Emerging landscape of molecular interaction networks: Opportunities, challenges and prospects

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Network biology finds application in interpreting molecular interaction networks and providing insightful inferences using graph theoretical analysis of biological systems. The integration of computational bio-modelling approaches with different hybrid network-based techniques provides additional information about the behaviour of complex systems. With increasing advances in high-throughput technologies in biological research, attempts have been made to incorporate this information into network structures, which has led to a continuous update of network biology approaches over time. The newly minted centrality measures accommodate the details of omics data and regulatory network structure information. The unification of graph network properties with classical mathematical and computational modelling approaches and technologically advanced approaches like machine-learning- and artificial intelligence-based algorithms leverages the potential application of these techniques. These computational advances prove beneficial and serve various applications such as essential gene prediction, identification of drug–disease interaction and gene prioritization. Hence, in this review, we have provided a comprehensive overview of the emerging landscape of molecular interaction networks using graph theoretical approaches. With the aim to provide information on the wide range of applications of network biology approaches in understanding the interaction and regulation of genes, proteins, enzymes and metabolites at different molecular levels, we have reviewed the methods that utilize network topological properties, emerging hybrid network-based approaches and applications that integrate machine learning techniques to analyse molecular interaction networks. Further, we have discussed the applications of these approaches in biomedical research with a note on future prospects.

Keywords. Centrality; disease mechanisms; hybrid network-based models; machine learning; molecular interaction networks; network topology; systems biology

1. Introduction

Molecular entities of biological systems interact with each other at various levels and cooperatively function together to exhibit specific cellular phenotypes. Essentially, every biological entity interacts with other biological entities, forming a network of interactions that maintains the proper functioning of biological systems. The network properties of these biological interactions provide us the opportunity to model biological systems as different types of networks such as protein–protein interactions (Kumar et al. 2020a, b; Tomkins and Manzoni 2021), gene regulatory (Grimes et al. 2019; Sinha et al. 2020a, b) and metabolic networks (Bidkhori et al. 2018; Toubiana et al. 2019). The availability of a vast expanse of molecular information with the increase in omics analyses has created avenues...
to better understand these molecular interactions (Hawe et al. 2019). In this context, systems biology aims to understand biological entities at the system level by studying them not only as discrete components but also as interacting systems with emergent properties. Network biology allows the representation and analysis of biological systems using tools derived from graph theory (Barabási and Oltvai 2004).

Different types of information of biological systems can be represented as networks of mutually interacting entities, where each entity has an effect on the overall function of these networks. The definition of the nodes and edges used in a network representation depends on the type of data used to build the network. In a molecular interaction network, the nodes can be represented by genes, proteins, enzymes, metabolites, transcription factors, etc., and the edges can be represented as the interaction between these nodes. Different types of data produce different network characteristics in terms of structure, connectivity, and complexity, where edges and nodes potentially mediate multiple layers of information (Grennan et al. 2014). The integration of constantly evolving complex high-throughput data, such as whole genome sequencing data, single-cell ribonucleic acid (RNA) sequencing, and clustered regularly interspaced short palindromic repeats (CRISPR-cas9) technology, and their ease of availability have further led to improvements and advances in newer techniques and approaches as well as upgradation of the traditional network biology approaches to carry out systems-level studies (Charitou et al. 2016; Koh et al. 2019; Ma and Zhang 2019).

Due to incomplete information, variability in data resources, and heterogeneity, multiple challenges have been consistently observed while carrying out systems-level studies. Prospects created by molecular interaction networks are instrumental in addressing these challenges (Imam et al. 2015). One of the prevalent challenges that limit the applicability of network models is the difficulty in identifying appropriate centrality measures due to variability in the type of molecular network. Universal acceptance of the centrality–lethality hypothesis remains inconsistent owing to the changing network structure and topology of the molecular interaction networks. The centrality measure that defines the central or the most influential nodes in the network changes with changing network structure. The challenge is to identify proper centrality measures that appropriately identify these central nodes (Oldham et al. 2019). Furthermore, uncertainty in model structure and parameters that affect the network inferences is an additional challenge in the case of gene regulatory networks (GRNs) (Saint-Antoine et al. 2020). One promising avenue is created by hybrid network-based modelling approaches in the analysis of these molecular interaction networks. These approaches are an improvement over the systems modelling methods as they integrate and use network topological properties and also implement advanced computational techniques, such as machine learning-based algorithms, to tackle the aforementioned challenges (Chowdhury et al. 2013; Chowdhury and Sarkar 2019; Kang et al. 2020; Nandi et al. 2020). These, in turn, provide an opportunity to scale up the dynamic genome-scale models for incorporation with network biology and are currently being explored (Stéphanou and Volpert 2016; Bardini et al. 2017). The emerging landscape of these molecular interaction networks has enabled better understanding of molecular systems and their prospects (Charitou et al. 2016). A comprehensive review that discusses this emerging landscape of molecular interaction networks in the light of the challenges faced and the new approaches and techniques developed in this area is missing.

Hence, in this review, we aim to thoroughly assess available network biology approaches and the progress made in them to decipher the understanding of the molecular interaction networks over the last two decades, with the advancement in biological and computational research. We begin with a brief introduction of the different types of molecular interaction networks, and their structural and topological properties, so as to provide a basic understanding of the molecular interaction networks. In section 3, we discuss several network topology-based methods and their progress in effectively drawing various types of inferences from different types of molecular interaction networks. In each of the following subsections, we briefly highlight how these methodological advances have helped to overcome different limitations and also introduce some recently developed methods that can be useful in future research. Next, we introduce the hybrid network-based approaches that combine traditional systems biology methods with graph theoretical techniques and briefly discuss their applications. We also explain how advanced statistical methods and machine learning (ML)-based computational frameworks help to overcome the limitations of these hybrid approaches. We briefly state a few applications where these recently developed methodologies successfully contribute to advance the molecular network analyses. In section 6, we discuss a few disease-specific studies where these network-based approaches have successfully contributed. We aim to highlight the increasing forte of
network topology-based techniques to analyse molecular-interaction networks with methodological advancements. Finally, we conclude this review by providing suggestions to the readers for possible future prospects in areas that hold scope for advanced molecular network analyses to improve biological and biomedical research. This review will be beneficial to systems biologists who can use emerging graph theoretical approaches, hybrid network-based models, and ML-based applications to study molecular interactions. Network biologists can also gain a holistic view of applications of these emerging approaches, such as deciphering drug–disease interactions, analysing perturbation patterns, characterizing regulatory genes, predicting gene essentiality, etc.

2. Types of molecular interaction networks

Various types of molecular interaction networks emerge from the combination of different interactions among molecular entities that determine the systems-scale behaviour of the cell (Barabási and Oltvai 2004; Han 2008). Some of the most common molecular interaction networks are: (i) protein–protein interaction networks, (ii) metabolic networks, (iii) gene regulatory networks, and (iv) signal transduction networks. In the graph $G (V, E)$ representation of molecular interaction networks, nodes $v \in V$ represents biological entities, i.e., genes, proteins, transcription factors, or miRNAs, and edges $e \in E$ represents interactions among these biological entities. Due to their importance in biological research, these molecular interaction networks are continuously revisited and updated with time. We provide a brief introduction of the different types of molecular interaction networks in the following subsections.

