Modelling Our System

Christin S. and Farah V.

Slide2: General Derivation

Assumptions:

- *Concentration of promoters is constant*
- *Promoter P and activator A are in equilibrium with their complex PA*
- *The reaction forming the protein Z is irreversible*
- *Note: Contrarily to Michaelis-Menten, the substrate is not used up & the protein Z rebinds the promoter*

$$P + A \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} PA \xrightarrow{k_2} Z$$

$$\frac{d[PA]}{dt} = k_1[P][A] - k_{-1}[PA] = 0 \quad \text{(steady state reached quickly if } k_1 \succ k_{-1})$$

$$(1) \quad [PA] = \frac{k_1[P][A]}{k_{-1}} = [P][A]K_D \quad \text{where } K_D \equiv \frac{k_1}{k_{-1}}$$

$$\text{Note: in Michaelis-Menten } K_m = \frac{k_{-1} + k_2}{k_1} \approx \frac{k_{-1}}{k_1} = \frac{1}{K_D} \quad \text{if } k_2 \prec\prec k_{-1}$$

As the total concentration of promoters is constant:

$$[P_0] = [P] + [PA] \qquad \therefore [P] = [P_0] - [PA]$$

Substituting into (1):

$$[PA] = \frac{[P][A]}{K_D} = \frac{([P_0] - [PA])[A]}{K_D} \qquad \therefore [PA] = \frac{[P_0]}{1 + K_D/[A]} = \frac{[A][P_0]}{[A] + K_D}$$

The rate of protein synthesis is described by:

$$\frac{d[Z]}{dt} = k_2[PA] = \frac{k_2[P_0][A]}{[A] + K_D} = \frac{V_{max}[A]}{[A] + K_D} \qquad \text{where } V_{max} \equiv k_2[P_0]$$

Modelling T9002

$AHL + LuxR \xleftrightarrow{k_{-\alpha},\ k_{\alpha}} A$ (Assuming all stochiometric numbers as shown)

$$\frac{d[A]}{dt} = k_{\alpha}[AHL][LuxR] - k_{-\alpha}[A] = 0 \quad \text{(steady-state)}$$

LuxR is constitutively produced and reaches steady state before AHL is added.

[LuxR] can be approximated as a constant: $[LuxR] \approx \lambda$

$$\therefore \frac{[AHL][LuxR]}{[A]} = \frac{[AHL]}{[A]} = \frac{k_{-\alpha}}{\lambda k_{\alpha}} = \frac{1}{\lambda K_{D\alpha}} \qquad \therefore (2) \quad [A] = \lambda K_{D\alpha}[AHL][LuxR]$$

Key:

P: Promoter pLuxR

A: AHL/LuxR complex

PA: pLuxR/AHL/LuxR complex

Z: GFP

$P + A \xleftrightarrow{k_{-1},\ k_{1}} PA \xrightarrow{k_2} Z$

The rate of protein synthesis is described by:

$$\frac{d[Z]}{dt} = k_2[PA] = \frac{k_2[P_0][A]}{[A] + K_D} = \frac{V_{max}[A]}{[A] + K_D} \qquad \text{where } V_{max} \equiv k_2[P_0]$$

(see Slide No. 2 for derivation)

The total change in protein concentration includes protein degradation:

$$\frac{d[Z]}{dt} = \frac{V_{max}[A]}{[A] + K_D} - \delta_2[Z]$$

Substituing Equation (2): $\frac{d[Z]}{dt} = \frac{V_{max}\lambda K_{D\alpha}[AHL]}{\lambda K_{D\alpha}[AHL] + K_D} - \delta_2[Z]$

Rearranging and substituting for Z:

$$(3) \quad \frac{d[GFP]}{dt} = \frac{V_{max}[AHL]}{[AHL] + K_D / \lambda K_{D\alpha}} - \delta_{GFP}[GFP]$$

Note:

$AHL + LuxR \leftrightarrow AHL/LuxR$

LuxR is present in excess of AHL.

The protein Z (AHL) associates with LuxR to form A. Thus, Z indirectly becomes the activator A.

