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Resolving the Rules of Robustness and Resilience in Biology Across Scales

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Synopsis Why do some biological systems and communities persist while others fail? Robustness, a system's stability, and resilience, the ability to return to a stable state, are key concepts that span multiple disciplines within and outside the biological sciences. Discovering and applying common rules that govern the robustness and resilience of biological systems is a critical step toward creating solutions for species survival in the face of climate change, as well as the for the ever-increasing need for food, health, and energy for human populations. We propose that network theory provides a framework for universal scalable mathematical models to describe robustness and resilience and the relationship between them, and hypothesize that resilience at lower organization levels contribute to robust systems. Insightful models of biological systems can be generated by quantifying the mechanisms of redundancy, diversity, and connectivity of networks, from biochemical processes to ecosystems. These models provide pathways towards understanding how evolvability can both contribute to and result from robustness and resilience under dynamic conditions. We now have an abundance of data from model and non-model systems and the technological and computational advances for studying complex systems. Several conceptual and policy advances will allow the research community to elucidate the rules of robustness and resilience. Conceptually, a common language and data structure that can be applied across levels of biological organization needs to be developed. Policy advances such as cross-disciplinary funding mechanisms, access to affordable computational capacity, and the integration of network theory and computer science within the standard biological science curriculum will provide the needed research environments. This new understanding of biological systems will allow us to derive ever more useful forecasts of biological behaviors and revolutionize the engineering of biological systems that can survive changing environments or disease, navigate the deepest oceans, or sustain life throughout the solar system.

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The problem

Life on Earth is shaped both by ancient and current events: no environment on Earth is fully invariant. Why particular biological systems, lineages, and communities persist while others fail is a question that spans multiple disciplines within and outside the biological sciences. Understanding how all levels of biological organization respond to perturbation is central to decoding the rules of life. All living systems, including humans, face rapid changes in climate and landscapes that bring significant biotic (e.g., availability and phenology of prey and food items) and abiotic impacts (e.g., frequency/severity of floods, droughts, wildfires; temperature extremes; König et al. 2020; Wintle et al. 2020). Revealing and applying common rules that govern the robustness and resilience of biological systems is an important and indispensable step toward finding solutions for preventing and curing diseases, for the ever-increasing need for food and energy, as well as for species survival. However, we lack an overarching understanding of the fundamental mechanisms that enable biological systems at various levels to appropriately respond to alterations in their environment and withstand or recover from perturbations. If researchers can decode universal rules of robustness and resilience, we can use these rules to predict how life on Earth will respond to rapidly changing conditions, to develop tools for ecosystem conservation, and to improve human conditions.

Shifting our conception of the natural world as many nested and interconnected networks (see Fig. 1, Cantor et al. 2017) will transform how we view the minutiae and grandeur of biodiversity, while understanding how biological systems respond to changing conditions over time and space has a multiplicity of broader applications. How biological systems react with current, rapidly changing environmental conditions will affect every living thing on Earth (e.g., Hammerschlag et al. 2019). Outcomes of these efforts have consequences for an array of applications that will improve the quality of life for humans. The study of robustness and resilience at sub-cellular, physiological and tissue levels has medical implications; research in this area can set the stage for advancements in disease biology and cancer treatments (Pienta et al. 2020; Rauter et al. 2020). The study of robustness and resilience can also be viewed through the lens of organismal biology and responses to environmental changes; outcomes from this area will influence conservation strategies for species in threatened ecosystems as well as providing a unique view of many potential and realized threats to biodiversity (e.g., Donelan et al. 2019). Finally, understanding resilient and robust biological systems can even facilitate improved design of energy storage/transport, urban transportation systems, and movement of resources across the globe (Ma et al. 2015; Wu et al. 2019; Tang et al. 2021).

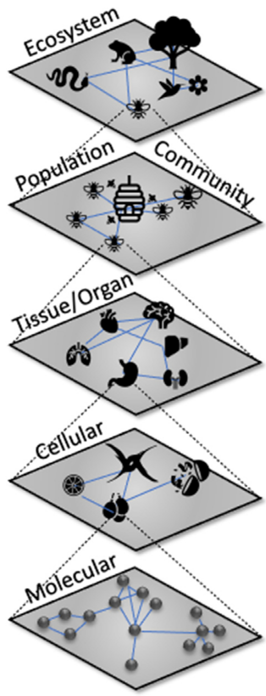

Fig. 1 Network theory can be applied to describe systems across biological levels of organization, and models linking these nested networks will ultimately allow us to understand and predict how biological systems respond to changing conditions over time and space

Concepts of robustness and resilience

Processes related to robustness and resilience are studied by scientists across biological and physical disciplines, as well as social sciences, computer science and engineering (Table 1). At the same time, research into the responses to perturbations is often siloed at molecular, cellular, organismal, and ecological scales or within a discipline. Here, we define robustness as the ability of a system to remain in or reach the same stable state despite diverse internal and external environments. Robustness underscores the ability of a biological system to maintain the original state even after encountering perturbations. In contrast, resilience (or resistance in ecological sciences) is the ability of a biological system to return to a previous state or establish a new state after significant perturbations. For example, a plant is robust and resilient if it grows normally across all different light conditions. A plant is resilient but not robust if it becomes dormant in the dark but restores growth rapidly once the desired light condition is met. A plant is robust but not resilient if it can grow under most light conditions but cannot handle switching between differ-

Table 1. Selected definitions of robustness and resilience and conceptually related terms that are commonly used across scientific disciplines, with citations. Note that in some cases, definitions of terms merge aspects of robustness and resilience and therefore, the location of the term within columns was arbitrary

