

# Engineering of Gene Circuits for Cellular Computation

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**ABSTRACT:** Living cells of organisms operate as highly complex biological computational systems which read and rewrite deoxyribonucleic acid (DNA) all the time and perform all the processing for the organism's activities. Computer scientists along with molecular biologist and biochemists are exploring the potential for computation of DNA molecules. Biological computation is needed to realize engineered biological systems that have sophisticated sense-and-respond behaviors. Synthetic biologists have applied engineering concepts to biological systems in order to construct progressively more complex gene circuits capable of processing information in living cells. Novel genetic circuits have been constructed through rational design and forward engineering for their useful applications. Such engineered living cells or microbes have the potential to perform a wide range of desirable tasks that require sophisticated computation and control. In this article, the current states of artificial gene circuits that perform digital and analog computation have been described.

**KEYWORDS:** Digital gene circuits, Analog gene circuits, DNA logic gates, cellular computation, Synthetic biology

## 1. INTRODUCTION

Information processing in living cells involves integrating multiple inputs, performing computations on these signals, storing information in memory and actuating outputs. These highly complex biological nanocomputers in living cells have the potential to dynamically interrogate and respond to the environment [1,2]. Synthetic biology involves the construction and manipulation of the biological systems from the molecules such as DNA and RNA to the functional cellular level [3,4]. The modeling component of synthetic biology allows the designing of biological circuits and the analysis of its expected behaviour. The experimental component merges models with real systems by providing quantitative data and sets of available biological units that can be used to construct circuits [5,6]. Sufficient progress has been made in the combined use of modeling and experimental methods, which reinforces the idea of using biological databases from different available genomes and engineered the microbes as a technological platform [7]. Integrated and extensible biological design cycles enable engineers/researchers to develop high-level conceptual designs, translate these designs into potential circuit implementations using libraries of well-characterized model/devices, construct the designs in an automated fashion and modulate the resulting constructs for proper operation [8].

Significant work has been carried out on the designing of synthetic digital logic gates in living cells, where biological signals such as chemical concentrations are artificially thresholded to represent '0' or '1' ('OFF' or 'ON', respectively) states. However, biological molecules do not generally exist at only two possible concentrations, but usually vary over a wide range of concentrations. Furthermore, achieving more complex computations with digital logic requires assembling together many simple digital logic units. Thus, it is challenging to construct large numbers of highly orthogonal parts in biological systems. Analog computation is an alternative to digital computation that utilizes inputs and outputs using the range of continuous values between these extremes and calculates mathematical functions on these signals using the laws that govern biochemical phenomena [3,9]. Thus, analog computation enables complex computations to be performed, without needing a large number of biological parts or resources, by taking advantage of the underlying physics of the components.

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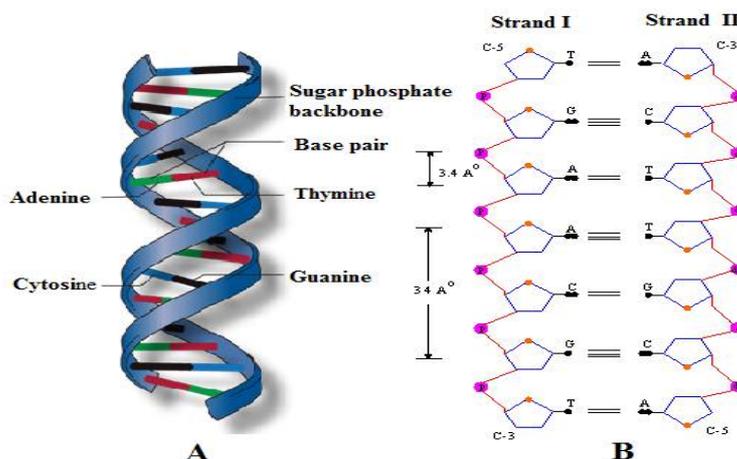
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Synthetic biology has often adopted an electrical engineering framework for the design of novel gene networks in which basic functional units are termed parts [10] and can be connected to build circuits and input-output responses described as having analogue or digital characteristics [11]. In these engineered genetic logic circuits, signal processing is often implemented using Boolean logic functions [12]. But, the predictions of how gene circuits will behave often fail when they are implemented in living cells because of the many undefined, complex and dynamic interactions that can occur between the circuit and its synthetic biological systems [13]. Functional circuits can still be produced, using iterative cycles of testing and refinement to correct failures [14], but the inability to accurately model circuit behaviour remains a major barrier to the construction of large gene circuits.

