Computational characterization of recombinase circuits for periodic behaviors

Recombinase-based oscillator

Highlights
We design dynamical recombinase-based oscillators operating at single copy
Our designs couple two stable negative feedback loops through mutual activation
We develop a computational approach to evaluate oscillation coherence
Computational characterization of recombinase circuits for periodic behaviors

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SUMMARY

Recombinases are site-specific proteins found in nature that are capable of rearranging DNA. This function has made them promising gene editing tools in synthetic biology, as well as key elements in complex artificial gene circuits implementing Boolean logic. However, since DNA rearrangement is irreversible, it is still unclear how to use recombinases to build dynamic circuits like oscillators. In addition, this goal is challenging because a few molecules of recombinase are enough for promoter inversion, generating inherent stochasticity at low copy number. Here, we propose six different circuit designs for recombinase-based oscillators operating at a single copy number. We model them in a stochastic setting, leveraging the Gillespie algorithm for extensive simulations, and show that they can yield coherent periodic behaviors. Our results support the experimental realization of recombinase-based oscillators and, more generally, the use of recombinases to generate dynamic behaviors in synthetic biology.

INTRODUCTION

Oscillatory behaviors drive essential processes in nature. For example, the mitotic oscillator drives cell division,1 the circadian oscillator drives the sleep-wake cycle,2 and the segmentation clock drives spatial pattern formation during vertebrate embryonic development.3 These numerous examples have motivated biologists, physicists, and mathematicians to look for the design principles required to build biomolecular oscillators from the bottom-up,4,5 and many decades of theoretical and experimental research have consolidated design principles for oscillator design, which primarily include the presence of a negative feedback loop and of mechanisms for local destabilization, such as positive feedback and delays.6–9 The implementation of these design principles within artificial genetic circuits has been shown to facilitate the emergence of periodic behaviors, in contexts spanning from single cell metabolism to multicellular environments,10 and tremendous progress has been made toward building robust synthetic oscillators using transcription factors.11,12 Capitalizing on these achievements, new oscillator architectures should take advantage of the rapidly expanding set of molecular parts and gene editing tools harnessed by synthetic biology.13

Recombinases are a class of proteins with major potential toward engineering cellular behavior.14 These enzymes cleave and rejoin DNA strands with high specificity for given genetic domains (sequences), and many orthogonal recombinases exist. By carefully placing these domains, recombinases can perform diverse operations such as DNA excision, insertion, and translocation to generate logic and regulatory circuits that are easy to scale, and can be implemented in a variety of organisms.15,16 Because recombinases make it possible to swap domains of DNA, they can “rewire” entire gene expression pathways. By simply inverting target promoter regions, recombinases can activate or deactivate expression with a nonlinear response that is comparable to a digital on/off switch. While this is an attractive feature toward building complex cellular circuits, the use of recombinases to generate periodic behaviors has received little attention. Creating periodic cycles of DNA site inversion using recombinases has a fundamental limitation posed by the difficulty to reverse-rearrange DNA. This limitation can be overcome by adopting serine inte- 

In this paper, we describe and compare various candidate architectures to design oscillators using recombinases that generate regulatory feedback loops with nonlinear, switch-like responses. The simplest recombinase-based oscillator could be described as the interconnection of two negative feedback loops.
We summarize the most important functions of serine integrases (panels A–C) and their relevant applications (panels D and E). There are two families of recombinases: tyrosine recombinases and serine recombinases. All recombinases are site-specific proteins that can rearrange DNA, performing, for example, excision/insertion, inversion, and translocation. Serine integrases are a subfamily of serine recombinases, each of which has a cognate Recombination Directionality Factor (RDF) that allows the serine integrase to reverse-rearrange DNA. We consider three examples involving the serine integrases Bxb1, φ C31, and TP901, with cognate attP and attB binding sites. When the attP and attB binding sites have the same orientation, Bxb1 monomers form a dimer, bind to the two specific sites, and excise the DNA segment in between, as shown in panel A (excision/insertion). When the attP and attB binding sites point in opposite directions, φ C31 binds to them and inverts the DNA between the binding sites, as shown in panel B (inversion). When the attP and attB binding sites are not in the same region of DNA, TP901 can bind to the attP and attB binding sites and translocate the DNA strands, as shown in panel C (translocation). In all the above examples, adding a recombination directional factor (RDF) enables the recombinases to recognize the binding sites attL and attR present after the rearrangements described, and therefore to reverse them. Tyrosine recombinases can perform the DNA rearrangements described, among others, but not reverse them, examples including Cre, Vre, and FLP. The exception to this is the pair of tyrosine recombinases FimE and HbiF that can reverse the DNA recombination completed by the other. Recombinases have been used within logic gate circuits, such as the AND gate shown in panel D. In this example, two transcription terminators are between the attP and attB binding sites for two different and orthogonal recombinases, Bxb1 and φ C31, shown in purple and green, respectively. In the absence of both recombinase inputs, the transcription of gene X is suppressed (OFF). When Bxb1 is added, it excises the first terminator. However, the transcription remains suppressed (OFF), because the second terminator is still present. When φ C31 is also added, it excises the second terminal, which finally activates the transcription of gene X (ON). Hence, two recombinase inputs (Bxb1 and φ C31) are needed to activate the circuit. One major disadvantage of recombinase-based logic gates is that the irreversibility of the DNA rearrangement means they can only be operated a single time. This circuit would require the cognate RDGs of these integrases to insert the DNA that was excised and thus be a multiple-use device. There are few demonstrations of multiple-use, dynamic devices built using recombinases. The first example was the engineering of a programmable switch, as shown in panel E. This pioneering work uses Bxb1 and its RDF to change the direction of the promoter controlling the production of X over multiple cell generations. An improved version of the programmable switch uses tyrosine recombinases FimE and HbiF, which are the only special cases of tyrosine recombinases that allow reversible DNA rearrangement. Other dynamical circuit designs based on recombinases include a negative feedback controller to track a ref. Some theoretical designs of toggle switches that incorporate multiple copies of the circuit, and a single-input counting circuit. The oscillatory behavior of a recombinase-based circuit has also been analyzed deterministically. Because recombinases can be used as a switch to turn on/off gene expression, they are well suited to build large Boolean logic circuits that can be hierarchically composed with a predictable response. In addition, self-excision recombinases were used to generate temporal responses such as pulses, and a cascade of self-excision mechanisms can create a sequential pulse behavior that operates once. Because leaky expression of recombinases can jeopardize circuit operation (only few protein copies are necessary to carry out their function), methods to tightly control their production are necessary, for example via light-induction.
Approach

Stochastic simulations

We used the Gillespie algorithm, implemented in Python, to generate stochastic trajectories of a set of species interacting according to a list of chemical reactions. The reaction rate constants (Table S1) associated with each reaction are converted to reaction propensities, and the algorithm simulates which reactions fire at each step of the simulation. The copy number increases or decreases one molecule at a time while the algorithm tracks the changes in all species over time. We study six distinct chemical reaction networks that model candidate oscillators based on recombinase interactions. Every design has a single copy of each promoter that inverts back and forth under the action of a serine integrase and the same serine integrase fused to its RDF, so the chemical reactions include promoter inversion, transcription, translation, and degradation. The left-pointing and right-pointing configurations of the promoter are considered different species: \( SL \) and \( SR \), respectively. The propensity for converting from one promoter species to the other is controlled by a function of recombinase concentration since recombinase is what physically inverts the promoter. For each design, we generated 500 stochastic trajectories using a reaction volume of 1 fL; initial conditions were set to zero for all species except one copy of left-pointing promoter \( SL = 1 \).

