BIOLOGICAL ROBUSTNESS

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Abstract | Robustness is a ubiquitously observed property of biological systems. It is considered to be a fundamental feature of complex evolvable systems. It is attained by several underlying principles that are universal to both biological organisms and sophisticated engineering systems. Robustness facilitates evolvability and robust traits are often selected by evolution. Such a mutually beneficial process is made possible by specific architectural features observed in robust systems. But there are trade-offs between robustness, fragility, performance and resource demands, which explain system behaviour, including the patterns of failure. Insights into inherent properties of robust systems will provide us with a better understanding of complex diseases and a guiding principle for therapy design.

LYSIS
Part of a bacteriophage life cycle in which its genome is expressed to cause dissolution of the bacterial host cell, leading to manufacture of more bacteriophage particles and subsequent infection of other cells.

LYSOGENY
Part of a bacteriophage life cycle, during which its genetic material is integrated into the genome of its bacterial host, where it remains in a latent state.

SEGMENTAL POLARITY
A pathway that regulates the anteroposterior identity of segments during insect development.

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The discovery of fundamental, systems-level principles that underlie complex biological systems is a prime scientific goal in systems biology[1,2]. Robustness is a property that allows a system to maintain its functions despite external and internal perturbations. It is one of the fundamental and ubiquitously observed systems-level phenomena that cannot be understood by looking at the individual components. A system must be robust to function in unpredictable environments using unreliable components. Understanding the origin and principles of robustness in biological systems will help us to put various biological phenomena into perspective; it will also catalyse the formation of principles at the systems level.

In this article, I argue that robustness is a fundamental feature of evolvable complex systems. Complex biological systems must be robust against environmental and genetic perturbations to be evolvable. Evolution often selects traits that might enhance robustness of the organism. Robustness is, therefore, ubiquitous in living organisms that have evolved. However, systems that are robust face fragility and performance setback as an inherent trade-off. Identification of the basic architecture for a robust system and the associated trade-offs is essential for understanding their faults and countermeasures — diseases and therapies, respectively.

Robustness as an organizational principle

Robustness enables the system to maintain its functionalities against external and internal perturbations. This property has been widely observed across many species, from the level of gene transcription to the level of systemic homeostasis. For example, fate decision of λ phage — the result of which is the activation of either LYSIS or LYSOGENY pathways — was once considered the result of fine tuning of the binding affinity of promoters to corresponding regulatory factors. However, it has been shown that it is the structure of the network, which involves both positive and negative feedback, that is responsible for making sustainable commitment, not the specific binding affinity — the fate-decision behaviour was shown to be robust against point mutations in the promoter region[3]. In addition, cooperative binding of repressors that forms implicit local positive feedback also contributes to the stability of the switch[4–6]. Many examples of robust properties can be observed in different biological systems.

Escherichia coli is capable of chemotaxis over a wide range of chemo-attractant concentrations owing to integral intracellular feedback that ensures perfect adaptation and that is independent of ligand concentration[7–9].

A biochemical network that is involved in the establishment of SEGMENTAL POLARITY in *Drosophila melanogaster* has been shown to be robust against changes in initial values and rate constants of molecular interactions, enabling stable pattern formation[10,11]. Similar observations have also been made for MORPHOGEN-pattern formations[10,12,13].

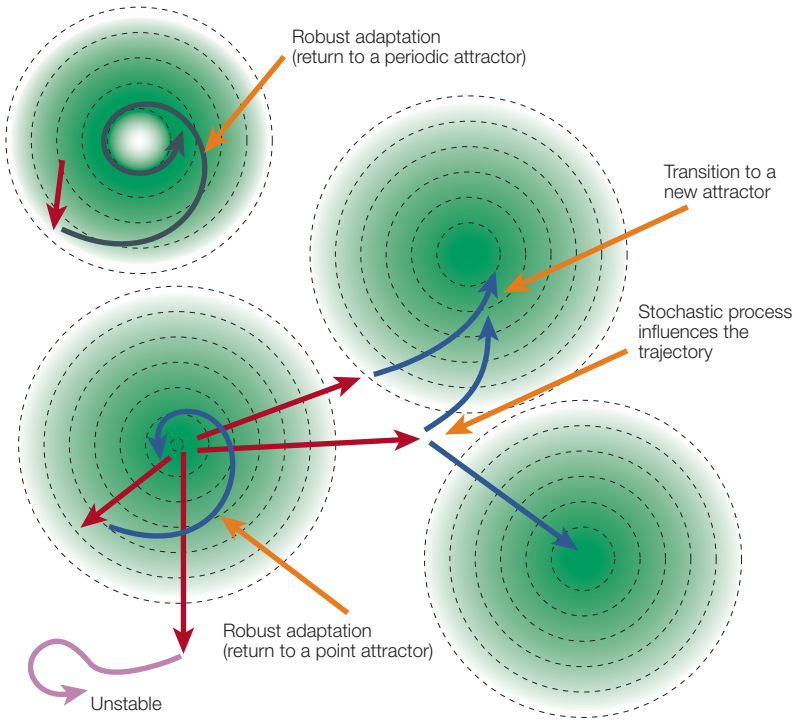

Figure 1 | **Robust reactions of the system: to stay or to change.** The state of a system can be shown as a point in the state space. In this case, the state space is simplified into two dimensions. Perturbations forcefully move the point representing the system's state. The state of the system might return to its original attractor by adapting to perturbations, often using a negative feedback loop. Bacterial chemotaxis is an example. There are basins of attractions in the state space within which the state of the system moves back to that attractor. If the boundary is exceeded, the system might move into an unstable region or move to other attractors. Positive feedback can either move the system's state away from the current attractor, or push the system towards a new state. The cell cycle involves a combination of positive and negative feedbacks that facilitate transition between two attractors (G1 and S/G2/M) creating a bistable system. Often, stochastic processes affect transition between attractors, as seen in λ-phage fate decision, but maintenance of a new state has to be robust against minor perturbations.

Diseases such as cancer and diabetes are manifestations of co-opted robustness, in which mechanisms that normally protect our bodies are effectively taken-over to sustain and promote the epidemic states[14–16]. As more studies are done, it is becoming important to provide an integrated perspective on the robustness of biological systems.

The robustness of a system can manifest itself in one of two ways: the system returns to its current ATTRACTOR or moves to a new attractor that maintains the system's functions (FIG. 1). A return to the current attractor is often called 'robust adaptation'. The attractor can be either static (a point attractor; a fixed point in the PHASE SPACE that the trajectory of the system state approaches asymptotically) or oscillatory (a periodic attractor; a cyclic orbit in the phase space that the trajectory of the system state approaches asymptotically).

