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Computational design of synchronous sequential structures in biological systems

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ABSTRACT

Numerous applications of synthetic biology require the implementation of scalable and robust biological circuits with information processing capabilities. Basic logic structures, such as logic gates, have already been implemented in prokaryotic as well as in eukaryotic cells. Biological memory structures have also been implemented either *in vitro* or *in vivo*. However, these implementations are still in their infancy compared to their electronic equivalents. Their response is mainly asynchronous. We may learn from electronic computer systems that robust and scalable computing devices can be implemented only with edge-triggered synchronous sequential structures. Implementation of such structures, however, has yet to be performed in the synthetic biological systems even on the conceptual level.

Herein we describe the computational design and analysis of *edge-triggered D flip-flop* in *master–slave* configuration based on transcriptional logic. We assess the robustness of the proposed structure with its global sensitivity as well as parameter sweep analysis. Furthermore, we describe the design of a robust *Johnson counter*, which can count up to $2n$ cellular events using a sequence of n flip-flops. Changing the state of the counter is edge-triggered either with synchronization, i.e. clock signal, or with a pulse, which corresponds to the occurrence of observed event within the cellular environment. To the best of our knowledge this represents the design of the first biological synchronous sequential structure on such level of complexity.

1. Introduction

First successful implementations of logic structures in *Escherichia coli* cells, such as logic gates [1] and oscillators [2], mark the beginning of the field of synthetic biology [3]. Further development of these structures received large attention in the last 15 years [4,5]. More complex processing systems, such as a simple biological computer, are together with one of their vital parts, i.e. scalable, robust and reliable memory structures, however, yet to be implemented. Memory structures are also of significant importance for the wider scope of synthetic biology applications. For example, memory could be used in order to identify cell populations responsive to specific events and track their progression through the cellular response [6,7]. Moreover, logic structures can be used in a combination with biological memory to select and maintain one of the possible states of the system with fundamentally different biological functions, e.g., to implement an effective multi-state treatment from inhibition of inflammatory processes (state 1) to tissue regeneration (state 2).

Attention towards the implementation of robust and scalable biological memory has therefore been increasing in recent years. In this context we can divide the current implementations of biological memory structures in two groups, i.e. *long-term memory circuits* with high density, which are based on DNA recombination, on one hand [8–11], and *transcriptional memory devices* with short-term storage capabilities, which are based on bistable genetic response, on the other hand.

Several *in-vitro* implementations of long-term memory circuits have been reported in the last decade [10,11]. Few realizations of *switchable and reversible long-term memory circuits* have also been reported recently [8,12,13]. These are able to interface the logic functions with the long-term memorisation of their outputs [9]. The complexity of such circuits together with relatively long access times, i.e. read and especially write times, make them however unsuitable for current information processing applications.

Transcriptional memory devices are on the other hand less complex and have significantly shorter access time in comparison to DNA recombination based circuits. Their implementations are based on a bistable transcriptional response of gene

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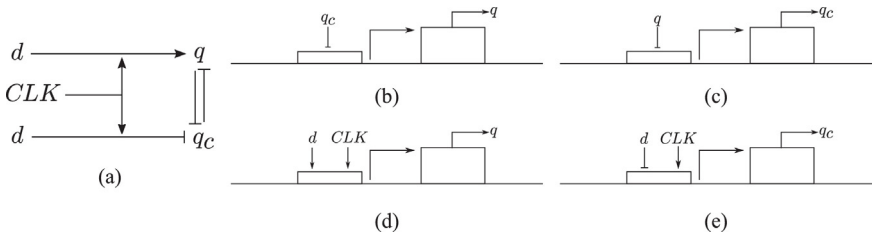

Fig. 1. Design of the basic biological clocked D flip-flop. (a) represents its logic scheme and (b–e) genes in its transcriptional implementation. In all figures *d* represents the data protein and *CLK* the clock protein, while *q* and q_c represent the complementary output proteins. Sharp end arrows indicate transcriptional activation, whereas blunt end arrows indicate transcriptional repression. In (b–e) small boxes indicate transcription factor binding sites, bent arrows indicate promoters and large boxes indicate protein coding regions.

regulatory interactions [6], which can be implemented in several ways, e.g. using single self-activating transcriptional factor (TF) [14], or either double-positive or double-negative feedback loop between two interacting TFs. An example of natural system which reflects bistable behaviour, and involves both positive autoregulation and double-negative feedback loop, is *phage lambda switch system* [15]. The first synthetic implementation of short-term storage, i.e. *toggle switch*, was performed in a similar manner using double-negative feedback loop [1]. This was later extended to a so called *push-on push-off switch* [16]. Short-term memory using autoregulatory transcriptional positive feedback was implemented in yeast cells [17]. Maintenance of active state of a signal pathway was achieved with the integration of autoregulatory positive feedback in the MAP kinase system [18]. Recently, yeasts cells have been used to implement multicellular short-term memory [19]. Short-term memory structures have also been implemented in mammalian [20] and even in human cells [7].

