

Simulation-Based Engineering of Time-Delayed Safety Switches for Safer Gene Therapies

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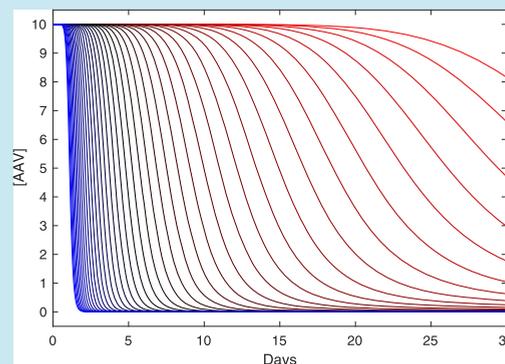
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ABSTRACT: CRISPR-based gene editing is a powerful tool with great potential for applications in the treatment of many inherited and acquired diseases. The longer that CRISPR gene therapy is maintained within a patient, however, the higher the likelihood that it will result in problematic side effects such as off-target editing or immune response. One approach to mitigating these issues is to link the operation of the therapeutic system to a safety switch that autonomously disables its operation and removes the delivered therapeutics after some amount of time. We present here a simulation-based analysis of the potential for regulating the time delay of such a safety switch using one or two transcriptional regulators and/or recombinases. Combinatorial circuit generation identifies 30 potential architectures for such circuits, which we evaluate in simulation with respect to tunability, sensitivity to parameter values, and sensitivity to cell-to-cell variation. This modeling predicts one of these circuit architectures to have the desired dynamics and robustness, which can be further tested and applied in the context of CRISPR therapeutics.

KEYWORDS: kill switch, time delay, gene therapy, CRISPR, simulation, SBOL



INTRODUCTION

CRISPR-based gene therapy adapts elements of prokaryotic immune systems to produce a powerful tool for programmable gene editing.¹ In a prototypical gene-editing application, the Cas9 nuclease and a targeting sgRNA are introduced into the organism to be edited, where the sgRNA binds with Cas9 to produce a complex that creates double-stranded DNA breaks targeted by the sequence encoded in the sgRNA, thus enabling targeted gene modification.² The therapeutic potential of this mechanism has been widely recognized and has been reported to permanently modify disease-relevant genes in a number of tissue types, including the liver,³ retina,⁴ brain,⁵ heart,⁶ and skeletal muscle.⁷ The order in which gene editing is carried out can also be regulated in vivo by incorporation of delay mechanisms.⁸

Therapeutic usage of CRISPR-based editing, however, must also address potential problematic side effects, notably off-target editing and immune response.^{9,10} One approach that has been proposed to address both of these issues is incorporation of a “safety switch,” with which the therapeutic system deletes itself by targeting its own sequence, thereby limiting the time, in which CRISPR-based editing is operating in a patient’s cells.¹¹ The efficacy of gene editing, however, can be compromised if self-deletion is too rapid.⁴

In order to better balance between these concerns, we investigate the potential for regulating the time delay of safety switches. An ideal safety switch would allow high expression levels of Cas9 for a specified period of time, tuned to the

expected requirements of a particular gene therapy treatment. After that time, the switch would activate self-deletion and rapidly eliminate the therapeutic construct.

In this paper, we perform a simulation-based analysis of the potential for building effective time-delayed safety switches using a transcriptional regulator and/or recombinase, two well-understood and readily engineered regulatory mechanisms. Combinatorial circuit generation identifies 30 potential architectures for delay circuits using either one or two regulators. Evaluating these circuits in simulation with respect to a range of biologically plausible parameters, we find that precisely one is capable of producing the desired dynamics: a sequential circuit, in which a transcriptional activator stimulates recombinase to turn on expression of the self-deletion sgRNA. Simulation indicates that this circuit should be tunable over a range of at least 30 days by modulation of the expression rate and stability of the activator. We further evaluate this circuit with respect to its sensitivity to parameter values and to cell-to-cell variation.

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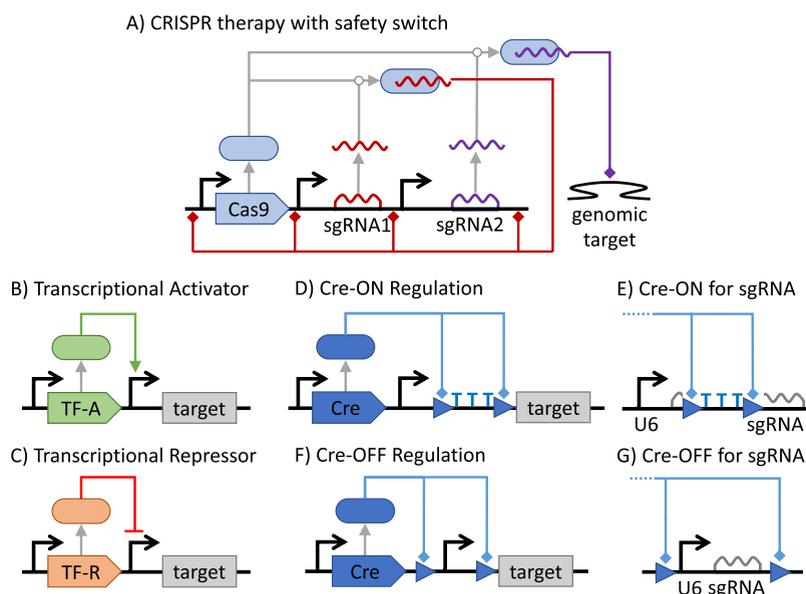


