Mathematical model of a serine inteegrase-controlled toggle switch with a single input

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Dual-state genetic switches that can change their state in response to input signals can be used in synthetic biology to encode memory and control gene expression. A transcriptional toggle switch (TTS), with two mutually repressing transcription regulators, was previously used for switching between two expression states. In other studies, serine inteegrases have been used to control DNA inversion switches that can alternate between two different states. Both of these switches use two different inputs to switch ON or OFF. Here, we use mathematical modelling to design a robust one-input binary switch, which combines a TTS with a DNA inversion switch. This combined circuit switches between the two states every time it receives a pulse of a single-input signal. The robustness of the switch is based on the bistability of its TTS, while inteegrase recombination allows single-input control. Unidirectional inteegrase-RDF-mediated recombination is provided by a recently developed inteegrase-RDF fusion protein. We show that the switch is stable against parameter variations and molecular noise, making it a promising candidate for further use as a basic element of binary counting devices.

1. Introduction

Genetic switches with two states (ON/OFF) are essential components of synthetic biology memory and counting devices, with potential application in biotechnology, biosensors and biocomputing [1–3]. The creation of these binary switches is, therefore, an important goal of synthetic biology. Here, we design a synthetic genetic switch, which switches between two states in response to a single-input signal. The response of the switch depends on its current state. If it is OFF when it receives an input signal, it switches to ON; if it is ON, it switches to OFF. An orthogonal set of single-input state-based toggle switches with this behaviour could be used to encode the digits in a binary ripple counter [2]. In such a counter, each switch represents a single binary digit, and N interconnected switches would be able to count up to 2N−1 occurrences of the same repeated signal. The counting of various intracellular or extracellular events can then be used to control intracellular processes, to track genetic lineage, or to count the occurrences of events [2,4]. No single-input switch capable of robust toggling between two states has been implemented to date.

The best-characterized bistable switch is the toggle switch, based on mutual repression of two inhibitors [5–8]. Transcriptional toggle switches (hereafter called TTS) are constructed in vivo and, therefore, can be directly used for intracellular applications. A TTS is based on the expression of two transcriptional repressors I1 and I2 [5,6,8]. Each repressor is expressed from a promoter...
regulated promoters (P1 and P2). Two different input signals (inducer 1 and 2) initiate the transition between the two states, by de-repressing the respective promoters. Activation of the inducible promoter Pind results in the expression of the currently unexpressed inhibitor, followed by the expression of (or switches that control gene expression, encode memory or logic gates, showed a damped response to repeated induction with two inputs [5,6]. However, the only single-input switch implemented to date, which combines a TTS and a DNA inversion switch. The switch between states can be brought about using two different inducers (input signals), be repressed by the other repressor (P1 or P2) (figure 1a), so that when I1 is expressed, transcription of I2 is turned off and vice versa. There are two steady states, with either I1 or I2 expressed. The switch between these two steady states can be brought about using two different inducers (input signals), such as IPTG and anhydrotetracycline (aTc), inducing transcription of the unexpressed repressor (figure 1a) [5]. Experimentally implemented TTS shows robust switching with two inputs [5,6]. However, the only single-input switch implemented to date, which combines a TTS and a logic gate, showed a damped response to repeated induction of the circuit [8].

Another class of genetic switch uses site-specific recombinases, enzymes that cut and rejoin DNA at specific recombination sites. Depending on the arrangement of these sites in the DNA, recombinases carry out fusion, deletion or inversion reactions. Inversion of a DNA segment flanked by two recombination sites in a ‘head-to-head’ orientation allows repeated switching between two alternative states. Placing a promoter on the invertible segment allows switching between expressions of two different genes (figure 1b). This has been used to make simple inversion switches that control gene expression, encode memory or carry out logical calculations [3,4,9–11]. Using serine integrases (int) for these genetic switches has the advantage of unidirectional recombination, and the ability to reverse this directionality by the addition of a recombination directionality factor (RDF) [10,12,13]. Int on its own carries out recombination on two specific DNA sequences called attP and attB sites (PB), producing attL and attR product sites (LR), each consisting of half of a P and half of a B site (figure 1b). The presence of the RDF reverses int directionality, so that LR recombines back to PB.