Discipline	Terminology and definitions of robustness and related terms/concepts	Terminology and definitions of resilience and related terms/concepts
Physics	**Robustness:** Maintenance of some desired system characteristics despite fluctuations in the behavior of its component parts or its environment (Carlson and Doyle 2002).	**Resilience:** The ability of an object to recover its original structure and shape after a deformation once the constraint causing the deformation is removed; it might exhibit some transient hysteresis or slow relaxation, but the initial state is finally restored (Lesne 2008).
Biochemistry	**Robust** biochemical networks are relatively insensitive to the precise values of biochemical parameters (Barkai and Leibler 1997). **Robustness** is a property that allows a system to maintain its functions against internal and external perturbations (Kitano 2004).	**Structural divergence:** Proteins that share evolutionary history and differ in tertiary structure but maintain biochemical functionality (Zhang et al. 2014).
Developmental biology	**Canalization:** Adjustment of developmental reactions so as to bring about one definite end-result regardless of minor variations in conditions during the course of the reaction (Waddington 1942). **Robustness:** The ability to produce a consistent phenotype regardless of environment or genetic variation (Wagner 2005; Sieriebriennikov and Sommer 2018); also called phenotypic stability (Nijhout et al. 2019).	**Adaptive developmental/phenotypic plasticity:** Phenotypic variation displayed by genetically identical individuals that develop under differing environmental conditions (Lafuente and Beldade 2019; Nijhout et al. 2019).
Neuroscience	**Robustness:** Reproducible emergence of a desired outcome, irreversible and unaffected by noise creating self-amplification of that outcome (Lesne 2008).	**Resilience** is a dynamic process whereby an individual can withstand challenging conditions while still maintaining relatively normal physical and physiological functioning (i.e., positive adaptation; Fletcher and Sarkar 2013).
Physiology	**Robustness** refers to one's ability to maintain physiological function and avoid persistent damage when a disturbance occurs. A robust individual would have a wide reactive scope which refers to the ability to physiologically adjust or acclimate and survive in a wide range of conditions (Ukraintseva et al. 2016; Wada 2019; Romero et al. 2009). **Homeostasis: "**The coordinated physiological processes which maintain most of the steady states" (Cannon 1932). [*Note: The emphasis on the maintenance of steady states in this definition relates to robustness, but the coordinated physiological processes that maintain these states refers to resilience*] ." The constancy of the internal environment is the condition for free and independent life" Claude Bernard, cited in Davies (2016).	**Resilience** refers to one's ability to recover from the damage and regain or re-establish normal physiological function (Ukraintseva et al. 2016; Wada 2019). **Adaptive homeostasis:** 'The transient expansion or contraction of the homeostatic range in response to exposure to sub-toxic, non-damaging, signaling molecules or events, or the removal or cessation of such molecules or events" (Davies 2016). **Heterostasis:** The establishment of a new steady state by exogenous (pharmacologic) stimulation of adaptive mechanisms through the development and maintenance of dormant tissue reactions" (Selye 1973). **Allostasis:** Achieving stability through change; the active process by which the body responds to daily events and maintains homeostasis (Sterling and Eyer 1988; McEwen 2008).
Social science/behavioral science	**Organizational robustness:** Organizations that are able to reduce the adverse consequences of externally generated failures (Dodds et al. 2003).	**Individual resilience:** Competence despite high-risk status, chronic stress, or following prolonged or severe trauma (Egeland et al. 1993). **Social resilience:** The ability of groups or communities to cope with external stresses and disturbances as a result of social, political, and environmental change (Adger 2000).
Community and ecosystem ecology	**Robust** communities maintain structural organization and functionality when responding to disturbances/uncertainty (Stenuit and Agathos 2015). **Resistance:** the ability of individuals or structures to tolerate or persist through disturbance, or the ease or difficulty of changing an ecosystem (Brand and Jax 2007; Falk et al. 2019); also referred to as persistence (Sutherland 1990); related to ecosystem stability (Elton 1958).	**Resilience:** Measure of the persistence of systems and of their ability to absorb change and disturbance and and reorganize while undergoing change so as to still retain essentially the same function, structure, identity, and feedbacks (Holling 1973; Walker et al. 2004) (*Note: combines concept of robustness and resilience*). The ability of the system to maintain its identity in the face of internal change and external shocks and disturbances (Cumming et al. 2005).

Table 1. Continued

Discipline	Terminology and definitions of robustness and related terms/concepts	Terminology and definitions of resilience and related terms/concepts
		Recovery: The re-establishment of the pre-disturbance population following mortality of the original individuals, through recruitment or colonization (Falk et al. 2019).
Evolutionary biology	**Mutational robustness:** The persistence of the phenotype, individual, or species (or lineage) in the face of deleterious mutations (Whitacre 2012).	**Evolutionary resilience/resistance**: A dynamic process in which lineages undergo evolutionary adaptation in response to changing environmental conditions; associated with the maintenance of biodiversity (Sgro et al. 2011).

ent light conditions. A plant is neither robust nor resilient if it only grows under one specific light condition and dies when that condition is not met. It is important to recognize in these definitions that one needs to carefully define variables into measurable characteristics or properties of a system (operationalize the system) maintaining stability, and identify what processes or mechanisms are conferring the ability to return to the steady state in the context in which each of these terms are used (Brand and Jax 2007; Whitacre 2012; Nijhout et al. 2019).

There are two overarching questions for examining robust and resilient systems: (1) how do biological systems maintain robustness and resilience in a continuously fluctuating and changing environment? (2) are there common rules that govern resilience and robustness across different scales of biological organization, from molecules to ecosystems? These questions can be addressed by examining biology as a multiscale, nested, hierarchical system, and considering how this complex system navigates changing conditions. We can then develop a holistic view of biological organization with more integrative approaches than the more discipline-specific or molecule-specific approach currently used. This approach will allow us to decode the complexity of biological systems and depict the hierarchical and network designs of biological systems more clearly. When we can deduce these rules, strategies, and mechanisms and any necessary variations, we will be better positioned to describe, model, and forecast resilience and robustness in systems across different levels. In addition, we will be able to create tools that allow us to “hack” biological systems, lending solutions to large problems involving disease, climate change, and threats to biodiversity.

Here, we propose that concepts from network theory provide a framework for universal mathematical models to describe robustness and resilience and their relationship. First, we review properties of networks that confer robustness and resilience and provide examples of systems in which network theory has been applied (review the current state of knowledge). We then identify barriers that need to be overcome before scientific investigation can embrace network theory approaches, and describe ways a reintegration of biology and potential technological advances will allow us to overcome those barriers to advance our understanding of mechanisms underlying robustness in biological systems. Lastly, we suggest open questions and research opportunities that remain to be addressed.

Review of network theory

How network theory relates to the study of robustness and resilience

The study of resilience and robustness is a transdisciplinary field that is amenable to a network science framework across different levels of biological organization. One network at a particular level of biological organization (e.g., within a cell) can become a node in a network at a different scale (e.g., across a cell population). Because networks are universal, scientists in all disciplines such as psychology, anthropology, social science, economics, and engineering can benefit from a network-based, unified theory of biological robustness and resilience.

