Beyond Reductionism: Systems Biology and Drug Discovery

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Introduction

The last decade has witnessed a significant decline in viable drug candidates,[1] while escalating costs are resulting in an average $1.8 billion price-tag of developing and bringing each new drug to market.[2] Faced with myriad challenges, the pharmaceutical sector is expressing an urgent need to re-evaluate current approaches to drug discovery.[3]

Reductionism has dominated 20th century drug discovery. A reductionist approach aims to reduce complex disease into a single molecular cause and then develop a drug exhibiting high specificity to that single target. This strategy is often referred to as the "one target - one drug - one disease" approach. Limitations of this strategy are two-fold: i) complex diseases are multi-factorial and irreducible; single molecular variants are unlikely to explain the cause and perpetuation of complex disease,[3] and ii) 'single-target' drugs often affect multiple *off-target* sites leading to unwanted side-effects.[3]

The limitations of modern pharmacology are tightly coupled with the limitations of reductionist bioscience. Werner Heisenberg, the great 20th century physicist, said: "What we observe is not nature herself but nature exposed to our method of questioning".[4] We only see what we ask to be shown. Reductionism, as a method of questioning, has led bioscience down a path of observing nature's parts. Reductionism is not wrong; it simply does not provide the whole story, for certain properties of biological function, such as the emergent property of robustness*, cannot be observed through analysis of molecules alone. Yet robustness of disease phenotype leads to the failure of many single-target drugs once they reach phase II and III clinical trials.[5] Robustness is not considered in the early stages of drug development because reductionist methods of questioning have no way to see or accommodate this property.

If we want to account for the properties that arise at the level of whole cells and organisms — properties that not only affect drug action but help explain the cause and perpetuation of complex disease — then a new approach is required. If we want to know the *whole* story of what biology is, then we need new, more holistic methods of questioning. For this reason, 21st century bioscience is moving towards *systems biology*.[6] Systems biology aims to understand biological processes as whole systems instead of collections of isolated parts. When the components of a cell are studied as a whole system, they reveal complex signalling *networks* that display emergent properties. A deeper understanding of these cellular properties brings us to the realization of why reductionist single-target drugs often fail in complex disease and guides us, alternately, towards the need for multi-component and multi-target medicines, not unlike the complex mixtures of medicines used in many natural and traditional medical practices.

Systems Biology: a new method of inquiry

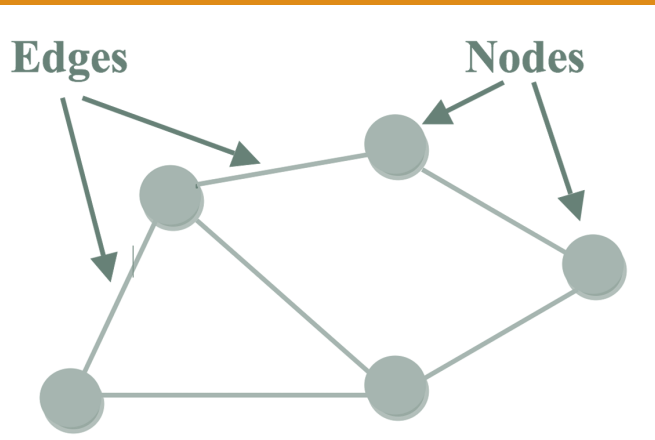
Systems biology has been identified as "the next wave" in the evolution of bioscience.[6] Concerned with the study of biological wholes: including whole cells, organs, organisms, and ecosystems, the fundamental premise of systems biology is that the organization and function of biological wholes cannot be understood through analysis of their individual components in isolation. Rather, all components must be analysed together as an integrative system to reveal a comprehensive understanding of the whole.

Systems biology utilizes advances in biotechnology such as *high-throughput sequencing* that allow biologists to rapidly sequence and screen cells for large numbers of active molecules, from RNA to proteins and metabolites. These large data sets are then subjected to *in silico* (computer) analysis to reveal patterns of expression and interaction among molecules. This technology enables biologists to bridge quantitative science with experimental biology to derive global views of biological systems.[7,8] The global view emerging is one of *networks*. Cell phenotype is not driven by individual genes or individual molecular pathways but by complex interactive *networks* of gene expression, protein interaction and metabolic intermediates.[9-12] This allows mathematical approaches like graph theory to be applied to biological networks to reveal their patterns of inter-relationship and to better predict their behaviour.