A transition to a new attractor has to be made robustly in response to stimuli so that the system behaves consistently against perturbations. As seen in λ-phage fate decision, the stochastic process often influences the trajectory of transition and the attractor on which the system eventually converges.

MORPHOGEN
A diffusible signal that acts at a distance to regulate pattern formation in a dose-dependent manner.

ATTRACTOR
A point or an orbit in the phase space where different states of the system asymptotically converge.

PHASE SPACE
A multi-dimentional space that represents the dynamics of a system. For a system with N-variables, a phase space is a 2N dimensional space composed of N-variables and their time derivatives.

Robustness is often misunderstood to mean staying unchanged regardless of stimuli or mutations, so that the structure and components of the system, and therefore the mode of operation, is unaffected. In fact, robustness is the maintenance of specific functionalities of the system against perturbations, and it often requires the system to change its mode of operation in a flexible way. In other words, robustness allows changes in the structure and components of the system owing to perturbations, but specific functions are maintained.

In the following sections, I outline the mechanisms that ensure the robustness of a system: system control, alternative (or fail-safe) mechanisms, modularity and decoupling.

System control. System control consists of negative and positive feedback to attain a robust dynamic response observed in a wide range of regulatory networks, including the cell cycle, the circadian clock and chemotaxis[7,17,18]. Negative feedback is the principal mode of control that enables robust response (or robust adaptation) to perturbations. Bacterial chemotaxis is one of the most studied examples of robust adaptation that uses negative feedback — INTEGRAL FEEDBACK in particular — to attain the perfect adaptation that allows chemotaxis to occur in response to a wide range of stimuli[7–9]. Integral feedback, a particular control strategy, is essential to maintain robust adaptation in both *E. coli* and *Bacillus subtilis*, despite the fact that the network topologies are not the same[19].

Positive feedback contributes to robustness by amplifying the stimuli, often producing bistability, so that the activation level of a downstream pathway can be clearly distinguished from non-stimulated states, and so that these states can be maintained. In *D. melanogaster* segment-polarity formation — repetitive stripes of differential gene expression — is observed along the antero-posterior axis of the developing embryo. The first stripe has to express *wingless* (*wg*), the second stripe has to express *engrained* (*en*), but the third stripe expresses neither. von Dassow and colleagues[10] created a computational model of this system, initially without positive autoregulatory feedback on *wg* and *en*, but the model failed to reproduce experimentally observed patterns. However, with two positive feedbacks on *wg* and *en* activations, robust pattern formation was reproduced[10]. Recently, Ingolia[11] analysed this model and showed that the bistability caused by positive feedback loops is responsible for robust pattern formation[11].

Positive feedback is also used in signal transduction and the cell cycle to form switch-like behaviour of the system by amplification of stimuli, and for fate decision (as seen in the λ phage), so that it initiates a transition and a new state of the system is made that is more robust against noise and fluctuations of stimuli[3,20–27]. Many biological subsystems use the combination of these system controls to perform their functions and to maintain robustness[27,28].

An alternative, or fail-safe, mechanism (redundancy and diversity). Robustness can be enhanced if there are multiple means to achieve a specific function, because failure of one of them can be rescued by others. Here, I call this mechanism 'alternative', or 'fail-safe'. This concept encompasses redundancy, overlapping function and diversity, as the differing degrees of similarity between the various alternative means that are available.

Redundancy generally refers to a situation in which several identical, or similar, components (or modules) can replace each other when another component fails. Diversity, or heterogeneity, represents the other extreme, whereby a specific function can be attained by different means available in a population of heterogeneous components. Some of these phenomena are well known as phenotypic plasticity[29–31].

In some tissues, cells are surrounded by similar neighbours, so that damaged cells are quickly replaced by other cells. However, having multiple identical components as alternatives is rare. An alternative, or fail-safe, mechanism is usually attained by having multiple heterogeneous components and modules with overlapping functions. For example, *Clb5* and *Clb6* of budding yeast are two relatively homologous genes encoding B-type cyclins. They share 49.7% identical residues and both gene products are involved in the entry into the cell cycle[32]. Deletion of *Clb6* has little or no effect on the progression of the cell cycle, and deletion of *Clb5* caused a prolonged S-phase. Deletion of both genes impedes timely initiation of DNA replication. Recent findings strongly demonstrate that gene duplication, particularly whole-genome duplication followed by extensive gene loss and specialization, is one of the crucial mechanisms of evolutionary innovation[33,34], providing support for the long-standing hypothesis proposed by Susumu Ohno[35]. If the function of duplicated gene pairs overlaps to some extent, the duplication acts as an evolutionary capacitor[36,37]. There is at least one indication from computational studies that under certain conditions these pairs could be evolutionary stable[38].

There are also numerous examples of alternative mechanisms at the network level. Text-book examples include glycolysis and oxidative phosphorylation[39]. Although both processes produce ATP, oxidative phosphorylation requires a constant supply of oxygen, whereas glycolysis can be either aerobic or anaerobic (although the latter is less efficient). DIAUXIC SHIFT in yeast causes a drastic change in metabolic pathways, depending on whether glucose or ethanol is available for energy metabolism[40]. The flux balance of metabolic pathways is readjusted according to the available resources in the environment, but either pathway can, ultimately, produce essential materials for survival and growth[41,42]. These are examples of phenotypic plasticity that are often considered to be the opposite of robustness. However, I argue that it is more consistent to view phenotypic plasticity as a part of robustness, because this plasticity enables organisms to robustly adapt to a changing environment.

It is interesting to note that although redundancy through duplicated genes is frequently observed, there is no reported case of duplicated circuits, despite the fact that there are many circuits with similar topologies. Investigation of networks that share similar topology, as far as the degree of homologous genes involved in each network is concerned, revealed that although genes might be duplicated, the network as a whole is not duplicated[43]. This indicates that similar network topologies represented in different contexts within and between species are the result of convergent evolution, rather than duplications[43,44]. This is consistent with the fact that circuit-level alternative mechanisms are attained by the differing implementation of overlapping functions.

It is important to understand that alternative mechanisms are coupled with system control to ensure robustness. First, the existence of alternative mechanisms at the system-component level allows regulatory feedback to remain intact despite mutations. Second, switching between alternative mechanisms has to be orchestrated by specific controls so that the system behaviour is properly maintained.

Modularity. Modularity is an effective mechanism for containing perturbations and damage locally to minimize the effects on the whole system. Modules are widely observed in various organisms, functioning as possible biological design principles[30,45,46] and as essential elements in engineering and industry[47,48]. Despite intuitive consensus, the concept is still ambiguous and therefore can be difficult to define[46].

