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BioFNet: biological functional network database for analysis and synthesis of biological systems

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Abstract

In synthetic biology and systems biology, a bottom-up approach can be used to construct a complex, modular, hierarchical structure of biological networks. To analyze or design such networks, it is critical to understand the relationship between network structure and function, the mechanism through which biological parts or biomolecules are assembled into building blocks or functional networks. A functional network is defined as a subnetwork of biomolecules that performs a particular function. Understanding the mechanism of building functional networks would help develop a methodology for analyzing the structure of large-scale networks and design a robust biological circuit to perform a target function. We propose a biological functional network database, named BioFNet, which can cover the whole cell at the level of molecular interactions. The BioFNet takes an advantage in implementing the simulation program for the mathematical models of the functional networks, visualizing the simulated results. It presents a sound basis for rational design of biochemical networks and for understanding how functional networks are assembled to create complex high-level functions, which would reveal design principles underlying molecular architectures.

Keywords: *biological database; simulator; functional network; network motif; rational design*

INTRODUCTION

The goals of systems biology and synthetic biology are to reveal the mechanisms of how large-scale complex biochemical networks generate responses to environmental stresses, stochastic fluctuations or genetic variations and to enable rational design of such networks for engineering purposes [1–5]. The biochemical network is a sound basis for a bottom-up approach to dynamic modeling for system analysis and rational design [6]. A number of dynamic models, from networks of a few components to whole-cell models with hundreds of components, have been constructed in a wide range of species from microbes to mammals [7].

It is difficult to understand the entire biochemical network of a cell because it is too large and complicated. An alternative method would be to decompose the whole network into subnetworks called 'building blocks' [8] in terms of topology or regulatory architecture and to simulate and analyze their associated mathematical models. The system is regarded as the hierarchical assembly of these subnetworks [9–11]. Biological parts or biomolecules [12] are assembled into building blocks, including network motifs [13]. These building blocks are combined to generate a complex high-level function. This synthetic approach is analogous to the standard strategy of engineering systems with a scalable

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hierarchical modular structure, where a set of off-the-shelf parts with operation specifications can be combined.

To analyze or design a biochemical network, it is critical to understand a variety of relationships between network structure and function (RNFs) [14], the mechanism by which biomolecules are assembled to form a functional network [15]. In this review, the functional network is defined as the subnetwork of biomolecules required to generate a particular function. An understanding of the RNFs of the functional network provides an analytical methodology for the structure of a large-scale network and rational guidance for how to design a robust biological circuit to carry out a target function.

Identifying network motifs has helped to illustrate fundamental building blocks and elementary networks [9, 16–18]. While it may be difficult to rigorously define the elementary network, it can be regarded as the minimal or small subnetwork responsible for specific biological functions such as ultrasensitive response, homeostasis, amplification, adaptation, noise filtration, pulse generation, oscillation and bistability. Note that elementary networks are a part of functional networks. As with LEGO blocks, elementary networks can be assembled into a hierarchy to synthesize a large-scale network for complex function.

To find elementary networks, exhaustive computational searches and theoretical analyses have been used to explore the full design space of 2- or 3-gene networks to enumerate every possible unique topology that is capable of executing a specific function [9, 19–21]. Although elementary networks are likely to have more than three nodes, many can be reduced to simpler 2- or 3-gene networks or low-resolution networks, assuming that multiple molecules often function in concert as a single virtual component [15, 22]. This sacrifice in resolution enables the exhaustive search of the full design space. Automatic modeling by combination of biomolecules has been proposed [22–25]. In this method, biomolecules are combined in silico to search the wide space of kinetic parameters to achieve a target function. A parameter-free method of chemical reaction network theory was presented to characterize the bistability function of enzyme reaction and gene regulatory networks [22, 26].

The RNFs of functional networks such as chemotaxis [22, 27–29], MAP kinase [22, 30], two-component signaling systems [22, 31, 32] and morphogen gradient-induced pattern formation [22, 33–36] have been described in detail. Those individual studies that focus on details of biochemistry and kinetics are effective at identifying the RNFs that coarse computational searches may miss.

