From molecular to modular cell biology

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Cellular functions, such as signal transmission, are carried out by 'modules' made up of many species of interacting molecules. Understanding how modules work has depended on combining phenomenological analysis with molecular studies. General principles that govern the structure and behaviour of modules may be discovered with help from synthetic sciences such as engineering and computer science, from stronger interactions between experiment and theory in cell biology, and from an appreciation of evolutionary constraints.

Although living systems obey the laws of physics and chemistry, the notion of function or purpose differentiates biology from other natural sciences. Organisms exist to reproduce, whereas, outside religious belief, rocks and stars have no purpose. Selection for function has produced the living cell, with a unique set of properties that distinguish it from inanimate systems of interacting molecules. Cells exist far from thermal equilibrium by harvesting energy from their environment. They are composed of thousands of different types of molecule. They contain information for their survival and reproduction, in the form of their DNA. Their interactions with the environment depend in a byzantine fashion on this information, and the information and the machinery that interprets it are replicated by reproducing the cell. How do these properties emerge from the interactions between the molecules that make up cells and how are they shaped by evolutionary competition with other cells?

Much of twentieth-century biology has been an attempt to reduce biological phenomena to the behaviour of molecules. This approach is particularly clear in genetics, which began as an investigation into the inheritance of variation, such as differences in the colour of pea seeds and fly eyes. From these studies, geneticists inferred the existence of genes and many of their properties, such as their linear arrangement along the length of a chromosome. Further analysis led to the principles that each gene controls the synthesis of one protein, that DNA contains genetic information, and that the genetic code links the sequence of DNA to the structure of proteins.

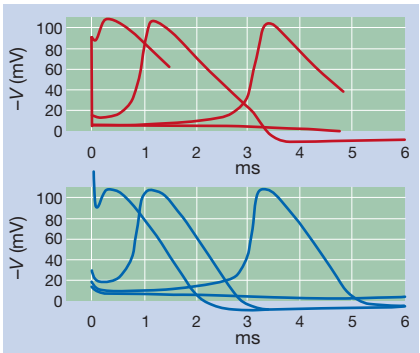
Despite the enormous success of this approach, a discrete biological function can only rarely be attributed to an individual molecule, in the sense that the main purpose of haemoglobin is to transport gas molecules in the bloodstream. In contrast, most biological functions arise from interactions among many components. For example, in the signal transduction system in yeast that converts the detection of a pheromone into the act of mating, there is no single protein responsible for amplifying the input signal provided by the pheromone molecule.

To describe biological functions, we need a vocabulary that contains concepts such as amplification, adaptation, robustness, insulation, error correction and coincidence detection. For example, to decipher how the binding of a few molecules of an attractant to receptors on the surface of a bacterium can make the bacterium move towards the attractant (chemotaxis) will require understanding how cells robustly detect and amplify signals in a noisy environment. Having described such concepts, we need to explain how they arise from interactions among components in the cell.

We argue here for the recognition of functional 'modules' as a critical level of biological organization. Modules are composed of many types of molecule. They have discrete functions that arise from interactions among their components (proteins, DNA, RNA and small molecules), but these functions cannot easily be predicted by studying the properties of the isolated components. We believe that general 'design principles' — profoundly shaped by the constraints of evolution — govern the structure and function of modules. Finally, the notion of function and functional properties separates biology

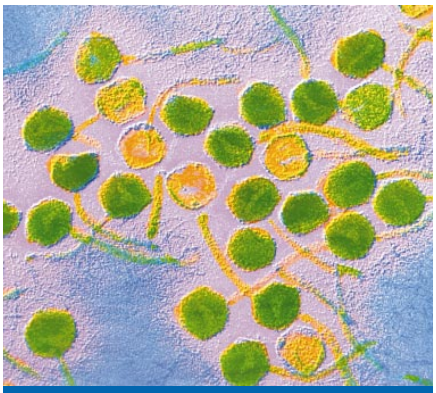
Box 1 Phenomenological analysis of action potentials in nerve cells

Action potentials are large, brief, highly nonlinear pulses of cell electrical potential which are central to communication between nerve cells. Hodgkin and Huxley's analysis of action potentials[29] exemplifies understanding through *in silico* reconstruction. They studied the dynamical behaviour of the voltage-dependent conductivity of a nerve cell membrane for Na^+ and K^+ ions, and described this behaviour in a set of empirically based equations. At the time, there was no information available about the channel proteins in nerve cell membranes that are now known to cause these dynamical conductivities. From (conceptually) simple experiments on these individual conductivities, Hodgkin and Huxley produced simulations that quantitatively described the dynamics of action potentials, showed that the action potentials would propagate along an axon with constant velocity, and correctly described how the velocity should change with axon radius and other parameters. Just as explanations of hydrodynamic phenomena do not require knowledge of the quantum chemistry of water, those who are interested in the behaviour of neural circuits need not know how the particular channel proteins give rise to the Hodgkin–Huxley equations.