A cell is an obvious example of a module that constitutes multi-cellular systems; it interacts with the environment and other cells. Modules are often hierarchically organized; a cell itself is composed of organelles, and, at the same time, it is also a part of larger modules such as tissues and organs.

Aside from physical modules such as a cell, there are functional, spatial and temporal modules that can be recognized as subsystems of metabolic networks, signal transduction and developmental regulatory networks. A bacterial flagellum and its control module, for example, represents both a physical and logical module that is robust and versatile[49]. Logical modules, as seen in segmental polarity networks[10,11] and elsewhere[50], are often less obvious than physically partitioned and engineering modules.

Decoupling. Decoupling isolates low-level variation from high-level functionalities. For example, Hsp90 not only fixes proteins that are mis-folded as a result of environmental stresses, but also decouples genetic variations from the phenotype using the same mechanism, therefore providing a genetic buffer against mutations[51–53]. These genetic buffers decouple the genotype from the phenotype, and they provide robustness to cope with mutation while maintaining a degree of genetic diversity. These buffers have been shown to underpin the robustness of developmental processes (as in the case of Waddington's CANALIZATION[54]). Importantly, the mutations

INTEGRAL FEEDBACK
A method of feedback control in which control is proportional to the integral of the systems' output.

DIAUXIC SHIFT
The process of switching from anaerobic to aerobic respiration.

CANALIZATION
The buffering or stabilization of developmental pathways against mutational or environmental perturbations, by several genetic factors.

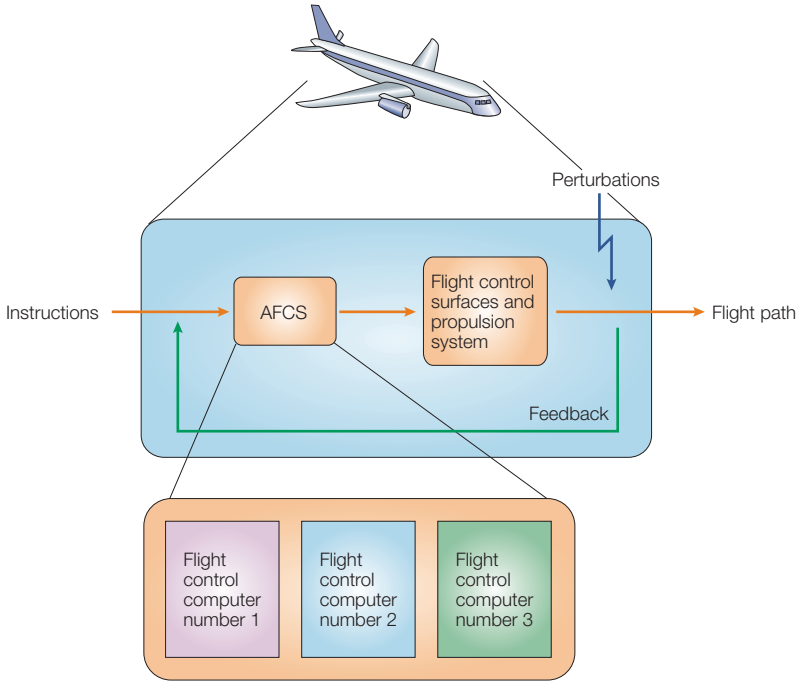

Figure 2 | **Explaining robustness — the aeroplane example.** The concept of robustness is best described using the example of modern aeroplanes. Many commercial passenger aeroplanes have an automatic flight control system (AFCS) that maintains a flight path (direction, altitude and velocity of flight) against perturbations in atmospheric conditions. This can be accomplished by a feedback control in which deviations from the defined flight path are automatically corrected. AFCS is the crucial component that allows the robust maintenance of the flight path by controlling the aeroplane's flight-control surfaces (rudder, elevator, flaps, aileron, etc) and the propulsion system (engines). AFCS is generally composed of three modules with the same functions, thereby creating redundancy, although each is designed differently (heterogeneity) to avoid a common mode failure. Three computers are made that are modular, so that failure in one module does not affect the functions of other parts of the system. This type of mechanism is implemented using digital technologies that decouple low-level voltages from digital signal (ON/OFF of pulses), thereby preventing noise from influencing its functions. Although this is a simplified explanation of the actual system, the concept applies to details of the basic system as much as it does to the more complex systems. Although there are differences between man-made systems and biological systems , the similarities are overwhelming. Fundamentally, robustness is the basic organizational principle of evolving dynamic systems, be it through evolution, competition, a market niche or society.

that are masked by genetic buffering are selectively neutral (one of the premises of Kimura's NEUTRAL THEORY OF EVOLUTION[55,56]), providing a source of material for the evolution of the system during extreme perturbations.

Feedback controls sometimes compensate for changes in rate constants of interactions within the network and changes in the initial state of the network, as is the case for bacterial chemotaxis[7–9] and *D. melanogaster* segmentation[10], or they might even mitigate the impact of loss-of-function mutation. A computational study of the cell cycle demonstrated that removing some genes does not necessarily block the cell cycle, it might only make it more fragile against perturbations[17].

Bistability created through positive feedback sometimes results in the decoupling of fluctuations at a molecular level; for example, the decoupling of a number of molecules that are involved in reactions from the committed state of the system. Therefore, dynamic networks often decouple genetic and environmental perturbations[57]. There is even a hypothesis, albeit computational, that claims that this buffering is an intrinsic property of complex networks[58–60].

Another example of decoupling might take place between information encoding and conversion of stimulus dosage into pulses of protein activations. When the DNA is damaged, p53–MDM2-feedback loops generate oscillatory behaviour; this behaviour was recently found to be a potential converter of graded stimuli (degree of DNA damage) to digital pulses (with a peak of p53 activation), so that it is only the number of pulses that matter after the conversion[61,62], not the subtle changes in concentration levels.

The mechanisms that underlie robustness can be understood using an example of a sophisticated engineering object, such as an aeroplane (FIG. 2). There are similar mechanisms in various other sophisticated engineering systems: they can be built on less than perfect components, but have to cope with unpredictable environmental pressures, thereby indicating the universal nature of robustness. It is interesting to consider whether there is a fundamental system architecture for successful robust systems, and what limitations and risks are associated with these systems.