Metrics for coherence/incoherence

To evaluate the consistency/inconsistency of the period of stochastic trajectories, we used an incoherence metric based on the autocorrelation function following the approach introduced by Yan and Paulsson. We focus strictly on the recombinase copy number and study the coherence of the oscillation period produced by each circuit design. Fully coherent oscillations not only have a regular period but also each cycle of the oscillations has comparable amplitude. In a stochastic context, these requirements for perfect coherence are not achievable, so we only consider fluctuations of the period from the start of the simulation. Given a stochastic trajectory like the one in Figure 2A, we first compute the autocorrelation function (shown in Figure 2B) of the simulated concentration trajectory. Then, we evaluate two kinds of features of the autocorrelation function: the time interval between the first peak and each consecutive
peak, termed $T_i$, $i = 1, 2, \ldots$; and the time intervals between subsequent peaks, defined as $\Delta T_i = T_i - T_{i-1}$ for $i \geq 2$, and $\Delta T_1 = T_1$. As an example, the intervals $\Delta T_i$ and $T_i$ are marked in Figure 2B for the first three peaks.

Given a collection of stochastic simulations, the histograms of the inter-peak time intervals $\Delta T_i$ provide direct information on the period variability; coherence in period would be associated with similar mean and variance for all the $\Delta T_i$. It is more advantageous however to consider statistics of the $T_i$ intervals, in particular their variance. While $T_i = \Delta T_i$, one expects the average of $T_i$, $i > 1$, to increase linearly with $i$. While the variance of $\Delta T_i$, $i > 1$, should not increase if oscillations are coherent, the variance of $T_i$ does linearly increase, as fluctuations in each period are added (over each cycle) in the computation. The variance should increase proportionally to the level of “incoherence” of the oscillations. Thus, we focus on the variance of the histograms of the $T_i$ intervals as exemplified in Figure 2C. The variance of each $T_i$ histogram is then plotted against the peak index, as shown in Figure 2D. Because the variance of $T_i$ is captured well by a linear fit, as shown in previous work,\textsuperscript{30} we use the slope of the variance plot as an incoherence metric for the period. The variance tells us how regular the autocorrelation period is because a high variance means there is a large variety of periods. In other words, these values are irregular when variance is high. Low variance in period is required for coherent oscillations, which is the case when the metric is low. This metric was computed with our simulations of the six different designs in different parameter regimes to compare their robustness to changes in the various parameters, including recombinase translation rate constant and degradation rate constant.

**RESULTS**

**Building a biomolecular oscillator by coupling two self-inhibiting recombinases**

Our basic design for achieving periodic behavior using recombinases is shown in Figure 3A (detailed schematic) and in Figure 3B (simplified schematic). It consists of a single promoter between two recombinase binding sites, that controls the expression of two genes encoding distinct recombinase homodimers: $X_1$, a serine integrase that targets attP and attB binding sites, and $X_2$, the same serine integrase homodimer, but fused to its recombination directionality factor (RDF), which targets attR and attL binding sites (see Figure 1). The expected operation of the circuit is the following: when the promoter points to the left, with
To computationally characterize the behavior of our circuits, we considered a set of chemical reactions that model transcription, translation, and sequestration interactions between recombinase monomers, and promoter inversion. The rates of these reactions were converted to reaction propensities, expressing the probability of a reaction event per unit time, and we used the Gillespie algorithm to simulate the system (see the approach section). Depending on its orientation, the promoter regulates the transcription of either mRNA $M_1$ (when it points to the left in configuration $S_L$) or mRNA $M_2$ (when it points to the right in configuration $S_R$), with rate constant $q$. Thus, $S_L + S_R = 1$ for a single-copy scenario (one could adopt $S_L + S_R = N$ for a multiple-copy scenario where the number of copies is $N$). Both mRNAs are assumed to dilute/degrade with a rate constant $d$. In addition, the mRNAs $M_1$ and $M_2$ respectively are translated to recombinase monomers $Z_1$ and $Z_2$ with rate constant $a$.

**Transcription:**
\[
S_L^0 + M_1 \xrightarrow{a_1} S_L^1, \quad S_R^0 + M_2 \xrightarrow{a_2} S_R^1
\]

**mRNA Degradation:**
\[
M_1 \xrightarrow{d} \emptyset, \quad M_2 \xrightarrow{d} \emptyset
\]

**Translation:**
\[
M_1 \xrightarrow{a} Z_1 + Z_1, \quad M_2 \xrightarrow{a} Z_2 + Z_2
\]

The serine recombinase monomers $Z_1$ and $Z_2$ can form the homodimers $X_1$ and $X_2$, respectively. Since $Z_2$ is fused to its RDF, the two species can also form the heterodimer $C$. For simplicity, we assume all dimers have an association rate constant $a_1$ and a dissociation rate constant $d_1$. In addition, we assume all proteins degrade/dilute with a rate constant $d$.

**Homodimerization:**
\[
Z_1 + Z_1 \xrightarrow{a_1} X_1, \quad Z_2 + Z_2 \xrightarrow{a_2} X_2
\]

**Heterodimerization:**
\[
Z_1 + Z_2 \xrightarrow{a_1} C
\]

**Protein Degradation:**
\[
Z_1, X_1, Z_2, X_2, C \xrightarrow{d} \emptyset
\]

The rate of promoter inversion is regulated by the recombinase dimers $X_1$ and $X_2$ with binding and unbinding rate constants $a_2$ and $d_2$, respectively, and the inversion rate constant $r$.

**Binding/Unbinding:**
\[
X_1 + S_L^0 \xrightarrow{a_2} S_L^1, \quad X_1 + S_R^0 \xrightarrow{a_2} S_R^1
\]
\[
X_1 + S_L^1 \xrightarrow{d_2} S_L^0, \quad X_1 + S_R^1 \xrightarrow{d_2} S_R^0
\]

**Binding/Unbinding:**
\[
X_2 + S_L^0 \xrightarrow{a_2} S_L^1, \quad X_2 + S_R^0 \xrightarrow{a_2} S_R^1
\]
\[
X_2 + S_L^1 \xrightarrow{d_2} S_L^0, \quad X_2 + S_R^1 \xrightarrow{d_2} S_R^0
\]

**Inversion:**
\[
S_L^1 \xrightarrow{r} S_R^0 + 2X_1, \quad S_R^1 \xrightarrow{r} S_L^0 + 2X_2
\]

where the subscript $L$ stands for a promoter oriented to the left and $R$ for a promoter pointing to the right. The superscript $S^0$ corresponds to a promoter without any recombinase dimers bound to it, $S^1$ indicates a
promoter has a recombinase dimer bound to the right binding site, $S^{10}$ represents a promoter with a recombinase dimer bound to the left binding site, and $S^{11}$ consists of a promoter with two recombinase dimers, one bound to each binding site. In total, we have eight possible states for the promoter, four where it points to the left and four where it points to the right. Then, $S_{L} = S_{L}^{00} + S_{L}^{10} + S_{L}^{01} + S_{L}^{11}$, and $S_{R} = S_{R}^{00} + S_{R}^{10} + S_{R}^{01} + S_{R}^{11}$. With this, it still holds that $S_{L} + S_{R} = 1$ for a single copy ($S_{L} + S_{R} = N$ for $N$ copies).

**Coherence of stochastic simulations**

We explored the emergence of periodic behaviors in the design described above via stochastic simulations. In particular, we wished to assess whether the circuit supports the occurrence of oscillatory solutions with a regular period. Figure 3C shows example trajectories of recombinase concentration computed using the parameters from Table S1: $X_{1}$ in gray and $X_{2}$ in orange. The light orange regions mark when the promoter points to the right (configuration $S_{R}$). The trajectories for $X_{1}$ and $X_{2}$ show oscillations with antiphase behavior, meaning that one level increases while the other decreases. Yet, since stochastic noise makes it challenging to identify a defined period, we examined coherence by computing the trajectory’s autocorrelation and then calculating the inter-peak times, $\Delta T_{i}$, of the autocorrelation function as shown in Figure 2B. As a metric to evaluate the incoherence of oscillations, we measured the time from the first peak of the autocorrelation function to six consecutive peaks in 500 trajectories. A small variance for these time intervals corresponds to trajectories with a small amount of change, while higher variance indicates oscillations with more change in period over time. Regardless, the variance increases approximately linearly with the peak index (See Figure 2D). The variance of the distribution of the times, $T_{i}$, was plotted against the peak index, $i$ (Figure 3D). We use the slope of this line as a metric to quantify the incoherence of oscillations (see STAR Methods). The lower the slope is, the more coherent the oscillations are. One can see that these lines have a very good fit since the $R^{2}$ value for each is very close to 1, as is expected for such data.30 Also see Figure 3D.

