Primary structure: linear amino acid sequence of the polypeptide chain, peptide bonds. Secondary structure: regular folding patterns such as alpha-helices and beta-sheets, hydrogen bonding. Tertiary structure: 3D fold of a single polypeptide chain, wide range of side-chain interactions. Quaternary structure: multiple polypeptide subunits in a functional protein complex, wide range of interactions. Marking (2 marks): 1 mark for correctly naming the four levels of protein structure, 1 mark for correctly identifying the types of bond which stabilises

them. Allow 1 mark for partially correct answer with 3 or more correct levels and bonds.

(ii) Explain the experimental approach you would take to generate and screen new enzyme variants. [5%]

Crib: Generation of genetic diversity (e.g. *de novo* design, random mutagenesis or targeted mutagenesis), expression of a library of variants, screening or selection for improved catalytic activity or stability. Marking (1 mark): 1 mark for describing a valid experimental approach.

(iii) Describe the main experimental steps in your approach and the appropriate controls to include. [15%]

Crib:

Expected steps include: Generation of variant library (e.g. error-prone PCR or site-directed mutagenesis); cloning into expression plasmids, expression in cells or cell-free systems; assay to measure catalytic activity and stability; identification and validation of improved variants; potentially mentioning feeding data back into a design-build-test-learn cycle. Appropriate controls may include: wild-type enzyme comparator; no-enzyme or inactive-enzyme negative controls; controls to distinguish improved activity from higher expression levels. Marking (3 marks): 3 marks for a correct answer i) listing valid experimental steps matching previous answer, ii) describing the experimental steps in sufficient detail to demonstrate knowledge of the techniques and iii) listing valid controls. 2 marks for a largely correct answer including all three components but with some minor errors. 1 mark for a largely correct answer including all three components but with one or more major errors, or a correct answer with no attempt to include one of the three components.

(iv) Briefly describe two technological reasons that successful engineering of enzymes with new-to-nature functions is more common than in previous decades. [10%]

Crib: Any two of the following, with explanation: advances in DNA synthesis and sequencing; high-throughput screening and selection technologies; improved structural biology and computational tools especially AI/ML approaches; automation, accelerating iterative design–build–test cycles. Marking (2 marks): 1 mark per reason from list above.

2 A transgenic strain of *Drosophila suzukii*, a major agricultural pest, has been developed for environmental release as part of a population-control strategy. The strain carries a transgene designed to reduce the viability of offspring when crossed with wild-type flies. This should, over time, reduce population size and crop damage in areas where releases take place. The transgene can be deactivated in the lab because the promoter is repressed by tetracyline added to the fly media, but it is active in the environment in the absence of antibiotic.

(a) After release, it is necessary to monitor the environmental spread of the transgene. PCR can be used on DNA extracted from *Drosophila suzukii* captured in the environment. You design primers that generate a 1546 bp product from transgenic flies and a 236 bp product from wild-type flies.

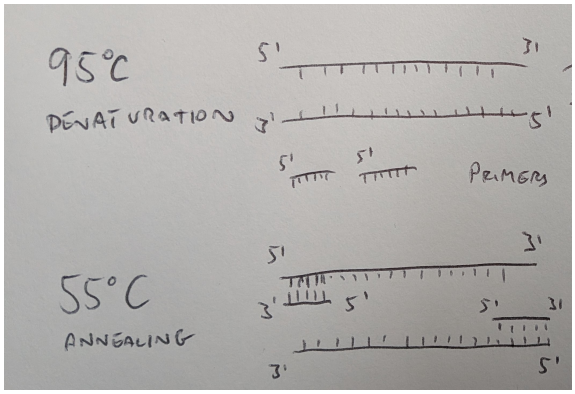
(i) Briefly explain the principles of PCR and why it is a suitable molecular assay for this application. [10%]

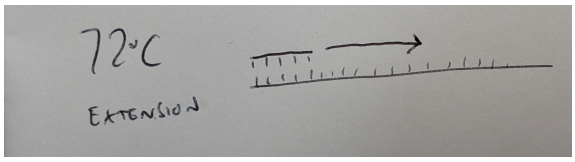
Crib: PCR uses cycles of denaturation, primer annealing, and DNA polymerase extension to exponentially amplify a specific DNA sequence defined by primer binding sites. It is suitable because it is sensitive, specific, and can detect low-abundance DNA in mixed environmental samples. Marking (2 marks): 1 mark for explaining that PCR employs thermocycling, primers and extension of DNA by an enzyme. 1 mark for describing at least two of the above reasons for its suitability.