In spite of significant progress in the field of synthetic biological memory, implementation of *synchronous sequential structures* in biological systems is yet to be performed. These are, however, essential for the implementation of more complex biological information processing structures, since they provide synchronisation between the logic elements, which results in a robust behaviour of the system. Herein we present the computational design and analysis of biological *master–slave D flip-flop*, which is *edge-triggered* by a *synchronisation (clock) signal*. We apply the proposed structure to the design of a *Johnson counter*, which reflects robust behaviour, and can count up to 2*n* events using a sequence of *n* flip-flops. Changing the state of the counter can be triggered either with synchronization, i.e. clock signal, or with a pulse which corresponds to the occurrence of the observed event. Correctness of the proposed counter's performance is not affected by the pulse length as is the case of the *state-of-the-art* biological counters [21]. All the code used in this paper is available at http://lrss.fri.uni-lj.si/bio/material/counter.zip under the Creative Commons Attribution license.

2. Computational design of biological D flip-flop

Edge-triggered memory structures are of a significant importance for the implementation of complex processing structures, but are yet to be implemented in biological systems. In this section we describe the computational design of the robust biological D flip-flop in a master–slave configuration.

2.1. Clocked D flip-flop

We propose the design of the biological clocked D flip-flop, which is based on a double-negative feedback loop as is also the case in its electronic equivalent [22]. Negative feedback loop is as in the toggle switch regulatory circuit [1] implemented with two complementary proteins (*q* and q_c) that serve also as outputs of the flip-flop. Their expression is additionally controlled by two input proteins, i.e. *data protein* (*d*) and *clock protein* (*CLK*), for which we presume an oscillatory behaviour. The design of proposed clocked D flip-flop is presented in Fig. 1. The first two genes expressing proteins *q* and q_c (see Fig. 1(b) and (c)) are active, when the complementary proteins (*q* for q_c and vice versa) are absent. The second two genes expressing proteins *q* and q_c (see Fig. 1(d) and (e)) are active only when the clock protein *CLK* is present. If the data protein *d* is active at the same time, *q* is expressed, otherwise q_c is expressed. Proteins *q* and q_c provide the state maintenance with the double-negative feedback loop while *d* and *CLK* enable the switching of the state. Expression of output proteins *q* and q_c is described in Table 1.

Table 1
Expression of output proteins *q* and q_c. (a) The expression of the protein *q* and (b) the expression of the protein q_c. The value 0 represents absence, i.e. low concentration, and 1 respectively presence, i.e. high concentration, of specific protein. Symbol x is used as a *don't care* value.

(a)

CLK	*d*	q_c	*q*
0	x	0	1
0	x	1	0
1	0	x	0
1	1	x	1

(b)

CLK	*d*	*q*	q_c
0	x	0	1
0	x	1	0
1	0	x	1
1	1	x	0

2.2. Master–slave D flip-flop

The clocked flip-flop as described in the previous section assumes that the switching of the state is triggered with a high level of clock signal. The duration of the high level clock pulses therefore needs to be precisely tuned with the dynamic response of each gene. The pulse needs to be long enough to perform the switch, but at the same time short enough to prevent more than one change of the flip-flop state [21]. The implementation thus requires a stable and robust clock signal, which is usually not the case in biological systems. The problem can be avoided using edge-triggered structures in which the state is never changed more than once in the same clock signal period, i.e. with a single pulse. Edge-triggered flip-flop can be implemented with a master–slave configuration using two clocked flip-flops as presented in Fig. 2. Proposed biological D flip-flop again uses two input proteins, i.e. *CLK* and *d*, and an additional pair of complementary proteins, i.e. *a* and a_c. We assume that each protein in the scheme is expressed by two independent genes as in the clocked flip-flop described in Section 2.1. The enhanced flip-flop also includes two additional negative feedback loops that