While the circuit design in Figure 3A (or its simple representation in Figure 3B) can exhibit coherent oscillations (as shown in Figure 3C for the nominal parameters in Table S1), it can also have a switch-like behavior with significant variability of the period when parameters deviate from the nominal values. By lowering the switching rate parameter to $r/5$, for example, the system’s switching behavior becomes less consistent. This means that promoter inversion becomes a random event, and we observe a regime that we call stochastic pulsing, illustrated with an example simulation in Figure 3E. Stochastic pulsing is characterized by high variance in period (as well as low variance in amplitude in some cases). Also, Figure 3F shows how fast the variance of $T_{i}$ increases: the slope is 9.5 while it is 2.4 for the example of periodic trajectory, thus confirming the effectiveness of the incoherence metric. The irregular frequency of the promoter position’s switching can be noted in Figure 3E by looking at the irregularly spaced, light orange bands, marking when the promoter is pointing to the right.

**A range of circuit architectures can yield coherent oscillations**

We next explore systematically the coherence of the oscillations when the architecture of the circuit and the values of specific parameters are varied. We focus in particular on the effects of adding different mechanisms that control the level of recombinase: repression, catalytic degradation, and activation. Because in the absence of sequestration the basic recombinase circuit did not yield coherent oscillations, we reasoned that mechanisms that introduce additional control over recombinase expression may help maintain consistent switching events in time.

We begin by considering a design that includes repressors of recombinase transcription (RR design) to push down the level of recombinase after inversion. We then consider removing recombinase molecules via proteolytic degradation (RP design). Both transcriptional repression and protease degradation, by reducing the available recombinase, have the result of delaying the next switching event.33 We then explore three additional designs: one that regulates recombinase concentration by means of a transcriptional activator (RA design), one that incorporates the sequestration of recombinase mRNA via complementary small RNA (sRNA) species that prevent translation (RS design), and another that incorporates negative feedback.

Each of these additional reactions changes the timing of promoter switching events, and thus can change the coherence of oscillations. We used the Gillespie algorithm to generate an ensemble of trajectories for each circuit, starting with the recombinase-based oscillator we have already proposed (see Figure 3A). We
then assess the corresponding coherence of oscillations as we vary the nominal parameters in a given range.

**R design: Analyzing the coherence of the recombinase-based oscillator**

The R design was illustrated in Figure 3A. In Figure 4D, we examine the circuit as multiple parameters are varied, and we compare it with other circuit variants. A lower translation rate constant, \( r \), results in lower recombinase concentration, which leads to stochastic pulsing as reflected in the higher values of the incoherence metric in the orange plot in Figure 4D (1). Similarly, a lower transcription rate, \( \theta \), gives rise to a higher variance in \( T_1 \). The orange plot in Figure 4D (1) also shows that a higher transcription rate reduces the incoherence metric for this circuit, thus indicating improved coherence of oscillations. However, it is worth keeping in mind that very high values of \( \rho \) can lead to very rapid switching.

Increasing the degradation rate constant, \( \delta \), can also cause stochastic pulsing by reducing the amount of recombinase in the system to very low levels, as is reflected in the high value of the incoherence metric in the orange plot in Figure 4D (2). A low degradation rate constant, \( \delta \), also corresponds to a high value of incoherence metric, also shown in the orange plot of Figure 4D (2), since it could lead to a high recombinase concentration and therefore to rapid switching. Overall, our incoherence metric indicates there is a range of \( \delta \) values that yield strong coherence of oscillations, while very low or very large values hinder coherence.

The incoherence metric remains fairly constant in a wide range of \( a_1 \) values shown in the orange plot of Figure 4D (3), which means that coherence is unaffected by reasonably small changes in the value of the dimer association rate. However, decreasing \( a_1 \) by a factor of 100 does affect the incoherence metric (not shown).

On the other hand, changing \( a_0 \) values does impact the coherence of the simulations. Lower \( a_0 \) values give rise to less coherent oscillations while the oscillations produced by a circuit with higher \( a_0 \) values are much more coherent. This can be seen in the orange plot in Figure 4D (4). Since this is the binding rate constant for the recombinase-DNA complex, this means that oscillations have a more regular period when the recombinase and DNA have a greater affinity for each other.

A low switching rate, \( r \), is associated with a high incoherence metric, and we observed this results in stochastic pulsing because the probability of inversion is lower. Conversely, increasing the value of \( r \) improves the coherence of this design (the incoherence metric is much lower), as can be seen in the orange plot in Figure 4D (4). However, higher values of \( r \) may lead to very fast switching, which may be too rapid to be classifiable as a suitable oscillation.

**RR design: Transcriptional repression of recombinase production**

The RR design, shown in Figure 4B (simplified schematic) along with an example trajectory, incorporates transcriptional repressors as a form of cascaded regulation. In this design, the transcription of recombinases \( X_1 \) and \( X_2 \) is regulated by transcriptional repressors \( Y_1 \) and \( Y_2 \), respectively. The production of \( Y_1 \) and \( Y_2 \) is controlled by an inverting promoter positioned between recombinase binding sites (as it was for the production of recombinases in the R design), while the genes for \( X_1 \) and \( X_2 \) have promoters regulated by \( Y_1 \) and \( Y_2 \), respectively, acting as repressors. When the promoter of the genes points to the right, \( Y_1 \) is produced, inhibiting the production of recombinase \( X_1 \). At the same time, the amount of recombinase \( X_2 \) increases because \( Y_2 \) is not being produced. This causes the promoter to invert to the left. As a consequence, the production of \( Y_2 \) increases while \( Y_1 \) decreases in concentration due to decay. The remaining \( Y_1 \) still blocks the production of \( X_1 \) until its level is too low for repression. When \( Y_2 \) is high in concentration and \( Y_1 \) is low, the amount of recombinase \( X_1 \) increases and eventually becomes sufficient to invert the promoter to the right. In addition, \( Y_2 \) delays the production of \( X_2 \) until the concentration of \( Y_2 \) becomes low enough, making it unable to repress the production of \( X_2 \). This sequence of steps is expected to repeat as the promoters invert back and forth. Overall, the repressors introduce a lag in the response time of the system because each repressor protein remains present after promoter inversion and continues to repress its associated promoter effectively. Additional time is required for the repressor to decay to levels where it no longer represses the production of its associated recombinase. The sequestration reaction between recombinases further helps keep the less abundant recombinase species inactive, thereby preventing stochastic pulsing and promoting more coherent oscillations.
We developed a model for the RR circuit (Figure 4B), reported here as a list of chemical reactions, including transcription and translation. Rate constants were converted to propensities to simulate the reactions using the Gillespie algorithm. We then studied the periodic behavior of this circuit under different parameter values.

The inverting promoter regulates the transcription of either of the two mRNA repressors, $W_1$ and $W_2$, with rate constant $\beta$ at any given time. $W_1$ is produced when the promoter is pointing to the right (configuration $S_R$) and $W_2$ is produced when the promoter is pointing to the left (configuration $S_L$). The mRNAs also decay with a rate constant $\delta$.

Transcription: $S_R^{\beta} \rightarrow S_R^{\beta} + W_1$; $S_L^{\beta} \rightarrow S_L^{\beta} + W_2$.

mRNA Degradation: $W_1 \xrightarrow{\delta} \emptyset$; $W_2 \xrightarrow{\delta} \emptyset$.

$W_1$ and $W_2$ are translated into repressors proteins $Y_1$ and $Y_2$ with a rate constant $r_2$ and decay with a rate constant $\delta$.
Translation: \[ W_1 \xrightarrow{\rho_1} W_1 + Y_1 \quad W_2 \xrightarrow{\rho_2} W_2 + Y_2 \]

Protein Degradation: \[ Y_1 \xrightarrow{} \varnothing \quad Y_2 \xrightarrow{} \varnothing \]

The repressors \( Y_1 \) and \( Y_2 \) bind and unbind to target promoters \( P_1 \) and \( P_2 \), making them promoters \( P_1^* \) and \( P_2^* \), at rates \( a_p \) and \( d_b \), respectively. The unbound promoters, \( P_1 \) and \( P_2 \), regulate the transcription rate of recombinase mRNAs \( M_1 \) and \( M_2 \) with parameter \( \theta \). Both mRNAs decay with a rate constant \( \varphi \). In addition, the mRNAs produce recombinase monomers \( Z_1 \) and \( Z_2 \) with a rate constant \( \rho_1 \).