2.1 Protein–protein interaction networks

These are mathematical representations of the molecular contacts between the proteins in a cell. These contacts are specific, occur between defined binding regions in the proteins, and have a particular biological meaning (i.e., they serve a specific function) (Schreiber 2021). Protein–protein interactions (PPIs) are essential to almost every process in a cell and play an important role in drug development (Mabonga and Kappo 2019). The interactome is the totality of PPIs that occur in a cell, an organism, or a specific biological context (Safari-Alighiarloo et al. 2014).

Knowledge of PPIs can be extended to a wide range of applications, such as understanding complex disease disorders, assigning putative roles to uncharacterized proteins (Lv et al. 2015; da Costa et al. 2018), and adding fine-grained details about the steps within a signalling pathway (Navlakha et al. 2012; Mei and Zhu 2015). Identifying active signalling pathways (Kabir et al. 2018), and characterizing the relationships between proteins that form multi-molecular complexes, such as the proteasome (Di Paola et al. 2015), are some additional areas where PPI networks find application. Some of the widely popular protein–protein interaction network (PPIN) resources actively used for mining PPI information include the Biological General Repository for Interaction Datasets (BioGRID) (Oughtred et al. 2019) and Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) (Szklarczyk et al. 2021) Database.

2.2 Metabolic networks

These consist of chemical reactions that involve the catalytic conversion of small biomolecules known as metabolites aided by enzymatic reactions. Construction of the network depends on several factors, most importantly the type of analysis to be performed on the network. The most common graph theoretical representation of metabolic networks is considering the metabolites as nodes and the reactions catalysing the conversion of one metabolite to another as edges. Another way is to represent the metabolic network as a reaction adjacency graph, where the nodes are formed by reactions and the connection/edges between the reactions is established if the product of one reaction is the substrate of the second reaction (Kim et al. 2019). Metabolite concentration and reaction fluxes are measurable quantities that have been used to infer the properties of the metabolic graph networks at the structural, kinetic, and regulatory levels (Beguerisse-Díaz et al. 2018).

2.3 Gene regulatory networks

A gene regulatory network (GRN) represents the complex mechanisms that regulate the expression of genes. Regulatory mechanisms occur at different stages of protein production from DNA, such as during the transcription, translation, and splicing phases. Proteins act as both the product and the controller of gene expression in these networks (Junker and Schreiber
In GRNs, each node represents a gene, and a directed link between two genes implies that one gene directly regulates the expression of the other without intervention by any other genes. GRNs are very important in understanding the mechanistic regulation of gene expression and the sequence of events that result in a phenotype. The graphical representation of these networks provide a visualization and intuitive explanation of these complex and interconnected mechanisms.

2.4 Signal transduction networks

These are essentially protein interaction networks, but the interaction between the proteins and flow of information is directional. Like the PPI network, the nodes in the signal transduction network are represented by proteins that usually belong to the phosphatase or kinase or similar protein family, e.g., protein tyrosine phosphatases (PTPs), protein serine phosphatases (PSPs), and mitogen-activated protein kinases (MAPKs) (Nguyen et al. 2013). The edges are determined by the interaction between two proteins where the first protein interacts to activate the second one, and hence the directionality. Signal transduction networks form the core of information flow for most of the signalling networks. These form the bridge between the receptor-mediated activation of protein complexes whose information is passed down for the activation of transcription factors via these signal transduction networks (Soyer et al. 2006).

3. Network topology-based approaches in the study of molecular interaction networks

Representation of molecular interaction data in the form of an interconnected network of biomolecules forms the topology of the information in these networks. This topology is created from the representative graphs, where nodes represent singular entities or the individual biomolecules of the process under study, and the edges represent the relation between them. Elucidation of the topology of these networks is effectively applied to gain a systems-level understanding of the interactive exchange between the entities of the biological system under study (Janjić and Pržulj 2012; Koutrouli et al. 2020; Masoomy et al. 2021). For example, a recent study used topological analysis on a systems-level curcumin-rewired PPI based on centralities like betweenness and degree, to identify key regulatory proteins that govern the molecular mechanisms, thereby aiding in understanding the anti-cancerous and anti-inflammatory properties of curcumin (Dhasmana et al. 2020).

Network topology-based approaches have also helped understand host–pathogen interplay during infection processes (Mulder et al. 2014; Saha et al. 2018). Recently, Panditrao et al. (2021) used betweenness centrality combined with shortest-path analysis to analyse phenotype-specific protein subnetworks of *Leishmania donovani* secretory proteins to delineate infection mechanisms and identify regulatory host proteins that could potentially act as immunomodulatory candidates. Also, the study of molecular networks of SARS-CoV2 during the COVID-10 pandemic has been instrumental in deciphering its viral pathogenesis through virus-host PPI networks (Díaz 2020; Gordon et al. 2020; Messina et al. 2020). The topological properties of virus–host protein interaction networks have aided in understanding the mechanisms of its pathogenesis. Centrality measures like PageRank, betweenness, eigenvector centrality along with weighted k-shell decomposition analysis have helped identify the most influential nodes of the viral proteins that interfere with the host nucleocytoplasmic trafficking, immune system, and cell cycle which facilitates pathogenesis (Kumar et al. 2020a, b). It has also been possible to identify candidate target viral genes for repurposing drugs for treating the COVID-19 infection through the analysis of the fused viral interaction network (VIN) and the drug–target interaction network (DTI) (Zambrana et al. 2021). In this analysis, the network structure topological information was utilized for data fusion and the graphlet degree vector (GDV) was used for capturing the local rewiring patterns for functional assessment of gene–drug interactions. Graph theoretical approaches reveal hidden properties and features in molecular interaction networks (Pavlopoulos et al. 2011). Thus, such topological network analyses enable several applications such as discovery of drug targets, evaluation of disease genes, and prediction of essential nodes. In this section, we discuss several emerging methodological advances that utilize topology-based approaches to draw various insightful inferences from molecular interaction networks.

3.1 Centrality measures

In the past two decades, the use of centrality measures in molecular interaction networks has gained momentum.
Centrality measures depend on the topology-centric parameter of the nodes in the network which would influence the structural properties of the graph. They are instrumental in deducing meaningful interpretations of molecular interaction networks that include PPINs (Ash- tiani et al. 2018), GRNs (Liseron-Monfils and Ware 2015), signal transduction networks (Alvarez-Ponce et al. 2017), and metabolic networks (Resendis-Antonio et al. 2012). The commonly used centrality measures include degree, betweenness centrality, closeness centrality, eccentricity, and eigenvector centrality, often referred to as the classical centrality measures (figure 1A). The basic definition and mathematical formulation of the classical centrality measures are provided in table 1.

The past two decades saw a surge in various newly minted concepts of calculating centralities (Jalili et al. 2015, 2016). Previous studies show that integration of omics data with topological features can develop improved centrality measures (Li et al. 2012, 2010). These centralities are developed through a combination of the classical centralities, utilizing topological features based on connectivity as well as integrating known biological information from experimental outputs. We provide an overview of these newly developed centrality measures in table 2. Figure 1B provides a pictorial representation of how these centralities have evolved from the classical centralities by including additional molecular information. Based on the methods used to derive these new centralities, they can be broadly classified. PageRank, marginal essentiality, subgraph centrality, motif-based centrality, bridging centrality, pairwise dis- connectivity index, flux centrality, leverage centrality, perturbation centrality and SSC (source/sink centrality) use solely topological features of the nodes in the network that are based on their connectivity patterns. Annotation transcriptional centrality, neighbourhood functional centrality, game theoretic centrality, DiffSLC and SCNRank (spectral clustering for network-based ranking) additionally integrate biological information in the form of omics data. Perturbation centrality and game centrality are the centrality measures that can be applied to dynamic networks.

