

# Supplementary Information - RNA compensator: A positive feedback insulation strategy for RNA-based networks

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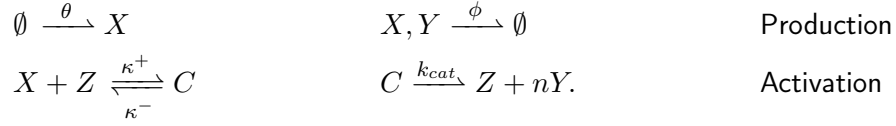
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# 1 STAR module analysis

## 1.1 Model derivation of the small transcription activating RNAs (STAR) system

In this section, we perform a steady state analysis of the STAR system. To help the reader, we summarize the chemical reaction,



We derive the Ordinary Differential Equations (ODEs) by using the law of mass action:

$$\dot{x} = \theta - \phi x - \kappa^+ x z + \kappa^- c \quad (1)$$

$$\dot{c} = \kappa^+ x z - \kappa^- c - k_{cat} c \quad (2)$$

$$\dot{y} = n k_{cat} c - \phi y \quad (3)$$

with a mass conservation of the total amount of DNA complex constant,  $z + c = z^{tot}$ .

**Steady state** To find the steady state, we make equations (1)-(3) equal to zero. From  $\dot{c} = 0$ , we obtain

$$\bar{c} = \frac{\bar{x}}{(\kappa^- + k_{cat})/\kappa^+} \bar{z} = \frac{\bar{x}}{K} \bar{z}, \quad (4)$$

where we define the dissociation constant  $K = (\kappa^- + k_{cat})/\kappa^+$ . Next, we use mass conservation,  $\bar{z} + \bar{c} = z^{tot}$ , and find that

$$\bar{c} = \frac{\bar{x}}{\bar{x} + K} z^{tot}. \quad (5)$$

From  $\dot{x} = 0$ , and  $\dot{y} = 0$ , we plug  $\bar{c}$  in these equations, and leads to

$$0 = \theta - \phi \bar{x} - k_{cat} z^{tot} \frac{\bar{x}}{\bar{x} + K} \quad (6)$$

$$0 = n k_{cat} z^{tot} \frac{\bar{x}}{\bar{x} + K} - \phi \bar{y} \quad (7)$$

Equation (6) leads to a second order polynomial,  $\bar{x}^2 + p_1 \bar{x} - p_0 = 0$ , where  $p_1 = K + (k_{cat} z^{tot} - \theta)/\phi$  and  $p_0 = \theta K/\phi$ . This result in a single positive real solution and we can find

$$\bar{x} = \frac{-p_1 + \sqrt{p_1^2 + 4p_0}}{2} \quad \text{and} \quad \bar{y} = n \frac{k_{cat} z^{tot}}{\phi} \left( \frac{\bar{x}}{\bar{x} + K} \right). \quad (8)$$

**Stability analysis** We compute the Jacobian matrix for the system (1)-(3), resulting in

$$J = \begin{bmatrix} -\phi - \kappa^+ \bar{z} & \kappa^+ \bar{x} + \kappa^- & 0 \\ \kappa^+ \bar{z} & -\kappa^+ \bar{x} - \kappa^- - k_{cat} & 0 \\ 0 & k_{cat} & -\phi \end{bmatrix}. \quad (9)$$

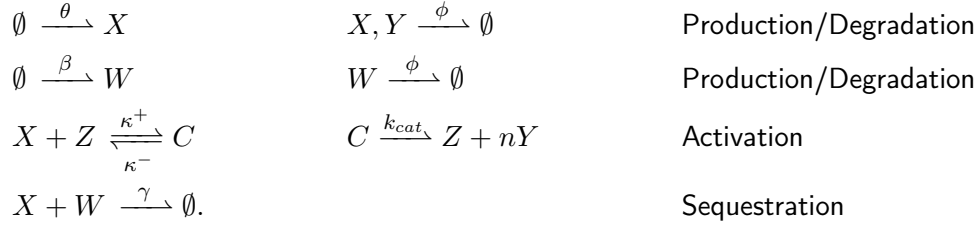
The system can only admit negative eigen values when the following condition is satisfied,

$$\phi k^+ \bar{x} + \phi k^- + \phi k_{cat} > -k_{cat} k^+ \bar{z}.$$

Since this condition is satisfied by all parameters, we conclude that the system is unconditional stable.

