BIOLOGICAL PROCESSES STUDIES THROUGH SOFTWARE TOOLS: DEVELOPMENT AND OPTIMIZATION IN THE SIMULATION OF SYNTHETIC BIOLOGICAL CONSTRUCTIONS

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***Abstract**: In this paper is aim to design a new software tool that allows to create synthetic biology complex systems and genetic circuit designs, as well as to carry out the development of these models using a wide set of biological and biochemical rules. Thus, it will keep a very close relationship respect to the real characteristics of the biological systems and their environment, obtaining then a high reproducibility in the progress and simulation systems in relation to the progress of the real experiment. It will describe a comparison between two software tools which allow us to design, build and simulate synthetic biology networks and biochemical processes. The progress, improvement and combination of the distinct possibilities that offer both software as well as the analysis of a joint usage of them will result the creation of the new software.*

***Keywords**: synthetic biology, natural computation, COPASI, SynBioSS, biological systems, synthetic biology,in silico, in vivo, in vitro, BioBricks, SBML, XML, repressilator.*

ACM Classification Keywords: D.2 SOFTWARE ENGINEERING, H.2 DATABASE MANAGEMENT J.3 LIFE AND MEDICAL SCIENCES, I.6 SIMULATION AND MODELING, J.2 PHYSICAL SCIENCES AND ENGINEERING.

Introduction

In this paper the comparative between two tools is discussed: SynbioSS and COPASI. The first one, SynBioSS, is available under the GNU General Public License at http://synbioss.sourceforge.net. For the second one, the complete software as well as the full source code is available under an open license from http://www.copasi.org. Both are platform-independent and user-friendly biochemical simulators that offer a software with universal rules and laws of molecular biology to generate models of any arbitrary synthetic biological system. In this case, has compared these two software tools and show a comparative between them, providing advantages to find better experimental approaches. There are numerous similar software tools to design and modeling biological systems, however have used SynBioSS and COPASI because are two tools which are based on very specifics biological rules that are more resemble to the reality than others software, so these have been chosen. Thus, will get extrapolate these synthetic biology systems from in silico to in vitro and in vivo with more efficiency. To explain the software foundations and subsequently it comparative, will use a representative synthetic biology construction, the Repressilator, that allow describes better the tools and it joint functionality.

The Repressilator

The Repressilator [Michael B. Elowitz and Stanislas Leibler, 2000] is a synthetic oscillating network of transcriptional regulators. In this case the network is formed by three transcriptional repressor systems which periodically induce the synthesis of green fluorescent protein as a read-out of its state in individual cells. Such “rational network design” may lead both to the engineering of new cellular behaviors and an improved understanding of naturally occurring networks.