These approaches have identified or suggested a vast number of RNFs, but they have not synthesized into a comprehensive model, despite their importance in systems and synthetic biology. To intelligibly illustrate the RNFs, we have developed the biological functional network database named BioFNet that has the capacity to cover the whole cell at the level of molecular interactions. To facilitate understanding of the RNFs, we interpret them in the context of engineering control systems. BioFNet takes advantage of a simulation program for mathematical models of functional networks, visualizing the simulated results. It provides a sound basis for rational design and engineering of biochemical networks and for an understanding of how functional networks are assembled to perform a complex high-level function, revealing the design principles underlying molecular architectures.

NETWORKS

A machine can be separated into modules and parts in a hierarchical manner. The parts are assembled to make basic functional modules such as power supply, sensor, actuator and controller, which are further assembled to form a complete system. Analogous to the machine, the biochemical network of a cell can be decomposed into biomolecules, elementary networks and combined networks, as shown in Figure 1. Biomolecules correspond to biological parts. The function of a biomolecule can be illustrated by the regulator and reactions [22, 24, 37, 38].

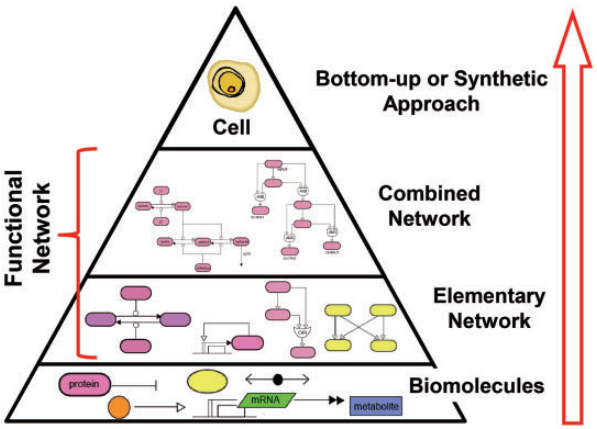

Figure 1: Hierarchical structure of biomolecules, elementary networks and combined networks.

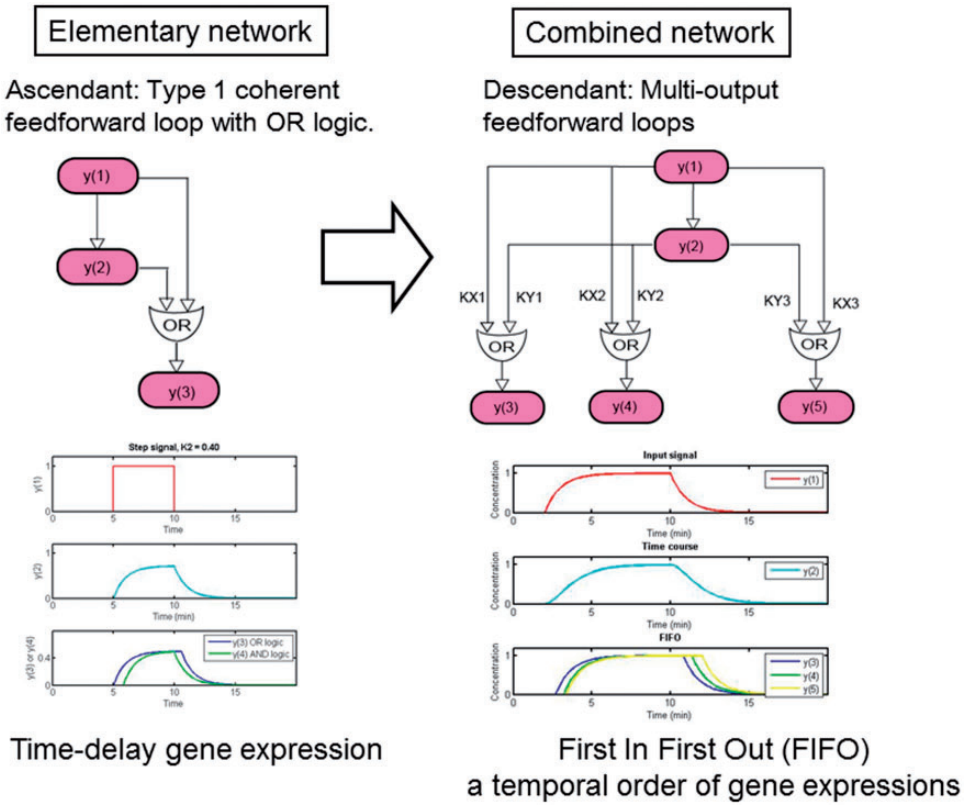

Figure 2: A functional network built by combination of elementary networks. Combination of FFLs generates multi-output FFLs with the FIFO function. The FFL network is the ascendant; the multi-output FFL network is the descendant.

