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RNA Inspired Genetic Logic Devices and Functional Verification based on Calculus for Communicating Systems (CCS) Approach using Formalisms.

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Abstract

Researchers are now building synthetic circuits for controlling gene expression and considering practical applications for engineered gene circuits. What can we learn from nature about design principles for gene circuits? A large body of experimental data, is readily available to test some important theoretical predictions, about how gene circuits could be organized, but the data also raises some intriguing and challenging questions. Synthetic regulatory circuits, can be readily built, owing to the advanced state of genetic engineering. Attention is turning towards, manipulating genetic regulatory circuits, for therapeutic and technological applications, gene circuits for Bio remediation, metabolic engineering and gene therapy are being constructed. Such applications require a thorough understanding of the functional consequences of genetic manipulations and of the general principles that can guide the design process. In our current research work, on Simulation of logic devices and circuits, we focused on RNA based derivation of gene logic devices and their effective utilization in the simulation of large scale circuits for subsequent assembling of Biochemical Circuits to fabricate future Gene Chips. We used efficient symbolic tools software in our current bio-inspired simulation work. Life could be better explained by synthesizing its “logical forms, inference and deduction” from simple machines.

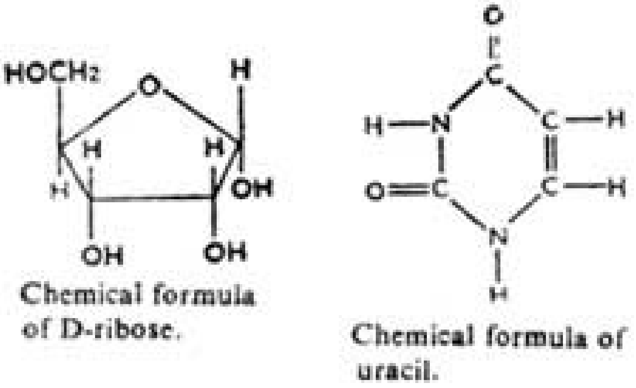
Keywords: RNA / Genetic Logic Devices / Functional verification / CCS / Simulation / EST.

Introduction

"Nucleic acids *were discovered in 1868 by* Friedrich Miescher*, who called the material 'nuclein' since it was found in the nucleus. Ribonucleic* acid (RNA) is a biologically important type of molecule that consists of a long chain of nucleotide units. Each nucleotide consists of a nitrogenous base, a ribose sugar, and a phosphate. RNA is very similar to DNA, but differs in a few important structural details: in the cell, RNA is usually single-stranded, while DNA is usually double-stranded; RNA nucleotides contain ribose while DNA contains deoxyribose (a type of ribose that lacks one oxygen atom); and RNA has the base uracil rather than thymine that is present in DNA. RNA is transcribed from DNA by enzymes called RNA polymerases and is generally further processed by other enzymes. RNA is central to protein synthesis". Here, a type of RNA called messenger RNA carries information from DNA to structures called ribosomes. These ribosomes are made from proteins and ribosomal RNAs, which come together to form a molecular machine that can read messenger RNAs and translate the information they carry into proteins. There are many RNAs with other roles – in particular regulating which genes are expressed, but also as the genomes of most viruses.(Bassler, 1999; Elowitz et al., 2000)

"RNA and DNA are both nucleic acids, but differ in three main ways. First, unlike DNA which is double-stranded, RNA is a single-stranded molecule in most of its biological roles and has a much shorter chain of nucleotides. Second, while DNA contains deoxyribose, RNA contains ribose (there is no hydroxyl group attached to the pentose ring in the 2' position in DNA)."These hydroxyl groups make RNA less stable than DNA because it is more prone to hydrolysis. Third, the complementary base to adenine is not thymine, as it is in DNA, but rather uracil, which is an unmethylated form of thymine.(Berg et al., 2002, Higgs2000, Nissan et al., 2000, Kobayashi etal., 2004)

Like DNA, most biologically active RNAs, including mRNA, tRNA, rRNA, snRNAs and other non-coding RNAs, contain self-complementary sequences that allow parts of the RNA to fold and pair with itself to form double helices. Structural analysis of these RNAs has revealed that they are highly structured."Unlike DNA, their structures do not consist of long double helices but rather collections of short helices packed together into structures akin to proteins. In this fashion, RNAs can achieve chemical catalysis, like enzymes.(Feng et al., 2004) For instance, determination of the structure of the ribosome—an enzyme that catalyzes peptide bond formation—revealed that its active site is composed entirely of RNA".(Ramachandra et al., 2001; Mattick, 2004)



Chemical formula of D-ribose.