## II. THE STRUCTURE OF DNA

DNA was recognized as the most important biological molecule of cellular organisms, wherein it serves to store genetic information. In living organisms, its proper replication and transmission to next progeny is crucial for maintaining the genotype characteristics [15]. The ability to store billions of data as nucleotide base sequences is an important feature of the DNA. DNA (a polymer of nucleotides) consists of adenine, guanine, cytosine and thymine bases, commonly abbreviated to A, G, C and T, respectively. The DNA double helix is stabilized primarily by hydrogen bonds between nucleotides and base-stacking interactions among nucleotide bases (Fig. 1). Each purine base is the Watson-Crick (WC) complement of a unique pyrimidine base (and vice versa) i.e., adenine and thymine form a complementary pair, as do guanine and cytosine. The nucleotides C and G pair through three hydrogen bonds, while A and T pair through two hydrogen bonds. The arrangement of nucleotides varies from one organism to the other and different bacterial species have different arrangement of nucleotides. The discrete segments of DNA coding for particular protein/polypeptide are called genes.

The hereditary information present in the nucleotide sequence is maintained intact by complex metabolism involving both replication and repair functions. The different nucleotides i.e., ATP, GTP, CTP and TTP are polymerized by the enzyme DNA polymerase using a template of the parent strand. Occasionally, mistakes may occur and it may result in alteration of a particular nucleotide in DNA. The change of even single base pair in particular gene of the DNA structure could lead to mutation and it may result in morphological and functional changes. These changes in DNA composition could result in evolution of new species. To encode information using DNA, one can choose an encoding scheme mapping the original alphabet onto strings over (A, C, G, T), and proceed to synthesize the obtained information-encoding strings as DNA single strands. Knowledge of DNA sequences has become indispensable for basic biological research, and in numerous applied fields such as diagnostic, biotechnology, forensic biology, virology and biological systematics.



**Figure 1.** Double helical structure of DNA (A). Two strands of DNA bond together through hydrogen bonding by the pairwise attraction of bases; A bonds with T (with two hydrogen bonds) and G bonds with C (with three hydrogen bonds) (B). Each strand has, according to chemical convention, a 5' and a 3' end polarity.

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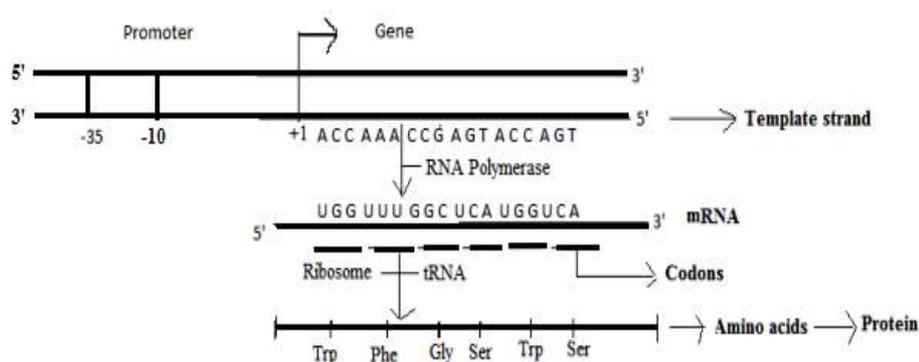
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## III. CONTROL OF GENE EXPRESSION

The biological information encoded in genes is made available by gene expression [16]. The information is transferred from DNA to mRNA by the process of transcription and this information is further translated into the protein by making use of the ribosomes. The technological advances in DNA synthesis and high-fidelity assembly of DNA fragments led to the developments and improvements of molecular biology and genetic engineering tools [17]. For metabolic engineering applications and the investigation of all biological functions, a finer control over the expression of genes in a given pathway could be designed and implemented [18]. Designed expression control for individual genes typically occurs either at the transcription or translation levels.