Depending on the problem of interest, a certain type of centrality measure may be more important than another. For example, the highest betweenness centrality node in the network has a more pronounced influence on control than the highest closeness centrality node in the network if one wants to control a chaotic metapopulation to the steady states (Meena et al. 2017), to protect the resilience of the dynamical networks (Rungta et al. 2018), irrespective of the dynamics on the nodes (Meena et al. 2020a, b).

Limitations in using these centralities exist in terms of the biological inferences in molecular interaction networks. For example, the universality of the centrality–lethality hypothesis becomes questionable if the measures to identify central nodes in the network are not chosen wisely. In 2005, Hahn and Kern showed that hub proteins in a PPIN were highly essential (Hahn and Kern 2005); however, shortly thereafter Mahadevan and Palsson showed that essentiality was not correlated to the connectivity of the node in GRNs (Mahadevan and Palsson 2005). This idea was further supported by studies which showed that in PPINs, low connectivity could also be considered essential (Tew et al. 2007). Hence, the centrality–lethality paradox still exists.

These above-mentioned new centrality measures are derived and continuously added to address the limitations previously faced by the classical centrality measures and to improve our ability to extract more useful information from complex molecular networks (Roy 2012). For example, leverage centrality is observed to be highly useful in analysing hierarchical networks, such as brain networks, which harbour assortative behaviour, as it helps to identify nodes which are critical for the function of the global network as well as local communities (modules) in that network (Joyce et al. 2010). This helps capture the assortative or disassortative behaviour of the network since it captures the nodes which control the quality of information received by its neighbours. Classical centralities like degree, betweenness, and eigenvector centrality fail to analyse this assortative behaviour. This is possible as leverage centrality relies on the principle that a node in the network is central if its immediate neighbours rely on that node for information. Game centrality is another example that effectively contributes to the identification of functionally and dynamically important network nodes, which has previously been a difficult task (Simko and Csermely 2013). It significantly outperforms the classical centrality measures in predicting genetic buffering of evolutionary changes, i.e., the contribution of a protein to the overall robustness of the cell. This was possible due to the ability of game centrality to precisely discriminate hubs with different dynamic parameters. Degree centrality, although able to capture essential proteins, is highly dependent on the degree-sorted nodes list and thus misses out on a few other known essential proteins that have fewer interactions. DiffSLC promotes potentially essential proteins with low node degree by elevating eigenvector centrality values with additional weights from co-expression data (Mistry et al. 2017). The source/sink centrality is a useful measure if one wishes to clearly distinguish and identify a priori important genes from pathways, as it accounts for the importance of
pathway elements with respect to the upstream and downstream positions (Naderi Yeganeh et al. 2020).

Thus, advancements in these centrality measures continue to prove to be valuable tools that can tackle the complexities and limitations in the emerging molecular networks and help to gain meaningful inferences.

3.2 Methods for integrating large-scale genomic screen data in PPINs

Large-scale genomic screens involve functional genomic approaches employing diverse experimental techniques such as transcriptome profiling, loss-of-function screening, RNA interference screens, and CRISPR...
Definition and mathematical representation of classical network centrality measures

| Centrality        | Definition                                                                 | Mathematical representation                                                                 | References |
|-------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|------------|
| Betweenness       | Ratio of the number of shortest paths passing through a node \( v \) out of all the shortest paths existing between all node pairs in a network | \( BC(v) = \frac{\sum_{i \neq j \neq v} \sigma_{ij}}{\sigma_{ij}} \) \( \sigma_{ij} \) = number of shortest paths between node \( i \) and \( j \) \( \sigma_{ij}(v) \) = number of shortest paths in \( \sigma_{ij} \) that pass through the node \( v \) | Freeman (1977) |
| Closeness         | A measure of the average farness of a node \( v \) from all other nodes belonging to \( V \)                                 | \( CC(v) = \frac{1}{\sum_{u \in V(v)} d(v,u)} \) \( d(v,u) \) = distance between the node \( v \) from \( u \) and \( i \in V \) | Freeman (1978) |
| Degree            | The most classical centrality measure that represents the number of connected neighbours of a node \( v \)                      | \( DC(v) = N(v) \) \( N(v) \) = number of nodes connected to the node \( v \in V \) | Proctor and Loomis (1951) |
| Eccentricity      | A measure of proximity of node \( v \) to all other nodes belonging to \( V \) in \( G \). A node \( v \) with high eccentricity means all other nodes are in proximity to node \( v \) | \( Ecc(v) = \frac{1}{\max_{i \in V(v)} dist(v,i)} \) \( dist(v,i) \) = distance between the node \( v \) and node \( i \), where \( v, i \in V \) | Hage and Harary (1965) |
| Eigenvector       | A measure of the influence of a node. EC of a node \( v \) is defined as the weighted sum of the centralities of all the nodes that are connected to \( v \) by an edge in an adjacency matrix \( A_{v,i} \) | \( EC(v) = \frac{1}{\lambda} \sum_{i \in V} A_{v,i} \cdot EC(i) \) \( A_{v,i} \) = (if node \( v \) and \( i \) are connected, then \( a_{v,i} = 1 \), otherwise \( 0 \)) \( EC(v) \) = eigenvector value of node \( v \) associated with the eigenvalue \( \lambda \) of \( A \) | Ruhnau (2000) |

Libraries to investigate gene functions. Molecular interaction networks potentiate the identification of functionally related biological components from large-scale genomic screen datasets. Genomic information is incorporated into network topology using the principle of guilt-by-association to develop network-based scoring methods that allow detection of false-positive and false-negative screens (Wang et al. 2009). These genome-scale interaction networks that harbour co-localized genes or proteins having similar topological roles are more likely to be functionally correlated. Thus, the ‘guilt-by-association’ process allows inferring properties of unknown proteins or genes by transferring knowledge from these similar co-localized genes or proteins. This principle can be effectively used for identification of drug–target interaction (Li et al. 2016), integrating gene regulatory pathways (Grimes et al. 2019). Furthermore, incorporation of network neighbour information improves the quality of functional genomic screens (Jiang et al. 2015), and the selection of an optimally functionally enriched network allows easier identification and interpretation of diagnostic or predictive gene signatures for diseases (Kairov et al. 2012). These strategies enable better utilization of genomic screening data in conjunction with more topological properties to associate the information with phenotypes. For example, recently, Rubanova and co-workers developed a new method called MasterPATH, which utilizes the results from functional screening data such as loss-of-function data and uses shortest path-based subnetwork extraction to elucidate members of molecular pathways that influence the studied phenotypes (Rubanova et al. 2020).