Figure 1. Base safety switch architecture (a) and regulatory elements under consideration for producing delay: (b) transcriptional activator providing delayed stimulation of a target, (c) transcriptional repressor providing delayed inhibition of a target, (d) Cre-ON recombination providing delayed removal of termination for pol II promoters or (e) for sgRNA, and (f) Cre-OFF recombination providing delayed removal of a pol II promoter or (g) both promoter and sgRNA. All circuits are shown using SBOL visual notation.¹²

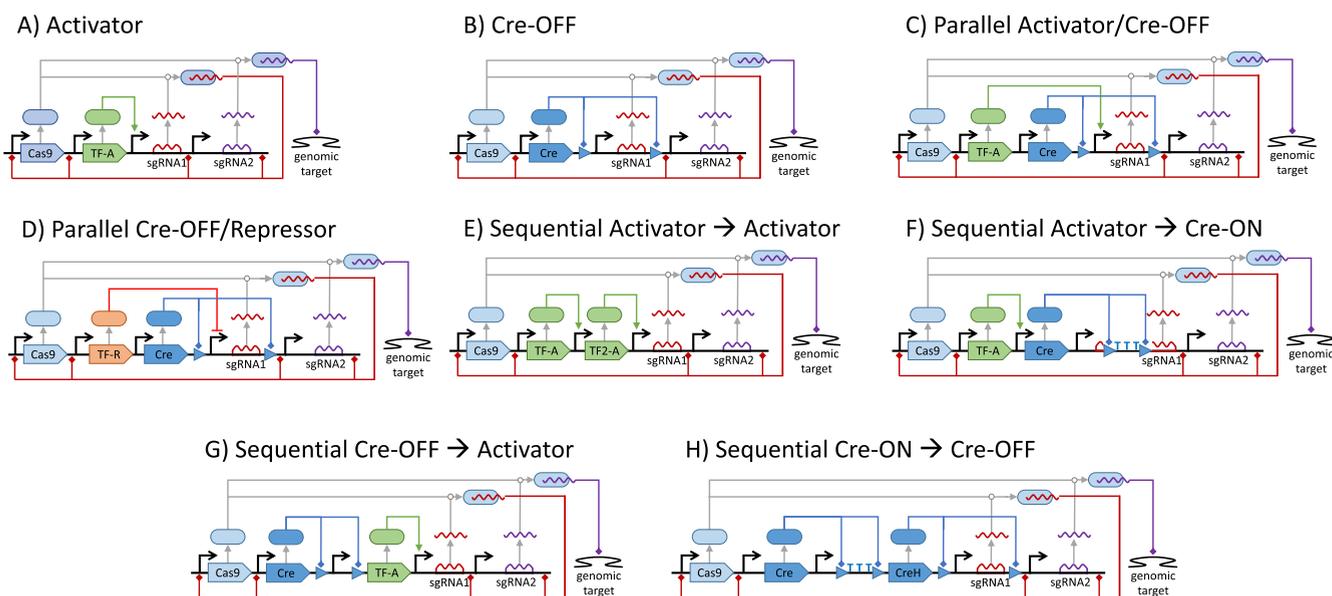


Figure 2. Representative examples from the generated collection of all 30 possible circuit topologies with one or two regulators that are considered as possible delay circuit architectures. Diagrams for all circuits are included in Figures S1–S31.

RESULTS

We first detail the class of delayed safety switch circuits under investigation. We then present an exploration of circuit behaviors with respect to biologically plausible parameters, followed by evaluation with respect to tunability, sensitivity to parameter values, and sensitivity to cell-to-cell variation.

Generation of Candidate Delay Circuits. The base self-deleting safety switch architecture, as adapted from Li et al.,¹¹ is shown in Figure 1a. The system consists of a genetic construct expressing the Cas9 nuclease and two sgRNAs, delivered via adeno-associated viruses (AAV). One of the sgRNAs, which we designate sgRNA1, implements self-deletion by targeting some number of sites on the construct itself. This self-targeting is

intended to create cleavages that compromise the integrity of delivery vector and disable its expression, thus clearing AAV from the cell over time. The other, sgRNA2, targets one or more genomic sites for therapeutic modification.

Note that as the focus of this investigation is regulation, we place potential issues with ordering of constructs and location of self-targeting sites out of scope, assuming that these potential location issues can be addressed if needed in a sequence-level final design. For purposes of this discussion, then, the ordering of functional units and number and placement of self-targeting sites are both notional, selected primarily for clarity of illustration.