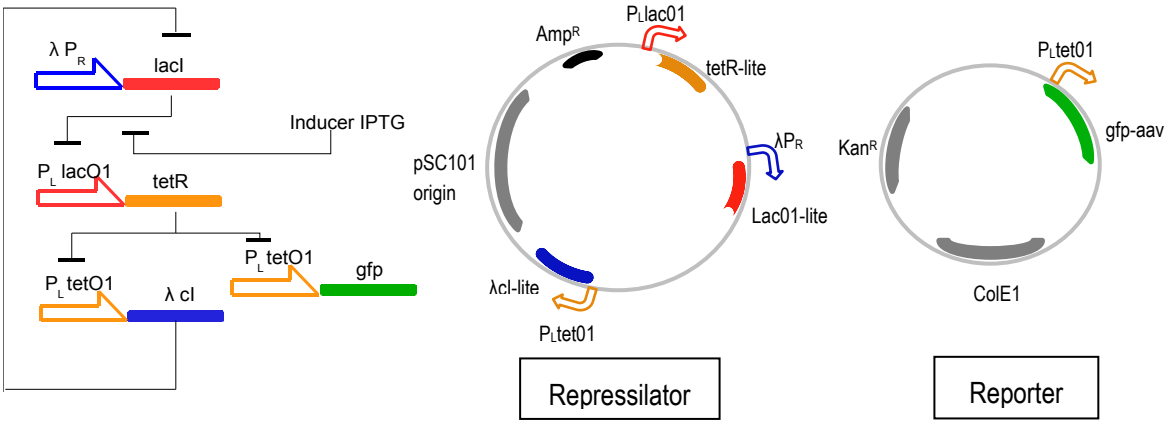


Figure 1. The Repressilator mechanism and foundations. There are three different repressors with their corresponding promoters. The repressor proteins are TetR, LacI, and λ CI, and their conjugate promoters are, respectively, pTet, pLac, and λP_R. So, pTet is repressed by TetR, pLac (from the *lac* operon) is repressed by LacI, and λP_R is repressed by CI. The first repressor protein, LacI from E. coli, inhibits the transcription of the second repressor gene, tetR from the tetracycline-resistance transposon Tn10, whose protein product in turn inhibits the expression of a third gene, cI from λ phage. Finally, λ CI inhibits lacI expression completing the cycle.

The repressilator is a negative transcription feedback cycle, which can generate oscillations of each one of its parts of the repressilator. There are numerous factors that have influence on the progress of the synthetic biological network, including the transcription rate on repressor concentration, the translation rate, environment conditions and the presence or absence of inducers. According to the values of these parameters, different behaviors are possible, resulting on distinct time-course of the fluorescence. To generate the synthetic oscillator network were used standard molecular biology techniques to construct a low-copy plasmid encoding the repressilator and a compatible, higher-copy reporter plasmid containing the tetR-repressible promoter pTet fused to an intermediate stability variant of green fluorescent protein. The design of the repressilator started with a simple mathematical model of transcriptional regulation to identify possible classes of dynamic behavior and determine what experimental parameters should be adjusted to obtain sustained oscillations. These mathematical models as well as the biological synthetic network and its components, included inducers, could be confronted and contrasted through SynBioSS and COPASI, fulfilling an exemplary comparative between these software.

SynbioSS Software

The Synthetic Biology Software Suite (SynBioSS) [Anthony D. Hill, et al., 2008] is a software for modeling, design and simulation of synthetic genetic constructs. SynBioSS facilitates computational synthetic biology and consists of three independent components: the Desktop Simulator (DS), the Wiki, and the Designer.

1. SynBioSS Designer: is a web service to create automatically kinetic model from a Biobricks construction [Michal Galdzicki et al., 2009], using a set of universal biological rules to get a biomolecular interactions network. SynBioSS uses the registry of standard biological parts, a database of kinetic parameters, and both graphical and command-line interfaces to multiscale simulation algorithms. The International Genetically Engineered Machine (iGEM) Foundation is dedicated to education and competition, progress of synthetic biology, and the development of an open community and collaboration. This organization promotes and fosters scientific research and education by establishing and operating the Registry of Standard Biological Parts, a community collection of

biological components which are used by SynBioSS to obtain the transcriptional units from the Biobricks system. SynBioSS Designer has the advantage that allows designs and builds distinct genetic circuits from these Biobricks, obtaining even biochemical and genetic complex buildings upon which could be able modify some of their characteristics to get the desire reaction network that will describe the created model, considering the biological rules and laws from which the software is based. SynBioSS Designer considers the behavior of the individual parts as well as the behavior of the connectivity spatial and temporal of each part, including the biochemical interactions resulting of the system, so it is accurately, then the biochemical reactions network resulting will become very likely respect to the reaction generation network that should occurs in vivo underneath biochemical approach. The types of reactions network generated through the Repressilator corresponding to transcription, translation, regulation, and induction are stored in the SynBioSS Wiki, so assigns for each reaction a rate law, according to the number of reactants or the nature of the reaction, and a default kinetic constant of an appropriate order of magnitude in relation to the nature of the reaction. However, must pay attention to these values having to seek in different sources as *SynBioSS Wiki* or relevant literature to get suitable information pertinent to the system, seeing the dynamic behavior based on these parameter values. Definitively the reaction network generation will depends on the circuit design provided through inputs and their properties where several of these can be changed.

Table1. The table shows a summary of inputs and their properties which can be introduced and changed into SynBioss Designer.

