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CHEME 5440 PRELIM

1. mRNA copy per cell for many promoters using smFISH in single dividing

E coli Plac promoter, expressing ⇒ lacZ gene

Assume (i) $T_d \simeq 40 min$, (ii) OD600 = 0.1 ⇒ $1\times10^{8} \frac{cell}{mL}$ (i) promoter function in terms of extracellular inducer (ignore inducer transport) (iv) assume lacZ gene is present in 2 $\frac{copies}{cell}$ (v) lacZ mRNA half-life 5 min, (vi) characteristic transcript length 1000nt.

(a) $\langle n \rangle$ values ($\frac{mRNA}{cell}$) to specific volume basis $B = \langle m_c \rangle \hat{N}_c V$

Sample size of 1 mL at $\underbrace{OD600}_{\text{optical density}} = 0.1 \rightarrow 1\times10^{8} \frac{cells}{mL}$ $B[=]\left(\frac{gDW}{cell}\right)\left(\frac{cell}{mL}\right)(mL) = gDW$

$\langle m_c \rangle [=] \frac{mass}{cell} [=] \frac{gDW}{cell}$ $\hat{N}_c [=] \frac{cell}{vol} [=] \frac{cell}{mL}$ $V = mL = 1 mL$ ← sample volume

$B = \left(433\times10^{-15} \frac{gDW}{cell}\right)\left(1\times10^{8} \frac{cells}{mL}\right)(1 mL) = \boxed{4.33\times10^{-5}\ gDW}$

specific units: $\frac{nmol}{gDW}$ $\frac{\langle n \rangle mRNA/cell}{\cancel{4.33\times10}\ gDW} \cdot \frac{1 mol\ mRNA}{6.02\times10^{23}\ mRNA} \cdot \frac{1\times10^{9} nmol}{1 mol}$

$\langle m_c \rangle = 433 fg = 433\times10^{-15}$ gdw / cell (from Bionumbers) ecoli cell with $T_D = 40$ min

(b) $\frac{dm_i}{dt} = \dot{m}_i = r_{x,i}\bar{u}_i - (\mu + \theta_{m,i})m_i$ $0 = r_{x,i}\bar{u}_i - (\mu + \theta_{m,i})m_i$

$$\boxed{m_i^* = \frac{r_{x,i}\bar{u}_i}{\mu + \theta_{m,i}} = \left(\frac{r_{x,i}}{\mu+\theta_{m,i}}\right)\bar{u}_i \quad K_x(G,\theta) = \left(\frac{r_{x,i}}{\mu+\theta_{m,i}}\right) \quad \bar{u}(I,t) = \bar{u}_i}$$

(c)

$u = \frac{\overbrace{N}^{\text{sum of microstates leading to trans}}}{\underbrace{D}_{\text{all possible microstates}}}$ $u(I) = \frac{w_1 + w_2 f_I}{1 + w_1 + w_2 f_I}$ $f_I = \frac{I^n}{K^n + I^n}$

$m^* [=] \frac{nmol}{gDW}$ $r_{x,i} = k_{E,i}^{x} R_{x,T}\left(\frac{G_i}{J_{x,i}K_{x,i} + (J_{x,i}+1)G_i}\right)$ μ = dilution

$K_{x,i} = \frac{k_- + k_I}{k_+}$ $J_{x,i} = \frac{k_E^x}{k_I}$ $\theta_{m,i}$ = degradation

Assume positive induction, assume $\mu = 0$

promoter constants: data in problem

when $u = 1$, $m^* = \frac{r_{x,i}}{\mu + \theta_{m,i}}$

when $I = 0$, no inductor, rate determiniting in initiation

$t_{1/2} = \frac{\ln 2}{D}$ $D = \theta_{m,i} = \frac{\ln 2}{5 min} = 0.139 \frac{1}{min}$

Assume μ dilution term is zero.

$k_{E,i} = \langle k_E^x \rangle \left(\frac{L}{L_i}\right) = e_x \frac{1}{L}$

$$\overbrace{m^*}^{\frac{nmol}{gDW}} = \underbrace{k_x(G,\theta)}_{\text{gain function}} \overbrace{\bar{u}(I,k)}^{\text{promoter function}} = \left(\frac{r_{x,i}}{\mu+\theta_{m,i}}\right)\bar{u}_i$$

I (inducer abundance)

G (gene abundance)

$$k_x(G,\theta) = \frac{r_{x,i}}{\theta_{m,i}+\mu}$$

Promoter constants from problem

$u = 1$ when mRNA $= 5.52\times10^{-9}$, IPTG = 1 mM

$I = 0$ when mRNA $= 1.127\times10^{-9}$, IPTG = 0 mM

$$u = \frac{w_1 + w_2 f_I}{1 + w_1 + w_2 f_I} \quad f_I = \frac{I^n}{k^n + I^n}$$

$n = 1.54$
$k = 0.204$
$w_1 = 0.26$
$w_2 = 167.53$

$$r_{x,i} = k_{E,i}^X R_{x,T}\left(\frac{G_i}{J_{x,i}K_{x,i} + (J_{x,i}+1)G_i}\right)$$

$$K_{x,i} = \frac{k_- + k_I}{k_+} \quad J_{x,i} = \frac{k_E^X}{k_I}, \text{ Assume } k_A = 0$$

$k_x(G,\theta) = 3.56\times10^{-9}$ Ideally

• Assuming the dilution term is zero

$$\left(\frac{2\text{ copies}}{\text{cell}}\right)\left(\frac{1}{4.33\times10^{-5}\text{ gDW}}\right)\left(\frac{1\text{ mol}}{6.02\times10^{23}\text{ copies}}\right)\left(\frac{10^9\text{ nmol}}{1\text{ mol}}\right) = 7.67\times10^{-11}\frac{\text{nmol}}{\text{gDW}} \Rightarrow$$

Bionumbers RNAPol elongation rate: $\left(3.72\frac{\text{kb}}{\text{min}}\right)\left(\frac{1000\text{ nt}}{1\text{ kb}}\right) = e_x$

$$k_E^X = \left(3720\frac{\text{nt}}{\text{min}}\right)\left(\frac{1}{1000\text{ nt}}\right) = 3.72\text{ min}^{-1} = \frac{e_x}{L_j}$$

Assume the rate constants are the same as those from the paper as is $R_{x,T}$ for A2 promoter. This is not a good assumption at all

$$\frac{1}{k_I} = 25\text{ sec} \qquad \left(\frac{k_- + k_I}{k_+ k_I}\right) = 0.12$$

$$k_I = 0.04\,s^{-1} \qquad \frac{1}{25}(K_{x,i}) = 0.12 \Rightarrow K_{x,i} = 0.0048$$

$$J_{x,i} = \frac{3.72}{25} = 0.1488$$

$$r_{x,i} = \left(\frac{7.67\times10^{-11}}{0.1488(0.0048) + (.1488+1)(7.67\times10^{-11})}\right) = (1.07\times10^{-7})$$

$$K = \frac{(1.07\times10^{-7})(3.72)\frac{\text{nmol}}{\text{gDW}}\text{ min}}{0.139\text{ min}^{-1}} = 2.87\times10^{-6}\frac{\text{nmol}}{\text{gDW}}$$