Previous switches used two inputs to control separate expression of int and int+RDF [10]. In this paper, we aim to design a robust single-input switch, which can be further used as a basic element of counters and memory devices. Our switch is based on a combination of two double-input switches (a TTS and a DNA inversion switch). The TTS, based on two mutually repressing inhibitors, controls whether int or int+RDF is synthesized (figure 1c). Expression of int or int+RDF in turn operates a DNA inversion switch, changing the orientation of an inducible promoter. Activation of the promoter by inducer (ind) provides a single-input signal, inducing expression of the currently inactive inhibitor and thus changing the state of FB state

Figure 1. Gene circuit of integrase-controllable inversion-and-transcriptional toggle switch (ITTS). (a,b) Basic elements of the switch. (a) Two states of the bistable transcriptional toggle switch (TTS), expressing I1 (left) or I2 (right). The TTS is regulated by mutual repression of expression of I1 and I2 inhibitors from P1 and P2. Two different input signals (inducer 1 and 2) initiate the transition between the two states, by de-repressing the respective promoters. (b) A DNA inversion switch that can switch between two DNA states (PB and LR), mediated by serine integrase int and its fusion protein with RDF (intRDF), which invert the DNA fragment located between P and B, or L and R attachment sites. (c) Scheme of the one-input ITTS, illustrating the two states of the switch, expressing I1 and intRDF in the PB state (blue box) and I2 and int in the LR state (red box). The switch between states is initiated by a pulse of an inducer, activating the inducible promoter Pind. This results in the expression of the currently unexpressed inhibitor, followed by the expression of int (or intRDF) and changing of the DNA state.
the switch. We use mathematical modelling to demonstrate that the inversion-and-transcriptional toggle switch (ITTS) is capable of robust switching between two DNA states over a broad range of parameters and is stable against molecular noise. We anticipate that the robustness of the switch should make it useful for further experimental implementations of single-input memory devices.

2. Model description

Here, we use mathematical modelling to develop a single-input DNA switch, the ITTS. Similar to previous work, our switch is designed to be implemented in Escherichia coli cells bearing plasmids with the switch gene circuit [10]. The ITTS integrates a TTS (figure 1a) and a DNA inversion switch operated by int and its RDF (figure 1b). It has been shown recently that LR-to-PB recombination is more efficient with an integrate-RDF fusion protein (intRDF). This fusion protein improves directionality compared to a mixture of int and RDF proteins, and expression of a single protein simplifies the switch design [14] (figure 1b). Our ITTS, therefore, uses intRDF to switch from LR to PB, and int to switch from PB to LR.

The TTS consists of two mutually repressing transcriptional initiators I1 and I2 expressed from P1 and P2 promoters (figure 1a). The int and intRDF genes are expressed from their own copies of the P2 and P1 promoters respectively, thus coupling the state of the inversion switch to the state of the TTS (figure 1c). When I1 is expressed and I2 is not, only intRDF will be expressed, putting the switch in the PB state (figure 1c, top). Similarly, when I2 is expressed, only int will be expressed and the switch will be in the LR state (figure 1c, bottom). Our switch design is not specific to any particular types of repressors I1 and I2. However, an essential requirement is that in order for the toggle switch to be bistable, the repressors have to bind their target promoters with cooperativity [5].

Switching between the two states of the ITTS is provided by periodic pulses of inducer ind, activating an inducible promoter Pind located between att sites of the DNA inversion switch (figure 1c). For example, the sugar arabinose could be used as ind to induce the arabinosin-inducible Pind promoter [15]. Experimentally, we envision testing the system using short 1–4 h pulses of inducer every 24 h. Therefore, we model ind mathematically using a suitable periodic function.

The orientation of Pind depends on the state of the inversion switch, which in turn is governed by the TTS (figure 1c). When I1 is on, induction of Pind will turn on expression of I2; when I2 is on Pind will express I1. Each pulse of inducer results in a cycle of events: (i) Pind-mediated transient expression of the currently repressed inhibitor; (ii) a change in the state of the TTS (switch from I1 to I2 or I2 to I1 expression); and (iii) a switch between int and intRDF expression, and thus a change in the orientation of the invertible DNA segment.

2.1. Model equations

The intracellular kinetics of int, intRDF, I1 and I2 protein production and decay is described by four ordinary differential equations (ODEs), corresponding to the scheme of figure 1c. Based on fast mRNA degradation [16,17], we assumed that mRNA levels are proportional to promoter activities. Therefore, the rates of protein expression are simply proportional to promoter activities. All proteins were assumed to be diluted due to cell growth and division. The equations for int, intRDF, I1 and I2 proteins are as follows:

\[
\frac{d[\text{int}]}{dt} = v_p \cdot \left[ D_{\text{ind}} \right] - k_{\text{dil}} \cdot [\text{int}],
\]

\[
\frac{d[\text{intRDF}]}{dt} = v_p \cdot \left[ D_{\text{ind}} \right] - k_{\text{dil}} \cdot [\text{intRDF}],
\]

\[
\frac{d[I_1]}{dt} = v_{\text{ind}} \cdot \left[ L_{\text{ind}} \right] + v_p \cdot \left[ D_{\text{att}} \right] - k_{\text{dil}} \cdot [I_1],
\]