In a network model, the components of the cell (i.e., genes, proteins, metabolites) are represented as "nodes", and the interactions between components are represented as "edges".

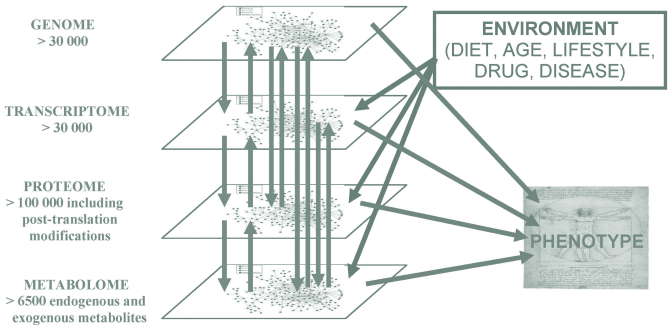
* Robustness is an emergent property of cellular networks. It will be further defined and discussed in the body of paper.

FIGURE 1
Example of a network graph with five nodes and six edges. In cellular systems, nodes represent components of the cell and edges represent their physical interactions.



Systems biology can be applied in the study of whole organisms to better understand how phenotypic expression arises out of the complex networks that span multiple levels of biological organization, from genes to environment. Figure 2 illustrates the complexity of these interactive networks.

FIGURE 2 (used under license by *Chem Soc Rev.*):
The complex interacting networks of the genome, transcriptome, proteome and metabolome in biological systems. Information flows bi-directionally among biological networks and is combined with environmental influence, to produce phenotype. [13]



Disease Networks

When systems biology is applied to the study of disease phenotype, we see that the majority of chronic diseases are the result of complex interactions of genetic, metabolic and environmental factors.[14] Even when a single genetic mutation is identified, it is often not enough to explain the cause and propagation of the disease. Huntington's Disease, for example, is a devastating neurodegenerative disease that has been traced to a mutation in a single gene.[15] Although this mutation has been known since 1993, no effective cure has been developed. Knowledge of the genetic mutation alone is not enough to explain the pathogenesis of this disease or to provide a viable single target for its treatment.[16]

Systems-level analysis can provide more comprehensive views of the complex networks involved in disease process. We can now see that the interconnectivity of cellular components means the impact of a genetic mutation is not limited to the function of the altered gene product, but can spread throughout the interaction network and alter the function of gene products that are otherwise unchanged.[14] Dr. Albert-Laszlo Barabasi and his team in Boston, MA, are pioneering this research. They conclude:

"Therefore, the phenotypic impact of a defect is not determined solely by the known function of the mutated gene, but also by the functions of components with which the gene and its products interact and of their interaction partners, i.e., by its network context." [14]

This means that the initial mutated gene and its products are not necessarily the molecules responsible for propagating or maintaining the disease state. Rather, the genes and proteins responsible for maintaining the disease state may be normal, yet their function has been changed by the altered context of the cell network. These molecules are difficult to identify through reductionist experimental approaches since they may have no physical abnormality and they often lie outside of the suspected molecules involved in the disease process. Network analysis can help identify these molecules by screening large sets of biological data and mapping their interconnections to identify "nodes" that occupy critical positions in the disease network.[17]

Network Drugs

The discovery of disease networks has implications for drug development. It means a shift away from single-target drugs aimed at the molecular 'cause', towards multi-component drugs (often called *network drugs*) that interfere with the functioning disease network by targeting multiple critical nodes simultaneously.[14,18-20] For example, Huang et al [21] are applying network analysis in the pathogenesis of glioblastoma, the most common adult brain cancer. Amongst thousands of possible molecules involved in the disease process, they have identified only a handful that occupy critical nodes in the disease network. Nodes such as those found within the SRC tyrosine kinase family (already well-known to be involved in the disease process) were recapitulated through network analysis, in addition to nodes that were formerly not suspected to be involved in the disease process such as the estrogen receptor, ESR1. Using this research, combinational agents that targeted multiple nodes in the Glioblastoma disease network had a greater effect on halting disease progression than targeting single nodes alone.[22]