Modelling action potentials. The upper trace shows three membrane action potentials, responding to different strengths of stimulus, calculated by Hodgkin and Huxley, while the lower trace shows a corresponding series of experimental recordings. (Adapted from ref. 29.)

Box 2 A decision-making module in bacteriophage lambda



False-colour transmission electron micrograph of lambda bacteriophages (× 13,500).

The bacterial virus lambda can exist in two states inside a bacterial cell. In the lytic state, the virus replicates, producing about 100 progeny virus particles, and releases them by inducing lysis of the host cell. In the lysogenic state, the viral DNA is integrated into the bacterial chromosome and the production of a single viral protein, the repressor, inhibits expression of the other viral genes. The physiology of the host cell and other factors regulate the probability that an infecting lambda virus will become a lysogen, instead of replicating and inducing lysis[30].

Elegant phenomenological experiments inferred the existence of bacteriophages and the existence of lytic and lysogenic states[31] well before the viruses could be seen as physical particles. The isolation of mutants that biased the switch between lysis and lysogeny defined genes whose products formed part of the switch and sites on the DNA at which these products bound. Sophisticated analysis of the interactions between the mutants led to proposals about the circuitry of the switch, and specific proposals for which DNA sites bound which regulatory proteins. These proposals were verified by molecular analyses that showed that the repressor bound to DNA[32] and produced a very detailed description of the biochemical interactions among repressor, other DNA-binding proteins and DNA. Key predictions of models of the switch were verified by reconstructing it in genetically engineered bacteria[33], and by simulating its behaviour using computer models derived from tools used to simulate the behaviour of electrical circuits[34].

from other natural sciences and links it to synthetic disciplines such as computer science and engineering.

Is cell biology modular?

A functional module is, by definition, a discrete entity whose function is separable from those of other modules. This separation depends on chemical isolation, which can originate from spatial localization or from chemical specificity. A ribosome, the module that synthesizes proteins, concentrates the reactions involved in making a polypeptide into a single particle, thus spatially isolating its function. A signal transduction system, on the other hand, such as those that govern chemotaxis in bacteria or mating in yeast[1–3], is an extended module that achieves its isolation through the specificity of the initial binding of the chemical signal (for example, chemoattractant or pheromone) to receptor proteins, and of the interactions between signalling proteins within the cell. Modules can be insulated from or connected to each other. Insulation allows the cell to carry out many diverse reactions without cross-talk that would harm the cell, whereas connectivity allows one function to influence another. The higher-level properties of cells, such as their ability to integrate information from multiple sources, will be described by the pattern of connections among their functional modules.

The notion of a module is useful only if it involves a small fraction of the cell components in accomplishing a relatively autonomous function. Are modules real? Several lines of evidence suggest that they are. Some modules, such as those for protein synthesis, DNA replication, glycolysis, and even parts of the mitotic spindle (the cellular machinery that ensures the correct distribution of chromosomes at cell division), have been successfully reconstituted *in vitro*. Others are intrinsically more difficult to reconstruct from purified components and, for these, other methods have established the validity of the module. One method is to transplant the module into a different type of cell. For example, the action potentials characteristic of nerve and muscle cells have been reconstituted by transplanting ion channels and pumps from such cells into non-excitable cells[4]. Another approach is to create theoretical models of the system and verify that their predictions match reality. This approach was used to describe the generation of action potentials long before a molecular description of membrane channels existed (see Box 1). This was the first example of '*in silico* reconstitution', which will have an increasingly important role in cell biology.

Functional modules need not be rigid, fixed structures; a given component may belong to different modules at different times. The function of a module can be quantitatively regulated, or switched between qualitatively different functions, by chemical signals from other modules. Higher-level functions can be built by connecting modules together. For example, the super-module whose function is the accurate distribution of chromosomes to daughter cells at mitosis contains modules that assemble the mitotic spindle, a module that monitors chromosome alignment on the spindle, and a cell-cycle oscillator that regulates transitions between interphase and mitosis.

