

Systems Proteomics for Translational Network Medicine

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Universal principles underlying network science and their ever-increasing applications in biomedicine underscore the unprecedented capacity of systems biology-based strategies to synthesize and resolve massive high-throughput generated data sets. Enabling previously unattainable comprehension of biological complexity, systems approaches have accelerated progress in elucidating disease prediction, progression, and outcome. Applied to the spectrum of states spanning health and disease, network proteomics establishes a collation, integration, and prioritization algorithm to guide mapping and decoding of proteome landscapes from large-scale raw data. Providing unparalleled deconvolution of protein lists into global interactomes, integrative systems proteomics enables objective, multimodal interpretation at molecular, pathway, and network scales, merging individual molecular components, their plurality of interactions, and functional contributions for systems comprehension. As such, network systems approaches are increasingly exploited for objective interpretation of cardiovascular proteomics studies. Here, we highlight network systems proteomic analysis pipelines for integration and biological interpretation through protein cartography, ontological categorization, pathway and functional enrichment, and complex network analysis.

Proteomic Cartography

Proteomics encompasses diverse methods by which to study proteins, their abundance, structure, posttranslational modifications, and physical or functional interacting partners to map the proteome, the protein complement of a genome. Although such methods can be applied to individual proteins, proteomics facilitates large-scale analysis of complete proteomes or targeted subproteomes (Table 1). Moreover, proteomics enables comparisons between distinct conditions/states, from defined biological sources, across discrete timelines. In general, a proteomic cartography pipeline involves sample acquisition, followed by protein isolation, separation, resolution, and identification (Figure). Along this continuum, each successive module harbors a multistep process, whereby proteomic methodologies can be implemented.

Once proteins are isolated from the source of interest, 2 major separation strategies have evolved to address proteome complexity.^{1,2} Traditional gel-based approaches, particularly 2-dimensional gel electrophoresis involving isoelectric focusing followed orthogonally by sodium dodecyl sulfate - polyacrylamide gel electrophoresis, were the earliest methods adopted for protein separation and resolution, comparative assessment of relative abundance, and initial reduction of protein complexity before mass spectrometry (MS). Although

2-dimensional gel electrophoresis remains common, the advent of quantitative MS techniques has led to increased application of various gel-based and gel-free alternatives, whereby protein or peptide complexity is addressed by separation and resolution before or in conjunction with MS, whereas quantification is subsequently measured from MS precursor or fragment ion spectra. Choice of separation strategy is influenced by study objectives and resources, often guided by advantages and limitations to each approach. Quantitative MS methods tend to be less time- and labor-intensive while offering greater potential throughput and data acquisition, yet in instances where 2-dimensional gel electrophoresis approaches are applied in parallel, use of duplicate approaches can provide independent confirmation^{3,4} or yield complementary, nonoverlapping information.^{5,6} Regardless of separation strategy, bottom-up or top-down MS analysis^{7,8} facilitates protein assignment, generating extensive lists that, depending on experimental design, typically include relative abundance between conditions or states (Figure). Several reviews provide greater technical detail on proteomic methodologies and their broad-based application to cardiovascular research.^{1,2,9-11}

A decade ago, an initial survey of the cardiovascular proteomics domain emphasized that successful incorporation of existing and new technologies would help to unravel intricacies of proteome dynamics.¹ Indeed, introduction of several enabling technologies has accelerated this emerging field. The utility of 2-dimensional gel electrophoresis was enhanced by improved gel reproducibility attained with immobilized pH gradient isoelectric focusing¹² and fluorescent-based difference gel electrophoresis allowing coresolution of paired samples.¹³ Numerous MS advances included algorithms for automatic spectral interpretation and quantitative MS strategies, such as isotope-code affinity tag peptide labeling¹⁴ or stable isotope labeling of amino acids.¹⁵ Subsequent instrumentation advances, such as development of high-resolution, high-mass accuracy Fourier transform-ion cyclotron resonance MS and introduction of Orbitrap MS technology for proteomic applications,^{16,17} expansion of quantitative MS techniques, such as isobaric tag and label-free methods,¹⁸⁻²⁰ development of selective posttranslational modification analysis strategies, eg, phosphopeptide enrichment,²¹ plus continued growth and extensive curation of protein databases for comparison and identification of peptides and proteins from acquired MS data, all contributed to expansion of proteomic studies.

An important corollary of these developments is the increasing magnitude of acquired information. Individual proteomic data sets are not simply more numerous; they are

Table 1. Systems Proteomics Lexicon

Subproteome	Proteome subset, corresponding to particular intra- or extracellular compartments, involvement in specific functions, or modulated by or dependent on particular biological contexts.
Metaboproteome	Subproteome involved in and supporting cellular metabolic function.
Systems biology	Analytical approach to investigate and model relationships among system components to understand and predict emergent properties.
Network/interactome	Mathematical representation of pairwise collections of proteins connected by interdependent interactions and relationships, the structure of which conveys emergent properties.
Network medicine/biology	Analysis of molecular (eg, protein, gene, metabolite) interaction networks through complex network theory, comprising topological features characterizing symmetric or asymmetric relationships among discrete network objects and their known or established interactions to guide medical or biological interpretation.
Node/vertex	Fundamental unit of a network, in network biology including one or more of the following: genes, proteins, metabolites, drugs, and endogenous small molecules.
Edge	In network biology, a relationship between 2 nodes or node self-interaction, involving physical, regulatory, or genetic interactions which may be undirected or directed (uni- or bidirectional). Edges can include information regarding relationship confidence (eg, node weighting) or function (eg, interaction/pathway kinetics).
Degree distribution	Node degree defines number of connections between a network node and other nodes, with degree distribution being the probability distribution of the degrees of all nodes.
Clustering coefficient	Node clustering coefficient is determined by proportion of nodes connected to it that also connect to one another, providing an indication of neighborhood relatedness, the degree to which nodes within a network cluster together.
Scale-free topology	Biological networks are postulated to exhibit power law distributions of node connectivity, which influences emergent network properties, such as robustness.
Path length	Shortest distance required to connect 2 nodes. Scale-free networks have extremely small average path lengths.
Network diameter	Maximum path length necessary to connect any 2 network nodes.
Network motif	Patterns of interconnections in complex networks occurring at significantly higher rates than expected in randomly assembled networks.
Network module	Collection of nodes with high internal connectivity but few connections with remainder of the network, typically comprising nodes sharing a particular function (eg, complex, signaling cascade).
Hub	Node or small number of nodes much more highly connected to other network constituents than expected randomly.
Bridging node	Node bridging the shortest path between 2 other nodes or modules in a network, which can be critical bottlenecks in network functionality.

growing ever larger. Regardless of whether gel-based or gel-free methodology is applied, if surveys are quantitative comparisons or qualitative cataloging or if they provide coverage of single or multiple time-point/phenotype dynamics, proteomic studies now regularly yield lists comprising hundreds

of identified proteins. Thus, a critical consideration for continued maturation of proteomic research is defining appropriate measures by which to collate, prioritize, and integrate expansive protein lists to maximize biologically meaningful data extraction. When confronted with overwhelming protein numbers, where traditional reductionist analysis of each no longer remains feasible, it becomes tempting to focus on a particular subset, such as a fraction of proteins exhibiting greatest differential expression, those with established links to the topic of interest, or another delimiter to focus interpretation and narrow downstream analysis.²² As a consequence, mechanistically relevant proteins, complexes, and functional modules may be inadvertently marginalized or ignored. In this context, analysis of proteomic and other high-throughput data acquired using objectively defined statistical criteria²³ should instead be inclusive.²²