Binding/Unbinding: \[ Y_1 + P_1 \xrightarrow{a_p} P_1^* \quad Y_2 + P_2 \xrightarrow{a_p} P_2^* \]

Transcription: \[ P_1 \xrightarrow{\theta} P_1 + M_1 \quad P_2 \xrightarrow{\theta} P_2 + M_2 \]

mRNA Degradation: \[ M_1 \xrightarrow{} \varnothing \quad M_2 \xrightarrow{} \varnothing \]

Translation: \[ M_1 \xrightarrow{\rho_1} M_1 + Z_1 \quad M_2 \xrightarrow{\rho_1} M_2 + Z_2. \]

The monomers \( Z_1 \) and \( Z_2 \) form homodimers \( X_1 \) and \( X_2 \) in addition to heterodimer \( C \), both with an association rate constant \( a_1 \) and a dissociation rate constant \( d_1 \). The heterodimer formation is possible because the second recombinase monomer is fused to its RDF. In addition, all protein complexes decay with a rate constant \( d \).

Homodimerization: \[ Z_1 + Z_1 \xrightarrow{a_1} X_1 \quad Z_2 + Z_2 \xrightarrow{a_1} X_2 \]

Heterodimerization: \[ Z_1 + Z_2 \xrightarrow{d_1} C. \]

Protein Degradation: \[ Z_1, X_1, Z_2, X_2, C \xrightarrow{} \varnothing \]

Finally, the switching rate of the promoter is regulated by the recombinase dimers \( X_1 \) and \( X_2 \) with binding rate constant \( a_2 \) and unbinding rate constant \( d_2 \).

Binding/Unbinding: \[ X_1 + S_{L}^{0} \xrightarrow{a_2} S_{L}^{10} \quad X_1 + S_{L}^{10} \xrightarrow{a_2} S_{L}^{11} \]
\[ X_1 + S_{L}^{10} \xrightarrow{d_2} S_{L}^{0} \quad X_1 + S_{L}^{10} \xrightarrow{d_2} S_{L}^{11} \]

Binding/Unbinding: \[ X_2 + S_{R}^{0} \xrightarrow{a_2} S_{R}^{10} \quad X_2 + S_{R}^{10} \xrightarrow{a_2} S_{R}^{11} \]
\[ X_2 + S_{R}^{10} \xrightarrow{d_2} S_{R}^{0} \quad X_2 + S_{R}^{10} \xrightarrow{d_2} S_{R}^{11} \]

Inversion: \[ S_{L}^{11} \xrightarrow{} S_{L}^{00} + 2X_1 \quad S_{R}^{11} \xrightarrow{} S_{R}^{00} + 2X_2 \]

As shown in the blue plot in Figure 4D (1), the RR design has a high incoherence metric for low values of translation rate constant, \( \rho_1 \), while coherence increases for higher \( \rho_1 \) values, but not to the same extent as it does for the R design. Similarly, high \( \rho_1 \) can lead to fast switching and low \( \rho_1 \) can give rise to stochastic pulsing. The blue plot in Figure 4D (2) shows that the RR oscillator is quite coherent for high values of protein degradation \( \delta \), but rather incoherent for low values of \( \delta \). This is because the coherent simulations for this oscillator have a large period, so a longer simulation is required to properly ascertain an accurate coherence metric. However, this was not provided to keep the comparisons in this paper consistent.

Coherence is unaffected by \( a_1 \) in the RR design, but is lower in general, as shown in the blue plot in Figure 4D (3) unless \( a_1 \) is decreased by a factor of 100 (not shown). The blue plot in Figure 4D (4) shows that, as for the R design, the incoherence metric becomes higher as the \( a_0 \) value of the RR design decreases, revealing poor coherence between periods because promoter inversion is induced poorly by the lack of a recombinase/DNA complex, \( S_{L}^{11} \) or \( S_{R}^{11} \). This design also has a high incoherence metric for low values of the switching rate, \( r \), while the incoherence metric gets lower when \( r \) is high, as shown in the blue plot in Figure 4D (5). There is also a small increase in the incoherence metric as the repressor translation rate, \( \rho_2 \), increases, as shown in the blue plot in Figure 4D (6).

**RP design: Introducing protease-based regulation**

Since slowly decaying recombinases lead to higher rates of inversion for the R design (Figure 4D (2), orange plot), we reasoned that regulating the decay rate could improve the switching behavior of an oscillator. Next, we analyze a circuit design that relies on proteases to control the amount of active recombinase present. Others have created similar circuit designs using protease in the past. The simple circuit schematic is...
shown in Figure 4C, along with an example trajectory. Proteases are proteins that can cleave a polypeptide at a specific, targeted amino acid sequence so as to inhibit its function. The RP circuit design consists of recombinases $X_1$ and $X_2$ that are cleaved by two orthogonal proteases $Y_1$ and $Y_2$, respectively. When the inverting promoter points to the right, it produces the protease $Y_1$, which selectively cleaves the recombinase $X_1$. At the same time, the amount of recombinase $X_2$ increases due to the lack of protease $Y_2$. Then $X_2$ inverts the promoter to the left, initiating the production of protease $Y_2$ and leaving $Y_1$ to decrease in concentration due to degradation. However, the remaining $Y_1$ continues to target and cleave $X_1$. This delays the increase in the amount of $X_1$. After this delay, $X_1$ eventually inverts the promoter to the right and stops the production of $Y_2$. The remaining amount of $Y_2$ will delay the production of recombinase $X_2$ before it can then effectively invert the promoter to the left. These interactions are expected to generate a repetitive switching behavior in which the promoter is inverted from right to left back and forth, but the half-cycle of each oscillation is expected to be longer when compared to the R design.

As in the previous cases, we built a model to evaluate the dynamics of the RP circuit design shown in Figure 4C. Constitutive transcription of recombinase mRNAs $M_1$ and $M_2$ occurs at a constant rate, $\theta$, and decay at a constant rate, $\phi$. In addition, the mRNAs produce recombinase monomers $Z_1$ and $Z_2$ with a rate constant $\rho_1$.

\begin{align*}
\text{Transcription:} & \quad \emptyset \xrightarrow{\rho_1} M_1, \quad \emptyset \xrightarrow{\rho_1} M_2 \\
\text{mRNA Degradation:} & \quad M_1 \xrightarrow{\phi} \emptyset, \quad M_2 \xrightarrow{\phi} \emptyset \\
\text{Translation:} & \quad M_1 \xrightarrow{\theta} Z_1 + Z_2, \quad M_2 \xrightarrow{\theta} M_2 + Z_2.
\end{align*}

The monomers $Z_1$ and $Z_2$ can form homodimers $X_1$ and $X_2$, respectively, as well as heterodimer $C$ with association rate constant $a$ and dissociation rate constant $d$. The heterodimer formation is possible because the second recombinase monomer is fused to its RDF. In addition, all protein complexes decay with a rate constant $\delta$.

\begin{align*}
\text{Homodimerization:} & \quad Z_1 + Z_1 \xrightarrow{d_1} X_1, \quad Z_2 + Z_2 \xrightarrow{d_2} X_2 \\
\text{Heterodimerization:} & \quad Z_1 + Z_2 \xrightarrow{d_3} C. \\
\text{Protein Degradation:} & \quad Z_1, X_1, Z_2, X_2, C \xrightarrow{\delta} \emptyset.
\end{align*}

The inverting promoter, located between recombinase binding sites, regulates the transcription of protease mRNAs $W_1$ and $W_2$ with rate parameter $\beta$. Protease mRNAs are also degraded/diluted with rate parameter $\phi$.

\begin{align*}
\text{Transcription:} & \quad S_b \xrightarrow{\beta} S_b + W_1, \quad S_b \xrightarrow{\beta} S_b + W_2 \\
\text{mRNA Degradation:} & \quad W_1 \xrightarrow{\phi} \emptyset, \quad W_2 \xrightarrow{\phi} \emptyset.
\end{align*}