BioBricks	Promoter →  RBS →  Coding Region →  Terminator → 
Effectors	Protein to which it binds Maximum times which it may bind to said protein
Operators	Location (Upstream of -35, Between -35 and -10, Downstream of 10)
Promoters	Constitutively ON or OFF Associated operator
Proteins	Activator →  Repressor →  Reporter→  Number of subunits in an active complex Constitutive or Non-Constitutive Acts on its own or in concert with an effector (for activators only)

Given this information, the Designer begins by extracting all of the “transcriptional units” from the sequence of BioBricks. In the Designer approach, a transcriptional unit is defined as a promoter followed by an RBS, coding DNA, and one or more terminators. In this case, is developed the Repressilator, whose shows as far as Designer, a sequence consist of “three transcriptional units” that generate a reaction network in accordance with the following reactions. Finally the output is a reaction network representing all the steps in gene expression and regulation. SynBioss Designer outputs either a NetCDF or SBML file [M. Hucka et al., 2003], which can then be loaded in simulation software such as *SynBioSS Desktop Simulator*.

2. SynBioSS Wiki: SynbioSS Wiki can be schematizes on two things: is a web interface based on Media Wiki packages and also a database for storing of molecular components as well as their corresponding biological information. SynbioSS Wiki makes possible and facilitates the creation of biochemical reaction networks through the scientific community that stores and retrieves information related to synthetic biology. Subsequently, these reactions can be modeled by *SynbioSS Desktop Simulator*. The Synbioss Wiki databases to be made up of two parts. The first part is the standard Media Wiki tables, necessary for the Media Wiki software to function properly. The second part of database contains the tables generated specifically for the SynBioSS Wiki project. Media Wiki is written in PHP and needs a database behind it and HTML language used to format how the wiki displays in a web browser. Synbioss Wiki also uses a SQL language database to communicate the PHP-scripted web pages with the database. The users will can customize the pages where are localizes the distinct components needed to create the model, adding, modifying and editing these components as well as their characteristics, aspects and parameters if its them have. Also allows transform the model seen on the Media Wiki into a SBML file, it may then be imported into a simulation program and consequently analyzed.

3. Desktop simulator: To simulate the reaction network which has generated previously by SynBioSS Designer first the file "SBML" or "NetCDF" generated is loaded to reproduce the progress of the reaction network that will be export as a set of ".csv" files whose values can be plotted by a graphing program to visualize the progress of one parameter respect to others. The ".csv" files are opened as an excel table where are displayed the values based on all parameters fixed on the Desktop Simulator previously. This simulator offers a set of features that can affect to reactions progress of the model. Can be changed the rate law, initial conditions and kinetic constant for each reaction, modifying their progress for each one in order to change the global process of the reaction network. Subsequently are modify specific simulation parameters among which are found the length of the simulation, the number of trajectories for simulate, the cell volume as well as the cell doubling time and their standard deviation. Also there is the possibility to choose the integration algorithm to solve the model created by SynBioSS Designer. To interpret the reactions networks and solve them, Desktop Simulator contains a set of mathematical algorithms [Howard Salis et al., 2006] and will use some or others depending on the reaction network developed. After the model has been characterized should be exported the model as a SBML file or run the simulation and then export data as a ".csv" file.

Registry of Standard Biological Parts

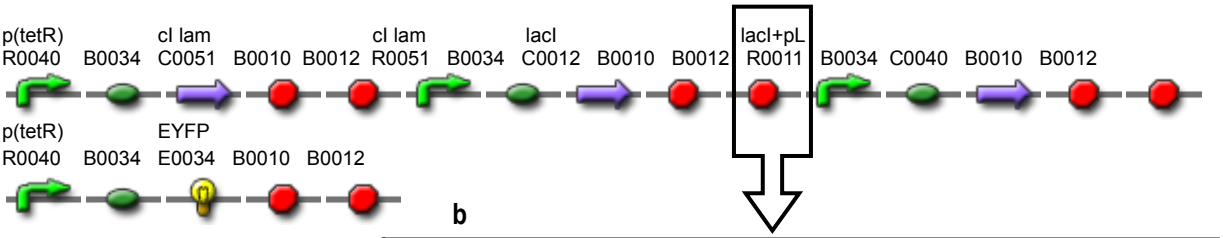
Is a database composed by a collection of genetic and biological parts can be used and combined by different software to design synthetic biological systems. The iGEM, academic labs and scientific institutes always are providing new biological parts and genetic devices so is continuously growing. This fact allows a progress and an improvement on the design and creation of biology synthetic systems by SynBioSS Designer, since is based on all of the parts and devices of this Registry to create the models. So, the usage of these parts provides a lot of possibilities approached to achieve the desired model.