Systems Algorithms for Proteome Comprehension

Network systems biology offers an integrative approach to achieve inclusivity and maximize comprehension. Network platforms incorporate a complement of identified proteins, together with their interactions and, where available, input of other high-throughput data, to better comprehend the system in its entirety.^{22,24–29} Various network analysis approaches are becoming increasingly prevalent in genomic,^{30–34} microRNA,^{35,36} proteomic,^{3,37–41} and metabolomic⁴² cardiovascular research.^{43–45}

Proteins function within intricate webs of interactions. Accordingly, derivation of biological interaction networks provides a rational means of assembling proteomic data in functional contexts. As an example, the minimal network required to incorporate all identified proteins via established interactions yields an immediate focal point of proteomic measurement within an experimental system, regardless of extent of data set size or breadth of constituent protein functional variability. Derived networks are amenable to compositional assessment, based on function/ontology overrepresentation and enrichment analysis, and to structural examination, such as architectural topology or positional relevance of components, eg, hubs or bridging nodes (Table 1).^{46,47} Moreover, network composition and structure may be exploited for mechanistic interpretation, hypothesis generation, and functional validation, enabling refinement of assembled networks.^{22,48} Network systems biology thus offers a logical, inclusive approach by which to process, prioritize, and interpret complex systems behavior from high-throughput data sets of theoretically unlimited size.

Network biology approaches to proteomic data assessment and interpretation typically involve data reduction, clustering or grouping acquired data through ontological categorization, and a combination of pathway and complex network analysis (Figure).^{29,37–40} Functional ontology classification provides initial biological context for individual proteins, whereas pathway and network analysis integrate composition and architecture of associated interrelationships from defined proteomic observations. Both biological context and network architecture serve as tools to scrutinize acquired proteomes and to develop informed follow-up investigation and validation of insights predicted from high-throughput data.^{3,29,37–40}

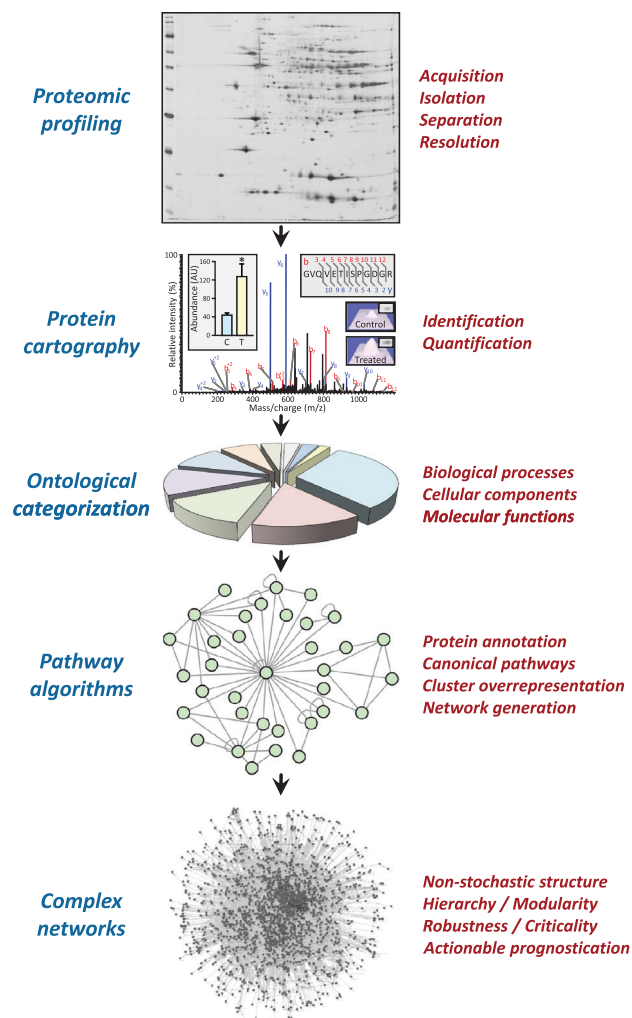


Figure. Network systems proteomic pipeline. Strategy extending proteomic profiling from mapping and quantification of protein identities to ontological categorization, pathway analysis and network generation for inclusive systems interpretation.

Ontological Categorization

Proteomic studies are proficient at detecting changes, yet biological interpretation is often limited by data complexity. When lists remain small, relevance can be anticipated and functionally validated on a protein-by-protein basis. For large-scale studies, this approach is untenable, and there is a need to define appropriate methodology for bioinformatic assessment of high-throughput data sets. Data comprehension is facilitated through clustering of proteins by biological processes, cellular components, or molecular functions (Figure).^{29,37–40,49}

Accurate categorization requires the ability to cross-reference protein database information with complementary sources, such as the Gene Ontology (GO) or pathway analysis applications. Despite progress, lack of conformity in nomenclature and computational systems biology data and language formats can limit cross-platform interoperability.

For spectral assignment during tandem MS (MS/MS) and initial protein characterization, the UniProt Knowledge Base (UniProtKB) is an established reference. Alternatives include or have included International Protein Index, Protein Information Resource, National Center for Biotechnology

Information nonredundant database, Swiss-Prot (from the Swiss Institute of Bioinformatics), and Translated European Molecular Biology Laboratory Nucleotide Sequence Database from the European Bioinformatics Institute. The UniProt Consortium initially combined Protein Information Resource, Swiss-Prot, and Translated European Molecular Biology Laboratory into the UniProtKB and now also includes the International Protein Index database. UniProtKB is curated and continuously updated to incorporate new entries and minimize redundancies, while maintaining a history of entry changes, such as consolidation, replacement, or protein/gene renaming.

UniProtKB entries often include multiple identifiers, increasing the ability to acquire complementary data from other repositories during subsequent analyses. They also contain a wealth of related information, such as interacting partners and residency in protein complexes or functional modules, GO information regarding associated molecular functions or biological processes, and hyperlinks to additional function- and interaction-related databases. Of the protein identifiers used in UniProtKB, the Human Genome Organization gene symbol ID is prioritized and universally applied across databases. Therefore, it serves as a pragmatic choice for cross-database and downstream querying of UniProtKB-derived proteomic information. Protein complexity, however, still occasionally leads to Human Genome Organization ID nomenclature issues during bioinformatic data assessment. This may occur when: (1) a single ID represents multiple variants of 1 protein, all of which may be subsumed by MS/MS data and thus indistinguishable from one another, eg, TNNT2 represents 10 human cardiac troponin T splice variants; (2) other highly homologous proteins are subsumed by spectral data, eg, actin isoforms; (3) MS/MS peptide matches are subsumed by protein isoforms of identical sequence arising through gene duplication, eg, calmodulins 1, 2, and 3; and (4) synonyms exist for a single protein, eg, histone 4 has 14 human and 12 mouse Human Genome Organization IDs. From each scenario, however, only 1 ID is assigned, in accordance with accepted parsimony standards.⁵⁰ Although indistinguishable isoforms and ID redundancy might be considered confounding issues during ontological classification and pathway/network analysis, their impact is negligible because they are variants of the same protein, and their occurrence is infrequent in the context of hundreds of additional proteins.