\[
\frac{d[I_2]}{dt} = v_{\text{ind}} \cdot \left[ PB_{\text{att}} \right] + v_p \cdot \left[ D_{\text{att}} \right] - k_{\text{dil}} \cdot [I_2],
\]

and

\[
\frac{d[v_P]}{dt} = \frac{k_i}{1 + (I_2/K_i)^2} + k_{\text{dil}}; \quad \frac{d[v_{PB}]}{dt} = \frac{k_i}{1 + (I_1/K_i)^2} + k_{\text{dil}};
\]

\[
v_{P\text{ind}} = k_0 \cdot \text{ind}(t),
\]

where \([\text{int}]\) and \([\text{intRDF}]\) are the concentrations of int and intRDF fusion protein; \([I_1]\), \([I_2]\) are the concentrations of I1 and I2; and \([PB_{\text{att}}] \) and \([LR_{\text{att}}] \) are the concentrations of plasmid DNA in the PB and LR state, respectively, determined by the recombination reactions described below. \([D_{\text{ind}}]\) is the total concentration of plasmid DNA \([D_{\text{ind}}] = [PB_{\text{att}}] + [LR_{\text{att}}]\). \([v_P], [v_{PB}] \) and \([v_{P\text{ind}}] \) are the rates of protein expression from P1, P2 and Pind, respectively. Orthogonal inhibitors from the TetR family [18] represent likely candidates for I1 and I2 in future experimental implementation of the ITTS. Therefore, based on the reported dimeric structure of TetR complexes [19], we used a Hill coefficient of 2 for the formation of the complex between PB and LR inhibitors.

The recombination reactions implementing the conversion between the PB and LR states are described based on our minimal model of \(\text{in vitro}\) recombination by φC31 integrase with or without RDF (electronic supplementary material, figure S1) [20]. To describe \(\text{in vivo}\) recombination, we have included in the present model the dilution of int and intRDF proteins from their complexes with DNA upon DNA replication (equations (2.5) and (2.6)). Additionally, because we use intRDF fusion protein instead of a mixture of int with RDF, our model does not have the equation for the formation of the complex between int and RDF, which was used in [20].

The equations for recombination reactions were derived in [20] assuming that recombination steps (r1, r2) and synaptic conformational change steps (syn, synr) are much slower compared to other steps. The slow-changing variables \(LR_{\text{int}}\), \(PB_{\text{int}}\) and \(PB_{\text{att}}\) (sum of all PB-containing complexes) are described by three ODEs (electronic supplementary material, figure S1):

\[
\frac{d[LR_{\text{int}}]}{dt} = k_{r1} \cdot [PB_{\text{int}}] - k_{r1} \cdot [LR_{\text{int}}] + k_{\text{syn}} \cdot [LR_{\text{int}}] - k_{\text{dil}} \cdot [LR_{\text{int}}],
\]

\[
\frac{d[PB_{\text{int}}]}{dt} = k_{r2} \cdot [LR_{\text{int}}] - k_{r2} \cdot [PB_{\text{int}}] - k_{\text{syn}} \cdot [PB_{\text{int}}] + k_{\text{dil}} \cdot [PB_{\text{int}}],
\]

and

\[
\frac{d[PB_{\text{att}}]}{dt} = k_{r2} \cdot [LR_{\text{int}}] - k_{r2} \cdot [PB_{\text{int}}] - k_{\text{syn}} \cdot [PB_{\text{att}}] + k_{\text{dil}} \cdot [PB_{\text{att}}].
\]
The algebraic equations for fast-changing variables were derived using rapid equilibrium approximations [20]:

\[
\begin{align*}
[PB\text{int}] &= \frac{[\text{int}]^4 \cdot [PB]}{K_{\text{int}}}, \\
[LR\text{int}]_2 &= \frac{[\text{int}]^4 \cdot [LR]}{K_{\text{LR}2}}, \\
[LR\text{intRDF}] &= \frac{[\text{intRDF}]^4 \cdot [LR]}{K_{\text{LR}3}}, \\
[PB\text{intRDF}]_2 &= \frac{[\text{intRDF}]^4 \cdot [PB]}{K_{\text{LR}4}}, \\
[PB\text{intRDF}] &= \frac{([\text{int}]^2 \cdot [\text{intRDF}]^2) \cdot [PB]}{K_{\text{LR}5}}. \\
[LR\text{intRDF}] &= \frac{([\text{int}]^2 \cdot [\text{intRDF}]^2) \cdot [LR]}{K_{\text{LR}6}}.
\end{align*}
\]  