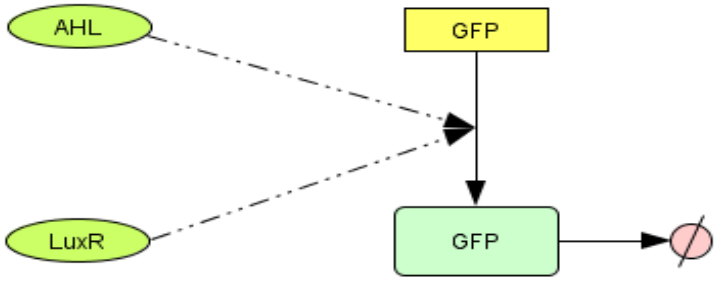

Fig.1a: *Diagram for model in cell designer*

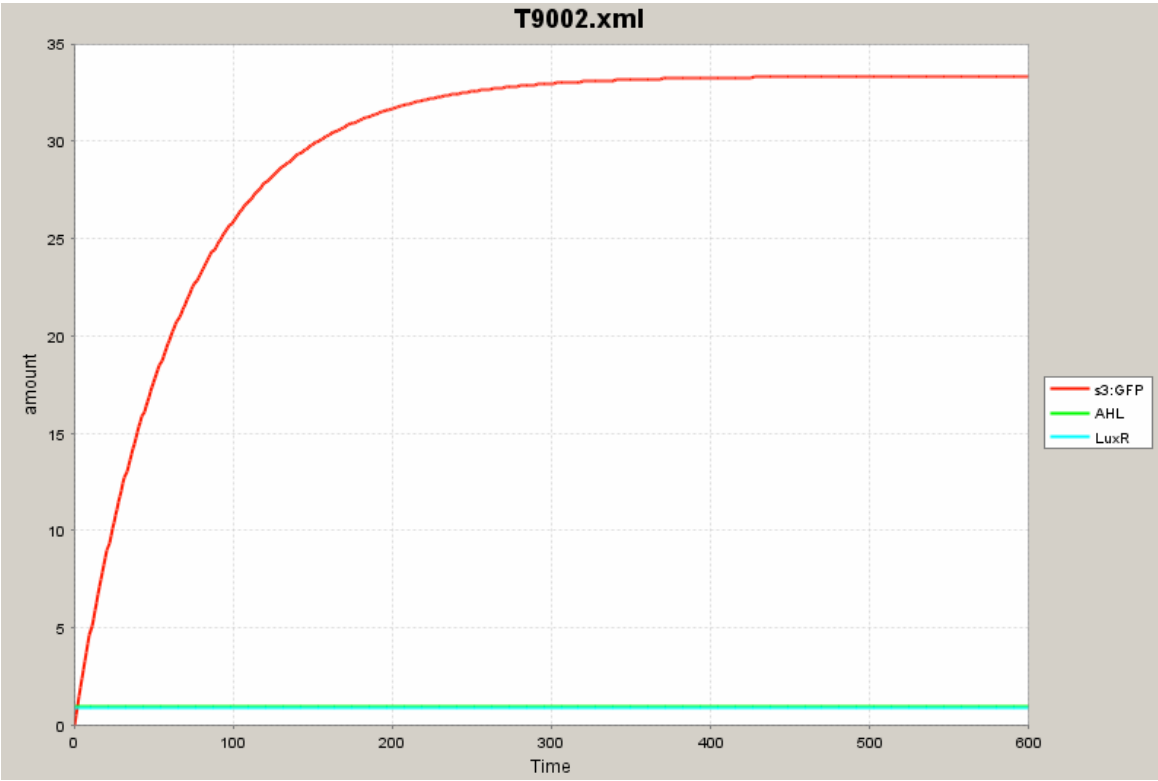

Fig.1b: *Output from the model in Fig.1a*

Equation:

$$\frac{d[GFP]}{dt} = \frac{V_{max}[AHL]}{[AHL] + K_D/\lambda K_{D\alpha}} - \delta_{GFP}[GFP]$$

Known or measurable parameters:

- [GFP], [AHL], GFP degradation

Parameters to extract from model:

- V_{max}, $K_D/\lambda K_{D\alpha}$

Input Values used to generate above graph:

$\delta_{GFP} = 0.015\,\text{min}^{-1}$, $K_D/K_{D\alpha} = 1.0$

$V_{max} = 1.0$, [AHL]=1.0 , [LuxR]=0.93

Modelling J37015

Key:

P: Promoter pLuxR

A: AHL/LuxR complex

PA: pLuxR/AHL/LuxR complex

Z: AHL

Note:

$AHL + LuxR \leftrightarrow AHL/LuxR$

LuxR is present in excess of AHL.

The protein Z (AHL) associates with LuxR to form A. Thus, Z indirectly becomes the activator A.

$AHL + LuxR \xleftrightarrow{k_{-\alpha},\ k_{\alpha}} A$ (Assuming all stochiometric numbers as shown)

$$\frac{d[A]}{dt} = k_{\alpha}[AHL][LuxR] - k_{-\alpha}[A]$$

LuxR is constitutively produced and reaches steady state before AHL is added.