Delays can be added to the safety switch function by regulation of the self-targeting sgRNA1. Figure 1b–g shows the potential delay elements that we consider. Specifically, a

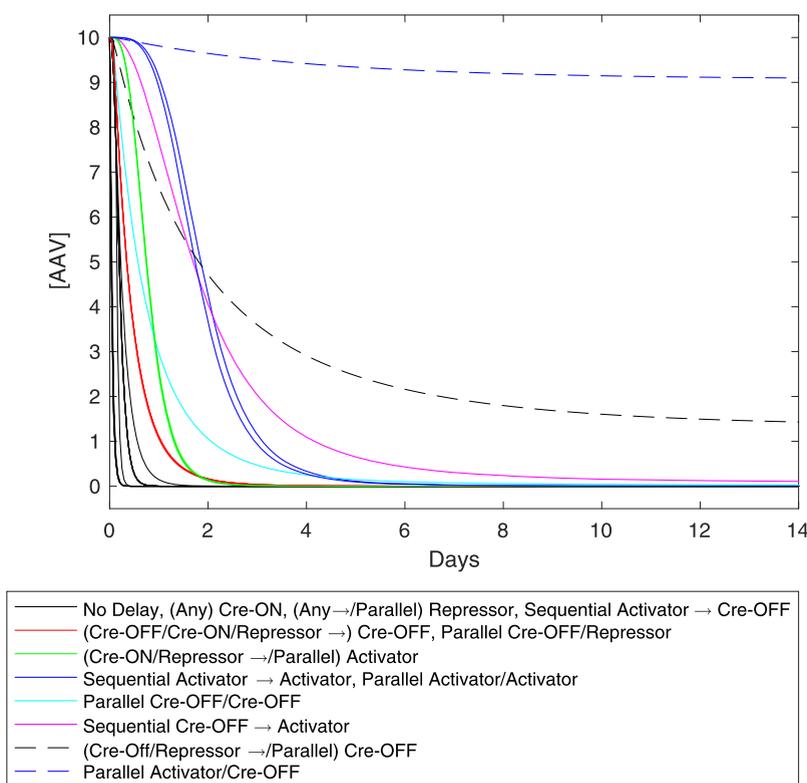


Figure 3. Simulation of AAV clearing by all candidate circuit architectures using base parameter values. The results for many circuits are identical or nearly identical, so they are grouped into clusters of similar behaviors. Cluster descriptions use parentheses to denote an optional second regulator and slashes to denote alternatives, for example, “(Cre-OFF/repressor → /Parallel) Cre-OFF” designates a collection of five circuit architectures: a Cre-OFF regulator alone or in parallel or sequential combination with either a repressor or another Cre-OFF, all of which have near-identical results.

transcriptional activator can be used to delay the start of a target’s expression, while a transcriptional repressor can be used to slow the action of a target by cutting off expression at a certain level. Likewise, a recombinase, such as the well-understood Cre recombinase or one of its natural or engineered orthogonal homologues,^{13,14} can be configured to remove a genetic region after a delay, thereby either initiating expression (Cre-ON) or cutting off expression (Cre-OFF) depending on the region targeted. Note that the specifics of the region modulated depends on the type of product: for a polymerase II promoter and protein coding sequence, we use removal of a set of terminators between promoter and coding sequence (Cre-ON) or removal of the promoter (Cre-OFF); for a gRNA product, on the other hand, with Cre-ON, we use the mechanism demonstrated in a study by Chylinski et al.⁸ that operates by removing a blocking region inserted in a stem-loop of the gRNA, and for Cre-OFF, we use removal of the promoter and gRNA as a unit. The AAV vector places strong limits on the size of genetic constructs that can be delivered, however, so we will only consider circuits with a maximum of two regulatory elements.

To investigate the potential for delayed safety switches, we first generated all of the possible regulatory circuit architectures that can be implemented using either one or two of these regulatory elements (see the methods: [model construction](#) section). In addition to the non-delayed base design in [Figure 1a](#), there are a total of 30 circuit topologies identified for consideration as potential delay architectures. Four of these circuits have a single regulatory element acting on sgRNA1, 16 instantiate all combinations of sequential regulation, in which a regulator targets the regulator of sgRNA1, and 10 instantiate all combinations, in which two regulators act in parallel on

sgRNA1. [Figure 2](#) shows a representative assortment of circuits from the generated set.

Analysis of Candidate Delay Circuits. We use simulation to evaluate the potential for each of the 30 candidate circuit architectures to be used as a delayed safety switch. Ordinary differential equation models were developed for the base CRISPR editing module and each of the regulatory elements following mechanistic models, as described in the [Supporting Information](#), then automatically linked following the topology for each circuit (see the methods: [model construction](#) section). Finally, a base set of parameter values were obtained from the literature, as described in methods: [parameter fitting](#) section. Note that while stochastic models would be more accurate, particularly for the operation of the recombinase, ordinary differential equations (ODEs) were selected for this investigation because the much lower computational cost of the continuous approximation also allowed us to investigate many more parameter combinations than would have been feasible with stochastic models. Note also that while one of the goals of a safety switch is to limit the impact of off-target effects, we do not explicitly model such effects; instead, off-target effects impact our models in the form of the target clearing time that would be determined by the designers of a therapy balancing risks within its particular medical context.

