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Tracing the footsteps of autophagy in computational biology

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Abstract

Autophagy plays a crucial role in maintaining cellular homeostasis through the degradation of unwanted materials like damaged mitochondria and misfolded proteins. However, the contribution of autophagy toward a healthy cell environment is not only limited to the cleaning process. It also assists in protein synthesis when the system lacks the amino acids' inflow from the extracellular environment due to diet consumptions. Reduction in the autophagy process is associated with diseases like cancer, diabetes, non-alcoholic steatohepatitis, etc., while uncontrolled autophagy may facilitate cell death. We need a better understanding of the autophagy processes and their regulatory mechanisms at various levels (molecules, cells, tissues). This demands a thorough understanding of the system with the help of mathematical and computational tools. The present review illuminates how systems biology approaches are being used for the study of the autophagy process. A comprehensive insight is provided on the application of computational methods involving mathematical modeling and network analysis in the autophagy process. Various mathematical models based on the system of differential equations for studying autophagy are covered here. We have also highlighted the significance of network analysis and machine learning in capturing the core regulatory machinery governing the autophagy process. We explored the available autophagic databases and related resources along with their attributes that are useful in investigating autophagy through computational methods. We conclude the article addressing the potential future perspective in this area, which might provide a more in-depth insight into the dynamics of autophagy.

Key words: autophagy mechanism; apoptosis; mathematical models; differential equations; network analysis; autophagy database

Background

The importance of autophagy in maintaining cellular homeostasis is ineffable. In Greek, the term autophagy means self-eating. This term was first coined by Christian de Duve, and this nomenclature was solely based on the observed degradation of mitochondria and other intracellular structures within the lysosome of rat liver perfused with glucagon [1]. Degradation of unwanted materials like damaged mitochondria and misfolded proteins are essential for the maintenance of cellular homeostasis. Autophagy engulfs these unwanted materials and facilitates the process of cellular filtration. However, the contribution of autophagy toward a healthy cell environment is not only limited to the cleaning process; it is indeed much more than that. The degradation of proteins in the lysosome results in amino acids, which assist the protein synthesis even if the system lacks the

inflow of amino acids from the extracellular environment due to diet consumptions [2]. Therefore, under stressed conditions like starvation, autophagy levels rapidly upregulate and initiate cellular degradation to provide nutrients to the cell [3–6].

Continuous divulgence of new autophagy-related genes (ATG), as well as pathways, have made autophagy a growing field. Deterioration of this aforementioned catabolic process is evidenced to be associated with various diseases (Figure 1). Autophagy acts as a Janus in cancer by playing a role both in tumor suppressor and tumor activator. Autophagy-related proteins are associated with the prevention of cancer cell growth in various cancers, including the likes of the colon, gastric, breast and prostate cancer [7–11]. However, autophagy also helps in tumorigenesis by promoting the proliferation of cancer-cell and tumor growth [12–14]. Again, abnormal lipid metabolism and the excessive accumulation of triglycerides stored in lipid droplets trigger the non-alcoholic fatty liver disease, which may eventually lead to non-alcoholic steatohepatitis (NASH) [15,16]. *In vitro* and *in vivo* studies have revealed that autophagy plays a protective role in NASH by selective degradation of these lipid droplets [17]. Hence, the autophagy pathway can be a potential target in the treatment of NASH. In various neurodegenerative diseases, including Parkinson's disease, Alzheimer's disease and Amyotrophic lateral sclerosis [18], misfolded protein accumulation is considered a pathological hallmark. Since the accumulation of misfolded proteins is directly affected by a decrease in the neuronal autophagy level, autophagy is considered as a target pathway in neurodegenerative diseases. The importance of autophagy can be mapped to insulin resistance and type 2 diabetes, as it plays an indispensable role in the physiology of beta cells. Autophagy takes part in the regulation of insulin homeostasis and is necessary for normal beta cell homeostasis [19–21]. The disrupted autophagic activity has been reported in the beta cells of type 2 diabetes mellitus (T2DM) patients [22]. Metformin has been widely used in type 2 diabetes clinical therapy and protects pancreas beta cells from injury through autophagy activation by the AMP-activated protein kinase (AMPK) pathway [23]. Due to its crucial role in cellular housekeeping, autophagy also plays a role in anti-aging mechanisms [24,25]. It also plays an essential role in cell remodeling during development [26] and in cellular defense against pathogens [27].

Nevertheless, despite playing a protective role in various diseases, uncontrolled autophagy may lead to excessive degradation of the cellular constituents and may cause cell death [28–31]. Hence, although important, the autophagy process needs to be strictly monitored for the smooth functioning of the cellular homeostasis [32,33]. To understand the significance of autophagy in cellular processes and its association with different diseases, we recommend reading two excellent reviews on autophagy [3,34].

There are three defined types of autophagy, viz. macro-autophagy, micro-autophagy and chaperone-mediated autophagy [34]. In this study, by autophagy, we mean macro-autophagy only (Figure 1). In macro-autophagy, the degradable constituent is first encompassed by a double membrane vesicle, which gradually extends to form autophagosome. The autophagosome then fuses with lysosome and forms autolysosome, where the degradation of the cytosolic cargo occurs. The mammalian target of Rapamycin complex 1 (mTORC1) and lysosome play a crucial role in macro-autophagy. A nutrient-rich environment within the cell enables mTORC1 to be located in the lysosome and promotes growth processes. Transcription factor EB (TFEB) is a protein that dictates the transcription levels of lysosomal and autophagy genes. mTORC1 suppresses autophagy by modulating the nuclear export of TFEB [35] and by the inhibition of the autophagy initiation complex [36]. This terminative effect of mTORC1 ceases when starvation triggers it to dissociate from the lysosome, which leads to autophagy induction. The process of autophagosome–lysosome fusion, i.e. the induction of autolysosomes, is facilitated by several proteins. Cooperative activity of soluble N-ethylmaleimide-sensitive factor attachment protein receptor proteins embedded in either membrane helps to overcome the high energy barrier of membrane fusion [37]. Again during fusion, the two vesicles must be kept close for which HOPS complex, PLEKHM115 and EPG5 simultaneously interact with proteins present on both the autophagosomal membrane and autolysosomal membrane [38–40]. Once the autolysosome forms, the inner autophagosomal membrane degrades, and more than 60 lysosomal hydrolases work simultaneously to digest the confined material [41].

This evolutionarily conserved process has always been an important research topic. A PubMed survey using the keyword 'autophag∗' in the Title category revealed that the intensity of autophagy in the research field had increased exponentially in the last few years. Though the literature exists from 1956, its bloom is observed only after the year 2004–05. The autophagic mechanism is highly sensitive to perturbations of the intracellular or extracellular microenvironment. Its increasing and decreasing level may play lethal or survival roles in biological functions. Hence, a better knowledge about the autophagy pathway and its part is essential for improving future therapy that might contribute to recognizing alternative cell death and cell survival mechanisms in the presence or absence of some apoptotic pathways.

Biological processes are governed by specific rules, and systems biology acts as a hatchet to unveil these underlying principles [42,43]. Every system possesses a hierarchical structure and a systematic study of it helps to find how components are organized, viz. what lies in the core, and what remains on the periphery of the system. Again, these structures are interlinked together, where each lower level in the hierarchy creates the level immediately above (for example, cell to tissue, tissue to organ, organ to the organ system and so on) by means of some linkages. Systems biology is nothing but the study of both these structures and the linkages. By implementing various algorithms to analyze a disease network, the core modulators of the disease can be detected, and the dynamics of these core sets of modulators can be studied through mathematical modeling. The enrichment analysis can help to identify the core pathways involved in a disease in the protein–protein interaction (PPI) network. In other words, mathematical modeling helps to study the system in parts while system biological approaches (network analysis and enrichment analysis) help to explore the entire system. Various systems biology methods have been applied to delineate the process of autophagy (we have considered macro-autophagy only) using mathematical modeling and network analysis. Mathematical models have been combined with experimental studies to decipher the complex dynamics of macro autophagy. An application of mathematical modeling is to study the effect of starvation-induced autophagy in cell (yeast) population dynamics [44]. Again, these models study the dynamics of the proteins that govern and modulate macro-autophagy at different phases. In various diseases like cancer, Alzheimer's, etc., the core set of proteins or pathways have been studied mathematically to capture the mechanism of the disease progression and to extract the influential parameter to restore the cell homeostasis in disease conditions [45–51]. The network analysis approaches have been
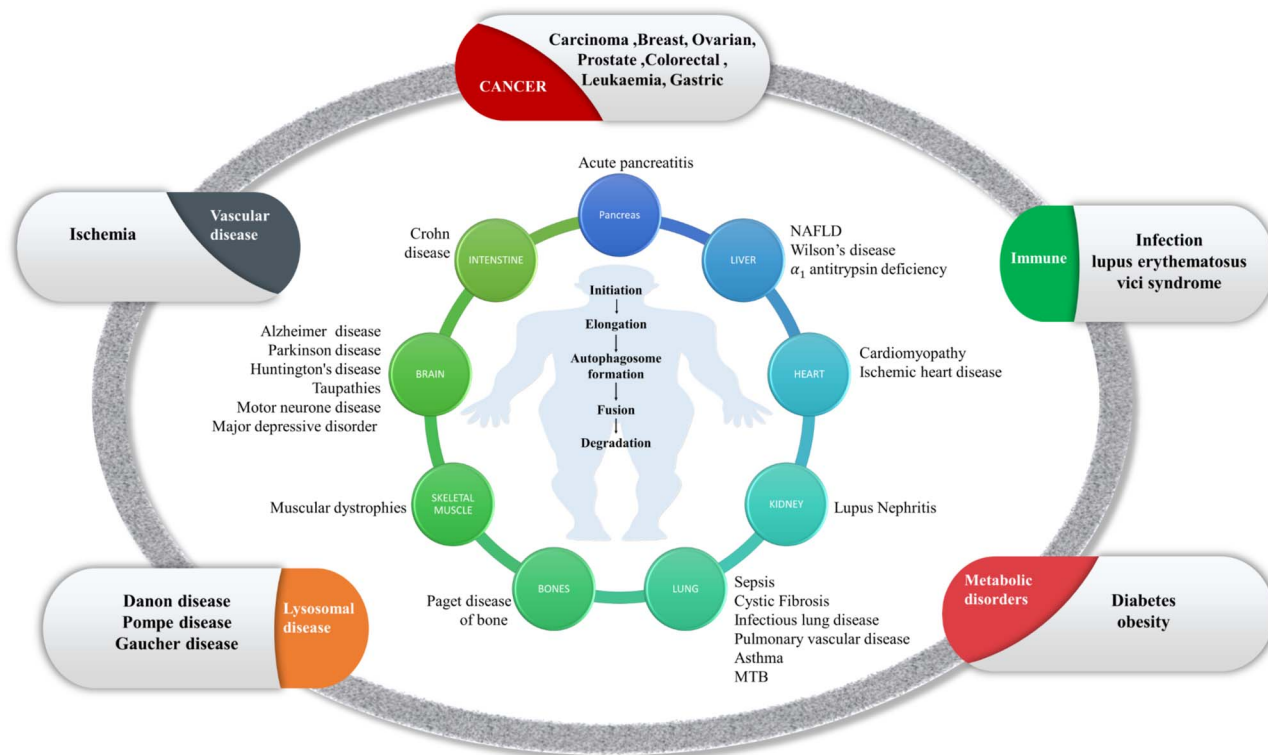
Figure 1. The process of autophagy is completed in five steps: initiation, elongation, autophagosome formation, fusion and degradation. Each step is monitored and modulated by a group of genes called ATG. Autophagy-related diseases can be categorized into two parts: organ-specific (shown in the inner circle) and multisystemic (shown in the outer circle). This figure shows the autophagy-disease interplay. The association with most human diseases, including varieties of cancer and immune disorders, has proved that autophagy is a quintessential process, and its manipulation can be targeted as a therapeutic strategy.

applied to observe the topological behavior of autophagy-related proteins in various diseases. Network analysis of differentially expressed genes (DEG) in multiple diseases such as leukemia, pancreatic cancer, etc. has shown the abundance of autophagy-related proteins and autophagic pathways in the disease [52,53]. Again, the implementation of network centrality measures such as degree, betweenness centrality, clustering, etc., help to identify the core set of proteins in the network [54,55]. Although these two approaches are often studied separately, these studies may complement each other. For example, for a better understanding of the underlying mechanism of the association of autophagy proteins in disease, mathematical modeling can be done on the core set of proteins obtained from PPI network analysis. It may identify potential parameters that otherwise could not be explained by network analysis alone. This methodology is shown in Figure 2. Hence, with the *in vivo* and *in vitro* studies of autophagy which have explored many novel discoveries, the systems biology, with the potential of decrypting the system's complexity both as a whole and in part, have significantly emerged and made tremendous contributions to the field of autophagy.