The origin of robustness

It is now increasingly recognized that robustness is ubiquitous. So, what are the principles and mechanisms that lead to the emergence of robustness in biological systems? My theory is that robustness is an inherent property of evolving, complex dynamic systems — various mechanisms incurring robustness of organisms actually facilitate evolution, and evolution favours robust traits. Therefore, requirements for robustness and evolvability are similar. This implies that there are architectural requirements for complex systems to be evolvable, which essentially requires the system to be robust against environmental and genetic perturbations.

Evolvability requires flexibility in generating diverse phenotypes by means of producing non-lethal mutations[45,63,64]. Kirschner and Gerhart define evolvability, or evolutionary adaptability, as a capacity to generate heritable and selectable phenotypic variations that consists of features that "…reduce the potential lethality of mutations and the number of mutations needed to produce phenotypically novel traits"[63]. They argue that flexible versatile proteins, WEAK LINKAGE, EXPLORATORY SYSTEMS and compartmentalization are central features that foster evolvability[63]. They also argue that the emerging architecture is composed of highly conserved core processes that are co-selected with various other processes, some of which bring about phenotypically novel traits, which is consistent with the BOW-TIE architecture.

These features can be translated into architectural requirements of the system that are consistent with robustness. First, mechanisms that preserve the components and interactions against mutation must be capable of generating genetic variation. Second, there

NEUTRAL THEORY OF EVOLUTION
A theory proposed by Motoo Kimura which states that most variations at the molecular level are neutral to selection.

WEAK LINKAGE
A property of a process that refers to the coupling of processes; in this case, a process depends minimally on other components or processes; example include neural relays or signal transduction pathways, in which individual components often have a switch-like capacity to exist in active or inactive states.

must be modules that robustly maintain their functions against external perturbations and mutations. Third, there must be highly conserved core processes, which are also modular, that have fundamental functions, such as metabolism, the cell cycle and transcriptional machinery, where various modules can be interfaced to create diverse phenotypes. The overall architecture that meets these requirements is probably a modularized nested bow-tie, or hour-glass, structure where various input and output modules are connected to a conserved core (FIG. 3); incidentally, the same architecture underlies the World Wide Web[65]. This bow-tie structure occurs in various aspects of biological systems, from global structure to specific mechanisms such as transcription and translation processes[66]. The architectural features of a modularized bow-tie structure are optimal for enhancing the robustness of the various aspects of a system.

Buffering

The first step towards robustness is to protect components and interactions from perturbations. As well as correcting misfolded proteins, chaperones also impose a particular conformation on some proteins that are genetically varied; masked genetic variation is exposed when these mechanisms are impeded[53]. Networks also contribute to this type of buffering[57]. This is particularly the case when a network is robust against external perturbations, because regulatory feedbacks can also provide robustness against perturbations of various internal parameters. The genetic buffering mechanism — also referred to as the evolutionary capacitor — attained either by chaperons or networks, is one of the fundamental mechanisms that provides robustness and evolvability. It has been increasingly noted that robustness against mutation might evolve as a side effect of robustness against environmental perturbations and EMERGENT PROPERTIES of complex networks[67].

Robust modules

In principle, a modular system allows the generation of diverse phenotypes by the rearrangement of its intermodule connections and relatively independent evolution of each module through mutations. If the system is well integrated without modular structure, change in any part of the system might have a significant impact on other parts of the system, and slight changes in stimuli or noise might result in an unexpected and undesirable outcome. The system will be intractable, and it is difficult for these systems to generate new phenotypes without lethal effects. Notably, modularization mitigates this problem by allowing each module to function autonomously. However, simply having modules is not enough to ensure evolvability. It must also be robust against various perturbations. This feature is essential because modules need to be able to cope with changes in stimuli from adjacent modules that might evolve independently or that function in a

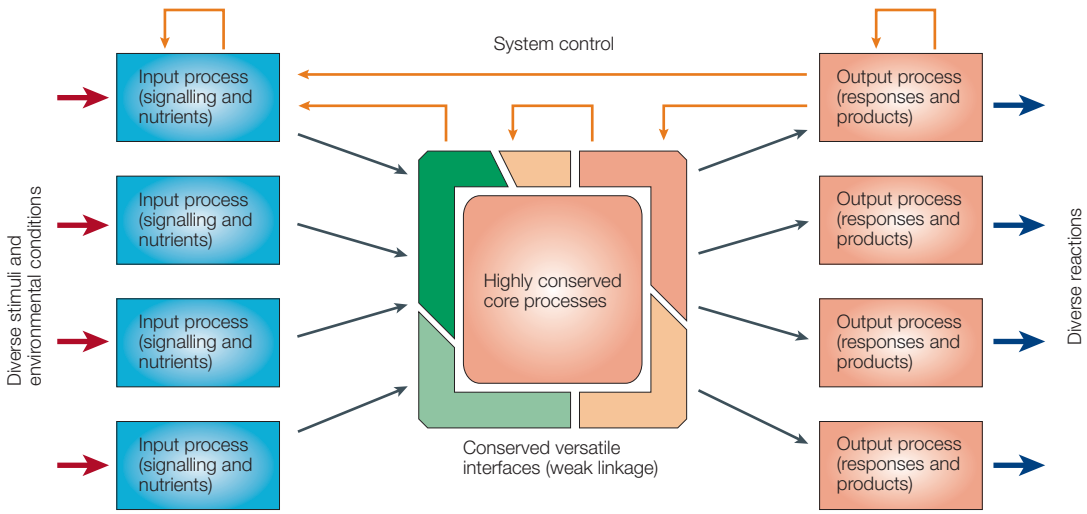

Figure 3 | **The architectural framework of robust evolvable systems.** The bow-tie (or hour-glass) structure has many inputs and outputs that are connected through a conserved core and versatile weak linkage with the extensive system control governing the system's dynamics. Core processes and versatile interfaces overlap or merge in some cases. This bow-tie structure appears at various levels in the system, such as metabolism, signal transduction, transcription and translation[66]. In signal transduction, diverse stimuli are initially received by receptors, different isoforms of G-proteins are activated, but converge mainly to second messengers that have a limited variety and cause weak linkage. Then, modulations in second messengers influence core processes to trigger expression of different genes and, ultimately, different reactions. However, this process is not a simple flow of information, as extensive local and global feedback regulations are imposed at every step. During metabolism, diverse nutrients are processed into precursors that core metabolic pathways then covert into basic cellular 'currencies' such as ATP and NADH, as well as activating biosynthesic pathways to produce amino acids, nucleotides, sugar, and so on. Transcription and translation also represent structures where common machineries are used to decode a wide range of genetic information and produce diverse proteins, but versatile mechanisms themselves make up the conserved core. Various processes are interfaced with core processes through versatile interfaces, so that novel processes can be added and removed easily without seriously affecting other parts of the system.

