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# Designing and Engineering of Biological Systems using Computational Tools

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**ABSTRACT**
The highly complex biological nanocomputers in living cells involve integration of multiple inputs, performing computations on these signals, storing the information in memory and responding to the environment. Computational tools and techniques have been developed for modification and designing of the DNA, RNA and proteins. Diverse paradigms have emerged for designing, modeling, constructing and characterizing of artificial genetic systems. By utilization of biological databases and computational tools, synthetic biologists have constructed novel genetic circuits through rational design and forward engineering that enable living systems to sense their dynamic environments, perform computation on the inputs and formulate appropriate outputs. Synthetic feedback loops or embedded biosensors can also be used as built-in control mechanisms for monitoring or triggering the cellular processes. Engineered living cells have the potential to perform a wide range of desirable tasks for biological applications in biotechnology, biomedical engineering and basic biology studies. Ultimately, the use of different computational tools and optimal integration of digital logic, analog computation as well as memory circuits will enable powerful next generation genetic circuits to drive innovation in the field of genome designing, cellular functions and synthetic biology. This paper focuses on computational tools and softwares used in metabolic engineering and synthetic biology for designing of genomes, microbial strains and cellular functions.
**Keywords:** Computation tools, Gene circuits, Biological databases, Softwares, Genome designing, Synthetic biology

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## I. INTRODUCTION

Synthetic biology involves the construction of biological systems from the minute functional unit to the functional cellular level through engineering and manipulating of biomolecular systems. The fabrication of biobricks, synthetic cells, genetic circuits and nonlinear cell dynamics, along with engineering of metabolic pathways, has occupied researchers in the field of synthetic biology. Thus, the main focus of synthetic biology is fundamental biological research facilitated by the use of synthetic DNA and genetic circuits [1] [2]. Computer-aided designs and concepts for standardization and hierarchies of parts, devices and systems provide a basis for efficient engineering in biology. Recently developed computational tools, for instance, enable rational and graphical composition of genetic circuits from standard parts and subsequent simulation for testing the predicted functions in silico. The computational design of DNA and proteins with predetermined quantitative functions has made similar advances [3].

The sequencing of whole genome and genomics technologies are also used to develop methods for utilizing genomic information to understand and predict phenotypic function. The rapid improvements in DNA synthesis and enhanced assembly techniques enabled the construction of entire genomes. Synthetic biology possesses the capabilities to design and redesign biological components and systems that will address global food and energy challenges, propel industrial transformations, improve the sustainable bio-engineered processes and offer new gene-based methods to target human medical conditions and insect-borne diseases (Fig. 1) [4] [5]. These include improvements in DNA synthesis (longer fragments and higher accuracy), reduced DNA synthesis and sequencing, new capabilities to read, edit and rewrite the genes and cells of organisms, advances in bio-engineering design and modeling techniques, enhanced tools for biological assembly and engineering, the development of standardized biological parts and the use of automated and data-

intensive methods to speed up discovery and testing [6] [7] [8] [9].