A network is defined as a collection of nodes and edges, which are abstract and universal to systems of all levels but can also be embodied with specific properties unique to individual systems. Each node in a network could be molecules, genes, cellular transduction pathways, individuals, or genotypes in a population, species in a community, or trophic levels in an ecosystem. For example, a biological community can be regarded as a network of interacting species within a geographic area. Within each species, different populations can have varying levels of interconnectivity, resulting in gene flow or isolation and constituting a dynamic network over time (Proulx et al. 2005; May 2006). Within each population, such as a colony of eusocial insects, individuals operate in a network to fulfill different functions of the colony (Wild et al. 2021). Within

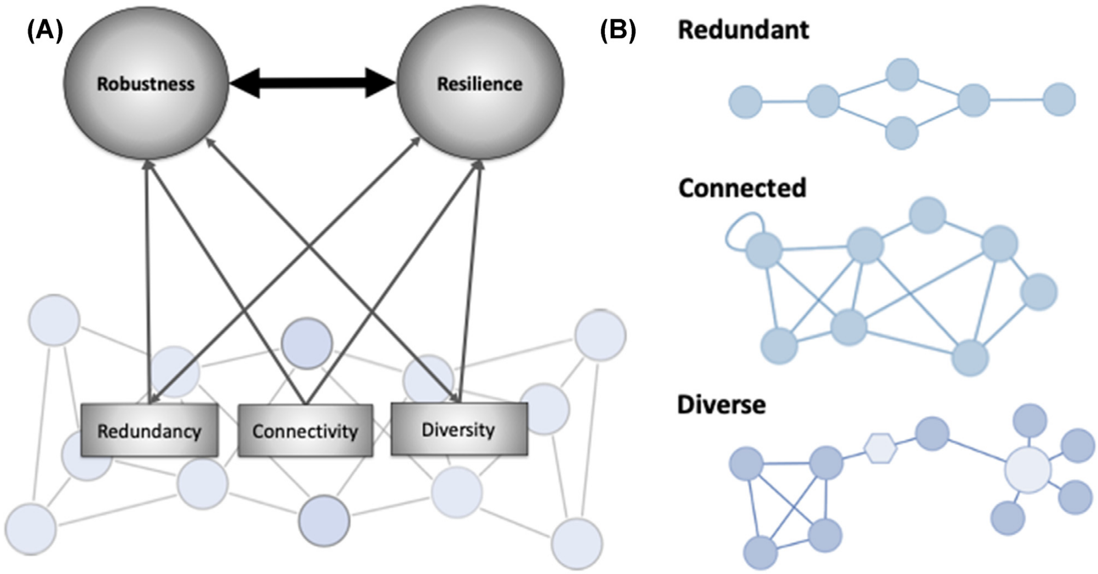

Fig. 2 Schematic of the properties relating to robustness and resilience of biological systems based on a network science framework. **(A)** In this model, robustness and resilience are emergent properties (blue circles) of the dynamic workings of networks that have redundancy, diversity, and connectivity, which includes functional feedbacks and lines of communication among nodes. **(B)** Examples of networks that represent redundant, connected, and diverse topologies are shown. By defining systems using this framework, biologists can use unifying experimental, mathematical, computational, and engineering approaches to understand how systems interact across levels of biological organization and respond to perturbations

the organism, physiological regulatory networks operate to adjust functionality of multiple systems depending on environmental conditions (Cohen et al. 2012; Nijhout et al 2019). Within an embryo, different cell populations connect and operate in a developmental regulatory network to pattern the body plan of an organism (Levine and Davidson 2005). Within each cell, functions are maintained by metabolic networks and cytoplasmic molecular networks, and in the nucleus, transcriptional networks are modulating cellular function (Gómez-Romero et al. 2020).

Borrowing from the mathematical theory of networks, we propose that key properties determining the robustness and resilience of biological systems at any organizational level are redundancy, diversity, and connectivity (see Fig. 2). Below we provide definitions and some examples of the relationship between these network properties and robustness and resilience.

Redundancy: Multiple nodes in a network could have the same or overlapping functions. If one or more nodes lose function, others can compensate. Similarly, there could be multiple routes of communication among nodes that confer the same functionality to a network. Redundancy is widely observed in developmental biology, where essential developmental events are often under the control of many genes that have similar or overlapping functions, and the expression of one gene compensates for the failure of another, up to a certain point. Redundancy is often used to explain how embryos tolerate developmental errors to result in the successful development of canalized body plans and morphogenesis (Lachowiec et al. 2018). Genetic knockout studies demonstrate the redundancy of many different molecular pathways (Salanga and Salanga 2021). Similarly, redundancy of neuroendocrine and genetic mechanisms regulating food intake are characteristics of a system regulating energy balance homeostasis (Schwarz et al. 2000). Lastly, food webs with overlapping ecological niches at different trophic levels are considered to confer stability to the system (Sanders et al. 2018).

Connectivity: We broadly define connectivity as the extent to which nodes communicate with each other, or specifically, the number and types of connections (edges) linking nodes in a network. Connectivity is a universal property of networks, but the specific connectivity depends on the structure of the network and mechanisms of communication and interaction. Networks can be described as distributed, decentralized, or centralized, each having different patterns of connectivity. An important concept is the idea of "scale-free" networks, describable by power law distributions of nodes with increasing connectivity degrees. Scale-free connectivity patterns are more likely to occur in biological systems than in informational or other technological systems (Broido and Clauset 2019), but the idea of universal scale-free network connectivity remains slightly contentious and requires more development (Holme 2019).

Connectivity plays a critical role in determining the robustness and resilience of a network. For example, distributed networks with high levels of edges connecting nodes confer stability, as demonstrated in the stability and persistence of metapopulations linked with migration (Hopf et al. 2019). During gastrulation, sheets of cells are robust against any "weak links" of individual cells in the population to allow for successful differentiation into germ layers. However, they are also resilient—they can bend in response to external forces, while enabling them to still maintain cohesion and function (Davidson 2012). System feedback (i.e., negative feedback or positive feedback) is an essential part of control theory of dynamic systems. In the context of biological networks, feedback mechanisms are encoded in connectivity. Feedbacks in a network allow upstream nodes to send out signals to downstream nodes in response to signals they receive from the downstream nodes. A network with feedback connections will sense the state it is in, compare the current state to a setpoint or desired state, and then adjust its output to meet the desired state. In the scenario where the original set state cannot be met, a network with the appropriate connectivity could activate different feedbacks to break old connections, make new connections to establish a new stable state. Feedback mechanisms allow a network to correct or repair nodes and links that are perturbed or become dysfunctional under certain conditions. Common examples include negative feedbacks in predator-prey systems that result in population oscillations (Li et al. 2011), gene regulation systems that lead to constant gene expression outputs (Gjuvsland et al. 2007; Hensel et al. 2012), or DNA proofreading and repair systems (Ashour and Mosammaparast 2021), and positive feedbacks in excitable organism behaviors (O'Boyle et al. 2020) or memories in gene regulatory networks (Qiao et al. 2020).