Chemical formula of uracil.

Figure 1: Chemical Structure of RNA Like DNA, RNA is polymeric nucleic acid of four monomeric ribotids or ribonucleotides.

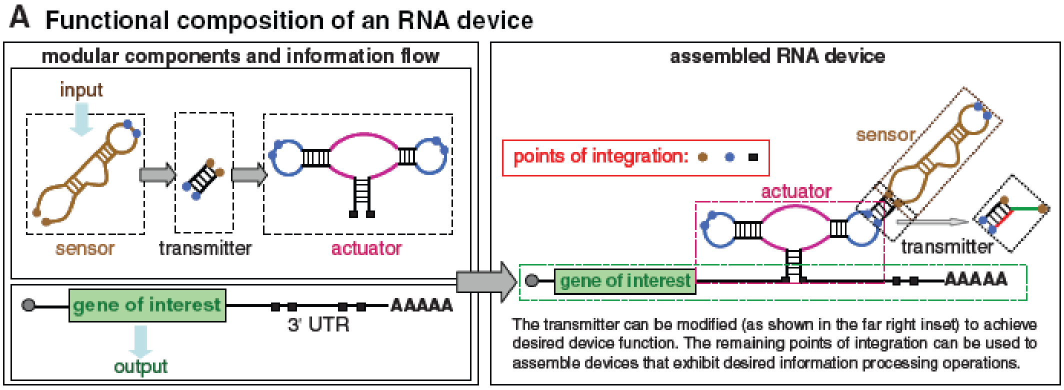
"Studies of gene circuits (both experimental and theoretical)are similar to many other areas of biological research — the principal aim is to understand the relationship between structure and function. For example, as we discuss below, patterns of regulation in elementary gene circuits can be understood in terms of the functional requirements for biosynthesis and catabolism. For natural systems, the important design features are those that can confer a selective advantage in an ecological context. This is in contrast to directed evolution and rational improvement of synthetic circuits, in which selection of features is an artifact of engineering".(Lee and Gutell 2004; Holley et al., 1965)

Description of the Simulation using RNA inspired Logic Formalisms

"The engineering of biological systems is anticipated to provide effective solutions to challenges that include energy and food production, environmental quality, and health and medicine. Our ability to transmit information to and from living systems, and to process and act on information inside cells, is critical to advancing the scale and complexity at which we can engineer, manipulate, and probe biological systems. We developed a general approach for assembling RNA devices that can execute higher-order cellular information processing operations from standard components". The engineered devices can function as logic gates (AND, NOR, NAND, or OR gates) and signal filters, and exhibit cooperativeness. RNA devices process and transmit molecular inputs to targeted protein outputs, linking computation to gene expression and thus the potential to control cellular function.

(Maung and Smolke, 2008; Feng et al., 2004; Weiss, 2001; Weiss and Basu, 2002; Weiss et al., 2003)

"Genetically encoded technologies that perform information processing, communication, and control operations are needed to produce new cellular functions from the diverse molecular information encoded in the various properties of small molecules, proteins, and RNA present within biological systems. For example, genetic logic gates that process and translate multiple molecular inputs into prescribed amounts of signaling through new molecular outputs would enable the integration of diverse environmental and intracellular signals to a smaller number of phenotype responses". Basic operations such as signal filtering, amplification, and restoration would also enable expanded manipulation of molecular information through cellular networks.(Sibert, 2006, Gardner et al., 2000; Ramachandra et al., 2001; Holley et al., 1965)



The transmitter can be modified (as shown in the far right inset) to achieve desired device function. The remaining points of integration can be used to assemble devices that exhibit desired information processing operations.

Figure 2: Functional Composition of an RNA device (Permission to reproduce requested, Maung and Smolke, 2008).