The mRNA species $W_1$ and $W_2$ yield proteases $Y_1$ and $Y_2$, each with a rate constant $\rho_2$. The protease proteins decay with a rate constant $\delta$.

\begin{align*}
\text{Translation:} & \quad W_1 \xrightarrow{\theta} W_1 + Y_1, \quad W_2 \xrightarrow{\theta} W_2 + Y_2 \\
\text{Protein Degradation:} & \quad Y_1 \xrightarrow{\delta} \emptyset, \quad Y_2 \xrightarrow{\delta} \emptyset.
\end{align*}

The protease $Y_1$ (respectively $Y_2$) targets the recombinase monomer $Z_1$ (respectively $Z_2$) as well as dimer $X_1$ (respectively $X_2$) for catalytic degradation. Since we have both a high protease and recombinase copy number, we decided to model the cleavage of recombinase by protease using Hill function-type propensities, consistently with previous literature; this choice also has the advantage of reducing the model complexity. Protease reactions occur with rate parameters $g_1$ and $g_2$, corresponding to a Hill-type function with Hill coefficient equal to 1.

\begin{align*}
\text{Catalytic Protein Degradation:} & \quad Y_1 + Z_1 \xrightarrow{g_1} Y_1, \quad Y_1 + X_1 \xrightarrow{g_1} Y_1, \\
\text{Catalytic Protein Degradation:} & \quad Y_2 + Z_2 \xrightarrow{g_2} Y_2, \quad Y_2 + X_2 \xrightarrow{g_2} Y_2,
\end{align*}

where $g_1 = \frac{1}{X_1 + X_2 + K_1}$ and $g_2 = \frac{1}{X_1 + X_2 + K_2}$.

Finally, the switching rate constant of the promoter is regulated by the recombinase dimers $X_1$ and $X_2$ with binding rate constant $a_b$ and unbinding rate constant $d_b$ as shown.
Like the previous designs, the incoherence metric of the RP design is high at low $\rho_1$ values, and it decreases as $\rho_1$ increases, as shown in the teal plot in Figure 4D (1). Hence, a large $\rho_1$ improves the coherence of oscillations. In contrast with other designs, this case has a remarkably low incoherence metric for low degradation rate constants, $d_1$, as shown in the teal plot in Figure 4D (2). This indicates that stochastic pulsing does not occur at low degradation rates. For this reason, this design is the most robust to changes in degradation rate constant, $d_1$.

The incoherence metric increases as $r$ increases, following the same trend observed when varying $\rho_1$ in the RR oscillator (repressor protein translation rate).

**Additional architectures**

Here, we briefly describe three additional architectures where recombinase levels are regulated with different approaches. The derivation of the model for each circuit design can be found in the STAR Methods.

**An activator-based circuit design (RA design)**

The RA circuit design incorporates an activator to regulate recombinase production. The corresponding schematic is reported in Figure 5A along with an example trajectory. The design consists of the transcriptional activators $Y_1$ and $Y_2$ that drive the production of recombinases $X_1$ and $X_2$. When the inverting promoter points to the left, it produces the activator $Y_1$, driving the production of $X_1$. These two steps in the cascade must occur before $X_1$ can be produced and hence slow down the production of the recombinase. Once $X_1$ increases in concentration, it can invert the promoter to the right. This leads to the production of $Y_2$ and stops the production of $Y_1$. Then, $Y_2$ increases the production of $X_2$. When $X_2$ increases in concentration, it can invert the promoter to the left. Overall, this leads to a switching cycle of inverting the promoter from left to right repeatedly. One challenge with this design is that, even when an activator is not being produced, the remaining transcription factor can still increase the production of its associated recombinase. This makes it difficult for the concentration of each recombinase to get very low, which causes the promoter to invert more irregularly.

As a result, the RA design is the worst-performing among the designs we tested. The incoherence metric is consistently higher than that of the other oscillators in nearly every parameter regime. When $\rho_1$ is varied, the incoherence metric follows the same pattern as that of previous designs, decreasing as $\rho_1$ increases (Figure 5D (1), blue plot). The window of $\delta$ values for which this oscillator is more coherent (Figure 5D (2), blue plot) is also the smallest.

Parameter $\rho_2$ also does not affect coherence in this design, as shown by the blue plot in Figure 5D (3), unless it is decreased by a factor of 100. However, the incoherence metric is higher overall for the RA oscillator compared to the other designs. The blue plot in Figure 5D (4) follows the general pattern of this plot for the other designs, the incoherence metric decreasing as $\rho_0$ increases. While the incoherence metric does decrease as $r$ increases, it again remains higher than for the other oscillators over the entire range of $r$ values we tested with the exception of the RR oscillator, which is fairly comparable to the RA oscillator in this regard (Figure 5D (5), blue plot).
Finally, the activator translation rate, $r_2$, does not affect the coherence of the oscillator very much (Figure 5D, blue plot), a pattern noticeably different from that shown by the other oscillators with an additional element in their cascade that we have discussed so far. We will see it is more similar to this pattern for the next oscillator we consider.

An sRNA-based circuit design (RS design)

Molecular sequestration can program temporal delays by setting concentration thresholds. The time that the system takes to reach this threshold defines the delay caused by sequestration. We take inspiration from this mechanism to design a circuit that sequesters recombinase mRNA with small RNA as shown in Figure 5B, which reports the schematic as well as an example trajectory. The circuit consists of small
RNA molecules $Y_1$ and $Y_2$ that target the recombinase mRNAs $X_1$ and $X_2$, respectively. When the inverting promoter points to the right, it produces the recombinase mRNA $X_2$, which is sequestered by the small RNA $Y_2$. Since only $X_2$ is being produced without $X_1$, the concentration of the recombinase transcribed from $X_2$ increasing to overcome this sequestration results in the inversion of the promoter to the left. Then, the amount of recombinase mRNA $X_1$ increases while $X_2$ decays. Once $X_1$ increases in concentration to overcome its sequestration by $Y_1$, the recombinase transcribed from $X_1$ inverts the promoter to the right. This results in the continuous inversion of the promoter from left to right where the delay is caused by the time it takes for recombinase mRNA concentration to reach the threshold where it overcomes its sequestration by sRNA.

The incoherence metric for varying $\rho_1$ values (Figure 5D (1), teal plot) is akin to that for the R, RR, and RP designs. Compared to the RA oscillator, the RS oscillator has a broader range of $\delta$ values for which the incoherence metric is low (Figure 5D (2), teal plot). Like in all other designs, $a_1$ does not affect coherence (Figure 5D (3), teal plot) unless it is decreased by a factor of 100. The incoherence metric follows the same overall pattern with the regard to $a_2$ as all of the oscillators, decreasing as $a_2$ increases (Figure 5D (4), teal plot). For the RS oscillator, the incoherence metric also decreases as $\gamma$ increases, which is similarly in line with the general behavior for the R, RR, and RP designs (Figure 5D (5), teal plot).

Interestingly, the incoherence metric increases slightly as $\theta$, the translation rate of the sRNA, increases (Figure 5D (6), teal plot). This follows the same pattern as all of the other oscillators with an additional regulatory component with regard to $\rho_2$, which controls the production rate of such factor.

**Negative feedback oscillator (NF design)**

The NF design, whose schematic is shown in Figure 5C along with an example trajectory, consists of a constitutive promoter controlling the production of recombinase $X_1$ and a self-inhibitory module regulating recombinase $X_2$. The presence of two distinct promoters differentiates this design from the circuit design in Figure 4A, which contains a single promoter. The regulation (or orientation) of the promoter controlling the production of $X_2$ experiences the effect of suppression by negative feedback, strengthened by heretodimer formation by recombinase monomers. This circuit can exhibit switching behavior with a consistent period, as well as stochastic pulsing behavior. When the total production rate of $X_2$ is larger than that of $X_1$, $X_2$ not only inhibits its own production by causing its promoter to point to the left but also limits the homodimer formation of $X_1$. When $Z_2$ monomers are no longer being produced, they become depleted by sequestration. Only then does the concentration of $X_1$ have the opportunity to increase, which inverts the promoter to the right (configuration $S_2\beta$). This results in periodic behavior with a consistent period. Conversely, stochastic pulsing occurs when the level of $X_1$ is very low and the inversion of the promoter to the right occurs randomly. On the other hand, when the concentration of $X_1$ is larger than that of $X_2$, the system yields very fast oscillations, because $X_1$ is readily available to flip the promoter to the state $S_\beta$. This fast promoter inversion may be the reason behind the circuit’s low incoherence metric when compared to all the other variants, as shown in Figure 5D. The effect of the various parameters is akin to that observed for the other circuits.