EXPLORATORY SYSTEMS
Systems that are based on epigenetic variations and selection; such as angiogenesis and nerve outgrowth.

BOW-TIE
A structure that has various inputs (fan-in) and outputs (fan-out) that are connected by a knot, resembling a bow-tie.

EMERGENT PROPERTY
A feature that is characteristic of system-level dynamics that cannot be attributed to any of its components. The existence of an emergent property indicates that the whole is more than just the sum of the parts.

different context. This robustness is attained through system control and alternative mechanisms that are inherent to each module.

The contribution of system control to module robustness is particularly important in development, whereby new morphologies can be explored during evolution. A similar type of robustness to that of the segment-polarity network in *D. melanogaster* was also identified through the establishment of the BMP (bone morphogenetic protein) morphogen gradient[68]. The organizing centre is a good example of a robust buffer against variation in development[69,70]. There is a subset of developmental processes that is tolerant against variations in initial values and that robustly forms patterns that serve as a basis for further elaborations in developmental patterning and that are amenable to reuse in a different context[71,72]. *Hox* genes exemplify the power of modularity where changes in the Hox cluster alone affects basic body plan — a network of genes downstream of the *Hox* gene direct development of a given part of the body autonomously from other parts of the body[73–78].

Signal-transduction pathways have an important role in generating phenotypic diversity during evolution. Hedgehog (Hh), wingless-related (Wnt), TGF-β, receptor tyrosine kinase (RTK), Notch, JAK-STAT (signal tranducers and activators of transcription) and nuclear hormone pathways are signal-transduction pathways that are widely used in various aspects of development. Co-option of existing signal-transduction pathways to new processes is considered one of the crucial features in evolution. For example, the Hh-signalling pathway that is used in wing-pattern formation is co-opted in butterfly-eyespot pattern formation[71]. Several signal-transduction cascades combine negative and positive feedback loops and are robust against perturbations so that normal cellular physiology and developmental processes can be maintained[25,26,28,79,80]. This intrinsic robustness of the pathway enables co-option, so that new morphologies can be generated.

The origin of modularity. The origin of modularity is still controversial. It is certainly an evolved property, not necessarily a selectable trait by itself, and it enhances flexible generation of various phenotypes during development[81]. At the same time, modular structures and modular regulatory networks within a single cell (including bacteria) that demonstrate enormous diversity and evolution indicate that flexibility of development is not the only reason for modularity. There are two possible reasons for why modularity might have emerged.

First, emergence of modularity of gene regulation might be required to handle diverse and complex stimuli and responses[49]. It is essential that signalling networks and reaction-related networks are modularized to some extent, to cope with various external perturbations without losing specificity to stimuli versus responses, and to prevent the effects of environmental perturbations from spreading system-wide.

Second, if a modularized phenotype has selective advantage owing to robustness against environmental perturbations, modular developmental processes could be co-selected because they are better at generating modular phenotypes. This hypothesis assumes that the modular phenotype has a selective advantage and the modular developmental processes are better at generating the modular phenotype; both assumptions have still to be tested. If these two reasons hold, modularity might have originated to ensure robustness against environmental perturbations, but congruent with flexibility of development.

The architectural framework

There are two architectural features that facilitate evolvability and robustness[63,64]: highly conserved core processes that are interfaced with diverse inputs (signalling and nutrients) and outputs (reactions and products); and versatile mechanisms that underlie essential processes of the system, so that any new processes that properly interface with these mechanisms can use them — this is also known as 'weak linkage'[63]. These features represent the bow-tie architecture at different levels, ranging from global topology to specific processes. Below, I argue that the bow-tie structure that is actually observed in biological systems facilitates robustness and evolvability.

Bow-tie structure in biological systems. Genome-wide analysis has revealed intriguing characteristics of the biological networks that support bow-tie structure. It has been proposed that metabolic networks and protein-interaction networks form a scale-free network (a property of scale-free networks that predicts that proteins prefer to form links with other proteins that already have the highest number of links)[82,83]. Scale-free networks tolerate random removal of nodes, but not systematic removal of nodes with high connectivity[84]. Ma and Zeng[85] considered the directionality of reactions in the analysis of metabolic networks of 65 fully sequenced genomes. They found that the these networks do not exhibit scale-free structure, but rather a 'bow-tie' structure, in which a large, highly connected core cluster is interfaced with less connected in- and out-clusters. The core of the network is a GIANT STRONG COMPONENT (GSC) SUB-NETWORK, the components of which are tightly connected[85]. Further comparisons of the metabolic networks between *Streptococcus pneumoniae* and *Pyrococcus furiosus* showed conservation of essential metabolic pathways, such as the TCA (tricarboxylic acid) cycle, pentosephosphate pathway and glycolysis pathway, within a GSC sub-network; the conserved core pathway is robust against perturbations[85].

Another indication that there is such a division into conserved core processes and those that provide novel functions comes from comparative studies that use functional annotation of 150 fully sequenced genomes[86,87]. The Gene Ontology's biological process hierarchy[88] was used to assign functional categories to each gene, and the proportion of genes in each functional category, for all 150 species, was counted. The

GIANT STRONG COMPONENT SUB-NETWORK
A sub-network in which there are a large number of components that have extensive internal connections.

results were presented in terms of a scaling exponent — 1.0 is when the total number of genes in a category is doubled, and less than 1.0 is when the number of genes in a category that have been increased is less than the increase in the total number of genes. A cross-species study carried out by van Nimwegen on 65 bacterial genomes indicates that basic biological processes have a scaling exponent of less than 1.0 (these include the cell cycle, DNA repair and replication, and protein biosynthesis), but processes that might generate evolutionary novelty tend to have a scaling exponent over 1.0 (these processes include transcriptional regulation, signal transduction, ion transport, two-component systems and cell communication)[86]. This tendency can also be seen in an extended study of 15 archaeal, 116 bacterial and 10 eukaryotic genomes by van Nimwegen[87]. These results show that there are highly conserved core processes, such as biosynthesis and DNA replication, and processes that have been added with increase in genome size, which might be responsible for the generation of various cell-types and morphological features, such as signal transduction, transcriptional regulation and intercellular communications. This implies that new pathways might be constantly added to the wings of the bow-tie structure as the genome size increases.