One must also ask how a cell integrates information and instructions that come from the many different modules that monitor its internal and external environment. Neurobiology has an analogous problem, where the central nervous system integrates information from different senses and dictates the organism's behaviour. Does cellular integration merely emerge from a web of pairwise connections between different sensory modules, or are there specific modules that act as a cellular equivalent of the central nervous system — integrating information and resolving conflicts?

Complete understanding of a biological module has depended on the ability of phenomenological and molecular analyses to constrain each other (see Box 2). Phenomenological models have fewer variables than molecular descriptions, making them easier to constrain with experimental data, whereas identifying the molecules involved makes it possible to perturb and analyse modules in much greater detail. Thus, the demonstration that genetic information for virulence could be transferred between bacteria prompted the identification of the information-carrying molecule as DNA, before the molecular processes involved in virulence and the structure of DNA were understood. The discovery that genetic information resided in the DNA encouraged structural studies, which then suggested how DNA encodes information and transmits it from generation to generation.

Modular structures may facilitate evolutionary change. Embedding particular functions in discrete modules allows the core function of a module to be robust to change, but allows for changes in the properties and functions of a cell (its phenotype) by altering the connections between different modules. If the function of a protein were to directly affect all properties of the cell, it would be hard to change that protein, because an improvement in one function would probably be offset by impairments in others. But if the function of a protein is restricted to one module, and the connections of that module to other modules are through individual proteins, it will be much easier to modify, make and prune connections to other modules. This idea is supported by the analogous observation that proteins that interact with many other proteins, such as histones, actin and tubulin, have changed very little during evolution,

and by theoretical arguments that proteins are difficult to evolve once they are participating in many different interactions[5].

Understanding the relatedness of modules is useful because knowledge about one member of a class can inform the study of the others. Relatedness by descent is often apparent from the homology of chemical components. For instance, the mitogen-activated protein kinase cascades that occur in many intracellular signalling pathways define a common functional class of signal transduction modules. Modules may also be related by shared design or functional principles, even if they are not related by descent. The pheromone-detection system of budding yeast and the chemotactic machinery of bacteria use unrelated components, but both pathways achieve a sensitive response over a wide range of pheromone or chemoattractant concentrations by using reactions that specifically turn off active forms of the signalling receptors[6–8].

Lessons from other sciences

We have argued that most functional properties of a module are collective properties, arising from the properties of the underlying components and their interactions. Collective properties have long been studied in statistical physics and share attributes that rise above the details[9]. For example, the melting of the surface of a solid can be induced in different ways: by changing the pressure or temperature or by adding impurities. Similarly, different organisms induce the transition between different patterns of microtubule organization that occurs during cell division by changing different members of the set of kinetic parameters that govern microtubule polymerization[10,11]. The concept of phase (or state) transitions from physics may help unify different observations and experiments. Moreover, many molecular details are simply not needed to describe phenomena on the desired functional level.

Biological systems are very different from the physical or chemical systems analysed by statistical mechanics or hydrodynamics. Statistical mechanics typically deals with systems containing many copies of a few interacting components, whereas cells contain from millions to a few copies of each of thousands of different components, each with very specific interactions. In addition, the components of physical systems are often simple entities, whereas in biology each of the components is often a microscopic device in itself, able to transduce energy and work far from equilibrium[12]. As a result, the microscopic description of the biological system is inevitably more lengthy than that of a physical system, and must remain so, unless one moves to a higher level of analysis.

Information flows bidirectionally between different levels of biological organization. For instance, the macroscopic signals that a cell receives from its environment can influence which genes it expresses — and thus which proteins it contains at any given time — or even the rate of mutation of its DNA[13], which could lead to changes in the molecular structures of the proteins. This is in contrast to physical systems where, typically, macroscopic perturbations or higher-level structures do not modify the structure of the molecular components. For example, the existence of vortices in a fluid, although determined by the dynamics of molecules, does not usually change the nature of the constituents and their molecular interactions.

