

(1) a)  $1000 \text{ L of } 3 \times 10^7 \text{ cells/mL} = 3 \times 10^{10} \text{ cells}$

$$\frac{dg_1}{dt} = -10^{-8}g_1 + 10^{-6}g_2 \approx 0 \Rightarrow \frac{10^{-8}}{10^{-6}} = \frac{g_2}{g_1} \Rightarrow 100 \times \text{ more } g_1 \text{ than } g_2 \text{ at "equilibrium"}$$

Assume that  $3 \times 10^{10}$  cells is the population after "equilibrium" has been reached

# cells with  $g_1 = 2.97 \times 10^{10}$  cells

# cells with  $g_2 = 2.97 \times 10^8$  cells

(b) equilibrium population fraction of damaged DNA:

D = damaged DNA T = total DNA  
N = normal

w/o UV light:  $\frac{dD}{dt} = k_1N - k_{-1}D = 0$  in equil

$T = N + D \Rightarrow N = T - D$

$k_1(T - D) - k_{-1}D = 0 \Rightarrow k_1T - k_1D - k_{-1}D = 0$

$k_1T = D(k_1 + k_{-1})$

$\frac{k_1}{k_1 + k_{-1}} = \frac{D}{T}$

with UV light:  $\frac{dD}{dt} = k_1N - k_2D + k'_1N = 0$  in equil  $T = N + D$

$0 = (k_1 + k'_1)(T - D) - k_2D$

$k_1D = (k_1 + k'_1)T - (k_1 + k'_1)D \Rightarrow D(k_1 + k_2 + k'_1) = (k_1 + k'_1)T$

$\frac{D}{T} = \frac{k_1 + k'_1}{k_1 + k_2 + k'_1} \quad \text{QED}$

(2)  $R_0$  = total amt of repressor protein

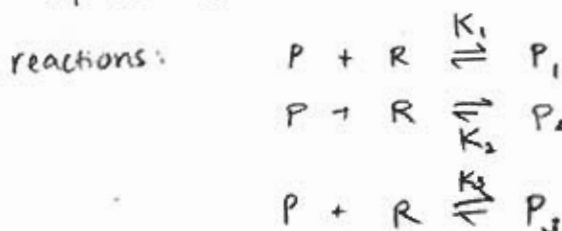
$P_0$  = # of  $P_{RO}$  sequences in the cell

4-species of promoter  $\mathcal{P}_R$  depending on if repressor is bound, and if so where

- $P$  = unbound
  - $P_3$  = repressor bound at  $O_{R3}$
  - $P_2$  = " " " at  $O_{R2}$
  - $P_1$  = " " " at  $O_{R1}$
- } accessible to RNA polymerase  
} inaccessible

$P_1 + P_2 + P_3 + P = P_0$

$P_1 + P_2 + P_3 + R = R_0$



$\frac{dP_1}{dt} = k_1[P][R] - k_{-1}[P_1] \approx 0$

$\frac{dP_2}{dt} = k_2[P][R] - k_{-2}[P_2] \approx 0$

$\frac{dP_3}{dt} = k_3[P][R] - k_{-3}[P_3] \approx 0$

$K_1, K_2, K_3$  as given are "association constants" b/c of units of  $M^{-1}$

$$\text{Thus: } K_1 = \frac{P_1}{PR}, \quad K_2 = \frac{P_2}{PR}, \quad K_3 = \frac{P_3}{PR}$$

$$P_1 = K_1 PR, \quad P_2 = K_2 PR, \quad P_3 = K_3 PR$$

$$P_0 = K_1 PR + K_2 PR + K_3 PR + P \Rightarrow P_0 = P(K_1 R + K_2 R + K_3 R + 1)$$

$$P = \frac{P_0}{K_1 R + K_2 R + K_3 R + 1}$$

$$R_0 = R + P_1 + P_2 + P_3 \Rightarrow R_0 = R + K_1 PR + K_2 PR + K_3 PR$$

$$R_0 = R(1 + K_1 P + K_2 P + K_3 P)$$

$$P = \frac{P_0}{1 + \frac{K_1 R_0 + K_2 R_0 + K_3 R_0}{1 + K_1 P + K_2 P + K_3 P}}$$

$$P + \frac{PK_1 R_0 + PK_2 R_0 + PK_3 R_0}{1 + K_1 P + K_2 P + K_3 P} = P_0 \Rightarrow \text{Solve for } P$$

