A Top-down Approach to Genetic Circuit Synthesis and Optimized Technology Mapping

Genetic logic circuits are becoming popular as an emerging field of technology. They are composed of genetic parts of DNA and work inside a living cell to perform a dedicated boolean function triggered by the presence or absence of certain proteins or other species.

Logic analysis and verification of n-input genetic logic circuits

Nature is using genetic logic circuits to regulate the fundamental processes of life. These genetic logic circuits are triggered by a combination of external signals, such as chemicals, proteins, light and temperature, to emit signals to control other gene expressions or metabolic pathways accordingly. As compared to electronic circuits, genetic circuits exhibit stochastic behavior and do not always behave as intended. Therefore, there is a growing interest in being able to analyze and verify the logical behavior of a genetic circuit model, prior to its physical implementation in a laboratory. In this paper, we present an approach to analyze and verify the Boolean logic of a genetic circuit from the data obtained through stochastic analog circuit simulations. The usefulness of this analysis is demonstrated through different case studies illustrating how our approach can be used to verify the expected behavior of an n-input genetic logic circuit.
Methods and Tools for the Analysis, Verification and Synthesis of Genetic Logic Circuits,

Synthetic biology has emerged as an important discipline in which engineers and biologists are working together to design new and useful biological systems composed of genetic circuits. The purpose of developing genetic circuits is to carry out desired logical functions inside a living cell. This usually requires simulating the mathematical models of these genetic circuits and perceive whether or not the circuit behaves appropriately. Furthermore, synthetic biology utilizes the concepts from electronic design automation (EDA) of abstraction and automated construction to generate genetic circuits with the aim to reduce the in-vitro (wet-lab) experiments. To address this, several automated tools have been developed to improve the process of genetic design automation (GDA) with different capabilities. This thesis attempts to contribute to the advancement of GDA tools by introducing capabilities which we believe that no other existing GDA tools support. First, we introduce a user-friendly simulation tool, called D-VASim, which allows user to perform virtual laboratory experimentation by dynamically interacting with the model during runtime. This dynamic interaction with the model gives user a feeling of being in the lab performing wet-lab experiments virtually. This tool allows users to perform both deterministic and stochastic simulations. Next, this dissertation introduces a methodology to perform timing analyses of genetic logic circuits, which allows user to analyze the threshold value and propagation delays of genetic logic circuits. In this thesis, it has been demonstrated, through in-silico experimentation, that the threshold value and propagation delay plays a vital role in the correct functioning of genetic circuit. It has also been shown how some circuit parameters effect these two important design characteristics. This thesis also introduces an automated approach to analyze the behavior of genetic logic circuits from the simulation data. With this capability, the boolean logic of complex genetic circuits can be analyzed and/or verified automatically. It is also shown in this thesis that the proposed approach is effective to determine the variation in the behavior of genetic circuits when the circuit’s parameters are changed.

In addition, the thesis also attempts to propose a synthesis and technology mapping tool, called GeneTech, for genetic circuits. It allows users to construct a genetic circuit by only specifying its behavior in the form of boolean expression. For technology mapping, this tool uses a gates library developed by the collective efforts of the researchers at MIT and Boston universities. It is shown experimentally that the tool is able to provide all feasible solutions, containing different genetic components, to achieve the specified boolean behavior. Finally, it has been shown how D-VASim can be used along with other tools for useful purposes, like model checking. With respect to this, an experimental workflow is proposed for checking genetic circuits using the statistical model checking (SMC) utility of the Uppaal tool and the timing analysis capability of D-VASim. We further demonstrated how the reliability of a simulation can be improved by using the real parameter values. In this regard, the relationship between the simulation parameters and real parameters have been derived.
Simulation Approach for Timing Analysis of Genetic Logic Circuits

Constructing genetic logic circuits is an application of synthetic biology in which parts of the DNA of a living cell are engineered to perform a dedicated Boolean function triggered by an appropriate concentration of certain proteins or by different genetic components. These logic circuits work in a manner similar to electronic logic circuits, but they are much more stochastic and hence much harder to characterize. In this article, we introduce an approach to analyze the threshold value and timing of genetic logic circuits. We show how this approach can be used to analyze the timing behavior of single and cascaded genetic logic circuits. We further analyze the timing sensitivity of circuits by varying the degradation rates and concentrations. Our approach can be used not only to characterize the timing behavior but also to analyze the timing constraints of cascaded genetic logic circuits, a capability that we believe will be important for design automation in synthetic biology.