In this review, we have encapsulated the overview of autophagy in computational biology explored via mathematical modeling and network analysis along with comprehensive insights about these approaches and their applications in the exploration of the autophagy process at various levels (molecules, cells, tissues). We have delineated several well-established methods such as mathematical models based on different types of differential equations, Petri net, agent-based models (ABM), enrichment analysis and centrality analysis to capture the dynamical behavior or the collective influencers in the network. Further, we have enlisted the available autophagic

databases and the related resources and their feature selection and epitomized some conventional software and tools used for visualization and analysis in computational biology. We believe that this review provides an in-depth and improved understanding of autophagy in systems biology and will help the researchers to select the appropriate method for new studies to find potential targets in a plethora of diseases, including cancer, Alzheimer's, metabolic and immune-related disorders.

Mathematical modeling for autophagy

Significance of mathematical models in studying biological systems

Biological systems are complex, and a mathematical model acts as a reflector of this complexity. The complexity of a biological system appears due to many reasons. One of them is the underlying hierarchy of the system that ranges from cells to the organism level. Each of the different hierarchy levels is dynamic. Even if they imitate regular and predictable behavior, abrupt major and stochastic shifts may arise anytime, even for a minute changes in the cellular environment. These uncertainties lead to complexities that are difficult to capture in experimental studies. Even if they do, it is challenging to grasp routes and patterns of the actual evolution of complexity. For example, to identify the functional role of a gene, scientists perform the knockout experiments [56]. However, such experiments overemphasize the role and importance of a single gene and are not ideal to understand the complex nature of the system. Mathematical models study these complexities of a system and portray an abstraction of the reality. These models study the crucial genes/proteins that
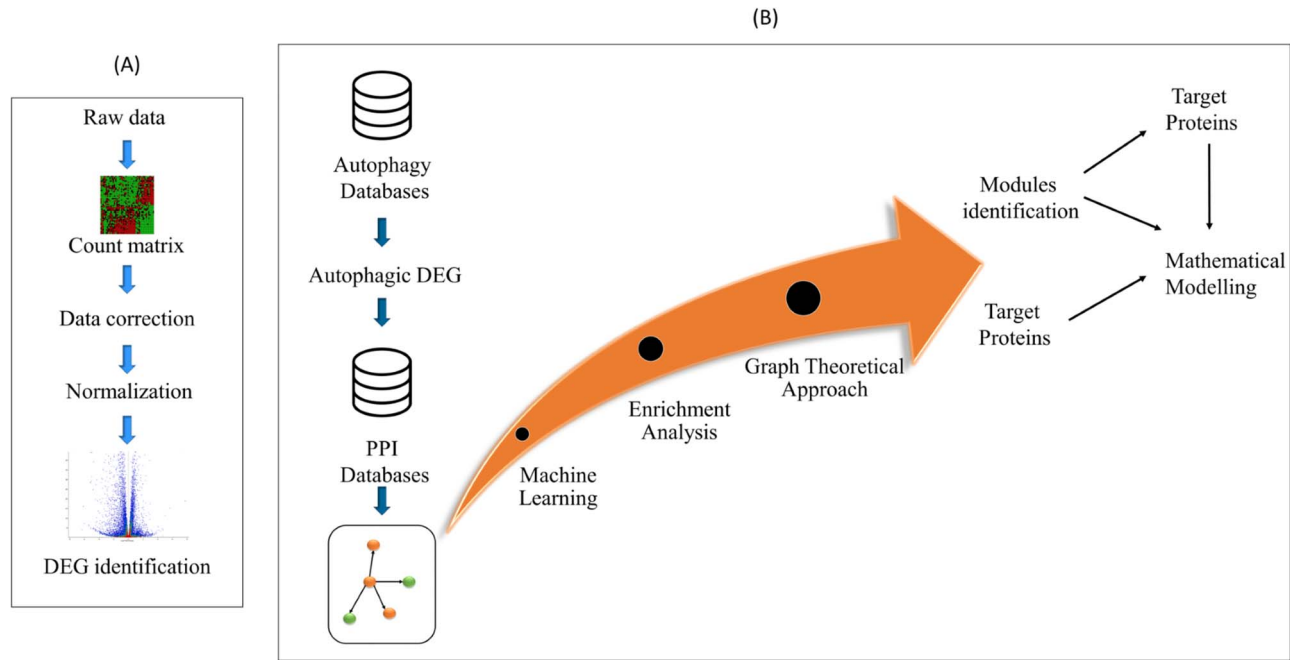
Figure 2. The journey of proteins from being inside the system to the arms of mathematical modeling. The palate (A) shows the processing of data. The raw gene expression data to study a particular disease is first corrected, including steps like dealing with the null values and the outliers. The data are then normalized, and the DEG are calculated. The palate (B) shows the autophagy specific study of the disease. The autophagic genes are first obtained from an autophagy database from where the differentially expressed autophagy genes are selected. Using PPI databases, a PPI network of the DEGs (which may be entirely autophagic DEGs or a mixture of autophagic and not-autophagic DEGs) is constructed. In the figure, the green color denotes the autophagic, and the orange color represents the non-autophagic genes. Implementing ML approaches, graph theoretical approaches or enrichment analysis (pathway analysis, disease analysis or gene ontology analysis), the significant modules or target proteins from the network are extracted. In the first case, the proteins driving the module can further be identified. Finally, the implementation of mathematical modeling approaches can explore the dynamics and underlying mechanism of the target proteins or the module.

are capable of driving the system and study the underlying dynamics of the association between the genes/proteins. These models have the audacity to identify crucial parameters that are capable to decide the fate of the system undertaken, which can be further proven by experimental validation. Among the various models available to investigate the biological processes, ordinary differential equations based models are the most commonly used. These equations are of the form

$$\frac{dx_i}{dt} = f_i(x_1, x_2, \ldots \ldots, x_n, \lambda_1, \lambda_1, \ldots \ldots \ldots, \lambda_k, t) \, ; i = 1 : n$$

and they describe how individual variables x_i vary over time, λ_k are the model parameters describing different rate constants. When explicit dependence of the dependent variables on the independent variable is absent, the system is called autonomous. The functions, along with the parameters, beautifully portray the dynamics of the state variables.

Another type of mathematical model, called delay differential equations (DDE) models, is used in representing biological phenomena. Time delay is an inherent property and occurs naturally in biological systems. The time delays in these models represent the duration of hidden processes between two major processes. The time between infection of a cell by some pathogens and the subsequent production of new pathogens can be taken as an example. A typical DDE is of the form

$$\frac{dX}{dt} = F(t, X(t - \tau_1), \ldots, X(t - \tau_n)) \, ; \tau_i, i = 1 : n \geq 0$$

$\tau_i, i = 1 : n$ are time delays. They are measurable and may be constant. Sometimes, the initial or boundary conditions may

not be sufficient to predict the future state of a system. For such a scenario, it is indispensable to know how the system behaved in the early stages, and hence, DDEs play a vital role in understanding a biological system, where the current state of some variables depends on the past states.

Both ODE and DDE are deterministic methods to map a biological system of equations. But, a biological system is always exposed to the uncertainty that is not entirely understood. An approach to model such systems is by adding stochastic influence or noise. Some stochastic behavior examples are hormonal oscillations, respiration, blood pressure variations, cellular metabolism, etc. The general form of a stochastic differential equation (SDE) can be expressed as

$$dx_t = f(t, x_t) \, dt + G(t, x_t) \, dw_t$$

Or with the equivalent integral form

$$x_t = x_{t_0} + \int_{t_0}^{t} f(s, x_s) \, ds + \int_{t_0}^{t} G(s, x_s) \, dw_s$$

with an initial value, x_{t_0}. Here, $f : [t_0, t] \, X \, R^d \to R^d, G : [t_0, t] \, X \, R^d \to R^{d \times m}$ and $\{w_t\}_{t \in [t_0, t]}$ denote an m-dimensional Wiener process (Brownian motion). R^d and $R^{d \times m}$ are d-dimensional and $d \times m$-dimensional Euclidian space, respectively.

The mathematical modeling is based on four crucial pillars, where the first pillar is a literature survey of the system. The second pillar is the construction of the model, where the relationship between the model variables will be established

using model parameters. This step is followed by the analysis of the model, and the last pillar is the validation part where the result of the model will be validated either by literature or by an experimental approach. If the model fails to deliver the appropriate output, the necessary changes will be implemented in the model until the desired outcome appears. Mathematical models are perfect examples of complexity and simplicity as they are ornamented with a set of equations, which are complex enough to replicate the properties of the system and concurrently simple enough to grab up the underlying phenomena of the system. Theoretically, these models can drive a system anywhere, but it has to follow some constraints in systems biology. For example, a negative concentration of a protein will make no sense, so as a species' negative population. Similarly, there must always be an upper bound, be it concentration of a protein inside a cell or population of a species. But, although restricted to biological constraints, a mathematical model can help to find out the crucial parameters responsible for deciding the fate of the system. In other words, for a specific cellular process full of many regulatory patterns, mathematical modeling paves the way to pick the right one.

Different tools and packages have been built across multiple platforms (MATLAB, Python, R, etc.) to support mathematical modeling [57–63]. A structural diagram editor, Cell Designer [64], has also been developed to draw gene-regulatory and biochemical networks to make mathematical modeling a feasible approach in systems biology. CellML is an XML-based language designed to describe mathematical models in a machine-independent form suitable for sharing between different authors and archiving in a model repository [65].

Why model autophagy?

The process of autophagy consists of five steps, and all these stages are easily observable [66]. Different steps in the autophagy pathway may exert a different effect on the system. However, the biochemical reactions in autophagy are mostly nonlinear, i.e. a minute change in any of its stages will not necessarily exert a proportional effect throughout the system. Mathematical modeling endorses simplified abstractions and approximations to identify the steps of autophagy that are responsible for a particular behavior in the system. Moreover, the constant shift in the behavior of the system exerts randomness in the autophagy process. Mathematical modeling of autophagy keeps track of these factors and allows the researchers to investigate the dynamics of the system following any environmental conditions that may arise due to various external or internal perturbations or signals. Autophagy is a bridge between cell survival and cell death. Depending on certain extracellular or intracellular signaling, the process of autophagy may decide cell fate. At the single-cell level, these events may be mutually exclusive, indicating that cell death and cell survival events are different attractors of the system. Mathematical modeling can be done to understand the crosstalk between these two events using attractors, fixed points and limit cycle concepts.

Cell types differ in their response to autophagy stimuli. Addressing this cell to cell variability, various therapies have targeted autophagy manipulation in cancer therapy [67–69]. Mathematical modeling can help in planning and predicting the parameters that could be targeted and its outcome on the cell population. For example, autophagy helps in tumor cell survival under various stress conditions [70]. On the contrary, increased autophagy may lead to excessive cellular degradation and thus, may initiate cell death [71]. A mathematical model can perfectly utilize these conditions to identify the biological parameters that increase the autophagy process in disease conditions, so that the tumor cell gets less benefit from the basal level of autophagy and cell death initiates.