## 1.2 Molecular sequestration tunes the dose response of the STAR system

From the description of main text, we summarize the chemical reaction,



We derive the Ordinary Differential Equations (ODEs) by using the law of mass action:

$$\dot{x} = \theta - \phi x - \kappa^+ x z + \kappa^- c - \gamma x w \quad (10)$$

$$\dot{c} = \kappa^+ x z - \kappa^- c - k_{cat} c \quad (11)$$

$$\dot{w} = \beta - \gamma x w - \phi w \quad (12)$$

$$\dot{y} = n k_{cat} c - \phi y, \quad (13)$$

with a mass conservation of the total amount of DNA complex constant,  $z + c = z^{tot}$ .

**Steady state** We follow similar steps as before, and by making  $\dot{c} = 0$ , we find that  $\bar{c} = \frac{\bar{x}}{\bar{x} + K} z^{tot}$ . Further, by plugging in the other equations  $\dot{x} = \dot{w} = \dot{y} = 0$ , this results in

$$0 = \theta - \phi \bar{x} - k_{cat} z^{tot} \frac{\bar{x}}{\bar{x} + K} - \gamma \bar{x} \bar{w} \quad (14)$$

$$0 = \beta - \phi \bar{w} - \gamma \bar{x} \bar{w} \quad (15)$$

$$0 = n k_{cat} z^{tot} \frac{\bar{x}}{\bar{x} + K} - \phi \bar{y}. \quad (16)$$

From equation (14)-(15), we can find  $\bar{w}$  as a function of  $\bar{x}$

$$\bar{w} = \frac{\theta - \phi \bar{x} - k_{cat} z^{tot} \frac{\bar{x}}{\bar{x} + K}}{\gamma \bar{x}} = \frac{\beta}{\gamma \bar{x} + \phi}. \quad (17)$$

This leads to a third order polynomial. However, a simple way to find the input-output is to find  $\theta$  as a function of  $\bar{x}$ . This results in

$$\theta(\bar{x}) = \frac{\gamma \bar{x}}{\gamma \bar{x} + \phi} \beta + \phi \bar{x} + k_{cat} z^{tot} \frac{\bar{x}}{\bar{x} + K}. \quad (18)$$

**Stability analysis.** We compute the Jacobian matrix for the system (10)-(13), resulting in

$$J = \begin{bmatrix} -\phi - \kappa^+ \bar{z} - \gamma \bar{w} & \kappa^+ \bar{x} + \kappa^- & -\gamma \bar{x} & 0 \\ \kappa^+ \bar{z} & -\kappa^+ \bar{x} - \kappa^- - k_{cat} & 0 & 0 \\ -\gamma \bar{w} & 0 & -\phi - \gamma \bar{x} & 0 \\ 0 & 0 & n k_{cat} & -\phi \end{bmatrix}. \quad (19)$$

The system  $J$  can admit negative eigenvalues if the following conditions are satisfied,

$$(2\phi + \gamma(\bar{w} + \bar{x}))(\kappa^+ \bar{x} + \kappa^- + k_{cat}) + (\phi + \kappa^+ \bar{z} + \gamma \bar{w})\phi + (\phi + \kappa^+ \bar{z})(\gamma \bar{x}) > -k_{cat} \kappa^+ \bar{z}$$

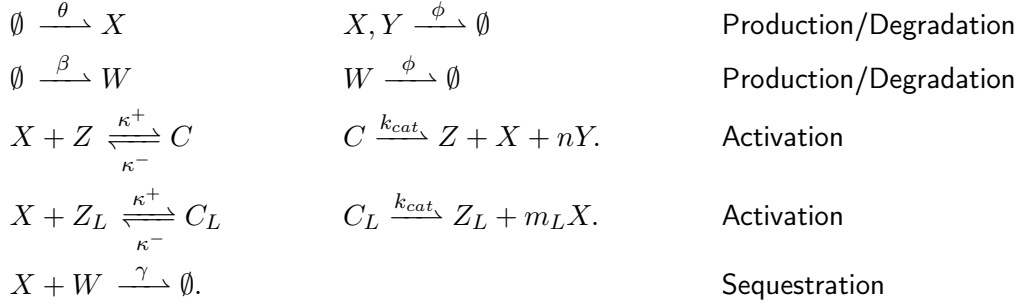
and

$$\phi(\phi + \gamma(\bar{x} + \bar{w}))(\kappa^+ \bar{x} + \kappa^- + k_{cat}) > -k_{cat} \kappa^+ \bar{z}(\phi + \gamma \bar{x}).$$

Then, we can conclude that the STAR system that incorporates sequestration is unconditional stable for any parameter value.