(ii) Using a diagram, describe the base-pairing and interactions of the target DNA and primers at each step in a PCR cycle. [10%]

Crib: Denaturation at 95 C separates strands; annealing at 55-60 C (typically) involves complementary base pairing between primers and template; extension at 72 C involves DNA polymerase extending from the primer 3'-OH in the 5' to 3' direction.

Example diagram:





Marking (2 marks): 2 marks for diagram showing correct base-pairing and polarity during three temperature stages of the thermocycling process, 1 mark for two out of three correct stages.

(iii) After gel electrophoresis, you observe bands at both 1546 bp and 236 bp in single *Drosophila suzukii* individuals sampled from the environment, while a lab-validated transgenic positive control produces a single band. Give two explanations for this result. [10%]

Crib: Heterozygous individuals that inherited one mutant allele (homozygotes would require two heterozygotes to mate and would have reduced survival, so less abundant in population and there is bias in capture); contamination in environmental samples or in the preparation of DNA in the lab. Marking (2 marks): 1 mark per correct explanation.

(b) Some genetic systems, such as gene drives, increase the spread of a transgene by ensuring that more than 50% of offspring inherit the gene (i.e. more than expected under standard Mendelian inheritance). This reduces the number of insects required for a successful release and thus the operations costs of the control programme.

(i) Describe one example of a gene drive mechanism. [5%]

Crib: Examples include: homing drives with CRISPR or homing endonuclease genes, where the drive cuts the wild-type allele and copies itself via homology-directed repair, biasing inheritance; *Wolbachia* or similar endosymbionts that confer mating (cytoplasmic) incompatability). Marking (1 mark): 1 mark per correct description, which must include a mechanism. This could be invented rather than an existing one, as long as it is logically sound.

(ii) Describe three biosecurity or public-perception objections that different stakeholders might raise regarding the release of insects carrying a gene drive system. [15%]

Crib: Concerns may include uncontrollable spread of the gene drive system, ecological impact, irreversibility, cross-border effects, dual-use or misuse, lack of public consent, or governance challenges. Marking (3 mark): 1 mark per correct objection. Description must demonstrate why it matters for oversight or regulation.

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(c) During the release phase, it is useful for transgenic flies to have survival advantages that increase their likelihood of successfully mating compared to wild-type flies. *Drosophila suzukii* can only tolerate relatively low levels of dietary ethanol. As it breeds on rotting fruit, increasing this tolerance might confer a survival advantage. You consider two ways to increase ethanol tolerance: i) directed evolution; ii) overexpression of recombinant alcohol dehydrogenase, which is an enzyme that metabolises ethanol.

(i) Briefly explain the concept of directed evolution. [5%]

Crib: Directed evolution involves applying strong selection pressure to a population that enriches phenotypes of interest. The underlying advantageous genotype is enriched without requiring detailed mechanistic understanding of what it is. Marking (1 mark): 1 mark for a correct definition.

(ii) Outline a directed-evolution strategy to increase ethanol tolerance in Drosophila suzukii. [15%]

Crib: Must include i) generation of variation (mutagenesis or standing variation), ii) application of selective pressure (increasing ethanol exposure), iii) population-level selection, and iv) iteration. Should explicitly link tolerance to fitness and reproductive success. Marking (3 marks): 2 marks for correctly identifying steps i-iv, 1 mark for a mostly correct strategy. 1 additional mark for explicitly linking tolerance to fitness and reproductive success.

(iii) How would you track the genetic changes introduced during the directed evolution campaign? [5%]

Crib: Whole-genome sequencing would be best answer, targeted sequencing of candidate loci is acceptable. Marking (1 mark): 1 mark for either answer provided in crib.

(iv) You decide to try the overexpression of alcohol dehydrogenase. This involves generating a new promoter-enzyme fusion in a plasmid that enables CRISPR-based integration into the *Drosophila suzukii* genome. Assuming that you are using a Type IIS Assembly-compatible plasmid and you *de novo* synthesised the DNA components with the appropriate overhangs and restriction sites, describe the key steps in the protocol to generate a sequence-validated plasmid. [15%]

Crib: Digest/assembly of plasmid and synthesised DNA using Type IIS enzymes to generate overhangs, ligation or one-pot assembly allowing correct ordering of the fragments via the overhang base pairing and sealing the nick, transformation into E. coli to enable plasmids to replicate, colony selection typically using marker on the plasmid e.g. antibiotic resistance, plasmid extraction, and sequence validation (e.g. Sanger sequencing). Marking (3 marks): 2 marks for correctly listing all key steps with description, 1 mark for correct order/sequence.