Biomolecules are assembled to form the fundamental building blocks or elementary networks. The elementary network, which includes network motifs such as feedforward loops (FFL), autoregulation, a single input module and a dense overlapping regulon [13, 22], is regarded as the small- or minimal-scale network that consists of a few interacting biomolecules and is responsible for generating particular functions such as sigmoid response, amplification, adaptation, bistability and oscillation. The elementary networks are assembled into a combined network to perform a complex function. For example, superposition of the FFL with OR logic produces a First In First Out (FIFO) function [22, 39] (ID128 in the database) (Figure 2). The combination number of elementary networks and biomolecules is extremely large, suggesting many potential functions. Note that the elementary networks and combined networks are a part of the functional networks (Figure 1).

DATABASE

To register the functional networks in an intelligent manner, we develop the BioFNet (Figure 3), where each functional network is characterized as shown in Figure 4. It enables keyword searching with biological and engineering terms (http://kurata22.bio.kyutech.ac.jp/db/pub/pub_main.php?Ver=3.4). Figure 3A shows the search panel where key words are input and the output panel where search results appear. Figure 3B shows the record of the selected functional networks. Figure 3C shows the calculation tool that simulates a mathematical model and visualizes the simulated results while changing the values of critical parameters. At present 181 records are registered. The instruction is provided by Supplementary data 1. The architecture of the BioFNet is shown in Figure 5. Its outstanding feature is that it implements the numerical simulation and visualization programs provided by the Matlab (Figure 3C).

Searches for functional networks of interest are entered in the left panel using key words associated with network topology, function, network name and engineering function. Search results appear in the right panel. Clicking a record of interest displays its contents. As shown in Figure 4, the 'Spec' tab

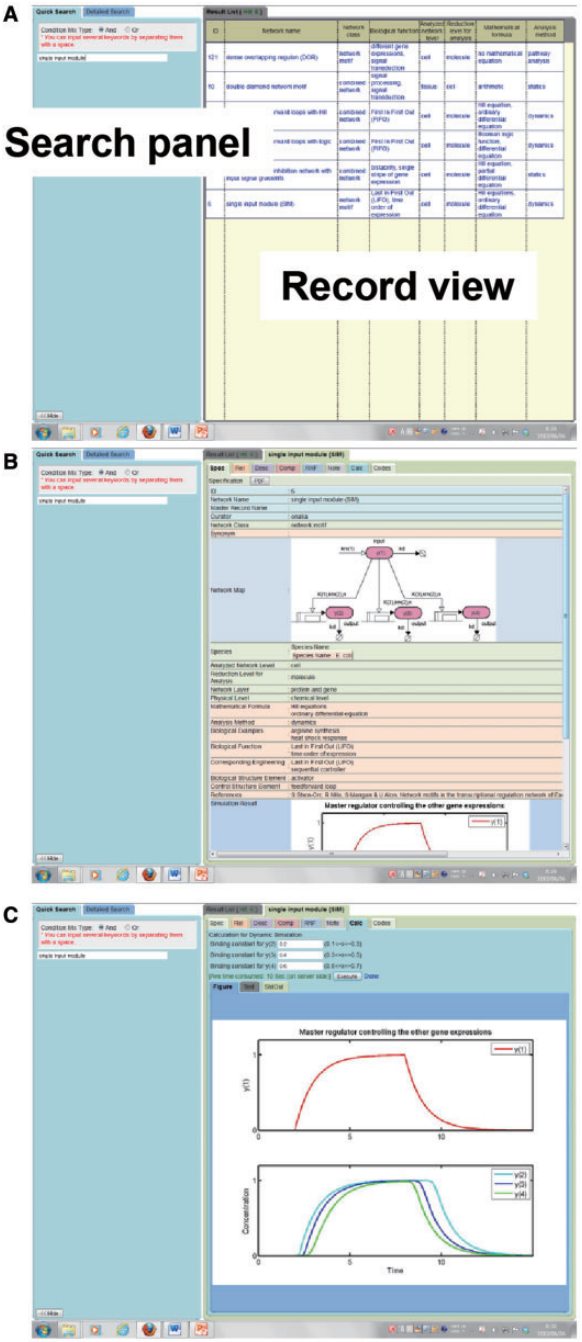

Figure 3: Specification of a functional network. Details are described in the DB.

