# Supporting Information

# Studies of drug resistance response of sensitive and drug-resistance strains in microfluidic system

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**Anitibiotic mechanism**

Because of the hydrophilic nature of β-lactam antibiotics, Ceftriaxone enter the cell through channel proteins on membrane. Ceftriaxone could bind to the active site of PBPs, and thus block transpeptidation during peptidoglycan synthesis. Although glycan chain polymerization occurs in the absence of transpeptidation[1], without transpeptidation, the health condition of cell wall decays. The study of the molecule organization of peptidoglycan shows that peptidoglycan consists of short glycan chains with oligopeptide crosslinks to others[2]. We define a glycan in the peptidoglycan network is healthy when it has enough crosslinks with other chains and we introduced a variable h to describe the health condition of cell wall, which means the ratio of healthy chains in the peptidoglycan network. We assume that the health condition h will affect the elongation rate and low health condition may cause cell death. β-lactam hydrolase produced by the drug-resist strain controls the antibiotic concentration inside the cell.

**Model**

We built a differential model based on the molecule mechanism to comprehend dose response on a single cell level.

$$\frac{dL}{dt} = KL \quad (1)$$

$$\frac{d[E]}{dt} = (K_0 - K)[E] \quad (2)$$

$$\frac{d[S]}{dt} = \frac{D_1([S_0]-[S])}{([S_0]+[S]+D_2)} - \frac{R_1[S][E]}{R_2+[S]} \quad (3)$$

$$[\mathrm{P}] = \frac{[P_0]}{1+R_3[S]} \quad (4)$$

$$\frac{dh}{dt} = -K(h+c) + b[P] \quad (5)$$

$$\mathrm{K} = K_0(1+R_4)\frac{h}{h+R_4} \quad (6)$$

Additionally, we build a simplified model to describe the death rate or the survival rate, and $\theta(x)$ represent the Heaviside step function. We assume that the probability of a cell dies in an unit time is proportional to the portion that the health condition h is lower than a threshold $h_m$ and the osmotic pressure which is proportional to $\frac{e^{K_0 t}}{L}$, hence we have the death rate change

$$\frac{\mathrm{d}P_m}{\mathrm{dt}} = \frac{K_m(1-P_m)\theta(h_m-h)(h_m-h)e^{K_0 t}}{L} \quad (7)$$

Where Variables:

L: Length of a cell

K: Elongation rate per unit length

[E]: the concentration of β-lactam hydrolase

[S]: Concentration of Ceftriaxone inside the cell

[P]: Concentration of active PBPs

h: Cell wall health condition

$P_m$: Cell death rate

**Constants:**

$K_0$: Elongation rate per unit length without antibiotics

$D_1, D_2$: Constants determined by the Ceftriaxone transmembrane transportation reaction

$R_1, R_2$: Constants determined by the Ceftriaxone hydrolysis reaction

$[S_0]$: Concentration of antibiotics Ceftriaxone where cells are exposed to, different values equal to corresponding environment concentration

$[P_0], R_3$: Constants determined by the Ceftriaxone PBPs binding reaction

c: Constant determined by cell wall health degradation caused by adding new nodes[3]

b: Constant determined by cell wall health increase caused by transpeptidation

$R_4$: Constant determined by glycan insertion reaction

$h_m$: A threshold of health condition, we assume that the probability of a cell dies in an unit time is proportional to the portion that the health condition h is lower than a threshold $h_m$

$K_m$: Proportionality coefficient in death rate equation

## Model derivation

1. Equation (1) is the definition of *K*.
2. Chemicals which are not influenced by Ceftriaxone follow an exponential growth.
3. Let Q represents channel proteins on membrane and [Q] is its concentration. The transmembrane transportation could be written as:

$$[Q] + [S_0] \rightleftharpoons [QS] \rightleftharpoons [Q] + [S]$$

$$v_{1+} = k_1[S_0][Q]$$

$$v_{1-} = k_2[QS]$$

$$v_{2-} = k_1[S][Q]$$

$$v_{2+} = k_2[QS]$$

Because $[Q]$ is much smaller than $[S_0]$ and $[S]$, we applied the quasi-equilibrium hypothesis that

$$v_{1+}+v_{2-}=v_{1-}+v_{2+}$$

Hence derived

$$\frac{d[S]}{dt}_{trans} = v_{2+} - v_{2-} = \frac{k_1([S_0]-[S])[Q_{total}]}{k_1([S_0]+[S])+2k_2} = \frac{D_1([S_0]-[S])}{([S_0]+[S]+D_2)}$$

Ceftriaxone hydrolysis rate follows Michaelis-Menten equation

$$\frac{d[S]}{dt}_{hydro} = -\frac{R_1[S][E]}{R_2+[S]}$$

Then we derived

$$\frac{d[S]}{dt} = \frac{d[S]}{dt}_{trans} + \frac{d[S]}{dt}_{hydro} = \frac{D_1([S_0]-[S])}{([S_0]+[S]+D_2)} - \frac{R_1[S][E]}{R_2+[S]}$$

4. Because Ceftriaxone PBPs binding reaction is much faster than cell elongation, we assume that [P] and [S] are under chemical equilibrium.

$$[\mathrm{P}] + [\mathrm{S}] \rightleftharpoons [\mathrm{PS}]$$

$$K_{ch} = \frac{[PS]}{[P][S]} = \frac{[P_0]-[P]}{[P][S]}$$

Where $K_{ch}$ represents the chemical equilibrium constant in this reaction and $[P_0]$ is the concentration of PBPs when [S]=0. Then we derived.

$$[\mathrm{P}] = \frac{[P_0]}{1+K_{ch}[S]} = \frac{[P_0]}{1+R_3[S]}$$

5. Peptidoglycan grows by inserting new chains and removing old chains. PBPs build crosslinks between glycan chains when they are inserted into peptidoglycan. Thus we assume that new inserted chains are unhealthy chains and they become healthy when PBPs build enough crosslinks for them. Variable h represents the ratio of healthy chains. Without antibiotics, h=1.