Most cardiovascular proteomic studies now include ontological categorization, clustering identified proteins by GO biological process, cellular component, or molecular function (Figure), with or without segregation into expression cohorts, ie, upregulated, downregulated, or unchanged. While providing initial biological context, assignment of a single function to each protein is often an oversimplification, because many proteins participate in multiple physiological processes. For instance, promiscuous enzymes influencing an array of other proteins and their activity, eg, oxidoreductases, kinases, and phosphatases, may carry out a specific function, such as phosphate transfer, yet this may impact numerous biological processes, confounding comprehensive protein categorization. In this regard, proteins with multiple GO associations are more appropriately accommodated and more effectively accounted

for by calculating overrepresentation and enrichment significance during subsequent pathway and network analysis.

Pathway Analysis Algorithms

Pathway analysis maps high-throughput data to, and assesses enrichment of, canonical pathways. Pathway algorithms facilitate data annotation, containing detailed descriptions of individual proteins and links to current knowledge. Pathway analysis software can also be used to calculate functional enrichment, by assessment of algorithm-specific functional terminology or GO molecular functions and biological processes. Some pathway algorithms enable biologically oriented network analysis, where subsets of proteins, represented as nodes or vertices, are connected by edges via established relationships, generating networks that may be further scrutinized for function or pathway overrepresentation. Thus, pathway analysis extends functional categorization by evaluating collective consequences of proteomic findings in the context of established pathways, functions, and interactions (Figure).

Pathway analysis is an expanding field, with a diversity of bioinformatic applications designed to address molecular pathways and interactions. A comprehensive list of applications available at Pathguide⁵¹ outlines over 300 existing pathway and molecular relationship resources. Collectively, they encompass 8 subcategories, including protein-protein interactions, metabolic pathways, signaling pathways, pathway diagrams, transcription factor/gene regulatory networks, protein-compound interactions, genetic interaction networks, protein sequence focused databases, and 14 repositories related to topics not covered by the 8 major categories. Pathguide provides information about each resource, facilitating informed decisions regarding algorithm selection.

Deconvolution of broad-based proteomic data is most readily accomplished with pathway resources that maximize information retrieval, such as Ingenuity Pathways Knowledge Base, archived in 5 Pathguide categories—protein-protein interactions, metabolic pathways, signaling pathways, transcription factors and gene regulatory networks, and protein-compound interactions. Ingenuity Pathways Knowledge Base is composed of data manually curated or automatically extracted from the literature and incorporates reviewed content from over 20 additional repositories. In turn, data are accessed and interrogated using the complementary bioinformatics tool, Ingenuity Pathways Analysis (IPA). Because Ingenuity Pathways Knowledge Base is a compilation of existing data, it is important to emphasize that IPA analysis is a pathway-oriented interpretation of current knowledge rather than inference of novel outcomes and interactions. As data acquisition increases, gaps in knowledge and limitations in scope will be reduced, expanding and refining pathway analysis interpretation. Together, IPA and Ingenuity Pathways Knowledge Base offer broad applicability and, as such, have become increasingly popular for cardiovascular proteomic studies,^{3,5,37–40,52–66} as have various alternative applications for ontology/enrichment analysis (eg, Gene Set Enrichment Analysis,⁶⁷ Ontologizer,⁶⁸ and the Database for Annotation, Visualization, and Integrated Discovery)⁶⁸ and dedicated pathway and network analysis (eg, MetaCore's GeneGo).⁶⁹

Pertinent considerations for implementing pathway analysis can be garnered from an overview of IPA search parameters. IPA accepts 21 different identifiers to process and interpret high-throughput datasets, from a number of gene/protein ID formats suitable for proteomic data, several mRNA microarray platforms, plus additional formats to facilitate microRNA or metabolomic studies, and, more recently, RNA-Seq and next generation sequencing experiments. Thus, pathway analysis can be carried out using proteomic data alone or integrated with other high-throughput data. Protein IDs uploaded for individual or multiple observations may include quantitative values such as fold change, *P* value, or log ratio. Additional user-specified variables include output data set size (eg, optional settings for maximum size and number of generated networks), desired stringency and scope (eg, species-specific information; consideration of direct and indirect relationships; inclusion of observed and predicted interactions; inclusion/exclusion of endogenous chemicals, together with genes/proteins as network nodes), whether or not to focus on a subset in isolation (eg, fold change and *P* value cutoffs to focus on up-, down-, or both up- and down-regulated proteins simultaneously; inclusion/exclusion of unaltered proteins from assessment), and selection of a baseline or standard reference set against which proteins of interest are compared for pathway and function enrichment or overrepresentation. To cast as wide a net as possible for detection of pathway and network interactions, both direct and indirect relationships across a variety of tissues and species should be included,^{37–40} as this will obviate annotation limitations. For instance, tissue-specific limits may be confounded by gene/protein annotation being based primarily on healthy tissues, which may not reflect observed expression under conditions of stress or disease, eg, fetal isoform expression in adult tissue, whereas setting species limits may overlook relevant knowledge of a particular protein that is more extensively documented in a species other than the one under investigation. Search stringency for proteomic data is better attained by limiting interpretation to include proteins only, by limiting interactions to those that are experimentally observed and by defining network generation parameters to minimize the number of networked molecules required to incorporate all submitted proteins.^{37–40}

As an evolving component of proteomic research, pathway analysis is best exploited as an interpretation tool facilitating hypothesis generation for subsequent follow-up, rather than as a confirmatory/validation step within the data analysis pipeline. The utility and potential of pathway analysis software are tempered by the fact that documented relationships only comprise partial biological information. Moreover, reliability of data supporting these interactions is variable, and it is impractical to assess interactions for quality control on a protein-by-protein basis when thousands of connections are generated. Finally, substantial effects on pathway software have been demonstrated by annotation changes, updates, additions, and errors, all of which impact prediction and significance outcomes.⁷⁰ Thus, pathway analysis searches run the risk of being incomplete or potentially misleading and are best validated by experimental follow-up.

Overrepresentation of normal and adverse biological functions assessed in IPA can be useful in guiding follow-up. In

a model of imposed stress in the context of ATP-sensitive K⁺ channel deficiency, IPA analysis of proteomic data exclusively predicted cardiac adverse effects, which were subsequently validated by *in vivo* assessment in the ATP-sensitive K⁺ channel-deficient cohort.³⁸ Adverse effect screening was also used in a study examining proteomic consequences of cardiomyopathy and structure/function remodeling mediated by stem cell therapy.⁴⁰ In this regard, cardiomyopathic proteome changes predicted several cardiac adverse effects and overrepresentation of cardiac disease, which were diminished or no longer evident when assessing proteomic remodeling of stem cell-treated cardiomyopathic hearts. Validation of the predicted spectrum of structural and functional benefits by stem cell therapy was demonstrated by multimodal imaging, indicating restoration of prestress cardiac functional levels and normalization of heart structure.⁴⁰ Thus, proteome-specified functional targets can be identified by pathway analysis for experimental follow-up and validation.

