

## MECHANISMS OF ROBUSTNESS

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**“There is nothing permanent but change”**

**-Heraclitus**

### Problem statement

No environment is invariant. Thus, how a biological system responds to perturbation underscores the persistence of its performance across generations. Despite the ubiquitous nature of change around us, we lack an overarching understanding of the fundamental mechanisms that enable organisms to appropriately respond to alterations in their environment. It is possible that there are some common mechanisms that promote robustness at the molecular, cellular, organismal, population, and ecosystem levels. We hypothesize that there are microscale to macroscale network dynamics which work together to facilitate robustness. To test this hypothesis, it is vital to determine how mechanisms of robustness at different biological scales fit together. Determining these interactions across scales has been a challenge because research into the responses to perturbations are often siloed at molecular, cellular, organismal, and ecological scales. The aims of this vision statement is to outline the potential impacts of biological robustness, review the current state of our knowledge, identify challenges, and ways to reintegrate biology to advance our understanding of mechanisms underlying robustness in biological systems.

Robustness implies the stability of biological outputs given diverse internal and external environmental states. It underscores the ability of biological systems to tolerate environmental change without incurring a significant alteration to fitness-related measures. In contrast, resilience is the ability to recover to a previous state after some time following an environmental perturbation. Our hypothesis is that resilience at lower levels of organization contribute to increasing robustness at higher spatial and biological scales. For instance, an organ may maintain its function with some damaged cells becoming apoptotic. Similarly, ecosystem robustness may be maintained when some populations thrive while others decline with an environment change. Thus the output, e.g. survival or appropriate development of an organism, may be robust to environmental insult by virtue of the resilience of underlying interaction networks.

Understanding the underlying principles of biological robustness and resilience will allow us to model and predict consequences of environmental changes across scales, and enable engineering or manipulation of biological systems to promote stabilization of desirable states or destabilization of undesirable states. For example, a state system that may be important to destabilize is the condition of persistent neural seizures resulting from epilepsy or neurotoxin

exposure, in which neural signals are persistently entrained. Similarly, we may want to model or predict consequences of anthropogenic effects such as pollution to promote stability of essential ecosystem. These models can also inform methods to stabilize or destabilize agri(aqua)culture improving ecological balance or reduce the impact of invasive species. They could also provide insight into disease development and progression.

This question is particularly timely for two reasons: 1) We now have access to tools that provide large data sets relating to genomics, transcriptomics, and other “omics.” Population and individual behaviors can be recorded and analyzed in almost real-time. More importantly, we are developing better tools for data acquisition, analysis, and transfer that will allow to bridge from atoms to stellar scales. 2) We need to understand biology as a multi-scale, hierarchical system and how this complex system navigates through change. With more integrative approaches, we can develop a multi-scale view of biological organization rather than the more discipline-specific or molecule-specific approach we currently use. This new approach will allow us to decode the complexity of biological systems and depict more clearly the hierarchical and network designs of the systems. To understand mechanisms of robustness across scales and disciplines, it is vital to provide common language and perspective. We have identified several key challenges to defining the metrics of robustness that need to be addressed in order to transition this concept to the larger scientific community.

### **Key challenges**

Here, we define mechanisms that could explain robustness in biological systems and the barriers associated with carrying out these measurements:

*Scale:* 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. The current state of the art techniques generally lack the ability to integrate information across length scales and time scales. How do we build techniques that are inherently measuring multiscale responses? How do these probes capture changes in cellular and organismal responses across length scales and time scales? How can we efficiently study these scales if they do not match current technological abilities or research mechanisms?

*Networks and redundancy:* On a molecular, cellular, or even microbial population level, protein and/or signaling networks can define responses to stimuli. Strengths or directions of connections within networks may change or connections may form or degrade in response to perturbations. This plasticity in signaling networks can help sustain individual and population survival in a changing environment. Networks of interactions can be envisioned at the levels of amino acids, protein-protein interactions, and gene-environment interactions, scaling up to organisms and their ability to forge ecological connections. Alternatively, plasticity in gene or protein responses can also be used to describe the instantaneous or adapted responses to challenges. Networks and plasticity can also be scaled to describe the interactions of populations. Yet, how the networks are defined and interactions quantified requires more

development. Some critical barriers to defining networks are: How can we efficiently define nodes, connectivity, and dynamics? What extent of damage/alteration is necessary to induce reconfiguration of networks and what outcome measure is needed to evaluate a response or lack thereof? Which parts of a network are more important for stabilizing or destabilizing a system, e.g. for experimentally testing networks or deliberately manipulating the overall state of system (organism)? What is the role of networks in robustness? Do redundancy in networks and mechanisms improve robustness, or constrain evolution?

