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Networks at the nexus of systems biology and the exposome

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Abstract

Research on the impact of environmental exposures on health has largely been reductionistic. The field is now complementing the mechanism-based work with systems-based approaches to better understand the effects of exposure on complex biology. Borrowing principles from systems biology, systems toxicology has emerged as a way to study complex biological responses to exposures. By using the exposome framework, we can capture the complex nature of exposures, as well, and determine the underlying structure of real-world exposures. We discuss recent advances in measuring the exposome and the use of systems approaches in toxicology. We believe the integration of systems biology principles, network science, systems toxicology, and the exposome can help synthesize the complex interplay between the environment and biology.

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Systems biology, Exposome, Network science.

Introduction

The past century has witnessed extraordinary advances in the resolution at which we can study cellular biology and physiology. From molecular biology to cryogenic electron microscopy, this reductionistic approach has been highly successful in advancing our understanding of cellular processes. At the same time, it has become necessary to adopt approaches that rebuild the molecular constituents into models that explain multisystem interactions at the molecular and cellular levels. The past two decades have seen a steady growth in the field of systems biology [1,2], which includes an increased focus on cellular networks rather than individual components of a cell. It is becoming evident that to truly understand cellular function, it is necessary to take a holistic approach. This need is especially true when trying to understand the environmental contributors to human disease.

The US National Research Council has recognized this need and pushed toward using high-throughput techniques in testing of environmental chemicals [3]. However, the use of high-throughput techniques has led to an explosion in the number of data points derived from a single sample, and approaches to synthesize and interpret these types of data are still developing. The emerging concept of the exposome provides an organizational framework to address the complexity of environmental contributors to health and disease. In this article, we review progress in use of principles of network science to merge information generated by systems toxicology and exposome science to better understand the complex interplay between the environment and human biology.

Networks

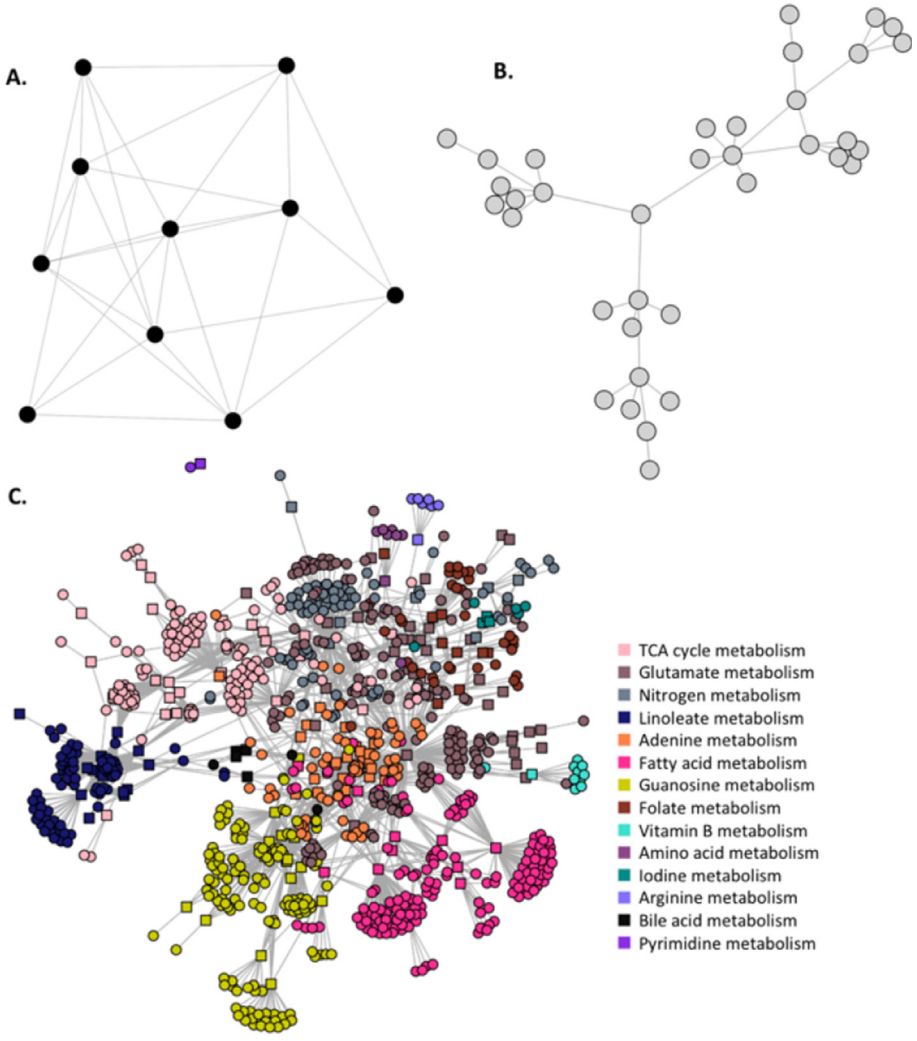
The advent of omics techniques that generate high-dimensional and unbiased measures of cellular function demands methods that can organize measures of many cellular molecules into interpretable functional and/or physical structures. Systems biology has leveraged principles from graph theory and network science to achieve this organization. Network science provides a framework that can help interpret the behavior of molecules that are correlated, covary, and form an organized structure: a network. These functional networks can be thought of as a collection of nodes, with a pair of nodes being connected to each other via edges. The edges may contain information about the strength, direction, or quality of the relationship between the two nodes. This overall structure of a network is similar across different disciplines, making it possible to borrow computational methods while requiring domain-specific interpretation of results [4,5]. Many have shown that networks in biology tend to be scale free and exhibit 'small-world' network characteristics [6,7]. This type of organization shows the presence of key hubs, that is, influential nodes that regulate many downstream processes. Biologists believe this affords some amount of resiliency through redundancy while highlighting the importance of key regulators. This organization is apparent in many