[LuxR] can be approximated as a constant: $[LuxR] \approx \lambda$

$$\frac{d[A]}{dt} = k_{\alpha}\lambda[AHL] - k_{-\alpha}[A] = 0 \quad \text{(steady-state)}$$

$$\therefore \frac{[AHL]}{[A]} = \frac{k_{-\alpha}}{\lambda k_{\alpha}} = \frac{1}{\lambda K_{D\alpha}} \qquad \therefore (4) \quad [A] = \lambda K_{D\alpha}[AHL]$$

$$P + A \xleftrightarrow{k_{-1},\ k_{1}} PA \xrightarrow{k_2} Z$$

The rate of protein synthesis is described by:

$$\frac{d[Z]}{dt} = k_2[PA] = \frac{k_2[P_0][A]}{[A] + K_D} = \frac{V_{max}[A]}{[A] + K_D} \qquad \text{where } V_{max} \equiv k_2[P_0]$$

(see Slide No. 2 for derivation)

The total change in protein concentration includes protein degradation:

$$\frac{d[Z]}{dt} = \frac{V_{max}[A]}{[A] + K_D} - \delta_1[Z]$$

Substituing Equation (4): $\frac{d[Z]}{dt} = \frac{V_{max}\lambda K_{D\alpha}[AHL]}{\lambda K_{D\alpha}[AHL] + K_D} - \delta_1[Z]$

Since AHL/LuxR complex is in equilibrium with AHL, we can approximate:

$$(5) \quad \frac{d[AHL]}{dt} = \frac{V_{max}[AHL]}{[AHL] + K_D/\lambda K_{D\alpha}} - \delta_{AHL}[AHL]$$