### 1.3 RNA compensator mitigates the loading effects

In section we will consider the activation of two downstream process. Now the activator can bind and unbind to  $Z$  and  $Z_L$ , forming complex  $C$  and  $C_L$ .  $C$  produces a copy of  $X$  and  $Y$ , which are separated by insulators such as ribozymes or endoribonuclease sites. Meanwhile,  $C_L$  produces  $m_L$  copies of  $X$  using same insulation strategy. We set  $m_L = 0$  to simulate loading effect and set  $m_L = 1$  when RNA compensator is implemented. Both  $X$  and  $Y$  degrade at a constant rate  $\phi$ . We summarize the chemical reaction as



We derive the Ordinary Differential Equations (ODEs) by using the law of mass action:

$$\dot{x} = \theta - \phi x - \gamma x w - \kappa^+ x z + \kappa^- c - \kappa^+ x z_L + \kappa^- c_L + m_L k_{cat} c_L \quad (20)$$

$$\dot{c} = \kappa^+ x z - \kappa^- c - k_{cat} c \quad (21)$$

$$\dot{c}_L = \kappa^+ x z_L - \kappa^- c_L - k_{cat} c_L \quad (22)$$

$$\dot{w} = \beta - \phi w - \gamma x w \quad (23)$$

$$\dot{y} = n k_{cat} c - \phi y \quad (24)$$

with a mass conservation of the total amount of DNA complex constant,  $z + c = z^{tot}$  and  $z_L + c_L = z_L^{tot}$ .

**Steady state analysis.** By making  $\dot{c} = \dot{c}_L = 0$ , following similar steps as before, we find that  $\bar{c} = x z^{tot} / (x + K)$ , and  $\bar{c}_L = x z_L^{tot} / (x + K)$ ,

$$0 = \theta - \phi \bar{x} - \gamma \bar{x} \bar{w} - k_{cat} z^{tot} \frac{\bar{x}}{\bar{x} + K} + (m_L - 1) k_{cat} z_L^{tot} \frac{\bar{x}}{\bar{x} + K} \quad (25)$$

$$0 = \beta - \phi \bar{w} - \gamma \bar{x} \bar{w} \quad (26)$$

$$0 = n k_{cat} z^{tot} \frac{\bar{x}}{\bar{x} + K} - \phi y \quad (27)$$

From equation (25)-(26), we can find  $\bar{w}$  as a function of  $\bar{x}$

$$\bar{w} = \frac{\theta - \phi \bar{x} - k_{cat} z^{tot} \frac{\bar{x}}{\bar{x} + K} + (m_L - 1) k_{cat} z_L^{tot} \frac{\bar{x}}{\bar{x} + K}}{\gamma \bar{x} + \phi} = \frac{\beta}{\gamma \bar{x} + \phi}. \quad (28)$$

This leads to a third order polynomial. However, a simple way to find the input-output is to find  $\theta$  as a function of  $\bar{x}$ . This results in

$$\theta(\bar{x}) = \frac{\gamma \bar{x}}{\gamma \bar{x} + \phi} \beta + \phi \bar{x} + \{z^{tot} + (1 - m_L) z_L^{tot}\} k_{cat} \frac{\bar{x}}{\bar{x} + K}, \quad (29)$$

and  $\bar{y} = n k_{cat} z^{tot} \frac{\bar{x}}{\bar{x} + K}$ .

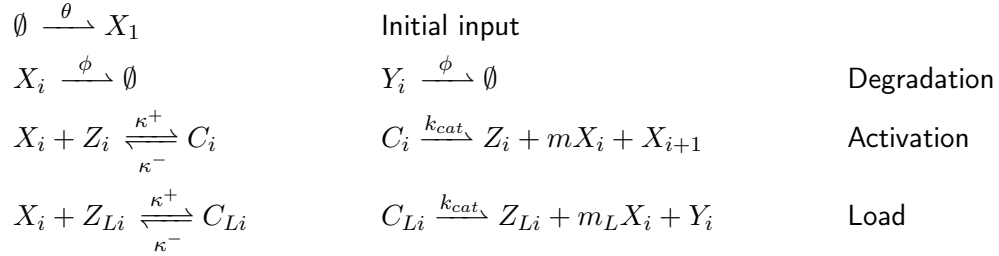
**Stability analysis.** We write down the Jacobian matrix for the system (20)-(24), resulting in

$$J = \begin{bmatrix} -\phi - \kappa^+(\bar{z} + \bar{z}_L) - \gamma\bar{w} & \kappa^- + k^+\bar{x} & \kappa^- + m_L k_{cat} + k^+\bar{x} & -\gamma\bar{x} & 0 \\ \kappa^+\bar{z} & -\kappa^- - k^+\bar{x} & 0 & 0 & 0 \\ \kappa^+\bar{z}_L & 0 & -\kappa^- - m_L k_{cat} - k^+\bar{x} & 0 & 0 \\ -\gamma\bar{w} & 0 & 0 & -\phi - \gamma\bar{x} & 0 \\ 0 & n k_{cat} & 0 & 0 & \phi \end{bmatrix}. \quad (30)$$