different biological networks generated using omics data, including transcription factor binding, protein–protein interactions, metabolic interactions, and genetic interaction networks [7–9] (Figure 1). Thus, network science enables organization of large omics data sets to characterize biology and thereby provides a means to understand systemic biological changes that associate with chemical exposures.

Complex exposures organize into the exposome

Human interactions with the environment are constant, complex, and spatiotemporally dynamic. An exposure occurs when chemicals in the environment reach a human–environment boundary. The dose of exposure becomes of interest if the chemical crosses the boundary and enters the body [10]. The definition of the exposome has seen many variations since Christopher Wild first coined the term in 2005, but the central theme remains the same. It is the measure of exposures over a life course, which produce quantifiable endogenous changes [11–15]. Systematically measuring the burden of chemicals inside a person offers a critical component of the exposome. Assessing the biological response to chemical exposures also represents an important measure. As noted by Hartung et al [16], measuring the biological 'footprint' of the chemical can serve as a marker of exposure. Importantly, biological responses can be transient or sustained, with the latter contributing to a cumulative, lifelong biological impact.

Figure 1



Illustrations of different network architectures. **(a)** shows organization of a random network where each node has an equal probability of being associated with another. **(b)** shows organization of a protein–protein interaction obtained from yeast recreated from curated data [58,59]. In **(c)**, we show an illustration of interactions between metabolites (square nodes) and proteins (circular nodes) with differential expression in cases of Alzheimer disease. Differential chemical features were mapped to pathways using *mummichog* [32]. The interactions between annotated metabolites and proteins were obtained using OmicsNet [60] and graphed using the igraph package [61] in R. The different colors indicate communities as determined by network properties.

There have been two main approaches to quantify the exposome: top-down and bottom-up. Both approaches have been gaining steadily over recent years. A top-down approach can be thought of as an 'outside-in' approach, where the estimates of exposure are determined by measures of the external environment [17]. Many techniques have successfully been able to measure the presence of multiple chemicals in a person's environment including the use of passive samplers [18,19], geographic information systems [20], satellite remote sensing [21], sensors [22], and personal monitors [23]. A recent study from the Snyder lab created an interaction network that used personal monitoring data collected over three years. Using active air sampling, they collected information on chemical constituents, plant-derived allergens, and microbiota. They assessed the relationship between different microbiota by creating an interaction network. This type of network was also used to understand the relationship between an individual's exposure to microbiota, chemicals, and plant allergens. They termed this interaction network a 'human-environment cloud' and found it to be diverse, distinct for individuals, and dynamic. Although this study had a small sample size, it serves as an excellent example that integrates various sources and techniques to measure an individual's exposome [24].