Networks generated during pathway analysis also yield biologically relevant contexts for functional prioritization, hypothesis generation, and experimental validation (Figure). IPA networks are ranked by calculated overrepresentation of the proportion of input proteins within each network relative to expected numbers if proteins were instead randomly selected from a background reference set. Included with these prioritized networks is predicted intranetwork functional enrichment, which can further guide hypothesis generation. For example, the top prioritized network generated from proteomic analysis of endoderm secretome-mediated cardiopoiesis was associated with cardiovascular growth and development.³⁷ Validation was carried out *in silico* by removal of the most highly connected network node from the list of input proteins in a parallel IPA assessment, which demoted cardiovascular development prioritization. Validation was independently conducted *in vitro* by targeted pharmacological inhibition, which abolished the potentiation of cardiac differentiation, thereby confirming network contributions to cardiopoiesis.³⁷

Default IPA constraints typically double the number of proteins from initial upload to network output,^{3,37–40} thereby increasing complexity, but this also provides additional targets for hypothesis generation by incorporating potentially relevant proteins not detected during initial proteomic screening that can be targeted, assessed, and validated.³⁷ Another case in point is an IPA-derived network assessing crosstalk between endothelial and vascular smooth muscle cells in response to differential shear stress.⁶⁴ Here, the primary proteomic network included several secretory proteins involved in cellular communication and function modulation. Some of these proteins were not detected during proteomic analysis, yet their presence in the network suggested criticality for intercellular crosstalk underlying observed vascular remodeling, which was subsequently assessed and validated.⁶⁴ Thus, pathway analysis provides actionable predictions for validation via network functional prioritization, including addition of target proteins networked with, but not initially observed among, acquired proteomic data.

Pathway algorithms are advantageous for enrichment analysis or function and ontology representation but lack tools

necessary to characterize network architectural topology and positional relevance of network components. Pathway analysis networks emphasize biological relationships over structure and are designed to be sufficiently large to provide biological interactions, yet small enough for rapid network inference and construction, and to readily visualize all nodes simultaneously. IPA network settings, for instance, range from 35 to 140 nodes, and although multiple networks can be combined, merges may comprise no more than 500 nodes at a time. Moreover, no provisions are included to assess functional enrichment within merged composite interactomes. To fully comprehend network structure and composition for inclusive systems interpretation of proteomic data, particularly as data sets continue to increase in magnitude, it is necessary to move beyond pathway software to applications dedicated to network visualization and interpretation.

Complex Network Analysis

Complex network analysis enables integrative proteome study across the spectrum of properties inherent within assembled protein-protein interrelationships. These mathematical representations comprise individual protein constituents, termed nodes or vertices in a derived network, held together by physical or regulatory interconnections, defined as edges (Table 1). Nodes and edges can be assessed for architectural connectivity, superimposed with qualitative or quantitative information, and, for some network algorithms, linked to external annotation or ontological information sources. Networks can also be modified or updated in an iterative fashion. Although popular for proteomic and other systems interpretation, network approaches are limited with respect to input data quality, output variability from differing algorithms, and lack of flexibility,^{22,71} with limited capacity to account for posttranslational or spatiotemporal dynamics, or when networking data acquired from multiple high-throughput sources. Metabolic networks, for example, may comprise protein nodes with metabolite edges, metabolite nodes with protein edges, or both proteins and metabolites as nodes connected by various interrelationships. Applied judiciously in light of such caveats, networks remain versatile platforms on which to integrate proteomic and other high-throughput data for systems comprehension.²²

Current understanding of the nonstochastic nature of networks arose from the demonstration that supposedly random networks from a variety of sources instead possess nonrandom connectivity.⁷² In these networks, the vast majority of nodes possess 1 or few edges, whereas an exceedingly small proportion are more highly connected. Node connectivity is defined as degree, the number of edges a node possesses, ie, how many other nodes it connects to in the network. Rather than exhibiting Gaussian node degree distributions, expected if randomly connected, networks approximate power law distributions that form characteristic scale-free topologies. Debate continues regarding whether network structures are scale-free, exponential, or adhere to more esoteric mathematical constraints, yet it is clear that their organization is not random. Nonstochasticity of network architecture is now known to be universally applicable, and this imparts emergent structural properties of biological relevance, such as network motifs, modularity, and functional robustness (Table 1).^{46,47} Proteome networks, too,

follow principles of nonstochasticity, reflecting nonrandom, ordered structure, consistently observed regardless of network scale, which may be exploited on the basis of these properties in a variety of ways not apparent when viewed simply as a list of protein identities.

To assess such properties for proteomic experiments, interaction networks are typically derived by impartial assembly from pathway analysis resources or by manual screening of the literature and can be generated *de novo* by assessing protein-protein interaction data. Alternative criteria may be used to delineate network connectivity, such as statistically on the basis of coexpression matrices and Pearson correlation thresholds³³ or by *ab initio* reverse engineering network models from gene expression dynamics.⁷³ Statistical approaches may yield novel relationships based on gene coregulation but require confirmation to determine whether or not connections are functionally meaningful.⁷⁴ Moreover, extensive expression profiling over numerous perturbations or time points is necessary to provide sufficient data for reliable network modeling. Until such time as proteomic data are produced in quantities or at scales similar to transcript profiling, proteomic network generation will rely primarily on current biological knowledge to establish node connectivity.

Just as Pathguide serves as a portal into pathway analysis, Graph Visualization Software References provides an online resource detailing over 80 network analysis algorithms to enlighten and guide selection of network software, including additional links to other sites and resources.⁷⁵ As with pathway analysis, those interested in network generation may avail themselves of specialized or generalized algorithms through Graph Visualization Software References. A comprehensive Graph Visualization Software References-listed application is Cytoscape, an open-source platform for visualization of proteomic molecular interaction networks and their integration with other state data.⁷⁶ At the core of Cytoscape is a network visualization tool, for which both developers and users alike can create and exploit specific peripheral tools, termed plug-ins, to facilitate network interpretation, including network and molecular analyses, additional layout options and file format support, and connections to external databases and annotation.

Network generation in Cytoscape requires no specialized knowledge of network theory or computer programming. In their simplest form, networks consist of a collection of pairwise interactions. Thus, Cytoscape generates networks from spreadsheets or simple interaction files (.sif extension) for which each row expresses an interaction and the nature of that interaction, eg, undirected, unidirectional, or bidirectional. Once pairwise interactions are compiled, eg, from pathway analysis sources, and uploaded, additional files containing expression attributes may be included and applied to network layouts and node or edge attributes for network visualization. For instance, node and edge shape, size, and color can be used to convey direction and extent of expression, node functional category, edge confidence, and so on, and layouts can be used to emphasize or highlight particular attributes, such as the most highly connected or functionally related nodes.^{37–40} For instance, network information can be accentuated by clustering nodes of common function via layout colocalization^{38–40} or by presenting common elements of adjacent networks by locating them in

the same relative location for both networks.⁴⁰ Unlike standard pathway analysis network settings that limit network size and scope, dedicated complex network analysis software can generate networks comprising hundreds of thousands of nodes, accommodating the largest proteomic interactomes.