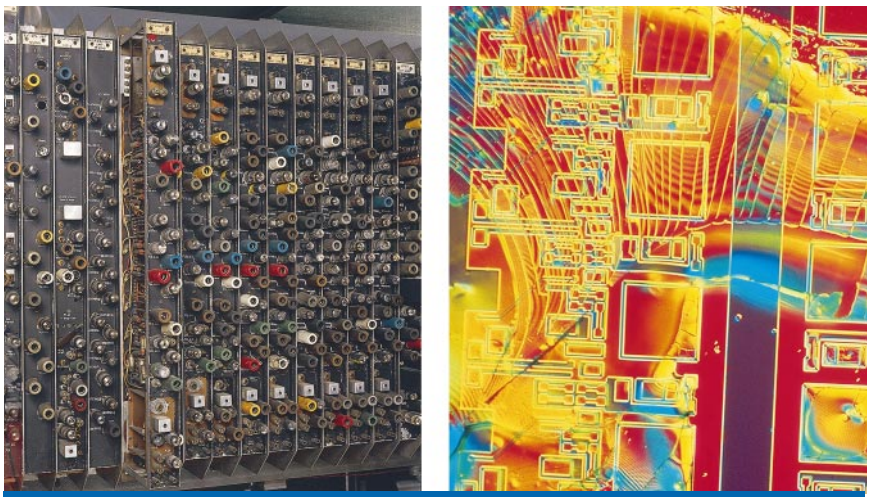
More importantly, what really distinguishes biology from physics are survival and reproduction, and the concomitant notion of function. Therefore, in our opinion, the most effective language to describe functional modules and their interactions will be derived from the synthetic sciences, such as computer science or engineering, in which function appears naturally.

The essence of computational science is the capacity to engineer circuits that transform information from one form into another on the basis of a set of rules. How might the lessons learned here apply to biology? Evolution selects those members of a genetically diverse population whose descendants proliferate rapidly and survive over many generations. One way of ensuring long-term survival is to use information about the current environment to predict possible future environments and generate responses that maximize the chance of survival and reproduction. This process is a computation, in which the inputs are environmental measurements, the outputs are signals that modulate behaviour, and the rules generate the outputs from the environmental inputs. For example, signals from the environment entrain circadian biological clocks to produce responses to predicted fluctuations in light intensity and temperature. Indeed, the history of life can be described as the evolution of systems that manipulate one set of symbols representing inputs into another set of symbols that represent outputs[14].

Just as electrical engineers design circuits to perform specific functions, modules have

Box 3 From atoms to modules in computers

Building a computer in the 1950s relied on understanding barium oxide, the material of choice for emitting electrons from the cathodes of vacuum tubes. A vacuum-tube module could then be designed whose function was the amplification of a signal, but whose functional description had no reference to barium oxide. These amplifiers were assembled into logical circuits, whose logical operation could be described without reference to vacuum tubes. The connecting wires in these logical circuits had insulation of many colours so that the circuits could be accurately hand-manufactured. Mathematical programs were then designed on the basis of these logic circuits. In the computer of today, there is no barium oxide or coloured wires. Instead, the properties of silicon and silicon dioxide are of primary importance in designing an amplifier, and the transistor replaces the vacuum tube. Both old and new computers have logic circuits based on the same elementary principles, but arranged rather differently as computers have become more sophisticated. Yet all this is unimportant to most users, whose computer program runs on either machine. At one level, barium oxide and coloured wires were the soul of the old machine, while at another level, they are irrelevant to understanding the essence of how a computer functions.



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An early stored-program computer (left), built around 1950, used vacuum tubes in logic circuits, whereas modern computers use transistors and silicon wafers (right), but both are based on the same principles.

evolved to perform biological functions. The properties of a module's components and molecular connections between them are analogous to the circuit diagram of an electrical device. As biologists we often try to deduce the circuitry of modules by listing their component parts and determining how changing the input of the module affects its output[15]. This reverse engineering is extremely difficult. Although an electrical engineer could design many different circuits that would amplify signals, he would find it difficult to deduce the circuit diagram of an unknown amplifier by correlating its outputs with its inputs. It is thus unlikely that we can deduce the circuitry or a higher-level description of a module solely from genome-wide information about gene expression and physical interactions between proteins. Solving this problem is likely to require additional types of information and finding general principles that govern the structure and function of modules.

A number of the design principles of biological systems are familiar to engineers. Positive feedback loops can drive rapid transitions between two different stable states of a system, and negative feedback loops can maintain an output parameter within a narrow range, despite widely fluctuating input. Coincidence detection systems require two or more events to occur simultaneously in order to activate an output. Amplifiers are built to minimize noise relative to signal, for instance by choosing appropriate time constants for the circuits. Parallel circuits (fail-safe systems) allow an electronic device to survive failures in one of the circuits.