In an attempt to address the challenges of accurately integrating cell-line and CRISPR-Cas9 data within the network structure, a method called SCNrank has been developed. This method prioritizes potential drug targets in tumour cell-line screens by combining expression profiles from tumour tissues, normal tissues and cell-lines, PPI network and CRISPR-cas9 data to construct tissue-specific networks that are aligned based on graph structure similarity (Liu et al. 2020). The Network-augmented Gene Set Enrichment Analysis (NGSEA) method has been developed to utilize the information from Gene Set Enrichment Analysis (GSEA) of functional networks by calculating the enrichment score for gene sets using expression difference not only for individual genes but also from
Table 2. Description of the new developed centrality measures

| New centrality measures          | Description                                                                 | Additional features                                                                 | Year of development | References                  |
|----------------------------------|-----------------------------------------------------------------------------|-------------------------------------------------------------------------------------|---------------------|-----------------------------|
| Page Rank Centrality             | A variant of eigenvector centrality which scores a vertex as a fraction of time spent visiting that vertex measured over time in a random walk over all vertices in the network with an additional probability for jumping to any vertex. | Uses directionality in the network                                                   | 1998                | Brin and Page (1998)        |
| Marginal Essentiality            | A quantitative measure that calculates the importance of no-essential gene to a cell using protein networks by using the topological information in the form of local interconnectivity measures. | Characterized by two classical centralities, namely, degree and clustering co-efficient | 2004                | Yu et al. (2019)            |
| Subgraph Centrality              | Quantifies the participation of a vertex in all subgraphs of a network. Subgraph centrality (SC) of a node is a weighted sum of the numbers of all closed walks of different lengths in the network starting and ending at the node. | Built over eigenvector centrality and uses edge weights                             | 2005                | Estrada and Rodríguez-Velázquez (2005) |
| Neighbourhood Function Centrality| Quantifies the extent to which a protein is surrounded by functionally consistent neighbouring proteins in a PPI network and thus help in mining lethal proteins. | Uses protein interactome, neighbourhood information and functional annotation         | 2007                | Tew et al. (2007)           |
| Motif-based Centrality           | Calculates the occurrence of motifs (subnetwork patterns in local interconnections) by calculating motif match sets to analyse gene regulatory and protein interaction patterns. | Uses directionality and edge weights                                                 | 2007                | Koschützki et al. (2007)    |
| Bridging Centrality              | Identification of a node or edge that is located between and connects modules (modular subregions) in a network. Based on the principle that the number of edges entering or leaving the direct neighbour subgraph of a vertex is high at the bridge. | Uses neighbour subgraph information and betweenness centrality                        | 2008                | Hwang et al. (2008)         |
| Pairwise Disconnectivity Index    | Calculated for a vertex as a fraction of those initially connected pair of vertices in a directed network which becomes disconnected after removal of the vertex from the network. It evaluates the importance of an individual element in a network for sustaining the communication ability between connected pairs of a directed network. | Built upon betweenness centrality and uses edge connectivity                        | 2008                | Potapov et al. (2008)       |
| Annotation transcriptional centrality | Delimits representative functional domains in co-expression networks and uses this information to identify key nodes (proteins/genes) that modulate the propagation of functional influences within the network. | Integrates gene expression profiles with co-expression network information for centrality calculation | 2010                | Prifti et al. (2010)        |
| Flux centrality                  | Developed on the concept of maximum flow which is defined by the largest flow that is observed for all possible paths between the two vertices in a network using shortest path closeness centrality. | Built upon the closeness centrality by integrating directionality and maximum flux flow closeness | 2010                | Koschützki et al. (2010)    |
| Leverage Centrality              | For a vertex in a network, it determines the extent to which its immediate neighbouring vertices reply on the vertex for information. | Built upon eigenvector and degree centrality by integrating neighbourhood information | 2010                | Joyce et al. (2010)         |
| New centrality measures     | Description                                                                                                                                                                                                 | Additional features                                                                 | Year of development | References                  |
|-----------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|---------------------|-----------------------------|
| Perturbation Centrality     | A measure of dynamic network centrality which uses weighted degree and is defined as the reciprocal of silencing time retrieved by using a Dirac delta type starting perturbation of 10n units, where n is the number of nodes in the network, using a dissipation value of 1. | Identifies intermodular hubs from dynamic networks                                    | 2013                | Szalay and Csermely (2013) |
| Game Centrality            | A dynamic centrality measure that measures the individually defecting nodes in the network to convert other nodes in the network to its own strategy.                                                         | Applies strategy update rule on the network dynamics data                              | 2013                | Simko and Csermely (2013)   |
| DiffSLC                    | Combines multiple centrality measures and exploits the advantage of eigenvector centrality and edge clustering co-efficient to identify essential genes/proteins. This centrality is a weighted combination of eigenvector centrality and co-expression biased degree centrality. | Gene co-expression values are used in conjunction with eigenvector and edge clustering co-efficient | 2017                | Mistry et al. (2017)        |
| Game Theoretic Centrality  | Determines a gene’s contribution to the overall connectivity of its corresponding node in the network by calculating the gene’s synergistic influence in a gene-to-gene interaction network.                                          | Uses differential gene expression profile (GWAS data) and is built upon game centrality | 2020                | Sun et al. (2020)          |
| Source-Sink centrality (SSC)| This centrality measures the importance of the node separately in the upstream and downstream of a pathway, as sender and receiver of biological signals.                                                     | Built upon the PageRank centrality by incorporating directionality                     | 2020                | Naderi Yeganeh et al. (2020) |
| Spectral clustering for Network-based Ranking (SCNrank) | Centrality developed for network-based target ranking based on spectral clustering. It calculates a target influential score by integrating PPI, CRISPR-cas9 and gene expression data to calculate the influence of target towards a cluster it belongs to for ranking and scoring each drug target. | Integrates data from three sources: gene expression, protein–protein interaction network and CRISPR-cas9 data | 2020                | Liu et al. (2020)          |
their neighbours. This method facilitates the repurposing of approved drugs with pathway interpretation of gene expression phenotypes (Han et al. 2019).

3.3 Methods for analysing metabolic networks

The inferences of graph theoretical analyses of metabolic systems are highly dependent on graph construction, which includes multiple options to define the nodes and edges of the network. Generally, in metabolic networks, nodes are represented as metabolites and edges as reactions, or vice versa, in reaction adjacency graphs. In bipartite networks, nodes can be metabolites or can also represent reactions. Metabolic graph networks are essentially directional. However, it was observed that directionality information, which was considered the sole defining factor of metabolic graph networks, might not be the only defining factor for the metabolic function (Wagner and Fell 2001). Previously, metabolic reaction graphs had limitations in analysing context-specific metabolic events under different growth conditions of the cell (Sauer et al. 1999).

In conjunction with this limitation is the challenge that arises from the reversibility of these metabolic graphs. The prior approaches do not ensure justice by generalizing the direction of reaction based on one condition and also do not take into consideration varying physiological conditions (Wagner and Fell 2001). Beguerisse-Díaz’s group addressed this complexity by emphasizing the inclusion of directionality information, as well as capturing environment-specific metabolic connectivity (Beguerisse-Díaz et al. 2018). This approach accounts for the utilization of metabolic directionality for representing the natural flow of chemical mass from reactants to products. It provides a flux-based strategy using Flux Balance Analysis (FBA)-based solutions to build different metabolic graphs under different growth conditions, creating opportunities to convert genome-scale metabolic models into directed graphs. Further, to improve upon the above-discussed challenges, several tools that employ network science to improve FBA pipelines have been consistently explored (Lewis et al. 2012). The integration of graph theory with FBA by constructing flux-weighted graphs is recently being proposed as a promising solution to overcome these previous shortcomings (Dusad et al. 2021) and holds application in several areas of industrial biotechnology such as maximizing production from metabolic cell factories and dynamic control of gene expression (Brockman and Prather 2015; de Lorenzo et al. 2018; Liu et al. 2018).

Several tools have been developed in the recent past in an effort to improve and provide a seamless experience in developing and executing pipelines for simulation of metabolic networks and also to integrate several other optimizing functions which include FBA-based solutions (Ebert et al. 2012; Rowe et al. 2018). A useful tool which can assist in carrying out FBA and several other network analysis for metabolic networks is MetaNET (Narang et al. 2014). Along with simulation studies, it also provides an option to conduct topological analysis.