**Transcription:** The first level of functional control for specific genes occurs during transcription. The transcriptional process involves a binding of RNA polymerase on a DNA sequence (promoter) to initiate biosynthesis of mRNA (Fig. 2). The analysis of naturally occurring promoter sequences showed the occurrence of conserved sequence motifs that physically bind to the sigma subunit of the RNA polymerase. Sequence variation in the promoter was found to affect transcriptional strength. In the early stages of the development of synthetic biology, transcriptional DNA parts were created and catalogued allowing the use of genetic variants for transcriptional control [19]. By dividing promoter sequences into 6 motifs (-35, spacer, -10, disc start, initial transcribed region), 60 different promoter sequences were characterized for promoter strength and used to evaluate the influence of specific genetic changes on function. Recently, synthetic promoters have been designed to produce a desired level of transcriptional strength with the recent modeling developments.

**Translation:** During translation, mRNA is translated into proteins. Translation initiates when a ribosome interacts with a ribosome binding site (RBS) and facilitates the subsequent tRNA binding to mRNA codons to produce polypeptides by the addition of amino acids. Translation involves three steps: initiation, elongation and termination. Of these three steps, translation initiation is the rate-limiting step and different rates of translation initiation are found due to variation in the DNA sequence of the RBS within each cell. Recently, a computational approach has been developed to predict translation initiation rates for all start codons in a given DNA sequence based upon a thermodynamic calculation of Gibbs free energy. Synthetic biology has now developed a complement of experimental and computational tools to design and control individual gene expression levels at both the transcriptional and translational levels [20]. These tools enable a finer level of design control for biological systems and can be implemented for metabolic engineering applications.



**Figure 2.** Promoter sequences on the DNA are recognized by RNA polymerase enzyme and synthesize mRNA using sense strand of double stranded DNA as template strand (transcription). The mRNA is translated to synthesize different aminoacids by the ribosomes in conjunction with tRNA. Different aminoacids get polymerized to make various proteins and enzymes in living cells.

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## IV. DNA LOGIC GATES

Logic gates are a vital part of how computer carries out functions that you command it to do. These gates convert binary code moving through the computer into a series of signals that the computer uses to perform operations. Logic gates interpret input signals from silicon transistors and convert these signals into an output signal. In DNA computers, these DNA logic gates rely on DNA code, instead of using electrical signals to perform logical operations. These gates are actually tiny DNA processing centers that detect specific fragments of the genetic blueprint as input and then splice together the fragments to form a single output. For example, a genetic gate called the 'And gate' links two DNA inputs by chemically binding them so they are locked in an end-to-end structure. Ogihara and Ray [21] suggested that these logic gates might be combined with DNA microchips to create a breakthrough in DNA computation.

## V. DIGITAL-LOGIC SYNTHETIC GENE CIRCUITS

The implementation of digital logic gates with synthetic gene circuits used both combinatorial approaches as well as rational design. A combinatorial library of gene networks was created based on the natural transcription factors (TFs) LacI, TetR, and lambda CI as well as promoters responsive to these TFs [22]. Networks within this library were found to behave as NOR, NAND, and NOT-IF two-input logic gates. Afterwards, digital logic gates have been constructed using a variety of regulatory mechanisms including regulation at transcriptional, translational and protein levels (Fig. 3). The combined action of both activating and repressing synthetic TFs in various combinations were used to build NOT-IF, NOT-IF-IF, NAND, OR, NOR and INVERTER gates [23]. The NOR gate was a sequential transcription cascade, in which a transcriptional activator binds to a promoter in the absence of the inducer erythromycin (EM) and induced expression of another transcriptional activator whose ability to induce the 'output' reporter gene could be repressed by pristinamycin (PI). The reporter was expressed (output = 1) when neither small molecules (EM or PI) were present. If EM, PI or both were present, then expression of the reporter was repressed (output = 0), thus forming a gate with NOR logic.