**Interpreting the coherence of oscillations through the analysis of single self-inhibiting circuits**

To interpret and explain the emergence of incoherent oscillations in our basic architecture (Figure 3B), which consists of two interconnected self-inhibitory modules, we examined the behavior of a single self-inhibitor. We reasoned that a single module contributes to half of each oscillatory cycle and its analysis could provide qualitative insights into the behavior of the full system. We computed the time it takes for inversion to occur, denoted $T_I$, and the recombinase concentration at inversion, denoted $X_I$. Both quantities have statistical properties that make it possible to determine the impact of noise propagation on the switching behavior. Furthermore, the analysis of $T_I$ and $X_I$ contributes to the identification of key network parameters that mitigate the phase change in the oscillations.

A schematic of the self-inhibiting module is in Figure 6A; the set of chemical reactions we modeled is in the STAR Methods.

All stochastic simulations of the self-inhibiting module produce a pulse in recombinase concentration, as shown by the gray trajectories in Figure 6B (top). This pulse can be viewed as half of an oscillatory cycle, and occurs when the promoter is in the on-state ($S_\alpha$), until the level of recombinase is sufficiently high to elicit
inversion of the promoter to the off-state ($S_L$). When the promoter is off, the recombinase level decreases due to dilution/degradation. Each trajectory presents a distinct promoter inversion time, $T_I$, and recombinase concentration at the point of inversion, $X_I$. We mark these quantities in Figure 6B for the trajectory in black. The top and bottom panels of Figure 6C respectively show the histograms of the relative frequency of $X_I$ values and of $T_I$ values for 500 simulations, using the nominal parameters in Table S1. While $X_I$ has small variance, $T_I$ has a large variance, indicating that the half-cycle of an oscillator using this particular transcription rate would have an irregular period. Thus, many consecutive, irregular half-cycles could cause the oscillator to become out of phase very quickly.

We sought to gain a more comprehensive understanding of how the histograms of $X_I$ and $T_I$ are affected by the circuit parameters. Figures 6D and 6E show heat maps representing the computed distribution of $X_I$ (top) and $T_I$ (bottom). Each row of these plots should be interpreted as a color-coded histogram of $X_I$ and $T_I$ corresponding to a different translation rate constant, $r$ (Figure 6D), and switching rate parameter, $r_s$ (Figure 6E). Higher translation rates reduce both the mean and the variance of $T_I$, but introduce a high variance for $X_I$. When two such self-inhibiting modules are interconnected to build an oscillator (Figure 3A), we see that a high recombinase translation rate yields a low incoherence metric in all architectures (Figures 4D (1) and 5D (1)), which indicates that a consistent switching time may be crucial for coherence. It is important to remember that a low incoherence metric may be associated with rapid switching, as suggested by the low mean of $T_I$. In contrast, small values of $r_s$ lead to a reduced variance for $X_I$ but to an increased variance for $T_I$ (Figure 6D). This larger variance of the inversion time may be the reason why in the complete oscillator (Figure 3A) a low recombinase translation rate constant causes stochastic pulsing and high incoherence.

In Figure 6E, we varied the switching rate parameter, $r_s$, which scales the inversion of the promoter. Overall, a lower value for $r_s$ leads to higher variance in the distribution of $T_I$, and hence the trajectories of the
corresponding full oscillator design in Figure 3A exhibit stochastic pulsing for low \( r \) values. In contrast, a high \( r \) value results in small mean and variance for both \( T_i \) and \( X_i \), which may improve the coherence of oscillations: this is consistent with the simulations of the full circuit variants in Figures 4D (5) and 3D (5). Overall, while not predictive, these simulations indicate that examining an individual self-inhibiting module is useful to interpret the behavior of the full circuit.

**Limitations of the study**

Coherent oscillations should have comparable period, phase, and amplitude throughout their duration. Our approach focuses exclusively on period consistency, and broad progress in this context will require the development of methods for examining phase and amplitude consistency as well. It is important to note that our notion of coherence does not provide information on the period duration. First, we note that small stochastic fluctuations in the recombinase trajectories are always captured when computing the autocorrelation function, and thus contribute to the incoherence metric; these fluctuations are due to the presence of multiple reaction steps like promoter inversion and recombinase dimerization. However, some parameter combinations result in a predominance of these rapid fluctuations in the recombinase level, due to very rapid cycles of promoter inversion. This fast switching occurs in particular when the switching rate and the dilution/degradation rates are large. In these particular cases of predominant fast switching, the distribution of inter-peak times of the autocorrelation function shows a small variance, so the slope of the coherence plot remains low, even though the peak levels of recombinase may be irregular. This points to the fact that our coherence analysis cannot discriminate between a dominant fast switching regime and slower, coherent oscillations. This is how the NF with its fast switching appears so successful by this metric. Another challenge in our approach is posed by the duration of the stochastic simulation. When examining stochastic signals, the peaks of the autocorrelation function depend significantly on the window of observation of the signal: the autocorrelation peaks obtained from a short observation window should differ from those computed from a long observation window, which are likely to be smaller and present more variability due to noise. In addition, computing the autocorrelation function of an ensemble of extended stochastic trajectories is computationally demanding. Driven by these observations, we used a 40 h simulation window, but we verified that 5-fold longer simulations are qualitatively similar and indicate that oscillations are sustained for all the circuits we examined (Figure S1).

**Summary of simulation results**

Overall, the key finding of our computational analysis is that all the architectures we proposed have the capacity to exhibit coherent oscillations in a range of parameters. This finding indicates that diverse experimental implementations of recombinase oscillators are likely to succeed. Table 1 summarizes the most salient features and behavior of the different designs. We defined an incoherence metric to assess the effect of individual parameters on the ability of the circuits to produce oscillations. We found that the protein dilution/degradation rate constant parameter \( \delta \) has a strong influence on the viability of our candidate oscillators; in particular, in all designs, a small \( \delta \) (slow degradation) is detrimental to achieving coherent oscillations, while a large \( \delta \) (fast degradation) may have either beneficial or detrimental effects depending on the circuit architecture. The R and NF designs have the smallest number of components and achieve coherent oscillations, with the NF design achieving the smallest incoherence; the R design is particularly sensitive to \( \delta \) and the coherence of oscillations degrades as this parameter increases. For both circuits, coherence improves with larger values of translation parameter \( \rho \). The RR and RP designs show incoherence trends comparable with the R circuit, except a large value of \( \delta \) decreases their incoherence. The RR and RA designs also allow for the simple integration of a coupled process via another gene that is either repressed or activated, respectively. Implementing a coupled process by creating a system that alternates between producing one and two RNAs in the other designs would be more difficult to implement because it would require the integration of an operon at the inverting promoter. All designs are essentially unaffected by changes in the association rate parameter of the dimers, \( a_{ij} \), unless the value is decreased by a factor of 100 or more. Overall, the RR, RP, RS, and NF designs, which are the ones with an inhibitory element explicitly present in their cascade with the exception of the NF design, have comparable performance in terms of incoherence.