Recent studies implement global centrality measures such as in-degree, out-degree, closeness centrality, and modularity for directed networks rather than a centralized focus on only the high-degree nodes, to identify targets in metabolic pathways (Newman 2006; Kim et al. 2019). For topological analyses, reaction-centric bipartite graphs that use centrality metrics independent of a node’s degree are being explored (Kim et al. 2019). These studies focus on calculating the influence of a node on the downstream flow of information in the network by calculating the bridging centrality and cascade number (Kim et al. 2019) (figure 2A). These newly developed approaches help in the analyses of directed reaction graphs to prioritize the nodes and their associated genes, which are essential for global and local connectivity and can help identify crucial targets in metabolic engineering. Development of network-based approaches for understanding the evolution of metabolic genes at different evolutionary timescales that tackle the challenge of procuring the gene’s likelihood to be under adaptive selection is another emerging application of network biology in understanding metabolic networks (Dobon et al. 2019).

3.4 Methods for integration of gene co-expression networks

The incorporation of omics datasets in computational networks using correlational analysis is gaining momentum. The development of the Weighted Gene Co-expression Network Analysis (WGCNA) (Langfelder and Horvath 2008) to identify highly correlated genes, or eigengene-based highly correlating gene clusters, has paved the way for transcriptomic data integration and analysis for relating modules to one another and for measuring module membership (Niu et al. 2019). The co-expression network analyses have garnered applications in uncovering various disease
mechanisms, from identification of rheumatoid arthritis-related diagnostic genes, potential disease genes, and vital microRNAs (Ren et al. 2021) to biomarkers and prognostic signatures in multiple cancers (Kadkhoda et al. 2020; Terkelsen et al. 2020). The previously used co-expression network analyses methods could not distinguish between the regulatory and regulated genes or provide information on

Figure 2. Schematic representations of recent methodological developments in molecular network analyses techniques. (A) Cascade number representing local controllability of the node demonstrated on a schematic metabolic network model which measures the influence of a node on its downstream flow of information and identifies the highest influencing reaction node. (B) Graphical workflow representation of the netImpute algorithm which employs diffusion of co-expression network to improvise the dropout issue in single-cell data. (C) Backbone extraction: A schematic representation through a toy transcription factor regulatory bipartite graph demonstrating the working of the backbone extraction technique to extract a subset of important regulatory transcription factors.
causality. Improvements have been made in these network studies by including differential co-expression analysis performed under different regulatory conditions (Chowdhury et al. 2020). Gene Sets Net Correlation Analysis (GSNCA) is another method that has enabled analysis of differentially co-expressed pathways by inferring differences in co-expression networks (Rahmatallah et al. 2014). Algorithm for the Reconstruction of Accurate Cellular Networks (ARACNE) (Margolin et al. 2006) and Gene Network Inference with Ensemble of trees (GENIE3) (Huynh-Thu et al. 2010) are popular tools used to construct regulatory networks from co-expression data. The Generalised Single Value Decomposition (GSVD) is another approach that relies on spectral decomposition to identify modules of co-regulated genes (van Dam et al. 2018). Furthermore, Higher-Order GSVD (HO-GSVD) (Ponnappalli et al. 2011) helps in multi-tissue analysis (Xiao et al. 2014).

Single-cell RNA sequencing technology plays an influential role in obtaining transcriptomes at single-cell resolution. One drawback of this technique is that only a small fraction of the transcript gets sequenced, resulting in dropout events (excess zero counts). Zand and Ruan (2020) have recently developed a gene co-expression network-based method called ‘netImpute’ to alleviate this dropout issue (figure 2B). A similar effort was made using a Bayesian factor model (Sekula et al. 2020). In the case of single-cell RNA-seq experiments where the dataset consists of several biologically distinct unknown sample groups, identifying differentially expressed clusters with similar expression patterns is challenging. An alternative method is biclustering, which can identify such patterns without prior sample classification (Cheng and Church 2000). Several new tools have also been recently developed for improving the analyses of co-expression networks, which include CoExp (García-Ruiz et al. 2021), Gene Whole Co-Expression Network Analysis (GWENA) (Lemoine et al. 2021), Translational Bioinformatics Tool Suite for Network Analysis and Mining (TSUNAMI) (Huang et al. 2021) and Conserved and Comparative Co-expression Network (CococoNet) (Lee et al. 2020a, b).

3.5 Prediction of perturbation patterns

The unavailability or lack of information about kinetic parameters while studying interactions between biochemical entities leads to loss of information. The consequences often reflect in the perturbation patterns. Collective perturbations affect disease states. The patterns of these perturbations help to understand differential expression patterns. The study of perturbation patterns in biochemical networks provides the opportunity for drug development, better understanding of drug combinations, and improved therapies (Santolini and Barabási 2018). Increasingly accurate topological models have provided us with improved confidence to approximate the impact of perturbation patterns (Santolini and Barabási 2018). Santolini and Barabási have proved that the topology-based linear response matrix or correlation matrix alone provides more than 65% accuracy in predicting these perturbation and biochemical influence patterns (Santolini and Barabási 2018). Their proposed method predicts perturbation patterns with higher accuracy for the networks that, upon link removal, can be decoupled into sparse networks (i.e., nodes with a low degree and link density). Additionally, the topology-based method, along with the integration and inference from experimental perturbation data, plays a key role in predicting physiological and phenotypic perturbations. However, the interplay between network topology and inherent dynamics predicts the emergent patterns in biochemical networks (Meena et al. 2020a, b).

The impact of the perturbations can be effectively studied by characterizing transient cell states that reflect in the cellular responses. Dynamic gene interactions and pathway behaviour are of central importance to characterize these transient populations. Dynamic modelling techniques are being developed that utilize the RNA velocity from single-cell RNA sequencing data that help observe dynamics of single genes and thus assist in interpreting the impact of perturbations (La Manno et al. 2018; Bergen et al. 2020). Network representations of this dynamic gene interaction data hold potential to improve the prediction of perturbation impact through dynamic network analysis.

3.6 Identification of clustering patterns in dynamic networks

Clustering patterns in molecular interaction networks find applications in several analyses, such as identifying similar gene expression patterns (Oyelade et al. 2016) and classifying cellular subtypes. Several algorithms have been developed that identify such clustering patterns in the form of community detection methods to analyse large biological datasets (Oyelade et al. 2016; Sharma and Ali 2017; Kanter et al. 2021). The concept behind network clustering is to partition...
networks into clusters or groups of ‘topologically related’ nodes expected to ‘correlate’ well in terms of their function or phenotype. Clustering in dynamic networks is considered to be challenging and complex due to the additional factor of timescales. In the case of dynamic networks, where the data essentially consist of a series of snapshots of networks through time, two popular approaches, snapshot clustering (Chi et al. 2007) and consensus clustering (Lancichinetti and Fortunato 2012), are used. Dynamic network clustering (DNC) approaches like Louvain and Infomap (Held et al. 2016) assume that topological similarity naturally implies dense interconnectedness. This disparity of assumption is being addressed by a newly developed approach called ‘ClueNet’ (Crawford and Milenković 2018), which evaluates the need for some dynamic networks to be partitioned based on topological similarity. Another method uses a combined approach of partitioning based on topological similarity combined with denseness (Crawford and Milenković 2018). Other clustering methods have also been developed where the metadata of the nodes in network are considered prior to forming clusters and not just used in post-clustering module segregation steps (Newman and Clauset 2016; Peel et al. 2017). Timely improvements in these heuristic methods would further remove current limitations such as the incorporation of overlapping clustering that are evident in real-world networks where one node can belong to multiple functional modules.