[Figure 3](#) shows the results of simulating all of the candidate circuit architectures for a 2 week time period using the base parameter values, starting from an initial circuit dosage of 10 AAV copies per cell. With these parameter values, most circuits produced offer little or no delay over an unregulated safety switch. Some degree of delay is observed, however, for a number of circuits, in which sgRNA1 is regulated by either an activator or

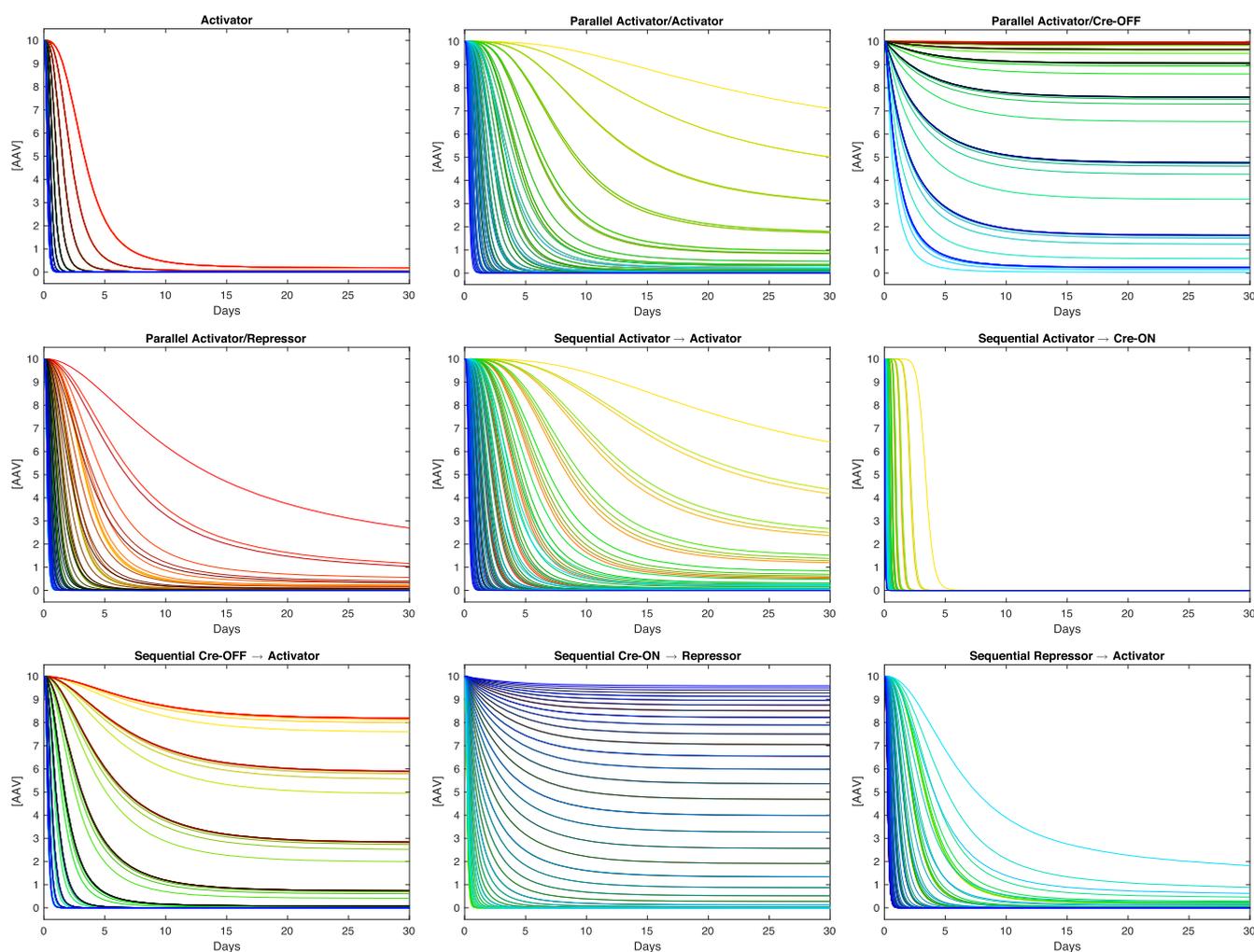


Figure 4. Adjusting the transcription/translation rate parameters for the circuit regulator product notable changes in safety switch behavior for nine of the 30 candidate circuit architectures. Of these, only the sequential activator to the Cre-ON architecture maintains the desired safety switch functionality as delay increases. Color indicates the parameter value, with the red to black to blue transition, indicating rising values of $\alpha_{p,1}$ (regulation of the first named element) and the green component scaling with $\alpha_{p,2}$ (regulation of the second named element, if any), that is, red is low for both parameters, blue is high $\alpha_{p,1}$ and low $\alpha_{p,2}$, yellow is low $\alpha_{p,1}$ and high $\alpha_{p,2}$, and cyan is high for both parameters. Since some trajectories are superposed, not all colors are visible.

Cre-OFF. Some of these, such as the sequential activator to activator circuit, provide a desirable delay behavior, in which the circuit persists at a high level for a significant time period, then rapidly decreases to zero. Others, such as the parallel activator/Cre-OFF circuit, fail to function correctly, with the safety switch ceasing to function before the AAV is cleared away.

While some parameters are difficult to adjust, particularly those relating to reaction dynamics, others are readily tuned, notably the rates of transcription and translation for the regulatory proteins. In our models, the transcription and translation rates for species i are combined into a joint $\alpha_{p,i}$ transcription/translation rate parameter (Supporting Information). In order to determine whether adjusting these rates can effectively adjust delay for any of the candidate circuit architectures, we adjust the $\alpha_{p,i}$ for each regulatory element across 4 orders of magnitude, up and down 100× from the base value logarithmically at two steps per decade, that is, a total of nine values for each parameter and 81 value combinations for circuits with two regulatory elements. Simulations for a 30 day time period with an initial 10 AAV dosage were run for each parameter value combination.

