NAR Parameterization Model

Table 01: List of NAR Model Parameters

#	Parameter	Description
P(1)	β_m	Transcription rate of mRNA
P(2)	K_1	Repression Coefficient of single piece antisense
P(3)	K_2	Repression Coefficient of double antisense
P(4)	K_{C1}	Repression coefficient of mismatched antisense due to crosstalk
P(5)	d_1	Degradation rate of single antisense
P(6)	r_{m1}	Maturation rate of single antisense
P(7)	r_{m2}	Maturation rate of double antisense
P(8)	r_b	Maturation and binding rate of MG
P(9)	β_R	Transcription rate of antisense
P(10)	k_t	Translational rate of SFGFP
P(11)	α	Maturation rate of SFGFP
P(12)	d_M	Degradation Rate of SFGFP mRNA
P(13)	β	Transcription rate of pre-cleaved MG RNA
P(14)	d_{MG}	Degradation rate of MG RNA
P(15)	d_2	Degradation rate of double antisense
P(16)	P_t	Auto-termination

Table 02: Model Species

Model Species	Definition
R_1^*	Immature single sRNA Repressor
R_1	Mature single sRNA Repressor
R_2^*	Immature single sRNA Repressor
R_2	Mature single antisense
M	mRNA of SFGFP
G	Immature SFGFP
G_M	Mature SFGFP
MG*	Pre-cleaved AS- MG
MG	Cleaved-off MG

1. **NAR-1**
   a. Construct:
   Att – STRSV – AS(H2) – STRSV – MG – T

   b. Equation:
   Four ODEs were used to describe the time varying concentrations of four species – Pre-cleaved RNA (P), Unfolded antisense (A_1^*), Matured antisense (A_1), and MG RNA (M):

$$\frac{dR_1^*}{dt} = \beta \cdot (1 - P_t) \cdot \left(1 - \frac{R_1}{K_1 + R_1}\right) - d_1 \cdot R_1^* - r_{m1} \cdot R_1^* \quad (1.1)$$

$$\frac{dR_1}{dt} = r_{m1} \cdot R_1^* - d_1 \cdot R_1 \quad (1.2)$$

$$\frac{dMG^*}{dt} = \beta \cdot (1 - P_t)^2 \cdot \left(1 - \frac{R_1}{K_1 + R_1}\right) - d_{MG} \cdot MG^* - r_b \cdot MG^* \quad (1.3)$$

$$\frac{dMG}{dt} = r_b \cdot MG^* - d_{MG} \cdot MG \quad (1.4)$$

1.1
Time varying concentration of pre-cleaved RNA depends on the rate of transcription β, transcriptional repression by matured antisense, rate of pre-cleave RNA degradation d, and the ribozyme cleave rate k_{cleave}. The transcriptional repression mechanism is described by a first order Hill function, with parameters {K} describing the concentration of antisense that's required to reach half of its maximum repression.

1.2
Concentration of unfolded antisense depends on the RNA cleavage rate, its own rate of degradation, and rate of maturation.

1.3
Concentration of folded and matured antisense depends on the maturation rate of unfolded antisense, and its rate of degradation.

1.4
Concentration of M-Green depends on the cleave rate of RNA and its rate of degradation d.

2. NAR-2

a. Construct:
Att – (STRSV – AS(H2)) x2 – STRSV – MG – T

b. Equation:
Four ODEs were used to describe the time varying concentrations of four species – Pre-cleaved RNA (P), Unfolded antisense (A_2^*), Matured antisense (A_2), and MG RNA (M):

$$\frac{dR_2^*}{dt} = \beta \cdot (1 - P_t) \cdot \left(1 - \frac{R_2}{K_2 + R_2}\right) - d_1 \cdot R_2^* - r_{m2} \cdot R_2^* \quad (2.1)$$

$$\frac{dR_2}{dt} = r_{m2} \cdot R_2^* - d_2 \cdot R_2 \quad (2.2)$$

$$\frac{dMG^*}{dt} = \beta \cdot (1 - P_t)^3 \cdot \left(1 - \frac{R_2}{K_2 + R_2}\right) - d_{MG} \cdot MG^* - r_b \cdot MG^* \quad (2.3)$$

$$\frac{dMG}{dt} = r_b \cdot MG^* - d_{MG} \cdot MG \qquad (2.4)$$

Equation has the same construct as 1.1-1.4, but A_2 here is for double antisense, which results in a stronger repression, slower degradation and slower maturation.