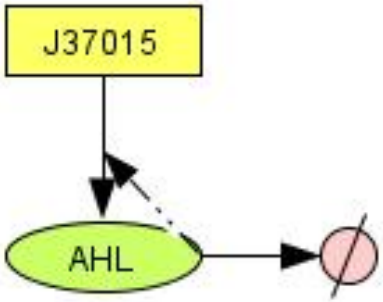

Fig.2a: *Diagram for model in cell designer*

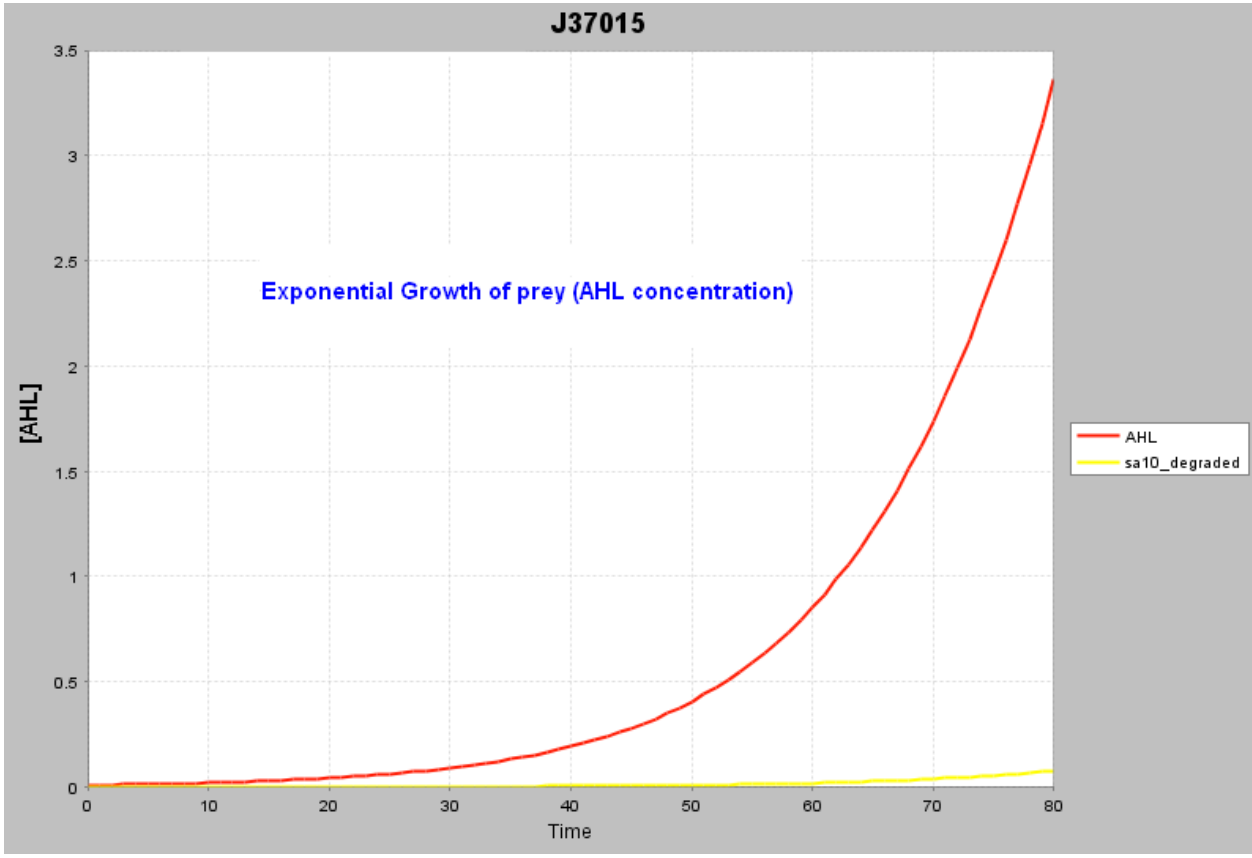

Fig.2b: *Output from the model in Fig.2a*

Equation:

$$\frac{d[AHL]}{dt} = \frac{V_{max}[AHL]}{[AHL] + K_D/\lambda K_{D\alpha}} - \delta_{AHL}[AHL]$$

Known or measurable parameters:

- [AHL], AHL degradation

Parameters to extract from model:

- V_{max}, $K_D/\lambda K_{D\alpha}$

Values used for graph:

V_{max}=1.245, $K_D/K_{D\alpha} = 15$, $\lambda = 1$, $\delta_1 = 0.0016\ s^{-1}$

Modelling J37016

Key:

P: Promoter pLuxR

A: AHL/LuxR complex

PA: pLuxR/AHL/LuxR complex

Z: GFP and LuxR

$AHL + LuxR \xleftrightarrow{k_{-\alpha},\ k_{\alpha}} A$ (Assuming all stochiometric numbers as shown)

$\frac{d[A]}{dt} = k_{\alpha}[AHL][LuxR] - k_{-\alpha}[A] = 0$ (because of steady state)

$\therefore \frac{[AHL][LuxR]}{[A]} = \frac{k_{-\alpha}}{k_{\alpha}} = \frac{1}{K_{D\alpha}}$ $\quad \therefore (6) \quad [A] = K_{D\alpha}[AHL][LuxR]$

$P + A \xleftrightarrow{k_{-1},\ k_{1}} PA \xrightarrow{k_{2}} Z$

The rate of protein synthesis is described by:

$\frac{d[Z]}{dt} = k_2[PA] = \frac{k_2[P_0][A]}{[A] + K_D} = \frac{V_{max}[A]}{[A] + K_D}$ where $V_{max} \equiv k_2[P_0]$

(see Slide No. 2 for derivation)

The total change in protein concentration includes protein degradation:

$\frac{d[Z]}{dt} = \frac{V_{max}[A]}{[A] + K_D} - \delta_2[Z]$

Substituing Equation (6): $\frac{d[Z]}{dt} = \frac{V_{max}K_{D\alpha}[AHL][LuxR]}{K_{D\alpha}[AHL][LuxR] + K_D} - \delta_2[Z]$

There are two different products being transcribed: LuxR and GFP.

Considering both products after another, keeping in mind they are measured at steady state:

(7) $\frac{d[LuxR]}{dt} = \frac{V_{max}[AHL][LuxR]}{[AHL][LuxR] + K_D / K_{D\alpha}} - \delta_{LuxR}[LuxR] = 0$

(8) $\frac{d[GFP]}{dt} = \frac{V_{max}[AHL][LuxR]}{[AHL][LuxR] + K_D / K_{D\alpha}} - \delta_{GFP}[GFP] = 0$

$\therefore$ (7) = (8) $\Rightarrow \delta_{LuxR}[LuxR] = \delta_{GFP}[GFP] \Rightarrow [LuxR] = \delta_{GFP} / \delta_{LuxR} [GFP]$