General information
State: Published
Organisations: Embedded Systems Engineering, Department of Applied Mathematics and Computer Science, Embedded Systems Engineering, Copenhagen Center for Health Technology
Authors: Baig, H. (Intern), Madsen, J. (Intern)
Number of pages: 11
Pages: 1169-1179
Publication date: 2017
Main Research Area: Technical/natural sciences

Publication information
Journal: A C S Synthetic Biology
Volume: 6
Issue number: 7
ISSN (Print): 2161-5063
Ratings:
Web of Science (2018): Indexed yes
Web of Science (2017): Indexed yes
Scopus rating (2016): CiteScore 4.7 SJR 2.736 SNIP 1.024
Web of Science (2016): Indexed yes
Scopus rating (2015): SJR 2.269 SNIP 1.049 CiteScore 4.41
Web of Science (2015): Indexed yes
Scopus rating (2014): SJR 3.783 SNIP 1.219 CiteScore 3.84
Web of Science (2014): Indexed yes
Scopus rating (2013): SJR 1.796 SNIP 0.859 CiteScore 3.42
ISI indexed (2013): ISI indexed yes
ISI indexed (2012): ISI indexed no
Original language: English
Genetic logic circuits, SBML, Stochastic simulation, Synthetic Biology, Threshold value analysis, Timing analysis, Virtual instrumentation
Electronic versions:
ACS.pdf. Embargo ended: 19/01/2018
DOIs:
10.1021/acssynbio.6b00296
Source: PublicationPreSubmission
Source-ID: 128708733
Publication: Research - peer-review › Journal article – Annual report year: 2017

Taming Living Logic using Formal Methods
One of the goals of synthetic biology is to build genetic circuits to control the behavior of a cell for different application domains, such as medical, environmental, and biotech. During the design process of genetic circuits, biologists are often interested in the probability of a system to work under different conditions. Since genetic circuits are noisy and stochastic in nature, the verification process becomes very complicated. The state space of stochastic genetic circuit models is usually too large to be handled by classical model checking techniques. Therefore, the verification of genetic circuit models is usually performed by the statistical approach of model checking. In this work, we present a workflow for checking genetic circuit models using a stochastic model checker (Uppaal) and a stochastic simulator (D-VASim). We demonstrate with experimentations that the proposed workflow is not only sufficient for the model checking of genetic circuits, but can also be used to design the genetic circuits with desired timings.

General information
State: Published
Simulation and behavioral analysis of genetic circuits is a standard approach of functional verification prior to their physical implementation. Many software tools have been developed to perform in silico analysis for this purpose, but none of them allow users to interact with the model during runtime. The runtime interaction gives the user a feeling of being in the lab performing a real world experiment. In this work, we present a user-friendly software tool named D-VASim (Dynamic Virtual Analyzer and Simulator), which provides a virtual laboratory environment to simulate and analyze the behavior of genetic logic circuit models represented in an SBML (Systems Biology Markup Language). Hence, SBML models developed in other software environments can be analyzed and simulated in D-VASim. D-VASim offers deterministic as well as stochastic simulation; and differs from other software tools by being able to extract and validate the Boolean logic from the SBML model. D-VASim is also capable of analyzing the threshold value and propagation delay of a genetic circuit model.

**D-VASim: An Interactive Virtual Laboratory Environment for the Simulation and Analysis of Genetic Circuits**

Simulation and behavioral analysis of genetic circuits is a standard approach of functional verification prior to their physical implementation. Many software tools have been developed to perform in silico analysis for this purpose, but none of them allow users to interact with the model during runtime. The runtime interaction gives the user a feeling of being in the lab performing a real world experiment. In this work, we present a user-friendly software tool named D-VASim (Dynamic Virtual Analyzer and Simulator), which provides a virtual laboratory environment to simulate and analyze the behavior of genetic logic circuit models represented in an SBML (Systems Biology Markup Language). Hence, SBML models developed in other software environments can be analyzed and simulated in D-VASim. D-VASim offers deterministic as well as stochastic simulation; and differs from other software tools by being able to extract and validate the Boolean logic from the SBML model. D-VASim is also capable of analyzing the threshold value and propagation delay of a genetic circuit model.
D-VASim: A Software Tool to Simulate and Analyze Genetic Logic Circuits

The Challenge:
Creating a software tool for the simulation and analysis of genetic logic circuits to help researchers performing wet lab experiments virtually, because the manual process of wet lab experimentation of genetic logic circuits is time consuming and a challenging task for early-stage researchers with limited experience in the field of biology.

The Solution:
Using LabVIEW to develop a user-friendly simulation tool named Dynamic Virtual Analyzer and Simulator (D-VASim), which is the first software tool in the domain of synthetic biology that provides a virtual laboratory environment to perform run-time interactive simulation and analysis of genetic logic circuits.