Thus, mathematical modeling can be used in a very effective way to decipher the process of autophagy and its role in various diseases or conditions. We have discussed below some of the modeling work done in the autophagy process to get an idea of the applicability of mathematical modeling in understanding the autophagy process.

Differential equations based models for autophagy

In 1975, Deter *et al.* [72] formulated the first mathematical model to delineate the glucagon induced autophagy in rat liver. This primitive study was based on experimental observations, collision theory and chemical kinetics, and mainly focused on studying the population of telolysosomes, autophagosomes and autolysosomes in rat liver. Thereafter, various studies on autophagy have incorporated different types of mathematical models, viz. ODE, DDE and SDE. The widely used ODE-based models are the simplest to study the process of autophagy. These models are entrenched on the assertion that the system considered is well-mixed, and there are sufficient numbers of components so that their numbers can be considered as continuous quantities. For the benefit of the readers, we have explained some terminologies associated with the model analysis in Table 1. Understanding the steady-state, stability, and other qualitative behavior of a model will unveil the system's underlying mechanism. For example, response to cellular starvation is an intrinsic property of autophagy and was mathematically addressed by Jin *et al.* [44]. They classified the cells to normal phase and autophagic phase, and by taking nutrition as the third variable, a logistic type (3D) model of yeast cell population was constructed and analyzed. The model considered in this example has one unstable trivial equilibrium point when the nutrient concentration in the input flux and nutrient loss rate by output flux is constant and a locally asymptotically stable positive equilibrium point when the system is considered without autophagy. The model analysis concluded that an efficient autophagy level might be adequate to sustain a population during a long duration of starvation. However, the author did not incorporate any molecular regulation in their study. A hybrid model consisting of cell population dynamics and molecular regulation could has provided a better insight into cell fate regulation by autophagy. Addressing this issue, the same group later developed a hybrid model [49] to understand the molecular regulation and population dynamics of yeast by incorporating molecular level interactions, the amino acid exchange between cells, and cell behavior.

ODE models are also built to predict optimal drug schedules to control autophagy. Shirin *et al.* [73] formulated a nonlinear ODE model to predict optimal drug schedules to control autophagy. Focusing on four autophagosome production influencers and their specific inhibitors, the model figured out various drug pairs that are more effective when taken together. Mathematical models can qualitatively estimate the protein levels that are capable of deregulating homeostasis, like Ouzounoglou *et al.* [74] formulated a model to understand the dynamics of Alpha-synuclein (ASYN) in Parkinson's disease.

Autophagy and apoptosis pathways are closely regulated, and some proteins, which regulate autophagy, can also regulate apoptosis [75–78]. Hence, proper knowledge of autophagy

Table 1. Some mathematical definitions

Equilibrium point or steady-state: An equilibrium point or a steady-state of a system of differential equations is the value of the state variables where the state variables do not change with time. In other words, it is a time-independent solution of the system.
Stability and instability of an equilibrium: An equilibrium is said to be stable if solutions starting close to the equilibrium point remain close, otherwise it is said to be unstable.
Bifurcation: Bifurcation in a system occurs when a small perturbation made to the parameter values of the system results in a sudden qualitative or topological change in its behavior. Such parameters are called bifurcation parameters.
Bistability: In a dynamical system, bistability means the system under consideration possesses two stable states.

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and apoptosis interconnections may help to stop or promote fatal cell decisions. Kapuy *et al.* [79] studied beclin1-mediated autophagy and caspases-mediated apoptosis by forming an ODE model. The model was built to address the B-cell lymphoma 2 (BCL2)-Beclin1-caspases minimal network. They have also considered the effect of stress on autophagy by taking it as a bifurcation input. Based on the observation, it was suggested that the autophagy apoptosis transition is adjudicated by a bistable switch and, depending upon the intensity and duration of stress levels, sequential activation of cellular response can be initiated by a combination of BCL2-dependent regulation and feedback loops between Beclin1 and caspases. Various other models have also been built to understand the autophagy–apoptosis interplay [80–82]. A key feature of autophagy is that it also plays a role in unfolded protein response (UPR). A literature study has revealed that the complex interactions between UPR, autophagy and apoptosis may determine cellular fate in response to drug treatment [83,84]. Cyto-protective or cyto-destructive UPR gets activated by anti-oestrogens or other drug therapies. Autophagy assists in the cyto-protective role of UPR, while the cyto-destructive role contributes to apoptosis [85]. Addressing these, a mathematical model of autophagy, apoptosis and UPR was proposed to understand the interactions that accomplish anti-estrogen resistance and the effects of GRP78 on both sensitive and resistant breast cancer cells [85]. The model provides a clear picture of interactions of autophagy, apoptosis and UPR to produce both sensitivity and resistance to antioestrogen therapy under various conditions.

The time delay associated with any biological process are not facilitated by ODE-based models. This is mainly addressed by DDE. These models address the time lags between biological processes and thus offer a better portrayal of biological systems. Time lag plays a vital role in autophagy, as in many biological processes. Various studies have implemented DDE-based mathematical modeling to understand the hidden mechanisms in the autophagy process. For example, in autophagy, the formation of autolysosome follows autophagosome formation indicating a time delay. Han *et al.* [47] formulated an 8D model using the delay to study the behavior of both resident (normal) and abnormal proteins along with the formation of autophagosomes and autolysosomes, the intracellular concentration of adenosine triphosphate (ATP), and amino acids. The study showed that intracellular levels of autophagosomes and autolysosomes display an oscillatory behavior. The same group later formed another mathematical model to explore the role of autophagy in the protein/organelle quality control when exposed to different physiological perturbations [86] and further extended their study to Alzheimer's disease [87].

ODE-based models do not consider the effect of noise, which is an inherent property in many dynamical systems. This property is addressed by the SDE models, as done by Martin *et al.* [46], who studied autophagy vesicle dynamics in a single cell.

They used live-cell fluorescent microscopy to measure the synthesis and lysosomal turnover of autophagic vesicles (AV). The data were used to build a 4D ODE model, followed by a 23D SDE model for the accurate prediction of AV dynamics in a cell. The SDE model has implemented a sequence of biochemical and physiological steps in the autophagic pathway from PtdIns3KC3 activation through LC3 conjugation that comprises of the nucleation of the phagophore, maturation of the AV and lysosomal degradation. The mechanistic model was a better portrayal of the autophagy dynamics in a cell. For example, correlating with the experimental data, the SDE model captured a time lag in the production of AV in response to treatment initiation, but no such behavior could be achieved with the deterministic model. The SDE model was also capable of accurately predicting that an 80% decrease in *ATG9* content would result in a corresponding reduction in vesicle synthesis rate. It also stated the correlation between AV size and LC3 levels across single cells. The study can be taken as an example to quote that although ODE models are less complicated and can portray biological behavior, SDE models are a better illustrator of biological phenomena.

Agent-based models for autophagy

ABM is an alternative approach that relies on a predefined logical programming language. In ABM, the system consists of a collection of interacting autonomous decision-making bodies known as agents. ODE modeling presupposes a homogeneous environment, while ABM is capable of simulating a transient and spatial evolution of a system that each participant in the model is represented as an individual agent per its laws. One of the fundamental aspects of ABM is the occurrence of complex behavior from a set of simple rules. It simulates the interactions between multiple independent agents and evaluates their effect on the overall system. It captures the emerging phenomena of a complex system from the perspective of its constituent components, making ABM a bottom-up approach [88]. The benefits of ABM include their flexibility, the natural way of description of the system and the capability of capturing the emergent phenomena due to the interactions of individual entities [88]. ABM facilitates both discrete and continuum mathematical modeling approaches. The study of tumor cell density, nutrient distribution, etc. comes within the radar of the continuum modeling approach, whereas cellular automation is a representation of discrete mathematical modeling.

ABMs have been used extensively to explain biological phenomena in various biological systems. For example, a 3D agent-based Voronoi–Delaunay hybrid model was developed by Schaller and Meyer-Hermann [89], where reaction–diffusion equations depicted the spatiotemporal distribution of oxygen and glucose. Their study was an effort to test the hypothesized functional dependence of the absorption rates of glucose and

oxygen, and to determine suitable mechanisms for necrosis induction. Another ABM was built by Engelberg *et al.* [90], where different spaces for tumor cells, oxygen, nutrient and toxic inhibitors were considered. The goal of the study was to create a model consisting of separate cells that fairly represents the behavior of an *in vitro* multicellular tumor spheroid.

However, the applications of ABM to study autophagy are very few. The creation, movement, fusion and deterioration of autophagy pathway vesicles are dynamic both temporally and spatially. To delineate the spatio-temporal aspects of autophagy regulation and its dynamic behavior, Borlin *et al.* [91] has constructed an ABM using the NetLogo ABM platform. The first agent being phagophore, which grows and matures to form the second agent autophagosome, which then fuses with the third agent lysosome to generate the last agent autolysosome. The newly formed autolysosomes can then either fuse with lysosomes, autophagosomes or other autolysosomes to grow. They inferred spontaneous motion for phagophores and autolysosomes to simulate organelle movements, while autophagosomes and lysosomes travel directly toward or directly away from the nucleus to replicate their active transport along the cytoskeleton, at a pace that is independent of its size. The key parameters of the model were fitted with an iterative method using a genetic algorithm and a predefined fitness function. The model, integrated with high-resolution fluorescence microscopy data, could successfully reproduce the short-term and long-term behavior and cell-to-cell variability.

Biological processes like autophagy involve complex mechanisms with many pathways and molecules that change over time and space, and understanding such systems ABM would play a vital role. These models can also help to mathematical portraiture of the biological phenomenon like the spatial and temporal requirement of autophagy-related protein to bacteria [92]. However, ABM has certain drawbacks. For instance, it demands more details to be provided about the system of interest, which may not always be reported in the literature. Another disadvantage of ABM is that it is more computationally expensive than partial differential equations (PDE) or ordinary differential equations.

Petri net

Petri net is the creation of Carl Adam Petri in his doctoral dissertation [93]. It is constructed using two types of nodes, viz. places, depicted as circles, and transitions represented as narrow black rectangles. In systems biology, places refer to chemical species such as metabolites, proteins, enzymes, DNA, RNA, etc. and transitions refer to chemical reactions such as activation, inhibition, phosphorylation, etc. Nodes are connected by arcs, which may only be directed from place to transition (input arcs) or transition to a place (output arcs). A Petri net is always bipartite. The stoichiometry of a reaction is indicated by the weight of the arc. Although initially designed to model only discrete processes, improvements have been made in Petri nets to deal with a continuous process [94,95].

Literature has witnessed many applications of Petri nets to different biochemical systems. For example, Koch *et al.* [96] built a metabolic Petri net (where the places represent metabolites and the transitions represent the biochemical reactions between metabolites) consisting of 17 places and 27 transitions, that qualitatively modeled the carbon metabolism in the potato tuber. Using this Petri net model as an example, the author has provided a method for model validation of metabolic networks using Petri net. Signal transduction pathways are commonly modeled with a set of ordinary differential equations,

but unknown parameter estimation is a problem inherent with ODE modeling. To deal with this problem, Sackmann *et al.* [97] implemented the Petri net theory to model and analyze signal transduction pathways. The authors put forward a systematic model validation method for signal transduction pathways that depends only on the network structure. This method is then illustrated using the mating pheromone response pathway in *Sacchromyces cerevisiae*.

Minimal literature is available on the use of Petri net in the study of autophagy. Jennifer *et al.* [98] studied the *Salmonella* xenophagy in epithelial cells by designing a Petri net model. The model includes all biochemically proven and published processes of *Salmonella* xenophagy in epithelial cells and comprises 61 places (proteins/macromolecular complexes/organisms/signals) and 184 arcs. The model consists of 16 T-invariants describing biological subpathways in steady-state and represents the fundamental dynamics of the system. The author has implemented *in silico* knockouts of specific proteins to investigate the model behavior and the corresponding biological effect. So, the Petri net model helps to combine different molecular processes like ubiquitination, binding of the autophagy receptor, etc., which can then be used for various analyses like *in silico* knockout experiment. This type of model is advantageous in the absence of quantitative data. So, in the field-like autophagy, where a lot of pathways are involved, we believe the Petri net model would play a vital role. However, it has the limitation that it will not capture the mechanism, which one can obtain with the help of differential equation-based models.

