

# Design for Improved Repression in RNA Replicons

Jacob Beal  
Raytheon BBN Technologies  
Cambridge, MA, USA  
jakebeal@bbn.com

Ron Weiss  
Massachusetts Institute of Technology  
Cambridge, MA, USA  
rweiss@mit.edu

## 1. MOTIVATION

RNA replicons are an emerging platform for synthetic biology, in which the infective capsid of a RNA virus is replaced with an engineered payload while its self-replication capability is retained [4, 3, 1, 6]. This self-replication capability allows RNA replicons entering a cell to amplify their engineered elements, providing strong expression from a low initial dose without integration into host DNA or propagation to other cells. Replicons thus offer an attractive platform for developing medical applications such as vaccines [2, 3] and stem-cell generation [7], combining both strong expression and relative genetic isolation. Development of RNA replicons to date has focused primarily on derivatives of alphaviruses, a well-characterized family of positive-strand RNA viruses, and most particularly the Sindbis and VEE vectors [4]. Protein expression from RNA replicons can be precisely predicted and controlled [1], and can support standard synthetic circuits such as cascades and toggle switches [6].

A key challenge for creating effective synthetic circuitry with RNA replicons, however, is that regulatory devices often perform less well when expressed from replicons. For example, L7Ae is a very strong RNA regulator, able to provide more than 200-fold repression when expressed from DNA plasmids, but was found to yield less than 30-fold repression when expressed from RNA replicons [6]. By examination of quantitative models derived from [1] and [6], we find that simple circuit adjustments, to exploit rather than oppose RNA replicon dynamics, should be able to reverse this problem and in fact produce significantly better circuit performance than is observed with DNA plasmids.

## 2. QUANTITATIVE EXPRESSION MODEL

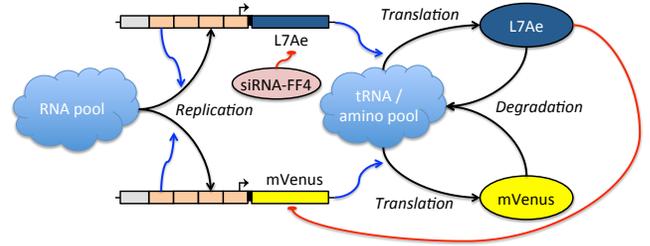
Figure 1 shows a diagrammatic model of the interactions in a two-replicon repression circuit modeled after [6]. In this circuit, the L7Ae RNA regulator suppresses expression of mVenus fluorescent protein and is in turn degraded by the small interfering RNA siRNA-FF4. When siRNA-FF4 is absent, L7Ae will not degrade and will repress mVenus, whereas when it is present L7Ae will rapidly degrade and mVenus should be high. This system may be simulated as an ODE using the following equations:

$$\frac{dR_i}{dt} = \alpha_i \cdot N \cdot R_i \quad (1)$$

$$\frac{dL}{dt} = \alpha_L \cdot A \cdot R_1 - \frac{\log 2}{t_L} \cdot \frac{1 + (S/D_S)^{H_S}}{1 + K_S^{-1}(S/D_S)^{H_S}} \cdot L \quad (2)$$

IWBDA 2016 Newcastle, UK

This work was sponsored by DARPA DSO under grant W911NF-11-054; the views and conclusions contained in this document are those of the authors and not DARPA or the U.S. Government.



**Figure 1: RNA replicon repression circuit: siRNA-FF4 degrades L7Ae, which in turn represses mVenus fluorescent protein.**

$$\frac{dV}{dt} = \alpha_V \cdot A \cdot R_2 \cdot \frac{1 + K_L^{-1}(L/D_L)^{H_L}}{1 + (L/D_L)^{H_L}} - \frac{\log 2}{t_V} \cdot V \quad (3)$$

$$\frac{dN}{dt} = -\sum_i \frac{dR_i}{dt} \quad (4)$$

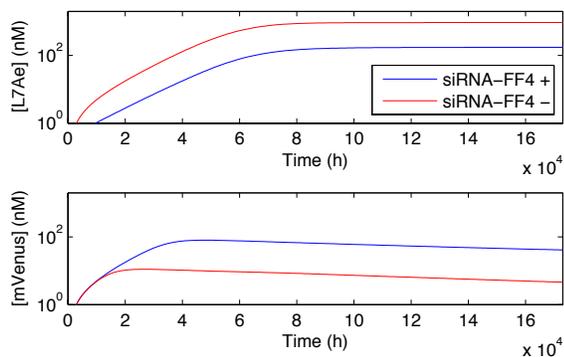
$$\frac{dA}{dt} = -\left(\frac{dL}{dt} + \frac{dV}{dt}\right) \quad (5)$$

where  $R_i$  is the number of copies of each replicon,  $N$  is the amount of available transcriptional resources,  $S$  is the amount of siRNA-FF4,  $L$  is the amount of L7Ae,  $V$  is the amount of mVenus,  $A$  is the amount of available translational resources,  $t_x$  is the decay half-life of species  $x$ , and  $\alpha_x$ ,  $K_x$ ,  $D_x$ , and  $H_x$  are standard Hill equation coefficients. When parameterized with best-fit values derived from [1] and [6]<sup>1</sup>, the system behaves as shown in Figure 2, producing a 9-fold repression: much less than the 63-fold it predicts from plasmid DNA and an underperformance ratio equal to that observed in [6]. The model suggests that poor performance is due to the high expression of L7Ae in its “off” state and the inability of L7Ae to sufficiently repress mVenus before a significant amount has built up in the system.