Free PB and LR concentrations were expressed from the mass balance equation for the PB- and LR-containing species [20]:

\[
\begin{align*}
[PB] &= \frac{[PB\text{tot}] - [PB\text{intRDF}]}{1 + \frac{[\text{int}]^4 \cdot [PB]}{K_{\text{LR}1}} + \frac{[\text{int}]^2 \cdot [\text{intRDF}]^2}{K_{\text{LR}2}}}, \\
[LR] &= \frac{[LR\text{tot}] - [LR\text{intRDF}]}{1 + \frac{[\text{int}]^4 \cdot [LR]}{K_{\text{LR}3}} + \frac{[\text{int}]^2 \cdot [\text{intRDF}]^2}{K_{\text{LR}4}}},
\end{align*}
\]

where \([PB\text{int}], [LR\text{intRDF}], [LR\text{intL}], [LR\text{int}], [PB\text{intRDF}], [PB\text{intRDF}], [PB\text{intRDF}])\) are the concentrations of the respective complexes and \([PB], [LR]\) are the concentrations of free PB and LR DNA (electronic supplementary material, figure S1). \([PB\text{tot}]\) and \([LR\text{tot}]\) are the sums of all LR- and PB-containing complexes \(([LR\text{tot}] + [PB\text{tot}] = [D\text{tot}])\), respectively. \(K_{\text{LR}1}, K_{\text{LR}2}, K_{\text{LR}3}, K_{\text{LR}4}, K_{\text{LR}5}\) are the dissociation constants for the respective complexes \((K_{\text{LR}1}, K_{\text{LR}2}, K_{\text{LR}3}, K_{\text{LR}4}\text{ are assumed to be equal to } K_{\text{LR}})\). The parameters \(k_{s+}, k_{s+y}, k_{s+\text{syn}}, k_{s+1}, k_{s+2}, k_{s+y}, k_{s-\text{syn}}\) stand for the forward and reverse rate constants of the slow recombination and synapsis \((s+, s+y, s-\text{syn})\) steps [20] (assuming \(k_{s+1} = k_{s+2} = k_{s+}\)), with the forward direction defined as \(PB \rightarrow LR\) for the \(\text{int}\) reaction and as \(LR \rightarrow PB\) for the \(\text{intRDF}\) reaction (electronic supplementary material, figure S1) [20].

All concentrations are expressed in \(\mu\text{M};\) the time units are hours.

### 2.2. Behaviour of the model components

\(I_1\) and \(\text{intRDF}\) proteins are expressed from copies of \(P_{I_1}\), while \(I_2\) and \(\text{int}\) are expressed from \(P_{I_2}\) promoters (figure 1c, equations (2.1)–(2.4)). The activities of \(P_{I_1}\) and \(P_{I_2}\) (\(v_{P_{I_1}}\) and \(v_{P_{I_2}}\)) are sums of two terms: the main activity, which is inhibited by \(I_2\) and \(I_1\), respectively, and the promoter leakages (background activities in the presence of saturated concentrations of inhibitors). The expression of \(I_1\) and \(I_2\) is also transiently induced from \(P_{\text{ind}}\) during pulses of the external signal \(\text{ind}(t)\). Expression of \(I_1\) and \(I_2\) is described as a sum of the expression from \(P_{\text{ind}}\) and from \(P_{I_1}\) or \(P_{I_2}\) (equations (2.3) and (2.4)). This assumption is based on observations of additive gene expression from tandem promoters [21,22]. We assume that transcription initiated by \(P_{\text{ind}}\) can read through the repressor-bound \(P_{I_1}\) and \(P_{I_2}\) [21].

The recombination mechanisms are described in detail in [20]. Briefly, PB-to-LR recombination starts from binding of four molecules of \(\text{int}\) to the PB substrate (binding step \(b_1\); electronic supplementary material, figure S1), followed by recombination (strand exchange, step \(r_1\)) leading to formation of the product synaptic complex \(\text{LRint}_1\). The \(\text{LRint}_1\) complex can also slowly de-synapase to form \(\text{LRint}_2\) complex (step \(\text{syn}\)), which can dissociate and release free LR product (step \(b_2\)). The last two steps are unfavourable (electronic supplementary material, figure S1) and \(\text{LRint}_1\) represents the main form of the LR product \(\text{in vitro}\) [20]. However, \(\text{intRDF}\) is described through a dilution of \(\text{int}\) from \(\text{LRint}_1\) (equation (2.5)), which decreases \(\text{LRint}_1\) concentration and thus increases free LR product (equation (2.15)). This increases the recombination efficiency of \(\text{in vivo}\) reactions (§3.1). Similarly, LR-to-PB recombination starts from binding of four molecules of \(\text{intRDF}\) to the LR substrate (step \(b_1\)), followed by recombination (step \(r_2\)) and the formation of the product synaptic complex \(\text{PBintRDF}_1\). The unfavourable steps include de-synapsis of \(\text{PBintRDF}_1\), producing \(\text{PBintRDF}_2\) (step \(\text{syn}\)) and release of the free PB product (step \(b_2\)). Dilution of \(\text{intRDF}\) from \(\text{PBintRDF}_1\) (equation (2.6)) decreases \(\text{PBintRDF}_1\) concentration and thus increases free PB product (equation (2.14)). The model also includes unproductive complexes \(\text{LRintRDF}_1\) and \(\text{PBintRDF}_1\) (equations (2.12), (2.13)), which form due to competition between \(\text{int}\) and \(\text{intRDF}\) dimers [20].
of the inducer