Of the 30 candidate circuit architectures, only nine show any notable change in safety switch behavior in response to changes in the transcription/translation rate (Figure 4), while all of the remainder produce little or no delay in all conditions (Figure S32). Most of the circuits with tunable delay, however, rapidly lose their ability to act as an effective safety switch as delay increases. For five of these (Cre-OFF regulating activator, Cre-ON regulating repressor, repressor regulating activator, parallel activator and Cre-OFF, and parallel activator and repressor), this is because the delay is created by a tension between positive and negative regulation, and as the relative strength of the negative regulation increases, the positive regulation is no longer able to drive the safety switch sufficiently to completely remove AAV. For the three activator-only circuits, delay is achieved by decreasing activator expression, which therefore also decreases expression of the safety switch, eventually reaching an equilibrium where sgRNA1 is expressed at a too low level for the safety switch to operate.

Only one circuit architecture produces tunable safety switch behavior throughout the whole range of parameters: sequential activator regulation of Cre-ON, the circuit shown in Figure 2f.

With the base parameters, no significant delay was observed relative to the unregulated safety switch. With a low Cre-ON expression rate $\alpha_{p,Cre}$, however, as activator expression $\alpha_{p,TF}$ decreases the delay increases, while the safety switch degradation of AAV continues to operate rapidly and completely. In this circuit architecture, as the activator builds up, it eventually hits a level where Cre-ON begins to operate on its target site. Once any target site has been edited, however, the Cre-ON regulated promoter can begin to express high levels of sgRNA1, and the safety switch rapidly removes all AAV. In effect, in this system, the slow accumulation of activator protein acts as an integrator “timer,” while the non-linearity of activation acts as a switch, and the Cre-ON acts as an amplifier on the output of the said switch.

The amount of delay that can be achieved with this circuit is strictly limited by the range over which activator accumulation can act as an approximately linear integrator. Once the desired delay is significantly longer than the half-life of the activator protein, the accumulation no longer rises sufficiently to switch on the Cre-ON, and the safety switch no longer operates as desired.

Fortunately, this analysis also indicates how delay should be able to be tuned without compromising safety switch functionality. Since the time span over which an activator can function as an integrator is regulated by the degradation rate δ_{TF} of the activator protein, stabilizing the protein to decrease this rate should allow it to function as an integrator over a longer time span, thereby allowing lower expression rates of the activator to produce longer delays.

Figure 5 shows a confirmation of this analysis in simulation, using a recombinase expression rate $\alpha_{p,Cre}$ reduced to 0.03 of the

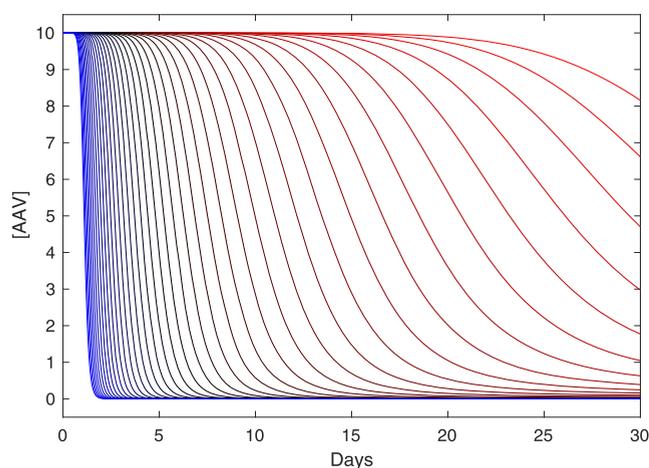


Figure 5. Tuning delay of the sequential activator to Cre-ON circuit by simultaneous adjustment of $\alpha_{p,TF}$ and δ_{TF} . Color shifts from red to black to blue as both values rise.

base parameter ($\alpha_{p,Cre} = 10^{1.5186}$) and jointly scanning the activator expression rate $\alpha_{p,TF}$ and degradation rate δ_{TF} logarithmically from 0.00001 to 0.1 and 0.001 to 10 times the base parameter, respectively, at 10 steps per decade, from an initial 10 AAV dosage. Here, we see that the desired safety switch behavior is indeed preserved across nearly the full range of delays achieved with these parameters.

The slope does become less steep as the delay increases, however, and as with the activator-only circuits, with a long enough delay, the simulation does begin to show a failure to clear

all AAVs from the system. For the sequential activator to Cre-ON circuit, however, the delay can be much longer before the simulation predicts a significant degradation in the performance of the safety switch. For example, setting a 30 day delay before half of AAV is degraded, the simulation predicts that more than 90% of the original AAV will still be operating on day 21 and that by 60 days, there will be less than one copy of AAV left in the system.

Finally, it is also important to note that the ODE models used necessarily under-represent the impact of a single recombination event. This means that in a real system, the actual rate at which AAV is cleared is likely to be much faster after long delays and effectively bring about complete clearing of AAVs from a cell once at least one recombination event has occurred. Stochasticity will also make the timing of that event more variable, but until the first recombination event occurs, the integration of the activator will continue, thereby increasing its probability and ensuring that eventually the safety switch will, in fact, be triggered.