AND gates at the transcriptional level have been built by relying on mechanisms other than activation or repression by inducible transcription factors. A T7 polymerase containing two amber stop codons in conjunction with the amber suppressor tRNA, supD was used [24]. One promoter of the gate drove expression of the T7 polymerase, while the other promoter drove expression of supD. If only the input signal to express the T7 polymerase was given, then translation of the T7 polymerase stopped prematurely at one of the amber stop codons. However, when the input to express the supD amber suppressor tRNA was also present, translation occurred through the amber stop codons, and full length, functional T7 polymerase was produced. T7 polymerase was then used to drive output gene expression from a T7-dependent promoter, thus forming an AND gate.

RNA-based parts are likely to increase gene circuit sophistication due to their ease of programmability and because the thermodynamic models that facilitate their design that can be adapted to predict behaviour at the circuit scale [25]. Regulation at the RNA level with RNA interference (RNAi) has been used for the construction of logic-based circuits. Leisner et al. [26] used RNAi to create a variety of 2-input and 3-input logic gates but produced the siRNA inputs to these gates within cells using transcriptional control. Another approach for was used for constructing logic gates at the RNA level [27]. They engineered synthetic ribozymes, where the catalytic cleavage action of the ribozyme could be controlled by a small molecule binding to a separate 'sensor' module of the RNA device. The 'sensor' and 'actuator' modules were connected by a short RNA stem called the 'transmitter' module. The binding of small molecule to the sensor module caused the transmitter module to hybridize with the actuator module in such a way that the ribozyme's catalytic activity was prevented. Without binding of small molecule to the sensor, the transmitter did not hybridize to the actuator module, thus allowing ribozyme cleavage to occur. By placing these synthetic ribozymes in the 3'UTR of a reporter gene, the expression or 'output' of the gene could be controlled. By combining synthetic ribozymes in various combinations, AND, NOR, NAND and OR logic gates could be achieved.

Tamsir et al. [28] connected together various versions of NOR, OR and NOT gates (with different inputs and outputs) to create all 16 2-input logic gates with populations of *Escherichia coli*, interconnected with diffusible signaling molecules. Specifically, separated colonies were used on an agar surface, where each colony contained a single logic gate and links between colonies were established through diffusible quorum-sensing molecules. Similar approach was followed in a mixed population of yeast with cellular communication based on yeast pheromones (alpha-factors from

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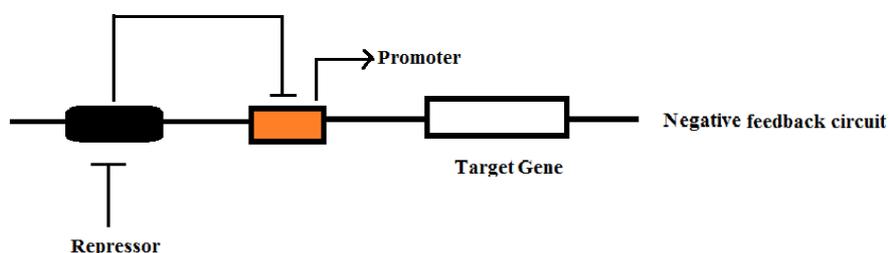
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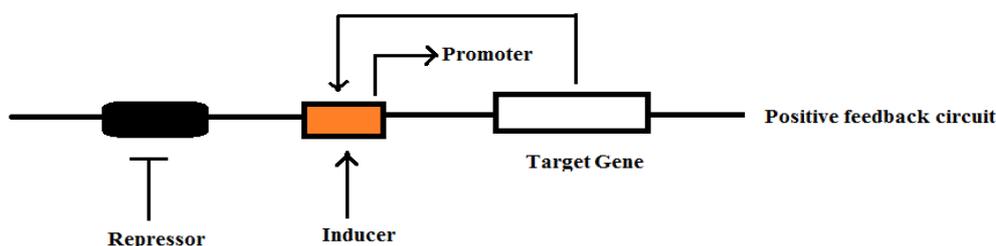
*Saccharomyces cerevisiae* and *Candida albicans*) [29]. Using this strategy, all 2-input logic functions, a 2-to-1 multiplexer and a half-adder circuit were constructed.