(a) fraction accessible of  $P_R$  promoters is

$$\frac{P_3 + P}{P_0} \quad \text{or} \quad \frac{P_0 - P_1 - P_2}{P_0}$$

3. growth kinetics:  $\mu = k_{\text{growth}} C_s \Rightarrow$  rewrite as  $\mu = k_{\text{growth}} S$ , define  $C = \#$  cells

- maximum dilution rate occurs when  $D = \mu_{\text{max}}$ . Above this dilution rate,  $D$  surpasses the growth (nutrients flowed faster than cells can use), and # of steady state cells is 0 in bioreactor.

$$D_{\text{max}} = \mu_{\text{max}} = k_{\text{growth}} S_{\text{in}}$$

-  $D$  for maximum output occurs when  $\frac{d(DP)}{dD} = 0$

$DP$  is maximized, when  $D_c$  is max, where  $D_c$  is the rate of cell production

- Find  $D_c$ : 2 steady state solutions to cell mass balance

$$0 = (-D + \mu(s))C \Rightarrow C_1 = 0, \text{ or } D = \mu(s) \text{ and } C_2 \text{ specified by nutrient eqn: } C_2 = Y_s(S_{\text{in}} - S_{\text{out}})$$

$$D = \mu(s) = k_{\text{growth}} S \Rightarrow S = \frac{D}{k_{\text{growth}}} = S_{\text{out}} \text{ assuming CSTR}$$

$$\text{thus, } C = Y_s \left( S_{\text{in}} - \frac{D}{k_{\text{growth}}} \right) \Rightarrow D_c = D Y_s S_{\text{in}} - \frac{D^2 Y_s}{k_{\text{growth}}}$$

$$\frac{dD_c}{dD} = Y_s S_{\text{in}} - \frac{2Y_s D}{k_{\text{growth}}} \Rightarrow \frac{dD_c}{dD} = 0 \text{ when } D = \frac{Y_s S_{\text{in}} k_{\text{growth}}}{2}$$

4. Assume symmetric exponential growth:  $C = C_0 e^{k_g t}$

$$t_1 = 3 \text{ hours, } C = 400/\text{mL}$$

$$400 = C_0 e^{3k_g}$$

$$t_2 = 10 \text{ hours, } C = 2000/\text{mL}$$

$$2000 = C_0 e^{10k_g}$$

$$\ln 400 = \ln C_0 + 3k_g$$

$$\ln 2000 = \ln C_0 + 10k_g$$

$$\left. \begin{array}{l} \ln 400 = \ln C_0 + 3k_g \\ \ln 2000 = \ln C_0 + 10k_g \end{array} \right\} 7k_g = \ln 2000 - \ln 400 \quad k_g = 0.23 \text{ hr}^{-1}$$

plug back in to find  $C_0$ :

$$C_0 = 200/\text{mL}$$

5.  $\frac{\mu_{\text{max}} S}{K_m + S} = \mu(s)$

$$\mu_{\text{max}} = 0.3 \text{ hr}^{-1}$$

$$K_m = 1.3 \text{ g/L}$$

$$Y_{x/\text{DIF}} = \frac{0.46 \text{ g cell}}{\text{g DIF}}$$

$$V = 10 \text{ L}$$

$$Q = \frac{1 \text{ L}}{\text{hr}}$$

$$S_{\text{in}} = \frac{5 \text{ g}}{\text{L}} \text{ DIF}$$

steady state cell mass balance:

$$V \frac{dc}{dt} = Q(C_{\text{in}} - C_{\text{out}}) + V\mu(s)C, \text{ Assume CSTR, } C_{\text{out}} = C$$

$$0 = \frac{Q}{V}(-C) + \mu(s)C \Rightarrow 0 = C \left( \mu(s) - \frac{Q}{V} \right)$$