3. NAR-Control (Single AS)

a. Construct:
Mutt Att –STRSV – AS(H2) – STRSV – MG – T

b. Equation:
The construct with mutated attenuator will be treated as the single antisense model described in NAR-1

Four ODEs were used to describe the time varying concentrations of four species – Pre-cleaved RNA (P), Unfolded antisense (A_1^*), Matured antisense (A_1), and MG RNA (M):

$$\frac{dR_1^*}{dt} = \beta \cdot (1 - P_t) \cdot \left(1 - \frac{R}{K_{c1} + R_1}\right) - d_1 \cdot R_1^* - r_{m1} \cdot R_1^* \qquad (3.1)$$

$$\frac{dR_1}{dt} = r_{m1} \cdot R_1^* - d_1 \cdot R_1 \qquad (3.2)$$

$$\frac{dMG^*}{dt} = \beta \cdot (1 - P_t)^2 \cdot \left(1 - \frac{R_1}{K_{c1} + R_1}\right) - d_{MG} \cdot MG^* - r_b \cdot MG^* \qquad (3.3)$$

$$\frac{dMG}{dt} = r_b \cdot MG^* - d_{MG} \cdot MG \qquad (3.4)$$

Equation has the same construct as 1.1-1.4. Except the antisense will have cross-talk repression on mut-att.

4. NAR-Control (Double AS)

a. Construct:
Mutt Att –STRSV – AS(H2) – STRSV-- AS(H2) – STRSV – MG – T

b. Equation:

$$\frac{dR_2^*}{dt} = \beta \cdot (1 - P_t) \cdot \left(1 - \frac{R_2}{K_{C2} + R_2}\right) - d_1 \cdot R_2^* - r_{m2} \cdot R_2^* \qquad (4.1)$$

$$\frac{dR_2}{dt} = r_{m2} \cdot R_2^* - d_2 \cdot R_2 \qquad (4.2)$$

$$\frac{dMG^*}{dt} = \beta \cdot (1 - P_t)^3 \cdot \left(1 - \frac{R_2}{K_{C2} + R_2}\right) - d_{MG} \cdot MG^* - r_b \cdot MG^* \qquad (4.3)$$

$$\frac{dMG}{dt} = r_b \cdot MG^* - d_{MG} \cdot MG \qquad (4.4)$$

*Kc2 needs to be scaled and extrapolated form Kc1

Parameterization Experiments

To parameterize the desired variables, five total TXTL experiments were proposed. The five experiments will have parameterizations based on reporter protein (SFGFP), involving more parameter estimations (protein maturation, degradation, etc.) in the process.

Proposed Parameterization Experiments

1. J23199 – att – SFGFP (JBL006)
2. J23119 – STRSV – AS(H2) – STRSV – Term (JBL3343) + JBL006
3. J23119 – [STRSV – AS(H2)]x2 – STRSV – Term (JBL5020) + JBL006
   4(CTRL) J23199 – mutt_att – SFGFP (JBL007)
4. J23119 – STRSV – AS(H2) – STRSV – Term (JBL3343) + JBL007
5. J23119 – AS (H2)—sTRSV--MG –Term (JBL3358)

Experiments 1

β_A is set to 0. Because there is no transcription of the antisense, this experiment will only give us information on the transcription, translation, maturation, and degradation rates of SFGFP.

1

$$\frac{d\boldsymbol{R_1^*}}{dt} = \boldsymbol{\beta_R} - \boldsymbol{d_1} \cdot \boldsymbol{R_1^*} - \boldsymbol{r_{m1}} \cdot \boldsymbol{R_1^*}$$

$$\frac{dX(1)}{dt} = P(9) - P(5) \cdot X(1) - P(6) \cdot X(1)$$

2

$$\frac{d\boldsymbol{R_1}}{dt} = \boldsymbol{r_{m1}} \cdot \boldsymbol{R_1^*} - \boldsymbol{d_1} \cdot \boldsymbol{R_1}$$

$$\frac{dX(2)}{dt} = P(6) \cdot X(1) - P(5) \cdot X(2)$$

3

$$\frac{d\boldsymbol{M}}{dt} = \boldsymbol{\beta_m} \cdot (1 - P_t) \cdot \left(\mathbf{1} - \frac{\boldsymbol{R_1}}{\boldsymbol{K_1} + \boldsymbol{R_1}}\right) - \boldsymbol{d_M} \cdot \boldsymbol{M}$$

$$\frac{dX(3)}{dt} = P(1) \cdot (1 - P(16)) \cdot \left(1 - \frac{X(2)}{P(2) + X(2)}\right) - P(12) \cdot X(3)$$