The available parts that are used to build the circuit has a great changeability, so for each part, there are variations of the same, providing them distinct characteristics will suppose other behaviors into the progress of the reaction network. This variability into the parts is shown as a variability of its characteristics such as LVA degradation tail, a sequence contained within the coding region which linked to the protein induces their degradation earlier. A same part can also contain little mutations, e.g. one amino acid variation into their sequence, showing one behavior or another according to the mutation. Can also found hybrid promoters which in contrast to simple promoters drives the expression of several genes, but at the same time have been designed to

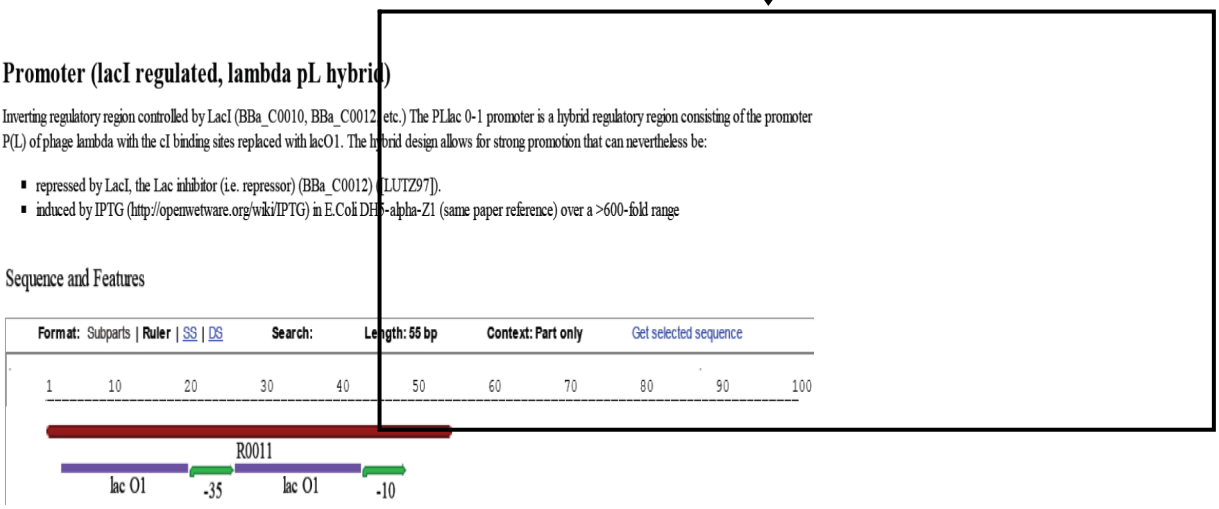
be repressed also by several genes. The registry also contains parts as plasmid backbones that allow the maintenance and propagation of the BioBrick parts or devices.

Thus, the experimental construction of BioBrick parts and devices usually requires working with plasmids and create these plasmids. Through genetic engineer are create the desire plasmids containing the BioBrick which belong to. Finally will be used on a lab to scientific purposes. Thus, It allows a great connection between the design systems and the application of these systems in vivo. Some parts of this database are in the planning stage, instead some others are available or has worked with them in at least one system as database shows, so could work in vivo with these parts with a high reliability and efficiency will assure good results in vivo, getting with a greater probability the purposes established previously. There are available distribution kits to build the BioBrick of interest by dried DNA and this DNA of the BioBricks are stored and maintained in plasmids in cells. The Registry carry out a quality control by sequencing to verify what is the qualitative value verifying if the DNA is suitable to work in vitro and in vivo. Furthermore restriction digests to introduce the BioBrick on plasmid running on gel say if the insert length and the plasmid length and quality are good or bad to work in vivo subsequently. The information for each part of the database is stored as XML format, offering these parts are available to be use through distinct software tools. The XML documents showed provide information about a great number of characteristics for each part. XML language offers support to databases, being useful when some applications must communicate between them.