illustrates many items to explain the features of the functional network, including network name, network map, function and simulated results. The 'Rel' tab presents the relationship between the ascendant and descendant functional networks. The 'Desc' tab provides an explanation of the background, network structure and simulated results shown in the 'Spec' tab. There are many synonyms for network architecture and function. The 'Comp' and 'RNF' tabs provide descriptions in the context of engineering and RNF, respectively. The 'Note' tab presents the mathematical equations, theory and detailed mathematical interpretations. In the 'Calc' tab, users can simulate the mathematical model encoded by the Matlab program, while changing the value of key parameters. The 'Codes' tab shows the corresponding Matlab programs.

TYPICAL FUNCTIONAL NETWORKS

The same function by different networks

The same biological function can be generated by different types of functional networks. Here we focus on specific functional networks to demonstrate that different network topologies can encode the same function.

Perfect adaptation

Perfect or exact adaptation, where the steady-state level of the output is independent of changes in the input signal after a transient response to the change, is achieved by different functional networks: combined linear reactions (ID 203) [16, 22], incoherent FFL (ID 12) and feedback loop (ID 15, 146, 147, 297) [22, 28, 40]. In the combined linear reactions, supplementing the simple linear reaction with a second signaling pathway (X) can create a response mechanism that exhibits perfect adaptation (R) to the signal (S) (See ID 203). The integral feedback control is a basic engineering strategy for ensuring that the output of a system robustly tracks its desired value independent of noise or variations in system parameters [28, 29]. The response to an extracellular stimulus returns to its prestimulus value even in the continued presence of the input signal.

Bistability

Bistability is a basic feature of many functional networks and is used as a toggle switch in the decision-making processes of cell-cycle progression, differentiation and apoptosis. Bistability is typically generated by positive feedback loops with ultrasensitive response, caused by cooperative transcription factor binding (ID 63, 65, 66, 67, 124, 171) [16, 22, 41–43]. On the other hand, a two-gene network with a positive feedback loop has been reported to produce bistability without cooperative transcription binding (ID 264) [22, 26]. In this network, one gene is the repressor and the other plays the dual functions