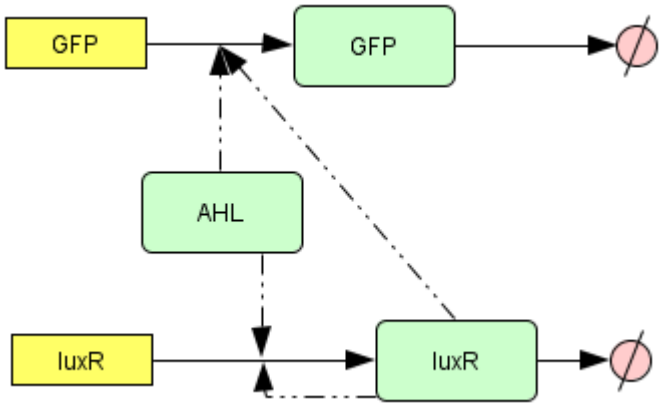

Fig.3a: *Diagram for model in cell designer*

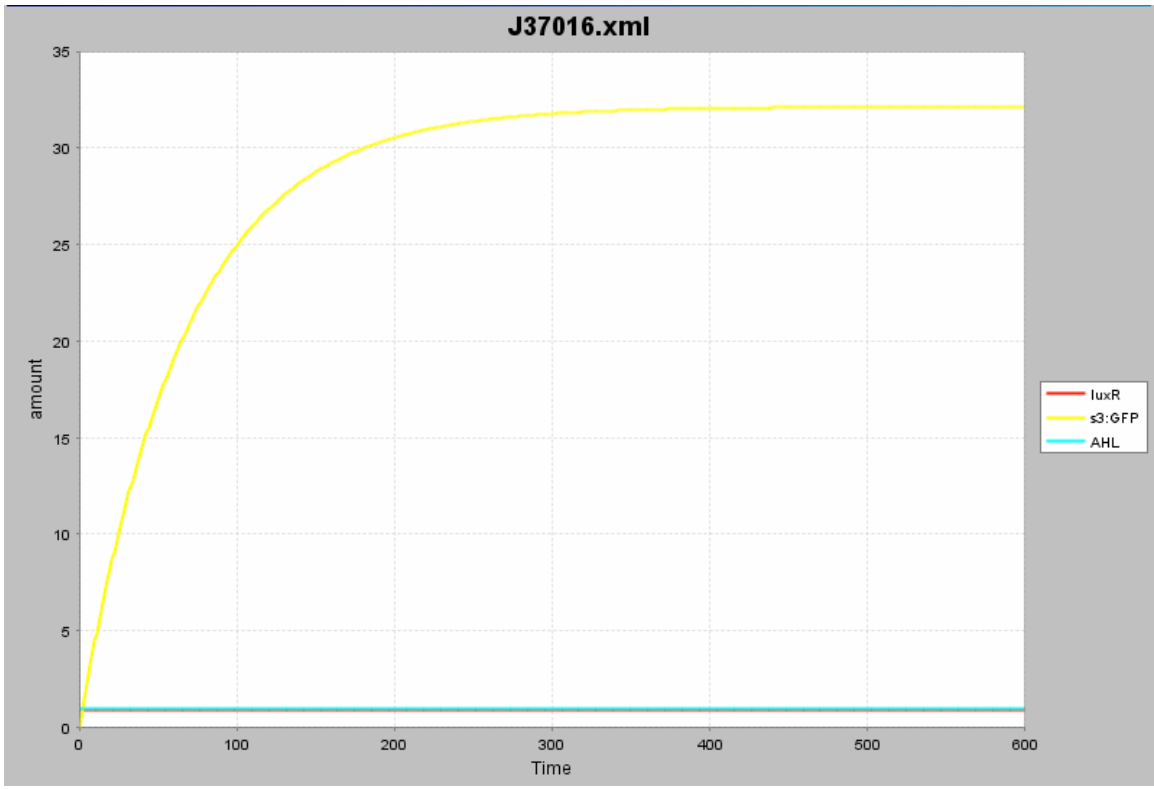

Fig.3b: *Output from the model in Fig.3a*

Equation:

$$\frac{d[GFP]}{dt} = \frac{V_{max}[AHL][LuxR]}{[AHL][LuxR] + K_D/K_{D\alpha}} - \delta_{GFP}[GFP]$$

Known or measurable parameters:

- [AHL], GFP degradation

Parameters to extract from model:

- V_{max}, $K_D/K_{D\alpha}$

Input values used to generate above graph:

$\delta_{GFP} = 0.015\,\text{min}^{-1}$, $K_D/K_{D\alpha} = 1.0$

$V_{max} = 1.0$, [AHL]=1.0 , [LuxR]=0.93

Modelling J37022 (AHL)

Key:

E: Enzyme AHL-lactonase

S: AHL

ES: aiiA/AHL complex

Z: Acyl-HS

True Michaelis-Menten :

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + Z$$

$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES] = 0$$

$$(9) \quad [ES] = \frac{k_1[E][S]}{k_{-1} + k_2} = \frac{[E][S]}{K_m} \quad \text{where } K_m \equiv \frac{k_{-1} + k_2}{k_1}$$