4

$$\frac{d\boldsymbol{G}}{dt} = \boldsymbol{k_t} \cdot \boldsymbol{M} - \boldsymbol{\alpha} \cdot \boldsymbol{G}$$

$$\frac{dX(4)}{dt} = P(10) \cdot X(3) - P(11) \cdot X(4)$$

$$\frac{\boldsymbol{dG_M}}{\boldsymbol{dt}} = \boldsymbol{\alpha \cdot G}$$

5

$$\frac{dX(5)}{dt} = P(11) \cdot X(4)$$

Experiments 2

The transcription of antisense is set to be $\beta_A = 8\beta$ in this experiment. Based on the SFGFP parameters obtained from experiment 1, antisense specific parameters such as degradation and maturation rate can be extrapolated from this experiment

$$\frac{\boldsymbol{dR_1^*}}{\boldsymbol{dt}} = \boldsymbol{\beta_R - d_1 \cdot R_1^* - r_{m1} \cdot R_1^*}$$

1

$$\frac{dX(1)}{dt} = P(9) - P(5) \cdot X(1) - P(6) \cdot X(1)$$

$$\frac{\boldsymbol{dR_1}}{\boldsymbol{dt}} = \boldsymbol{r_{m1} \cdot R_1^* - d_1 \cdot R_1}$$

2

$$\frac{dX(2)}{dt} = P(6) \cdot X(1) - P(5) \cdot X(2)$$

$$\frac{\boldsymbol{dM}}{\boldsymbol{dt}} = \boldsymbol{\beta_m \left(1 - \frac{R_1}{K_1 + R_1}\right) - d_M \cdot M}$$

3

$$\frac{dX(3)}{dt} = P(1) \cdot (1 - P(16)) \cdot \left(1 - \frac{X(2)}{P(2) + X(2)}\right) - P(12) \cdot X(3)$$

$$\frac{\boldsymbol{dG}}{\boldsymbol{dt}} = \boldsymbol{k_t \cdot M - \alpha \cdot G}$$

4

$$\frac{dX(4)}{dt} = P(10) \cdot X(3) - P(11) \cdot X(4)$$

$$\frac{\boldsymbol{dG_M}}{\boldsymbol{dt}} = \boldsymbol{\alpha \cdot G}$$

5

$$\frac{dX(5)}{dt} = P(11) \cdot X(4)$$

Experiment 3

Here, double antisense is used in place of single antisense, resulting in stronger repression. However, because ribozymes are ineffective at cleaving long strands of RNA, the double antisense would have very different

maturation rates compared to just multiplying the single antisense parameter by a factor of two. From this experiment, accurate values of double antisense degradation and maturation rates will be obtained.

$$\frac{\boldsymbol{dR_2^*}}{\boldsymbol{dt}} = \boldsymbol{\beta_R} - \boldsymbol{d_2} \cdot \boldsymbol{R_2^*} - \boldsymbol{r_{m2}} \cdot \boldsymbol{R_2^*}$$

1

$$\frac{dX(1)}{dt} = P(9) - P(15) \cdot X(1) - P(7) \cdot X(1)$$

$$\frac{\boldsymbol{dR_2}}{\boldsymbol{dt}} = \boldsymbol{r_{m2}} \cdot \boldsymbol{R_2^*} - \boldsymbol{d_2} \cdot \boldsymbol{R_2}$$

2

$$\frac{dX(2)}{dt} = P(7) \cdot X(1) - P(15) \cdot X(2)$$

$$\frac{\boldsymbol{dM}}{\boldsymbol{dt}} = \boldsymbol{\beta_m} \cdot (1 - P_t) \cdot \left(\mathbf{1} - \frac{\boldsymbol{R_2}}{\boldsymbol{K_2} + \boldsymbol{R_2}}\right) - \boldsymbol{d_M} \cdot \boldsymbol{M}$$