Limitations of mathematical modeling

Despite being an excellent approach to study biological system dynamics, mathematical modeling possesses certain limitations and difficulties. These limitations must be taken into account in capturing the characteristics of a certain biological process with the help of mathematical modeling. Equations in a mathematical model contain parameters, and mathematical models are driven by these parameters. These parameters can be determined by experimental studies. However, many parameters still remain unknown because either the relevant experimental data are not available or the parameter values obtained in the literature are not from the system addressed by the model. For example, in a lung cancer model, the rate of degradation of beclin1 is a parameter, but in literature, this parameter value is reported in pancreatic cancer. Another difficulty in mathematical modeling is the different functioning time of various components of a pathway. For example, genetic regulatory processes are caused by metabolic reactions, but while the time taken by metabolic reactions is in seconds or minutes, the regulatory processes could occur for several hours or days. A mathematical model of a biological system should always be abided by biological constraints. The findings of the model need to be validated according to the objectives of the model. Hence, a qualitative or quantitative association of model output and biological data is very much necessary. But quantitative experimental data on the time course of interaction between model variables are often very limited. Biological systems possess hierarchical layers (cells–tissue–organs etc.). To understand a system, it is necessary to understand the dynamics of each layer. However, it is hard to model the entire system as a whole as the model formulated would be non-computable. Hence, modeling is limited to study the system in parts that necessitate the emergence of system biology approaches like network analysis, which can study the entire system as a whole.

Systems biology approach for autophagy

Importance of systems biology and network analysis

Biological systems can be portrayed as networks, and these networks depict the physical and spatial organization of the organism. Systems biology employs a pragmatic approach to elucidate the emergent properties of such networks with the aim of quantitative explanation and to foresee the biological processes occurring at molecular, cellular, tissue, organ and whole-body level. It focuses on a holistic analysis of biological networks of various processes, including autophagy. The process of autophagy is governed by a large number of proteins as being a protective and life-sustaining process; it is associated with various physiological processes and pathological conditions. Systems biology quests for the understanding of the extent to which the intermodular connectivity modulates the autophagy process. The systems biological approaches, especially the network analysis, are necessary to decipher the crosstalk between autophagy and various diseases.

Network analysis investigates the entire system as a whole. It is like a snapshot of the entire system at a particular time, where we can see all the nodes and their interactors. In systems biology, there are various types of the network depending on the nodes studied, such as protein– PPI network, where nodes are proteins, and the edges are the interaction between them; metabolic network, where the nodes are metabolites and the edges are the reactions between them; gene regulatory networks, where nodes are genes and edges are the physical and/or regulatory relationships between the genes; ecological networks, where species are nodes and edges are the interactions which can be either trophic or symbiotic. In this review, we have focused on PPI networks. They are scale-free networks in nature, i.e. the majority of proteins possess only a few interactions with other proteins. In contrast, some proteins are connected to many other proteins in the network and are coined hub proteins. The degree distribution of a PPI network follows a power law. The diameter of a PPI network (the shortest distance between two distant nodes) is smaller. This confirms the fact that these networks show a small world effect. Another essential property of PPI networks is transitivity, which is the proneness of proteins to form clusters together.

In systems biology, network analysis can be divided into two categories. Enrichment analysis belongs to the first category. Some genes possess similar biological attributes like involvement in disease, common pathways or localizations. Enrichment analysis helps to classify these genes and points out essential processes, pathways or diseases in a gene list generated from genome-scale experiments. The second category is based on algorithm-related works where the goal is to find potential targets, i.e. to find important nodes in a network. Since the word importance is vague, it gives rise to the centrality analysis, where many methods have been used to find the important nodes of the system. Some of these methods include degree centrality, betweenness centrality, radiality, clustering coefficient, etc. Novel algorithms have also been developed to find important nodes in a PPI network [99–101]. Although distinguished, these studies are not mutually exclusive. In literature, evidences of both these categories studying together have also been reported [99,102].

Many packages across various platforms have also been used to perform the network-based study. We have enlisted a few useful and most used packages in Table 2. Proper visualization of data is crucial for understanding the biological network. There is various visualization software that exist [103–111], but four of the most widely used visualization and analysis software are Cytoscape [112], Gephi [113], Tulip [114] and Pajek [115]. Web tools like CellNetVis [116], Biographer [117] are also used for network visualization purposes. A detailed review of different types of visualization software in biological networks can be found in the study of Pavlopoulos *et al.* [118]. Table 3 contains some useful Cytoscape plugins used in network analysis, visualizations and enrichment analysis. A detailed review of the Cytoscape plugins can be found in [119]. The next section will provide a concise description of network analysis studies done in autophagy (in humans only).

Omics technologies and autophagy

The emergence of omics technologies such as genomics, epigenomics, transcriptomics, proteomics, metabolomics, microbiomics, etc. has embraced new possibilities to study a biological system to an extraordinary detailed level. Genomics is the study of the genome of an organis, epigenomics aims at exploring global epigenetic changes that offer crucial insights to mechanisms and function of gene regulation across several genes in a cell or organism, transcriptomics relies on the qualitative and quantitative genome-wide study of RNA levels, while proteomics facilitates the study of the whole proteome of an organism [120]. Mass spectrometry-based proteomics is an indispensable approach to delineate protein expression, PPI, subcellular localization and post-translational modifications. Similarly, metabolomics is the large-scale study of metabolites within cells, biofluids, tissues or organisms [121]. The study of the microorganism in a given community comes under the focus of microbiomics [120]. Throughout the times, new dimensions have been added to omics such as lipidomics, nutrigenomics, etc. Omics methods produce data to provide biological understanding based on methodological inferences from large (in most cases) datasets. These technologies allow different molecular level investigations of the system that, too in a highly parallelized manner. For example, the microarray analysis measures the expression of almost all the genes in the system at a time. This parallelization encourages scientists not only to track an organism's anticipated but also unexpected responses.

The integrated method of these omics strategies and PPI networks would enable a better understanding of the autophagy process. There are studies that incorporate a large-scale multi-omics approach to study the broad framework of autophagy and its association with other biological processes. These studies have deciphered the role of autophagy in host–pathogen interactions, tumor growth, various cancers, nervous systems etc. [122–125]. Considering that 'omics'-based studies are a pivotal area of current research to provide a more systematic view of biological processes, these approaches have driven our insights into the regulation of autophagy.

Network analysis for autophagy

Throughout the decades, the advancement of high-performing data collection technology has resulted in a large number of autophagy-related data. Network analysis approaches have been implemented in these data to delineate the association of autophagy with various diseases and biological processes. Network analysis also helps to uncover the organizing principles of a disease and identifies the potential targets accountable for the pathogenesis of the disease.

Table 2. List of useful packages and software for the network-based study of systems biology

S. no.	Package/software and Link	Platform	Description	Ref.
1	dplyr https://CRAN.R-project.org/package=dplyr	R	The dplyr is a powerful R package that facilitates the manipulation, cleaning and summarizing unstructured data. It comes with many functions that perform widely used data manipulation operations such as specific column selection, applying a filter, data sorting, addition or deletion of columns, and aggregating data. The functions of dplyr are very user friendly and are very easy to learn.	[155]
2	ggplot2 https://CRAN.R-project.org/package=ggplot2	R	ggplot2 is an excellent data visualization package and is based on the grammar of graphics. It is a flexible, mature and complete graphics system, but it has some cons like it is not capable of 3D graphics.	[156]
3	Bioconductor https://www.bioconductor.org/	R	The Bioconductor project is a collaborative effort to create computational biology and bioinformatics extensible packages and software. It uses the R programming platform and is open source and open development. A total of 1903 software are available in the latest release of bioconductor (3.11)	[157]
4	mlr https://CRAN.R-project.org/package=mlr	R	mlr is an R package to perform ML tasks. This package offers a standardized, object-oriented and extensible framework for classification, regression, survival analysis and clustering.	[158]
5	linear models for microarray data (limma) https://bioconductor.org/packages/release/bioc/html/limma.html	R	This R package is used for the analysis of gene expression data coming from microarray or RNA-seq technologies.	[159]
6	Weighted correlation network analysis https://CRAN.R-project.org/package=WGCNA	R	WCGNA is a popular R analytical package that constructs a gene co-expression network and identifies gene modules. Biologically or clinically significant modules are then determined and topological properties of the network are evaluated.	[160]
7	biomaRt https://CRAN.R-project.org/package=biomartr	R	This open-source R package incorporates easy and user-friendly functions to capture all genomic data or data for selected proteomes, genomes, coding sequences and annotation files contained in the databases hosted by the National Center for Biotechnology Information (NCBI) and European Bioinformatics Institute (EMBL-EBI).	[161]
8	DESeq2 https://bioconductor.org/packages/DESeq2/	R	DESeq2 is a widely used method for differential expression analysis of count data and freely available through Bioconductor. The differential expression tests of DESeq2 are based on a negative binomial generalized linear model. DESeq2 has several new features that allow for more quantitative analysis of RNA-seq comparative data with shrinkage estimators for dispersion and fold change.	[162]
9	CFinder http://www.cfinder.org/	–	CFinder is a platform-independent, stand-alone application that locates overlapping groups of densely interconnected nodes in a network with the aid of the clique percolation method. It can predict the function(s) of a protein and can also be used to discover novel modules.	[163]
10	Enrichr https://amp.pharm.mssm.edu/Enrichr/	–	This is a useful web-based and mobile software application to perform gene enrichment analysis and is facilitated by various interactive visualization approaches to display enrichment results. Enrichr uses different databases for the enrichment analysis and displays results obtained by each database separately.	[164]
11	PyPathway https://pypi.org/project/pypathway/#files	Python	PyPathway is a free and open-source python package that performs functional enrichment analysis, network modeling and network visualization.	[165]

The network analysis is well supported by autophagic databases, which play a crucial role in delineating the role of autophagy in various diseases. Various studies have been done by the implementation of the specific autophagic information obtained from these databases [123,126,127]. Lin *et al.* [127] carried out a comprehensive study of ATG and associated noncoding RNAs and transcription factors to investigate the association of autophagy with digestive system tumors (DST).

Table 3. List of useful Cytoscape plugins. Among these, BINGO, MCODE, Agilent Literature Search and jActiveModules are the four most downloaded Cytoscape plugins in the order they are mentioned [119]. All these plugins are freely available at Cytoscape app store (https://apps.cytoscape.org/)

S. no.	Plugin	Description	References
1	BiNGO	Quantifies GO terms that have been overrepresented in the network and portrays them as a network of relevant GO terms.	[166]
2	Mosaic and cerebral	These two are visualization plugins for Cytoscape and can compartmentalize the genes/proteins in a network according to their subcellular localization.	[167,168]
3	PathLinker	This package reconstructs signaling pathways from PPI networks.	[169]
4	CytoNCA	Perform centrality analysis of weighted and unweighted networks.	[170]
5	ClueGO	It helps to create and visualize a functionally grouped network of terms/pathways.	[171]
6	GeneMANIA	Uses public databases to import interaction networks from a list of genes with their annotations and putative functions.	[172]
7	BiNoM	It helps to access and analyze pathways.	[173]
8	PiNGO	Helps to locate candidate genes in a network that are linked with user-defined target GO terms.	[174]
9	MCODE	Create clusters in a given network based on the topology to identify densely connected regions.	[175]
10	ConsensusPathDBplugin	Retrieves interaction evidence for a given pair of genes or proteins.	[176]
11	AgilentLiteratureSearch	Mines scientific literature to find publications associated with the search term and to create an interaction network based on the search result.	[177]
12	jActiveModules	Detects clusters where nodes show significant changes in expression levels.	[178]
13	cytoHubba	By using various topological algorithms, this Cytoscape plugin can predict and find important nodes and subnetworks in a given network.	[179]

The Cancer Genome Atlas database was used to get the digestive tumor transcription details. The autophagy genes were extracted from the Human autophagy modulator database. The study, facilitated by WGCNA, crosstalk connection, pivot analysis and functional analysis, revealed that the autophagic genes control the pathogenesis of DST and highlighted the potential role of autophagy in the treatment of DST. Wang *et al.* [123] constructed a disease autophagy network where disease genes were taken from online Mendelian inheritance in man (OMIM) [128] and autophagic genes were extracted from the human autophagy database (http://www.autophagy.lu/), the autophagy database [129] and the autophagy regulatory network database [130]. The autophagy genes were observed to act as a bridge between diseases and were found to be topologically important in the disease–autophagy network.