\[
\text{ind}(t) = 0.5 \cdot \tanh \left( \frac{t - \text{per} \cdot \text{floor}(t/\text{per}) - \text{ind}_{\text{on}}}{k_{i1}} \right) - \tanh \left( \frac{t - \text{per} \cdot \text{floor}(t/\text{per}) - \text{ind}_{\text{off}}}{k_{i1}} \right),
\]

(2.16)

where \(\text{ind}_{\text{on}}\) and \(\text{ind}_{\text{off}}\) determine the times of the beginning and end of each pulse of inducer, administrated with a period \(\text{per}\) (\(\text{per}\) is chosen to be 24 h for the convenience of the future experimental design); \(k_i\) is a characteristic time of the inducer’s decay (\(k_i = 0.3\) h based on a 20 min cell doubling time).

The equilibrium constants of recombination reactions satisfy the energy conservation equations for PB-to-LR and LR-to-PB transitions (electronic supplementary material, figure S1) [20]:

\[
\frac{K_{i1} \cdot K_{\text{syn}} \cdot K_{\text{int}}}{K_{\text{dis1}}} = 1 \quad \text{and} \quad \frac{K_{i2} \cdot K_{\text{syn}} \cdot K_{\text{int}}}{K_{\text{dis2}}} = 1,
\]

(2.17)

where \(K_{i1}, K_{i2}, K_{\text{syn}}, K_{\text{int}}\) are the equilibrium constants (\(k_+/k_-\)) of the respective steps and \(K_{\text{dis1}}, K_{\text{dis2}}, K_{\text{int}}, K_{\text{int}}\) are the dissociation constants (\(k_/-k_+\), where \(k_+\) and \(k_-\) are rate constants of binding and dissociation of integrase or \(\text{intRDF}\) from DNA). The modelling of \(\text{int}\) with reduced efficiency (§3.2) was done by decreasing the equilibrium constants of the recombination steps \(K_{i1}, K_{i2}\) 10-fold, with compensating 10-fold increases of the dissociation constants \(K_{\text{dis1}}, K_{\text{dis2}}\) of \(\text{int}\) binding to DNA products, to comply with energy conservation (equation (2.17)). The model parameters are presented in electronic supplementary material, table S1.

3. Results and discussion

During the construction of the ITTS, we initially considered a simpler scheme with \(\text{int}\) and \(\text{intRDF}\) expressed from a constitutive promoter in an invertible DNA segment (electronic supplementary material, figure S2). The switch was expected to be bistable due to the expression of \(\text{intRDF}\) in the PB state, converting any LR product back to PB and expression of \(\text{int}\) in the LR state, maintaining the DNA in the LR state. This switch would operate by induction of expression of \(\text{int}\) or \(\text{intRDF}\) from an oppositely oriented inducible promoter within the invertible DNA segment. However, we found that the switch could not alternate between the two states in response to inducer pulses.

Instead, over a broad parameter range, the switch always ends up in the LR state, due to the higher efficiency of PB-to-LR conversion. The inability to switch state was caused by rapid initiation of recombination during the inducer pulse, leading to overlapping production of \(\text{int}\) and \(\text{intRDF}\) proteins. In order for the switch to make reliable transitions on inducer pulse, expression of \(\text{int}\) and \(\text{intRDF}\) from the inducible promoter must be temporally distinct from integrase-mediated inversion. This is difficult to achieve due to the rapid nature of transcriptional induction and site-specific recombination. The simultaneous expression of \(\text{int}\) and \(\text{intRDF}\) is avoided in our final design (figure 1c) due to the tight control of \(\text{int}\) and \(\text{intRDF}\) expression by the TTS, as described below.