Diversity: Diversity within a network can be regarded as the number, variations, and complexity of nodes of differential identities or functions. While the redundancy of nodes provides "backups" that can compensate for potential failures in any one node, the diversity within a set of nodes provides variations in responses to heterogenous challenges that can enable the system to function under different conditions. For example, genetic variations or differential gene expression states in microbial populations allow for the survival of resistant and persistent cells that could revive the entire population upon the termination of antibiotic treatment. High viral mutation rates create variants that escape host immune systems, resulting in robust viral infections (Drake 1993; Fitzsimmons et al. 2018). Genetic recombination and non-genetic memory (histone modifications, DNA methylation, and prion-based inheritance mechanisms) are critical for adaptation to unexpected environment changes. They provide the molecular ingredients for a heritable response, fixing these changes in phenotype within a population (Payne and Wagner 2019). Animals in unpredictable or highly variable environments produce eggs of various sizes or offspring with diverse phenotypes (or genotypes) so that at least some of the offspring are suited for the environment (bet-hedging; Olofsson et al. 2009; Morrongiello et al. 2012). Communities with more diverse species composition and larger population sizes are more stable and resistant to invasive species than those with smaller sizes (Hopf et al. 2019).

Box 1. Tandem gene duplication providing a resilient defense and a robust organism

Organisms have multiple pathways to defend themselves against foreign chemicals. These multiple pathways exist in some generalized form across all domains of life. These systems are often co-regulated, forming integrated networks of genes and pathways as orchestrated defenses against toxic chemicals (Goldstone et al. 2006; De Marco et al. 2017; Tayyrov et al. 2019; Mareya et al. 2019).

Many of these defending enzymes exhibit tandem duplication—that is, rather than just one copy at a genomic locus, there are many similar copies. This property, of multiple copies of extremely similar proteins collocated at a genomic locus, with duplicated regulatory regions in the case of eukaryotes, allows an organism to be resilient to both genomic insult (point mutations) and to slightly changing chemical environments. These molecular duplications are found also in other types of proteins, providing molecular resilience for a robust organism. Tandem duplication of xenobiotic-metabolizing genes allows selected individuals to survive a population-level chemical "attack," providing (eventually) a robust population from resilient individuals.

Prominent examples include the rise of insecticide resistance in mosquitoes due to the resilience of selected individuals with duplicated insecticide-metabolizing enzymes (Milesi et al. 2017; Weetman et al. 2018). Even at the cellular level, certain types of human cancers survive targeted chemotherapies by using an uneven distribution of extrachromosomal circular plasmids with duplicated genes critical for cellular survival (Turner et al. 2017).

We propose that once the redundancy, connectivity, and diversity of networks at any level of biological organization are understood, common rules of robustness and resilience will emerge

Each level of biological organization is conceptualized as a network consisting of nodes and edges, with the emergent collective behavior of the network as a node for the network of the next higher level of organization. With this framework, we can ask interesting questions such as how robustness and resilience are related across scales; i.e., are there microscale to macroscale network dynamics that work together to facilitate robustness?

One important hypothesis that can be tested is whether resilience at lower levels of organization contributes to increasing robustness at higher spatial and biological scales. For example, ecosystem robustness may be maintained when some populations thrive while others decline during an environment change. Thus the output, e.g., survival or appropriate development of a species or an organism, may be robust to environmental insult by virtue of the resilience of underlying interaction networks (see Box 1).

Concept of evolving networks

While a network with appropriate levels of redundancy, diversity and connectivity could confer robustness and resilience under set conditions, these networks must also be able to adjust under fluctuating and changing environments and evolve over time. Here, we broadly define evolvability as the ability of the system to change functions in response to significant perturbations, either by maintaining the original stable state but with enhanced stability, or by moving to a new stable state with changed properties. An evolved network may have broken or established new connections, or connections that have increased/decreased in strength, or direction relative to the remaining connections. An evolvable network can provide the potential to sustain individual and/or population survival in hostile environments, such as what was shown in signaling networks (e.g., Pimpinelli and Piacentini 2020). This concept is commonly referred to as physiological acclimation, phenotypic plasticity, or evolutionary adaptation depending on the level of biological organization. For an example of how robustness, resilience and evolvability play out in metabolic networks in living cells, see Fig. 3 and Box 2.

Evolvability can both contribute to and result from robustness and resilience under dynamic conditions. Variation in ecological niches can also promote the evolution of organismal specialization (Cordeiro et al. 2020). Organismal specialization can involve a gain or loss of a response to particular environmental conditions, depending on the dynamism of the environmental stressor (e.g., Saiz et al. 2021). The frequency, magnitude and type of environmental changes experienced by a lineage contribute to the evolution of robustness-supporting networks. The resilience of a system to environmental change is associated with the introduction of novelties into it, or the systems' adaptive capacity (Allen and Holling 2010). However, ecological, physiological, or evolutionary constraints may limit a system's response during exposure to extreme conditions that are significantly different than those previously encountered (Dutta et al. 2021). Even so, there may be biological factors that contribute to a species' population robustness even in the face of rapid human-driven changes (e.g., Reid et al. 2016).

Linking the changes that promote robustness or resilience in a particular environment to a single gene or small set of genes (or a small set of organisms) may artificially limit our understanding of the nature of these emerging properties. Evolutionary history shapes responses to environmental conditions; understanding these changes in broader terms that incorporate network changes or community changes is important. It is also important to note that phenotypic plasticity within a generation that can be transmitted to the next generation via epigenetic or non-genetic changes contribute to gain or loss of robustness in an organism (Payne and Wagner 2019). Regardless of whether its origin is genetic or epigenetic, study of flexible networks that occur at different levels of organization is needed to understand generalizable strategies. These strategies can then be modeled across scales to show how robustness or resilience at one level relates to those at another. Evolutionary biologists can help us understand how stability and resilience of systems change in response to selection different pressures or how diverse mechanisms create systems that confer stability and control.