Network-based studies often facilitate the identification of hubs and modules. Modularity is an essential property of a network. It refers to the organization of nodes in clusters. Module-based analyses can contribute to a deeper understanding of biological systems. Hub proteins are also crucial in maintaining the global network structure. A study carried out by Durocher and co-researchers [55] to elucidate the gene network in the peripheral blood transcriptome associated with human intracerebral hemorrhage. Using the WGCNA package in R, they identified the hubs and the modules in the network, and used ingenuity pathway analysis (IPA) and the DAVID Bioinformatics Database [131] to find the associated pathways and processes.

Various studies [53,54,132] have performed a network-based analysis on autophagy by using the dataset obtained from the Gene Expression Omnibus (GEO) repository [133]. After following the preliminary analysis, the WGCNA package in R has been used to identify significant modules and hubs in the network. Databases like OMIM and the cancer genome atlas (https://www.cancer.gov/tcga) have also been used to facilitate the network-based study of autophagy processes.

Although network analysis approaches have been applied extensively to study autophagy, methods like network stability, control theory, percolation, etc., are yet to be integrated to study the autophagy process. Given the importance of these methods, their implications will surely help to identify novel targets and pathways related to autophagy. The lack of sufficient temporal data to understand a disease progression has also limited the network-based study of autophagy processes. Nonetheless, with time the data are growing, and we believe in the coming years, we will have enough data to make better and more accurate predictions.

Artificial intelligence associated research of autophagy

With the recent explosion of data, the applicability of artificial intelligence (AI) is getting excellent attention and has emerged as a promising field in systems biology. It creates algorithms that help in the classification, pattern recognition and predictions using available data. As in many other biological processes, AI-based approaches have also been incorporated in the field of autophagy. In a recent study, Zhaoyue *et al.* [134] applied machine learning (ML) techniques to classify renal cell carcinoma (RCC) subtypes using autophagy proteins. The expression data of the key autophagy proteins in RCC were measured by immunohistochemical images. The data were then normalized with mean and standard deviation. The ML algorithm, K-Nearest Neighbor (KNN) algorithm, was applied to the normalized data for classification. Their study identified the basal level of autophagy as a potential measurement for discrimination of RCC. In an early work, by Janos and co-researchers [135], an image analysis pipeline was developed using the support vector machine for the determination of novel selective pharmacological inducers of autophagy in human cancer cell lines. A variety of software

Table 4. Some of the most used databases in autophagy. The features of these databases are shown in Figure 3

S. no.	Name	Full form	URL	Ref.
1	HAMDb	Human Autophagy Modulator Database	http://hamdb.scbdd.com	[180]
2	ARN	Autophagy Regulatory Network	http://autophagyregulation.org/	[130]
3	Autophagy database	Autophagy database	http://www.tanpaku.org/autophagy	[129]
4	ncRDeathDB	The noncoding RNA (ncRNA)-associated cell death database	http://www.rna-society.org/ncrdeathdb	[181]
5	ACDB	Autophagic compound database	http://www.acdbliulab.com	[182]
6	THANATOS	Autophagy, Necrosis, Apoptosis OrchestratorS database	http://thanatos.biocuckoo.org	[183]
7	HADb	Human Autophagy Database	http://www.autophagy.lu/	–
8	AutophagySMDB	Autophagy Small Molecule Database	http://www.autophagysmdb.org	[184]
9	ATD	Autophagy to disease	http://auto2disease.nwsuaflmz.com	[185]
10	iLIR	*In silico* identification of functional LC3 interacting region motifs database	https://ilir.warwick.ac.uk	[186]
11	ATdb	Autophagy and Tumor Database	http://www.bigzju.com/ATdb/#/	[187]

incorporating a broad range of ML algorithms has been developed recently. For example, Serrano *et al.* [136] have used the software Scikit-learn [137] to study the effect of mRNA alterations of some autophagic genes, one proapoptotic gene and one antiapoptotic gene in HIV-infected patients effectively treated with combined antiretroviral therapy.

In the past two decades, the pharmacological modulation of autophagy has gathered a great deal of attraction. The process of autophagy gets manipulated by various autophagy modulators. ML methods can be blended to study the mechanism of actions of these autophagy modulators to gain knowledge on various factors that include side effects, drug repurposing and development of novel polypharmacological strategies [138]. AI approaches are powerful tools that associate important molecular changes with an observed phenomenon. However, these approaches remain silent on the underlying mechanism for such observations. To capture the possible mechanism, we need to take help from differential equation-based models.

Databases with the information related to autophagy

Biological databases play a central role in systems biological studies. They offer the opportunity to access a wide variety of biologically relevant data, which include PPI information, disease-protein association information, microarray, next-generation sequencing, protein localization, post-translational modification, the structural details of a protein or compound and pathways associated with proteins, etc. However, databases containing exclusively autophagic information are very few. In Table 4, we have enlisted 11 most used databases in autophagy. These databases contain various information like disease associations, pathways, the specific effect on autophagy, etc. In Figure 3, we have compared the features of these databases.

Data repositories like GEO [133] and Array Express [139] can also be used for mining information such as microarray, next-generation sequencing and other forms of high-throughput functional genomic data. There are currently 510, and 181 studies on autophagy are available in GEO and ArrayExpress, respectively. The information regarding the biological pathways and diseases can be derived from databases such as the Kyoto Encyclopedia of Genes and Genomes (KEGG) [140] and reactome [141].

One single process and various computational approaches: which door to choose?

The Mathematical modeling and network analysis approaches can grasp the underlying dynamics and topology of any biological system. We have summarized the applications of mathematical and computational biology tools to study autophagy with differential environmental conditions (Figure 4). Nevertheless, the complexity and the choice of the approach can vary from system to system, depending on the perspective of the study. A mathematical model, as we have already stated, unveils the fundamental nature of a biological phenomenon. From the initiation to degradation, the process of autophagy comes under the influence of many proteins and stresses. Taking a few or all of them together, a mathematical model helps to understand how the dynamics of these sets of proteins influence the progression of autophagy by taking a deterministic approach. These models can predict cellular fate through autophagy by using a suitable set of parameters and a core set of autophagy modulators. They can also be used to study the randomness in the process of autophagy occurring due to the variability of the stress and frequent changes in the cell's energy requirements. ABM can range from continuous to discrete based on the requirement. Petri nets facilitate both the qualitative and quantitative models and hence can be used to model the involvement of autophagy in the cellular biochemical reactions.

On the other hand, network theory can be used to identify crucial autophagy-related proteins responsible for the progression of diseases. Different sets of targets will be obtained for the same disease owing to the method applied, which hence will further require a biological validation. For example, if the intention is to select only the most connected proteins, the proper method will be to measure the degree centrality. But, if the goal is to find the proteins that can disperse information very effectively, the closeness centrality would be the best approach to consider. Contrary to the analysis of the topology of the system by network analysis, enrichment analysis focuses on extracting the pathways, localization and functions of the proteins present in the disease network. These pathways can then further be studied by constructing an autophagy specific PPI network to detect influential proteins in that pathway.

In the conclusion of this section, the choice of a computational approach depends on the perspective of the study. Although modeling methods always possess limitations in terms of the realistic portrayal of the biological phenomenon, they

Figure 3. Comparison of some of the well-explored autophagy databases in literature. The columns contain the name of the databases, and features are placed in the row. The orange color means that the particular feature is present in the database, and the blue color means it is absent. Here, the agent feature includes drugs, chemicals and small molecules. Only autophagy-related proteins are considered for the ARN database. (## autophagy related genes extracted using text mining, # human autophagy related proteins).
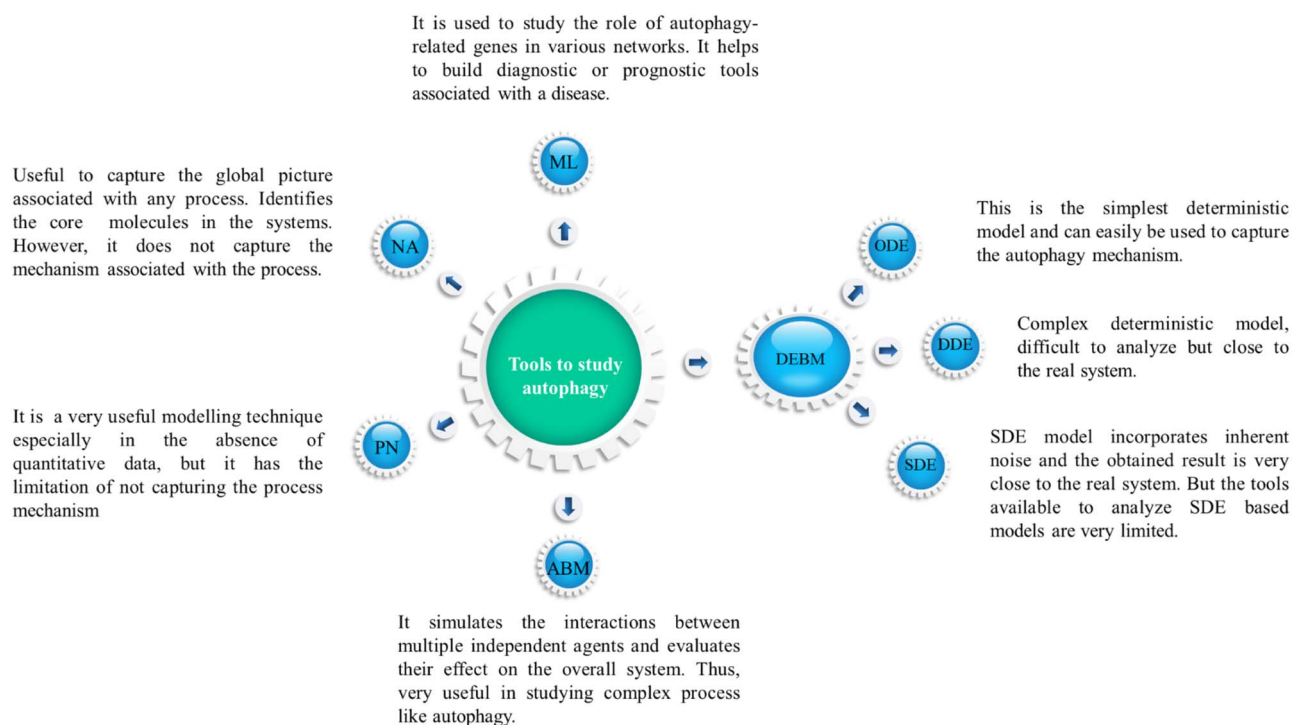
Figure 4. Application of mathematical and computational biology tools to study autophagy in different environmental conditions. Abbreviations: DEBM, differential equation-based mathematical models; ABM, agent-based model; PN, Petri net; NA, network analysis and ML, machine learning.

can still postulate conditions, parameters or factors that are necessary to govern the system.