(v) Explain why directed evolution is likely to be more effective than rational design for improving ethanol tolerance in this system. [10%]

Crib: Rational design like targeted overexpression is limited by incomplete system knowledge and the multiple steps in the metabolic pathway. Directed evolution exploits selection across many loci. Ethanol tolerance is unlikely to be a single gene trait. Marking (2 marks): 1 mark for describing how rational design and directed evolution differ, 1 mark for linking explanation to genotype of ethanol tolerance.

3 A well-studied synthetic genetic circuit is the repressilator, a ring of transcriptional repressors arranged in a negative-feedback loop that generates oscillations in gene expression. You are given a bacterial chassis strain growing exponentially in a defined medium with a doubling time of 30 minutes. A 3-protein repressilator implemented in this chassis produces precise oscillations with a period of 480 minutes. A modified design of this oscillator uses a 5-protein repressilator, increasing circuit complexity and burden. Oscillations are monitored using a fluorescent protein reporter with finite maturation and dilution kinetics.

(a) Explain qualitatively how the expression of a synthetic gene circuit can affect the doubling time and volume increase rate of the carrier cell, and interpret the observed period for the 3-protein repressilator. [10%]

Crib: Metabolic Burden (1 Mark): The synthetic circuit imposes a metabolic burden by competing with the host cell for limited shared resources (e.g., RNA polymerase, ribosomes, and ATP). This diversion of resources away from endogenous processes reduces the cell's volume increase rate and results in a longer doubling time compared to the chassis's baseline.

Period Interpretation (1 Mark): A 480-minute period in a chassis with a 30-minute nominal doubling time suggests an oscillation spanning roughly 16 generations. Since the circuit presence causes burden, the actual doubling time of the carrier cell is likely slightly slower than 30 minutes (e.g., 32 minutes per generation for a 15-generation cycle, as shown in the class).

(b) You can use time-resolved microscopy to quantify the rate change of volumes of individual cells over time as they grow and divide.

(i) Describe the expected pattern of cell volume (or length) change versus time when a single lineage of the chassis strain is imaged at 5-minute time resolution over several generations. [10%]

Crib: Over several generations, the volume (or length) of an individual cell will exhibit a sawtooth pattern. Within a single generation, the volume $V(t)$ increases exponentially, following the relationship $V(t) = V_0 e^{\mu t}$. At the point of cytokinesis, the volume decreases sharply (by approximately 50%) as the mother cell divides into two daughter cells, before the exponential increase resumes. Marking (2 Marks): 1 Mark: Identification of exponential increase in volume/length and 1 Mark: Identification of the sharp decrease/reset during division into daughter cells.

(ii) Explain how the presence and operation of a repressilator circuit within these

cells could modify these single-cell growth trajectories. [10%]

Crib: The fluctuating expression of the repressilator proteins and the fluorescent reporter creates a dynamic burden. This causes the exponential growth exponent (μ) to vary over time rather than remaining constant. Additionally, the reduced overall growth rate causes the inter-division points (the time between division events) to be further apart compared to the chassis strain. Marking (2 Marks). 1 mark for noting the non-constant growth exponent due to reporter expression; 1 mark for noting the increased time between division events.

(iii) Explain why using a much slower imaging interval (e.g. one image every 20 minutes) would be problematic for interpreting both growth dynamics and oscillator behaviour in these cells. [5%]

Crib: A 20-minute interval leads to temporal aliasing because the sampling frequency is too low relative to the 30-minute doubling time. This results in poor resolution of division timing, leading to significant errors in estimating growth rates and making it impossible to accurately correlate circuit dynamics with the cell cycle. 1 mark for discussing sampling issues/aliasing and the resulting errors in growth-rate or division estimates.

(c) Fluorescence time traces from single cells carrying the repressilator are measured.

(i) Do you expect the oscillations to be symmetric and sinusoidal, like a harmonic oscillator? Why or why not? [10%]

Crib: The oscillations are not expected to be symmetric or sinusoidal. Unlike a harmonic oscillator, these biological pulses are characterized by a distinct build-up phase driven by protein synthesis and a separate dilution/decay phase. This results in a non-symmetric waveform where the rates of increase and decrease in concentration are governed by different kinetic processes. Marking: 1 mark for stating they are not symmetric/sinusoidal and 1 mark for explaining the distinct build-up and dilution phases.