Synergy of Network Drugs

Network models are also revealing synergistic mechanisms of multi-component drug combinations.[23] Synergy occurs when multi-component drugs have a greater overall outcome on disease process than the outcome achieved by *adding together* the effects of each individual component on the disease. Synergistic drug combinations are helping to overcome the unwanted side effects, toxicity and drug

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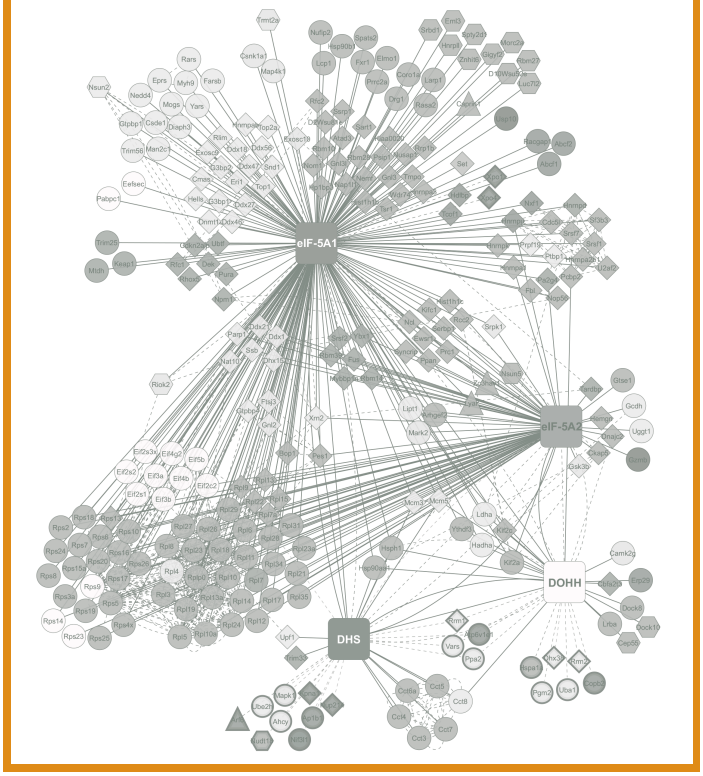
resistance often associated with high-dose single-target drugs while also demonstrating greater selectivity towards disease networks and superior therapeutic efficacy.[24,25] Lehár et al[24] provide numerous examples of drug synergy including the antibacterial synergy of ribivarin combined with disulfiram that resulted in greater selectivity of bacterial cells over host cells than each drug demonstrated on its own.

Two additional properties of cell networks, *modularity* and *robustness*, are particularly relevant to multi-target drug design.

Modularity in Cell Networks

In randomly connected networks, all nodes have, on average, the same number of connections ("edges"). Such networks have very low modularity. However, the nodes of biological networks are proteins and other biochemical intermediates whose interactions with other molecules are not at all random. Whereas the vast majority of biological nodes have few connections, a small number of nodes form very highly connected "hubs" that nucleate various regions of the cell network.[26-28] Networks containing such hubs are said to exhibit high modularity.

FIGURE 3 (used with permission from original author): An example of modularity in protein-protein interaction networks. Note that most nodes only have a few connections while some nodes are highly connected "hubs"[29]



Modular patterning is an important feature in the identification of complex disease because it helps to overcome the issue of molecular heterogeneity. Reductionism has revealed a high degree of molecular heterogeneity in cells associated with complex disease.[30] Cancer cells are a prime example. No two cancer cells exhibit the same molecular phynotype.[31,32] Even cells taken from the same tumour show differences in genome structure, distribution of mutations, repertoire of protein variants and activity along metabolic pathways.[33] This widespread variation has made the identification of single molecular targets difficult, often leading to disappointingly limited efficacy and safety of single-target anticancer drugs.[34] However if we shift our attention away from the parts and on to the whole, we become aware of living systems as interconnected networks of molecules in interacting pathways rather than just a list of individual molecules and cell pathways. We begin to see *patterns* emerging at the level of the whole cells that are not apparent at the level of the molecules.