As the total concentration of enzyme is constant:

$$[E_0] = [E] + [ES] \qquad \therefore [E] = [E_0] - [ES]$$

Substituting into (1):

$$[ES] = \frac{[E][S]}{K_m} = \frac{([E_0] - [ES])[S]}{K_m} \qquad \therefore [ES] = \frac{[S][E_0]}{K_m + [S]}$$

The rate of degradation of substrate (activity of enzyme) is described by:

$$-\frac{d[S]}{dt} = k_2[ES] = \frac{k_2[E_0][S]}{K_m + [S]} = \frac{V_{max}[S]}{K_m + [S]} \qquad \text{where } V_{max} \equiv k_2[E_0]$$

The total rate of degradation of AHL (activity of aiiA) is described by:

$$(10) \quad \frac{d[AHL]}{dt} = -\frac{V_{max}[AHL]}{K_m + [AHL]} - \delta_{AHL}[AHL] = -\frac{k_2[E_0][AHL]}{K_m + [AHL]} - \delta_{AHL}[AHL]$$

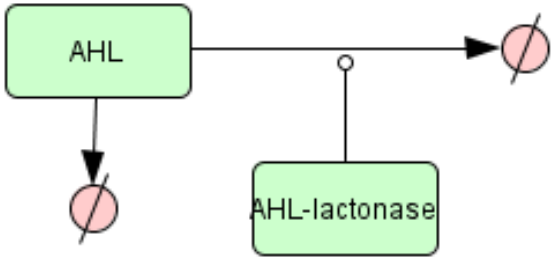

Fig.4a: *Diagram for model in cell designer*

Fig.4b: *Output from the model in Fig.4a*

Equation:

$$\frac{d[AHL]}{dt} = -\frac{k_2[E_0][AHL]}{K_m + [AHL]} - \delta_{AHL}[AHL]$$

Input values used to generate above graph:

$$\delta_{AHL} = 0.00048 \quad , \quad K_m = 331.95$$

$$V_{max} = 1.0 \quad , \quad [AHL] = 1.0$$

$$k_2 = 1000.0 \quad , \quad E_O = 1.0$$

Known or measurable parameters:

- IPTG, aiiA degradation

Parameters to extract from model:

- V_{max}, K_D

Modelling J37022 (aiiA)

Key:

P: Promoter LacI

A: IPTG

PA: LacI/IPTG complex

Z: aiiA

$$P + A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} PA \xrightarrow{k_2} Z$$

The rate of protein synthesis is described by:

$$\frac{d[Z]}{dt} = k_2[PA] = \frac{k_2[P_0][A]}{[A] + K_D} = \frac{V_{max}[A]}{[A] + K_D} \qquad \text{where } V_{max} \equiv k_2[P_0]$$

(see Slide No. 2 for derivation)

The total change in protein concentration includes protein degradation:

$$\frac{d[Z]}{dt} = \frac{V_{max}[A]}{[A] + K_D} - \delta_{aiiA}[Z]$$

$$(11) \quad \frac{d[aiiA]}{dt} = \frac{V_{max}[IPTG]}{[IPTG] + K_D} - \delta_{aiiA}[aiiA]$$