Future direction

The number of papers published in autophagy is growing year after year, showing its increasing importance. But the ratio of computational studies to experimental studies is meager. For a better insight into the dynamics of the autophagy pathways, these gaps need to be narrowed down. Computational methods are rapidly evolving. Although the literature has reported the application of various computational methods in systems biology, many methods are yet to be appended to the autophagy domain, like models based on PDE and image-based modeling (IBM).

PDE-based modeling is well established in many biological fields. Crucial insights into biological systems can be obtained by the implementation of PDE as they are used to classify the spatiotemporal evolution of biological systems. PDE, unlike ODE, investigates spatial patterns of inherently heterogeneous systems with regionally varying fields. A literature study has revealed the application of these models in various fields, including bone remodeling [142], dengue [143] and cancer [144]. Studies complimented with PDE and ML approaches have also opened a new window for disease treatment [145,146]. The same goes for IBM, which relies on interpreting images as quantitative measurements. It acts as a bridge between systematic quantitative image data collection and spatiotemporal systems modeling. The first step in IBM is to collect images and to quantify objects, shapes, intensities or motion trajectories [147]. This step is succeeded by the model formulation part. The domain of the models can be divided into four categories, viz. discrete/stochastic, discrete/deterministic, continuous/stochastic and continuous/deterministic [148]. This step is followed by a simulation phase. Different types of models require various forms of simulations, which can be either custom made or some predefined methods/software packages [149]. The last phase of IBM is the parameter estimation and validation part to check whether the output of the formulated model correlates with the desired output or not. A lot of studies have been done on various diseases with IBM [150–152].

The process of autophagy has been a prominent focus of research as it is still a puzzle with various missing pieces due to its complex mechanism in numerous biological processes and diseases. AI-based approaches could be the ideal tools to locate these missing pieces. Such methods can be used to capture the association of autophagy with various other diseases to gain a more comprehensive insight. For example, the complex role of autophagy in cancer is yet to be fully elucidated. Integration of ML in cancer to study the role of autophagy can help a detailed insight to the process as well as to cancer prognosis and personalized medicine approach. The ML methods can also help to identify autophagy related genes and pathways which have not been addressed before.

From a network analysis perspective, temporal analysis of genes is done in diseases like obesity [100], but none has been reported in autophagy. Such a study will help to decipher the change in behavior of ATG with respect to time and identify potential drug targets. A structure-based study can be done to determine the possible binding sites of the targets. The identification of binding sites of a protein can help in the rational designing of the therapeutic agents [153,154]. This study can also be facilitated by the mathematical modeling of the identified targets. Such a pipeline based study in autophagy is lacking and will surely help to provide fruitful insights into the autophagy process.

Conclusion

Autophagy is a quintessential biological process that breaks down the unwanted cellular constituents and thus plays a crucial role in maintaining cellular homeostasis. Due to its immense importance toward the modulation of cellular fate, the process of autophagy remains at the crossroads of various cellular processes and signaling pathways. The tracking of signals that modulate autophagy, and genes, which have a role in the autophagy process, has encouraged detection and controlling of the autophagy pathway, and any hindrance to either of them may lead to various diseases. In the last decade, there is eminent progress in understanding the role of autophagy in different processes, but the underlying mechanism that leads to the observed phenomenon is still far from being captured. This is because autophagy is a very complex process and exhibits different behaviors depending upon the situation. To understand the underlying complexity, comprehensive knowledge of autophagy is important, necessitating a joint effort of experimental and theoretical biologists to unravel this complexity.

Decades of research on autophagy have resulted in considerable accumulation of experimental data. Comprehensive information on the core set of proteins that govern the process of autophagy and their underlying dynamics can be availed by the implementation of system biology approaches on these data. In this review, we attempted to elucidate how these approaches are being used for the study of the autophagy process, both from modeling and network perspectives. For the benefit of the readers, we have provided multiple examples from different types of mathematical models and computational tools that are applied to understand the autophagy process. We have shown that mathematical modeling is a way to illustrate the underlying complex dynamics of the autophagic process emanating from the interaction of the individual components of the system. However, modeling approaches possess various limitations like complexity, parameter values and lack of experimental data for model validations. We have also shown that to get a global perspective of the process, we need to take the help of systems biology tools such as network analysis that will decipher the crosstalk between autophagy and various diseases. Despite various systems biology tools, the retrieval of valuable information from omics data is still a challenging task in computational biology, and AI associated research has exhibited an unprecedented performance in doing so. These approaches can detect and predict the association of autophagy genes with various diseases leading to a more detailed understanding of the disease. By the identification of crucial autophagy-related proteins, AI can provide researchers more holistic insight into the complex autophagy process. We have discussed the different environmental conditions in autophagy and illustrated that the choice of analysis depends upon the question that a researcher wants to address.

Our present review is aimed at reaching a broader audience and supporting researchers with and without prior expertise on mathematical and computational tools. We have, therefore, provided some essential tools, plugins and software that are being used by researchers to study and visualize biological systems. The hope is that, by such means, this review will help researchers working in the field of autophagy to analyze their generated data in a better way. We have also drawn attention to some of the possible and helpful applications of computational methods that

are yet to be integrated to study autophagy. In other words, our study limits autophagy with computational tasks that have been done before and can be performed in the near future, and thus serves as the footsteps of autophagy in computational biology.

> ### Key Points
>
> - Autophagy is a quintessential evolutionary conserved process and plays a crucial role in maintaining cellular homeostasis through the degradation of unwanted cellular materials.
> - Computational biology approaches like mathematical modeling and network analysis have been applied to delineate the underlying dynamics of complex autophagy mechanisms.
> - These approaches are bound up with constraints such as complexity, realistic description of the biological system, etc., and thus the method to be implemented should be chosen very carefully.
> - Although there is a plethora of PPI databases available in the literature, databases containing exclusively autophagic information are very few.
> - With the rapid development of the field, current challenges lie in how to choose the appropriate computational method that will address the problem one is studying.
> - Mathematical modeling enhances the mechanistic understanding of a system but works better in parts, while a network studies the changes associated with the whole system. For a better understanding of the process of autophagy, it is of utmost importance to link these two approaches and study the pipeline connecting them.

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Conflicts of interest

No potential conflicts of interest were disclosed.

References

1. Deter RL, De Duve C. Influence of glucagon, an inducer of cellular autophagy, on some physical properties of rat liver lysosomes. *J Cell Biol* 1967;**33**:437–49.
2. Singh R, Cuervo AM. Autophagy in the cellular energetic balance. *Cell Metab* 2011;**13**:495–504.
3. Levine B, Kroemer G. Autophagy in the pathogenesis of disease. *Cell* 2008;**132**:27–42.
4. Dice JF, Walker CD, Byrne B, *et al*. General characteristics of protein degradation in diabetes and starvation. *Proc Natl Acad Sci USA* 1978;**75**:2093–7.
5. Mortimore GE, Pösö AR. Intracellular protein catabolism and its control during nutrient deprivation and supply. *Annu Rev Nutr* 1987;**7**:539–64.
6. Greabu M, Giampieri F, Imre MM, *et al*. Autophagy, one of the main steps in periodontitis pathogenesis and evolution. *Molecules* 2020;**25**:4338. doi: 10.3390/molecules25184338.
7. He S, Zhao Z, Yang Y, *et al*. Truncating mutation in the autophagy gene UVRAG confers oncogenic properties and chemosensitivity in colorectal cancers. *Nat Commun* 2015;**6**:7839.
8. Takahashi Y, Coppola D, Matsushita N, *et al*. Bif-1 interacts with Beclin 1 through UVRAG and regulates autophagy and tumorigenesis. *Nat Cell Biol* 2007;**9**:1142–51.
9. Kim MS, Jeong EG, Ahn CH, *et al*. Frameshift mutation of UVRAG, an autophagy-related gene, in gastric carcinomas with microsatellite instability. *Hum Pathol* 2008;**39**:1059–63.
10. Kung C-P, Budina A, Balaburski G, *et al*. Autophagy in tumor suppression and cancer therapy. *Crit Rev Eukaryot Gene Expr* 2011;**21**:71–100.
11. Liang T-T, Shao Q, Deng Z-C, *et al*. Systemic expression analysis reveals prognostic significance of WIPI3 in hepatocellular carcinoma. *Front Genet* 2020;**11**:847. doi: 10.3389/fgene.2020.00847.
12. Luo T, Fu J, Xu A, *et al*. PSMD10/gankyrin induces autophagy to promote tumor progression through cytoplasmic interaction with ATG7 and nuclear transactivation of ATG7 expression. *Autophagy* 2016;**12**:1355–71.
13. Liu M, Jiang L, Fu X, *et al*. Cytoplasmic liver kinase B1 promotes the growth of human lung adenocarcinoma by enhancing autophagy. *Cancer Sci* 2018;**109**:3055–67.
14. Gremke N, Polo P, Dort A, *et al*. mTOR-mediated cancer drug resistance suppresses autophagy and generates a druggable metabolic vulnerability. *Nat Commun* 2020;**11**:4684.
15. Kawano Y, Cohen DE. Mechanisms of hepatic triglyceride accumulation in non-alcoholic fatty liver disease. *J Gastroenterol* 2013;**48**:434–41.
16. Pei K, Gui T, Kan D, *et al*. An overview of lipid metabolism and nonalcoholic fatty liver disease. *Biomed Res Int* 2020;**2020**:4020249.
17. Czaja MJ. Function of autophagy in nonalcoholic fatty liver disease. *Dig Dis Sci* 2016;**61**:1304–13.
18. Ciechanover A, Kwon YT. Degradation of misfolded proteins in neurodegenerative diseases: therapeutic targets and strategies. *Exp Mol Med* 2015;**47**:e147.
19. Ebato C, Uchida T, Arakawa M, *et al*. Autophagy is important in islet homeostasis and compensatory increase of beta cell mass in response to high-fat diet. *Cell Metab* 2008;**8**:325–32.
20. Riahi Y, Wikstrom JD, Bachar-Wikstrom E, *et al*. Autophagy is a major regulator of beta cell insulin homeostasis. *Diabetologia* 2016;**59**:1480–91.
21. Lee Y-H, Kim J, Park K, *et al*. β-Cell autophagy: mechanism and role in β-cell dysfunction. *Mol Metab* 2019;**27S**:S92–103.
22. Yang J-S, Lu C-C, Kuo S-C, *et al*. Autophagy and its link to type II diabetes mellitus. *Biomedicine* 2017;**7**:8.
23. Jiang Y, Huang W, Wang J, *et al*. Metformin plays a dual role in MIN6 pancreatic β cell function through AMPK-dependent autophagy. *Int J Biol Sci* 2014;**10**:268–77.
24. Bergamini E. Autophagy: a cell repair mechanism that retards ageing and age-associated diseases and can be intensified pharmacologically. *Mol Aspects Med* 2006;**27**:403–10.
25. Barbosa MC, Grosso RA, Fader CM. Hallmarks of aging: an autophagic perspective. *Front Endocrinol* 2018;**9**:790.

26. Levine B, Klionsky DJ. Development by self-digestion: molecular mechanisms and biological functions of autophagy. *Dev Cell* 2004;**6**:463–77.

27. Mizushima N, Levine B, Cuervo AM, *et al*. Autophagy fights disease through cellular self-digestion. *Nature* 2008;**451**: 1069–75.

28. Dang S, Yu Z-M, Zhang C-Y, *et al*. Autophagy promotes apoptosis of mesenchymal stem cells under inflammatory microenvironment. *Stem Cell Res Ther* 2015;**6**:247.

29. Zhang M, Su L, Xiao Z, *et al*. Methyl jasmonate induces apoptosis and pro-apoptotic autophagy via the ROS pathway in human non-small cell lung cancer. *Am J Cancer Res* 2016;**6**:187–99.

30. Denton D, Shravage B, Simin R, *et al*. Autophagy, not apoptosis, is essential for midgut cell death in drosophila. *Curr Biol* 2009;**19**:1741–6.