a



b



c

```
<!--
Parts from the MIT Registry of Standard Biological Parts
-->
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<part_id>10710</part_id>
<part_name>BBa_I724006</part_name>
```

```
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<part_short_desc>
Standard Elowitz Repressilator with Degradation Tag Addition
</part_short_desc>
<part_type>Composite</part_type>
<part_status>Planning</part_status>
<part_results/>
<part_nickname/>
<part_rating/>
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<part_entered>2007-10-26</part_entered>
<part_author>Susan Westfall</part_author>
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<specified_subscars>...</specified_subscars>
<sequences>...</sequences>
<features>...</features>
<parameters>...</parameters>
<categories/>
<samples>...</samples>
<references>...</references>
<groups>...</groups>
</part>
</part_list>
</rsbpml>
```

Figure 2. Standard Elowitz Repressilator with Degradation Tag Addition Composite. **a,** the subparts of the Repressilator and the Reporter such as is shown on the Registry, where each subpart is defined by a code. **b**, The Registry displays the information for each subpart, e. g. placI + pL, their properties, sequence and availability, so that we can check if that subpart is suitable for the Repressilator or, on the other hand, if is better change it for other subpart for the system of interest. **c**, the Repressilator Composite in XML language by entering a URL like the Repressilator in this case, Providing information about their subparts and specified of them, sequence, parameters and more.

XML language organizes and labels the documents but moreover make support to database being useful to integrate the information between some software. The XML documents have the advantage that can be modified easily to transform one part in another very similar at the first with changes that it will be more suitable to work in vivo and achieve the objectives.

Copasi

COPASI [Stefan Hoops et al., 2006] is a tool aided to help biologists, biochemists and scientists in other related fields to write and simulate biochemical networks. This tool has proven its validity to model genetic circuits, as it has been included as the modeling platform running under GenoCAD [Y. Cai et al., 2006], an open source web application for synthetic biology (cite). The program offers four different sections in order to construct the model, named as: model, tasks, output specifications and functions.

COPASI allows the biological model to be written in several different ways. However, it is intended for scientists having some knowledge of biochemistry, as it includes a set of biochemical functions 'ready-to-use' and implement in the model. In order to write the model, there are several parameters common to all types of model construction that COPASI accepts. First of all, the user defines the timescale of the model, the compartments

involved and their volumes, and the units to be used in the simulations. Whether the model is using particle number or particle concentrations has to be defined as well. A special feature of COPASI is that it permits to consider the growth of all compartments. This growth might be important in some models as it changes concentration of particles, although the number of particles is not changing. Therefore, the user can set the volume as constant or define a formula or differential equation that specifies its change. However, a functionality that is not included in COPASI (and, to our knowledge, in no other program publicly available) is the capability of considering local concentrations inside a cell, and diffusion mechanisms.

Once the compartments have been defined, the species involved in the model have to be declared in the menu 'Species' inside the biochemical section of the 'Model' area, and their changes along time must be specified. These changes can be defined by biochemical reactions. COPASI includes 38 biochemical functions to create the models, and the user can define new ones. These can be seen at the 'functions' section. Other forms to determine the changes in particle number or concentration include formulas specified by user ('assignment') and ordinary differential equations ('ODE'). Furthermore, species may also have a fixed concentration or particle number. Following, the user has to define some global values, the parameters of the model. These might be reaction constants or parameters for the differential equations. These values, as occurred with species and compartment volumes, can be fixed or change along time with a given formula or differential equation.

Thus, in order to construct the repressilator, a set of species has to be defined. Then, all parameters must be specified and the reactions or differential equations that join all these parts must be written. A major feature of COPASI is its ability to import SBML files to define the models. This can be used to design the circuits and models in more intuitive tools and then import them to COPASI so as to perform the proper simulations. It is also possible to export a model in this same format. We used this functionality in order to import the oscillator model available at the GenoCAD website, a model describing the behavior of the repressilator.

With this model, we tested some of the functionalities that COPASI offers in order to perform simulations and estimate and/or optimize unknown parameters of the model. One of the most useful functions that this program offers is the possibility to simulate a time-course experiment, to see the change of other species. By defining the time of the simulation and the timestep, it is possible to obtain a plot like the one in figure 3, showing the change in concentration of the different species of the repressilator involved.

It is possible, within the time-course task, to set sliders of the different parameters involved, or the initial concentrations of the species. Moving this sliders it is possible to see very quickly the effect on the behavior of the circuit, as it is not necessary to change the model each time.