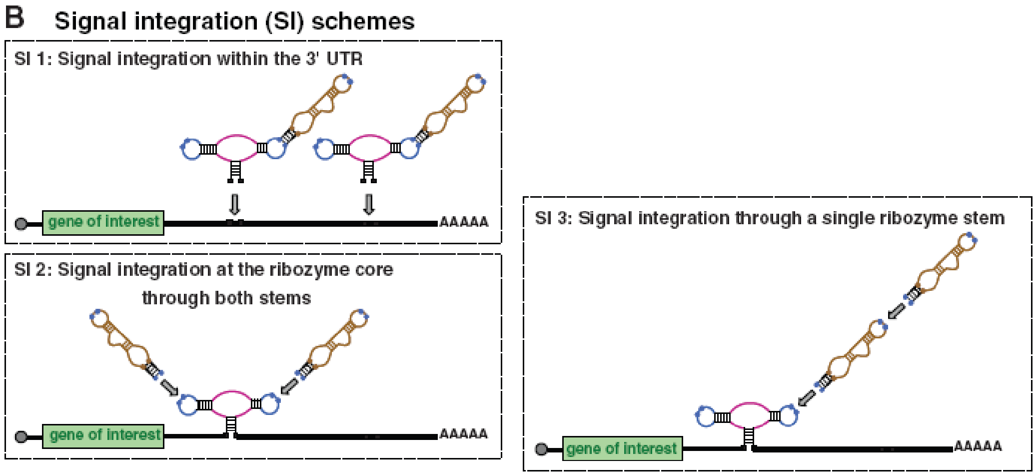
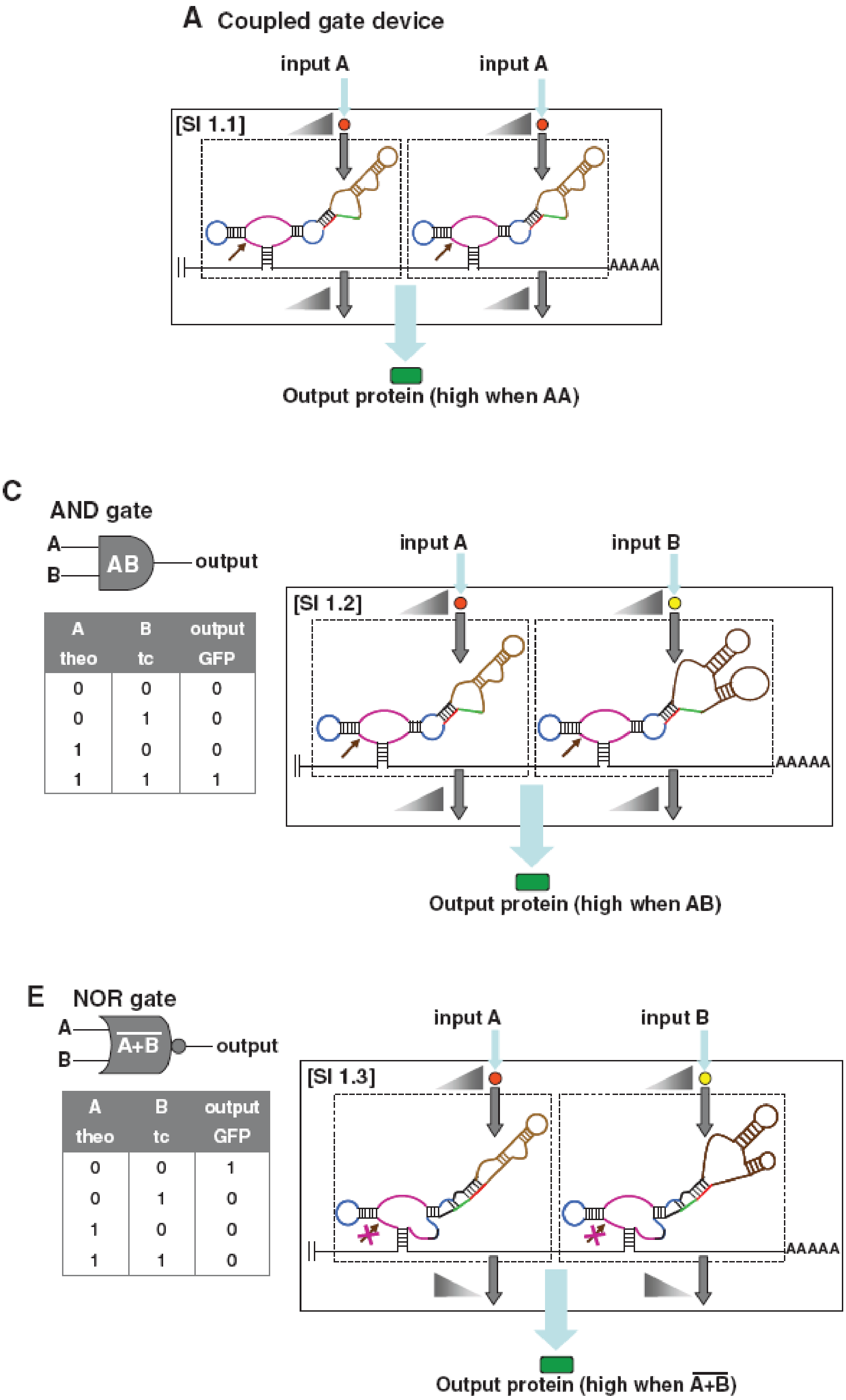