Designs such as these are common in biology. For example, one set of positive feedback loops drives cells rapidly into mitosis, and another makes the exit from mitosis a rapid and irreversible event[16]. Negative feedback in bacterial chemotaxis allows the sensory system to detect subtle variations in an input signal whose absolute size can vary by several orders of magnitude[17]. Coincidence detection lies at the heart of much of the control of gene transcription in eukaryotes, in which the promoters that regulate gene transcription must commonly be occupied by several different protein transcription factors before a messenger RNA can be produced. Signal transduction systems would be expected to have their characteristic rate constants set so as to reject chance fluctuations, or noise, in the input signal. DNA replication involves a fail-safe system of error correction, with proofreading by the DNA polymerase backed up by a mismatch repair process that removes incorrect bases after the polymerase has moved on. A failure in either process still allows cells to make viable progeny, but simultaneous failure of both is lethal. In both biological and man-made systems, reducing the frequency of failure often requires an enormous increase in the complexity of circuits. Reducing the frequency at which individual cells give rise to cancer to about 10^{-15} has required human cells to evolve multiple systems for preventing mutations that could generate cancer cells, and for killing cells that have an increased tendency to proliferate.

Biological systems can both resist and exploit random fluctuations, or noise. Thus, evolutionary adaptation depends on DNA being mutable, but because most mutations are neutral or deleterious, the rate of mutation is under rigorous genetic control. Many systems for specifying the polarity of cells or groups of cells rely on a mechanism known as 'lateral inhibition', which causes adjacent cells to follow different fates. This process can amplify a small, often stochastic, initial asymmetry causing adjacent cells or adjacent areas within cells to follow different fates.

Other aspects of functional modules are less familiar to engineers. Several can be subsumed under the idea that the rules for a module's function are rigidly encoded in the structures of its proteins, but produce messy, probabilistic intermediates that are then refined to give unique solutions. This principle seems to hold across an enormous range of scales, from the folding of protein

molecules to the evolution of organisms. The principle arises from a combination of three mechanisms: exploration with selection (trial and error), error-correction mechanisms, and error-detection modules that delay subsequent events until a process has been successfully completed. These are present to different extents in different examples.

Exploration with selection (trial and error) is a fundamental principle of biology and acts on timescales from milliseconds to aeons and at organizational levels from single molecules to populations of organisms. In single molecules, kinetic funnels direct different molecules of the same protein through multiple, different paths from the denatured state to a unique folded structure[18]. Within cells, the shape of the mitotic spindle is partly due to selective stabilization of microtubules whose ends are close to a chromosome[19]. At the organismal level, the patterning of the nervous system is refined by the death of nerve cells and the decay of synapses that fail to connect to an appropriate target. Within populations, differential reproductive success alters the structure of gene pools, giving rise to evolution.

The use of exploration with selection on short timescales as a design principle in intracellular modules may make them especially easy to modify on evolutionary timescales. The lack of a rigidly programmed sequence of intermediates should allow such modules to survive incremental modifications and incorporate evolutionary additions such as error detection and correction. Similar messy and probabilistic intermediates appear in engineering systems based on artificial neural networks — mathematical characterizations of information processing that are directly inspired by biology. A neural network can usefully describe complicated deterministic input–output relationships, even though the intermediate calculations through which it proceeds lack any obvious meaning and their choice depends on random noise in a training process[20].

Constraints from evolution

One approach to uncovering biological design principles is to ask what constraints they must obey. Apart from the laws of physics and chemistry, most constraints arise from evolution, which has selected particular solutions from a vast range of possible ones.

Today's organisms have an unbroken chain of ancestors stretching back to the origin of life. This constraint has been successfully used to understand protein functions, by comparing existing protein sequences from related species, finding conserved parts and inferring their roles. Comparing modules of common function from different organisms should be a similarly useful tool for understanding their operation. One has also to remember that today's modules were built by tinkering with already functional modules, rather than by starting from scratch, and may not be the optimal way of solving a particular problem[21]. This evolutionary history is similar to that of man-made devices. Particular solutions in computing, or for any engineered object, are the result of an elaborate historical process of selection by technological, economical and sociological constraints. A familiar example is the less than optimal QWERTY keyboard, originally invented to prevent jammed keys on early manual typewriters. It can be viewed as a living fossil.

The survival of living systems implies that the critical parameters of essential modules, such as the accuracy of chromosome segregation or the periodicity of a circadian clock, are robust: they are insensitive to many environmental and genetic perturbations. Evolvability[22], on the other hand, requires that other parameters of modules are sensitive to genetic changes. They can then be modified over many generations to alter the function of a module, or its connections to other modules, in a way that allows organisms to adapt to new challenges. It is important to understand how robustness and flexibility can be reconciled for each functional module.