Technological and computational advances enabling a network theory paradigm shift

Now is an opportune time to establish a framework that enables the modeling of complex systems across scales to understand biological robustness and resilience. We have access to many state-of-the-art, enabling technologies that can generate expansive molecular-level data sets, including all of the 'omics" at the molecular levels. Population-wide and individual behaviors at the large can be recorded remotely and analyzed in near real-time, through large-scale phenomics systems or satellite images. Most importantly, we are developing better tools for data acquisition, analysis, and transfer that will allow us to bridge data from atomic to stellar scales. We

Box 2. Cellular metabolic networks: Robustness, resilience and evolvability

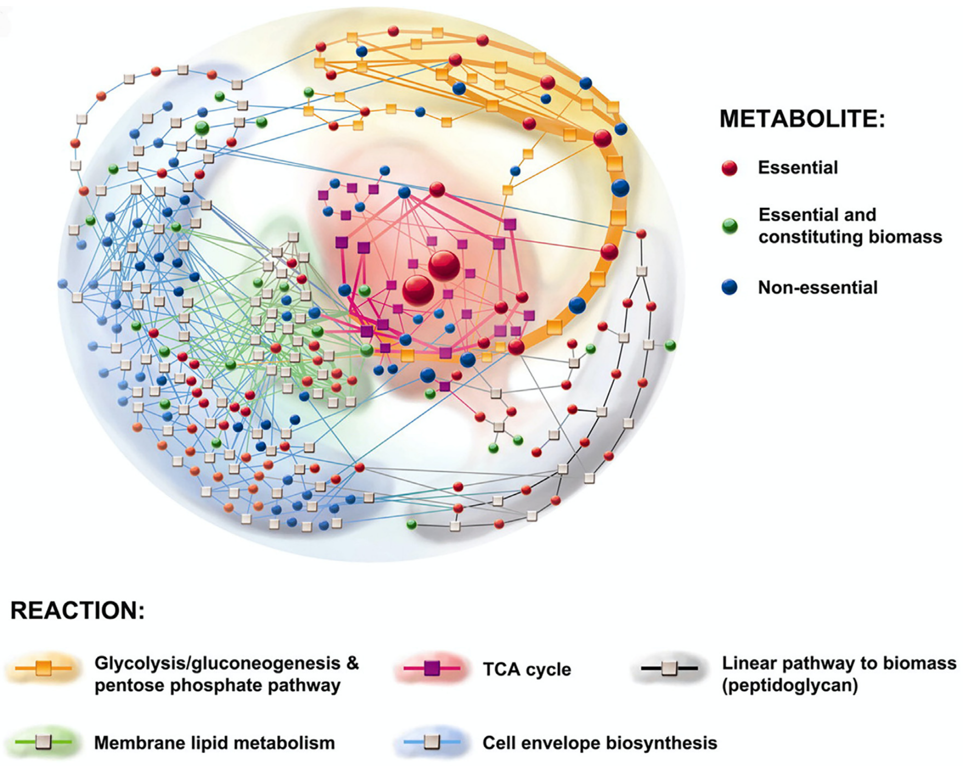

Fig. 3 Cellular metabolic network of *Escherichia coli* illustrating the characteristics of essential and nonessential metabolites obtained from a combined gene knock-out, computational modeling, and physiological analysis (adapted from Kim et al. 2007)..

Robustness, resilience, and evolvability of metabolic networks makes possible the maintenance of cellular functions in the face of different internal and environmental perturbations. Robustness in metabolic networks emerges from at least two fundamental properties: The first derives from the kinetic properties of the individual enzymes of the network in conjunction with the steady state-pool sizes of the set of metabolites the enzymes are operating upon. Pool sizes tend to hover at the K_m value of the enzyme, which is the linear portion of the saturation behavior of the enzyme, such that the rate of the enzyme changes maximally in response to fluctuations in pool size. This even applies to metabolite pools that have extremely high rates of turnover due to high rates of metabolic flux through the pathway.

The second property that contributes to the robustness of networks relates to the fact that intermediates often have several pathways of production and/or consumption resulting in a balancing effect on their accumulation. Overall, this situation results a robust maintenance of metabolic pool sizes throughout the network occurring in shorter time domains (e.g., seconds to minutes). Resilience, likewise, has evolved though the properties contributing to robustness mentioned a moment ago, plus a myriad of homeostatic mechanisms modulating enzyme abundance and allosteric feedback mechanisms adjusting enzyme activities according to changing conditions. These are especially valuable for adjusting to persistent stressors and perturbations, which last for hours or longer.

Evolvability is apparent in metabolic networks as observed in long-term natural and laboratory experiments that are revealing adaptive genetic changes fixed in populations that have transitioned to new environmental conditions (Baez and Shiloach 2014; La Rosa et al. 2018). The description of these networks has been made possible by the development of computational modeling approaches and the integration of large datasets. For example, the field of metabolomics has hybridized advanced analytical biochemistry, genomics, and mathematical modeling. With advances in large-scale experimental metabolic analysis, it is possible to trace hundreds of metabolites simultaneously in a single experiment and, thus, it is now becoming possible to quantitatively evaluate fluctuations in metabolite concentrations for key metabolites across entire metabolic networks (Orth et al. 2010; Basan et al. 2015, Sauer 2006; Neilson 2017).

Developments in genomics and computational modeling have led to a renaissance in the understanding of metabolism, leading to new understandings of metabolic system resilience and robustness. It is already clear that the robustness of metabolic networks is in large part due to the network topology, with highly interconnected metabolites in "modules" such as the TCA cycle, connected by a much smaller number of common intermediates, including ATP (Kim et al. 2007). Similar considerations apply to network models that maximize biomass production (Braddrick et al. 2016; see Fig. 3, inset). Some of the mathematical formalism for this (linear programming of simultaneous reactions, network analysis) shares features in common with biological networks at different scales of time and space. For example, mathematical descriptions of low apparent HIV viral titers with high viral turnover rates are described in the same form as findings that metabolites present in cells at very low concentrations often correspond to pathways that have the highest flux (traffic) through them (Xiong et al. 2015; Liang and Lindblad 2016). In both cases, low steady state levels reflect high turnover due to high rates of production matched by high rates of consumption. No doubt ecological and population dynamic process have parallel dynamical features. This illustrates how entirely different biological processes, studied using very different experimental techniques, and by scientists in different disciplines can find common ground in describing and integrating different phenomena.

now possess technologies to manipulate, observe, analyze and synthesize our understanding of model and non-model systems in controlled lab environments as well as in the field, even up to the global scale. Much is now known about the mechanisms of life, including the biochemical reactions of information and energy processing within microbial cells, programs that define the development and evolution of multicellular organisms from plants to humans, and interactions among diverse life forms that contribute to ecosystem emergence and dynamics.