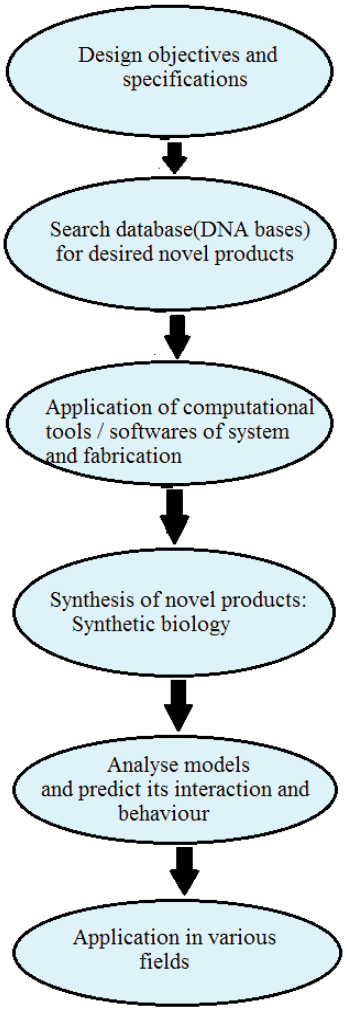


**Fig. 1.** Steps involved in designing synthetic engineered product

## II. APPLICATIONS OF COMPUTATIONAL TOOLS AND TECHNIQUES

Synthetic genomics is useful for the synthesis of DNA. It gives the methods which involves the combination of chemical and computational techniques. It is possible to design and assemble the whole genome by using synthetic genomics. These methods allow scientists to construct genetic material that would be impossible or impractical to produce using more conventional biotechnological approaches. Functional genomics uses genomic data to study about gene and protein expression and its function on a global scale (genome-wide or system-wide). It focuses on gene transcription, translation and protein-protein interactions, and often involves high-throughput method, including the identity of genes and the factors that control differential expression. Potential possible applications of synthetic genomics are biological production of fuels and vaccines generation against emerging microbial disease.

Bioinformatics is the field that develops the methods and software tools for understanding biological data. Research in bioinformatics includes method development for storage, retrieval and analysis of the data. Bioinformatics obtains the knowledge from computer analysis of biological data. It consists of the information stored in the genetic code, but also experimental results from various sources, patient statistics and scientific literature. The techniques like image and signal processing allow extraction of results from large amount of raw data. Bioinformatics tools help to compare genetic and genomic data. It also plays a role in the analysis of gene regulation and protein expression.

The initial genome-scale models were constructed based upon genomic data (DNA sequence) information and biochemical data (reaction stoichiometry) in conjunction with linear

programming to apply mass balancing principles to a whole-cell system. These models range from understanding the underlying structure of networks by using model-building approaches [10] and progressively more cellular details including transcriptional regulation [11] and signaling pathways [12]. All of these models have contributed to improve the predictive capability and accuracy of genome-scale metabolic models and can be used to study a variety of aspects of cellular systems.

A broader challenge in synthetic biology is to engineer existing genomes for bio-manufacturing or to decipher the principles that govern the operation of biological systems [13] [14]. Recently, synthesis capabilities have progressed from a Mycoplasma genome of 582,970 base pairs to a 1.08-mega-base-pair Mycoplasma genome transplanted into a recipient cell lacking a genome [15] [16] [17]. Dymond et al. [18] reported the remarkable synthesis of the right arm of chromosome IX in yeast and a portion of chromosome VI. These genomes were integrated into yeast cells with minimal phenotypic variation in growth and gene expression. This work provided a valuable method of studying the yeast genome and adapting yeast to specific applications such as biosynthesis.

Next-generation sequencing (NGS) has revolutionized the field of biology over the last decade. The Genomes OnLine Database (GOLD) that monitors sequencing projects worldwide has grown from just 1575 sequencing projects in 2005 to over 70,000 in 2015 [19]. This is partly caused by a rapid drop in the price of high-throughput sequencing [20], but also an increase of free user-friendly bioinformatical tools such as MG-RAST [21], MEGAN [22] and user fora such as seqanswers.com, biostars.org etc. In recent publications up to 100 Gbases have been sequenced [23], which allowed even a partly reconstruction of genomes of single microbes from the obtained reads [24]. Vestergaard et al. [25] discussed some basic guidelines for the experimental design of metagenomic surveys to characterize community composition and function of soil microbiomes, without losing the environmental context.

## III. CONTROL OF GENE EXPRESSION AND GENETIC ENGINEERING

Genes are a stretch of nucleotides which code for different polypeptide sequences. Genes are isolated and amplified artificially by polymerase chain reaction (PCR) with gene specific primers if the DNA sequence of the gene is known. The desired sequence may also be synthesized artificially by solid phase DNA synthesis. Artificial gene synthesis in synthetic biology is used to create artificial genes in the laboratory. It differs from molecular cloning and PCR in that the user does not have to begin with preexisting DNA sequences. It is possible to make synthetic double stranded DNA molecule with no size limits. Oligonucleotides are synthesized by phosphoramidite nucleosides artificially and used nucleosides can be natural or artificial. 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 [26] [27]. Designed expression control for individual genes typically occurs either at the transcription or translation levels.