31. Denton D, Nicolson S, Kumar S. Cell death by autophagy: facts and apparent artefacts. *Cell Death Differ* 2012;**19**:87–95. doi: 10.1038/cdd.2011.146.

32. Chen Z-F, Li Y-B, Han J-Y, *et al*. The double-edged effect of autophagy in pancreatic beta cells and diabetes. *Autophagy* 2011;**7**:12–6. doi: 10.4161/auto.7.1.13607.

33. Galluzzi L, Pietrocola F, Levine B, *et al*. Metabolic control of autophagy. *Cell* 2014;**159**:1263–76. doi: 10.1016/j.cell. 2014.11.006.

34. Glick D, Barth S, Macleod KF. Autophagy: cellular and molecular mechanisms. *J Pathol* 2010;**221**:3–12.

35. Napolitano G, Esposito A, Choi H, *et al*. mTOR-dependent phosphorylation controls TFEB nuclear export. *Nat Commun* 2018;**9**:3312.

36. Lawrence RE, Zoncu R. The lysosome as a cellular centre for signalling, metabolism and quality control. *Nat Cell Biol* 2019;**21**:133–42.

37. Han J, Pluhackova K, Böckmann RA. The multifaceted role of SNARE proteins in membrane fusion. *Front Physiol* 2017;**8**:5.

38. Wang Z, Miao G, Xue X, *et al*. The Vici syndrome protein EPG5 is a Rab7 effector that determines the fusion specificity of autophagosomes with late endosomes/lysosomes. *Mol Cell* 2016;**63**:781–95.

39. McEwan DG, Dikic I. PLEKHM1: adapting to life at the lysosome. *Autophagy* 2015;**11**:720–2.

40. Jiang P, Nishimura T, Sakamaki Y, *et al*. The HOPS complex mediates autophagosome–lysosome fusion through interaction with syntaxin 17. *Mol Biol Cell* 2014;**25**:1327–37. doi: 10.1091/mbc.e13-08-0447.

41. Schröder BA, Wrocklage C, Hasilik A, *et al*. The proteome of lysosomes. *Proteomics* 2010;**10**:4053–76.

42. Kitano H. Computational systems biology. *Nature* 2002; **420**:206–10. doi: 10.1038/nature01254.

43. Kitano H. Systems biology: a brief overview. *Science* 2002; **295**:1662–4. doi: 10.1126/science.1069492.

44. Jin H, Lei J. A mathematical model of cell population dynamics with autophagy response to starvation. *Math Biosci* 2014;**258**:1–10. doi: 10.1016/j.mbs.2014.08.014.

45. Deter RL. Analog modeling of glucagon-induced autophagy in rat liver. *Exp Cell Res* 1975;**94**:122–6. doi: 10.1016/0014-4827(75)90538-8.

46. Martin KR, Barua D, Kauffman AL, *et al*. Computational model for autophagic vesicle dynamics in single cells. *Autophagy* 2013;**9**:74–92. doi: 10.4161/auto.22532.

47. Han K, Kwon HW, Kang H, *et al*. Dynamics of macroautophagy: modeling and oscillatory behavior. *Physica A:* *Stat Mech Appl* 2012;**391**:686–92. doi: 10.1016/j.physa.2011. 08.046.

48. Han K, Kim J, Choi M. Autophagy mediates phase transitions from cell death to life. *Heliyon* 2015;**1**:e00027.

49. Jin H, Lei J. A hybrid model of molecular regulation and population dynamics for yeast autophagy. *J Theor Biol* 2016;**402**:45–53. doi: 10.1016/j.jtbi.2016.04.019.

50. Schwartz-Roberts JL, Cook KL, Chen C, *et al*. Interferon regulatory factor-1 signaling regulates the switch between autophagy and apoptosis to determine breast cancer cell fate. *Cancer Res* 2015;**75**:1046–55.

51. Kapuy O, Papp D, Vellai T, *et al*. Systems-level feedbacks of NRF2 controlling autophagy upon oxidative stress response. *Antioxidants (Basel)* 2018;**7**:39.

52. Mazza T, Fusilli C, Saracino C, *et al*. Functional impact of autophagy-related genes on the homeostasis and dynamics of pancreatic cancer cell lines. *IEEE/ACM Trans Comput Biol Bioinform* 2015;**12**:667–78. doi: 10.1109/tcbb.2014. 2371824.

53. Wang F, Cho WCS, Chan LWC, *et al*. Gene network exploration of crosstalk between apoptosis and autophagy in chronic Myelogenous leukemia. *Biomed Res Int* 2015;**2015**: 1–9. doi: 10.1155/2015/459840.

54. Ahmed M, Hwang J, Lai T, *et al*. Co-expression network analysis of AMPK and autophagy gene products during adipocyte differentiation. *Int J Mol Sci* 2018;**19**:1808. doi: 10.3390/ijms19061808.

55. Durocher M, Ander BP, Jickling G, *et al*. Inflammatory, regulatory, and autophagy co-expression modules and hub genes underlie the peripheral immune response to human intracerebral hemorrhage. *J Neuroinflammation* 2019;**16**:56.

56. Xiong X, Tao R, DePinho RA, *et al*. Deletion of hepatic FoxO1/3/4 genes in mice significantly impacts on glucose metabolism through downregulation of gluconeogenesis and upregulation of glycolysis. *PLoS One* 2013;**8**:e74340.

57. Dhar P, Meng TC, Somani S, *et al*. Cellware: a multi-algorithmic software for computational systems biology. *Bioinformatics* 2004;**20**:1319–21. doi: 10.1093/bioinformatics/ bth067.

58. Smith RW, van Rosmalen RP, VAP MDS, *et al*. DMPy: a python package for automated mathematical model construction of large-scale metabolic systems. *BMC Syst Biol* 2018;**12**: 72.

59. Lopez CF, Muhlich JL, Bachman JA, *et al*. Programming biological models in python using PySB. *Mol Syst Biol* 2013;**9**:646. doi: 10.1038/msb.2013.1.

60. Schmidt H, Jirstrand M. Systems biology toolbox for MATLAB: a computational platform for research in systems biology. *Bioinformatics* 2006;**22**:514–5. doi: 10.1093/bioinformatics/bti799.

61. You L, Hoonlor A, Yin J. Modeling biological systems using Dynetica: a simulator of dynamic networks. *Bioinformatics* 2003;**19**:435–6. doi: 10.1093/bioinformatics/btg009.

62. Mendes P. GEPASI: a software package for modelling the dynamics, steady states and control of biochemical and other systems. *Bioinformatics* 1993;**9**:563–71. doi: 10.1093/bioinformatics/9.5.563.

63. Emonet T, Macal CM, North MJ, *et al*. AgentCell: a digital single-cell assay for bacterial chemotaxis. *Bioinformatics* 2005;**21**:2714–21. doi: 10.1093/bioinformatics/bti391.

64. Funahashi A, Matsuoka Y, Jouraku A, *et al*. CellDesigner 3.5: a versatile Modeling tool for biochemical networks. *Proc IEEE* 2008;**96**:1254–65. doi: 10.1109/jproc.2008.925458.

65. Cuellar AA, Lloyd CM, Nielsen PF, *et al*. An overview of CellML 1.1, a biological model description language. *Simulation* 2003;**79**:740–7. doi: 10.1177/0037549703040939.

66. Zhang Z, Singh R, Aschner M. Methods for the detection of autophagy in mammalian cells. *Curr Protoc Toxicol* 2016;**69**:20.12.1–26.

67. Thorburn A, Thamm DH, Gustafson DL. Autophagy and cancer therapy. *Mol Pharmacol* 2014;**85**:830–8.

68. Pérez-Hernández M, Arias A, Martínez-García D, *et al*. Targeting autophagy for cancer treatment and tumor chemosensitization. *Cancer* 2019;**11**:1599. doi: 10.3390/cancers11101599.

69. Antunes F, Erustes AG, Costa AJ, *et al*. Autophagy and intermittent fasting: the connection for cancer therapy, e814s. *Clinics* 2018;**73**.

70. Eskelinen EL. The dual role of autophagy in cancer. *Curr Opin Pharmacol* 2011;**11**:294–300. doi: 10.1016/j.coph.2011.03.009.

71. Bialik S, Dasari SK, Kimchi A. Autophagy-dependent cell death – where, how and why a cell eats itself to death. *J Cell Sci* 2018;**131**:jcs215152. doi: 10.1242/jcs.215152.

72. Deter RL. Analog modeling of glucagon-induced autophagy in rat liver. *Exp Cell Res* 1975;**94**:122–6. doi: 10.1016/0014-4827(75)90538-8.

73. Shirin A, Klickstein IS, Feng S, *et al*. Prediction of optimal drug schedules for controlling autophagy. *Sci Rep* 2019;**9**:1428.

74. Ouzounoglou E, Kalamatianos D, Emmanouilidou E, *et al*. In silico modeling of the effects of alpha-synuclein oligomerization on dopaminergic neuronal homeostasis. *BMC Syst Biol* 2014;**8**:54. doi: 10.1186/1752-0509-8-54.

75. Thorburn A. Apoptosis and autophagy: regulatory connections between two supposedly different processes. *Apoptosis* 2008;**13**:1–9. doi: 10.1007/s10495-007-0154-9.

76. Crighton D, Wilkinson S, O'Prey J, *et al*. DRAM, a p53-induced modulator of autophagy. Is critical for apoptosis. *Cell* 2006;**126**:121–34. doi: 10.1016/j.cell.2006.05.034.

77. Fan Y, Ullman E, Zong W-X. The cellular decision between apoptosis and autophagy. *Beyond Apoptosis* 2008;**32**:127–41. doi: 10.5732/cjc.012.10106.

78. Chen Q, Kang J, Fu C. The independence of and associations among apoptosis, autophagy, and necrosis. *Signal Transduct Target Ther* 2018;**3**:18. doi: 10.1038/s41392-018-0018-5.

79. Kapuy O, Vinod PK, Mandl J, *et al*. A cellular stress-directed bistable switch controls the crosstalk between autophagy and apoptosis. *Mol Biosyst* 2013;**9**:296–306.

80. Tavassoly I, Parmar J, Shajahan-Haq AN, *et al*. Dynamic modeling of the interaction between autophagy and apoptosis in mammalian cells. *CPT Pharmacometrics Syst Pharmacol* 2015;**4**:263–72. doi: 10.1002/psp4.29.

81. Yang B, Liu Q, Bi Y. Autophagy and apoptosis are regulated by stress on Bcl2 by AMBRA1 in the endoplasmic reticulum and mitochondria. *Theor Biol Med Model* 2019;**16**:18.

82. Kapuy O, Vinod PK, Bánhegyi G. mTOR inhibition increases cell viability via autophagy induction during endoplasmic reticulum stress - an experimental and modeling study. *FEBS Open Bio* 2014;**4**:704–13.

83. Cook KL, Shajahan AN, Wärri A, *et al*. Glucose-regulated protein 78 controls cross-talk between apoptosis and autophagy to determine antiestrogen responsiveness. *Cancer Res* 2012;**72**:3337–49.

84. Bernales S, McDonald KL, Walter P. Autophagy counterbalances endoplasmic reticulum expansion during the unfolded protein response. *PLoS Biol* 2006;**4**:e423.

85. Parmar JH, Cook KL, Shajahan-Haq AN, *et al*. Modelling the effect of GRP78 on anti-oestrogen sensitivity and resistance in breast cancer. *Interface Focus* 2013;**3**: 20130012.

86. Han K, Kim J, Choi M. Computer simulations unveil the dynamics of autophagy and its implications for the cellular quality control. *J Biol Syst* 2014;**22**:659–75. doi: 10.1142/s0218339014500260.

87. Han K, Kim SH, Choi M. Computational modeling of the effects of autophagy on amyloid-β peptide levels. *Theor Biol Med Model* 2020;**17**:2.