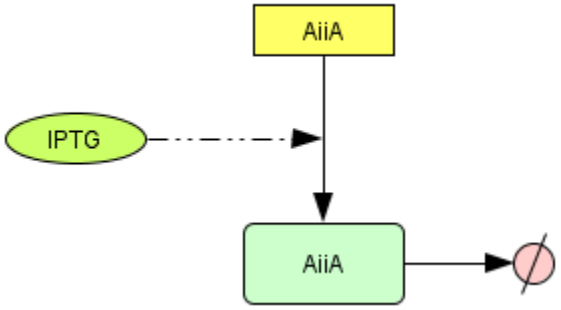

Fig.5a: *Diagram for model in cell designer*

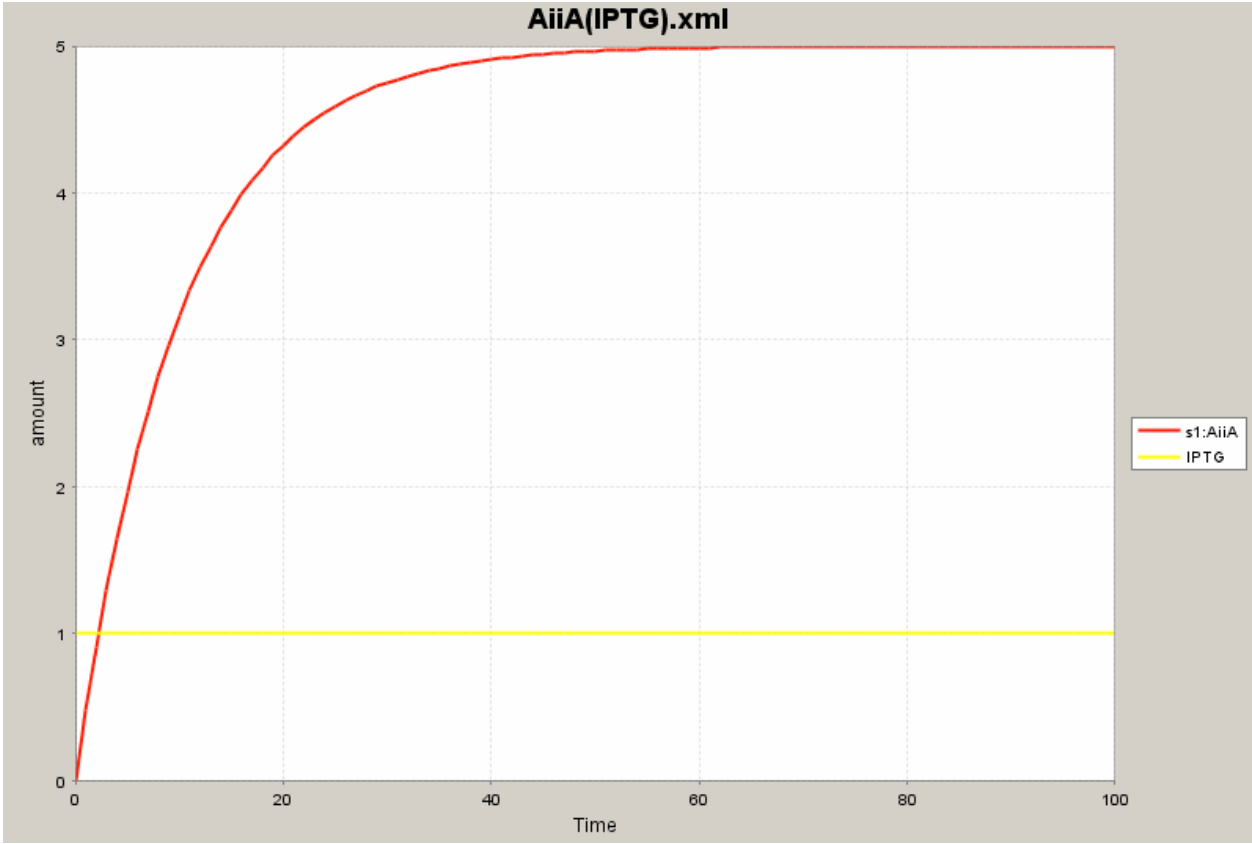

Fig.5b: *Output from the model in Fig.5a*

Equation:

$$\frac{d[aiiA]}{dt} = \frac{V_{max}[IPTG]}{[IPTG] + K_D} - \delta_{aiiA}[aiiA]$$

Known or measurable parameters:

- IPTG, aiiA degradation

Parameters to extract from model:

- V_{max}, K_D

Input values used to generate above graph:

$\delta_{Aiia} = 0.1$ (real value to be found)

$K_D = 1.0$ (real value to be found)

$V_{max} = 1.0$ (real value to be found)

$[IPTG] = 1.0$

Modelling the Overall System

Assumptions:

AHL is diffusing freely throughout the system

Three resulting equations describing the Overall System:

(from the previously derived equations 5, 7, 8, 10, 11)

Production of AHL

Degradation of AHL by enzyme

"Natural" degradation of AHL

$$\frac{d[AHL]}{dt} = \frac{V_{max}[AHL]}{[AHL] + K_D / \lambda K_{D\alpha}} - \frac{k_2[aiiA][AHL]}{K_m + [AHL]} - \delta_{AHL}[AHL]$$

$$\frac{d[aiiA]}{dt} = \frac{V_{max}[AHL][LuxR]}{[AHL][LuxR] + K_D / K_{D\alpha}} - \delta_{aiiA}[aiiA]$$

$$\frac{d[LuxR]}{dt} = \frac{V_{max}[AHL][LuxR]}{[AHL][LuxR] + K_D / K_{D\alpha}} - \delta_{2LuxR}[LuxR]$$