'Weak linkage'[64] enables the addition of new processes to the existing core process using common versatile mechanisms that operate on diverse inputs and outputs, such as ion channels, G-proteins and transcription machinery. If transcription machinery is different for each gene, addition of new genes and new transcriptional regulations requires the invention of customized transcription machinery that makes evolution almost impossible. GTP-binding proteins and the downstream cyclic AMP and calcium dynamics, as well as ion channels also represent systems with common underlying mechanisms that allow new repertoires to be added[64,89]. Inputs from various receptor channels, such as RTK and GPCR (G-protein-coupled receptor), converge mainly on second messengers, such as cAMP and calcium ions, which mediates various cellular responses, such as cell movement, cell growth, metabolism and so on[89]. Cdc42, a member of the Rho-family of GTPases, is another common regulator on which various RTK and GPCR pathways converge and mediate different cellular responses[90]. Most signal-transduction cascades converge to modulate limited numbers of second messengers, but signalling pathways are often diverse and include cross-talk[91,92].

Bow-tie is robust. Whether bow-tie structure provides robustness against external perturbations depends on the robustness of the conserved core and the global regulations imposed. Ma and Zeng argue that the GSC, a conserved core of the metabolic network, is robust against mutations because there are multiple routes between any pair of nodes within the GSC[85].

Various stimuli activate signal-transduction pathways that converge to modulate second messengers, which in turn activate various cellular responses. Here, second messengers are the conserved core of the bow-tie architecture and have to be maintained robustly. For example, cAMP is produced from adenylyl cyclases by ATP. Various adenylyl cyclase isoforms are involved in multiple pathways, possibly creating alternative pathways. ATP is supplied by a robust metabolic core mechanism that is also a bow-tie structure. Here, the bow-tie structure at the metabolic level supports the bow-tie structure at the level of signal transduction.

In addition to the robustness of the conserved core, the bow-tie architecture might provide an advantage in generating coordinated response to various stimuli. So, a bow-tie architecture improves robustness against external perturbation by having many inputs connecting to the robust core where numerous reactions are mediated. Direct association between stimuli and reactions, without the use of the robust core, requires extensive individual controls to achieve a coordinated response, and disruption of any such regulation could seriously undermine system behaviour. Unless each stimulus reaction can be regarded as independent, making coordination unnecessary, control through the common robust core might provide better system robustness.

However, do these mechanisms allow for the accommodation of phenotypic diversity? In fact, versatile mechanisms are essential to accommodate various possible input stimuli and reactions in a consistent manner. For example, the addition of new signal transductions only needs to be interfaced with existing machinery without inventing an entire cascade. Also, recent findings of differential tissue and cell-type specific expression of GPCRs in the human and mouse[93] provide explanations on how various cells might use the conserved-core and weak-linage mechanisms. Expression-pattern analyses using real time PCR on 100 randomly selected endoGPCRs (GPCRs for ligands of endogenous origins) revealed that each endoGPCR is expressed in numbers of different tissues, with each tissue having a unique combination of endoGPCRs[93]. This indicates the possibility that a relatively small set of second messengers are used in different contexts to allow differentiation into various cell types and a range of cellular responses. The same argument applies to a limited set of versatile genetic networks, also referred to as a toolkit[72], because they can be used in different contexts.

Robustness trade-offs

I have argued that robustness is a fundamental feature that enables complex systems to evolve, and that evolution enhances robustness of organisms. One possibility following on from this, is to increase the complexity of organisms through successive addition of regulatory systems, such as diverse regulation, signal-transduction pathways, RNA regulation[94–96] and histone modifications[97], to enhance robustness against specific environmental perturbations and to allow exploration into unoccupied niches[98]. However, the introduction of various control feedback loops generates trade-offs by causing instability when unexpected perturbations are encountered, leading to catastrophic failure. Carlson and Doyle tried to generalize these

issues in their HIGHLY OPTIMIZED TOLERANCE (HOT) THEORY by arguing that systems that have evolved to be robust against general perturbations are extremely fragile against certain types of rare perturbations[99,100]. In dynamic systems, these evolutionary optimizations are achieved by successively adding feedback controls to the system. It is important to recognize that although the HOT theory describes behaviour of complex systems designed or evolved towards optimality against perturbations, other self-organization models such as a scale-free network[83,101] by preferential attachment and SELF-ORGANIZED CRITICALITY[102] describe systems with stochastic additions of complexity without design or evolution involved. The nature of robustness and fragility that is predicted by the HOT model is generally consistent with observed properties of designed complex systems and is notably different from other models[99,100]. Csete and Doyle further argued that trade-offs between robustness and fragility indicate that robustness is a conserved quantity, a concept that also applies to biological systems[103].

Trade-offs between robustness and fragility can be intuitively understood by using the aeroplane example again. The Wright brothers' aeroplane is not robust against atmospheric perturbations, unlike modern commercial aeroplanes. However, modern aeroplanes are extremely fragile against unusual perturbations, such as total power failure — because their flight-control system is totally dependent on electricity. The Wright brothers' aeroplane, on the other hand, is not affected by this type of failure as it does not use electrical controls in the first place. Although they might seem simple, it is important to appreciate these trade-offs because diseases are often manifestations of fragility.

Fragility is not the only cost of improved robustness. In electronic-circuit design, the use of negative-feedback control achieves an improved fidelity or amplification for a certain range of inputs by reducing overall gain of the amplifier. So, the use of negative-feedback control achieves robustness within a certain range of inputs at the cost of some aspects of performance and with the creation of fragility elsewhere. The trade-offs between robustness, fragility and performance can be observed in biological systems at different levels. Bacteria, for example, should be able to swim faster without negative feedback, but this would sacrifice their precision in following a chemical gradient: the use of negative feedback improves the bacteria's ability to follow a chemical gradient, at the cost of reduced swim speed.

Alternative mechanisms and modularity also enhance robustness, but at the cost of increased resource demands. For example, the probability that a function with a single component will fail is p. The probability of the function with two components that fail to back up each other will be reduced to $(1-p)^2$. This reduction is accompanied by doubling of the resource requirement. This type of trade-off is effectively mitigated in biological systems because components are not identical copies, but tend to have overlapping functions. Having identical copies as alternatives is only efficient when failure rate or turn-over rate, is expected to be high. Modularity also causes trade-off between robustness, flexibility and resource demands. Merging modules to share common circuits and components reduces resource needs. However, preventing the spread of perturbation and flexibility of rearrangement in a robust way is seriously compromised.

Although there are several trade-offs in robust complex systems, trade-offs involving system controls are the most important ones. System controls define dynamic properties of the biological systems that illustrate how complex biological systems behave when perturbed and coordinate how alternative components and modules are re-routed to ensure robustness of the whole system. Owing to the intrinsic trade-offs that have been discussed, it is not possible to simply increase general robustness of the system without a sacrifice in performance and increased resource demands.