The first level of functional control for specific genes occurs during transcription (Fig. 2). The transcriptional process involves binding of RNA polymerase on a DNA sequence (promoter) to initiate biosynthesis of mRNA. 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 [28]. Base-by-base changes in the promoter or transcriptional modulating sequences called UP elements [29] could be accurately synthesized and tested in synthetic DNA constructs.

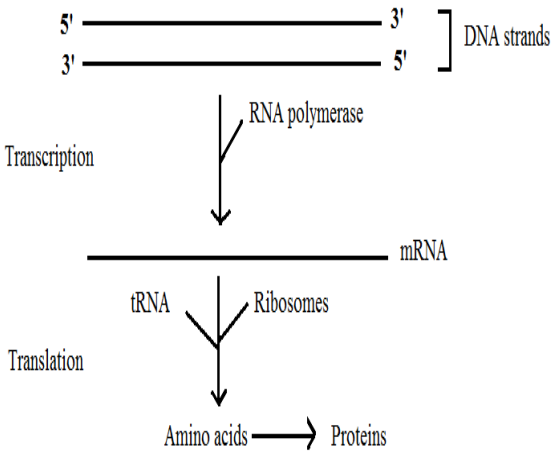


**Fig. 2.** Promoter sequences on the DNA are recognized by the RNA polymerase and sense strand of double stranded DNA acts as template strand for synthesis of mRNA (transcription). The message in mRNA is translated in synthesis of different aminoacids by the ribosomes in conjuction with tRNA. Aminoacids get polymerized to make different proteins in living cells.

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. Due to variation in the DNA sequence of the RBS within each cell, different rates of translation initiation are found. 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 [30]. This calculation specifically considers the interaction of the 30S ribosomal subunit with a specific mRNA sequence.

## IV. COMPUTATIONAL TOOLS AND SOFTWARES USED IN SYNTHETIC BIOLOGY

Historically, natural products have been the major source of lead compounds for antimicrobial drugs, but also are used in other application fields, such as anti-cancer drugs, insecticides, anthelmintics, pain killers, flavors, cosmeceuticals and crop protection. With the broad availability of 'omics technologies', we currently experience a paradigm shift in natural product research [31]. For decades, the only way to get access to new compounds was to cultivate antibiotics-producing microorganisms, mainly fungi and bacteria, under different growth conditions, and then isolate and characterize the compounds with sophisticated analytical chemistry. Nowadays, 'omics approaches offer complementary access to natural products; by identifying natural product/secondary metabolite biosynthetic gene clusters (BGCs). It is now possible to assess the genetic potential of producer strains and to more effectively identify previously unknown metabolites using the available databases (Table 1). This information will also be the basis to rationally engineer molecules or develop "designer molecules" using synthetic biology approaches in the future [32].

Public access databases such as KEGG [33], MetaCyc [34] and RHEA [35] were found useful for the designing of metabolic pathways. A database containing molecular and biochemical data of enzymes, BRENDA can be useful to select the core pathway capable to produce the metabolite of interest [36]. Web servers, such as From-Metabolite-To-Metabolite (FMM) [37] and Metabolic Route Search and Design (MRSD) [38] can also be used for designing synthetic and unique metabolic pathways in cell-free systems.

Using the natural ability of genome-scale metabolic models to simulate the behaviour of cellular metabolism, cellular designs for maximizing chemical production can be predicted. A genome-scale model of *Escherichia coli* was demonstrated to predict strain designs for the over-production of lactic acid [39], which set the stage for genome-scale models as powerful computational tools for strain design. Controlled sequence-to-function relationships could then be extrapolated using mathematical correlation methods such as a position weight matrix (PWM) (Table 2). Recently, PWMs have been used to quantitatively describe the sequence-to-function relationship for promoters in *E. coli* [28]. Recently, synthetic promoters have been designed to produce a desired level of transcriptional strength with the recent modeling developments. To calculate the relative contribution of each enzymatic step in the pathway when optimization of particular objective function is required, Flux Balance Analysis (FBA) is commonly used [40]. The "Ribosome Binding Site Calculator" can be used not only to predict translation initiation rates for existing sequences, but also to design de novo RBS sequences for synthetically controlling translated protein levels. 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 [41]. These tools enable a finer level of design control for biological systems and can be implemented for metabolic engineering applications.