Network analysis reveals changes in modular patterning within cell networks associated with complex diseases including neurological disease, cardiovascular disease and cancer.[14,35] Moreover, similar modular changes take place within cell networks of a given disease, irrespective of the molecular heterogeneity among these cells.[36] Awareness of changes in network modularity offers a potential breakthrough for drug discovery. It means that instead of screening immense molecular heterogeneity looking for consistent variation in single nodes, we can now document reproducible changes in *modularity*, that is the *organization patterns of interconnected molecules,* and screen disease networks for the molecules responsible for maintaining these changes.

This network approach is already advancing cancer research and drug design. A recent study of patients with sporadic breast cancer showed a significant and consistent change in cellular network modularity between patients with poor prognosis compared to those who were disease-free after extended follow-up.[37] Iyanger and Hansen conclude:

"*Therefore, it appears that the analysis of modularity can be used to characterize disease states. The origins and progression of diseases might not only involve a change in the activity of individual pathways…but could also involve the re- or disorganization of functional modules. Successful treatment might depend on the ability to go back to a prior network organization or to go to a new organization characteristic of normal physiology."*[8]

In summary, screening diseased cells for single molecular variants is proving to be difficult due to the molecular heterogeneity of disease. However, screening disease states for changes in modular *patterning* is providing new, more consistent network-based targets for the treatment of complex disease. This calls for approaches that address and treat the identifiable *pattern(s)* of disease states rather than attempting to address and treat individual molecules of disease.

Robustness of Cell Networks

In some instances, sorting through the molecular heterogeneity of complex disease *has* led to the successful identification of single molecular targets. The HER2 receptor, for example, is over-expressed in a subset of metastatic breast cancers, distinguishing them from

healthy cells. The chemotherapy drug trastuzumab (Herceptin) is a monoclonal antibody designed to act specifically on this receptor. However, the majority of patients who initially respond well to this drug will also build resistance to it.[38] Cancer cells are persistently adaptive to disturbances from drug perturbation. Cancer cells, in fact, are *robust.* Robustness is often a quality attributed to healthy cells, defined as an intrinsic property that enables cells to maintain their function in the face of various perturbations.[39,40]

Dr. Paul Weiss, a pioneer in systems biology, gave a lecture in 1968 marveling at the ability of biological form to spontaneously re-organize in the face of disturbance:

"Since any movement or change of any part of the system deforms the structure of the whole complex, the fact that the system as a whole tends to retain its integral configuration implies that every change of any one part affects the interactions among the rest of the population in such a way as to yield a net countervailing resultant; and this for every part. Couched in anthropomorphic language, this would signify that at all times every part 'knows' the stations and activities of every other part and 'responds' to any excursions and disturbances of the collective equilibrium as if it also 'knew' just precisely how best to maintain the integrity of the whole system in concert with the other constituents." [41]

Flexibility and adaptability are properties of healthy networks; they are also properties of many disease networks. Kitano[39] points out: *"Disease can be viewed as a breakdown of the robustness of normal physiological systems and the re-establishment of robust, and potentially progressive, disease states."* Disease networks are highly robust, able to re-assign protein functions and rewire circuits to allow for persistence of function in the presence of perturbation.[42] As a result, drugs have little therapeutic benefit when the robustness of the system that is being targeted compensates for any changes caused by drugs. Disease networks are especially robust to the removal of components by single-target drugs.[39,43-45]

To overcome the robustness of disease networks, drugs need to interfere with the compensatory mechanisms of the cell. This is achieved using multi-component drugs that target not only the molecular variants but the mechanisms responsible for maintaining robustness. In the case of HER2 expressed breast cancers, numerous mechanisms contribute to the resistance to trastuzumab.[46] For example, HSP90 (heat shock protein 90) is a molecular chaperone and key player in stabilizing denatured client proteins including HER2. Studies show that combining HSP90 inhibitors with trastzumab results in increased downregulation of HER2, less resistance to trastzumab, and greater efficacy of treatment.[47]