Figure 3: Signal integration schemes (Permission to reproduce requested, Maung and Smolke, 2008).

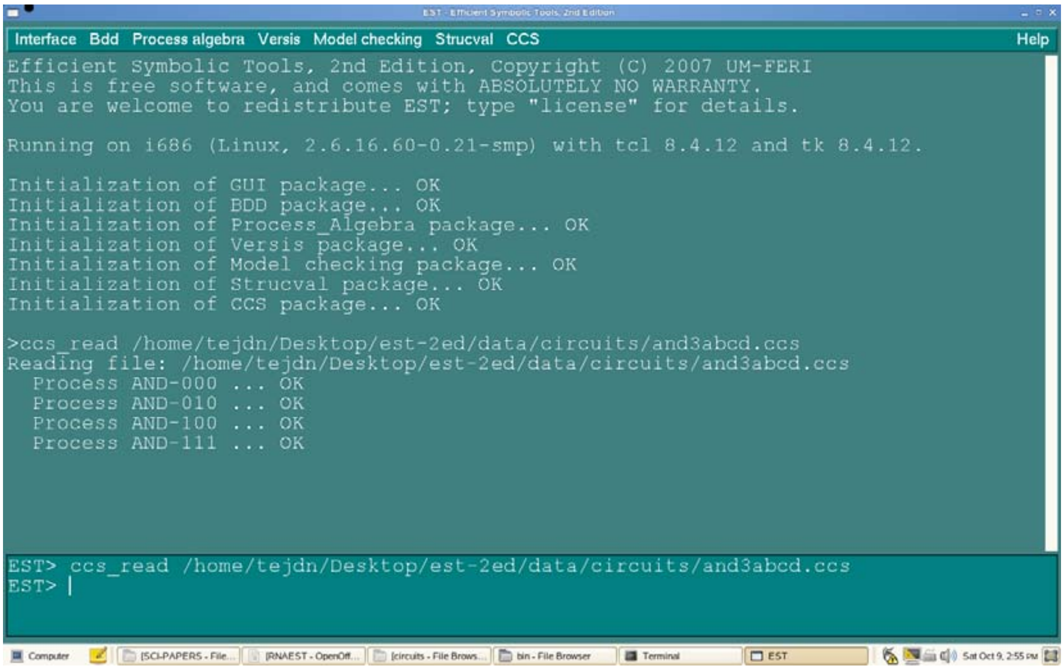


A	B	output
theo	tc	GFP
0	0	0
0	1	0
1	0	0
1	1	1

A	B	output
theo	tc	GFP
0	0	1
0	1	0
1	0	0
1	1	0

Figure 4: Logical deductions based on RNA inspired configuration (Permission to reproduce requested, Maung and Smolke, 2008)