To complement network assembly, Cytoscape users can presently select from 155 plug-ins to suit particular needs, including analysis of existing networks, import of networks and network attributes, network inference, functional enrichment, communication and scripting applications, and a miscellany of additional applications. Download statistics indicate that the most popular Cytoscape plug-in is the Biological Network Gene Ontology (BiNGO) tool.⁷⁷ BiNGO assesses biological network overrepresentation for various GO categories—biological process, cellular component, and molecular function—by comparison to a species-specific reference, similar to enrichment analysis tools in pathway algorithms. Other top downloads include plug-ins for assessment of network structural attributes, such as highly clustered or activated network modules (Molecular Complex Detection [MCODE] and jActive Modules, respectively), and those facilitating access to other databases (National Center for Biotechnology Information Entrez Gene User Interface and National Center for Biotechnology Information Client) and manuscript searches (Agilent Literature Search) for network annotation (Table 2). Another popular plug-in is Network Analyzer,⁷⁸ a useful tool for assessing network topology, the benefits of which become more apparent as network size increases. This application computes and displays a wide array of topological parameters for both directed and undirected networks. These include number of nodes, edges, connected components, network diameter, degree distribution, clustering coefficient, and shortest path lengths, among others (Tables 1 and 2).⁷⁸ Due to the versatility of Cytoscape, this network visualization and interpretation tool has become increasingly useful for complex network analysis in cardiovascular proteomic studies.^{3,37–40,67}

Complex network analysis ontological overrepresentation extends observations available during pathway analysis by providing enrichment assessment for entire network neighborhoods rather than partial subnetworks. Although functional enrichment analysis of merged composite networks is not available within IPA proper, they can be assembled and scrutinized elsewhere, such as within Cytoscape. For example, widespread metabolic remodeling in response to ATP-sensitive K⁺ channel deficiency was apparent by IPA functional ontology classification but was reinforced and expanded by BiNGO analysis of the derived composite network with Cytoscape.³⁹ BiNGO assesses protein or gene networks for ontological representation, generating a hierarchical network of GO terms as nodes to visualize overrepresented functions, with connecting edges comprising nested connections within the GO hierarchy (Table 2). Specifically, BiNGO analysis extended the ATP-sensitive K⁺ channel-dependent metabolome (Table 1) by generating an ontology network from the proteomic network, demonstrating that from all 962 network-associated biological processes, the 55 that were overrepresented were all metabolic processes, associated with protein catabolism, glycolysis, the tricarboxylic acid cycle, fatty acid, and other substrate metabolism.³⁹ Enrichment

Map offers an alternative plug-in for Cytoscape functional interpretation.⁷⁹ This tool creates an ontological network with nodes again comprising overrepresented functions, but with connecting edges showing a weighted intensity based on the extent of proteomic data set overlap between nodes, and an automated layout situating connected nodes closer to one another to facilitate comprehension of closely related enrichment terms (Table 2).⁷⁹ By including all detected protein changes rather than a portion of those most differentially expressed, Enrichment Map was combined with Gene Set Enrichment Analysis to expand biological consequences evident from cardiomyopathy proteomic profiling.⁶⁷

Network topological analysis provides yet another advantage of moving beyond pathway analysis enrichment, facilitating actionable prognostication of emergent structural properties (Figure). Because most nodes in scale-free networks possess only 1 or at most a few connections and a small number are highly connected hubs, nonstochastic networks are functionally robust.⁸⁰ Thus, random node removal would result in a high probability of targeting low connectivity nodes and a small likelihood of radically disrupting network connectivity and functionality by removing a critical network hub.⁸⁰ Therefore, assessing functional integrity in the context of specific hub inhibition could provide understanding of network biological relevance. This approach was used to confirm the potentiating effect of an endodermal secretome network on cardiac differentiation by targeted inhibition of either the primary or secondary hub of the derived network.³⁷ Thus, understanding network mathematical architecture enables interrogation of constituent node positional relevance. Together, these examples demonstrate how network-oriented analysis algorithms extend pathway analysis data interpretation, apply alternative sources of network assembly to comprehend function (ie, GO terms rather than proteins as nodes), make use of all protein IDs rather than a small proportion of the most extensively observed differences for data interpretation (ie, inclusivity), and extract testable hypotheses from emergent structural properties of complex networks.

Future Prospects

As the science of proteomics advances, network-based systems analysis is becoming increasingly integral to high-throughput protein deconvolution, with the ultimate purpose of furthering fundamental knowledge and translational ramifications.^{81,82} Rigor in proteomic experimental design and stringency in protein-protein interaction studies will be paramount for confidence in data interpretation and network protein interaction quality. Network systems medicine approaches offer unprecedented opportunity for integration of proteomic data with other high-throughput sources to better comprehend function in health and disease. Accurate systems interpretation ultimately requires assessment of function or activity, such as biochemical rate constants or metabolic flux analysis, to facilitate prognostic modeling of prioritized pathways and cellular functions, requiring proteomic studies to transition from static state analyses examining change in protein abundance toward dynamical systems analysis. This necessitates incorporation of modalities facilitating state space comprehension to track both

Table 2. Examples of Popular and Useful Network Support Applications

Application	Network Function Addressed
Network Analyzer	Calculates an extensive array of network topological parameters to facilitate identification of nodes of interest and relevant network structural characteristics.
MCODE	Detects and prioritizes highly interconnected regions within a network.
jActive Modules	Identifies expression activated subnetworks, connected network regions that exhibit significant changes in expression.
ReOrient Plugin	Facilitates visual comparison of adjacent networks by locating common nodes in the same relative location.
BiNGO	Determines GO category statistical over- or underrepresentation from a set of genes/proteins within a network or subnetwork of interest, visualized as a GO hierarchy network to map significant themes by biological process, cellular component, or molecular function.
Enrichment Map	Visualizes networks of gene set enrichment analysis results, where nodes represent gene sets and edges their mutual overlap (rather than connecting nodes by nested GO classification, as done in BiNGO), clustering highly redundant gene sets to improve enrichment analysis navigation and interpretation.
NCBI Entrez Gene User Interface/NCBI Client	Imports gene/protein interactions, annotations, and attributes from NCBI Entrez Gene for network derivation.
Agilent Literature Search	Queries multiple text-based search engines to obviate the need for manual search and extraction of gene/protein interactions for network generation.

MCODE indicates Molecular Complex Detection; BiNGO, Biological Network Gene Ontology; NCBI, National Center for Biotechnology Information.

deterministic and stochastic effectors influencing complex cellular outcomes.⁸³ The established capacity of network-based systems templates to account for and incorporate a diversity of input data is well suited for this evolutionary transition at the forefront of emerging fields of translational network medicine.^{84,85}

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References

- Arrell DK, Neverova I, Van Eyk JE. Cardiovascular proteomics: evolution and potential. *Circ Res*. 2001;88:763–773.
- McGregor E, Dunn MJ. Proteomics of the heart: unraveling disease. *Circ Res*. 2006;98:309–321.
- Behfar A, Perez-Terzic C, Faustino RS, Arrell DK, Hodgson DM, Yamada S, Puceat M, Niederländer N, Alekseev AE, Zingman LV, Terzic A. Cardiopoietic programming of embryonic stem cells for tumor-free heart repair. *J Exp Med*. 2007;204:405–420.