$$\text{meaningful solution when } \mu(s) = \frac{Q}{V} = \frac{\mu_{\text{max}} S}{K_m + S}$$

$$\frac{Q}{V} K_m + \frac{Q}{V} S = \mu_{\text{max}} S$$

$$\frac{Q}{V} K_m = \mu_{\max} S - \frac{Q}{V} S$$

$$S = \frac{\frac{Q}{V} K_m}{\mu_{\max} - \frac{Q}{V}}, \quad \text{Again, assume CSTR, } S_{\text{out}} = S$$

$$Q = 1 \frac{\text{L}}{\text{hr}}, \quad V = 10 \text{ L}, \quad K_m = 1.3 \frac{\text{g}}{\text{L}}, \quad \mu_{\max} = 0.3 \text{ hr}^{-1}$$

$$S = \frac{\frac{1 \frac{\text{L}}{\text{hr}}}{10 \text{ L}} \cdot 1.3 \frac{\text{g}}{\text{L}}}{0.3 \text{ hr}^{-1} - \frac{1 \frac{\text{L}}{\text{hr}}}{10 \text{ L}}} = \frac{0.13 \frac{\text{g}}{\text{L}} \cdot \text{hr}}{0.2 \text{ hr}^{-1}} = \boxed{0.65 \frac{\text{g}}{\text{L}}}$$

$$\text{concentration of cells: } C = Y_s (S_{\text{in}} - S_{\text{out}})$$

$$= \frac{0.46 \frac{\text{g cells}}{\text{g DIF}}}{\text{g DIF}} \left( 5 \frac{\text{g DIF}}{\text{L}} - 0.65 \frac{\text{g DIF}}{\text{L}} \right) = \boxed{2.001 \frac{\text{g cells}}{\text{L}}}$$

6. Assume synchronous division for both:  $k_{g,e}$  = growth of endothelial cells  
 $k_{g,f}$  = growth of fibroblasts

$$t_{D,e} = 40 \text{ hours}, \quad k_{g,e} = \frac{\ln 2}{40 \text{ hr}} = 0.0173 \text{ hr}^{-1}$$

$$t_{D,f} = 20 \text{ hours}, \quad k_{g,f} = \frac{\ln 2}{20 \text{ hr}} = 0.0347 \text{ hr}^{-1}$$

$$\text{endothelial cell \#}: \quad n_e(t) = n_{e,0} e^{0.0173 t}$$

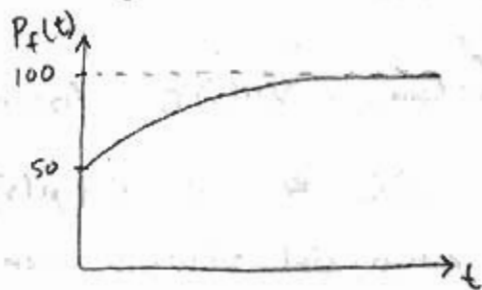
$$\text{fibroblast cell \#}: \quad n_f(t) = n_{f,0} e^{0.0347 t}$$

$$n_{e,0} = n_{f,0} = n_0$$

(a) % of cells that are fibroblasts:

$$P_f(t) = \frac{n_f(t)}{n_f(t) + n_e(t)} = \frac{n_0 e^{k_{g,f} t}}{n_0 e^{k_{g,f} t} + n_0 e^{k_{g,e} t}} = \frac{e^{k_{g,f} t}}{e^{k_{g,f} t} + e^{k_{g,e} t}}$$

graph of  $P_f(t)$  starts at 50%, and saturates at 100%



(b) time required to reach 90% fibroblasts

$$0.90 = P_f(t) = \frac{e^{k_{g,f}t}}{e^{k_{g,f}t} + e^{k_{g,e}t}} \Rightarrow 0.90 e^{k_{g,f}t} + 0.90 e^{k_{g,e}t} = e^{k_{g,f}t}$$

$$0.90 e^{k_{g,e}t} = 0.1 e^{k_{g,f}t}$$

$$k_{g,e}t = \ln\left(\frac{0.1}{0.9}\right) + k_{g,f}t \Rightarrow -\ln\left(\frac{1}{9}\right) = t(k_{g,f} - k_{g,e})$$

$$t = 126.3 \text{ hours}$$

7. fractional saturation.