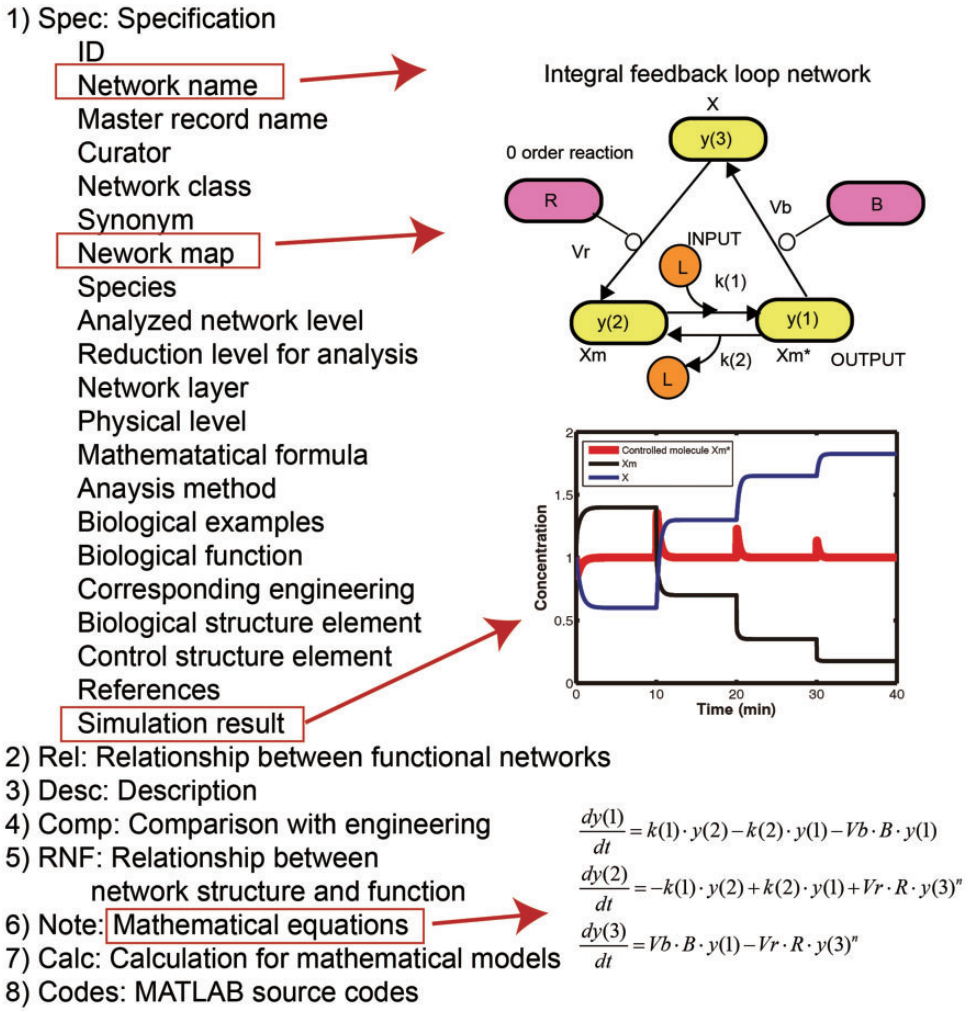

Figure 4: BioFNet database. (**A**) Search panel. (**B**) Record content. (**C**) Simulation tool and simulated results.

of self-activation and suppression of the repressor. An essential mechanism is the competitive binding of repressor and activator to the promoter.

Some functional networks show bistability without explicit positive feedback loops. A chain of phosphorylation reactions can generate bistability (ID 174) [22, 30], where the same kinase consecutively phosphorylates the non- and mono-phosphorylated kinases and the same phosphatase dephosphorylates the mono- and double-phosphorylated substrate forms. In addition, commonly used enzymatic reactions for a single overall reaction, involving one or two substrates, are capable of bistability, suggesting that it is rooted in simple chemistry (ID 271) [22, 44].

Different functions by a unique network architecture

Functions in unique network architecture often depend on reaction kinetics or the value of kinetic parameters. By changing the kinetic values, a positive feedback loop can generate different responses such as slow response, ultrasensitivity and bistability (ID 1, 48, 63, 66, 67, 69, 114, 124, 183, 190, 192) [9, 16, 41, 44–46]; positive and negative feedback loops can produce oscillation (ID 129) or pulse generation (ID 185) [41, 44]; and a three-layer structure of phosphorylation chain reactions can generate ultrasensitivity, bistability or oscillation (ID 174, 249, 250, 251, 253, 259, 281) [30, 44].

Complex functions generated by combined networks

A combination of functional networks can produce a complex high-level function by additive, synergistic and emergent effects, which increases the designability of a biochemical network.

Additive effect

Assembly of functional networks can superimpose their functions. A combination of fast and slow