High-resolution mass spectrometers (HRMSs) have improved our ability to measure multiple chemical features in a biological matrix. The field of metabolomics, the study of the sum of all small molecules in a biological system, has gained substantially from these instruments. It has been the driving force for approaching the exposome from the bottom up, or inside out. For example, the Environmental Protection Agency is leveraging the power of HRMSs through 'non-targeted analysis' methods to rapidly characterize chemicals present in different media. This characterization of all chemicals in a medium is often referred to as 'exposomics.' These large environmental data sets produce complex coexposure structures that can be organized into interaction networks using correlation information for visualization and characterization. Because exposures co-occur, combinations of exposures can be predicted by the occupation, geography, socioeconomic status, diet, and urbanicity index of an individual. This structure can be expanded to include data on disease state to deduce clusters of chemicals that are most associated with a disease or biological response. The non-targeted analysis framework as proposed by the Environmental Protection Agency plans to use this 'exposomic' approach to make broad measurements of chemicals that can be detected in different matrices. Others have shown the power of this technique to detect chemicals in human serum [25]. Untargeted high-resolution metabolomics offers an unbiased approach in comparison with data accumulated through several targeted analyses such as those curated through the National health and nutrition examination survey [26,27].

Differentiating exogenous chemicals from endogenous metabolites is a challenge in mass spectrometry—based metabolomics because a large number of the chemical features have not yet been identified. To overcome this limitation, analytical and computational methods have been developed using probabilistic and chemoinformatic techniques [28—30] to measure the exposome. With this approach, high-resolution mass spectrometry data have been used to link the possible source and identity of a chemical feature with measured exposure, as well as to determine the biological relevance of features to measure biological outcomes [31]. For example, the pathway enrichment tool, *mummichog* [32], uses Fisher's exact tests to determine which metabolic pathway is most likely enriched based on the differential presence of chemical features. The algorithm uses previously curated information on metabolic maps as a reference to make these predictions. A new chemoinformatic tool *BioTransformer* [29] is a software package that uses machine learning approaches with a knowledge-based approach to predict small-molecule metabolism in human tissues and the environment via a prediction tool. These predictions can help in accurate, rapid, and comprehensive *in silico* compound identification. However, some chemicals can be derived from both sources and generated endogenously and through environmental exposures, posing a challenge in determining the source of such a chemical detected on the mass spectrometer.

Systems approaches to understand big toxicological data

Hartung and McBride (2011) urged toxicologists to curate Pathways of Toxicity that they defined as 'a molecular definition of cellular processes shown to mediate adverse outcome of toxicants.' These pathways focus on modes of actions instead of the end point of toxicity. Curation of Pathways of Toxicity created the 'human toxome,' a collection of finite number of pathways that are involved in a toxic response [33]. The hope is that such a compilation will bolster regulation and reduce reliance on animal testing [34]. A similar idea was proposed by the organization for economic cooperation and development, where the adverse outcome pathway serves as 'a conceptual construct that portrays existing knowledge concerning the linkage between a direct molecular initiating event (e.g., a molecular interaction between a xenobiotic and a specific biomolecule) and an adverse outcome at a biological level of organization relevant to risk assessment.' It has been proposed that most real-world exposure scenarios are likely a combination of multiple adverse outcome pathways which form networks with shared key molecular events [35]. These concepts provide a framework that can be used to develop tools that let us leverage the power of networks that help decipher interactions between different layers of biomolecules, as they fall under the central dogma.