(ii) The fluorescent protein reporter has finite maturation time and is diluted by growth. Explain how these properties modify the observed fluorescence signal relative to the underlying protein dynamics of the oscillator. [10%]

Crib: The finite maturation time of the fluorescent reporter introduces a time delay between the synthesis of the protein and the detection of the signal, causing the observed fluorescence peaks to shift to later times. Additionally, the combination of maturation delays and dilution by cell growth prevents all synthesized proteins from reaching a fully fluorescent state before being diluted, which reduces the observed signal amplitude. Marking: 1 mark for identifying the peak shift/delay due to

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maturation; 1 mark for identifying the reduction in signal amplitude.

(iii) For the 5-protein repressilator, what is the expected inter-peak interval in any particular colour channel (assume that the expression of each fluorescence reporter is being regulated by individual repressors from the repressilator circuit)? [10%]

Crib: The period of a repressilator scales with the number of components in the loop. Since the 3-protein repressilator has a period of 480 minutes (160 minutes per repressor stage), a 5-protein repressilator increases the circuit length and total delay. Therefore, the expected inter-peak interval should increase proportionally to at least 800 minutes (160×5). Marking: 2 marks for calculating or stating the expected interval of at least 800 minutes based on the increased complexity.

(iv) How do you expect the inter-peak interval estiamte to change, if we add a degradation tag to the reporter to speed up its removal rate? [5%]

Crib: Adding a degradation tag to the reporter is expected to have little to no effect on the inter-peak interval. The period of the oscillator is determined by the dynamics of the repressilator proteins themselves, not the reporter. Furthermore, given the very long period (800 minutes), the removal rate of the reporter does not significantly influence the timing of the underlying circuit oscillations. Marking: 1 mark for stating that the interval will not change significantly because the period is dominated by the circuit dynamics rather than reporter dilution/removal.

(d) Cells carrying the 5-protein repressilator are plated sparsely on agar to form colonies derived from single cells.

(i) Describe the spatial or temporal fluorescence patterns you might observe as the colony expands. [10%]

Crib: As the colony expands radially from a single cell, the oscillations in gene expression are captured in space. This results in the formation of concentric rings of fluorescence. Each ring represents a period of the oscillator, where cells at a certain distance from the center were at the same phase of expression during the colony's growth. Marking: 2 marks for describing the formation of concentric rings in the colony images.

(ii) Would you expect cells within a single colony to remain phase-synchronized or gradually desynchronize? Why? [10%]

Crib: The cells are expected to gradually desynchronize over time. This is due to intrinsic and extrinsic biological noise, including stochasticity in gene expression and variations in individual cell growth rates. Even if they start from a single synchronized progenitor, these small fluctuations accumulate, leading to a loss of phase coherence across the colony. Marking: 2 marks for stating they will slowly

desynchronize and attributing this to biological noise in expression and growth.

(iii) You acquire time-lapse images of colonies from cells plated from the liquid culture at regular intervals. How can we estimate the doubling time of cells in the culture from colony images? [10%]

Crib: The doubling time can be estimated by analyzing the phase shift of the concentric rings as a function of the plating intervals, as shown in the class from the paper by Rigler at al 2019. By comparing the phase of the oscillator (the position of the rings relative to the colony center) across colonies started at different known times, the underlying growth rate and doubling time of the cells in the source culture can be back-calculated. Marking: 2 marks for explaining that the phase shift of the rings relative to plating intervals allows for the estimation of doubling time.

4 Some temperate bacteriophages (e.g., Lamba phage) can follow two alternative developmental pathways upon infecting a bacterial cell. In the lytic state, the phage expresses genes that replicate its genome and lyse the host. In the lysogenic state, the phage integrates into the host bacteira's chromosome and expresses a repressor that keeps the lytic genes off while the host continues to grow. Entry into lysogeny is controlled by a genetic toggle switch consisting of two mutually repressing proteins (e.g., CI and Cro in Lamba phage). This circuit produces bistability, allowing the phage genome to stably maintain one of the two states.