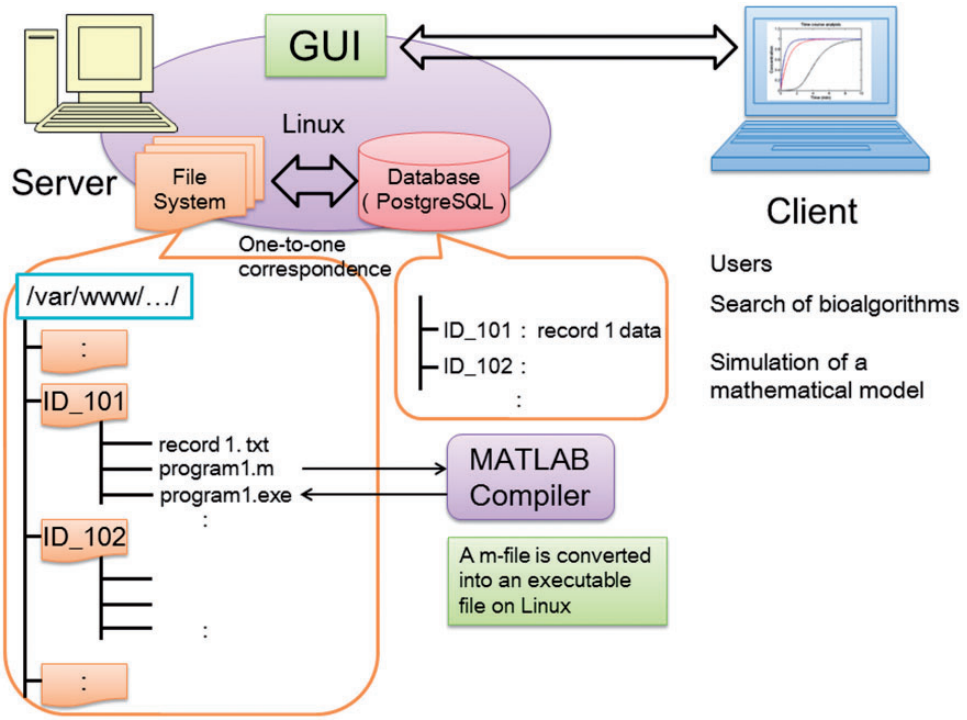

Figure 5: Architecture of the BioFNet. The client–server model is accessed through Internet Explorer 8, Internet Explorer 9 and Firefox 13/15 in Windows XP/Vista/7 and through Firefox 13/15 in Linux. A personal computer [CPU: Intel(R) Celeron (R) 450, 2.20 GHz, RAM: 1 GB] is used as the server machine, running LINUX CentOS5.5. The GUI program is written in PHP 5.2, JavaScript, CSS2 and HTML4. The database can be queried using standard SQL to retrieve functional networks that may be relevant to given key words. PostgreSQL (version 8.4.6) is used to register the functional network data. The mathematical simulation programs are written in Matlab (R2009a). All m-files are converted into executable files by the Matlab compiler and are controlled through PHP. Data are automatically backed-up by Redundant Arrays of Inexpensive Disks (RAIDI). The entirety of each record can be downloaded as PDF or text files.

positive feedback loops generates a dual-time switch that is rapidly inducible and resistant to noise (ID 173) [44, 47]. The output is generated rapidly as a consequence of the kinetic properties of the fast loop, while it turns off slowly as a consequence of the kinetics of the slow loop. The combined network allows for independent tuning of the activation and deactivation rates. A combination of type-1 coherent feedforward loops (C1-FFLs) can generate a FIFO order (ID 128) by separately tuning the threshold value of each switch for C1-FFLs [39, 44]. The interlocked FFL network consists of the type-1 incoherent FFLs that produce the gene expression pulse and the C1-FFLs responsible for a time delay between pulses. Thus, the interlocked FFL network can generate gene expression pulses in temporal order (ID 52) by independently tuning the threshold values for switching gene expression [22, 48]. A combination of diamond network motifs forms a perceptron model, integrating multiple input signals into a variety of outputs (ID 11) [22, 49]. This network is similar to the information processor of multilayer perceptrons. As shown in the record for ID 11, combination of input signals X1 and X2 calculates the values of Y1 and Y2 in the second layer. Y1 and Y2 generate the output of Z in the third layer. Combination of X1 and X2 can generate various output patterns: AND, OR, XOR, NOT, NAND and NOR by independently tuning the parameter values.

Synergistic effect

Addition of a functional network to an existing network can enhance the function of the existing one. Addition of a positive feedback loop to a negative feedback loop network enhances the oscillatory behavior generated by the negative feedback (ID 129, 184) [22, 47]. An increase in the number of positive feedback loops enhances bistability (ID 65) [22, 45].

Emergent effect

A sequential chain of phosphorylation reactions is expected to generate ultrasensitivity. Interestingly, such chains of phosphorylation reactions can create bistability despite the absence of an explicit positive feedback loop. A three-layer structure of the phosphorylation chain reactions can create unexpected oscillations despite the absence of an explicit negative feedback loop (ID 174, 249, 250, 251, 253, 259, 281) [22, 30].

Loss of function

The combined network may cause loss of function of the ascendant networks. Addition of a positive feedback loop to a bistable switch network can form a more digital-like response, providing robustness against external perturbation, but may reduce robustness to internal perturbation owing to inherent properties of the positive feedback loop. The *Escherichia coli* ammonia assimilation system exemplifies such loss of function [22, 50]. The assimilation system consists of complex but highly structured modules: the glutamine synthetase (GS) activity feedback control module with bidirectional reactions catalyzed by bi-functional enzymes (UTase/UR, PII, GlnK) (ID 132) and the GS synthesis feedback control module that implements negative and positive feedback loops (ID 124, 165) with a two-component phosphorelay system comprising NRI and NRII (ID 200) [22, 51]. The GS activity module presents a fast response that is robust to internal perturbation; the GS synthesis module amplifies GS activity with respect to ammonia depletion. The GS activity module was added to the GS synthesis module to improve the transient response to ammonia depletion, but the robustness to internal perturbation was lost. A combined network can enhance a specific function, while triggering the loss of other functions.

