# Model equations

Indicators: $\mathbf{1}_k^{\mathrm{a}}$ live bacterium in cell $k$, $\mathbf{1}_k^{\mathrm{inj}}$ root injection zone.

## 1 Differential equations

### Resveratrol (extracellular)

$$\frac{\partial A_k}{\partial t} = D_A\,(\nabla^2 A)_k - \delta_A A_k - u_k + \sigma_A\,\mathbf{1}_k^{\mathrm{inj}} \tag{1}$$

### TtgR (shared sensor)

$$\frac{dT_k}{dt} = \alpha_T\,\mathbf{1}_k^{\mathrm{a}} - \delta_T T_k \tag{2}$$

$$\frac{dA_{\mathrm{in},k}}{dt} = \rho\,u_k - \delta_{\mathrm{in}} A_{\mathrm{in},k} \tag{3}$$

### Toxin–antitoxin

$$\frac{dF_k}{dt} = \alpha_F\,\mathbf{1}_k^{\mathrm{a}} - \delta_F F_k^{\mathrm{free}} - \delta_{FE}\big(\mathrm{FE}_k + 2\,\mathrm{FEF}_k\big) \tag{4}$$

$$\frac{dE_k}{dt} = \beta_E\,\frac{K_E^{\,n}}{K_E^{\,n} + T_{\mathrm{free},k}^{\,n}}\,\mathbf{1}_k^{\mathrm{a}} - \delta_E E_k^{\mathrm{free}} - \delta_{EF}\big(\mathrm{FE}_k + \mathrm{FEF}_k\big) \tag{5}$$

### CRISPR–Cas9

$$\frac{dL_k}{dt} = \beta_L\,\frac{K_L^{\,n_c}}{K_L^{\,n_c} + T_{\mathrm{free},k}^{\,n_c}}\,\mathbf{1}_k^{\mathrm{a}} - \delta_L L_k \tag{6}$$

$$\frac{dg_k}{dt} = \beta_g\,\frac{K_g^{\,m_c}}{K_g^{\,m_c} + L_k^{\,m_c}}\,\mathbf{1}_k^{\mathrm{a}} - \delta_g g_k - k^{+} C_k g_k + k^{-} g C_k \tag{7}$$

$$\frac{dC_k}{dt} = \beta_C\,\mathbf{1}_k^{\mathrm{a}} - \delta_C C_k - k^{+} C_k g_k + k^{-} g C_k \tag{8}$$

$$\frac{d\,gC_k}{dt} = k^{+} C_k g_k - k^{-} g C_k - k_{\mathrm{cleave}} g C_k - \delta_{gC} g C_k \tag{9}$$

$$\frac{d\,\mathrm{DSB}_k}{dt} = k_{\mathrm{cleave}}\,gC_k - k_{\mathrm{repair}}\,\mathrm{DSB}_k \tag{10}$$

## 2 Algebraic equations

### Uptake

$$u_k = \mathbf{1}_k^{\mathrm{a}}\,\frac{V_{\max} A_k}{K_m + A_k} \tag{11}$$

**Free TtgR**

$$T_{\mathrm{free},k} = \frac{T_k}{1 + (A_{\mathrm{in},k}/K_R)^m} \tag{12}$$

**Toxin–antitoxin binding, $F \equiv F^{\mathrm{free}}$**

$$D(F) = 1 + \frac{F}{K_{D1}} + \frac{F^2}{K_{D1}K_{D2}}, \qquad u(F) = \frac{F}{K_{D1}} + \frac{2F^2}{K_{D1}K_{D2}} \tag{13}$$

$$F + \frac{E_{\mathrm{tot}}\, u(F)}{D(F)} - F_{\mathrm{tot}} = 0 \tag{14}$$

$$E^{\mathrm{free}} = \frac{E_{\mathrm{tot}}}{D(F)}, \qquad \mathrm{FE} = \frac{E^{\mathrm{free}} F}{K_{D1}}, \qquad \mathrm{FEF} = \frac{\mathrm{FE}\, F}{K_{D2}} \tag{15}$$

$$F_{\mathrm{tot}} = F^{\mathrm{free}} + \mathrm{FE} + 2\,\mathrm{FEF}, \qquad E_{\mathrm{tot}} = E^{\mathrm{free}} + \mathrm{FE} + \mathrm{FEF} \tag{16}$$