At the molecular scale, we can access large quantities of genomic and transcriptomic information in near real time across phenotypes, populations, species, and lineages through NGS, single-cell sequencing and RNA-seq approaches (Iacono et al. 2019; Estermann and Smith 2020). Advanced mass spectroscopic techniques provide quantitative proteomic and metabolomic analyses to address a wide range of biological questions. Cryo-electron microscopy and tomography can visualize structures of macromolecular complexes in native or near native environments with atomic resolutions. Super-resolution and single-molecule imaging push the detection of molecules and cellular structures in live cells beyond the diffraction limit of light microscopy. We also possess incredible powers in manipulating organisms through genome editing and targeted perturbations. At the organismal level, it is feasible to build synthetic cells and grow organoids that recapitulate essential features of life, and now even sustain mammalian development *in vitro* (Aguilera-Castrejon et al. 2021). At the population level, the most advanced tracking technologies are able to monitor the dynamics of large populations of animals and changes in ecosystems (Barnas et al. 2019). Various social media outlets offer new platforms to gather and disseminate information at the societal level. Growing computational and mathematical power, coupled with mechanistic modeling, machine learning, and artificial intelligence algorithms, have the potential to describe systems and predicate outcomes at different scales, across different levels of biological organization (molecules to ecosystems), spanning broad time scales (nanoseconds, seconds, minutes, and hours), or by some metric of complexity (e.g., reaction, pathway, network, and hairball). We have an abundance of in-depth data not only from model systems, but also from diverse, non-lab adapted systems. If coalesced into standardized, user-accessible databases (as exemplified by Pangeo for geoscientific data; http://pangeo.io), these data can be used to systems and examine strategies universal to different scales. The substantial amount of historical genetic and ecological data can be integrated with current data to develop algorithms of hindcasts to forecast robustness and resilience of systems.

Barriers to progress: Challenges to the adoption of a network theory framework

While there are many advances that make this paradigm shift possible at this time, there are also many barriers that need to be overcome before a wide range of scientists are able to embrace applying network theory for robustness and resilience across all biological scales. As described in more detail below, engineers, computer scientists, and biologists in different research communities lack a common language for describing the meaning of robustness or resilience across different levels of biological organization, although the field of systems biology has adapted many of the ideas of network theory for some biological systems, typically focused at the molecular, cellular, and tissue levels (e.g., Goldman et al. 2015). In addition, there are many institutional and structural barriers to be overcome. For a unified theory of robustness and resilience to emerge, meaningful incentives to promote collaborative research must be implemented, and traditional divisional barriers must be bridged.

Language

There is a lack of a common language for describing robustness or resilience across different levels of biological organization (see Table 1). Terms like "resilience" and "robustness" depend on context (molecular, cellular, multi-cellular, and population) and differ depending on scientific training or field (math/systems/engineering versus molecular/cell/biology/ecology). Developing a common language across fields provides an opportunity to identify unifying threads across biological levels and across scientific fields (e.g., Davies 2018). Different fields and training have hypotheses and constructed models of "resilience" or "robustness" for certain systems, but scientists outside the field (or approach) may struggle to adopt these models to novel areas, or they may toil to adapt powerful methods of another field to test hypotheses in their own. Common terms will allow scientists to find relevant concepts and empirical data in other fields through literature searches and increase opportunities to collaborate across fields. We propose that the language of network theory (see above) could take a first step toward unifying how researchers from diverse fields conceptualize and communicate information about complex systems.

Another general problem when integrating information across subdisciplines in the biological sciences is the use of jargon, such that the same phenomena are studied independently, preventing the integration of these disciplines. For example, we have amazing tools for searching primary literature that combine sources of information across diverse scientific disciplines (e.g., Web of Science), but literature searches are restricted to the terms used. Unless this terminology is standardized, or "smart" searches that translate those terms into others that are known to vary across fields are utilized, relevant information will be forever segregated in the minds of researchers of different fields. For example, evolutionary biologists interested in "transgenerational plasticity" may also be interested in "developmental programing" studies in the biomedical literature or "carry-over effects" in the ecological literature. As shown in Table 1, there are terms of similar meaning related to the concepts of robustness and resilience across fields, although in each case there are specific nuances, connotations or usages that differ among terms. Creating interdisciplinary educational programming will enhance this merging of language and terminology so that discipline-specific jargon will be eased.

Lack of technology and experimental testing

A process that is altered and returns to a previous state (resilient) may exhibit a robust response at a higher level of temporal, spatial, or organismal integration. Measures need to be relevant both to the physical and temporal scale of perturbation and must subsequently transmit a signal associated with this perturbation to adjacent levels. Despite access to huge sets of molecular, behavior, and population data, the current state-of-the-art techniques generally lack the ability to integrate information across length scales and time scales; how networks are defined and interactions quantified requires more development, including new technologies to measure how networks respond to perturbations across scales. It is also unclear which experimental systems best serve as case studies in which this technology can be tested and optimized.

Logistics

Even when there is a desire to collaborate across fields, finding potential colleagues with similar interests and willingness to collaborate can be challenging. Most scientific conferences are field-specific; thus, it is challenging for scientists to find opportunities to meet and discuss ideas with others in different fields. Even after finding a collaborator, there are logistical hurdles in carrying out a project such as grant administration and international access to sensitive data. In addition, there are institutional barriers that prevent scientists from gaining access to the physical infrastructure and tools needed to study transdisciplinary robustness and resilience across scales. Often funding opportunities and financial incentives that promote the formation of novel transdisciplinary collaborations are limited. When inter- or transdisciplinary proposals are submitted to traditional funding mechanisms, the small pool of reviewers who have discipline-specific expertise but also appreciate the novelty of transdisciplinary collaborations could limit the funding of such proposals.

Strategies to overcome barriers to progress

Reintegration of biology

Robustness is a concept that crosses many levels of biological organization; a fuller understanding of this characteristic requires the integration of many different disciplines so that a common language emerges. A multidisciplinary team approach would eliminate the inherent scale and model bias, allowing for broader perspectives into the rules of life. We therefore need platforms for researchers who are interested in understanding robustness and resilience from biophysics, mathematics, molecular biology, physiology, population genetics, and ecosystem biology, etc. who do not otherwise interact to brainstorm ideas. This could be done in workshops resulting in new collaborations and possible research coordination networks. Funding mechanisms that promote the formation of new multidisciplinary research teams will also broaden participation of researchers from different backgrounds and institutional types (e.g., primarily teaching institutions, medical schools, and research-intensive universities). Funding agencies such as the National Science Foundation have acknowledged that they can play a major role in promoting cross-disciplinary training of a new generation of scientists by changing funding schemes, paradigms and training programs. These changes will promote cross-disciplinary training of a new generation of scientists who have the skills to discover and describe the important overarching questions of life on Earth. For example, we might harness existing big data and integrate insights from available models of community and population dynamics that are successfully used for metabolism, viruses, microbiomes, and ecosystems (Cantor et al. 2017) to construct mathematical models to elucidate common rules underlying resilience and robustness.