4. Folmes CD, Nelson TJ, Martinez-Fernandez A, Arrell DK, Lindor JZ, Dzeja PP, Ikeda Y, Perez-Terzic C, Terzic A. Somatic oxidative bioenergetics transitions into pluripotency-dependent glycolysis to facilitate nuclear reprogramming. *Cell Metab.* 2011;14:264–271.
5. Fu Z, Wang M, Gucek M, Zhang J, Wu J, Jiang L, Monticone RE, Khazan B, Telljohann R, Mattison J, Sheng S, Cole RN, Spinetti G, Pintus G, Liu L, Kolodgie FD, Virmani R, Spurgeon H, Ingram DK, Everett AD, Lakatta EG, Van Eyk JE. Milk fat globule protein epidermal growth factor-8: a pivotal relay element within the angiotensin II and monocyte chemoattractant protein-1 signaling cascade mediating vascular smooth muscle cells invasion. *Circ Res.* 2009;104:1337–1346.
6. Gundry RL, Tchernyshyov I, Sheng S, Tarasova Y, Raginski K, Boheler KR, Van Eyk JE. Expanding the mouse embryonic stem cell proteome: combining three proteomic approaches. *Proteomics.* 2010;10:2728–2732.
7. Chait BT. Chemistry. Mass spectrometry: bottom-up or top-down? *Science.* 2006;314:65–66.
8. Zhang H, Ge Y. Comprehensive analysis of protein modifications by top-down mass spectrometry. *Circ Cardiovasc Genet.* 2011;4:711.
9. Macri J, Rapundalo ST. Application of proteomics to the study of cardiovascular biology. *Trends Cardiovasc Med.* 2001;11:66–75.
10. Arab S, Gramolini AO, Ping P, Kislinger T, Stanley B, van Eyk J, Ouzounian M, MacLennan DH, Emili A, Liu PP. Cardiovascular proteomics: tools to develop novel biomarkers and potential applications. *J Am Coll Cardiol.* 2006;48:1733–1741.
11. Van Eyk JE. Overview: the maturing of proteomics in cardiovascular research. *Circ Res.* 2011;108:490–498.
12. Sanchez JC, Rouge V, Pisteur M, Ravier F, Tonella L, Moosmayer M, Wilkins MR, Hochstrasser DF. Improved and simplified in-gel sample application using reswelling of dry immobilized pH gradients. *Electrophoresis.* 1997;18:324–327.
13. Unlü M, Morgan ME, Minden JS. Difference gel electrophoresis: a single gel method for detecting changes in protein extracts. *Electrophoresis.* 1997;18:2071–2077.
14. Gygi SP, Rist B, Gerber SA, Turecek F, Gelb MH, Aebersold R. Quantitative analysis of complex protein mixtures using isotope-coded affinity tags. *Nat Biotechnol.* 1999;17:994–999.
15. Ong SE, Blagoev B, Kratchmarova I, Kristensen DB, Steen H, Pandey A, Mann M. Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. *Mol Cell Proteomics.* 2002;1:376–386.
16. Håkansson K, Emmett MR, Hendrickson CL, Marshall AG. High-sensitivity electron capture dissociation tandem FTICR mass spectrometry of microelectrosprayed peptides. *Anal Chem.* 2001;73:3605–3610.
17. Hu Q, Noll RJ, Li H, Makarov A, Hardman M, Graham Cooks R. The Orbitrap: a new mass spectrometer. *J Mass Spectrom.* 2005;40:430–443.
18. Ross PL, Huang YN, Marchese JN, Williamson B, Parker K, Hattan S, Khainovski N, Pillai S, Dey S, Daniels S, Purkayastha S, Juhasz P, Martin S, Bartlett-Jones M, He F, Jacobson A, Pappin DJ. Multiplexed protein quantitation in Saccharomyces cerevisiae using amine-reactive isobaric tagging reagents. *Mol Cell Proteomics.* 2004;3:1154–1169.
19. Dayon L, Hainard A, Licker V, Turck N, Kuhn K, Hochstrasser DF, Burkhard PR, Sanchez JC. Relative quantification of proteins in human cerebrospinal fluids by MS/MS using 6-plex isobaric tags. *Anal Chem.* 2008;80:2921–2931.
20. Fang R, Elias DA, Monroe ME, Shen Y, McIntosh M, Wang P, Goddard CD, Callister SJ, Moore RJ, Gorby YA, Adkins JN, Fredrickson JK, Lipton MS, Smith RD. Differential label-free quantitative proteomic analysis of *Shewanella oneidensis* cultured under aerobic and suboxic conditions by accurate mass and time tag approach. *Mol Cell Proteomics.* 2006;5:714–725.
21. Edwards AV, Cordwell SJ, White MY. Phosphoproteomic profiling of the myocyte. *Circ Cardiovasc Genet.* 2011;4:575.
22. Arrell DK, Terzic A. Network systems biology for drug discovery. *Clin Pharmacol Ther.* 2010;88:120–125.
23. Levin Y. The role of statistical power analysis in quantitative proteomics. *Proteomics.* 2011;11:2565–2567.
24. Hood L, Heath JR, Phelps ME, Lin B. Systems biology and new technologies enable predictive and preventative medicine. *Science.* 2004;306:640–643.
25. Weston AD, Hood L. Systems biology, proteomics, and the future of health care: toward predictive, preventative, and personalized medicine. *J Proteome Res.* 2004;3:179–196.
26. Hood L, Perlmutter RM. The impact of systems approaches on biological problems in drug discovery. *Nat Biotechnol.* 2004;22:1215–1217.
27. Goh KI, Cusick ME, Valle D, Childs B, Vidal M, Barabási AL. The human disease network. *Proc Natl Acad Sci USA.* 2007;104:8685–8690.
28. Kohl P, Crampin EJ, Quinn TA, Noble D. Systems biology: an approach. *Clin Pharmacol Ther.* 2010;88:25–33.