$$\text{PFOA: } y = \frac{[L]_0}{K_d + [L]_0}$$

$$\text{full closed form: } y = \frac{[L]_0 + [R]_0 + K_d - \sqrt{([L]_0 + [R]_0 + K_d)^2 - 4[R]_0[L]_0}}{2[R]_0}$$

Assume  $K_d$  for c973 mutant same as wild type EGFR,

from Hendrick et al. Cancer Research. 63:1130-1137 (2003)

$$\left. \begin{array}{l} \text{EGF } k_f = 9.7 \times 10^7 \text{ M}^{-1} \text{ min}^{-1} \\ k_r = 0.24 \text{ min}^{-1} \end{array} \right\} K_d = 2.47 \times 10^{-9} \text{ M}$$

$$[R]_0 = \frac{6000 \text{ EGFR}}{\text{cell}} \times \frac{1 \text{ mole}}{6.022 \times 10^{23} \text{ EGFR}} \times \frac{5000 \text{ cells}}{\text{mL}} \times \frac{1000 \text{ mL}}{1 \text{ L}} = 4.98 \times 10^{-14} \text{ M}$$

$$(a) [L]_0 = 0.1 \text{ nM} \gg [R]_0, \text{ use PFOA: } y = \frac{0.1 \text{ nM}}{0.1 \text{ nM} + 2.47 \text{ nM}} = \boxed{3.89\% \text{ occupied}}$$

$$(b) [L]_0 = 1 \text{ nM} \gg [R]_0, \text{ PFOA: } y = \frac{1 \text{ nM}}{1 \text{ nM} + 2.47 \text{ nM}} = \boxed{28.82\% \text{ occupied}}$$

$$(c) [L]_0 = 10 \text{ nM} \gg [R]_0, \text{ PFOA: } y = \frac{10 \text{ nM}}{10 \text{ nM} + 2.47 \text{ nM}} = \boxed{80.2\% \text{ occupied}}$$

8. (a) no ligand ( $L=0$ ), assume no recycling,

$k_{er} = 2 \times 10^{-2} \text{ min}^{-1}$   
(from 4/24/06 lecture)

total cell receptors:  $R_T = V_R \left( \frac{1}{k_{er}} + \frac{1}{k_b} \right)$

$V_R = \frac{R_T}{\frac{1}{k_{er}} + \frac{1}{k_b}} = \frac{3 \times 10^4 \text{ \# / cell}}{\frac{1}{0.02} + \frac{1}{0.01 \text{ min}^{-1}}} = 200 \text{ \# / cell-min}$   
← estimate from lecture

(b) 10% fibroblast by volume

Assume avg. cell dimension =  $30 \mu\text{m} \times 30 \mu\text{m} \times 10 \mu\text{m}$   
L ← width, w ← width, thickness

in  $0.1 \times 1 \times 1 \text{ cm}$  volume:  $V = 0.1 \text{ cm}^3$  cell vol =  $(30 \times 10^{-4})(30 \times 10^{-4})(10 \times 10^{-4}) = 9 \times 10^{-9} \text{ cm}^3$

total cells =  $1.1 \times 10^7$  cells, 10% fibroblasts =  $1.1 \times 10^6 \text{ fibs} / 0.1 \text{ cm}^3$

concentration:  $1.1 \times 10^6 \frac{\text{#}}{0.1 \text{ cm}^3} \times \frac{1 \text{ cm}^3}{1 \text{ mL}} \times \frac{1000 \text{ mL}}{\text{L}} \times \frac{1 \text{ mol}}{6.022 \times 10^{23}} = 1.84 \times 10^{-14} \text{ M}$

growth rate required for 0.1% fibroblasts to populate to 10%:

0.1% fibroblasts =  $1.1 \times 10^4$  cells initially

$f = f_0 e^{k_g t}$

$\ln f - \ln f_0 = k_g t$

$k_g = \frac{\ln f - \ln f_0}{t}$ ,  $t = 1 \text{ week} = 168$

$\frac{\ln(1.1 \times 10^6) - \ln(1.1 \times 10^4)}{168} = 0.0274 \text{ hr}^{-1}$

$k_g = \frac{\mu_{max} C}{K_m + C} \rightarrow C = \frac{k_g K_m}{\mu_{max} - k_g} = \frac{(0.0274 \text{ hr}^{-1})(10^4 \text{ \# / cell})}{0.03 \text{ hr}^{-1} - 0.0274 \text{ hr}^{-1}} = 1.05 \times 10^6 \text{ \# / cell} \rightarrow \text{NOT reasonable!}$

(c) ligand depletion:  $L + R_s \xrightleftharpoons[k_r]{k_f} C_s \rightarrow C_i \xrightarrow{\text{recycling}}$   $L \rightarrow \text{degradation}$

$\frac{dL}{dt} = -(k_f LR_s - k_r C_s) \frac{P}{N_{av}} - k_{deg} L$   $L(0) = L_0$

$\frac{dC_s}{dt} = k_f LR_s - k_r C_s - k_{ec} C_s$

Assume  $C_s$  at steady state,  $\frac{dC_s}{dt} \approx 0$

$k_{ec} C_s = k_f LR_s - k_r C_s$

$\frac{dL}{dt} = -k_{ec} C_s \frac{P}{N_{av}} - k_{deg} L$

Assume maximum rate:  $C_s = R_{s0}$  ← # of receptors on the surface

$$R_{so} = \frac{V_R}{k_{eR}} \Rightarrow \frac{dL}{dt} = \frac{-k_{ec}}{k_{eR}} V_R \frac{P}{N_{av}} - k_{deg} L \quad A = \frac{-k_{ec}}{k_{eR}} V_R \frac{P}{N_{av}}$$

$$\frac{dL}{dt} + k_{deg} L = A \quad P(t) = k_{deg} \quad Q(t) = A$$

integrating factor:  $u(t) = e^{\int P(t) dt} = e^{k_{deg} t}$

$$u(t)L = \int u(t)Q(t) dt \Rightarrow e^{k_{deg} t} L = \int e^{k_{deg} t} A dt$$

$$e^{k_{deg} t} L = A e^{k_{deg} t} \frac{1}{k_{deg}} + C$$

$$L(t) = \frac{A}{k_{deg}} + C e^{-k_{deg} t} \Rightarrow L(0) = L_0 = \frac{A}{k_{deg}} + C$$

$$C = L_0 - \frac{A}{k_{deg}}$$

$$L(t) = \frac{A}{k_{deg}} + \left(L_0 - \frac{A}{k_{deg}}\right) e^{-k_{deg} t} = \frac{A}{k_{deg}} (1 - e^{-k_{deg} t}) + L_0 e^{-k_{deg} t}$$

complete solution:

$$L(t) = \frac{-k_{ec}}{k_{eR} k_{deg}} \frac{P}{N_{av}} V_R (1 - e^{-k_{deg} t}) + L_0 e^{-k_{deg} t}$$

$$\begin{aligned} k_{ec} &= 0.2 \text{ min}^{-1} \\ k_{eR} &= 0.02 \text{ min}^{-1} \\ k_{deg} &= 0.01 \text{ min}^{-1} \\ V_R &= 200 \text{ \% cell} \cdot \text{min} \end{aligned}$$

(d)

$$\mu(c) = \frac{\mu_{max} C}{K + C}$$

$$C_T = C_s + C_i$$

ignore recycling:  $\frac{dC_i}{dt} = k_{ec} C_s - k_{deg} C_i$

$$\frac{dC_s}{dt} = k_f L R_s - (k_r + k_{ec}) C_s$$

- substitute  $L(t)$  from part (c) into  $\frac{dC_s}{dt}$ , solve for  $C_s(t)$ ,  $C_i(t)$ , and finally  $\zeta(t)$

- This can then be plugged into  $\mu(c)$  to get time transient response of cell growth to 56F