We can also leverage our understanding of the evolution to advance our understanding of robust and resilient systems. With large-scale, multidimensional networks, comparative analysis of network interactions over time will allow the role of evolutionary pressure to be examined in biological robustness. This analysis would move beyond our current reliance on gene or protein networks, to incorporate communications between nearest neighbors (intra- and inter-habitat) and entire communities over time. Then specific nodes or network strategies to overcome challenges and promote robustness that recur over time could then be used to re-engineer robust and scalable networks from gene to community levels.

Development of new tools

To overcome technological barriers, we need to develop suitable metrics and tools to measure robustness and resilience (or lack thereof) across space and time scales. Ideally such a tool would measure or provide a measure of the response of a system at one scale and seamlessly measure the propagation of the response across multiple scales. For example, noise in the production of RNA during the activation of gene expression can contribute to cellular heterogeneity, resulting in a robust response to perturbations across a population of cells. It is unclear how heterogeneity that is generated at the cellular level affects higher-order processes. Real-time readouts would enable us to capture events that happen throughout the life of the organism. One method of obtaining this type of data would be using optical methods, requiring the development of stable reporters that are not susceptible to bleaching or degradation biases. Optical or other readouts of behavior, neural status, and molecular reporters could then be integrated across scales to provide networks in context. Eventually, to support the development of full molecular networks in context, real-time molecular sampling of a freely-responding (super)-organism will be necessary.

At the most ambitious level, advanced technologies would be deployed to generate and analyze network data in real time. These technologies might include real-time analysis of transcriptomes, proteomes, metabolomes, neural readouts, and behavior in an environmental context. Not all of these technologies are ready, but many are very close, enhanced by the current growth in computational power (data analytics), real-time sequencing, and computer vision. Assuming no limitations, we could have all the experimental data possible to build dynamic networks. This will require integrated hypotheses that probe networks and additional strategies to address evolutionary selection, particularly the survival of an individual and a population.

To move toward this integrative network-based analysis of robustness, in the next few years we would need to implement model test systems across multiple life scales with scientific teams to develop testable hypotheses that validated network development. understand In addition to the development of new sensing and measurement technologies, we need to develop new data analytics and computational methods to transform current data streams into multidimensional networks. Enormous, affordable computational capacity is needed in hardware for storage, fast CPU/GPU, parallel processing, and freely available open software. With these developments, we could not only test network robustness but analyze redundancy. Exploring redundancy and determining essential nodes for stability and robustness of networks at multiple levels would provide essential insight into robustness that has been inaccessible due to the lack of global monitoring systems capable of collecting data at sufficient scales. Infrastructure will also need to be created to host these databases, enable user contributions and make databases searchable and available to the public, much like NCBI databases.

Education

In order to realize a reintegration of biology and generate the workforce needed to create the technologies needed to advance network-level study of biological systems, we need to reform science and math education. Critically, science education from K-12 through the post-doctoral level should be designed to foster problem-based scientific thinking not siloed by discipline. Integration of knowledge from different scientific disciplines needs to become a common way of thinking for the next generation of scientists and innovators. In addition, curricula should include requirements that emphasize analytical reasoning and quantitative skills. Network theory and computer science courses could be included as standard biological science curricula in addition to algebra, calculus, and statistics. It is important to impress upon students how mathematical tools applied in modeling and engineering fields can be employed to derive potential solutions to important societal problems (NRC 2009; see Box 3).

Box 3. Stepping quantitatively toward robustness and resilience through mathematical modeling

Mathematical models are built for a variety of reasons: making sense of measured systems, testing assumptions in complex phenomena, or for applied forecasting. Mathematical models are reduced descriptions, retaining aspects of a phenomena that are thought to be important, and often the process of modeling reveals gaps in understanding. Experimental and observational biology is typically lacking in sufficient data in certain areas necessary to create robust models like those in the physical sciences. One quantitative modeling approach to mechanisms of robustness or resilience is akin to balancing our banking account:

$$\text{Current balance} = \text{initial deposit} + \text{amount deposited} - \text{amount withdrawn} \quad (1)$$

$$\text{Rate of change of balance} = \text{rate of deposit} - \text{rate of withdraw.} \quad (2)$$

Importantly, all terms of the equation must be in the same units, a key issue if accounting is to be applied to biology, tracking for example, levels of biomolecules, cells, or humans. By applying basic calculus, the change in the current balance over time and other parameters can be extracted. Just as with a bank account, equations of change can be written for biological parameters such as molecule concentrations or cells in a culture: for example, the change in the number of cells (X) over time is expressed as a function of a growth rate, r:

$$\frac{d\,(X)}{dt} = r\;(X)\,. \quad (3)$$

Normally, culture conditions have finite resources for growth, so cells stop growing as they expend the available nutrients. Equation (2) can be adapted to account for finite growth resources by including an addition term, to create a logistic growth equation:

$$\frac{d\,(X)}{dt} = r\left[1 - \frac{(X)}{K}\right](X)\,. \quad (4)$$

Notice that the finite resources that limit cell growth are described by the parameter *K*; when cell concentrations are very small, or *(X)* $<<$ *K*, then the term in the brackets [] is close to one, Equation (4) is approximated by Equation (3), and the cells can grow exponentially with rate *r*. However, as the cells grow, their concentration *(X)* increases, and eventually approaches the value of *K*, and the term in the brackets approaches zero. So as *(X)* approaches the value of *K*, right hand side of Equation (4) approaches zero, and the solution to the equation of change indicates *(X)* is a constant with the value of *K*. This equation is useful here for showing how the unlimited or unbounded growth model of Equation (3) can be modified to introduce limits on growth. To determine the parameters *r* (growth rate) and *K* (finite capacity) from experiments, one would ideally make measurements of *(X)* versus time over a wide range of *(X)* values, where d*(X)*/dt spans from its maximum value, when *(X)* is small, to zero, as *(X)* approaches *K*.

These equations provide a foundation to start thinking quantitatively about mechanisms of robustness or resilience, where systems with potentially multiple changing inputs achieve states or outputs that are stable over time. Stability over time implies a balance of positive and negative terms, deposit and withdrawn in the case of our bank account, or growth and stasis for living cells.

In a mathematically related example in cell biology, one can express the decay kinetics of a certain molecule in a cell with an equation similar to Equation (3), but with a negative term, such that we are examining the decay rate k of a molecule Y, rather than a growth rate. Usually the kinetics of a molecule also includes positive terms that represent synthesis and/or activation of the molecule (analogous to deposits to the bank account):

$$\frac{d\,(Y)}{dt} = k'\,(Z,t) - k\,(Y)\,. \quad (5)$$

The arguments *Z* and *t* in the positive term in Equation (5) imply that the synthesis/activation process may be controlled by other molecules in the cell, or a stimulus to the cell. By writing out equations like Equation (5) for the key molecules involved in a molecular regulatory network, one can build models for investigating the robustness or resilience behaviors of the network and its associated cellular function.