**Table 1**. Computational Databases used for Designing of Metabolites in Synthetic Biology

| **Databases** | **Description** | **Databases** | **Description** |
|---|---|---|---|
| BLASTN | Used to perform fast similarity searches among DNA nucleotides | MetaCyc | Contains information about metabolic pathway of model organism |
| FASTA | Uses 'hashing' strategy to find database similarity | BioModels | Contains published quantitative models |
| KEGG | Contains information about gene function | GLIMMER | Used for finding genes in microbial DNA |
| ClusterMine360 | Web accessible database of BGCs | StreptomeDB | Web accessible database on compounds produced by streptomycetes |
| ClustScan Database | Web accessible database of PKS/NRPS BGCs | ChemSpider | Web accessible database on structures and properties of over 35 million structures |
| Recombinant ClustScan Database | Database of in silico recombined BGCs | PubChem | Web accessible database on compounds and bioactivities |
| BRENDA | Contains information about properties and function of enzymes | RHEA | Comprehensive resource of expert-curated biochemical reactions |

| | | | |
|---|---|---|---|
| Alliance for cellular signaling (AfCS) | Contains information for studying signal process | Pathway tools | For creating model organism databases |
| GENSCAN | Identifies complete gene structures in genomic DNA | ERGO (WIT) | Contains information for comparative analysis |

By utilizing transcriptomic data of the experimental strain, algorithmic analysis predicted specific genes to be targeted for synthetic regulation with increased or decreased expression [42]. In this approach, the experimental data was translated to a binary present/absent scoring for each individual transcript/protein. The scored experimental data was then algorithmically integrated with the metabolic model framework using mixed integer linear programming (MILP) to calculate a flux state that is in concurrence with the experimental data (Table 2). The formulation of the Expression matrix or E matrix was major conceptual advances in the constraint-based modeling methodology in *E. coli* [43]. The stoichiometric matrix provided the basis for all simulations utilizing flux balance analysis (FBA). The E matrix represents a major advancement in prediction as it explicitly accounts for all mechanisms required for transcription, translation and modification of each gene product. Based upon the stoichiometric matrix, dynamic flux balance analysis (DFBA) was initially developed [44].

A multi-level optimization computational framework known as OptCom was developed for studying microbial communities and the interactions within those communities [45]. To facilitate the design process, a growing number of algorithms have been developed that expand the predictive capabilities of genome-scale models to simulate different strain design parameters. OptKnock was one of the first strain design algorithms developed for use with genome-scale metabolic models [46] that formulated a bi-level optimization, where gene deletions were found to increase the production of a desired chemical while maintaining cellular growth.

**Table 2.** Computational Tools used In Product (Metabolite) Designing in Synthetic Biology

| **Tools** | **Description** | **Tools** | **Description** |
|---|---|---|---|
| PWM | Prediction of DNA sequence variation on promoter strength | RBS calculator | Prediction of protein translation initiation rates |
| E matrix | Prediction of gene and protein expression levels | Virtual Cell | For modeling and testing biological networks |
| OptCom | Multi-level optimization for modeling microbial consortia | BioJake | Visualization tools for manipulating metabolic pathways |
| OptKnock | Bi-level optimization for strain design using gene deletions | STOCKS & Stoch-Sim | Stochastic kinetic simulation tool used for biochemical process and chemical reaction |
| FBA | Flux balance analysis | CellWare | For deterministic and stochastic cellular events |
| DFBA | Dynamic flux balance analysis | COPASI | For simulation of biochemical events |
| MILP | Refined flux state predictions based upon high-throughput experimental data | Dynetica | To study kinetic model of dynamic network |
| PRODORIC | a database and tool platform for the analysis of gene regulation in prokaryotes | GeneDesign | a web-based suite of tools/modules aiming to aid both the analysis and design of synthetic genes. |