The change of h caused by cell elongation

$$\frac{dh}{dt}_{el} = -Kh$$

Where K is elongation rate per unit length.

The change of h caused by the removal of old healthy chains

$$\frac{dh}{dt}_{rm} = -Kc$$

Where c is a constant.

The change of h caused by tranpeptidation in the participation of PBPs

$$\frac{dh}{dt}_{PBP} = b[P]$$

Where b is a constant. Thus

$$\frac{dh}{dt} = -K(h + c) + b[P]$$

6. Although the mechanism of insertion of new glycan chains is unsettled[1], based on the experimental fact that despite different $[S_0]$, K didn't change immediately, but decreased after a comparable time, we propose that K is influenced by the health condition h. We made a hypothesis that insertion of new glycan chains could only initiates on healthy chains. This hypothesis may be understood as crosslinks are significant for the bind of peptidoglycan polymerases, but we don't know the real mechanism. Thus K is proportional to polymerases that bind to healthy chains. The polymerases binding reaction

$$[\mathrm{Pp}] + [\mathrm{Hc}] \rightleftharpoons [\mathrm{PpHc}]$$

Where Pp is peptidoglycan polymerases and Hc is healthy chains.

The chemical equilibrium equation

$$K_{ch} = \frac{[PpHc]}{[Pp][Hc]} = \frac{[PpHc]}{([Pp]_{total} - [PpHc])[Hc]}$$

$$[\mathrm{PpHc}] = \frac{K_{ch}[Pp]_{total}[Hc]}{K_{ch}[Hc] + 1}$$

Where $K_{ch}$ represents the chemical equilibrium constant for this reaction and $[Pp]_{total}$ represents the total concentration of peptidoglycan polymerases. Because [Hc] is proportional to h and when h=1, K=$K_0$, we have

$$\mathrm{K} = K_0(1 + R_4)\frac{h}{h + R_4}$$

Where $R_4$ is constant.

**Description of simulation parameter**

| | |
|---|---|
| K0=0.02 | Initial value of the experimental results |
| D1=0.13 | |
| D2=100 | |
| R1=0.8 | |
| R2=0.01 | Values of D1, D2, R1 and R2 are all derived from data fitting. |
| P0=1 | Initial value of the concentration of active PBPs |
| R3=1000 | Chemical equilibrium constant of the Ceftriaxone PBPs binding reaction, which is derived from data fitting and represents tight combination of Ceftriaxone PBPs binding |
| c=0.25; | Synthesis of 1 mol new chain consumes c mol original health chain and according to the "three for one" model[3]. |

Although the value should be 0.5 in this model, the experimental results fit better taking the value c = 0.25 than the value c = 0.5 and the results when the value c = 0.5 are shown below (Fig. S1). Since the results when importing the value 0.5 show no significant difference and the "three for one" model of elongation is also not clearly established, we definite this constant value as 0.25.

| | |
|---|---|
| b=K0*(1+c) | This term ensures that the value of the term h equals to 1. |

R4=0.01 The value is derived from data fitting.

$K_m$=0.15 The value is derived from data fitting.

$h_m$=0.03 This is a set value according to the relationship between model result of health condition and experimental result of death rate.

**References**

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2. Gan, L.; Chen, S.; Jensen, G. J., Molecular organization of Gram-negative peptidoglycan. *Proceedings of the National Academy of Sciences* **2008,** *105* (48), 18953-18957.

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Figures

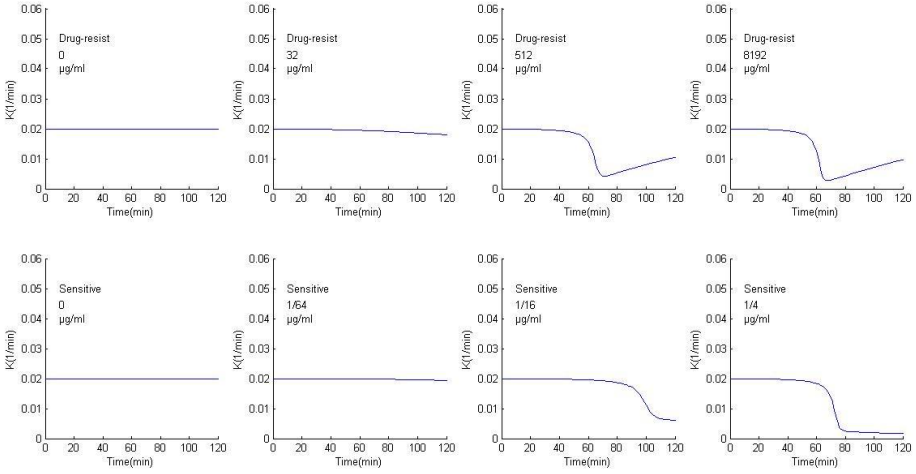


**Fig. S1** The simulation results of growth rate under different concentrations when importing c=0.5