A system-level view of disease and therapy

Properties of robust evolvable systems have direct consequences on our understanding of diseases and therapy design. First, robust systems, whether biological or engineered, are most vulnerable when the system's fragility is exposed. For example, *Diabetes mellitus* can be thought of as an exposed fragility of the system that has acquired robustness against near-starvation, a high energy-demand lifestyle and high risk of infection, but it is unusually perturbed by over-nutrition and a low energy-demand lifestyle[16]. Second, the system is relatively tolerant of the removal of some components or cells, because of available alternative mechanisms and the robustness of the bow-tie architecture. However, the system is vulnerable when components behave inappropriately but are not being removed. Third, the epidemic state might exhibit robustness against natural and therapeutic countermeasures if intrinsic mechanisms for robustness in our bodies are co-opted. The worst-case scenario is when components of the system behave inappropriately without being removed, and malfunctioning components and their behaviour are robustly maintained against countermeasures: the mechanisms that support robustness of the host are also used to protect the failed components. This is important because the intrinsic dynamics of diseases and appropriate countermeasures are different depending on which scenario the system failure follows. For example, cholera toxin interacts with the Gs_{α} subunit to trigger the symptoms of the disease[105,106]. It can be easily removed by antibiotics, because the intrinsic robustness of the host has not been hijacked by the pathogen and is not involved in the infection.

By contrast, cancer and HIV infection represent a worse scenario — they are maintained and even promoted through the intrinsic robustness mechanisms of host system. Counter measures for these diseases could include: actively perturbing specific interactions or components to maintain or reduce robustness, finding a point of fragility (an 'Achilles' heel') that is inherent in robust systems and re-establishing control of the epidemic state by introducing a counter-acting decoy (a 'Trojan horse') or a new regulatory feedback.

HIGHLY OPTIMIZED TOLERANCE THEORY
A theory about the dynamic properties of systems that are designed, or evolved, to be optimal (either towards a global optimum or sub-optimum). The theory predicts whether systems that are robust against certain perturbations are fragile against unexpected perturbations.

SELF-ORGANIZED CRITICALITY
A phenomenon whereby certain systems reach a crucial state through their intrinsic dynamics, independently of the value of any control parameters.

Cancer is a highly robust disease in which the tumour proliferates and metastasizes, in some cases despite much therapeutic effort. Although anti-cancer drugs might temporarily reduce tumour mass, it relapses in many cases and cure is still rare. The difficulty of treating tumours is attributed to acquired robustness, partly owing to the co-option of intrinsic mechanisms for robustness[14,15]. The high level of genetic heterogeneity in the tumour forms a fail-safe through genetic diversity and multiple feedback loops in both the cellular and the tumour-host environment and accounts for the robustness of cancer. The genetic heterogeneity within a tumour cluster, caused by chromosome instability, generates a high level of genetic heterogeneity in survival and proliferation. This genetic heterogeneity allows some malignant cells to tolerate therapy and re-form a tumour.

Mechanisms that maintain normal functions of the body also function to enhance tumour robustness against therapy. For example, drug resistance is caused by upregulation of *MDR1* and other genes, the products of which pump out toxic chemicals from the cell; so a function that protects us under normal conditions, is exploited by the tumour to protect the malignant cells. Low oxygen supply (hypoxia) during the tumour-cluster development is countered by metabolic shift from oxygen-dependent TCA cycle to glycolysis, as well as activating a feedback loop by upregulating *HIF1*, which upregulates VEGF (vascular endothelial growth factor)to promote angiogenesis, and MMP (matrix metalloproteases), uPAR (urokinase-type plasminogen activator) and CRCX4 (a chemokine receptor) to promote tumour-cell metastasis[107]; a series of responses that protects the body against the effects of hypoxia.

From the robustness perspective, possible clinical strategies that could be used against cancer are the control of the robustness of the tumour and finding out the point of fragility that is inherent in the robust system. To control robustness, therapy should be directed to induce tumour dormancy by selectively inducing cell-cycle arrest, rather than aiming at tumour eradication, because one source of robustness is genetic heterogeneity created through somatic recombination. Apart from specific cases where tumour cells are relatively homogeneous, tumour-mass reduction might not be an appropriate therapeutic goal, because of the high risk of relapse if the reduced tumour gained a greater level of heterogeneity, including those cells that are highly resistant to therapeutic efforts. Controlling tumour robustness would be a genuine measure of therapeutic efficacy, so that the risk of relapse could be well controlled. The other approach is to find the fragility of tumours. Tumours acquire robustness against a range of therapies, which must be accompanied by the development of extreme fragility.

The question remains how to find fragility that is therapeutically effective and practical. It is important to investigate what the system has been optimized for and to identify sources of robustness. As fragility is a by-product of robustness, the fragile point of the system must be associated with a mechanism that gives rise to enhanced robustness. For example, the robustness of a tumour is sustained by chromosome instability, intracellular feedback loops and host-tumour interactions. Possible countermeasures include control of the cell cycle through the combined use of several drugs, possibly using RNAi, selectively destabilizing or stabilizing unstable chromosomes, selectively delivering engineered genes[108] to re-establish control of host-tumour interactions or introducing artificial genetic circuits[109] to conditionally express tumour-suppressor genes, should be considered. Importantly, these countermeasures have to be carefully designed to specifically explore fragility or to control robustness.

HIV predominantly infects CD4-positive T cells and replicates when the cell activates its anti-virus responses[110–113]. Infected cells are not removed because infection is hardly detectable. This is a typical case of what happens when malfunctioning components (the infected cell, in this case) are not removed from the system. In addition, HIV creates diverse genetic alternation. HIV's strategy is to hijack the robust immune-response mechanism, which makes AIDS difficult to cure. Robust persistence of the epidemic state through generation of genetic diversity and various feedback loops is similar to the strategy found in cancer. Possible therapeutic approaches already discussed might apply to the effective treatment of AIDS. Interestingly, one strategy proposed to combat HIV involves forcing HIV into a latent state, instead of trying to remove it, by introducing a decoy — a conditionally replicating HIV-1 vector (crHIV-1)[114–116]. crHIV-1 contains *cis*, but not *trans*, elements that are necessary for virus packaging, and carries antiviral genes that inhibit wild-type HIV-1 functions[116]. If successful, crHIV-1 might re-establish control of the system and put HIV-1 virus in a state of prolonged latency.