3.7 Gene prioritization methods

Identifying causal genes and refining candidate genes for experimental verification is an important step in high-throughput analyses, specifically in studying diseases. One of the most widely used prediction servers is GeneMANIA (Warde-Farley et al. 2010). The prioritization of genes in this server is highly dependent on network topological properties and the likeliness of shared phenotypes. New approaches like Hybrid-Ranker exploit topological properties to prioritize genes based on their proximity to the causal genes of a particular disease of interest and information on its corresponding co-morbid disease (Razaghi-Moghadam and Nikoloski 2020). Arete is a similar tool incorporated as an app in the Cytoscape graph analysis suite (Lysenko et al. 2017). Novel gene prioritization tools like GenePANDA (Yin et al. 2017) and TopControl (Nazariieh and Helms 2019) use additional features like the relative distance of the candidate disease gene to the known disease genes and dominating sets on co-regulatory networks instead of high-degree nodes. Target gene prioritization has also been demonstrated through the identification of the important regulatory modules within large GRNs, such as transcription factors regulating the downstream target genes in regulatory pathways usually represented as bipartite graphs. A recent methodological development to identify these regulatory target modules in such bipartite graphs is being explored through the technique of backbone extraction (Pavlopoulos et al. 2018). Backbone extraction delivers a subgraph composed of the most significant nodes and edges in a network. It has been used in projected graph networks obtained from the bipartite projection of regulatory and target nodes, which helps to identify important regulatory modules within the large regulatory network (figure 2C).

3.8 Methods for analysing amino acid networks

Amino acid networks (AANs) or protein topological networks (PTNs) are used for the graphical representation of functional domains of proteins. The edges represent interactions based on the amino acid distance cut-off set at their primary, secondary, or tertiary structural arrangement levels. Topological parameters effectively represent the structural and functional properties of protein networks (Bagler and Sinha 2007). The structural organization of these networks shows small-world network properties (Bagler and Sinha 2005). These networks are assortative and have a hierarchy and are limited to the subnetwork of hydrophobic amino acids (Yan et al. 2014). These networks are helpful in distinguishing the folding states of the protein structures from the decoys (Zhou et al. 2014), predicting protein fold (Bhavani et al. 2011) and understanding disease mutational landscapes such as identifying the epitopes of topological importance for rational immunogen design (Yan et al. 2014). The Protein Topological Graph Library (PTGL) was developed to provide a fast search for secondary structure classification and characterization of proteins by abstracting the structure in the form of undirected labelled graphs (May et al. 2004). Network Analysis of Protein Structures (NAPS) (Chakrabarty and Parekh 2016; Chakrabarty et al. 2019) and Amino acid Network Construction and Analysis (ANCA) (Yan et al. 2020) are web servers developed that facilitate the qualitative and quantitative topological analyses and visualization to study residue–residue relationships and
help gain insights into structure–function relationships. A protocol developed by Sinha et al. effectively determined the allosteric residues regulating drug binding activities by constructing a protein-contact network (PCN) and subsequently employed the network propagation theory based on a heat diffusion model (Sinha et al. 2020a, b) (figure 3). Additional applications of AANs include studying mutation patterns to effectively design HIV vaccine target T-cell epitopes (Gaiha et al. 2019) and to study allosteric changes leading to protein stability (Srivastava and Sinha 2014), designing of thermostable mutants (Kandhari and Sinha 2017), and understanding the molecular basis for resistivity and specificity of proteins in drug resistance (Sinha et al. 2020a, b).

4. Emerging hybrid network-based approaches

Mathematical models based on interaction graphs allow the investigation of complex biological systems (Sinha 1997). However, with increasing size of these systems, their dynamics and complexity grows exponentially, consequently making the screening of possible interventions infeasible (Cohen and Harel 2007). Static network topology-based analysis of large biological systems allows the identification of dynamically relevant components of the whole network. Systems biology approaches unravel different intracellular and intercellular signalling mechanisms and metabolisms to study emerging molecular systems. Hybrid network-based models are designed by combining graph theoretical analysis with two or more systems biology modelling methods such as Boolean modelling (Chowdhury and Sarkar 2019), FBA-based metabolic modelling (Dusad et al. 2021), ODE (ordinary differential equation) (Kang et al. 2020) and PDE (partial differential equation)-based models (Bardini et al. 2017). Integration of high-throughput data into these hybrid models has helped to overcome previous limitations and challenges, such as identification of system-level continuous and discrete dynamic functional modules, timescale integration, handling data heterogeneity, and elucidation of complete pathway topology. In this section, we briefly explain some recent hybrid network-based modelling approaches developed to understand the temporal dynamics of regulatory molecular mechanisms, identification of tumour heterogeneity, and elucidation of pathway topological modules.

(i) Regulatory mechanisms: Integrative measures to identify dynamically relevant modules from large-scale systems-level molecular interaction networks have been employed. A sequential evaluation of the hedgehog signalling pathway in different types of cancer using network topology-based approach followed by Boolean analysis of the important regulatory modules provided a promising measure to predict the dynamic behaviour of biological networks (Chowdhury et al. 2013). Boolean models account for genes as either specific activators or repressors of the target genes, due to which the analysis of gene regulatory behaviour at the subfunction level is compromised. This limitation has been recently addressed by the data-driven Fundamental Boolean Model (FBM), which facilitates subfunction-level analysis over a period of time by generating dynamic trajectories. This model is implemented in R for use as a package, ‘FBNNet’, and can be effectively used to study dynamic gene regulatory behaviour (Chen et al. 2018).

(ii) Tumour heterogeneity: Mechanistic models with prior knowledge derived from topological measures have contributed to improved understanding of intra-tumour heterogeneity and dynamic regulations behind the emergence of tumorigenic phenotype lineages and regulation of plasticity in cancer (Chowdhury and Sarkar 2019). The protocol provides a platform for personalized and target-based glioblastoma tumour therapy (figure 3). Dynamic graphical model frameworks are developed for comprehensive analyses of tumour heterogeneity by integrating different genome-level datasets. These frameworks are useful to understand the role of mutations in conferring heterogeneity at different stages of cancer progression and additional complexities in tumour evolution (Lysenko et al. 2017).

(iii) Pathway topology: In the current era of precision medicine, a system-wide pathway-level understanding plays a crucial role. Elucidation of the entire pathway topology has been a challenge in systems biology for quite some time. In this context, a recent approach developed by Liang and co-workers handles the property of mutual exclusivity in the pathway perturbation of tumours by applying an OR-gated network that infers modules of patient-specific dysregulated pathways (Liang et al. 2021). The Boolean variables for generating OR-gate functions in this model are obtained from mutation and gene expression data, which are then converted to a OR-gated network and thus it effectively handles
co-occurrence of genes, mutual exclusivity, and other properties that effectively contribute to elucidate patient-specific pathway modules.

Hence, we observe that the hybrid network-based approaches contribute to the understanding of complex system behaviour and generate testable hypotheses for experimental validation. However, these hybrid network-based approaches face issues in obtaining phenotype-specific data as well as incorporating disparate variable types in the networks (Walker et al. 2014). Although the hybrid modelling techniques ameliorate the classical methods, their extensive application is limited by complexities during in silico implementation. The scope and scalability of each modelling approach, like the Boolean, constraint-based, ordinary and partial differential equation-based, or network-based approaches, are different. Assimilating the information generated by one into another approach is often challenged by loss of information. Furthermore, the availability of appropriate data and parameterization of the model are factors adding to the challenges. Since the hybrid models concatenate the information generated by tools and techniques with different scalability, the integration, calibration, and evaluation of the model outcomes using appropriate experimental evidence is more challenging than in the classical techniques. For example, parameterizing all variables obtained from a subnetwork of hub protein interactions for an ordinary differential model is challenged by the availability of adequate information. Although the parameter estimation techniques can be helpful to deduce unknown parameters, their reliability depends on the preciseness of the experimental data used for calibrating the biological context to be studied. In the case of a hybrid network-based approach for metabolic modelling, the connectivity between the metabolic components in the network method is defined solely by the metabolic stoichiometry, whereas in the FBA...