The software bridges the gap between the kinds of instructions biological designers would like to use for designing a synthetically biological compound (Table 3). Java Codon Adaptation Tool (Jcat) is a web-based application featuring codon usage optimization based on CAI score, restriction enzyme binding site elimination, rho-independent transcription terminator elimination, and prokaryotic ribosome binding site elimination [47]. Eugene is a stand-alone tool developed for multi-objective gene optimization [48]. The program uses an intuitive user interface that is straightforward and easy to use. Eugene also automatically loads relevant database data (KEGG, NCBI databases) upon loading a gene to the workspace using identifier information provided in the gene's FASTA/GenBank file. D-Tailor is another stand-alone tool (written in Python) that employs multi-objective optimization and modularity in creation of synthetic genes [49]. Codon Optimization OnLine is another web-based utility that can optimize for multiple objectives [50]. Optimization functions that the program can perform include codon usage optimization based on CAI and Individual Codon Usage (ICU), codon context bias optimization, hidden stop codon optimization, G/C content adjustment, and restriction site and other pattern elimination.

**Table 3**. Softwares used for Designing Synthetic Biological Compound

| **Softwares** | **Description** | **Softwares** | **Description** |
|---|---|---|---|
| Java Codon Adaptation Tool (Jcat) | Web-based application featuring codon usage optimization | Codon Optimization OnLine | Used in codon optimization |
| ORBIT, PRODART | For biomolecular designing | Geneetdes, RoVerGeNe | For automated circuit design |
| Gepasi | For modeling chemical and biochemical reaction networks | BioSilico | Integrated web-based system for studying metabolic process and pathways |
| BioSPICE | To access computational tools | Cell Designer | For diagrammatic editing of biological networks |
| Gene Designer | Facilitates the construction of novel genetic material; to add and remove genetic elements. | Visual Gene Developer | Utilizes modular optimization components, enabling user-accessible programing and addition of new functionality. |
| D-Tailor | Used in creation of synthetic genes | Eugene | Tool developed for multi-objective gene optimization |

Engineered strains have been constructed for usage as sensors to detect small molecule environmental stimuli in the mammalian gut [51]. Tools are being developed for engineering species of gut bacteria already well suited for colonizing the gut; these include members of the well-represented Bacteroidetes and Firmicutes [52]. Synthetic biologists have developed a toolkit amenable for engineering of the commensal *Bacteroides thetaiotamicron* comprising characterized promoters, RBS, inducible systems and the CRISPRi platform [53]. Saeidi et al. [54] engineered *E. coli* to sense *Pseudomonas aeruginosa*, a bacterium causing infections in the lung, urinary tract, gastrointestinal tract and skin. Quorum sensing was linked to expression of genes for pyocin (a bacteriocin) and a lysis protein E7. When grown in the presence of *P. aeruginosa*, the engineered *E. coli* accumulated intracellular pyocin and E7. The sufficient levels of E7 protein lysed the cell and the release of pyocin killed the pathogen, and also inhibited formation of biofilm.

## V. CONCLUSIONS

Using recent biological databases, genome-scale models, optimization of algorithms and metabolic network analysis, engineered molecules/metabolites can be produced within cells of diverse bacteria [55]. Designed tools coupled with rapid DNA synthesis and assembly technologies have accelerated the prototyping, tuning and deployment of synthetic biological systems for various applications. Moreover, synthetic DNA construct could be transferred in a microbial strain/living cell and these designed DNA sequences could provide desired levels of transcription and translation to achieve enhanced protein production [56]. Novel genetic circuits with useful applications have been constructed through rational design and forward engineering by the synthetic biologists. Efficient strategies have been described for rapidly identifying and correcting causes of failure and fine-tuning of genetic circuit characteristics [57, 58].

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