# Appendix A: Biological Questions and Model Overview

## A1. Biological Questions

Antimicrobial resistance is often treated as a binary property, with cells classified as either resistant or sensitive. However, resistance may also emerge dynamically as a time-dependent intracellular phenotype, shaped by feedback between antimicrobial exposure, cellular growth, and the expression of resistance determinants.

The central question of this project is:

> *Can weak intrinsic host efflux activity sustain sufficient growth under antimicrobial pressure to permit plasmid copy-number amplification and delayed expression of plasmid-encoded efflux pumps, ultimately leading to full resistance, even when plasmid-encoded resistance alone is initially insufficient?*

More specifically, this work addresses the following questions:

- Under what conditions does a resistance plasmid invade or go extinct within a single cell?
- How do intracellular drug accumulation, growth inhibition, and plasmid burden interact to determine this outcome?
- Does intrinsic host efflux activity reduce intracellular drug concentration sufficiently to permit plasmid amplification and delayed resistance expression?

This work focuses on intracellular dynamics within a single cell, treating resistance emergence as a race between antimicrobial toxicity and plasmid-driven efflux expression.

## A2. Model Assumptions

To address this question, a mechanistic intracellular model is constructed with the following assumptions:

- The cell is treated as a well-mixed compartment.
- The extracellular antimicrobial concentration is constant in time.
- Antimicrobial action is bacteriostatic, acting by reducing growth rate.
- Plasmid replication is coupled to host growth and incurs a fitness cost.
- Efflux pump expression is proportional to plasmid copy-number and introduces a delay between plasmid amplification and resistance.
- Plasmid copy-number is treated as a continuous variable in the deterministic model, but stochastic effects at low copy-numbers are introduced later.

Under these assumptions, resistance is an emergent property of the intracellular dynamical system, rather than a fixed trait.

## A3. State Variables

The intracellular system is described by the following state variables:

| Variables | Description |
|---|---|
| $A_{(t)}$ | Intracellular antimicrobial concentration |

| | |
|---|---|
| $P_{(t)}$ | Plasmid copy-number |
| $M_{(t)}$ | Efflux pump mRNA |
| $Q_{(t)}$ | Efflux pump protein |

---

**A4. Full ODE System**

The intracellular dynamics are governed by the following system of ordinary differential equations:

Antimicrobial dynamics:

$$\frac{dA}{dt} = k_{in}A_{ext} - k_{out}E(Q)A$$

where total efflux activity is

$$E(Q) = E_h + \beta Q$$

Growth inhibition

$$G(A) = G_{max}\frac{1}{1 + \left(\frac{A}{IC_{50}}\right)^h}$$

Plasmid copy-number dynamics

$$\frac{dP}{dt} = r_P G(A)P - c_P P - \gamma P^2$$

Efflux pump gene expression

$$\frac{dM}{dt} = k_m P - \delta_m M$$

$$\frac{dQ}{dt} = k_q M - \delta_q Q$$

---

**A5. Parameter Definition**

Antimicrobial transport and efflux:

| Parameter | Description |
|---|---|
| $k_{in}$ | Antimicrobial influx rate |
| $k_{out}$ | Efflux rate per unit efflux activity |
| $E_h$ | Intrinsic host efflux activity |
| $\beta$ | Efflux activity per efflux protein molecule |
| $A_{ext}$ | Extracellular antimicrobial concentration |

Growth inhibition

| Parameter | Description |
|---|---|
| $G_{max}$ | Maximum growth rate |
| $IC_{50}$ | Intracellular antimicrobial concentration causing 50% growth inhibition |
| $h$ | Hill coefficient |

Plasmid replication and cost

| Parameter | Description |
|---|---|
| $r_P$ | Plasmid replication rate per unit host growth |
| $c_P$ | Linear plasmid-associated fitness cost |
| $\gamma$ | Nonlinear copy-number control parameter |

Gene expression dynamics

| Parameter | Description |
|---|---|
| $k_m$ | Transcription rate per plasmid copy |
| $\delta_m$ | mRNA degradation rate |
| $k_q$ | Translation rate |
| $\delta_q$ | Protein degradation or dilution rate |

---

**A6. Experimental Scenarios**

To explore resistance emergence, model parameters are divided into control parameters and fixed parameters.

Primary control parameters:

- Intrinsic host influx activity, $E_h$
- Intracellular antimicrobial concentration, $A_{ext}$

Experimental scenarios considered:

- No antimicrobial ($A_{ext} = 0$), plasmid present
- Antimicrobial present ($A_{ext} > 0$) plasmid present, no intrinsic efflux ($E_h = 0$)
- Antimicrobial present ($A_{ext} > 0$) plasmid present, intrinsic efflux ($E_h > 0$)