Kitano reflects on what these findings mean for the future of cancer treatment:

"This recognition shifts our attention from the magic bullet approach of anti-cancer drugs to a more systematic control of cancer as complex dynamical phenomena. This leads to the view that a complex system has to be controlled by complex interventions." [48]

In similar reflection, Dr. Nathan Price, the associate director of the Institute for Systems Biology in Seattle, states:

"Cancer isn't one disease. It represents many, many different ways cells in our body go awry. The cure is unlikely to be a simple drug; it's much more likely to be a complex adaptive system that can help identify and eradicate cancerous cells. It would probably have to be a system that evolves and changes just like cancer evolves and changes." [49]

Simple problems require simple solutions. Complex problems require complex solutions. To overcome the robust mechanisms of complex disease networks, drugs must be as complex and multifaceted as the disease network itself, targeting not only the molecular variants but also the robust mechanisms of these networks. By a like-cures-like principle, a more effective treatment approach may be to work *with* the complexity of biology rather than against it. Rather than using a single-target drug aimed at controlling or suppressing complex disease, we may achieve greater therapeutic benefit by using complex multi-component drugs that can *participate* in the complex mechanisms of disease, effectively shifting it into a state of health.

Systems Pharmacology

In summary, the application of systems biology to drug development is resulting in a new field of pharmaceutical research called *Systems Pharmacology*.[50] Systems pharmacology aims to develop drugs that are multi-component, multi-target, synergistic, and able to shift complex disease networks towards states of health more effectively and with less toxicity than single-target drugs. Supported by a new, more holistic science, systems pharmacology is shifting drug-design from reductionism towards holistic approaches. This new paradigm is also supported by more holistic treatment principles. (Table 1 summarizes these principles). Challenges do exist in the translation of this new science into clinical practice, such as cultural barriers to developing integrated and holistic models, lack of expertise in translational therapeutics, and challenges in developing test models that can scale from molecular interactions to organismal physiology.[3]

TABLE 1: Discoveries of Systems Biology translated into Treatment Principles

SYSTEMS BIOLOGY DISCOVERY	TREATMENT PRINCIPLE
Disease Networks: The "root cause" of complex disease is not a *single causative factor* but a *multi-scale interacting network of factors.*	**Treat the *whole*, not the *part:*** treat the whole cell network, not a single gene/protein; treat the whole person, not only the part that appears to be symptomatic; address disease *in-context,* not *in-isolation* (ie: address predisposition, environmental and psychosocial factors, personal and molecular individuality).
Modular Patterning: The *relationships* among biological objects have become diseased, not necessarily the objects themselves.	**Identify diseased *patterns*, not only diseased *objects*** (eg: identify the altered interaction patterns among molecules, not only the altered molecules themselves); treat the altered *patterns of relationship;* use medicines that *engage and shift* the diseased relationships, not medicines that attempt to *control and suppress* diseased objects.
Robustness: States of health and disease are maintained by innate adaptive mechanisms that allow function to persist in the presence of perturbation.	**Remove the obstacles to health and establish the conditions for healing:** aim to destabilize the robust mechanisms of disease while re-establishing the robust mechanisms of health; support the innate healing potential of the body. **Like-cures-like:** use medicines that are as complex and adaptable as the disease itself. Rather than aiming to *control* complex disease with single-target drugs, aim to *shift* complex disease through complex and adaptable multi-target medicines.

Systems Pharmacology is Looking to Natural Medicine for Guidance

Drug discovery based on natural products is receiving renewed interest in the age of systems biology.[51-53] Systems biology provides an evidence-based foundation for the use of complex multi-component medicines such as those used in herbal an nutritional medicine. In particular, systems biology validates the wisdom of using *whole*-herbal mixtures and *whole*-foods, as opposed to the extraction and administration of single bioactive compounds. Additionally, the discoveries of systems biology can be translated into treatment principles that are in accordance with many of the principles of traditional medical systems: treat the whole person, treat the root cause of disease, treat the underlying *pattern* of disharmony, re-establish balance and harmony, remove the obstacles to health and establish the conditions necessary for healing, and, among others, utilize the healing power of nature.