**DISCUSSION**

We described and computationally modeled different molecular circuit architectures to induce periodic behaviors using recombinases and we found that coherent oscillators are achievable by all the
architectures we considered. At the core of all our designs, two self-inhibiting loops are coupled through a target DNA site that undergoes periodic inversion (switching) cycles induced by recombinase expression. We examined six variants of this basic architecture that use various mechanisms to introduce tighter control of recombinase levels in the circuit with the goal of achieving regular oscillations. Each of these oscillators was examined using stochastic simulations assuming that a single copy of each genetic component is present, capturing a realistic scenario for genomically integrated circuits. To evaluate the incoherence of oscillations, we used an approach that examines the statistics of the autocorrelation function of stochastic trajectories, and we used this metric to identify which kinetic rate constants improve oscillation coherence. Our simulations indicate that combining recombinases and mechanisms to suppress or delay recombinase expression, such as sequestration, transcriptional repressors, and proteases, allows to maintain or improve coherence. The positive effect of sequestration on the coherence of oscillations of the well-known repressor circuit was previously observed experimentally and computationally through analysis of incoherence similar to ours. 11

Our circuit designs are unique in that they couple two stable negative feedback loops through mutual activation, enabled by recombinase-mediated inversion of a target DNA site. This strategy departs from the traditional approach of designing molecular oscillators by including an overall negative feedback loop that can be destabilized via high gain, delays, or positive feedback. 4–9 Our computational simulations indicate that the parameters related to each individual self-inhibiting module influence the coherence of oscillations for the full circuit by determining the statistics of the peak amplitude and duration of each half-cycle (Figure 6). We speculate that, in experiments, these recombinase-based self-inhibiting modules may first be tuned individually, facilitating the synthesis and characterization of the complete oscillators. We also conjecture that nested clocks with multiple synchronized periods may be built by coupling different self-inhibiting components. Our computations suggest that periodic behaviors are likely to occur in a broad range of parameter values, supporting experimental realization of recombinase oscillators.

### STAR METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Data and code availability
- EXPERIMENTAL MODELS AND SUBJECT DETAILS
- METHOD DETAILS
  - Reactions modeling an individual self-inhibiting module
  - Activator-recombinase oscillator: AR design
  - Recombinase and small-RNA: RS design
  - Negative feedback: NF design
- QUANTIFICATION AND STATISTICAL ANALYSIS
- ADDITIONAL RESOURCES

| Circuit          | Number of genes | Max Incoherence | Sensitivity to degradation $\delta$ | Sensitivity to rec. translation $\rho$, $\rho_1$ | Sensitivity to TX/TL of regulators $\theta$, $\rho_2$ |
|------------------|-----------------|-----------------|-------------------------------------|-----------------------------------------------|-----------------------------------------------|
| R Design (Figure 3A) | 2               | 13              | IM increases with $\delta$           | IM decreases with $\rho$                       | N/A                                           |
| RR Design (Figure 3B) | 4               | 11              | IM decreases with $\delta$           | IM decreases with $\rho_1$                     | IM increases with $\rho_2$                     |
| RP Design (Figure 3C) | 4               | 9.5             | IM insensitive with $\delta$         | IM decreases with $\rho_1$                     | IM increases with $\rho_2$                     |
| RA Design (Figure 4A) | 4               | 12              | IM increases with $\delta$           | IM decreases with $\rho_1$                     | IM insensitive with $\rho_2$                   |
| RS Design (Figure 4B) | 4               | 15              | IM increases with $\delta$           | IM decreases with $\rho_1$                     | IM insensitive with $\theta$                  |
| NF Design (Figure 4C) | 2               | 8.5             | IM increases with $\delta$           | IM decreases with $\rho$                       | N/A                                           |

Table 1. Summary of the features of the architectures we considered. Incoherence metric is abbreviated as IM.
SUPPLEMENTAL INFORMATION
Supplemental information can be found online at https://doi.org/10.1016/j.isci.2022.105624.

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AUTHOR CONTRIBUTIONS
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STAR METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Software and algorithms | Python Core Development Team | https://python.org |
| NumPy | NumPy Development Team | https://numpy.org |
| SciPy | SciPy Development Team | https://scipy.org |
| GitHub | Github Development Team | https://github.com |

RESOURCE AVAILABILITY

Lead contact
Further information should be directed to Elisa Franco (Email: efranco@seas.ucla.edu).

Data and code availability
Stochastic simulations were implemented using custom scripts in Python and biocircuits package using a workstation with the following specifications: 8 GB RAM and a 2.9 GHz, dual-core Intel Core i5. Scripts are available on GitHub https://github.com/ccubasam-code/Recombinase-Oscillators.

EXPERIMENTAL MODELS AND SUBJECT DETAILS
None.

METHOD DETAILS

Reactions modeling an individual self-inhibiting module

Transcription:

\[ S_{R}^{0} \xrightarrow{\delta} S_{R}^{0} + M \]

mRNA Degradation:

\[ M \xrightarrow{4} \emptyset \]

Translation:

\[ M \xrightarrow{r} M + Z \]

Homodimerization:

\[ Z + X \xrightarrow{\sigma} X \]

Protein Degradation:

\[ Z, X \xrightarrow{\tau} \emptyset \]

Binding/Unbinding:

\[ X + S_{R}^{0} \xrightarrow{d_{1}} S_{R}^{10} \quad X + S_{R}^{10} \xrightarrow{d_{1}} S_{R}^{11} \]

\[ X + S_{R}^{0} \xrightarrow{d_{2}} S_{R}^{11} \quad X + S_{R}^{11} \xrightarrow{d_{2}} S_{R}^{10} \]

Inversion:

\[ S_{R}^{11} \xrightarrow{\iota} S_{L}^{00} + 2X. \]

Activator-recombinase oscillator: AR design

An inverting promoter regulates the transcription of either of the two mRNA activators, \( W_1 \) and \( W_2 \), with rate \( \delta \) at any given time. \( W_1 \) is produced when the promoter is pointing to the right (configuration \( S_0 \)), and \( W_2 \) is produced when the promoter is pointing to the left (configuration \( S_0 \)). The mRNAs also decay with a rate constant \( \varphi \).

Transcription:

\[ S_{R}^{00} \xrightarrow{\delta} S_{R}^{00} + W_1 \quad S_{L}^{00} \xrightarrow{\delta} S_{L}^{00} + W_2 \]

mRNA Degradation:

\[ W_1 \xrightarrow{\varphi} \emptyset \quad W_2 \xrightarrow{\varphi} \emptyset. \]

\( W_1 \) and \( W_2 \) are translated into activators proteins \( Y_1 \) and \( Y_2 \) with a rate constant \( \rho_2 \), and decay with a rate constant \( \delta \).
The activators \(Y_1\) and \(Y_2\) bind and unbind to target promoters \(P_1\) and \(P_2\) with association rate constant \(a_r\) and dissociation rate constant \(d_r\), respectively. The bound promoters \(P_r^1\) and \(P_r^2\) regulate the transcription of recombinase mRNAs \(M_1\) and \(M_2\) with rate \(\theta\). Both mRNAs decay with a rate constant \(\varphi\). In addition, the mRNAs produce recombinase monomers \(Z_1\) and \(Z_2\) with a rate constant \(p_1\).

**Translation:** \(W_1 \overset{\rho_1}{\rightarrow} W_1 + Y_1\) \(W_2 \overset{\rho_2}{\rightarrow} W_2 + Y_2\)

**Protein Degradation:** \(Y_1 \overset{\gamma_1}{\rightarrow} \emptyset\) \(Y_2 \overset{\gamma_2}{\rightarrow} \emptyset\).

The monomers \(Z_1\) and \(Z_2\) form homodimers \(X_1\) and \(X_2\) in addition to heterodimer \(C\), both with an association rate constant \(a_i\) and a dissociation rate constant \(d_i\). The heterodimer formation is possible because the second recombinase monomer is fused to its RDF. In addition, all protein complexes decay with a rate constant \(d\).

**Homodimerization:** \(Z_1 + Z_1 \overset{a_i}{\rightarrow} X_1\) \(Z_2 + Z_2 \overset{a_i}{\rightarrow} X_2\)

**Heterodimerization:** \(Z_1 + Z_2 \overset{a_i}{\rightarrow} C\).

**Protein Degradation:** \(Z_1, X_1, Z_2, X_2, C \overset{d}{\rightarrow} \emptyset\)

Finally, the switching rate of the promoter is regulated by the recombinase dimers \(X_1\) and \(X_2\), which bind to the promoter with an association rate constant of \(a_0\) and a dissociation rate constant of \(d_0\).