We evaluate the stability of the system by computing the eigenvalues of J.

## 2 Derivation of equilibrium points in RNA cascades

A multi-layer RNA cascade is described as following reactions:



These reactions are converted to ODEs:

$$\dot{x}_i = k_{cat}c_{i-1} - \phi x_i - \kappa^+ x_i z_i + \kappa^- c_i - \kappa^+ x_i z_{Li} + \kappa^- c_{Li} + m k_{cat} c_i + m_L k_{cat} c_{Li} \quad (31)$$

$$\dot{c}_i = \kappa^+ x_i z_i - \kappa^- c_i - k_{cat} c_i \quad (32)$$

$$\dot{c}_{Li} = \kappa^+ x_i z_{Li} - \kappa^- c_{Li} - k_{cat} c_{Li} \quad (33)$$

$$\dot{y}_i = k_{cat} c_{Li} - \phi y_i \quad (34)$$

where we define  $z_i^{tot} = c_i + z_i$ ,  $z_{Li}^{tot} = c_{Li} + z_{Li}$ , and  $K = \frac{\kappa^- + k_{cat}}{\kappa^+}$ . By setting (32) equal to 0 we derive:

$$\bar{c}_i = \frac{\bar{x}_i \bar{z}_i^{tot}}{\bar{x}_i + K} \quad \text{as well as} \quad \bar{x}_i = \frac{K \bar{c}_i}{\bar{z}_i^{tot} - \bar{c}_i} \quad (35)$$

Setting (31) equal to 0, we get

$$k_{cat} \bar{c}_{i-1} = \phi \bar{x}_i + (1 - m) k_{cat} \bar{c}_i + (1 - m_L) k_{cat} \bar{c}_{Li} \quad (36)$$

When there is no compensation ( $m = 0$ ) nor additional loads ( $z_{Li}^{tot} = 0$ ,  $\bar{c}_{Li} = 0$ ) in the cascade, the relation between the transcription output of  $i^{th}$  layer and  $i - 1^{th}$  layer is

$$k_{cat} \bar{c}_i = k_{cat} \bar{c}_{i-1} - \phi \bar{x}_i \quad (37)$$

Consider that the initial input  $k_{cat} c_0 = \theta$ , the equation above can be written as

$$k_{cat} \bar{c}_i = \theta - \sum_{n=1}^i \phi \bar{x}_n \quad (38)$$

This suggests that in an RNA cascade without compensation, the maximum of signal output is limited by the initial input  $\theta$ . The longer the cascade is, the lower the output signal will be due to the depletion of  $X_i$  in each layer of cascade.

Next, we solve  $k_{cat}\bar{c}_i$  with (35) and (37), and we get:

$$k_{cat}\bar{c}_i = k_{cat}\bar{c}_{i-1} - \frac{\phi K \bar{c}_i}{z_i^{tot} - \bar{c}_i} \quad (39)$$

$$k_{cat}\bar{c}_i = \frac{p_1 - \sqrt{p_1^2 - 4k_{cat}^2\bar{c}_{i-1}z_i^{tot}}}{2} \quad \text{where} \quad p_1 = k_{cat}\bar{c}_{i-1} + k_{cat}z_i^{tot} + \phi K \quad (40)$$

Then we add the compensator by setting  $m = 1$  in (36) and replacing  $x_i$  with (35). Then, the transcription output becomes:

$$k_{cat}\bar{c}_i = k_{cat} \frac{\bar{c}_{i-1}k_{cat}z_i^{tot}}{\bar{c}_{i-1}k_{cat} + \phi K} \quad (41)$$

This is a convergent sequence because  $(\bar{c}_{i-1}k_{cat})/(\bar{c}_{i-1}k_{cat} + \phi K) < 1$  and  $k_{cat}\bar{c}_i < k_{cat}z_i^{tot}$ . So we can find the limit by letting the  $k_{cat}\bar{c}_i = k_{cat}\bar{c}_{i-1}$ , and we get the equilibrium output for a long cascade

$$k_{cat}\bar{c}_i = k_{cat}z_i^{tot} - \phi K \quad (42)$$

Supposing that  $z_i^{tot}$  is a fixed number  $z^{tot}$  for all layers and the cascade is long enough, the output of the system will finally stabilize at  $k_{cat}z^{tot} - \phi K$  (if  $z^{tot}k_{cat} > \phi K$ ) or zero (if  $z^{tot}k_{cat} \leq \phi K$ ).