## 3. PARAMETER OPTIMIZATION

Given the issues identified by the model and the nature of this RNA replicon circuit, there are four tuning mechanisms that offer ready means of adjusting performance. The high L7Ae “off” expression can be decreased by decreasing the relative initial dose of its expressing replicon or by decreasing per-replicon expression by decreasing the strength of its subgenomic promoter (a well-established mechanism for controlling replicon expression, e.g. [5]). Likewise, the high ini-

<sup>1</sup>Note that due to the insufficiency of available experimental data, some parameters are poorly constrained.

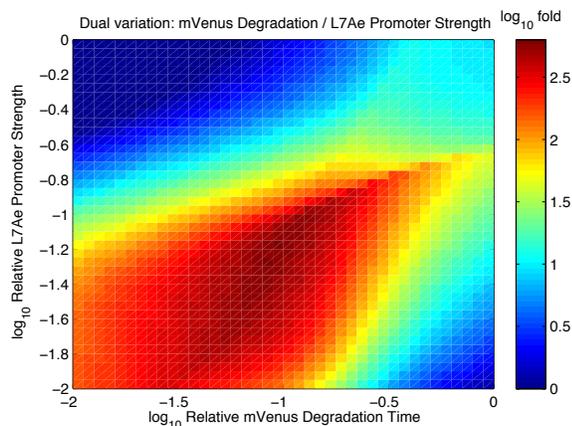


**Figure 2: Unoptimized circuit has poor dynamic range due to leaky L7Ae “off” and early unexpressed expression of mVenus.**

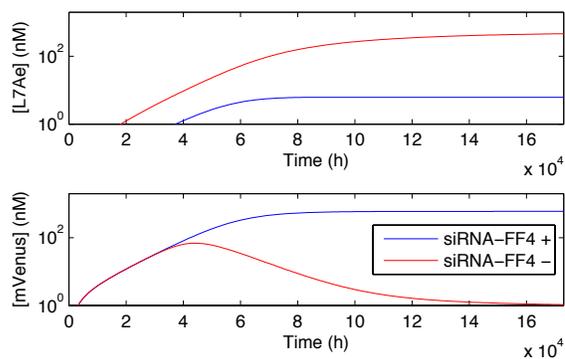
tial expression of mVenus can be decreased by decreasing the strength of its subgenomic promoter or by adding degradation tags to decrease its half-life.

To investigate the potential of these mechanisms, we performed single-parameter scans, running simulations of each adjustment across two orders of magnitude at 20 values per decade. These simulations indicate that the two L7Ae modifications have a near-equivalent effect in significantly amplifying the dynamic range of this circuit. Decreasing the half-life of mVenus can also improve dynamic range by affecting different dynamics, while adjusting mVenus promoter strength does not improve dynamic range but only shifts expression linearly.

Based on these single-parameter results, we conducted a detailed two-parameter scan for both decreasing L7Ae promoter strength and decreasing mVenus half-life. Figure 3 shows the results of this experiment, including an asymmetric region in which the combination of the two modifications is predicted to provide more than 500-fold dynamic range. The combination of decreasing L7Ae dose and decreasing mVenus half-life (not shown) produces very similar results. Intuitively, in this area decreased L7Ae expression means that unexpressed mVenus outcompetes L7Ae for resources



**Figure 3: Decreasing L7Ae promoter strength and mVenus half-life can markedly improve the predicted dynamic range of repression.**



**Figure 4: Optimized circuit with 7% L7Ae expression and 10% mVenus half-life has more than 50-fold improvement in predicted dynamic range.**

and decreases its “off” level, while decreased mVenus half-life means that even a high initial transient can be extinguished in the repressed state. Together, these predict expression patterns such as in the example in Figure 4, predicting much greater dynamic range for both L7Ae and mVenus.

## 4. CONTRIBUTIONS AND FUTURE WORK

Having predicted modifications to markedly improve the performance of repression in replicon circuits, a clear next step is for these modifications to be implemented in the lab and tested experimentally to see whether the predicted improvements materialize (which may require combining SGP and ratio manipulation to get sufficient range). Importantly, precise quantitative prediction and design has previously been demonstrated in replicons [1] and the predicted region of high performance is fairly broad. These models may also be extended to predict a larger range of systems, including more modes of regulation and more complex replicon architectures, thereby increasing the range of replicon applications that may be more effectively engineered.

## 5. REFERENCES

- [1] J. Beal et al. Model-driven engineering of gene expression from rna replicons. *ACS Synthetic Biology*, 4(1):48–56, 2015.
- [2] A. J. Geall et al. Nonviral delivery of self-amplifying rna vaccines. *Proceedings of the National Academy of Sciences*, 2012.
- [3] K. Lundstrom. Alphavirus vectors in vaccine development. *J. Vaccines Vaccin.*, 3, 2012.
- [4] S. Perri et al. An alphavirus replicon particle chimera derived from venezuelan equine encephalitis and sindbis viruses is a potent gene-based vaccine delivery vector. *J. Virol.*, 77:10394–10403, 2003.
- [5] R. Raju and H. V. Huang. Analysis of sindbis virus promoter recognition in vivo, using novel vectors with two subgenomic mrna promoters. *Journal of virology*, 65(5):2501–2510, 1991.
- [6] L. Wroblewska et al. Mammalian synthetic circuits with rna binding proteins for rna-only delivery. *Nature Biotechnology*, 2015.
- [7] N. Yoshioka et al. Efficient generation of human ipscs by a synthetic self-replicative rna. *Cell Stem Cell*, 13(2):246–254, 2013.