29. Arrell DK, Zlatkovic Lindor J, Yamada S, Terzic A. K_{ATP} channel-dependent metaboproteome decoded: systems approaches to heart failure prediction, diagnosis, and therapy. *Cardiovasc Res.* 2011;90:258–266.
30. Faustino RS, Behfar A, Perez-Terzic C, Terzic A. Genomic chart guiding embryonic stem cell cardiopoiesis. *Genome Biol.* 2008;9:R6.
31. Faustino RS, Chiriac A, Niederländer NJ, Nelson TJ, Behfar A, Mishra PK, Macura S, Michalak M, Terzic A, Perez-Terzic C. Decoded calreticulin-deficient embryonic stem cell transcriptome resolves latent cardiophenotype. *Stem Cells.* 2010;28:1281–1291.
32. Chiriac A, Nelson TJ, Faustino RS, Behfar A, Terzic A. Cardiogenic induction of pluripotent stem cells streamlined through a conserved SDF-1/VEGF/BMP2 integrated network. *PLoS ONE.* 2010;5:e9943.
33. Dewey FE, Perez MV, Wheeler MT, Watt C, Spin J, Langfelder P, Horvath S, Hannenhalli S, Cappola TP, Ashley EA. Gene coexpression network topology of cardiac development, hypertrophy, and failure. *Circ Cardiovasc Genet.* 2011;4:26–35.
34. Civelek M, Manduchi E, Riley RJ, Stoeckert CJ Jr, Davies PF. Coronary artery endothelial transcriptome in vivo: identification of endoplasmic reticulum stress and enhanced reactive oxygen species by gene connectivity network analysis. *Circ Cardiovasc Genet.* 2011;4:243–252.
35. Parikh VN, Jin RC, Rabello S, Gulbahe N, White K, Hale A, Cottrill KA, Shaik RS, Waxman AB, Zhang YY, Maron BA, Hartner JC, Fujiwara Y, Orkin SH, Haley KJ, Barabási AL, Loscalzo J, Chan SY. MicroRNA-21 integrates pathogenic signaling to control pulmonary hypertension: results of a network bioinformatics approach. *Circulation.* 2012;125:1520–1532.
36. Zampetaki A, Willeit P, Drozdov I, Kiechl S, Mayr M. Profiling of circulating microRNAs: from single biomarkers to re-wired networks. *Cardiovasc Res.* 2012;93:555–562.
37. Arrell DK, Niederländer NJ, Faustino RS, Behfar A, Terzic A. Cardioinductive network guiding stem cell differentiation revealed by proteomic cartography of tumor necrosis factor alpha-primed endodermal secretome. *Stem Cells.* 2008;26:387–400.
38. Zlatkovic J, Arrell DK, Kane GC, Miki T, Seino S, Terzic A. Proteomic profiling of K_{ATP} channel-deficient hypertensive heart maps risk for maladaptive cardiomyopathic outcome. *Proteomics.* 2009;9:1314–1325.
39. Arrell DK, Zlatkovic J, Kane GC, Yamada S, Terzic A. ATP-sensitive K^+ channel knockout induces cardiac proteome remodeling predictive of heart disease susceptibility. *J Proteome Res.* 2009;8:4823–4834.
40. Zlatkovic-Lindor J, Arrell DK, Yamada S, Nelson TJ, Terzic A. ATP-sensitive K^+ channel-deficient dilated cardiomyopathy proteome remodeled by embryonic stem cell therapy. *Stem Cells.* 2010;28:1355–1367.
41. Barallobre-Barreiro J, Didangelos A, Schoendube FA, Drozdov I, Yin X, Fernández-Caggiano M, Willeit P, Puntmann VO, Aldama-López G, Shah AM, Doménech N, Mayr M. Proteomics analysis of cardiac extracellular matrix remodeling in a porcine model of ischemia/reperfusion injury. *Circulation.* 2012;125:789–802.
42. Stegmann C, Drozdov I, Shalhoub J, Humphries J, Ladroue C, Didangelos A, Baumert M, Allen M, Davies AH, Monaco C, Smith A, Xu Q, Mayr M. Comparative lipidomics profiling of human atherosclerotic plaques. *Circ Cardiovasc Genet.* 2011;4:232–242.
43. Lusis AJ, Weiss JN. Cardiovascular networks: systems-based approaches to cardiovascular disease. *Circulation.* 2010;121:157–170.
44. Sperling SR. Systems biology approaches to heart development and congenital heart disease. *Cardiovasc Res.* 2011;91:269–278.
45. MacLellan WR, Wang Y, Lusis AJ. Systems-based approaches to cardiovascular disease. *Nat Rev Cardiol.* 2012;9:172–184.
46. Barabási AL, Oltvai ZN. Network biology: understanding the cell's functional organization. *Nat Rev Genet.* 2004;5:101–113.
47. Newman MEJ. The structure and function of complex networks. *SIAM Rev.* 2003;45:167–256.
48. Pawson T, Linding R. Network medicine. *FEBS Lett.* 2008;582:1266–1270.
49. Arrell DK, Elliott ST, Kane LA, Guo Y, Ko YH, Pedersen PL, Robinson J, Murata M, Murphy AM, Marbán E, Van Eyk JE. Proteomic analysis of pharmacological preconditioning: novel protein targets converge to mitochondrial metabolism pathways. *Circ Res.* 2006;99:706–714.
50. Nesvizhskii AI, Aebersold R. Interpretation of shotgun proteomic data: the protein inference problem. *Mol Cell Proteomics.* 2005;4:1419–1440.
51. Bader GD, Cary MP, Sander C. Pathguide: a pathway resource list. *Nucleic Acids Res.* 2006;34(Database issue):D504–D506.
52. Mayr M, Zampetaki A, Sidibe A, Mayr U, Yin X, De Souza AI, Chung YL, Madhu B, Quax PH, Hu Y, Griffiths JR, Xu Q. Proteomic and