Then we further investigate the loading effect on cascade with external loads ( $z_{Li}^{tot} > 0$ ). When no compensation is implemented ( $m_L = 0$ ) in (36), signal output of  $i^{th}$  layer become

$$k_{cat}\bar{c}_i = \frac{p_1 - \sqrt{p_1^2 - 4k_{cat}^2\bar{c}_{i-1}z_{Li}^{tot}}}{2 \frac{z_{Li}^{tot}}{z_i^{tot}}} \quad \text{where} \quad p_1 = k_{cat}\bar{c}_{i-1} + k_{cat}z_{Li}^{tot} + \phi K \quad (43)$$

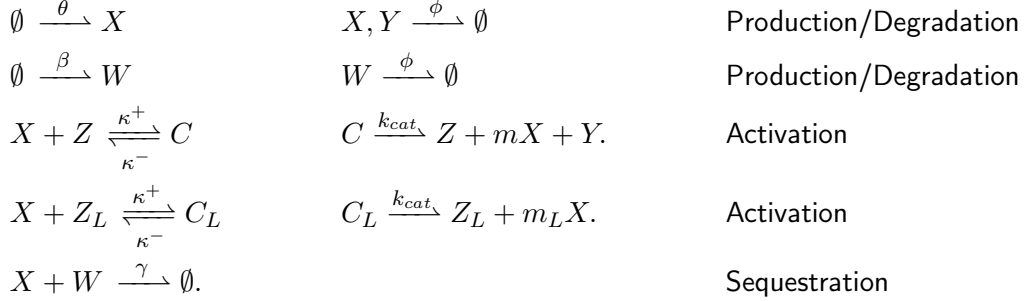
Similarly, we get the equilibrium point

$$k_{cat}\bar{c}_i = k_{cat}z_i^{tot} - \frac{\phi K}{1 - \frac{z_{Li}^{tot}}{z_i^{tot}}} \quad (44)$$

The value of  $k_{cat}\bar{c}_i$  in (44) is lower than the value computed from (42), indicating that the cascade is disturbed by the external load. When the external load is high ( $k_{cat}z_{Li}^{tot} \geq k_{cat}z_i^{tot} - \phi K$ ), the equilibrium point becomes zero. By implementing the compensation ( $n = 1$ ), the system is recovered to (41) and (42), result in fully mitigation of loading effect in equilibrium.

### 3 Modeling of an RNA-based memory system

To help the readers with the description of the RNA-based bistable design, we summarize the chemical reactions



We derive the Ordinary Differential Equations (ODEs) by using the law of mass action:

$$\dot{x} = \theta - \phi x - \gamma x w - \kappa^+ x z + \kappa^- c + m k_{cat} c - \kappa^+ x z_L + \kappa^- c_L + m_L k_{cat} c_L \quad (45)$$

$$\dot{c} = \kappa^+ x z - \kappa^- c - k_{cat} c \quad (46)$$

$$\dot{c}_L = \kappa^+ x z_L - \kappa^- c_L - k_{cat} c_L \quad (47)$$

$$\dot{w} = \beta - \phi w - \gamma x w \quad (48)$$

$$\dot{y} = k_{cat} c - \phi y \quad (49)$$

with a mass conservation of the total amount of DNA complex constant,  $z + c = z^{tot}$  and  $z_L + c_L = z_L^{tot}$ .