Table 2. Important biological and societal questions that could be addressed with a unifying understanding of the rules of robustness and resilience across scales

1.	Do systems that promote robustness and resilience reinforce or antagonize each other?
2.	Are there a key principle governing robustness across all biological organizations, such as redundancy or a noise-buffering network? What is the minimal requirement for such a key factor? For example, how redundant a system or how big a network is needed in order to maintain the robustness of the system?
3.	Are there a key principle governing resilience across all biological organizations, such as diversity in properties? What is the minimal requirement for such a key factor? For example, how many populations, genoytpes or number of variants in genetic mutations is needed in order to maintain the resilience of the system?
4.	How does the connectivity (and feedbacks therein) among different components of a system, such as that in metabolic networks, gene-regulatory networks, cytoskeletal networks, social networks, and contagion networks contribute to the robustness and resilience of the system?
5.	With a better understanding of the mechanisms underlying robustness, can we manipulate the performance or persistence of individuals, populations, species, and ecosystems to enhance or suppress robustness?
6.	Can we design and test synthetic systems that recapitulate specific functions of living systems, and how does a designed system differ from one that came about through natural processes?
7.	Can we manipulate existing systems or even engineer new systems that will thrive under even the most undesired conditions? For example, can we build new ecosystems to help balance global processes and address the climate crisis?
8.	Can we use unifying principles of robustness and resilience to our understanding of medicine, social or economic systems, and applied engineering?

Reorganization of institutional funding mechanisms and infrastructure

To overcome logistical barriers to advancing research on robustness and resilience, it is important for both funding agencies and research institutions to facilitate and incentivize interdisciplinary interactions among scientists. This can be best accomplished with specialized funding mechanisms that call for such interdisciplinary teams, such as the joint National Institutes of Health and National Science Foundation Ecology and Evolution of Infectious Disease mechanism, and the newly established NSF Integrative Research in Biology (IntBIO) and the Biology Integration Institutes mechanisms. However, it is still a challenge for researchers to establish relationships with collaborators, especially biomathematicians and bioinformaticians with allied interests and expertise. Within research institutions, increasing internal funding opportunities to encourage interdisciplinary collaborations, cluster hiring around interdisciplinary research themes, and encouraging young investigators to engage in collaborative research through established (or new) institutional interdisciplinary or transdisciplinary centers could increase research in robustness and resilience.

Open questions and research opportunities

Studying biological systems within a unifying framework as living and interacting networks will allow us to address some of the most important biological and social questions of our time (see Table 2). Understanding the underlying principles of biological robustness and resilience will allow us to model and anticipate consequences of environmental changes across scales and enable controlling of biological systems for most beneficial outcomes. For example, it is desirable to destabilize the state of persistent neural seizures resulting from epilepsy or neurotoxin exposure, in which neural signals are persistently entrained. Similarly, we may want to model or forecast consequences of anthropogenic effects such as an oil spill and develop ways to return ecosystems to its healthy state. Models of robustness and resilience can inform methods to stabilize or destabilize agri- and aquaculture, improving sustainability or reducing the impact of invasive species. They could also provide insight into disease development and progression, either in natural or modified systems. In a world with a rapidly changing climate, such interventions may be essential for organismal survival and to prevent a sixth extinction but will require significant ethical restraint in their applications.

Collaboration among researchers from experimental, mathematical, computational, and engineering fields will allow the application of developed models to improve the health of the ecosystem and human lives. For example, useful experimental datasets, mathematical models, and computational tools for validating and understanding behaviors of complex systems may be generated. New software incorporating improved parameter definitions and modeling techniques could facilitate the investigation and understanding of intra- and inter-level connections of complex biological systems. Synthetic datasets with standardized format could also result from this research to allow downstream applications for other multiscale studies.

A greater understanding of the theoretical mechanisms of robust or resilient networks will also help develop better computation tools and more reliable artificial intelligence (AI) algorithms. By identifying essential networks and nodes that promote robustness, we can implement them to perform complex AI-driven tasks such as self-driving vehicles, rover navigation undersea, or on Mars, or exploration of oceans and moons. Robustness and resilience theory will provide new algorithms for implementing complex tasks in constantly changing environments. Understanding the role of robustness in evolution will also enable artificial systems to learn how to rapidly navigate new and complex environmental contexts.

Finding common rules of robustness and resilience across scales in natural systems will accelerate new discoveries and progress on elucidating the rules of life on Earth, transform the way we understand biological systems and revolutionize synthetic biology. We will begin elucidating design and engineering principles of living systems and use them to deploy stable and viable synthetic systems. As biological systems of different organization levels are interconnected across scales, we may be able to forecast how changes at one organization level affect the other levels, contributing to a holistic understanding of all biological systems.

Glossary

Connectivity
The extent of connections among nodes/vertices of a network; also a metric to describe how well parts of the network are connected to one another.

Diversity
The property of a network that describes the existence of multiple independent routes of communication among nodes or diverse nodes with unique connections and feedbacks.

Evolvability
The ability of the system to change in response to perturbations, either maintaining the original stable state but with better stability or moving to a new stable state with changed properties. Networks can evolve by altering routes of communication and utilization of nodes. Networks may autonomously configure, monitor, and maintain structure, depending on data acquisition and communication path use.

Feedback
A circuit or loop in which the output of the system is routed back as input to become part of a chain of cause-and-effect. Often employed in systems that control network behaviors.

Network edges
Connections between the nodes of a network. Also called links in graph theory.

Network nodes
Connection points of a network. The specific character of a node depends on the nature of the network, but it is generally capable of creating, receiving, transmitting, or blocking information. Also called vertices in graph theory.

Network topology
The structure and makeup of the network as a whole, a collection of interconnected nodes and edges.

Network theory
The study of complex interacting systems that can be represented mathematically as sets of equations or visually as graphs.

Redundancy
The property of a network of having multiple independent means of connecting nodes or the existence of alternative nodes that have similar connections. The greater the redundancy of nodes and edges, the greater the availability of the network, and the less the risk of failure of the network.

Resilience
The ability to recover to a previous state or a new establish a new baseline after some time following an environmental perturbation. Synonymous with "resistance" in ecological literature (see Table 1).

Robustness
The stability of biological outputs given diverse internal and external environmental states.

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