Organisms have been selected for two properties: rapid reproduction in optimal conditions and the ability to survive rarely encountered extreme conditions. Because environments tend to fluctuate over time, most modules are likely to have been selected for their ability to contribute to both reproduction and survival. These considerations imply that understanding the full function of modules may require us to measure small differences in reproductive ability, as well as studying the performance of modules under extreme perturbations. Some components of an *in vivo* module that are 'nonessential' in normal laboratory conditions are likely to have important roles in the assembly, fidelity, robustness and dynamic characteristics of modules that produce small advantages in long-term survival probability. It may be very difficult, however, to measure such contributions directly.

Survival of a gene pool, as opposed to an individual organism, is favoured by diversification, as the simultaneous presence of multiple phenotypes in a population increases the possibility that some individuals will survive and reproduce in a heterogeneous and changing environment. Diversification can be achieved by epigenetic mechanisms that enable a single genotype to produce more than one phenotype, by genetic mechanisms that maintain multiple genotypes in a population, and by speciation, which splits a single gene pool into two independently evolving pools. It is thus important to consider the function of modules not only in the context of an organism, but also from the point of view of the population of organisms, and to ask how modular construction facilitates the maintenance and selection of diversity.

Towards modular biology

A major challenge for science in the twenty-first century is to develop an integrated understanding of how cells and organisms survive and reproduce. Cell biology is in transition from a science that was preoccupied with assigning functions to individual proteins or genes, to one that is now trying to cope with the complex sets of molecules that interact to form functional modules[23,24]. There are several questions that we want to answer. What are the parts of modules, how does their interaction produce a given function, and which of their properties are robust and which are evolvable? How are modules constructed during evolution and how can their functions change under selective pressure? How do connections between modules change during evolution to alter the behaviour of cells and organisms?

The number of modules that have been analysed in detail is very small, and each of these efforts has required intensive study. Biologists need to study more functions at the modular level and develop methods that make it easier to determine the relationship of inputs to outputs of modules, their biochemical connectivity, and the states of key intermediates within them. Three complementary approaches can help in this task: better methods for perturbing and monitoring dynamic processes in cells and organisms; reconstituting functional modules from their constituent parts, or designing and building new ones; and new frameworks for quantitative description and modelling of modules.

The first approach is illustrated by efforts to find small organic molecules that can perturb and report on the activity of modules. Calcium-binding dyes have been used to follow the activities of neurons with high spatial and temporal resolution. Light-activated chemicals can perturb function on the timescales that characterize changes within the modules, thus giving them an important advantage over the slower perturbations produced by classical and molecular genetics[25]. Another example of a new method for monitoring cellular processes is genome-wide analysis of gene expression[26,27]. But techniques for collecting information about the entire genome will be only as powerful as the tools available to analyse it, just as our ability to infer protein structure and function from protein sequence data has increased with the sophistication of tools for sequence analysis. We need better methods of finding patterns that identify networks and their components, of identifying possible connections among the components, and of reconstructing the evolution of modules by comparing information from many different organisms.

Another approach to discerning module function is that of 'synthetic biology'. Just as chemists have tested their understanding of synthetic pathways by making molecules, biologists can test their ideas about modules by attempting to reconstitute or build functional modules. This approach has already been used to construct and analyse artificial chromosomes made by assembling defined DNA elements[28], and cellular oscillators made from networks of transcriptional regulatory proteins (M. Elowitz and S. L., unpublished results). Seeing how well the behaviour of such modules matches our expectations is a critical test of how well we understand biological design principles.

The main difficulty in reconstructing the evolution of modules is our ignorance about past events. One solution to this problem is to examine the evolution of module function in the laboratory. Analysing multiple repetitions of such experiments may tell us how much the path of future change is restricted by the current structure and function of modules, and should help us to understand how evolutionary pressures constrain biological design principles.

Finally, we emphasize the importance of integrating experimental approaches with modelling and conceptual frameworks. The best test of our understanding of cells will be to make quantitative predictions about their behaviour and test them. This will require detailed simulations of the biochemical processes taking place within the modules. But making predictions is not synonymous with understanding. We need to develop simplifying, higher-level models and find general principles that will allow us to grasp and manipulate the functions of biological modules. The next generation of students should learn how to look for amplifiers and logic circuits, as well as to describe and look for molecules and genes (Box 3). Connecting different levels of analysis — from molecules, through modules, to organisms — is essential for an understanding of biology that will satisfy human curiosity.

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