### 3.1. The kinetics of the inversion-and-transcriptional toggle switch

The model of our single-input switch ITTS is described in §2 (figure 1c). The switch has two steady states (§3.2) and is capable of robust switching between the two states, as we show below. The single-input signal to the ITTS is provided by pulses of an external inducer, described by periodic step function \(\text{ind}(t)\) (equation (2.16)). Surprisingly, the model predicts that the switch of the DNA state is completed only after the inducer pulse finishes, due to the interactions between the ITTS components. Thus, if the switch was initially in the PB state, expressing \(I_1\) and \(\text{intRDF}\) (figure 1c top; figure 2a), then the addition of inducer causes an increase of \(I_2\), which downregulates \(I_1\) and \(\text{intRDF}\) expression from the \(I_2\)-inhibited \(P_1\) promoters. Decreased expression results in decreased protein levels, due to protein dilution during cell growth and division. The initial decrease in \(I_1\) initiates a minor increase of \(\text{int}\) (figure 2a). The decrease of the \(\text{intRDF}/\text{int}\) ratio causes slight increase of LR (at approx. 2 h on figure 2a, when \(\text{int} \sim \text{intRDF}\)). But in the presence of inducer this leads to a secondary wave of \(I_1\) expression from the \(P_{\text{ind}}\) promoter in the LR state. This prevents further increase of the \(\text{int}\) concentration and thus PB-to-LR conversion (figure 2a). Under induction with relatively strong \(P_{\text{ind}}\) (figure 2c), concentrations of both inhibitors are high enough during the pulse to prevent production of \(\text{int}\) and \(\text{intRDF}\). Therefore, the PB-to-LR transition is completed only after the inducer pulse finishes (figure 2a). \(I_1\) and \(I_2\) both decrease after the pulse, but the TTS falls into the \(I_2\) steady state because \(I_2 \gg I_1\) (figure 2a). The concentration of \(\text{int}\) is initially low after the pulse; it starts to increase only when \(I_1\) falls below the critical level required for the release of the repressed \(P_2\) promoter (half-released at 0.01 \(\mu\)M [23]). The inversion switch follows the TTS after the minimal \(\text{int}\) concentration required for recombination (0.1 \(\mu\)M) is achieved (approx. 5 h on figure 2a). When the ITTS is in the LR state, a pulse of inducer produces a switch to PB by a similar mechanism due to the symmetry of the ITTS design (figure 1c, figure 2b–d).

\(\text{int}\) recombination efficiencies observed experimentally in vivo [14] are typically higher than those observed in vitro [25]. Our previous models for \(\text{int}\) recombination [20,25] fit the \(\text{in vitro}\) data, predicting 80% and 70% recombination of PB-to-LR and LR-to-PB, respectively. To mimic the \(\text{in vivo}\) situation, the model was modified to include stripping of \(\text{int}\) and \(\text{intRDF}\) from DNA during DNA replication, accelerating the release of free DNA from reaction products (§2; electronic supplementary material, figure S1). The modified model predicts highly efficient intracellular conversion of PB-to-LR and LR-to-PB (100% and 97%, respectively) (figure 2c), in agreement with the \(\text{in vivo}\) data.

### 3.2. The robustness of the inversion-and-transcriptional toggle switch to parameter variations

Two characteristics are important for the ITTS operation: (i) coexistence of two steady states in the absence of inducer (bistability) and (ii) ability to switch between the two states in response to the inducer pulse. The bistability of the ITTS is determined by the TTS parameters, while the ability to switch depends on the parameters of \(P_{\text{ind}}\) induction (pulse duration and \(P_{\text{ind}}\) strength) and parameters of the inversion switch, as discussed below.
The bistability of the ITTS is based on the bistability of its TTS. Figure 3 shows the ITTS dynamics in the absence of inducer on a phase diagram, showing trajectories in the $I_1$/$I_2$ phase plane. Different initial concentrations of $I_1$ and $I_2$ produce different trajectories, and all the trajectories end up in one of the two stable steady states with high $I_1$ (blue) or high $I_2$ (orange) concentrations. We used the model to explore the dependence of the bistability range on the

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**Figure 2.** Intracellular kinetics of the ITTS. (a) The concentrations of $I_1$ (light blue), $I_2$ (orange), int (green), intRDF (black) and the relative (normalized to total) concentrations of LR (red) and PB (blue) DNA during the first hours of the PB-to-LR transition. (b–d) The long-term kinetics of the ITTS, with 3-h pulses of inducer repeated every 24 h. (b) The concentrations of $I_1$ (blue) and $I_2$ (orange). (c) The relative concentrations of LR (red) and PB (blue). (d) The concentrations of int (green) and intRDF (black). The inducer kinetics (in relative units) is shown on all panels by magenta dotted lines. The half-time of inducer decay is $k_t = 0.3$ h on (b–d) and $k_t = 0.1$ h on (a), for sharper transition (for clarity of the figure). All calculations were done for the equal strengths of $P_{ind,1}$ and $P_{ind,2}$.