3

$$\frac{dX(3)}{dt} = P(1) \cdot (1 - P(16)) \left(1 - \frac{X(2)}{P(3) + X(2)}\right) - P(12) \cdot X(3)$$

$$\frac{\boldsymbol{dG}}{\boldsymbol{dt}} = \boldsymbol{k_t} \cdot \boldsymbol{M} - \boldsymbol{\alpha} \cdot \boldsymbol{G}$$

4

$$\frac{dX(4)}{dt} = P(10) \cdot X(3) - P(11) \cdot X(4)$$

$$\frac{\boldsymbol{dG_M}}{\boldsymbol{dt}} = \boldsymbol{\alpha} \cdot \boldsymbol{G}$$

5

$$\frac{dX(5)}{dt} = P(11) \cdot X(4)$$

Experiment 4

In this experiment, mutated attenuator is paired with single antisense. Although no repression should be seen with this mutated attenuator and antisense pair since they are non-orthogonal, small level of crosstalk always exists. Thus, the purpose of this experiment is to parameterize the level of crosstalk and the repression coefficient of the mutt-att and antisense pair.

$\beta_A = 8\beta$

$$\frac{\boldsymbol{dR_1^*}}{\boldsymbol{dt}} = \boldsymbol{\beta_R} - \boldsymbol{d_1} \cdot \boldsymbol{R_1^*} - \boldsymbol{r_{m1}} \cdot \boldsymbol{R_1^*}$$

1

$$\frac{dX(1)}{dt} = P(9) - P(5) \cdot X(1) - P(7) \cdot X(1)$$

$$\frac{\boldsymbol{dR_1}}{\boldsymbol{dt}} = \boldsymbol{r_{m1}} \cdot \boldsymbol{R_1^*} - \boldsymbol{d_1} \cdot \boldsymbol{R_1}$$

2

$$\frac{dX(2)}{dt} = P(7) \cdot X(1) - P(5) \cdot X(2)$$

$$\frac{\boldsymbol{dM}}{\boldsymbol{dt}} = \boldsymbol{\beta_m} \cdot (1 - P_t) \cdot \left(\mathbf{1} - \frac{\boldsymbol{R_1}}{\boldsymbol{K_{C1}} + \boldsymbol{R_1}}\right) - \boldsymbol{d_M} \cdot \boldsymbol{M}$$

3

$$\frac{dX(3)}{dt} = P(1) \cdot (1 - P(16)) \left(1 - \frac{X(2)}{P(4) + X(2)}\right) - P(12) \cdot X(3)$$

$$\frac{\boldsymbol{dG}}{\boldsymbol{dt}} = \boldsymbol{k_t} \cdot \boldsymbol{M} - \boldsymbol{\alpha} \cdot \boldsymbol{G}$$

4

$$\frac{dX(4)}{dt} = P(10) \cdot X(3) - P(11) \cdot X(4)$$

$$\frac{\boldsymbol{dG_M}}{\boldsymbol{dt}} = \boldsymbol{\alpha} \cdot \boldsymbol{G}$$

5

$$\frac{dX(5)}{dt} = P(11) \cdot X(4)$$

Experiment 5

In this experiment, K_cleave is estimated

$$\frac{\boldsymbol{dMG^*}}{\boldsymbol{dt}} = \boldsymbol{\beta} \cdot (1 - P_t) - \boldsymbol{d_{MG}} \cdot \boldsymbol{MG^*} - \boldsymbol{r_b} \cdot \boldsymbol{MG^*}$$

$$\frac{dX(1)}{dt} = P(13) \cdot \big(1 - P(16)\big) - P(14) \cdot X(1) - P(8) \cdot X(1)$$

$$\frac{\boldsymbol{dMG}}{\boldsymbol{dt}} = \boldsymbol{r_b} \cdot \boldsymbol{MG^*} - \boldsymbol{d_{MG}} \cdot \boldsymbol{MG}$$

$$\frac{dX(2)}{dt} = P(8) \cdot X(1) - P(14) \cdot X(2)$$