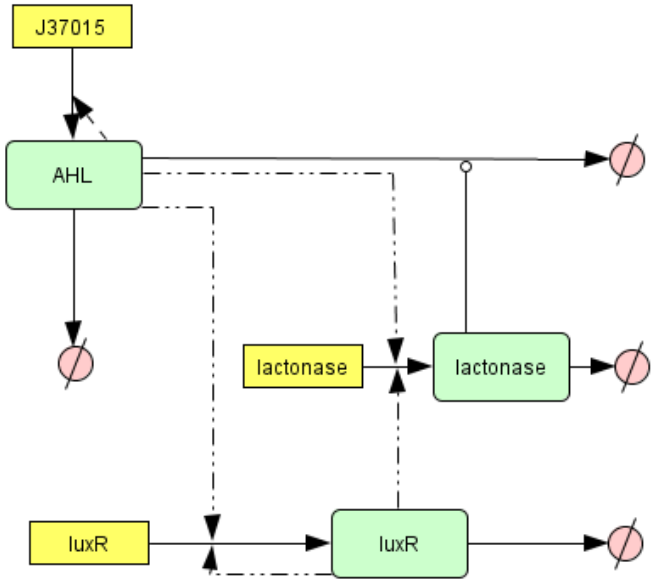

Fig.6a: *Diagram for model in cell designer*

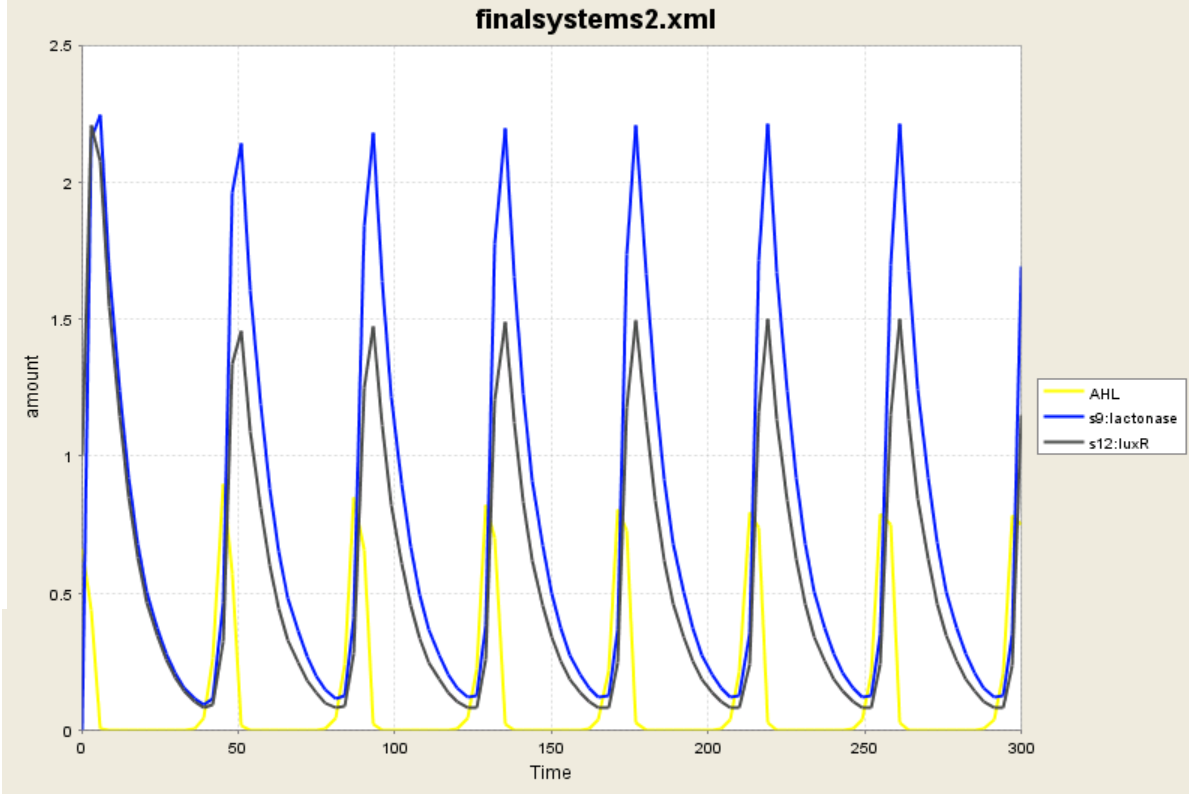

Fig.6b: *Output from the model in Fig.6a:*

We are getting oscillations !!!

- To gain some qualitative insight we will initially work under the rapid equilibrium approximation. This approximation assumes that that the timescale of protein-protein and protein-DNA interactions are significantly faster than the other chemical reactions and thus we can consider these protein reactions to be at equilibrium