Figure 3. Schematic workflows of emerging hybrid network models. Overview of emerging integrative approaches where network biology is combined with different molecular interaction data resources such as gene regulatory interactions, signalling and metabolic networks, and amino acid interaction in proteins. The implementation of different systems biology techniques such as Boolean formalism, flux balance analysis, heat diffusion, and integration of ML is demonstrated as various applications in deciphering molecular mechanisms.
model, it depends on the choice of objective functions (Dusad et al. 2021). As a result, while translating the FBA model into a network, it can be constructed as various types of graphs, such as bipartite graphs, graphs where nodes represent metabolites, or graphs where nodes represent a reaction. This creates a lack of consensus on the type of graph that can be built for that metabolic model and strongly influences the conclusions drawn from their network analyses (Beguerisse-Díaz et al. 2018). Another challenge arises while constructing networks that consider the direction of reaction fluxes in the metabolic models. However, these limitations are increasingly being addressed by recent developments of flux-weighted graphs and mass-flow graphs (Beguerisse-Díaz et al. 2018). In the case of a hybrid network-based approach that integrates Boolean modelling, the Boolean equations can often incorporate timescale-dependent behaviour, but this information might not be translated to the model’s graph representation, as, at one time point, the network can only represent a static interaction for one specific condition. These limitations and challenges further press the need for advanced statistical tools to augment non-continuous data and variables. With the surge in the approaches that automatically learn to encode network structure into low-dimensional representations, the use of transformation techniques with ML-based approaches and their hybridization with other first-principle modelling techniques have gained momentum (Lee et al. 2020a, b). In the next section, we will discuss some of the areas where network topology-integrated ML-based approaches find extensive application in analysing molecular interactions.

5. Applications of emergent machine learning-based approaches in molecular networks analysis

The above discussions provide a brief overview of the hybrid network-based approaches that are being constantly updated to incorporate network topological information with different systems biology approaches. With avenues of dimensionality reduction of the vast genome-wide association data for automated extraction of information, the ML-based techniques have revolutionized the prospects of network biology in understanding molecular interaction networks (Mochida et al. 2018). ML techniques, such as semi-supervised algorithms, effectively contribute to the classification problems with limited availability of data and parameters, which often remains as a limitation of the hybrid network-based approaches, as discussed in the previous section. They also enable easy automation of integration of multi-level heterogenous data with network models, thus enabling the use of a vast spectrum of information in an automated fashion. The use of network topological features in semi-supervised ML algorithms has enabled automation even with limited availability of relevant information (Nandi et al. 2020). In this section, we briefly state areas where these emerging ML techniques that amalgamate molecular network information find applications.

5.1 Gene essentiality prediction

Predicting essential genes through the analysis of molecular networks has significantly contributed to drug development and understanding of synthetic biology (Hwang et al. 2009). Single-gene knockout studies and genome-wide RNAi screens have unveiled the multifaceted nature of gene essentiality that is context-dependent and evolvable rather than just binary and static (Rancati et al. 2018). This provides scope for developing predictive models for identifying essential genes using genome-wide data by applying advanced computational techniques such as machine learning on genome-wide association studies (GWAS), e.g., EssRank (Xu et al. 2019). Integration of gene expression data and network topological features has been employed for predicting gene essentiality (Zhong et al. 2021).

ML algorithms use gene expression, functional annotation, sequence, and network topology as features to identify gene essentiality (Zhang et al. 2016). Along with PPINs, transcriptional and metabolic network features have also been increasingly incorporated into the ML models (da Silva et al. 2008; Plaimas et al. 2010). Nandi et al. (2020) have recently addressed the shortcomings of limited availability of experimental data and, thus, the lack of labelled data by developing a semi-supervised ML strategy. This Laplacian support vector machine (SVM)-based strategy revealed topological measures of reaction networks as one of the important determining features for classifying essential and non-essential genes in prokaryotes and eukaryotes (figure 3). These ML strategies contribute to identifying the deterministic features that help distinguish class labels (e.g., essential and non-essential gene), which was previously challenging due to limited availability of data. DEEPLYESSENTIAL is another method that uses deep neural network architecture to predict essential microbial genes using sequence information (Hasan
Prediction of drug–disease interactions

Drug repurposing is known to accelerate drug discovery research and development processes (Novac 2013). Identification of drug–disease interactions plays an important role in drug repurposing and thus accelerates de novo drug discovery. The advantage of using network-based models for identifying drug–disease interactions is that it utilizes complete large-scale high-throughput data to build complex biological interaction networks. Several network-based models to identify these drug–disease interactions have been developed in the recent past (Wang et al. 2014; Martinez et al. 2015; Luo et al. 2016). The prediction of potential drug–disease interactions by integrating multiple layers of network data has been helpful in assessing molecular actions and studying disease implications (Oh et al. 2014). Recent developments using ML-based prediction models designed for drug–disease association studies employ a number of methods ranging from logistic regression-based methods (Gottlieb et al. 2011) to Laplacian regularized sparse subspace learning (LRSSL)-based methods (Liang et al. 2021). Wu et al. (2017) proposed a semi-supervised graph cut (SSGC) algorithm to predict drug–disease pairs by integrating the information on drug substructures, disease phenotypes, and gene–gene interactions with known drug–disease interaction treatment relationships in a hierarchical framework. This proposed algorithm enabled the integration of three different layers of disease phenotype, treatment and gene mechanism data, and optimally identified drug–disease similarity associations. Network similarities have also shown to contribute to drug–disease associations and can be effectively used in ML-based training algorithms to improve predictions. A novel method was recently developed that combines network similarities of drugs and diseases with their chemical and semantic similarities to predict novel drug–disease interactions and effectively handles the unwanted disease interaction pairs, which have been a challenge in some previously developed methods (Cui et al. 2019). Integration of similarity measures in heterogenous networks and deep learning models to predict drug–disease interactions can further significantly benefit drug repurposing. A novel framework was recently proposed by Jarada and co-workers that uses similarity selection and similarity network fusion combined with neural network deep learning model to efficiently predict drug–disease interactions (Jarada et al. 2021). This method resolves the challenge of limited availability of known interactions by integrating similarity information along with tackling data noise and redundancy issues which were previously faced by other methods, and thus has improved prediction accuracy. Another recently developed methodology of ensemble-based strategy uses weighted K-nearest known neighbours to construct drug and disease similarity networks (Wang et al. 2021). Such statistically improved methods are increasingly being proposed to improve accurate drug–disease interaction predictions by developing novel strategies using molecular network information, which will advance the development towards precision medicine (Zhu et al. 2018; Yu et al. 2019).