(a) The phage toggle switch consists of two transcriptional repressors that inhibit each other. Explain why temperate phages use two repressors rather than two activators for the switch. [10%]

Crib: A circuit consisting of two mutual activators generally produces a "lock-on" behavior where the stable states are either both proteins "on" or both proteins "off" (on-on/off-off). In contrast, a toggle switch consisting of two mutual repressors creates an "on-off" or "off-on" logic. For a bacteriophage, the lytic and lysogenic pathways must be mutually exclusive; the cell cannot be in both states simultaneously. Therefore, mutual repression is required to ensure that the expression of one state's master regulator actively prevents the expression of the other. Marking: 2 marks for comparing the on-on/off-off behavior of two activators with the on-off/off-on exclusive behavior of two repressors.

(b) Explain (with a sketch) why a single activator with a high Hill coefficient (large n) cannot reliably replace the two-repressor toggle switch. [10%]

Crib: While a single activator with positive autoregulation and a high Hill coefficient can theoretically produce bistability, it is difficult to tune reliably. Depending on the promoter strength and the degradation rate, the system often collapses into monostability where the protein either cannot activate itself at all (remaining "off") or becomes stuck in the "on" state. Furthermore, a single activator lacks the active mutual exclusion provided by a second repressor, making the switch highly sensitive to parameter fluctuations and leaky expression, which could inadvertently trigger the irreversible lytic pathway. Marking: 2 marks for explaining that positive feedback can lead to monostability or parameter-sensitive bistability compared to the more robust two-repressor toggle.

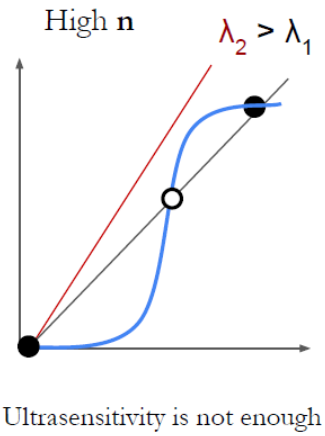

Fig. 1

(c) Consider a simplified deterministic model of mutual repression:

(i) Write the expressions for the model for each repressor. [10%]

Crib: The system can be described by two coupled ordinary differential equations (ODEs). The rate of change for each repressor concentration (X and Y) is defined by a production term following a Hill function (representing the repression by the opposing protein) and a first-order decay term (representing dilution and degradation):

$$\frac{dX}{dt} = \frac{\alpha_1}{1+Y^n} - d_1 X$$

$$\frac{dY}{dt} = \frac{\alpha_2}{1+X^m} - d_2 U$$

Where α represents the maximum production rate, d is the dilution/degradation rate, and n, m are the Hill coefficients representing the cooperativity of repression. Marking: 1 mark for providing the ODEs with a repression-based production term (e.g., Hill function); 1 mark for including the linear dilution/degradation term.

(ii) Qualitatively sketch or describe the nullclines (where one variable's rate of change is zero, giving you plots of each repressor's abundance as a function of the other repressor) when n = 1 and discuss whether we expect bistable states to appear. [10%]

Crib: For $n = 1$, the nullclines are derived by setting the ODEs to zero, resulting in $X = \frac{\alpha_1}{d_1(1+Y)}$ and $Y = \frac{\alpha_2}{d_2(1+X)}$. These curves are hyperbolas that are convex toward the origin. When $n = 1$, the nullclines intersect at only a single stable fixed point. Consequently, bistability cannot occur with $n = 1$; the system is monostable.

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Marking: 1 mark for describing or sketching that the nullclines intersect at only one point; 1 mark for stating that bistability does not occur when $n = 1$.

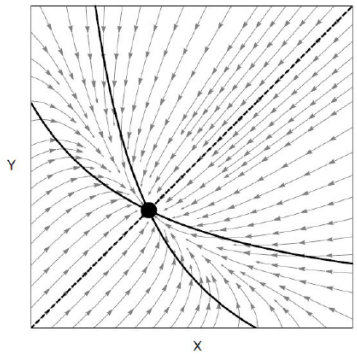

Fig. 2

(iii) Describe (with a qualitative sketch) how the nullclines change when n is increased (e.g., n more than 3) and if we expect bistability to appear. [10%]

Crib: As the Hill coefficient n increases, the nullclines become increasingly sigmoidal (S-shaped), exhibiting a sharper transition between high and low states. This increased non-linearity allows the two nullclines to intersect at three distinct points rather than just one. Under appropriate parameter conditions for α and d, the two outer intersection points represent stable fixed points (bistability), while the middle intersection point represents an unstable steady state (the saddle point).