**Binding/Unbinding:** \(X_1 + S_1^0 \overset{a_0}{\rightarrow} S_1^1\) \(X_1 + S_1^0 \overset{d_0}{\rightarrow} S_1^0\)

\(X_2 + S_2^0 \overset{a_0}{\rightarrow} S_2^1\) \(X_2 + S_2^0 \overset{d_0}{\rightarrow} S_2^0\)

**Binding/Unbinding:** \(X_1 + S_1^0 \overset{a_0}{\rightarrow} S_1^1\) \(X_1 + S_1^0 \overset{d_0}{\rightarrow} S_1^0\)

**Inversion:** \(S_1^1 \overset{S_1^0}{\rightarrow} S_2^0\)

### Recombinase and small-RNA: RS design

The promoter regulates the transcription of either of the two small RNAs, \(W_1\) and \(W_2\), with rate \(\beta\) at any given time. The mRNAs also decay with a rate constant \(\varphi\).

**Transcription:** \(\emptyset \overset{b_r}{\rightarrow} W_1\) \(\emptyset \overset{b_r}{\rightarrow} W_2\)

**mRNA Degradation:** \(W_1 \overset{\varphi}{\rightarrow} \emptyset\) \(W_2 \overset{\varphi}{\rightarrow} \emptyset\).

\(W_1\) and \(W_2\) sequester the mRNA \(M_1\) and \(M_2\), respectively, at a rate constant \(\psi\).

**mRNA Sequestration:** \(W_1 + M_1 \overset{\psi}{\rightarrow} \emptyset W_2 + M_2 \overset{\psi}{\rightarrow} \emptyset\).

The promoter pointing to the left and right up-regulates the transcription of recombinase mRNAs \(M_1\) and \(M_2\) with rate \(\theta\). Both mRNAs decay with a rate constant \(\varphi\). In addition, the mRNAs produce recombinase monomers \(Z_1\) and \(Z_2\) with a rate constant \(p_1\).

**Transcription:** \(S_1^0 \overset{\theta}{\rightarrow} S_1^0 + M_1\) \(S_2^0 \overset{\theta}{\rightarrow} S_2^0 + M_2\)

**mRNA Degradation:** \(M_1 \overset{\varphi}{\rightarrow} \emptyset\) \(M_2 \overset{\varphi}{\rightarrow} \emptyset\)

**Translation:** \(M_1 \overset{\rho_1}{\rightarrow} M_1 + Z_1\) \(M_2 \overset{\rho_1}{\rightarrow} M_2 + Z_2\).

The monomers \(Z_1\) and \(Z_2\) form homodimers \(X_1\) and \(X_2\) in addition to heterodimer \(C\), both with an association rate constant \(a_i\) and a dissociation rate constant \(d_i\). The heterodimer formation is possible because the second recombinase monomer is fused to its RDF. In addition, all protein complexes decay with a rate constant \(d\).
Homodimerization: \[ Z_1 + Z_1 \overset{a_1}{\rightleftharpoons} X_1 \quad Z_2 + Z_2 \overset{a_2}{\rightleftharpoons} X_2 \]

Heterodimerization: \[ Z_1 + Z_2 \overset{a_3}{\rightleftharpoons} C. \]

Protein Degradation: \[ Z_1, X_1, Z_2, X_2, \overset{\lambda}{\longrightarrow} \]

Finally, the switching rate of the promoter is regulated by the recombinase dimers \( X_1 \) and \( X_2 \) which bind and unbind to the promoter with rate constants \( a_9 \) and \( d_9 \), respectively.

\[
\begin{align*}
\text{Binding/Unbinding:} & \quad X_1 + S_2^0 \overset{a_{10}}{\leftarrow} S_1^0, \quad X_1 + S_2^0 \overset{d_{10}}{\rightarrow} S_1^1 \\
& \quad X_1 + S_R^0 \overset{a_{11}}{\leftarrow} S_1^0, \quad X_1 + S_R^0 \overset{d_{11}}{\rightarrow} S_1^1 \\
\text{Binding/Unbinding:} & \quad X_2 + S_2^0 \overset{a_{12}}{\leftarrow} S_2^0, \quad X_2 + S_2^0 \overset{d_{12}}{\rightarrow} S_2^1 \\
& \quad X_2 + S_R^0 \overset{a_{13}}{\leftarrow} S_2^0, \quad X_2 + S_R^0 \overset{d_{13}}{\rightarrow} S_2^1 \\
\text{Inversion:} & \quad S_L^1 \overset{r}{\rightarrow} S_R^0, \quad S_R^0 \overset{r}{\rightarrow} S_L^1
\end{align*}
\]

Negative feedback: NF design

dRNA \( M_1 \) is produced constitutively at a rate of \( \theta_1 \) while the dRNA \( M_2 \) is only produced when the promoter points to the right at a rate of \( \theta_2 \). Both dRNAs are assumed to dilute/degrade with a rate constant \( \varphi \). In addition, the dRNAs \( M_1 \) and \( M_2 \) are translated to recombinase monomers \( Z_1 \) and \( Z_2 \), respectively, with rate constant \( \rho \).

\[
\begin{align*}
\text{Transcription:} & \quad \varnothing \overset{\alpha}{\rightarrow} M_1, \quad S_R^0 \overset{\delta}{\leftarrow} S_R^0 + M_2 \\
\text{mRNA Degradation:} & \quad M_1 \overset{\varepsilon}{\rightarrow} M_1, \quad M_2 \overset{\varepsilon}{\rightarrow} M_2 \\
\text{Translation:} & \quad M_1 \overset{\lambda}{\rightarrow} M_1 + Z_1, \quad M_2 \overset{\lambda}{\rightarrow} M_2 + Z_2
\end{align*}
\]

The serine recombinase monomers \( Z_1 \) and \( Z_2 \) can form the homodimers \( X_1 \) and \( X_2 \), respectively. Since \( Z_2 \) is \( Z_1 \) fused to its RDF, the two species can also form the heterodimer \( C \). For simplicity, we assume all dimers have an association rate constant \( a_4 \) and a dissociation rate constant \( d_4 \). In addition, we assume all proteins degrade with a rate constant \( \delta \).

\[
\begin{align*}
\text{Homodimerization:} & \quad Z_1 + Z_1 \overset{a_5}{\rightleftharpoons} X_1 \quad Z_2 + Z_2 \overset{a_5}{\rightleftharpoons} X_2 \\
\text{Heterodimerization:} & \quad Z_1 + Z_2 \overset{a_6}{\rightleftharpoons} C. \\
\text{Protein Degradation:} & \quad Z_1, X_1, Z_2, X_2, \overset{\lambda}{\longrightarrow}
\end{align*}
\]

The rate of promoter inversion is regulated by the recombinase dimers \( X_1 \) and \( X_2 \) with binding and unbinding rates \( a_6 \) and \( d_6 \), respectively, and the inversion rate \( r \).

\[
\begin{align*}
\text{Binding/Unbinding:} & \quad X_1 + S_2^0 \overset{a_7}{\leftarrow} S_1^0, \quad X_1 + S_2^0 \overset{d_7}{\rightarrow} S_1^1 \\
& \quad X_1 + S_R^0 \overset{a_8}{\leftarrow} S_1^0, \quad X_1 + S_R^0 \overset{d_8}{\rightarrow} S_1^1 \\
\text{Binding/Unbinding:} & \quad X_2 + S_2^0 \overset{a_9}{\leftarrow} S_2^0, \quad X_2 + S_2^0 \overset{d_9}{\rightarrow} S_2^1 \\
& \quad X_2 + S_R^0 \overset{a_{10}}{\leftarrow} S_2^0, \quad X_2 + S_R^0 \overset{d_{10}}{\rightarrow} S_2^1 \\
\text{Inversion:} & \quad S_L^1 \overset{r}{\rightarrow} S_R^0, \quad S_R^0 \overset{r}{\rightarrow} S_L^1
\end{align*}
\]

**QUANTIFICATION AND STATISTICAL ANALYSIS**

Statistical analyses were performed using the mean over 500 trajectories from the stochastic simulations.

**ADDITIONAL RESOURCES**

None.