Analysis of Delay Circuit Sensitivity. In the sequential activator to Cre-ON circuit, we have identified a safety switch architecture that can, in theory, be tuned to operate as an effective safety switch across a wide range of time delays. Any physical realization of this circuit, however, will of course not precisely match the parameters that we have used in our analysis. First, the parameter values used are imperfectly known and represent abstractions of more complex processes. Second, the safety switch will need to operate in individual cells of many different types, with differing cell sizes, states, and resources. It is thus important to evaluate the sensitivity of the sequential activator to Cre-ON circuit to variation in parameter values, individually or in combination. The less sensitivity the circuit exhibits, the simpler it is likely to be to realize and to tune through adjustment of the three critical engineerable parameters identified above, $\alpha_{p,TF}$, $\alpha_{p,Cre}$, and δ_{TF} .

To evaluate the sensitivity of the sequential activator to Cre-ON circuit to parameter uncertainty, we performed both single parameter perturbation and random perturbation of multiple parameters. For this purpose, we again used $\alpha_{p,Cre} = 10^{1.5186}$ and selected three sets of values for $\alpha_{p,TF}$ and δ_{TF} from the ones explored in Figure 5: one set for 50% reduction of AAV after approximately 5 days ($\alpha_{p,TF} = 10^{-0.0585}$; $\delta_{TF} = 10^{-3.1}$), one for approximately 10 days ($\alpha_{p,TF} = 10^{-0.7585}$; $\delta_{TF} = 10^{-3.8}$), and one for approximately 20 days ($\alpha_{p,TF} = 10^{-1.4585}$; $\delta_{TF} = 10^{-4.5}$). We then perturbed parameter values individually, independently, and jointly with respect to these three delay configurations.

To investigate the sensitivity of the circuit with respect to each individual parameter, we modulated each of the 15 parameters of the circuit model up and down by ± 2 standard deviations with 1.5-fold log-normal uncertainty (i.e., across a slightly more than 5-fold range), while holding all others the same. Figure 6 shows representative results from these perturbations for the 10 day delay circuit, while results for the full set of parameters for all three delays are shown in Figures S33–S35. The majority of the 15 parameters modulated, such as the degradation rate of Cas9 ($\delta_{p,Cas9}$), had little to no effect on simulation results. Most of the rest of the parameters cause moderate variation in circuit delay, such as the degradation rate of Cre ($\delta_{p,Cre}$), with a proportionally greater range of variation for the 10 day and 20 day delays relative to the 5 day delay. There is only one parameter, the value of the Hill coefficient n , for which the simulation indicates a high degree of sensitivity. Critically, however, in every case, the modulation is a quantitative change in delay rather than a

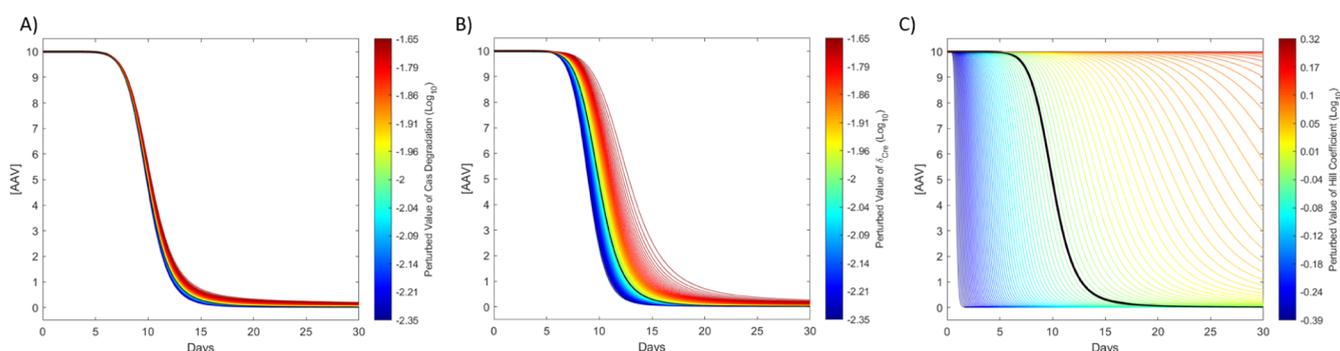


Figure 6. Examples of perturbation response for modulating individual parameters in the sequential activator to Cre-ON circuit with a 10 day delay. Some parameters, such as the degradation rate of Cas9-gRNA complex δ_{CG} , have little or no impact on the circuit (a); most, such as the degradation rate of Cre $\delta_{p,Cre}$, have moderate impact (b), but circuit behavior is only highly sensitive to the Hill parameter n (c). Results for other parameters and delays are provided in Figures S33–S35.