Combination of functional networks with spatial constraint

Spatial gradients of morphogen generally involve a variety of pattern formations [22, 36, 52]. Combination of an elementary network with spatial gradients generates an emergent function. Pattern formation by spatial gradients has been built on Turing's original model and the 'activator–inhibitor' models of Meinhardt and Gierer (ID 106, 107). The emergence of ultrasensitive (switch-like) responses to input signal provides a versatile mechanism for the design of a biochemical switch. The simple first-order kinetic system can exhibit ultrasensitivity in combination with the exponential dependence of spatial location of a diffuse molecular signal (ID 8) [22, 53]. Any two-state system with transition rates that are exponentially dependent on an input signal can be ultrasensitive with respect to the input signal. Morphogen-based spatial patterning is a two-step process: morphogen gradient formation by diffusion followed by morphogen interpretation. The incoherent type-1 FFL (ID 266), positive and negative feedback loops (ID 268) and regulated mutual inhibition network (ID 265) emerge to create a single stripe of expression in combination with input signal gradients [20, 22].

Importance of biochemical and kinetic details

Biological functions not only depend on network topology but also on details of the biochemistry or kinetics. Perfect adaptation by the integral feedback control network can be determined from the biochemical details such as a zero-order reaction, linear response or logarithmic input functions (ID 12, 146,147). Dynamics generated by a single negative feedback loop depend on the kinetics of suppression described by different mathematical formulas: linear, power-law and Michaelis–Menten type equations [22, 41]. Use of the linear equation can provide adaptation, a robust property with respect to a change in input signal (ID 165). Use of the power-law formula limits output with high-intensity input signals, but does not limit output with low-intensity noise (ID 188). Use of the Michaelis–Menten equation provides homeostasis to the output with low-intensity input or noise removal (ID 187).

Stochastic behaviors

Analogous to an engineering system that exclusively pursues the removal of noise, biochemical systems manage to reduce noise. Negative feedback loops are the typical mechanism to suppress noise on the molecular level (ID 102, 103). Other mechanisms such as fast turnover [22, 54, 55] (ID 105) and increase in the number of molecules within a cell (ID 104) also remove noise. Interestingly, some functional networks use noise to survive stochastic environments, suggesting cells have evolved to use stochastic noise rather than remove it.

Many bistable networks (ID 66, 67, 124, 174, 249, 250, 264, 271) are described by deterministic equations. Addition of noise can cause a monostable

network described by deterministic equations to show bistability or a bimodal response. Noise can enforce the values of some parameters within the monostable range to the bistability range, generating a bimodal response in a system where bistability appears within a certain range of parameters but its current parameters place the system in a monostable range (ID 274) [22, 56]. Even in the systems that are monostable for all parameter ranges, noise can promote emergence of bistability or bimodal response (ID 295) [20, 22, 46]. The noise-induced emergence of bistability is exemplified by the enzymatic futile cycle, which represents a recurring control motif in many processes from energy metabolism to signal transduction (ID 295) [46, 57–60]. The enzymatic futile cycle is a bidirectional reaction catalyzed by different monofunctional enzymes, described by the Michaelis–Menten equations. Its deterministic model never directly results in bifurcation, oscillation and other complex behaviors, but noise serves to confer bimodality, bistability or stochastic amplification/signaling.

Noise-induced heterogeneity of gene expression within a cell is also critical to biological design. As shown in noise filter-induced bimodality (ID 278) and bimodality due to transcriptional pulsing (ID 294), noise can generate spatial heterogeneity of gene expression in cell populations and temporal heterogeneity of gene expression [61]. In the NF-κB signaling system, dual-delayed negative feedback loops induce heterogeneous timing of oscillations between individual cells by using different delay times (ID 273) [55, 61].

COMPARISONS WITH ENGINEERING

Specifically designed network

Comparisons between biology and engineering improve our understanding of biological systems. At the system level, despite extremely different physical implementations, similar regulatory strategies such as feedback, feedforward and redundancy are widely used in engineering and in biological systems. Functional networks seem to be specifically designed to generate a variety of functions necessary for cellular systems, just as electric circuits are rationally designed as a combination of fundamental elements such as an amplifier, sensor, switch and oscillator.