Certain herbal medicines, for example, are utilized by traditional medicine for their ability to re-establish balance in biological systems and/or to strengthen the resilience of organ systems to environmental perturbation. Referred to as adaptogens and/or tonics these medicines contain complex mixtures of bioactive constituents that work synergistically to support the *robustness* of their targeted organ system.[54] The botanical formula ADAPT-232 is a mixture of *Eleutherococcus senticosus*, *Schisandra chinensis*, and *Rhodiola rosea* was shown to target multiple nodes in the metabolic network of neurogial cells, resulting in increased robustness of these cells through inhibition of stress-induced catabolic reactions.[55] Moreover, this complex mixture produced *synergetic* effects, deregulating genes that none of the individual botanicals on their own could affect.[55]

Using the technology of systems biology we can now, more comprehensively, screen botanical medicines and derive maps of the bioactive networks responsible for their medicinal actions, as well as provide organism-wide models that detail the synergistic and multi-scale mechanisms of botanicals throughout the human body.[56-61]

The results from these studies are piquing the interests of the pharmaceutical industry:

"Now, it is possible to link the network-based treatment principle of herbal medicine with the pathological target network and optimize the combined-dosage of the essential components. All in all, network-based drug discovery is taking the pharmaceutical industry into a new age where efficient use of systems biology and computational technologies for medicinal herbs investigation will function as a powerful engine for multi-target drug discovery and development of network medicine." [56]

Perhaps the area of greatest collaboration is occurring at the interface of systems biology and Traditional Chinese Medicine (TCM).[62-68] Systems biology can translate the principles and practices of TCM into western scientific language, providing a bridge for deep collaboration with western medicine.[69] For example, the Ottawa Institute for Systems Biology, located within the department of medicine at Ottawa University, has recently partnered with the Shanghai Institute of Materia Medica to use systems biology to better understand the mechanism of action of Chinese herbal medicine in neurological disease, with an initial focus in Alzheimer's disease.[70]

The benefits of these collaborations are mutual: systems pharmacology receives valuable insight into network-based drug design with less toxicity and greater efficacy, while the indigenous wisdom and medicine of traditional healing practices receives valuable scientific validation through holistic models that honor the complex medicines rather than attempting to break them down into reductionist explanations.

Conclusion

Modern pharmaceutical development has reached an impasse. Reductionism, as the underlying bioscience of pharmacology, no longer provides a model able to cope with the emerging view of biological complexity. As a result, the reductionist "one target - one drug - one disease" approach is failing to treat complex disease. A new approach is required. Systems biology offers a promising new approach. With advances in biotechnology and computational analysis, systems biology is allowing us to observe, more comprehensively, the biological whole. Through systems analysis, disease is understood to arise from interacting networks that span multiple levels of biological organization. These networks display emergent properties such as modularity and robustness. This new understanding of disease is resulting in a new approach to the treatment of disease that involves treating the whole disease network using multi-component, multi-target and synergistic medicines. This approach is not new, however. An epistemological approach that embraces the biological whole and a treatment approach that utilizes the healing power of complex synergistic medicines is as old as time and is still alive today in many traditional medical systems. Western botanical medicine, ayurvedic medicine, traditional Chinese medicine, and naturopathic medicine are among these medical systems. Systems pharmacologists have begun to recognize the wisdom of ancient medical practices and are now turning to nature and traditional medicine for guidance.

About the Author

Laura Batson, MSc, ND, graduated from CCNM and runs a family practice in Ottawa, Ontario. Prior to becoming a naturopathic doctor, Laura completed a Master's in holistic science with a focus on systems biology and complexity theory, from Schumacher College, UK. Laura is developing curriculum to integrate holistic science with the naturopathic medical program and profession. She is also a passionate advocate for the re-integration of science with spirit, love, and art. Additional work can be found at www.LauraBatsonND.com.

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