Marking: 1 mark for sketching or describing sigmoidal nullclines that intersect at three points; 1 mark for identifying that two of these points are stable, leading to bistability.

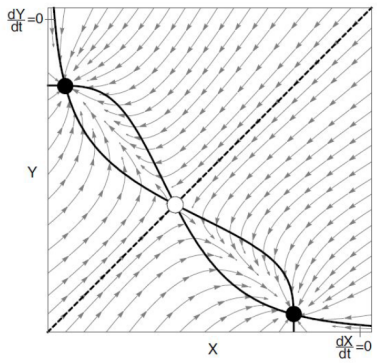

Fig. 3

(d) During lysogeny, the lysogenic repressor must be constitutively expressed, imposing a metabolic burden on the cell. Assume the lysogen produces 20 molecules per min of repressor protein, and the dilution/degradation rate is 0.02 per min.

(i) Estimate the steady-state copy number of the lysogenic repressor. [10%]

Crib: At steady state, the rate of production equals the rate of dilution/degradation. Using the relationship $S = \frac{k}{d}$, where $k = 20$ molecules/min and $d = 0.02$ min^{-1}:

$$S = \frac{20}{0.02} = 1000 \text{ molecules}$$

Marking: 2 marks for using the ratio of production to dilution rates to correctly estimate 1000 molecules.

(ii) If the cell contains 100,000 total protein molecules, estimate the percentage of proteome occupied by the repressor. [5%]

Crib: The proteome fraction is calculated by dividing the steady-state repressor count by the total protein count:

$$\text{Percentage} = \frac{1000}{100,000} \times 100 = 1\%$$

Marking: 1 mark for dividing the steady-state count by the total protein count to reach 1%.

(iii) Discuss how this proportion of proteome allocation could affect the growth rate of lysogens and their competitive fitness in mixed populations containing uninfected cells. [10%]

Crib: Model Answer: Allocating 1% of the proteome to a non-essential repressor protein reduces the resources available for host-specific functions, particularly for ribosomes. Since the growth rate is proportional to the ribosomal fraction of the proteome, this metabolic burden leads to a decrease in the growth rate of the lysogen. In a mixed population, uninfected cells (which do not bear this cost) will grow faster and eventually outcompete the lysogens.

Marking: 2 marks for explaining that reduced proteome allocation for ribosomes slows growth, allowing uninfected cells to outgrow the carrier cells.

(e) Imagine you are engineering a next-generation therapeutic phage virus whose infection decision is controlled by a synthetic toggle switch. The phage must: a) remain dormant (lysogenic) in healthy carrier cell and b) switch to lytic growth only under infection-associated signals.

(i) Propose necessary modifications to the canonical two-repressor toggle switch described above to achieve this behaviour. [10%]

Crib: To make the switch responsive to a specific external stimulus, one of the repressors in the canonical toggle must be replaced with a repressor that is allosterically regulated by the infection-associated signal. Specifically, the promoter controlling the lytic state should be regulated by a repressor that loses its DNA-binding affinity (or is degraded) in the presence of the signal. This allows the switch to flip from the stable lysogenic state to the lytic state only when the infection signal is detected.
Marking: 2 marks for discussing the replacement of a repressor with a signal-sensing repressor-operator pair that depends allosterically on the infection signal.

(ii) If the infection associated signalling molecule cannot pass through the membrane of the chassis cell, what design changes are necessary? [10%]
Crib: If the signal molecule is membrane-impermeable, a two-component signaling system (TCS) must be integrated into the design. This would involve a membrane-bound sensor kinase that detects the external signal and subsequently phosphorylates an internal response regulator. This response regulator would then act as the actuator for the toggle switch, either by repressing or activating the master regulators of the lytic/lysogenic states.
Marking: 2 marks for discussing the incorporation of a sensor module/two-component system to relay the signal to an internal response regulator.

(iii) How would you acquire the necessary parts for this new design? [5%]
Crib: The necessary genetic parts, such as the sensor kinase and response regulator genes, can be sourced from established synthetic biology parts libraries (e.g., iGEM Registry of Standard Biological Parts). One would specifically look for characterized sensing modules from relevant pathogenic strains, such as the Salmonella-derived environmental sensors discussed in class, and synthesize or clone these sequences into the phage vector.
Marking: 1 mark for mentioning the use of parts libraries to find infection-sensing modules (e.g., from Salmonella or other specific strains).

END OF PAPER

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