## VI. ANALOG SYNTHETIC GENE CIRCUITS

Analog processing represents an alternative to the conventional digital approach for cellular computation. Synthetic genetic circuits were constructed that perform analog computation using small molecule inducers to represent continuous input values and proteins to represent continuous output values [30]. An open-loop circuit with an inducible transcriptional activator (AraC) was used that displayed a sigmoidal, Hill-function-type input-output transfer function with a wide dynamic range. This was achieved by introducing a positive-feedback loop into the circuit such that the addition of inducer generated additional transcriptional activator proteins (Fig. 3). Furthermore, the positive-feedback loop circuit was constructed on a low-copy plasmid and engineered a high-copy plasmid to contain extra promoters for the AraC transcriptional activator (i.e. the pBAD promoter driving expression of the mCherry fluorescent protein). These two effects thus delayed the saturation of the input-output transfer function with the addition of inducer arabinose and increased the input dynamic range. When the input-output transfer function was plotted on a log-linear graph, it transitioned almost linearly between low and high output levels. Thus, over this range, the circuit exhibited an output fluorescence which was a scaled logarithmic transformation of the input inducer concentration.



A



B

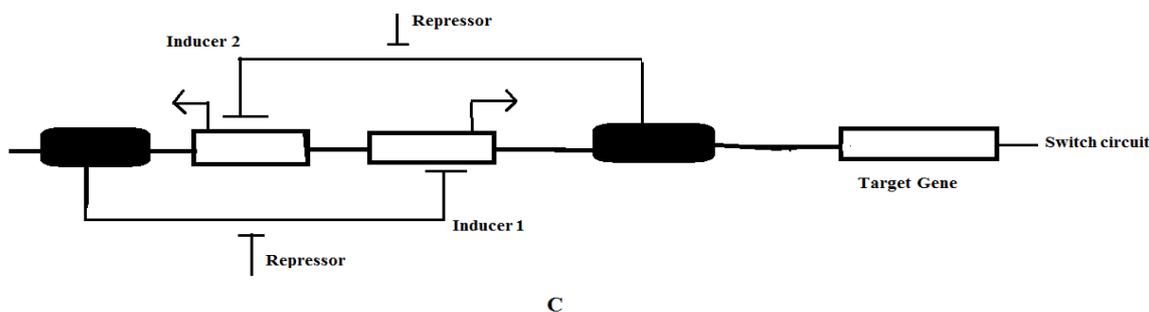


Figure 3. Techniques used for producing synthetic engineered gene circuits. A. Design of negative feedback circuit, in which transcription of the target gene is prevented by the repressor molecule. B. In positive feedback loop circuit, inducer/transcription factor activates its own transcription. C. In switch circuit, different inducer molecules are involved in neutralizing the effect of repressor protein molecules leading to activation or synthesis of target gene product.

This same motif was applied to another class of transcriptional activator proteins (LuxR), resulting in two wide-dynamic-range circuits which implemented positive-slope logarithms. By combining the two circuits, which accept two different inducer inputs but produce the same output protein, a circuit capable of addition was created. One of the positive-logarithm circuits was modified to produce a transfer function with a wide-dynamic-range negative slope instead of a positive slope, thus implementing a negative logarithm. By combining a positive-logarithm and negative-logarithm circuit together, circuits that could perform subtraction as well as division were created. Finally, using just two transcription factors, a feedback circuit was created, which displayed a power-law relationship between its input and output. An advantage of analog computation is that circuits can be built with far fewer components than their digital counterparts but achieve complex computational functions by using the inherent behaviors of the underlying components. Leveraging analog computation can therefore help to reduce the size of genetic circuits and alleviate the burden on cellular resources. However, analog systems are more susceptible to intrinsic noise than digital systems when working with lower molecule numbers [31].