- metabolomic analysis of smooth muscle cells derived from the arterial media and adventitial progenitors of apolipoprotein E-deficient mice. *Circ Res*. 2008;102:1046–1056.
53. Grant JE, Bradshaw AD, Schwacke JH, Baicu CF, Zile MR, Schey KL. Quantification of protein expression changes in the aging left ventricle of Rattus norvegicus. *J Proteome Res*. 2009;8:4252–4263.
 54. Li Z, Liu N, Zhang LS, Gong K, Cai Y, Gao W, Liu Z, Liu S, Han Q, Zhang Y. Proteomic profiling reveals comprehensive insights into adrenergic receptor-mediated hypertrophy in neonatal rat cardiomyocytes. *Proteomics Clin Appl*. 2009;3:1407–1421.
 55. Mayr M, Liem D, Zhang J, Li X, Avliyakov NK, Yang JI, Young G, Vondriska TM, Ladroue C, Madhu B, Griffiths JR, Gomes A, Xu Q, Ping P. Proteomic and metabolomic analysis of cardioprotection: Interplay between protein kinase C epsilon and delta in regulating glucose metabolism of murine hearts. *J Mol Cell Cardiol*. 2009;46:268–277.
 56. Agnetti G, Kaludercic N, Kane LA, Elliott ST, Guo Y, Chakir K, Samantapudi D, Paolucci N, Tomaselli GF, Kass DA, Van Eyk JE. Modulation of mitochondrial proteome and improved mitochondrial function by biventricular pacing of dyssynchronous failing hearts. *Circ Cardiovasc Genet*. 2010;3:78–87.
 57. Bugger H, Schwarzer M, Chen D, Schrepper A, Amorim PA, Schoepe M, Nguyen TD, Mohr FW, Khalimonchuk O, Weimer BC, Doenst T. Proteomic remodelling of mitochondrial oxidative pathways in pressure overload-induced heart failure. *Cardiovasc Res*. 2010;85:376–384.
 58. Farina A, Chambery A, Esposito S, Agozzino L, Cotrufo M, Della Corte A, Parente A. Proteomic profiling of medial degeneration in human ascending aorta. *Clin Biochem*. 2010;43:387–396.
 59. Parguina AF, Fernández Parguina A, Grigorian-Shamajian L, Agra RM, Teijeira-Fernández E, Rosa I, Alonso J, Viñuela-Roldán JE, Seoane A, González-Juanatey JR, García A. Proteins involved in platelet signaling are differentially regulated in acute coronary syndrome: a proteomic study. *PLoS ONE*. 2010;5:e13404.
 60. Angel PM, Nusinow D, Brown CB, Violette K, Barnett JV, Zhang B, Baldwin HS, Caprioli RM. Networked-based characterization of extracellular matrix proteins from adult mouse pulmonary and aortic valves. *J Proteome Res*. 2011;10:812–823.
 61. Fogle RL, Hollenbeak CS, Stanley BA, Vary TC, Kimball SR, Lynch CJ. Functional proteomic analysis reveals sex-dependent differences in structural and energy-producing myocardial proteins in rat model of alcoholic cardiomyopathy. *Physiol Genomics*. 2011;43:346–356.
 62. Hammer E, Goritzka M, Ameling S, Darm K, Steil L, Klingel K, Trimpert C, Herda LR, Dörr M, Kroemer HK, Kandolf R, Staudt A, Felix SB, Völker U. Characterization of the human myocardial proteome in inflammatory dilated cardiomyopathy by label-free quantitative shotgun proteomics of heart biopsies. *J Proteome Res*. 2011;10:2161–2171.
 63. Hanzu FA, Palomo M, Kalko SG, Parrizas M, Garaulet M, Escolar G, Gomis R, Díaz-Ricart M. Translational evidence of endothelial damage in obese individuals: inflammatory and prothrombotic responses. *J Thromb Haemost*. 2011;9:1236–1245.
 64. Qi YX, Jiang J, Jiang XH, Wang XD, Ji SY, Han Y, Long DK, Shen BR, Yan ZQ, Chien S, Jiang ZL. PDGF-BB and TGF- β 1 on cross-talk between endothelial and smooth muscle cells in vascular remodeling induced by low shear stress. *Proc Natl Acad Sci USA*. 2011;108:1908–1913.
 65. Li W, Rong R, Zhao S, Zhu X, Zhang K, Xiong X, Yu X, Cui Q, Li S, Chen L, Cai J, Du J. Proteomic analysis of metabolic, cytoskeletal and stress response proteins in human heart failure. *J Cell Mol Med*. 2012;16:59–71.
 66. de la Cuesta F, Barderas MG, Calvo E, Zubiri I, Maroto AS, Darde VM, Martín-Rojas T, Gil-Dones F, Posada-Ayala M, Tejerina T, Lopez JA, Vivanco F, Alvarez-Llamas G. Secretome analysis of atherosclerotic and non-atherosclerotic arteries reveals dynamic extracellular remodeling during pathogenesis. *J Proteomics*. 2012;75:2960–2971.
 67. Isserlin R, Merico D, Alikhani-Koupaei R, Gramolini A, Bader GD, Emili A. Pathway analysis of dilated cardiomyopathy using global proteomic profiling and enrichment maps. *Proteomics*. 2010;10:1316–1327.
 68. Cammarato A, Ahrens CH, Alayari NN, Qeli E, Rucker J, Reedy MC, Zmasek CM, Gucek M, Cole RN, Van Eyk JE, Bodmer R, O'Rourke B, Bernstein SI, Foster DB. A mighty small heart: the cardiac proteome of adult *Drosophila melanogaster*. *PLoS ONE*. 2011;6:e18497.
 69. Sun Z, Biela LM, Hamilton KL, Reardon KF. Concentration-dependent effects of the soy phytoestrogen genistein on the proteome of cultured cardiomyocytes. *J Proteomics*. 2012;75:3592–3604.
 70. Henderson-Maclennan NK, Papp JC, Talbot CC Jr, McCabe ER, Presson AP. Pathway analysis software: annotation errors and solutions. *Mol Genet Metab*. 2010;101:134–140.
 71. Merico D, Gfeller D, Bader GD. How to visually interpret biological data using networks. *Nat Biotechnol*. 2009;27:921–924.
 72. Barabasi AL, Albert R. Emergence of scaling in random networks. *Science*. 1999;286:509–512.
 73. Khalil I, Brewer MA, Neyarapally T, Runowicz CD. The potential of biological network models in understanding the etiopathogenesis of ovarian cancer. *Gynecol Oncol*. 2010;116:282–285.
 74. MacCannell KA, Shohet RV. Toward a holistic view of transcriptional regulation. *Circ Cardiovasc Genet*. 2011;4:2–3.
 75. Pinaud B, Kuntz P. GVSr: an on-line guide for choosing a graph visualization software. pp. 400–401. In: Brandes U, Cornelsen S, eds. *Graph Drawing: 18th Int. Symp., GD 2010, Lecture Notes in Computer Science*. Berlin, Germany: Springer; 2011.
 76. Cline MS, Smoot M, Cerami E, Kuchinsky A, Landys N, Workman C, Christmas R, Avila-Campilo I, Creech M, Gross B, Hanspers K, Isserlin R, Kelley R, Killcoyne S, Lotia S, Maere S, Morris J, Ono K, Pavlovic V, Pico AR, Vailaya A, Wang PL, Adler A, Conklin BR, Hood L, Kuiper M, Sander C, Schmulevich I, Schwikowski B, Warner GJ, Ideker T, Bader GD. Integration of biological networks and gene expression data using Cytoscape. *Nat Protoc*. 2007;2:2366–2382.
 77. Maere S, Heymans K, Kuiper M. BiNGO: a Cytoscape plugin to assess overrepresentation of gene ontology categories in biological networks. *Bioinformatics*. 2005;21:3448–3449.
 78. Assenov Y, Ramírez F, Schelhorn SE, Lengauer T, Albrecht M. Computing topological parameters of biological networks. *Bioinformatics*. 2008;24:282–284.
 79. Merico D, Isserlin R, Stueker O, Emili A, Bader GD. Enrichment map: a network-based method for gene-set enrichment visualization and interpretation. *PLoS ONE*. 2010;5:e13984.
 80. Albert R, Jeong H, Barabasi AL. Error and attack tolerance of complex networks. *Nature*. 2000;406:378–382.
 81. Loscalzo J, Barabási A-L. Systems biology and the future of medicine. *Wiley Interdiscip Rev Syst Biol Med*. 2011;3:619–627.
 82. West GB. The importance of quantitative systemic thinking in medicine. *Lancet*. 2012;397:1551–1559.
 83. Huang S. Cell lineage determination in state space: A systems view brings flexibility to dogmatic canonical rules. *PLoS Biol*. 2010;8:e1000380.
 84. Waldman SA, Terzic A. Molecular therapeutics from knowledge to delivery. *Clin Pharmacol Ther*. 2010;87:619–623.
 85. Barabási A-L, Gulbahce N, Loscalzo J. Network medicine: a network-based approach to human disease. *Nat Rev Genet*. 2011;12:56–68.

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