For the simulations, stochastic and deterministic methods can be chosen. However, stochastic (such as Gibson and Bruck) and also hybrid methods (Runge-Kutta) can only be selected when the model has been defined with the biochemical reactions and not with ODEs. Furthermore, the number of particles of each species has to be low, as otherwise simulations with deterministic methods are good approximations that run faster and spend less memory. In order to show the results, a variety of plots and tables can be obtained.

Other functionalities available in COPASI permit the user to define the variable space, explore the parameters of the reactions and optimize their values. These functions are present in the "Tasks" area, and are 'Parameter scan', 'Optimization' and 'Parameter estimation'. The function 'Parameter scan' is able to perform the simulations as in the time-course experiment, changing one or more of the parameters of the model in each simulation, in a range of values specified by the user. It overplots the results of each single simulation so that it is possible to visualize the effect of the change of the parameters.

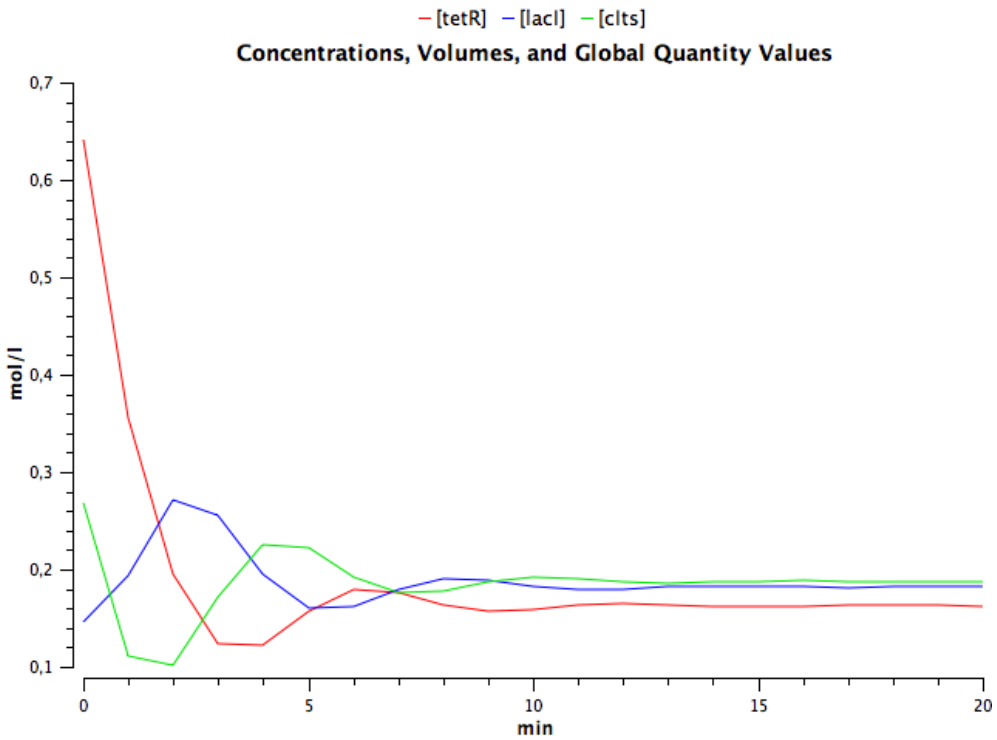

Figure 3: Output of the time-course simulation run in COPASI. Three different species and their concentrations are shown. It was observed that by changing the initial concentrations of each of the species, it was possible to obtain different patterns of this oscillations that decay with time

'Optimization' allows the search for the optimal parameters in order to maximize a value, a concentration of some of the species or a user-given function. From a given parameter and a range of possible values, it will return the value that best fits the conditions imposed. Up to 14 different optimization functions and algorithms are available, from random searches to evolutionary approaches, a variety that cannot be found in other tools.

Finally, if there are experimental data available, the function 'Parameter estimation' searches for the best values for the given parameters that make the model fit the data. One or more experiments can be evaluated in order to try to fit several parameters.

There are other functions available in the 'Tasks' section of COPASI, which we do not discuss, as they are less relevant for our further analysis and conclusions. These functions include metabolic control and steady-state analyses and, as for the rest of functionalities here introduced, a complete documentation can be found on the website of the software.