*Variance and diversity.* These critical ingredients span scales ranging from the molecular to the population level. Variance in molecular structure and interactions may contribute to an overall robustness of an organism and diversity among individuals or population may contribute to the species' robustness. Mechanisms that can create variation may be critical for generating robustness. Examples of such mechanisms include alternate splicing at the molecular level and bet hedging of offspring phenotype at the individual level. Further, genetic recombination and non-genetic memory (histone modifications, DNA methylation, prion based inheritance mechanisms) are critical for adaptation to unexpected changes in the environment. They provide the molecular ingredients for a heritable response thus fixing these changes in phenotype within a population. Therefore, variance can stem from interrelated mechanisms that are currently considered separately in the current research silos. To understand variance and diversity in populations, we need to ask to what extent does the variance produced from a given genotype contribute to the maintenance of high reproductive success and survival? When does variance in critical biological pathways (e.g. development) provide robustness? Can we quantify how variance within and between populations provides robust environmental responses?

*Evolutionary history and experiences and their role in plasticity and robustness:* Variation in ecological niches promote the evolution of niche-specific mechanisms. This can be a gain or loss of responses to particular environmental conditions, depending on the dynamism of the environmental stressor. The frequency, magnitude, and type of environmental changes that a lineage experienced contribute to the evolution of robustness-supporting networks. However, limits to responses may come in a system exposed to changes that are more extreme or significantly different than those previously 'encoded' by evolutionary or historical responses. Linking the changes that promote robustness in a particular environment to a single gene or small set of genes may reduce our understanding of the nature of robustness. Since 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. Does the time scale of relevant evolutionary processes affect the mechanism of robust responses? If organisms cycle between different environments do they exhibit enhanced robustness? Do their networks of protein-protein interactions become more resilient to changing environments? What is the role of plasticity in robustness?

## **Strategies to overcome these challenges**

*Development of new tools:* To overcome these barriers, we need to develop suitable metrics and tools to measure robustness. We need to convey robustness (or lack thereof) across length 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, the activation of gene expression is a digital event, although the magnitude of a response is not. Noise in the production of RNA can contribute the cellular heterogeneity, resulting in a robust response to cellular perturbations. It is unclear whether any 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 optical or sonographic 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.

*Integrating data into networks:* With our advanced tools, we will need data analytics and computational methods to develop the data stream into networks. These networks would ideally encompass four dimensions to include temporal changes. Enormous computational capability is needed, in hardware for storage, fast CPU/GPU, parallel processing, and in 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.

*Leveraging evolution:* 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.

*Long-term outlook:* 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. We will require integrated hypotheses that probe networks, and

additional strategies to address evolutionary selection, particularly the survival of an individual and a population.

### **Impact**

With a better understanding of the mechanisms underlying robustness, we will have the ability to manipulate the persistence and performance of populations and ecosystems to enhance or suppress robustness. 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.

Mechanism of robustness and understanding of networks will also help us develop better computation tools and more reliable artificial intelligence (AI) algorithms. By identifying essential networks and nodes that promote robustness, we can implement them in AI algorithms to complete complex tasks such as self-driving vehicles, rover navigation undersea, or on Mars, or exploration of ocean moons. Building in robustness will provide new algorithms for implementing complex tasks in environments where there is constant change. Understanding the relationship between robustness and evolvability also enables artificial systems to learn how to rapidly navigate new and complex environmental contexts.

From a health perspective, our understanding of molecular, cellular and ecological robustness may enable us to develop new classes of drugs to combat disease. The ability to manipulate otherwise robust networks may provide methods to intervene in stable health states that are detrimental, such as repeated seizures.

*Reintegration of Biology:* Robustness is a concept that crosses many levels of biological organization, and a fuller understanding requires the integration of many different disciplines. A multidisciplinary team approach would eliminate the inherent scale and model bias we currently experience and will inject a broader perspective. 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 in aims to lead to new working networks and possible RCNs. Thus we need funding mechanisms that allow these new networks to form. This will also broaden participation of researchers from different backgrounds and institutional types (e.g., primarily teaching institutions, medical schools, and research-intensive universities).

### **Implementation**

To move toward this integrative network-based analysis of robustness, in the next 5 years we would need to implement model test systems across multiple life scales, with scientific teams to develop testable hypotheses that validated network development. Additional technological development will be needed to support and obtain the data needed to develop and analyze these complex networks. In the next 10 years, we would need to move toward integrating the temporal component to understand evolutionary impact.