Simulation and Logical analysis using CCS Software



```
Efficient Symbolic Tools, 2nd Edition, Copyright (C) 2007 UM-FERI
This is free software, and comes with ABSOLUTELY NO WARRANTY.
You are welcome to redistribute EST; type "license" for details.

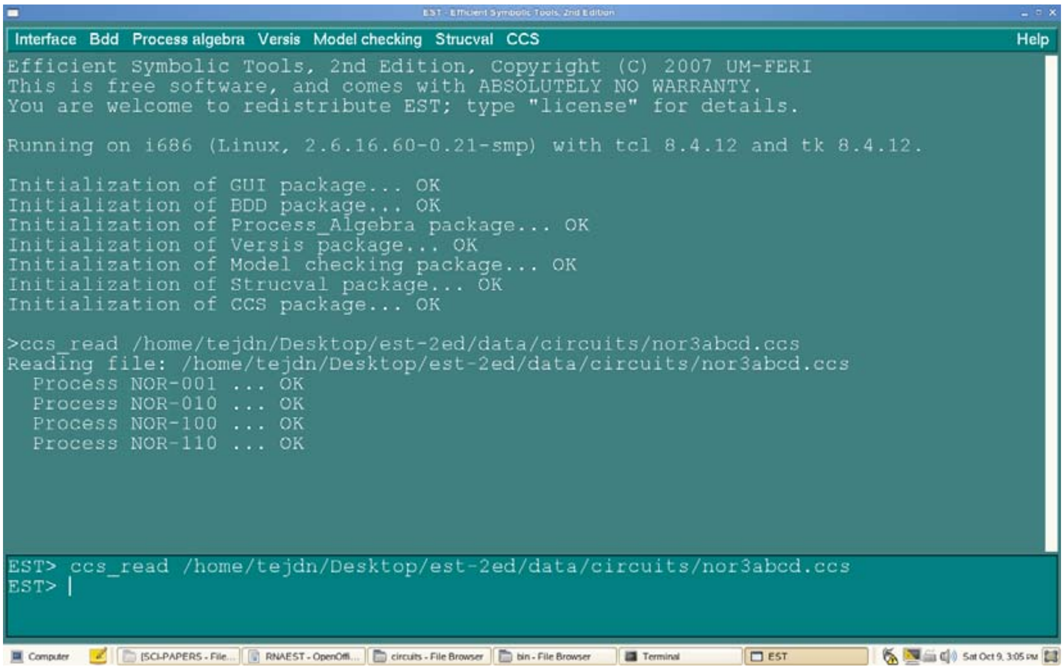
Running on i686 (Linux, 2.6.16.60-0.21-smp) with tcl 8.4.12 and tk 8.4.12.

Initialization of GUI package... OK
Initialization of BDD package... OK
Initialization of Process_Algebra package... OK
Initialization of Versis package... OK
Initialization of Model checking package... OK
Initialization of Strucval package... OK
Initialization of CCS package... OK

>ccs_read /home/tejdn/Desktop/est-2ed/data/circuits/and3abcd.ccs
Reading file: /home/tejdn/Desktop/est-2ed/data/circuits/and3abcd.ccs
  Process AND-000 ... OK
  Process AND-010 ... OK
  Process AND-100 ... OK
  Process AND-111 ... OK
```

```
EST> ccs_read /home/tejdn/Desktop/est-2ed/data/circuits/and3abcd.ccs
EST> |
```

Figure 5: Simulation of AND Gate, Screen Shot of the EST Simulation.



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Running on i686 (Linux, 2.6.16.60-0.21-smp) with tcl 8.4.12 and tk 8.4.12.

Initialization of GUI package... OK
Initialization of BDD package... OK
Initialization of Process_Algebra package... OK
Initialization of Versis package... OK
Initialization of Model checking package... OK
Initialization of Strucval package... OK
Initialization of CCS package... OK

>ccs_read /home/tejdn/Desktop/est-2ed/data/circuits/nor3abcd.ccs
Reading file: /home/tejdn/Desktop/est-2ed/data/circuits/nor3abcd.ccs
  Process NOR-001 ... OK
  Process NOR-010 ... OK
  Process NOR-100 ... OK
  Process NOR-110 ... OK
```