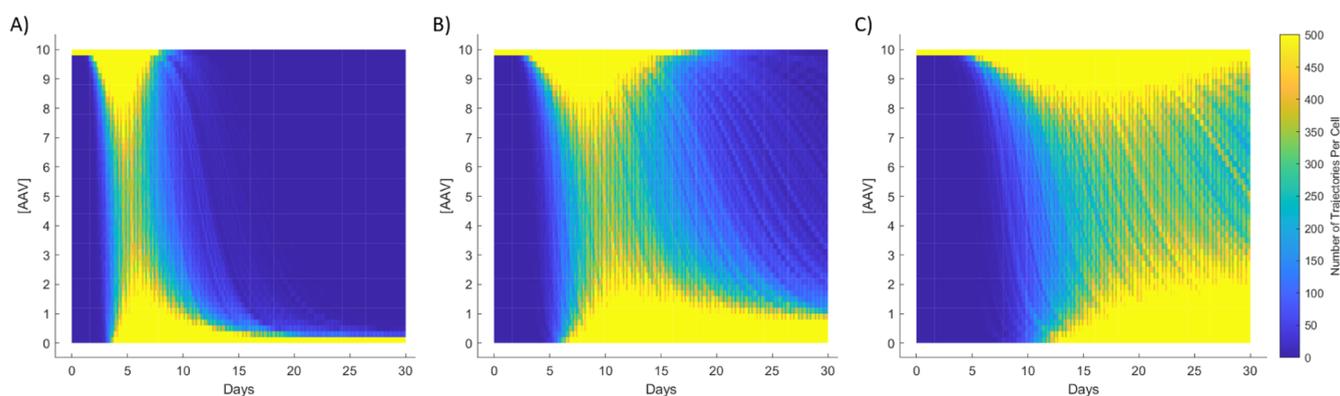


Figure 7. Distribution of trajectories generated by random perturbation of all parameters for the sequential activator to Cre-ON circuit configured for (a) 5 day delay, (b) 10 day delay, or (c) 20 day delay.

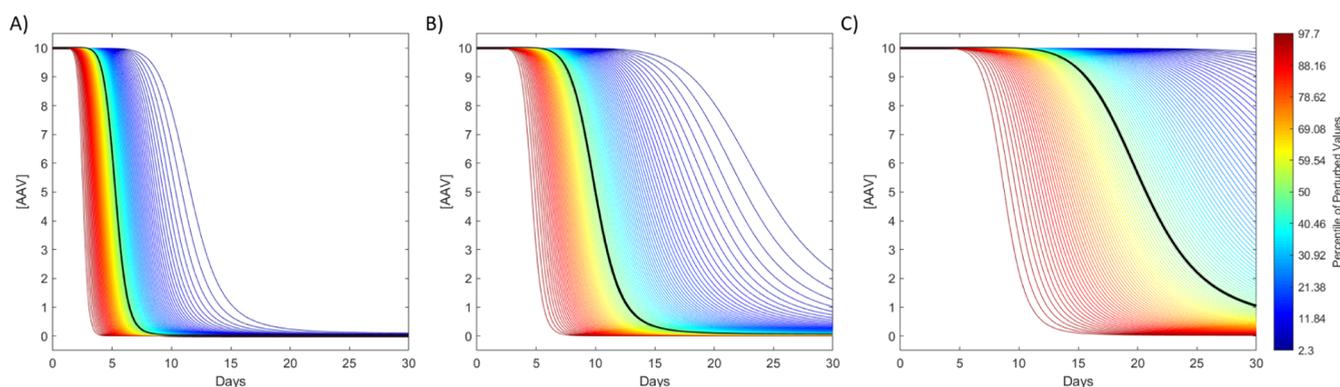


Figure 8. Distribution of trajectories generated by joint perturbation of each α expression rate parameter in the sequential activator to Cre-ON circuit configured for (a) 5 day delay, (b) 10 day delay, or (c) 20 day delay.

qualitative change in the behavior of the circuit. In short, these simulations predict a circuit that remains an effective delayed safety switch, but which would need parameter tuning to adjust back to the desired delay.

Complementary to the single parameter analysis, we also perform perturbation analysis, in which all parameters are simultaneously and independently varied. Here, we multiply each parameter by a log-normal random factor drawn with a standard deviation of 1.1-fold (i.e., with ± 2 standard deviations spanning a range of just under 1.5-fold). Figure 7 shows the distribution of trajectories from 10,000 such random parameter simulations for each of the 5 day delay, 10 day delay, and 20 day

delay configurations. As with single parameter perturbation, although the delay spreads, and spreads more for higher delays, the overall circuit behavior always remains that of a delayed safety switch as desired.

Variation from cell to cell, on the other hand, will typically involve correlated changes to similar parameters. Specifically, variation in circuit behavior due to variation in the cell state and resources can, in many cases, be modeled in terms of a multiplicative factor modulating all expression rates.¹⁵ We thus evaluate sensitivity to cell-to-cell variation by perturbing all α parameters of the model jointly by the same amount across the same approximately 5 fold range as for Figure 6. Figure 8 shows

the results of these perturbations for the three tuned circuit models. All tunings show a similar pattern: cells with low RNA and protein production rates (such as cells that are more dormant or in resource poor environments) show a slow, and in extreme cases potentially incomplete, removal of the system. As with the single and complete parameter perturbations mentioned above, as the circuit is tuned to persist for longer, the spread of behavior widens.

From these perturbations, we find that our analysis of the sequential activator to Cre-ON circuit as an effective delayed safety switch is generally resilient with respect to parameters that are not precisely known, with the one critical parameter being the Hill coefficient, and should also be relatively resilient across differences between cells.