Along these lines, Hartung et al. [16] describe the need for measurement of omics profiles as responses to perturbations from chemical exposures. They argue chemicals can leave a 'footprint' in a biological system that can be captured using omics techniques such as metabolomics and transcriptomics, which measure the layer closest to a functional response. Furthermore, interactions between different omics layers will uncover regulatory processes and help identify key hubs in biological networks. These hubs can provide information relevant for identifying vulnerabilities and possible targets of intervention. Computational methods that allow for this integration have been developed [36,37], but they have their limitations [38]. Integration of data obtained from different studies poses further challenges and requires rigorous inclusion criteria. One study analyzed epigenomics, transcriptomics, and proteomics data gathered from 12 cohorts of arsenic exposure. They found common genes and pathways affected across all 12 studies and reported tumor necrosis factor as the top upstream regulator of gene expression changes due to prenatal arsenic exposure [39].

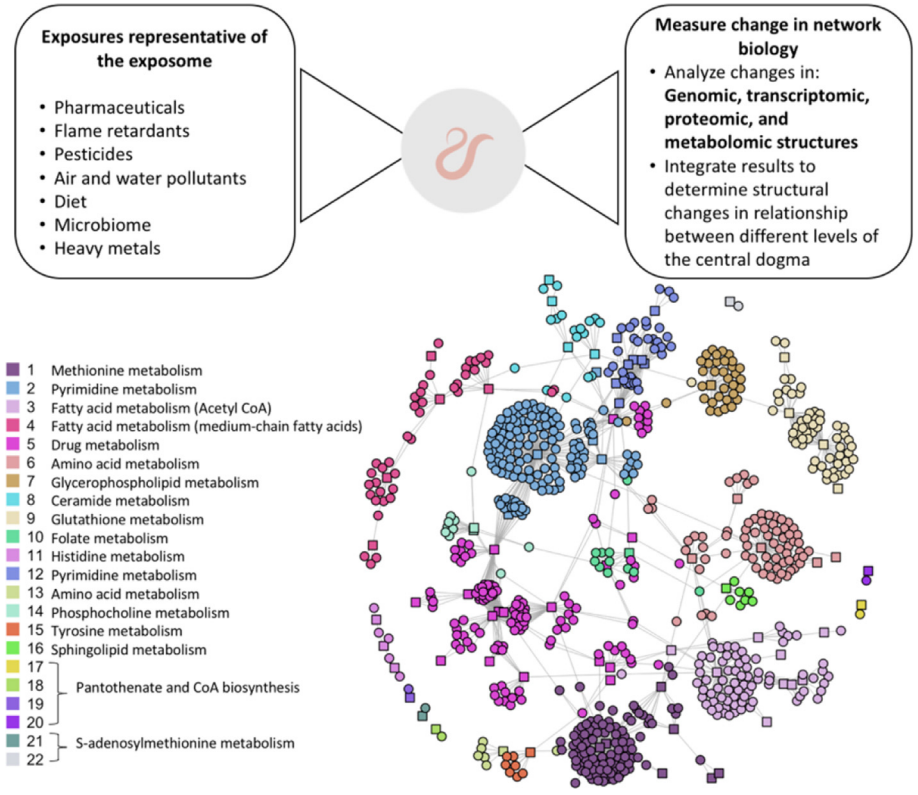
Many researchers have used the power of HRMSs in metabolomics to uncover systemic biochemical changes that accompany a disease state [40,41]. A recent report measured chemical constituents of interstitial fluid which can be derived from blisters or engineered microneedle patches. The measurements showed good correlation with constituents of plasma measured using a high-resolution liquid chromatography-mass spectrometry platform [42]. Petrick et al. [43] show the feasibility of using dried blood spots obtained from children right after birth as sources of metabolomic information in early life that can be used to develop novel biomarkers and to study childhood disease initiation and progression. Others have reported the use of inductively coupled plasma mass spectrometry to measure the amount of metals stored in deciduous teeth, which serve as biomarkers of in utero and early life metal metabolic dysregulation, and shed light on mechanisms underlying autism spectrum disorder [44]. Although the blood metabolomic profile can reveal metabolic changes associated with a disease process, it is likely confounded by changes in the metabolome associated with the disease process itself. This issue of temporality in assigning cause and effect can be controlled by using prospective, longitudinal cohort designs in epidemiological studies of the exposome.