**Laplacian (zero on fence and root)**

$$(\nabla^2 A)_k = A_{k-L} + A_{k+L} + A_{k-1} + A_{k+1} - 4A_k \tag{17}$$

**Hazards**

$$h_k^{\mathrm{TA}} = \frac{\left(F_k^{\mathrm{free}}\right)^p}{K_{\mathrm{mort}}^p + \left(F_k^{\mathrm{free}}\right)^p}, \qquad h_k^{\mathrm{CR}} = \frac{\mathrm{DSB}_k^q}{K_{\mathrm{DSB}}^q + \mathrm{DSB}_k^q} \tag{18}$$

$$P_k^{\mathrm{death}} = 1 - \exp\Bigl(-\Delta t \sum_{\mathrm{modules}} h_k^{(\cdot)}\Bigr) \tag{19}$$

**Movement and division**

$$P_k^{\mathrm{act}} = 1 - \exp\bigl(-r_k \Delta t\bigr), \qquad r_k \in \{\, k_{\mathrm{move}},\ \mu_{\max} \,\} \tag{20}$$

$$\mu_k = \frac{\mu_{\max}}{1 + \left(F_k^{\mathrm{free}}/K_{\mathrm{mort}}\right)^p} \qquad \text{(bacteriostatic)} \tag{21}$$

$$w_{k\to c} \;\propto\; \exp\bigl[\chi\,(A_c - A_k)\bigr] \tag{22}$$

**Division inheritance**

$$X'_{\mathrm{m}} = X'_{\mathrm{d}} = \tfrac{1}{2} X_{\mathrm{m}} \quad \text{(concentrations)}, \qquad X'_{\mathrm{m}} = X'_{\mathrm{d}} = X_{\mathrm{m}} \quad \text{(DSB)} \tag{23}$$

**Steady states**

$$T^{\mathrm{ss}} = \frac{\alpha_T}{\delta_T}, \qquad T^{\mathrm{ss}}_{\mathrm{free}}(R) = \frac{\alpha_T/\delta_T}{1 + (R/K_R)^m} \tag{24}$$

$$L^{\mathrm{ss}} = \frac{\beta_L}{\delta_L}\,\mathrm{rep}_L, \qquad \mathrm{rep}_L = \frac{K_L^{n_c}}{K_L^{n_c} + \left(T^{\mathrm{ss}}_{\mathrm{free}}\right)^{n_c}}, \qquad \mathrm{rep}_g = \frac{K_g^{m_c}}{K_g^{m_c} + \left(L^{\mathrm{ss}}\right)^{m_c}} \tag{25}$$

$$k_{\mathrm{off}} = k^- + k_{\mathrm{cleave}} + \delta_{gC} \tag{26}$$

$$gC = \frac{k^+ C g}{k_{\mathrm{off}}}, \qquad C = \frac{\beta_C - (k_{\mathrm{cleave}} + \delta_{gC})\, gC}{\delta_C}, \qquad g = \frac{\beta_g\,\mathrm{rep}_g - (k_{\mathrm{cleave}} + \delta_{gC})\, gC}{\delta_g} \tag{27}$$

$$\mathrm{DSB}^{\mathrm{ss}} = \frac{k_{\mathrm{cleave}}\, gC^{\mathrm{ss}}}{k_{\mathrm{repair}}} \tag{28}$$

### Integration

$$y_{n+1} = y_n + \frac{h}{6}\big(k_1 + 2k_2 + 2k_3 + k_4\big), \qquad h = \Delta t / S \tag{29}$$

$$\lambda = \max\Big\{\delta_T,\ \delta_L,\ \delta_g + k^+\tfrac{\beta_C}{\delta_C},\ \delta_C + k^+\tfrac{\beta_g}{\delta_g},\ k^- + k_{\text{cleave}} + \delta_{gC}\Big\} \tag{30}$$

$$S = \min\Big(64,\ \max\big(1,\ \lceil \Delta t\,\lambda / 1.5 \rceil\big)\Big) \tag{31}$$

## 3 Parameters

### State variables

| Symbol | Quantity | Compartment |
|---|---|---|
| $A$ | resveratrol | extracellular |
| $T$ | total TtgR | intracellular |
| $A_{\text{in}}$ | intracellular resveratrol | intracellular |
| $F$ | total toxin (MazF) | intracellular |
| $E$ | total antitoxin (MazE) | intracellular |
| $L$ | LacI | intracellular |
| $g$ | gRNA | intracellular |
| $C$ | Cas9 | intracellular |
| $gC$ | gRNA–Cas9 complex | intracellular |
| DSB | accumulated genomic damage (counter) | intracellular |

Derived, not integrated: $F^{\text{free}}$, $E^{\text{free}}$, FE, FEF (binding equilibrium) and $T_{\text{free}}$ (fast sensor equilibrium).

