

**Model for the relationship between IPTG concentration and optical density (OD) value**

**iGEM Tsinghua 2015**

1. Model for IPTG-inducible ccdB expression.

(1) Given that the concentration of CAP is stable, that is to say only to consider the negative regulation from lac operon. Here we simply consider the influence of IPTG on the gene expression regulated by lac operon. Therefore we can approximately assume that the transcriptional level of mRNA is proportion to the translational level of its protein.

(2) Since that the expression of lacI is regulated by constitutive promoter, i. e. the concentration of all states of lacI, including free lacI, lacI·IPTG, lacI·promoter and lacI·IPTG·promoter, are consistent. It does no harm to denote that:

$$CT_{lacI}$$

(3) Provided the gene downstream the lacI-bound operator is not expressed, whereas the gene downstream the available operator is expressed. Denote the transcriptional level per unit time when lac operator per unit concentration is not inhibited by lacI is  $m_{mRNA}$ . According to the above assumption, we know that the translational level of the protein when the lac operator per unit concentration is not inhibited by lacI is:

$$m_{ccdB} = K_1 * m_{mRNA}$$

(4) Since that the concentration of the plasmid containing lac operator is constant for a period of time, we de note the concentration as:

$$CT_{plasmid}$$

Besides, the concentration of lac operator is proportional to its plasmid concentration, therefore:

$$CT_{operon} = \theta * CT_{plasmid}$$

(5) There are two states of lac operator, the one bound and in turn inhibited by lacI (or lacI·IPTG) and the one unbound, the concentration of which are denoted as:

$$\begin{aligned} & [operon_{lacI+}] \\ & [operon_{lacI-}] \end{aligned}$$

respectively.

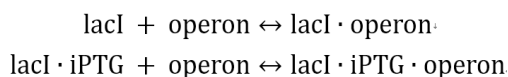
In addition,

$$CT_{operon} = [operon_{lacI+}] + [operon_{lacI-}]$$

Therefore, the total translational level of the protein per unit time is  $M_{mRNA}$ :

$$M_{ccdB} = K_1 * m_{mRNA} * [operon_{lacI-}]$$

(6) Given that lacI and lacI·IPTG will compete for binding to lac, we can therefore deem it as two thermal equilibrium:



Denote the equilibrium constant  $K_{lacI}$  and  $K_{lacI \cdot IPTG}$ , we therefore have:

$$\frac{[\text{lacI} \cdot \text{operon}]}{[\text{lacI}] * [\text{operon}_{\text{lacI}^-}]} = K_{\text{lacI}}$$

$$\frac{[\text{lacI} \cdot \text{iPTG} \cdot \text{operon}]}{[\text{lacI} \cdot \text{iPTG}] * [\text{operon}_{\text{lacI}^-}]} = K_{\text{lacI-iPTG}}$$

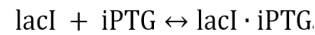
Notice that because iPTG will cause the conformation of lacI to change, and in turn significantly inhibit its binding capacity to lac operator, we have:

$$K_{\text{lacI-iPTG}} \ll K_{\text{lacI}}$$

Due to the above definition:

$$[\text{operon}_{\text{lacI}^+}] = [\text{lacI} \cdot \text{operon}] + [\text{lacI} \cdot \text{iPTG} \cdot \text{operon}]$$

(7) The binding of lacI to iPTG can be regarded as an equilibrium:



Denote the equilibrium constant as  $K_{\text{iPTG}}$ , then:

$$\frac{[\text{lacI} \cdot \text{iPTG}]}{[\text{lacI}] * [\text{iPTG}]} = K_{\text{iPTG}}$$

Also denote the total concentration of iPTG in the solution as  $CT_{\text{iPTG}}$ :

$$CT_{\text{iPTG}} = [\text{iPTG}] + [\text{lacI} \cdot \text{iPTG}]$$

According to assumption (2):

$$CT_{\text{lacI}} = [\text{lacI}] + [\text{lacI} \cdot \text{iPTG}] + [\text{lacI} \cdot \text{operon}] + [\text{lacI} \cdot \text{iPTG} \cdot \text{operon}]$$

We can derive the relationship between  $CT_{\text{iPTG}}$  and  $M_{\text{ccdb}}$ :

2. Model for the relationship between cell death and cell concentration.

3. Model for the relationship between cell concentration and cell optical value.

$$OD \propto C_{E.coli}$$

Reference data:

Dilution time	OD600(1)	OD600(2)	OD600(3)	Mean $\pm$ SD
1	1.357	1.371	1.377	1.368 $\pm$ 0.008
2	0.733	0.738	0.742	0.738 $\pm$ 0.008
3	0.518	0.522	0.525	1.368 $\pm$ 0.008
4	0.392	0.394	0.393	1.368 $\pm$ 0.008
5	0.299	0.303	0.306	1.368 $\pm$ 0.008
6	0.258	0.259	0.264	1.368 $\pm$ 0.008
8	0.2	0.201	0.208	1.368 $\pm$ 0.008
10	0.157	0.160	0.162	1.368 $\pm$ 0.008

The fit curve is:

$$OD = \lambda * C_{E.coli}$$

$$OD600 \approx 1.76 - \frac{1.81}{1 + e^{-0.29 \times (C_{\text{iPTG}} - 12.74)}}$$