Characterization of regulatory genes

ML methods contribute significantly in predicting and inferring GRNs using transcriptomic data (Mochida et al. 2018). GRN inferences face limitations due to noise, low sample size and incomplete characterization of regulatory dynamics, leading to networks with missing and anomalous links (Banf and Rhee 2017). A semi-supervised network reconstruction algorithm has been developed that enables the synthesis of information from partially known GRNs with time course gene expression data (Nguyen and Braun 2018). This method successfully identifies novel and anomalous connections. A recent advancement addresses the problem of two potential regulators in GRNs having high correlation or matching expression patterns, making it challenging to differentiate between them. A novel method called linear profile likelihood (LiPLike) predicts gene-to-gene regulation with high accuracy by selecting interactions that are uniquely inferred by measured data (Magnusson and Gustafsson 2020). A recent supervised-learning-based method, GRADIS, incorporates graph distance profiles from transcriptomic data to reconstruct GRNs (Razaghi-Moghadam and Nikoloski 2020). This approach offers the possibility to use network representations of large-scale data that help characterize cellular networks and analyse GRNs effectively.
5.4 Prediction of protein abundance and protein complexes from PPINs

ML models are being implemented to predict protein abundance from single-cell RNA-Seq data using PPI and prior knowledge embedded into neural graph networks (Niu et al. 2020; Dai et al. 2021). The PIKE-R2P (PPIN-based knowledge embedding with graph neural network for single-cell RNA to protein prediction) model proposed by Dai and co-workers uses graph neural networks (GNN) which enable multi-label modelling. The information from PPIs in these GNNs thus help in the cross-modality prediction of protein abundances at the single-cell level. DeepHE, a network embedding method, automatically learns features from PPINs and additionally uses sequence features. These two types of feature data are used to train multi-layer neural networks and address the imbalanced learning problem using a cost-sensitive technique (Zhang et al. 2020). New algorithms have been proposed for mining the best topological features to predict protein complexes from PPINs. The Sequential Forward Feature Selection (SFFS) algorithm, recently proposed by Younis and co-workers, uses random forest-based Boruta feature selection to integrate a wide variety of topological and biological features as well as protein interaction information (Younis et al. 2021).

6. Understanding disease mechanisms and identification of potential therapeutic targets

Network topology-based approaches have contributed to the development of advanced therapeutic applications to curb metastasis-driven cancer progression. Topology-based approaches have been applied to classifying breast cancer subtypes by searching for significant sub-networks (Chuang et al. 2007). Their application has expanded to a broader pan-analysis perspective with experimental and theoretical advancements in cancer diagnosis. Multi-layer frameworks combining network topology and spectral graph theory have enabled the study of the cancer complexome to identify important proteins across multiple cancers (Ramadan et al. 2016; Rai et al. 2017; Hari et al. 2020; Buffard et al. 2021). WGCNA on the PPI networks has been used to identify gene co-expression modules between the differentially expressed genes (DEGs) through hierarchical clustering to identify gene expression signatures associated with acquired gefitinib resistance (Lee et al. 2015). The potential network analysis techniques applied explicitly for developing precision cancer medicine have been thoroughly reviewed by Ozturk et al. (2018).

Evaluation of the inter-species heterogeneity in molecular interaction networks has contributed significantly towards delineating infection mechanisms by analysing cause–effect relationships in treatment strategies. For example, Singh et al. (2020) applied exhaustive topological analysis using the parameters in-degree, out-degree, and directed and undirected average path lengths to study the comprehensive transcriptional regulatory network of Mycobacterium tuberculosis (MTB) H37rv. Furthermore, global proteomic datasets analysis of virus-infected patients with human immunodeficiency virus (HIV) and hepatitis C virus (HCV) demonstrated that using degree and betweenness for identifying pathogen interactions was more effective and accurate than using differential regulation alone (McDermott et al. 2012; Soto-Girón and García-Vallejo 2012). Ackerman and co-workers proposed a new method of combining host PPI networks with virus–host PPI data to identify host target proteins and demonstrated the method in influenza infection by extracting virus-specific subnetworks (Ackerman et al. 2018). This study revealed that network position within the virus–host subnetwork offers an advantage in prioritization of drug targets. Controllability analysis using virus–host networks captured the dynamic properties without the knowledge of experimentally derived concentration parameters and helped identification of antiretroviral targets with higher potential (Ackerman et al. 2019).

The current global COVID pandemic has led to a surge in research techniques that can rapidly process the information generated for the newly identified SARS coronavirus 2. The immediate requirement to identify potent remedial solutions and understand the virulence mechanism of rapidly evolving strains to development of vaccines has led researchers towards network biology approaches. Construction and analysis of biomolecular networks in the form of PPINs, transcriptional, and gene co-expression networks have led to rapid assessment of concurrent effects (Nashiry et al. 2021), analysing viral host associations (Das et al. 2021; Terracciano et al. 2021) and predicting miRNAs associated with viral pathogenesis, elucidating neurological manifestations (Prasad et al. 2021).

7. Concluding remarks and future prospects

The present review thoroughly evaluates the insightful inferences that can be drawn using graphical networks of biological systems and their integration into different
hybrid network-based modelling techniques to provide additional details about complex system behaviour. With increasing advances in high-throughput technologies in biological research, attempts have been made to incorporate this information into network structures, leading to a continuous update of network biology approaches in the last two decades. New centrality measures like pairwise disconnectivity index, leverage centrality, bottleneckness, bipartivity, etc., along with classical centrality measures like betweenness, Katz centrality, and eigenvector centrality, can be used to predict the influence of genomic regulators on target gene networks and the impact of their deletion on target genes. Advances in topology-based approaches have paved the way to successfully identify perturbation patterns, gene prioritization, and clustering of dynamic networks. The computational advances in terms of amalgamation of machine learning (ML) and artificial intelligence (AI) in using network graph properties have proved beneficial with several applications such as essential gene prediction, drug–disease interaction identification, and prediction of protein abundance from single-cell data. Pertaining to the current knowledge of the available methodological advances in studying these molecular interaction networks, we suggest certain areas where advanced computational approaches incorporating network properties can be developed and applied in future:

Development of a testable hypothesis for disease diagnostics: With a demonstrated application of graph networks in deriving useful inferences about different disease conditions including both infectious disease and cancer, network biology approaches provide the opportunity to elucidate condition-specific network structures depending on the details of the specific disease systems under study. Empirical analysis of the network structure and topology of disease case-specific conditional differences and comparison with the network structure of the normal physiological conditions can help identify prognostic targets and modules for therapeutic benefits.

Identification of gene regulatory targets: Computational analysis of omics and high-throughput data on the translational and post-transcriptional regulators of gene expression has been successful in establishing a cause-and-effect relationship between differential expression of the gene expression regulators and target genes (figure 3C). Graph network analysis is a suitable choice to study these regulatory networks as these approaches can provide conclusions based on holistic analysis of large-scale information. Regulatory targets can be predicted from the analyses that can be further tested through in vivo and in vitro studies to test their feasibility as therapeutic targets under diseased conditions.

Automated identification of essential genes and prioritized molecular targets: Hybrid network-based models, which make use of network inferences have paved the way for the development of automated ML and artificial network-based (Bayesian, neural) tools with the ability to predict and identify essential genes and prioritize molecular targets even with limited experimental data availability. These integrative approaches can be continuously updated to improve the quality of prediction with corroboration of additional heterogeneous data. Semi-supervised algorithms can further be improved to increase the prediction accuracy when limited essential gene information as well as GRN data are available.

Continuous improvement in statistical methodologies in the area of deep learning and recurrent neural networks can be effectively applied to improve the use of molecular network data in the context of personalized medicine development. Advances in classification algorithms and improved sensitivity to phenotype-specific classification utilizing network topological information can accelerate research in personalized medicine development and improve our understanding of causal mechanisms in diseases.

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