General information
State: Published
Organisations: Department of Applied Mathematics and Computer Science, Embedded Systems Engineering
Authors: Baig, H. (Intern), Madsen, J. (Intern)
Publication date: 2016

Publication information
Type: Case study
Source/Publisher: National Instruments
Main Research Area: Technical/natural sciences
Electronic versions:
Logic and Timing Analysis of Genetic Logic Circuits using D-VASim

**General information**
State: Published
Organisations: Department of Applied Mathematics and Computer Science, Embedded Systems Engineering
Authors: Baig, H. (Intern), Madsen, J. (Intern)
Pages: 77-78
Publication date: 2016

**Host publication information**
Title of host publication: Proceedings of the 8th International Workshop on Bio-Design Automation (IWBDA 2016)
Main Research Area: Technical/natural sciences
Workshop: 8th International Workshop on Bio-Design Automation, Newcastle upon Tyne, United Kingdom, 16/08/2016 - 16/08/2016
Electronic versions: IWBDA_2016.pdf
Links: http://www.iwbdaconf.org/2016/program/
Source: PublicationPreSubmission
Source-ID: 127113795
Publication: Research - peer-review › Conference abstract in proceedings – Annual report year: 2016

Timing Analysis of Genetic Logic Circuits using D-VASim

A genetic logic circuit is a gene regulator network implemented by re-engineering the DNA of a cell, in order to control gene expression or metabolic pathways, through a logic combination of external signals, such as chemicals or proteins. As for electronic circuits, timing and propagation delay analysis may play a very significant role in the designing of genetic logic circuits. In this demonstration, we present the capability of D-VASim (Dynamic Virtual Analyzer and Simulator) to perform the timing and propagation delay analysis of genetic logic circuits. Using D-VASim, the timing and propagation delay analysis of a single as well as cascaded genetic logic circuits can be performed. D-VASim allows users to change the circuit parameters during runtime simulation to observe its effect on the circuit’s timing behavior. The results obtained from D-VASim can be used not only to characterize the timing behavior of genetic logic circuits but also to analyze the timing constraints of cascaded genetic logic circuits.

**General information**
State: Published
Organisations: Department of Applied Mathematics and Computer Science, Embedded Systems Engineering, Copenhagen Center for Health Technology
Authors: Baig, H. (Intern), Madsen, J. (Intern)
Number of pages: 1
Publication date: 2016
Event: Abstract from 19th Conference and Exhibition on Design, Automation and Test in Europe Conference and Exhibition (DATE 2016), Dresden, Germany.
Main Research Area: Technical/natural sciences
Electronic versions: 37908.pdf
Source: PublicationPreSubmission
Source-ID: 127113984
Publication: Research - peer-review › Conference abstract for conference – Annual report year: 2016

D-VASim: Dynamic Virtual Analyzer and Simulator for Genetic Circuits

A genetic circuit represents a gene regulator network that is triggered by a combination of external signals, such as chemicals, proteins, light or temperature, to emit signals to control gene expression or metabolic pathways accordingly. In order to match the intended behavior, genetic circuits are either assembled from a standard library of well-defined genetic gates or from parts of an available library, for instance, BioBricks. The obtained behavior can be validated through in-silico analysis, solving reaction kinetics using ordinary differential equations (ODEs) or by stochastic simulation, with the aim to reduce the number of required in-vitro experiments. We present a behavioral simulation and analysis tool that allows the biologist to carry out virtual lab experiments as an
interactive process during simulation of the genetic circuit, rather than a batch process, which is current practice. We believe that this increases the insights gained from the analysis and allows for exploring more parameters in an intuitive manner.

General information
State: Published
Organisations: Department of Applied Mathematics and Computer Science, Embedded Systems Engineering, Copenhagen Center for Health Technology
Authors: Baig, H. (Intern), Madsen, J. (Intern)
Pages: 48-49
Publication date: 2015

Host publication information
Title of host publication: Proceedings of the 7th International Workshop on Bio-Design Automation (IWBDA 2015)
Main Research Area: Technical/natural sciences
Workshop: 7th International Workshop on Bio-Design Automation, Seattle, United States, 19/08/2015 - 19/08/2015
Electronic versions:
D_VASim_Camera_ready_Hasan_and_Jan_.pdf
Links:
http://www.iwbdaconf.org/2015/program/#proceedings (Link to proceedings at the conference web-site)
Source: PublicationPreSubmission
Source-ID: 118030132
Publication: Research - peer-review › Conference abstract in proceedings – Annual report year: 2015

Projects:

A top-down approach to genetic circuit synthesis
Department of Applied Mathematics and Computer Science
Period: 15/08/2014 → 15/11/2017
Number of participants: 5
Phd Student:
Baig, Hasan (Intern)
Main Supervisor:
Madsen, Jan (Intern)
Examiner:
Hansen, Michael Reichhardt (Intern)
Bhatia, Swapnil (Ekstern)
Myers, Chris John (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: Institut stipendie (DTU)

Relations
Publications:
Methods and Tools for the Analysis, Verification and Synthesis of Genetic Logic Circuits,
Project: PhD

Activities:

International Synthetic and Systems Biology Summer School
Period: 5 Jul 2015 → 9 Jul 2015
Hasan Baig (Participant)
Department of Applied Mathematics and Computer Science
Embedded Systems Engineering

Description
Presented a poster in SSBSS (Synthetic and Systems Biology Summer School) 2015 in Taormina, Italy.
Related event

International Synthetic and Systems Biology Summer School
15/06/2014 → 19/06/2014
Taormina, Italy
Activity: Attending an event › Participating in or organising workshops, courses, seminars etc.