DISCUSSION

In this paper, we focus on Cas9 nuclease activity and examine different circuit topologies of possible safety switches, in which CRISPR cleaves within its own coding sequence in order to compromise the integrity of a delivery vector and disable its expression. Our emphasis is particularly on CRISPR delivery using AAVs, one of the most common viral delivery platforms for clinical application. As AAV can linger in cells, especially the non-dividing cells, for a long period of time, engineering strategies to remove it after the therapeutic mission is accomplished will be highly desirable for safe applications in humans.

Our investigation of potential delay mechanisms for CRISPR safety switches has determined that out of all possible configurations of one or two transcriptional or recombinase regulators, only the sequential activator to Cre-ON architecture is able to produce effective safety switch operation with a readily tunable delay. In particular, we find that delay can be tuned over a range of at least 30 days by modulation of the activator expression and decay rates, allowing the concentration of the activator to act as an integrator “timer,” while the non-linearity of activation acts as a switch, and the Cre-ON acts as an amplifier on the output of the said switch. Evaluation of the sensitivity of these results to both parameter uncertainty and cell-to-cell variation reveals that this circuit architecture is not unduly sensitive to the parameter value, except for the Hill coefficient of the activator, and should not affect tunability.

One potential area for future investigation is expanding the set of potential regulatory mechanisms to consider beyond the two that we investigated in this paper. Likewise, developments in the delivery technology may also allow more complex circuits to be considered. For further development of the sequential activator to Cre-ON circuit, next steps include more accurate simulation of dynamics via stochastic models and implementation and validation of the circuit in the laboratory. If this mechanism does bear out in practice, it may be a valuable addition to a wide range of therapeutic treatments, and further refinement and validation will be needed to bring safety switches to a point where they could be safely and confidently deployed in patients. Finally, this architecture may also be valuable to consider for adaptation to other application areas where safety switches are of interest, notably including limiting the escape potential of environmentally deployed genetically modified organisms.

METHODS

Model Construction. All of the models investigated were represented using SBOL3.^{16,17} Specifically, we constructed

SBOL generators for each of the modular elements of the circuit: the base CRISPR editing and safety switch, transcriptional regulation, and recombination regulation. These elements were then assembled combinatorially to generate a genetic regulatory network model for each of the possible configurations. Both LaTeX equations and Matlab code were then programmatically generated for each such genetic regulatory network.

Complete information about the models used, including full ODE equations for all systems, is provided in the [Supporting Information](#). The SBOL files for all safety switch designs are provided in the [Supporting Information](#). Matlab simulation files are also provided in the [Supporting Information](#). Model generators and products are also available on GitHub at <https://github.com/TASBE/CRISPR-safety-switches>.

Parameter Fitting. As detailed in the [Supporting Information](#), there are three sets of parameters needed for simulation:

- Cas9 and sgRNA expression, binding, editing, and degradation
- Cre-recombinase expression, recombination, and degradation
- Transcription factor expression, Hill function, and degradation

Parameter fitting for Cas9, sgRNA, and Cre recombinase used data extracted from a study by Chylinski et al.⁸ (Figures 1 and S2b). Specifically, we performed a least-squares fit for parameters in a logarithmic parameter value space in Matlab using the ODE model for a Cre-ON safety switch.

Transcription factor parameters are taken from the LmrA ODE model in a study by Wang et al.,¹⁸ which is based in turn on the data in a study by Davidsohn et al.¹⁹

The complete set of parameter base values determined in this manner is shown in [Table 1](#).

Table 1. Parameter Base Values as Determined by Parameter Fitting

variable	meaning	base value
V	initial vector (AAV) count	10
H	initial host genome target count	1
δ_{Cg} , $\delta_{p,Cas9}$, $\delta_{p,TP}$, $\delta_{p,Cre}$	Cas9 and Cas9-gRNA complex degradation rate	$10^{-2.0}$
k_{Cg}	Cas9 and gRNA-binding rate	$10^{-4.2577}$
$\alpha_{r,sgRNA1}$, $\alpha_{r,sgRNA2}$	gRNA transcription rate	$10^{3.3090}$
$\alpha_{p,Cas9}$, $\alpha_{p,TF}$, $\alpha_{p,Cre}$	coupled transcription/translation rate	$10^{3.0415}$
δ_g	gRNA degradation rate	$10^{0.0003}$
k_{cat}	Cas9-mediated DNA cleavage rate	$10^{-4.7518}$
n	Hill coefficient	0.92
K_A , K_R	Hill activation/repression dissociation constant	$10^{6.3692}$
k_{cre}	Cre DNA recombination rate	$10^{-7.1535}$

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acssynbio.1c00621>.

Derivation of equations for CRISPR safety switches (presentation of the equations and architectures used for modeling the various designs for CRISPR safety switch circuits, parameter adjustment simulation results for all 30 candidate circuit architectures, and sensitivity analysis for the sequential activator to Cre-ON circuit with respect to

each model parameter when configured for 5 day, 10 day, or 20 day delay) (PDF)

SBOL3 models of safety switch designs (TXT)

Matlab simulation files containing the ODE models for each CRISPR safety switch circuit (ZIP)

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Notes

The authors declare the following competing financial interest(s): Dr. Kiani is a founder of Genexgen Inc., and a preliminary patent has been filed regarding the application of the best discovered circuit architecture to gene therapy.

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