## VII. SYNTHETIC GENE CIRCUITS EXHIBITING MEMORY

The computational circuits usually have outputs that are transient i.e., if the inputs are removed, the outputs will not be sustained over a long timescale. Just as in electronic computing and neural processing, the development of sophisticated genetic programs will require that intermediate results (or ‘system states’) and outputs are stored in cellular memories for downstream use. The toggle switch [32] exhibited bistable behavior and was an early example of a synthetic gene circuit displaying memory. Subsequently, the work has focused on using bistable switches in memory-based circuits [33,34]. However, many of these subsequent circuits have used positive feedback in contrast to the original toggle switch, which relied on negative feed-back. Specifically, these circuits have involved a single gene whose protein product activates its own expression. At low protein levels, the rate of expression from the gene is too low to overcome the degradation rate of the protein product and the protein concentration is stably maintained at a low level. At high protein levels, the rate of expression is high enough to balance the degradation rate of the protein, allowing the protein concentration to be stably maintained at a high level. In order to switch the positive-feedback network between these two stable states, another input is required. This can either be one that activates the level of gene expression, pushing the system from the low to the high stable state [35] or one that represses gene expression, pulling the system down from the high to the low stable state.

Both logic and memory must be integrated such that the results of logical operations can be stored and operated upon at later times. A NOR logic gate was coupled to a bistable switch [36], which acted as a ‘memory module’. This allowed them to create a ‘push-on push-off’ switch in which the same sequential input (ultraviolet light) caused the system to alternate between expressing GFP or RFP, and the state of the system (i.e. whether GFP or RFP is expressed) was ‘held’ in the memory until the next pulse. The optimized circuit was able to switch back and forth three times, although with each subsequent switching event, progressively less cells successfully switched.

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Siuti et al. [37] created all 16 2-input logic gates using the unidirectional recombinases Bxb1 and phiC31 in combination with gene regulatory elements such as promoters, terminators and output genes. For example, an OR gate can be constructed by having two inverted promoters in tandem, one surrounded by recombinase-recognition sites for Bxb1 and the other by sites for phiC31, upstream of an output gene. When either Bxb1 or phiC31 is expressed by orthogonal inducer inputs, one of the promoters flips and can then drive downstream gene expression. A similar approach can be adopted to create all other logic functions. On the other hand, the majority of the logic gates relied on flipping only terminators [38]. For example, an AND gate comprised two tandem unidirectional terminators present between a promoter and a reporter gene, where each terminator was flanked by recombination sites recognized by one of two different unidirectional recombinases (Bxb1 and TP901-1). For the promoter to express the output gene, both recombinases had to be expressed in order to flip the terminators into their inverted positions. For the promoter to express the output gene, both recombinases had to be expressed in order to flip the terminators into their inverted positions.

## VIII. CONCLUSION

Genetic digital logic gates have been the focus of intensive study over the last decade. Analog circuits have been a more recent development and further investigation will lead to construction of more complex analog circuits and alternative circuit topologies. With the increase of complexity in biological computations, it is unlikely that these circuits can be solely based on digital logic due to their burden on host cells and challenges in identifying sufficient numbers of orthogonal digital parts. Thus, important decisions in the design process for synthetic circuits will be when and how to combine digital and analog processing to balance this trade-off. Thus, hybrid computation, in which analog processing is used for the majority of the computations and digital logic is used to set thresholds, make decisions, and mitigate noise, may be a useful compromise between the two approaches. Furthermore, integrating memory into these circuits will be another step towards increased sophistication and will enable a powerful next generation of genetic circuits.

Biological computation has to compete with silicon-based computing strategies and computed gene circuits will be useful for biotechnology, biomedical engineering and basic biology studies. Synthetic gene circuits could be designed that can sense intracellular and extracellular signals in a biological process and optimize the production of a heterologous pathway or protein in response [39,40]. In addition, artificial sensors could be engineered into cells which are then used to detect signals in the human gut and respond by producing the appropriate therapeutics. Furthermore, integrating memory into these circuits will be another step towards increased sophistication. Ultimately, the optimal integration of digital logic, analog computation and memory circuits will enable a powerful next generation of genetic circuits to drive innovation in the field of synthetic biology.

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