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**Figure 3.** Bistability range of the ITTS. (a) Phase diagram of $I_1$ and $I_2$ trajectories, starting from different $I_1$ and $I_2$ concentrations, with arrows showing the direction of the time. All trajectories end up in one of two steady states with high $I_1$ or high $I_2$ (indicated by blue and orange dots, respectively). Black and red trajectories show the transitions between steady states after the addition of inducer (1 h pulse). (b) Dependence of the steady-state LR levels (normalized to total DNA) on the fold difference in the strength of $P_2$ relative to $P_1$. Two steady states with high and low LR levels are shown by red and black lines, respectively. The lower and upper margins of the bistable region are marked by the symbols $f_1$ and $f_2$, respectively. (c) Dependence of the bistability range (values of $f_1$ and $f_2$) on the values of $P_1$ and $P_2$ leakages. Graphs in (a,b) were calculated with leakages in $P_1$ and $P_2$ equal to 1% of maximal activity. All calculations, except black and red dashed lines in (a), were done in the absence of inducer.

The bistability of the ITTS is based on the bistability of its TTS. Figure 3a shows the ITTS dynamics in the absence of inducer on a phase diagram, showing trajectories in the $I_1$/$I_2$ phase plane. Different initial concentrations of $I_1$ and $I_2$ produce different trajectories, and all the trajectories end up in one of the two stable steady states with high $I_1$ (blue) or high $I_2$ (orange) concentrations. We used the model to explore the dependence of the bistability range on the
strengths of $P_1$ and $P_2$ promoters. The simulations were run in the absence of inducer, starting from different initial concentrations of $I_1$ and $I_2$ (as on figure 3a). Both maximal activities and leakages (background expression from fully repressed promoter) affect the bistability range. When leakages in $P_1$ and $P_2$ promoters are relatively high (1% of the activities of unrepressed promoters), bistability is observed only for relatively similar promoter strengths (up to 2.5-fold difference in $P_1$ and $P_2$ strengths; figure 3b). The promoters of the TetR family have relatively high leakages and similar strengths [18], and so could be appropriate. Additionally, the ITTS is predicted to maintain its bistability when the promoters have substantially different strengths, providing that leakages are low. Thus, a 10-fold decrease in $P_1$ and $P_2$ leakages extends the bistability range up to 10-fold difference in $P_1$ and $P_2$ strengths (figure 3c). We conclude that the ITTS is bistable over a broad parameter range of promoter strengths and leakages.

In addition to being bistable, the ITTS is able to switch between the two states in response to the addition of inducer, as shown in figure 3a by black and red dashed lines. Figure 4 shows that the ITTS is capable of operating over a broad range of inducer pulse lengths and strengths of $P_{\text{ind}}$. Thus, for a relatively high strength of the $P_{\text{ind}}$ promoter ($P_{\text{ind}}$ strength greater than 20% of $P_1$ strength, with equal strengths of $P_1$ and $P_2$), the ITTS operates in both directions with any duration of inducer pulse longer than 4 min (figure 4a) and the DNA transitions happen only after the inducer pulse finishes, as described in §3.1. Therefore, a switch with strong $P_{\text{ind}}$ promoter is not sensitive to pulse duration. However, reduction of the $P_{\text{ind}}$ strength narrows the range of useful inducer pulses. Thus, for a $P_{\text{ind}}$ with 10% of the strength of $P_1$ and $P_2$, the inducer pulse duration required for the efficient switching is between 0.5 and 9 h (figure 4b). For a $P_{\text{ind}}$ with 2% of the $P_1$ strength, the range of effective pulses narrows to 3–5 h (figure 4c).

The narrower range of permitted pulse lengths with a weak $P_{\text{ind}}$ is due to low and comparable concentrations of the induced inhibitors during the pulse (figure 5a,b). Thus, if the ITTS was initially in the $PB$ state, $I_2$ is induced by $\text{ind}$ (figure 5a), but to much lower levels than with the strong $P_{\text{ind}}$ (figure 5b). $I_1$ slowly decreases, increasing the $\text{int}$ to $\text{intRDF}$ ratio and initiating the $PB$-$\text{to-LR}$ transition (figure 5a). $I_1$ is expressed from $P_{\text{ind}}$ in the LR state, but only to low levels compared to the strong $P_{\text{ind}}$ (figure 5a,b), allowing near-complete transition to the LR state during a long pulse (figure 5a,c). The conversion to LR causes $I_1$ concentration to increase again (figure 5c,d). For long enough pulses, $I_1$ eventually becomes higher than $I_2$ (figure 5d), reverting the transition back to the $PB$ state (figure 5f). For shorter pulses, $I_1$ remains lower than $I_2$ throughout the pulse (figure 5a), allowing the TTS to complete the transition to LR after the pulse (figure 5c).