Towards a theory of biological robustness

Given the importance of robustness for the understanding of the principles of life and its medical implications, it is an intriguing challenge to formulate a mathematically solid, and possibly unified theory of biological robustness that might serve as a basic organizational principle of biological systems (early attempts date back to the middle of the last century[117,118]). Such a unified theory could be a bridge between the fundamental principles of life, medical practice, engineering, physics and chemistry. This is a difficult challenge in which a number of issues have to be solved, particularly to establish mathematically well-founded theories. However, the impact would be enormous.

First, the solid quantitative index of robustness has to be established. This index has to be able to equate with experimentally measurable quantities, so that hypotheses on the degree of robustness can be tested experimentally. There are several concepts on the stability of the system in CONTROL THEORY[119], and some attempt has been made to apply these ideas to biological robustness[120]. The practical application of these concepts to biological systems has been limited, mainly owing to the enormous degree

CONTROL THEORY
The theory about the design of optimal control methods for engineered objects. It is one of the most successful fields in which mathematical principles are directly applied to practical products, such as aeroplanes, hard disks, automobiles, robotics and chemical plants, and enables them to function properly. Usually, the theory is concerned with how feedback control can be used in various cases to attain optimal design behaviour.

of dimensionality and non-linearity that is intrinsic to biological systems. Measuring perturbations for all these dimensions is impractical. A practical, theoretically solid index needs to be developed to provide guidelines for selecting smaller numbers of parameters to be perturbed so that the system's response can be measured. Haken pointed out that only a few parameters are important to describe system behaviour near the bifurcation point[121], but this approach is too limited to analyse various types of behaviour of biological systems. A number of mathematical methods are being developed that might be of benefit for the mathematical analysis of the robustness of biological systems[122,123]. On the experimental front, comprehensive mutant creation and dosage-controlled perturbations are already feasible for some organisms. As there are often practical aims to investigate robustness of the system — for example, to induce cell-cycle arrest — experiments can be carefully designed to perturb relevant genes and parameters, and published data can be systematically collected to obtain practical, sufficient indeces of robustness for specific aspects of the system[124].

Second, a theory that embraces the various aspects of robustness has not yet been formulated. Control theory is often used to explain robustness that involves feedback regulation, but this only covers one aspect of robustness. Furthermore, the control theory assumes that there is a certain set point, determined by the designer, that the system's state will approach, even when perturbed. Of course, there is no such designer in biological systems, and set point is implicit in the equilibrium state of the system, which often changes dynamically. A new theory is required to reflect these features of biological systems.

Recent efforts to integrate control theory and SHANNON'S CHANNEL-CODING THEOREM might provide an interesting framework for feedbacks[125,126]. This theorem tries to formalize cases where information capacity in the feedback loop is limited, so that the feedback signal is potentially impeded by fidelity, noise and delay. This better reflects the reality of biological systems, in which signals are transmitted with noise, delay and compromised fidelity.

Third, attempts to relate the dynamics of life to thermodynamics have been of limited success. The behaviour of physical and chemical systems near equilibrium have been investigated in the past. LE CHATELIER–BRAUN'S PRINCIPLE provides the basis for response against perturbations for systems in equilibrium[127]. This principle indicates the emergence of a compensatory feedback to cope with perturbations. Efforts have been made to extend thermodynamics from equilibrium to explain biological phenomena[128–130], but apply only to relatively simple chemical reactions under a medium, such as in BELOUSOV-ZHABOTINSKI (BZ) REACTIONS. Signal transduction, for example, brings about dramatic changes in the state of the cell depending on the intensity and types of stimuli, resembling transition from the near-equilibrium state to a state that is far from equilibrium. However, this is not the result of simple chemical reactions under an unstructured medium, as seen in BZ reactions. Cells are highly structured and have explicit interactions and physical structures that are controlled by gene expression, cytoskeleton and other regulatory systems that are optimized to be robust and evolvable. The greatest challenge will be to formulate theories that account for thermodynamics in heterogeneous and structured systems. Some efforts are being made to find a theoretical framework by assuming network structure as a basis of mathematical description[131]. However, the results are still too abstract to be practically applied to biological systems. Progress in this field will help to connect biology, chemistry, physics and mathematics in a coherent manner.

Conclusion

Robustness is a fundamental property of biological systems. It facilitates evolvability, and evolution selects robust traits. I have argued that there are specific architectural features required to make organisms robust and that they might be universal to any robust and evolvable complex system. System controls, modularity, alternative mechanisms and decoupling serve as basic mechanisms to provide robustness to the system, but these mechanisms need to be organized into coherent architecture to be effective at the level of the organism. Enhancement of robustness against perturbations can be made through the combination of these mechanisms, but system control is the prime mechanism for coping with environmental perturbations that require proper dynamics. Therefore, evolution of organisms can be viewed, at least in one aspect, as evolution of control systems. Modularity, alternative mechanisms and decoupling, in part, support the robust maintenance of control loops, but are also controlled by control loops either explicitly or implicitly. There is an intriguing possibility that genetic buffering and modularity originated from robustness against environmental perturbations, and subsequently evolved to have wider applicability. It is important to realize that systems that are evolved to be robust against certain perturbations are extremely fragile to unexpected perturbations. This robust yet fragile trade-off is fundamental to complex dynamic systems. It is important to understand architectural features of robust and evolvable systems and the intrinsic nature of robustness and fragility because they dictate a mode of system failures and effective countermeasures, which, as previously discussed, has direct implications for understanding disease and devising effective therapies. Failures and viable countermeasures of these systems are often counter-intuitive, which implies that a theoretically motivated robustness perspective might provide new therapeutic approaches. The emerging field of systems biology has been trying to identify system-level properties, but simple use of large data sets and computation would not effectively provide insights into biological systems. The perspective on biological robustness would provide effective guiding principles for understanding many biological phenomena, and for therapy design.

SHANNON'S CHANNEL-CODING THEOREM
A theorem by Claude Shannon which indicates that for a given channel there exists a code that will permit the error-free transmission across the channel at a rate R, provided R≤C, where C is the channel capacity. This means that the probability of error will not equal zero when R>C, that is, transmission is larger than channel capacity.

LE CHATELIER–BRAUN'S PRINCIPLE
A thermodynamics principle which states that if a dynamic equilibrium is disturbed by changing the conditions, then the system tends to adjust to a new equilibrium counteracting the change.

BELOUSOV-ZHABOTINSKI REACTIONS
This is a chemical reaction that is widely used to demonstrate transition from the near-equilibrium state to the far-from-equilibrium state. When a low level of heat is applied, it is dissipated without affecting the qualitative characteristics of the medium, but when additional heat is applied, the system undergoes a drastic change, and a circulating flow of chemicals emerges.

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Competing interests statement

The author declares no financial interests.

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