### Parameters exposed in the interface

| Symbol | Meaning | Default |
|---|---|---|
| $\sigma_A$ | resveratrol secretion by the root | 1.0 |
| $D_A$ | resveratrol diffusion rate (explicit stability: $< 0.25$) | 0.2 |
| $\delta_A$ | resveratrol degradation rate | 0.01 |
| $\alpha_T$ | TtgR synthesis (constitutive) | 5.0 |
| $\delta_T$ | TtgR degradation | 0.1 |
| $m$ | TtgR–resveratrol cooperativity | 1 |
| $K_R$ | resveratrol at which half the TtgR is sequestered | 10.0 |
| $K_E$ | free TtgR at which antitoxin synthesis halves | 20.0 |
| $n$ | TtgR–antitoxin cooperativity | 2 |
| $\beta_E$ | antitoxin synthesis | 1.0 |
| $\alpha_F$ | toxin synthesis | 0.5 |
| $K_{D1}$ | $F_2 + E_2$ dissociation constant | 0.01 |
| $K_{D2}$ | $F_2E_2 + F_2$ dissociation constant | 0.01 |
| $\delta_F$ | free toxin degradation | 0.1 |
| $\delta_{FE}$ | complexed toxin degradation | 0.01 |
| $\delta_E$ | free antitoxin degradation | 0.1 |
| $\delta_{EF}$ | complexed antitoxin degradation | 0.01 |

| Symbol | Meaning | Default |
|---|---|---|
| $\beta_L$ | LacI synthesis | 10.0 |
| $\delta_L$ | LacI degradation | 0.1 |
| $K_L$ | free TtgR at which LacI synthesis halves | 20.0 |
| $n_c$ | TtgR–LacI cooperativity | 2 |
| $\beta_g$ | gRNA synthesis | 5.0 |
| $\delta_g$ | gRNA degradation | 0.2 |
| $K_g$ | LacI at which gRNA synthesis halves | 30.0 |
| $m_c$ | LacI–gRNA cooperativity (LacI tetramer) | 4 |
| $\beta_C$ | Cas9 synthesis | 2.0 |
| $\delta_C$ | Cas9 degradation | 0.05 |
| $k^+$ | gRNA–Cas9 binding | 0.01 |
| $k^-$ | gRNA–Cas9 dissociation | 0.05 |
| $\delta_{gC}$ | complex degradation | 0.02 |
| $k_{\text{cleave}}$ | cleavage rate (complex consumed on cutting) | 0.1 |
| $k_{\text{move}}$ | motility (jumps per unit time) | 0.5 |
| $\mu_{\max}$ | maximum division rate | 0.01 |
| $\chi$ | chemotaxis ($> 0$ toward resveratrol) | 0 |

## Fixed internal constants

| Symbol | Meaning | Value |
|---|---|---|
| $V_{\max}$ | maximum resveratrol uptake rate | 1.0 |
| $K_m$ | uptake half-saturation | 1.0 |
| $\rho$ | extracellular/intracellular volume ratio | 50 |
| $\delta_{\text{in}}$ | intracellular resveratrol degradation | 0.5 |
| $K_{\text{mort}}$ | free toxin at which the TA hazard is 1/2 | 1.0 |
| $p$ | TA hazard cooperativity | 2.0 |
| $k_{\text{repair}}$ | DNA repair rate (sets $\text{DSB}^{\text{ss}}$) | 0.05 |
| $K_{\text{DSB}}$ | damage at which the CRISPR hazard is 1/2 | 10 |
| $q$ | CRISPR hazard cooperativity | 4.0 |
| $R_{\text{seed}}$ | resveratrol of the source culture (inoculum) | 50 |
| $L_{\text{b}}$ | board side, in cells ($N = L_{\text{b}}^2$; the stride $L$ in the Laplacian) | 100 |
| $\Delta t$ | integration time step | 0.1 |
| $S$ | RK4 substeps (adaptive) | $\geq 1$ |

## Switches

| Switch | Effect |
|---|---|
| TtgR mutant | $\alpha_T \to 0$: both circuits lose the sensor at once |
| Bacteriostatic | free toxin also slows division, not only kills |
| Division split | concentrations halved on division; DSB copied whole |
| Preconditioned | inoculum born at the steady state of $R_{\text{seed}}$, DSB = 0 |
| Auto substeps | $S$ chosen from $\lambda$ instead of fixed |

### Notes

- Production terms are gated by $\mathbf{1}_k^{\mathrm{a}}$; degradation and binding are not.
- $T_{\mathrm{free}}$ is a single shared pool: the two containment systems are coupled through it and share one failure mode.
- The TA hazard reads an instantaneous concentration and is reversible; the CRISPR hazard reads an accumulated counter and is not.
- Hazards add and are exponentiated; probabilities are never summed.
- Without $k_{\mathrm{repair}}$ the damage has no steady state and every bacterium eventually crosses any threshold.