Next, we explored the effect of the parameters of DNA inversion on the ITTS operation. Figure 6a shows the operation of the ITTS with low-efficiency $\text{int}$ and $\text{intRDF}$, simulated by 10-fold decreases in the equilibrium constants of the recombination steps ($K_{\text{sc}}$ and $K_{\text{sr}}$). The efficiency of conversion from LR to PB with these altered parameters is reduced to 79% (compared with 97% with the high-efficiency $\text{int}$ and $\text{intRDF}$), while the PB-$\text{to-LR}$ conversion is reduced from 100 to 97% (figure 6a). However, switching between the two states is still robust over a broad range of pulse durations (figure 6a).

In addition to the variations in the efficiency of $\text{int}$-mediated recombination, the inversion switch might be affected by the expression rates of $\text{int}$ and $\text{intRDF}$. However, our analysis demonstrates that the ITTS operates over a broad range (approx. 100-fold variation) of $\text{int}$ and $\text{intRDF}$ production rates (figure 6b). Very low rates of $\text{int}$ and $\text{intRDF}$ expression were insufficient to promote transition between the $PB$ and LR states. Excessive levels of $\text{int}$ and $\text{intRDF}$ expression led to more than 50% transition during the pulse (electronic supplementary material, figure S3). This reduced the working range of pulse durations by the same mechanism as for low $P_{\text{ind}}$ (figure 5), due to competition between the two inhibitors expressed from $P_{\text{ind}}$ in the $PB$ and LR states.

We conclude that the ITTS is very stable against variation in the parameters of the recombination reactions, in contrast to a previously developed inversion switch [10].
to the coupling of the inversion switch to the bistable TTS in our ITTS design, ensuring that only one of int and intRDF proteins is expressed (figure 2d). In addition, the inversion switch is stabilized by the use of the intRDF fusion protein, increasing the efficiency of the LR-to-PB transition compared to a mixture of integrase and RDF [10].
The ITTS is designed to be implemented in *Escherichia coli* cells. In each cell, the circuit is predicted to switch efficiently between the two states in response to each inducer pulse. However, due to potential differences in initial conditions when the circuit is first introduced into cells, the switch might start in the PB state in some cells and the LR state in others. Therefore, in future experimental implementations of the ITTS, the cells might need to be synchronized initially by adding an inducer to activate either P1 or P2 [26] (figure 1a).

The switch can be used to express different genes, depending on the desired applications. For example, expression of two different fluorescent reporters (e.g. GFP and RFP) in the two switch states would allow monitoring of the switch kinetics. Alternatively, the switch could be used to control expression of further integrases to build more complex circuits, for instance, a ripple counter as discussed in the Introduction and Conclusion.

### 3.3. Effects of molecular noise

Our simulations demonstrate that ITTS behaviour is very robust to variations in the *P*\textsubscript{ind} strength (figure 4) and recombination efficiency (figure 6), while changes in P1 and P2 cause more drastic changes in the working range of the ITTS (figure 3). In particular, the leakages in P1 and P2 (i.e. expression from fully repressed promoters) strongly affect the bistability range of the ITTS (figure 3c). The levels of these leakages in P1 and P2 are expected to be noisy due to the low probability of RNA polymerase binding to P1 or P2 in the presence of high repressor concentrations. To simulate the potential effects of the noise on the ITTS kinetics, we replaced the leakages in P1 and P2 (parameter *k*\textsubscript{to} in equations (2.1)–(2.4)) with the Poisson-distributed variables with a mean of 3.6 h\textsuperscript{-1} (equal to the leakages in the deterministic system) or 7.2 h\textsuperscript{-1} (in simulations with twofold increased noise). The noise was applied every minute. This results in noisy expression of *I*\textsubscript{LR}, *I*\textsubscript{PB}, *int* and *intRDF* proteins from P1 and P2. Our simulations demonstrate that even with relatively noisy leakages (with a mean of 3.6 h\textsuperscript{-1}, figure 7a) the switch between the PB and LR states is robust to the noise (figure 7b). However, a further increase of the noise destabilizes the switching (figure 7c), leading to unpredictable switching when the noise is twofold higher than leakages in the deterministic system (figure 7d).

### 4. Conclusion

We present here a mathematical model of a single-input binary switch (ITTS), formed by combining a TTS and an inversion switch based on serine integrase-mediated site-specific recombination. The model predicts that the combined bistability of the TTS and unidirectionality of integrase-mediated recombination ensures nearly 100% efficiency of switching between two DNA states using repeated pulses of a single inducer. The ITTS is predicted to be robust to parameter perturbations and molecular noise. We envision that several ITTS modules built with orthogonal recombinases and repressors could be connected together sequentially to form a binary ‘ripple counter’. Each module represents a single binary digit and would signal the next module with...
a pulse of integrase expression every time it makes the transition from LR to PB. This would generate a counter, which would count sequentially through all binary numbers, to keep track of potentially large numbers of inter- or extracellular events [2].

Data accessibility. There are no data associated with this paper.

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