**Steady state analysis.** By making  $\dot{c} = \dot{c}_L = 0$ , following similar steps as before, we find that  $\bar{c} = x z^{tot} / (x + K)$ , and  $\bar{c}_L = x z_L^{tot} / (x + K)$

$$0 = \theta - \phi \bar{x} - \gamma \bar{x} \bar{w} + (m - 1) k_{cat} z^{tot} \frac{\bar{x}}{\bar{x} + K} + (m_L - 1) k_{cat} z_L^{tot} \frac{\bar{x}}{\bar{x} + K} \quad (50)$$

$$0 = \beta - \phi \bar{w} - \gamma \bar{x} \bar{w} \quad (51)$$

$$0 = k_{cat} z^{tot} \frac{\bar{x}}{\bar{x} + K} - \phi y \quad (52)$$

From equation (50)-(51), we can find  $\bar{w}$  as a function of  $\bar{x}$

$$\bar{w} = \frac{\theta - \phi \bar{x} + (m - 1) k_{cat} z^{tot} \frac{\bar{x}}{\bar{x} + K} + (m_L - 1) k_{cat} z_L^{tot} \frac{\bar{x}}{\bar{x} + K}}{\gamma \bar{x}} = \frac{\beta}{\gamma \bar{x} + \phi}. \quad (53)$$

This leads to a third order polynomial. However, a simple way to find the input-output is to find  $\theta$  as a function of  $\bar{x}$ . This results in

$$\theta(\bar{x}) = \frac{\gamma \bar{x}}{\gamma \bar{x} + \phi} \beta + \phi \bar{x} + \{(1 - m) z^{tot} + (1 - m_L) z_L^{tot}\} k_{cat} \frac{\bar{x}}{\bar{x} + K}, \quad (54)$$

and  $\bar{y} = k_{cat} z^{tot} \frac{\bar{x}}{\bar{x} + K}$ .

**Stability analysis.** We write down the Jacobian matrix for the system (45)-(49), resulting in

$$J = \begin{bmatrix} -\phi - \kappa^+(\bar{z} + \bar{z}_L) - \gamma \bar{w} & \kappa^- + m k_{cat} + k^+ \bar{x} & \kappa^- + m_L k_{cat} + k^+ \bar{x} & -\gamma \bar{x} & 0 \\ \kappa^+ \bar{z} & -\kappa^- - m k_{cat} - k^+ \bar{x} & 0 & 0 & 0 \\ \kappa^+ \bar{z}_L & 0 & -\kappa^- - m_L k_{cat} - k^+ \bar{x} & 0 & 0 \\ -\gamma \bar{w} & 0 & 0 & -\phi - \gamma \bar{x} & 0 \\ 0 & k_{cat} & 0 & 0 & \phi \end{bmatrix}. \quad (55)$$

We inspect the stability of the system numerically by finding the eigenvalues of  $J$ . In addition, we can find some conditions for stability when  $z_L = 0$ , this results in

$$(2\phi + \gamma(\bar{w} + \bar{x}))(\kappa^+ \bar{x} + \kappa^- + k_{cat}) + (\phi + \kappa^+ \bar{z} + \gamma \bar{w})\phi + (\phi + \kappa^+ \bar{z})(\gamma \bar{x}) > (m - 1)k_{cat}\kappa^+ \bar{z}$$

and

$$\phi(\phi + \gamma(\bar{x} + \bar{w}))(\kappa^+ \bar{x} + \kappa^- + k_{cat}) > (m - 1)k_{cat}\kappa^+ \bar{z}(\phi + \gamma \bar{x}).$$

#### 4 Table 1 - General parameters

Parameter	Value	Annotation	Other studies
$\phi$ (1/s)	$1.2 \times 10^{-3}$	Degradation RNA	$10^{-4} - 10^{-3}$ [1]
$k^+$ (/M/s)	$2.1 \times 10^4$	STAR and target binding	$10^4 - 10^6$ [2, 3]
$k^-$ (1/s)	$6.3 \times 10^3$	STAR and target unbinding	
$k_{cat}$ (1/s)	0.011	STAR-target complex transcription	0.05 - 0.2 [4]*
$\theta$ (M/s)	$0 - 2.8 \times 10^{-10}$	STAR-target complex transcription	$2.8 \times 10^{-11} - 2.8 \times 10^{-8}$ [5, 6]
$\beta$ (M/s)	$1.4 \times 10^{-10}$	STAR-target complex transcription	$2.8 \times 10^{-11} - 2.8 \times 10^{-8}$ [5, 6]
$\gamma$ (/M/s)	$2.1 \times 10^5$	STAR and sequester binding	$10^4 - 10^6$ [2, 3]
$m$	5	STAR repeats in self-activator	
$A$ (M/s)	$2.5 \times 10^{-10}$	Amplitude of pulse input in self-activator	
$T$ (h)	3	Width of pulse input in self-activator	

\* Estimated from the average pause-free velocity of RNAP at saturating concentrations of nucleotide triphosphates (NTP) for an RNA length of 200bp.



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