Modularity

Engineering sciences exploit the properties of modular designs. A new module is superimposed or combined with an existing module through an interface according to standardized protocols that demonstrate efficiency, reliability, safety and robustness. Modularity guarantees that the complexity of a design is hidden in 'black boxes' that possess well-defined inputs, outputs and functionality. At the same time, standardized interfaces guarantee the plug-and-play addition of new modules, without the need for extensive fine adjustments.

Analogous to engineering systems, the functional networks would undoubtedly be crucial for rational design of a large-scale biochemical network. The large-scale network will be built by complex combinations of functional networks and can be understood in terms of a hierarchical modular structure. Is it possible to regard the functional networks as the black boxes of engineering systems? Although the functional networks seem to exhibit expected dynamical behaviors, it is not yet known to what extent and how they interact with each other. They would also experience considerable interference from other networks through biomolecules.

Designability

Use of BioFNet may enable more efficient, predictable, design-driven genetic engineering, which allows for reasonable selection from a vast list of components that meet a given function. For example, a bistable switch or a bistability network (ID 63, 65, 66, 67, 124, 171) can be built with positive feedback loops or phosphorylation cascades (ID 174). To identify the most suitable component, it is necessary to characterize the robustness of the bistability function with respect to parameter uncertainty and environmental changes, and to estimate the interactive effects between the embedded functional network and its surrounding networks.

Combination of functional networks increases our ability to design different behaviors. They can be rationally assembled for a given function, analogous to control engineering architecture, as indicated in previous studies [10, 11, 22, 50], while considering the additive, synergistic, emergent effects and loss of function. In addition, the combination of functional networks often produces a global loop that passes through them, changing the control architecture [11, 62, 63]. This requires readjustment of the kinetic

parameters such that the combined network functions properly.

Kinetic adjustment

In silico, we can readily modify, design and assemble functional networks because the kinetic parameters can be arbitrarily optimized or changed. *Invivo*, however, a serious practical problem emerges with biomolecule kinetics. Assembly of biomolecules requires kinetic adjustments so that the assembled molecules can act in concert. This requires quantitative kinetic information regarding the biomolecules and their interactions. If the kinetic parameters were arbitrarily adjusted *in vivo*, synthetic biology could yield the profound benefits seen in engineering sciences, which have not been realized in biology yet. The quantitative standards of biological parts have been discussed elsewhere [11, 64].

Design principles

Engineering systems have used biology-inspired algorithms such as fuzzy systems, neural networks, genetic or evolutionary algorithms for optimization and autonomous distributed systems, while biology often uses engineering terms such as robustness, stability, amplifier, sensor, feedback and feedforward. Thus, the gap between biology and engineering is being filled. What are the principles of biological design? *In vivo*, the number of biomolecules and their kinetics stochastically vary with time and fluctuating environments, greatly differing from the engineering systems that precisely specify their components and minimize parameter uncertainty and noise [11, 56, 65]. In this context, biological design is characterized by the fact that cells must coexist with such parameter uncertainty and noise. In fact, some biochemical systems use noise to enhance oscillation and bistability or to generate heterogeneity of gene expression. Noise-generated heterogeneity can increase the chances for some parts of the cell population to adapt to fluctuating environments. They may be advantageous for survival in consistently fluctuating environments.

TOWARD CELL DESIGN

The essence of synthetic biology is to make biology predictable, controllable and design-ready. The development of BioFNet would enable better understanding of biological design principles and would lead to advances in rational design of biochemical systems. We advocate a 'bottom-up' approach in which the assembly of functional networks comprises the whole cell. A deep understanding of this concept can dramatically increase the speed of design and reduce the cost of development. Our ever-expanding database will contribute to the design of robust biological systems in silico before fabrication, just as aeronautic engineers use computer-aided design tools to build airplanes.

SUPPLEMENTARY DATA

Supplementary data are available online at http://bib.oxfordjournals.org/.

Key Points

- Functional networks, which can be defined as the biochemical subnetwork of biomolecules assembled to generate a particular function, are presented and reviewed.
- We developed a biological functional network database with the capacity to cover the entire cell at the molecular interaction level.
- The outstanding feature of the database is that it implements the numerical simulation and visualization programs provided by Matlab.
- The database presents a sound basis for understanding how functional networks are assembled and for the rational design of biochemical networks.

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