Comparative between SynbioSS-COPASI

Have checked that it is possible to export the SBML file provided by SynBioSS Designer to COPASI. It supposes is achieved a generation reactions network based on a set of biological rules and laws so SynBioSS Designer operates as a biological rule-based algorithm. However, SynBioSS Designer lacks other properties that contain the COPASI software as well as some limitations. When is begun the simulation parameters assignment the user will can see that COPASI software is better than SynBioSS software, thus COPASI could provide more accurately and specific results when run the simulation if is desire, being a simulation will consider more

parameters present in the experimental environment than SynBioSS. Furthermore allows introduce differential equations as describing the behavior of the model as it has been designed previously. So, in this case the Repressilator model behavior could be directed by these equations modifying their parameters values to get the desire result through COPASI software, where each parameter represent the distinct components of the model. While would not be possible carry out this direction by SynBioSS because don't have the option to introduce diferential equations. Consequently, COPASI simulator is more accurate and extended than the Desktop Simulator of SynBioSS, and could then achieve better results corresponding to the directed progress of the model through changes on COPASI simulator parameters values. COPASI software allows also introduces other factors that do not belong to the model but have influence upon the behavior of the same, e. g. inducers that affect to promoters, and control these inducers through the differential equations, generating one development or another according to the presence or absence of these factors or their own concentrations. Adding these factors is provided a change on the progress of the model that will be reflected in the run simulation as a different development of the model, predicting through the software a more accuracy of the behavior system corresponding to these factors.

Instead, COPASI generate the model according to other biological rules whose seem less like to the experimental reality, obtaining a less accuracy model compared to the experimental model. Whereas SynBioSS Designer is based on biological rules and laws whose will allow a development of the model to obtain a reaction network more likely and realty in relation to how would be obtained in vitro or in vivo. SynBioSS Designer identifies the type of each input of the model that has added and has different characteristics for each one of them, generating then that the progress is developed for one pathway or another according to the modifications realized on each input (promoters and coding regions). In addition, SynBioSS uses The Registry of Biological Parts to construct the model, which implies that could become in a changeable and accurate designer, so is possible create almost any model want design. This database contains a big collection of genetic parts that can be combined to build complex synthetic biology systems and devices. As well as having a wide collections, it is noteworthy that a lot of these genetic parts have been verified to be functional, so, these two points allow any model will can be designed and created in vivo more easily.

The new software tool. Joint implementation SynBioSS-COPASI.

Both design and development of the model as the simulation is improved combining the two software tools, increasing the relationship between the progress and results obtained with two software combination and the experimental progress and results for the same model. In conclusion have proved that the design part is better make it on SynBioSS Designer, instead the simulation is more accuracy and suitable with COPASI. To realize the new software must pay close attention to the changeable corresponding to the formats of input and output files that the software can accept and provide respectively. It will allow that a combination of the best advantages of each one would be possible. The XML format allows communicate some applications between them as well as provides the exchange structured information between platforms and this language can be used in a lot of database and facilitates the SynBioSS-COPASI implementation, also uses then MathML, the XML language for storing mathematical expressions to represent complex rate laws. As priority to get more useful and reality models is necessary a suitable unification between SynbioSS Wiki and the implementation SynBioSS-COPASI through a gradual growth of the amount data on the Wiki. Also should be established a relationship between databases and parts design that use SynBioSS and COPASI, Registry Standard of Biological Parts and GenoCAD respectively, made up a joint applicability of them to use in any software. However SynBioSS Designer lacks of certain biological rules and has some limitations [Emma Weeding, et al., 2010] that are important from a biological point view, thus, to create the new software, should be programmed new biological

and biochemical rules into the new software, getting a software which displays robustness, modularity and complexity to explain the biological processes and generate them with a high reproducibility [Michal Galdzicki et al., 2009].

Conclusions

It has been proposed to combine the usage of these tools to obtain suitable results that can be reproduce reliably on laboratories, obtaining consequently better experimental results. These researches as well will allow to design and discover different experimental approaches that will allow laboratories to make great improvement into distinct fields as bioinformatics, medicine… The studies of these software, their properties, changeability and compatibilities, as well as the comparison among themselves according to simulate the biology processes have been directed in order that provide a new suitable software to perform the simulations of the designs optimizing the experimental costs. At the same time, it will allow scientists to introduce and predict the new variables related to biological processes which will suppose a valuable progress on research projects that are soon starting up.

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