```
EST> ccs_read /home/tejdn/Desktop/est-2ed/data/circuits/nor3abcd.ccs
EST> |
```

Figure 6: Simulation of NOR Gate, Screen shot of the EST Simulation.

Open Source Efficient Symbolic Tools Software based on Formalisms and Process Algebra

About the EST Software/SUSE Linux OS Used in our Simulation work

Efficient symbolic tools software package is an Research and academic open source tool available from University of Maribor, Slovenia, Europe, developed by Dr. Robert Meolic.EST is a new tool for the verification of concurrent systems, which has not been widely presented yet. EST is a relatively small package written in C with a Tcl/Tkuser interface. Main advantages are flexibility, portability and an efficient memory management.

Discussion

Formal reasoning is the most powerful and hardest technique to use, but already there is a long tradition of building tools for verifying properties of concurrent systems. Typical activities in this area are checking behavioral equivalence between different systems, or between different abstraction levels of the same system, including now biological systems While computational approaches to biology and other sciences are now common, several of the techniques outlined above are unique to computer science and virtually unknown in other fields; hopefully they will bring useful tools and perspectives to biology.

Model checking is now used routinely in the analysis of hardware and software systems that have huge state spaces; it is based on the state and transition model we emphasized during the discussion of abstract machines. Model checking consists of a model description language for building models, a query language for asking questions about models (typicallytemporal logic), and an efficient state exploration engine. The basic technology is very advanced, and is beginning to be applied to descriptions of biological systems too, in various flavors.(Garima, 2007)

Logic gates are the basic building blocks in electronic circuits that perform logical operations. These have input and output signals in the form of 0’s and 1’s; ‘0’ signifies the absence of signal while ‘1’ signifies its presence. Similar to the electronic logic gates, cellular components can serve as logic gates. We infer from the bio-informatic publications that, a typical biological circuit consists of a coding region, its promoter, RNA polymerase, the regulatory proteins with their DNA binding elements and small signaling molecules that interact with the regulatory proteins.Many aspects of biological organization are more akin to discrete hardware and softwaresystems than to continuous systems, both in hierarchical complexity and in algorithmic-likeinformation-driven behavior. These aspects need to be reflected in the modeling approaches and in the notations used to describe such systems, in order to make sense of the rapidly accumulating experimental data.(Elowitz and Lebler, 2000; Maung and Smolke, 2008)

Simulations using Efficient Symbolic Tools Package based on CCS-Calculus of Communicating Systems.

Configuration of AND Logic Gene Gate

```
/* AND3---Logic Gate Simulation Code */
/* Nirmal, March 2010 */
/*Jiangnan University. China*/
/*Ben-Gurion University, Israel*/
/* input-rejective model */
AND-000 = a?.AND-100 + b?.AND-010 + c?.AND-001
AND-010 = a?.AND-110 + b?.AND-000 + c?.AND-011
AND-100 = a?.AND-000 + b?.AND-110 + c?.AND-101
AND-111 = a?.d!.AND-011 + b?.d!.AND-101 + c?.d!.AND-110
```

Configuration of NOR Logic Gene Gate

```
/* NOR3---Logic Gate Simulation Code */
/* Nirmal, March 2010 */
/* Jiangnan University, China*/
/*Ben-Gurion University, Israel*/
/* input-rejective model */

NOR-001 = a?.NOR-101 + b?.NOR-011 + c?.d!.NOR-000
NOR-010 = a?.NOR-110 + b?.d!.NOR-000 + c?.NOR-011
NOR-100 = a?.d!.NOR-000 + b?.NOR-110 + c?.NOR-101
NOR-110 = a?.NOR-010 + b?.NOR-100 + c?.NOR-111
```

Conclusion & Future Perspectives

This publication highlights, an emerging field known as synthetic biology, that envisions integrating designed circuits into living organisms in order to instruct them to make logical decisions, based on the prevailing intracellular and extracellular conditions and produce a reliable behavior. The attempt is to design cells capable of sensing a bio-weapon or an environmental pollutant, activate its degradation pathway and perform bio-remediation or carry out programmed cell death or synthesize complex bio-materials. Within the living cell, a complex interplay of networks formed by genes and proteins, mediate all cellular processes. The networks in a system can be dissected, into small regulatory gene circuit modules. Synthetic biology attempts to construct and assemble such modules step by step, plug the modules together and modify them, in order to generate a desired behavior. This paper gives an insight, into the creation of gene circuits and discusses the potential applications in the field of biotechnology, medicine and environmental sciences. Expectations include a focus on wider scientific understanding, of entertaining alternative scenarios of life challenging the dogmas of biology and finally to discover novel design principles of life.

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