Integrating toxicological and exposomic data to observe biological response

While techniques that can collect big data have been gaining steadily, so have methods to integrate biological and environmental data. One approach uses quantitative and qualitative measures to describe a tripartite network consisting of interactions among environmental exposures, biological pathways, and human phenotypes to determine the contributions of environmental and biological changes toward a disease outcome [45]. Multiscale, multifactorial response networks also provide an opportunity to understand the relationship between variables across multiple networks using tools such as partial least squares regression [46]. An occupational health study used a combination of occupational exposure data, HRMS, and phenotypic data to show an interaction between exposure to trichloroethylene and markers of renal function. They report the presence of previously unidentified chlorinated metabolites that were strongly correlated with markers of renal dysfunction [31].

In Europe, the Human Early Life Exposome study is a prospective study that is designed to collect biological data and exposure assessments from gestation to early adolescence in more than 30,000 mother–child pairs living in 6 countries [47]. This birth cohort is designed to collect omics profiles and exposure measures made using biomarkers and environmental measurements. Although the use of prospective cohort studies in exposome research reduces uncertainties concerning causal association, they are expensive and may still have biases introduced by exposure misclassification and selection bias. We believe that experimental toxicologists have an important role to play in uncovering the relationship between complex biological responses and the exposome. Using animal models, toxicologists can systematically begin to explain the network changes associated with exposure to multiple chemicals. Evolution has conserved various pathways and molecular machinery across species, making model organisms great sources of biological information, with human relevance. Using organisms such as *Caenorhabditis elegans* (worms), *Danio rerio* (zebra fish), and *Drosophila melanogaster* (fruit fly), toxicologists and biologists have made important discoveries, including apoptosis, RNA interference, several developmental genes, cellular transporters, and nervous system receptors [48,49]. Because they have been widely used in biology and toxicology, curation of many aspects of their genome, transcriptome, proteome, and metabolome is available. Their short lifespan makes it possible to study lifelong effects of exposure and makes it possible to study the 'health span' [50]. Many researchers have used worms and zebra fish as models to study omics-level changes in metabolism and gene expression as a result of exposure to toxicants [51,52] (Figure 2). Furthermore, cross-species network analyses can be used to uncover evolutionarily conserved pathways that are perturbed by environmental exposures or an underlying disease process [53]. Apart from model organisms, organoids and organs on chips provide avenues to study cellular function with tightly controlled environmental conditions. We believe they offer opportunities to assess targeted questions that may arise from untargeted, network approaches applied in measuring the effects of the exposome [54,55].

Figure 2



The exposome at the bench. Leveraging small organisms provides an opportunity to ascertain effects of exposure, ranging from single chemical to mixtures of chemicals, on changes in network biology. Here, we see changes in the metabolomic network of *Caenorhabditis elegans* because of exposure to a known neurotoxicant, MPP^+. This was conducted in the laboratory using wild-type worms, exposed to 1 mM MPP^+. High-resolution metabolomics was performed on a Thermo Q-Exactive. Pathway level changes were determined using *mummichog*, and network changes were determined in the same way, as in Figure 1c.

Conclusion

Research in environmental health and toxicology has generated overwhelming amounts of data and evidence that point to toxic effects of several environmental exposures. These data have led to policy changes and regulatory implementation that have saved many lives and improved quality of life. However, much of this work has been based on a small number of high-use chemicals, for example, lead and mercury, whereas our current concern is on the multitude of low-abundance chemicals. Using the more holistic exposome framework in systems toxicology will provide a new level of understanding that considers the complex nature of real-world exposures to hundreds to thousands of chemicals and the corresponding biological responses. This endeavor is dependent on access to high-quality databases that are amenable to computational manipulation. Various consortia have made information available from omics-level investigations made in different organisms [56,57]. Biosynthetic experiments and chemoinformatic algorithms are required to fill databases on environmentally relevant chemicals, which remain sparse and need inclusion of metabolic products of parent toxicants [29]. Although we still face limitations in our ability to interpret omics data, advances in data curation and generation will improve our ability to identify new links between exposure and response. Integrating systems biology and the exposome using a network science framework will provide a measure of the environmental basis and contribution to human disease.

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Conflict of interest

The authors declare no conflict of interest.

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* of special interest
** of outstanding interest

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