88. Bonabeau E. Agent-based modeling: methods and techniques for simulating human systems. *Proc Natl Acad Sci* 2002;**99**:7280–7. doi: 10.1073/pnas.082080899.

89. Schaller G, Meyer-Hermann M. Multicellular tumor spheroid in an off-lattice Voronoi-Delaunay cell model. *Phys Rev E* 2005;**71**:051910. doi: 10.1103/physreve.71.051910.

90. Engelberg J, Ganguli S, Anthony HC. Agent-based simulations of in vitro multicellular tumor spheroid growth. In: *Proceedings of the agent-directed simulation symposium*, 2005, 141–8.

91. Börlin CS, Lang V, Hamacher-Brady A, *et al*. Agent-based modeling of autophagy reveals emergent regulatory behavior of spatio-temporal autophagy dynamics. *Cell Commun Signal* 2014;**12**:56. doi: 10.1186/s12964-014-0056-8.

92. Weddle E, Agaisse H. Spatial, temporal, and functional assessment of LC3-dependent autophagy in Shigella flexneri dissemination. *Infect Immun* 2018;**86**:8. doi: 10.1128/IAI.00134-18.

93. Petri C. *Kommunikation mit Automaten*, Ph. D. diss. edn. Germany: Technische Universitat Darmstadt, 1962.

94. Goss PJE, Peccoud J. Quantitative modeling of stochastic systems in molecular biology by using stochastic petri nets. *Proc Natl Acad Sci* 1998;**95**:6750–5. doi: 10.1073/pnas.95.12.6750.

95. Matsuno H, Tanaka Y, Aoshima HD, *et al*. Biopathways representation and simulation on hybrid functional petri net. *In Silico Biol* 2003;**3**:389–404.

96. Koch I, Junker BH, Heiner M. Application of petri net theory for modelling and validation of the sucrose breakdown pathway in the potato tuber. *Bioinformatics* 2005;**21**:1219–26. doi: 10.1093/bioinformatics/bti145.

97. Sackmann A, Heiner M, Koch I. Application of petri net based analysis techniques to signal transduction pathways. *BMC Bioinform* 2006;**7**:482.

98. Scheidel J, Amstein L, Ackermann J, *et al*. In silico knockout studies of xenophagic capturing of salmonella. *PLoS Comput Biol* 2016;**12**:e1005200. doi: 10.1371/journal.pcbi.1005200.

99. Vinayagam A, Gibson TE, Lee H-J, *et al*. Controllability analysis of the directed human protein interaction network identifies disease genes and drug targets. *Proc Natl Acad Sci* 2016;**113**:4976–81. doi: 10.1073/pnas.1603992113.

100. Anand R, Sarmah DT, Chatterjee S. Extracting proteins involved in disease progression using temporally connected networks. *BMC Syst Biol* 2018;**12**:78.

101. Sambaturu N, Mishra M, Chandra N. EpiTracer - an algorithm for identifying epicenters in condition-specific biological networks. *BMC Genomics* 2016;**17**:543. doi: 10.1186/s12864-016-2792-1.

102. Boltz TA, Devkota P, Wuchty S. Collective influencers in protein interaction networks. *Sci Rep* 2019;**9**:3948. doi: 10.1038/s41598-019-40410-2.

103. Bikakis N, Liagouris J, Krommyda M, *et al*. graphVizdb: a scalable platform for interactive large graph visualization.

IEEE 32nd International Conference on Data Engineering (ICDE) IEEE 2016;**2016**:1342–5. doi: 10.1109/ICDE.2016.7498340.

104. Kohler J, Baumbach J, Taubert J, *et al*. Graph-based analysis and visualization of experimental results with ONDEX. *Bioinformatics* 2006;**22**:1383–90. doi: 10.1093/bioinformatics/btl081.

105. Iragne F, Nikolski M, Mathieu B, *et al*. ProViz: protein interaction visualization and exploration. *Bioinformatics* 2005;**21**:272–4. doi: 10.1093/bioinformatics/bth494.

106. Hu Z, Hung J-H, Wang Y, *et al*. VisANT 3.5: multi-scale network visualization, analysis and inference based on the gene ontology. *Nucleic Acids Res* 2009;**37**:W115–21. doi: 10.1093/nar/gkp406.

107. Adar E. GUESS: a language and interface for graph exploration. *Proceedings of the SIGCHI Conference on Human Factors in Computing Systems*. New York, NY, USA: Association for Computing Machinery; 2006. 791–800.

108. Demir E, Babur O, Dogrusoz U, *et al*. PATIKA: an integrated visual environment for collaborative construction and analysis of cellular pathways. *Bioinformatics* 2002;**18**:996–1003. doi: 10.1093/bioinformatics/18.7.996.

109. Pavlopoulos GA, Hooper SD, Sifrim A, *et al*. Medusa: a tool for exploring and clustering biological networks. *BMC Res Notes* 2011;**4**:384. doi: 10.1186/1756-0500-4-384.

110. Secrier M, Pavlopoulos GA, Aerts J, *et al*. Arena3D: visualizing time-driven phenotypic differences in biological systems. *BMC Bioinformatics* 2012;**13**:45. doi: 10.1186/1471-2105-13-45.

111. Breitkreutz B-J, Stark C, Tyers M. Osprey: a network visualization system. *Genome Biol* 2003;**4**:R22.

112. Shannon P. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* 2003;**13**:2498–504. doi: 10.1101/gr.1239303.

113. Bastian M, Heymann S, Jacomy M. Gephi: an open source software for exploring and manipulating networks. *Third International AAAI Conference on Weblogs and Social Media* 2009.

114. Auber D. Tulip — a huge graph visualization framework. *Graph Drawing Software* 2004;105–26. doi: 10.1007/978-3-642-18638-7_5.

115. Mrvar A, Batagelj V. Analysis and visualization of large networks with program package Pajek. *Complex Adaptive Systems Modeling* 2016;**4**:6. doi: 10.1186/s40294-016-0017-8.

116. Heberle H, Carazzolle MF, Telles GP, *et al*. CellNetVis: a web tool for visualization of biological networks using force-directed layout constrained by cellular components. *BMC Bioinform* 2017;**18**:395. doi: 10.1186/s12859-017-1787-5.

117. Krause F, Schulz M, Ripkens B, *et al*. Biographer: web-based editing and rendering of SBGN compliant biochemical networks. *Bioinformatics* 2013;**29**:1467–8. doi: 10.1093/bioinformatics/btt159.

118. Pavlopoulos GA, Paez-Espino D, Kyrpides NC, *et al*. Empirical comparison of visualization tools for larger-scale network analysis. *Adv Bioinformatics* 2017;**2017**:1278932.

119. Saito R, Smoot ME, Ono K, *et al*. A travel guide to cytoscape plugins. *Nat Methods* 2012;**9**:1069–76.

120. Hasin Y, Seldin M, Lusis A. Multi-omics approaches to disease. *Genome Biol* 2017;**18**:83. doi: 10.1186/s13059-017-1215-1.

121. Akiyama M. Multi-omics study for interpretation of genome-wide association study. *J Hum Genet* 2020. doi: 10.1038/s10038-020-00842-5.

122. Lu G, Rao D, Zhou M, *et al*. Autophagic network analysis of the dual effect of sevoflurane on neurons associated with GABARAPL1 and 2. *Biomed Res Int* 2020;**2020**:1587214.

123. Wang J-Y, Yao W-X, Wang Y, *et al*. Network analysis reveals crosstalk between autophagy genes and disease genes. *Sci Rep* 2017;**7**:44391.

124. Qiu J, Sun M, Wang Y, *et al*. Identification and validation of an individualized autophagy-clinical prognostic index in gastric cancer patients. *Cancer Cell Int* 2020;**20**:178.

125. Liu B, Bao J-K, Yang J-M, *et al*. Targeting autophagic pathways for cancer drug discovery. *Chin J Cancer* 2013;**32**:113–20.

126. Yue P, Zhu C, Gao Y, *et al*. Development of an autophagy-related signature in pancreatic adenocarcinoma. *Biomed Pharmacother* 2020;**126**:110080.

127. Lin L, Xia L, Tang D, *et al*. Analysis of autophagy-related genes and associated noncoding RNAs and transcription factors in digestive system tumors. *Future Oncol* 2019;**15**:4141–54. doi: 10.2217/fon-2019-0341.

128. Amberger JS, Bocchini CA, Scott AF, *et al*. OMIM.org: leveraging knowledge across phenotype-gene relationships. *Nucleic Acids Res* 2019;**47**:D1038–43.

129. Homma K, Suzuki K, Sugawara H. The autophagy database: an all-inclusive information resource on autophagy that provides nourishment for research. *Nucleic Acids Res* 2011;**39**:D986–90. doi: 10.1093/nar/gkq995.

130. Türei D, Földvári-Nagy L, Fazekas D, *et al*. Autophagy regulatory network — a systems-level bioinformatics resource for studying the mechanism and regulation of autophagy. *Autophagy* 2015;**11**:155–65. doi: 10.4161/15548627.2014.994346.

131. Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* 2009;**4**:44–57.

132. Zhang G, Xu S, Yuan Z, *et al*. Weighted gene coexpression network analysis identifies specific modules and hub genes related to major depression. *Neuropsychiatr Dis Treat* 2020;**16**:703–13.

133. Barrett T, Wilhite SE, Ledoux P, *et al*. NCBI GEO: archive for functional genomics data sets—update. *Nucleic Acids Res* 2012;**41**:D991–5. doi: 10.1093/nar/gks1193.

134. He Z, Liu H, Moch H, *et al*. Machine learning with autophagy-related proteins for discriminating renal cell carcinoma subtypes. *Sci Rep* 2020;**10**:720. doi: 10.1038/s41598-020-57670-y.

135. Kriston-Vizi J, Lim CA, Condron P, *et al*. An automated high-content screening image analysis pipelinefor the identification of selective autophagic inducers in human cancer cell lines. *J Biomol Screen* 2010;**15**:869–81. doi: 10.1177/1087057110373393.

136. Serrano A, El Haddad S, Moal F, *et al*. Dysregulation of apoptosis and autophagy gene expression in peripheral blood mononuclear cells of efficiently treated HIV-infected patients. *AIDS* 2018;**32**:1579–87. doi: 10.1097/qad.0000000000001851.

137. Pedregosa F, Varoquaux G, Gramfort A, *et al*. Scikit-learn: machine learning in python. *J Mach Learn Res* 2011;**12**:2825–30.

138. Shi Q, Pei F, Silverman GA, *et al*. Mechanisms of action of autophagy modulators dissected by quantitative systems pharmacology analysis. *Int J Mol Sci* 2020;**21**:2855. doi: 10.3390/ijms21082855.

139. Athar A, Füllgrabe A, George N, *et al*. ArrayExpress update–from bulk to single-cell expression data. *Nucleic Acids Res* 2019;**47**:D711–5. doi: 10.1093/nar/gky964.

140. Ogata H, Goto S, *et al*. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res* 1999;**27**:29–34. doi: 10.1093/nar/27.1.29.

141. Croft D, Mundo AF, Haw R, *et al*. The reactome pathway knowledgebase. *Nucleic Acids Res* 2014;**42**:D472–7. doi: 10.1093/nar/gkt1102.

142. Baldonedo J, Fernández JR, Segade A. Analysis of a bone remodeling model with myeloma disease arising in cellular dynamics. *Int J Numer Meth Biomed Eng* 2020;**36**:e3333. doi: 10.1002/cnm.3333.

143. Gulbudak H, Browne CJ. Infection severity across scales in multi-strain immuno-epidemiological dengue model structured by host antibody level. *J Math Biol* 2020;**80**:1803–43. doi: 10.1007/s00285-020-01480-3.

144. da Silva JG, da Silva JG, de Morais RM, *et al*. Mathematical models applied to thyroid cancer. *Biophys Rev* 2019;**11**:183–9. doi: 10.1007/s12551-019-00504-7.

145. Alber M, Tepole AB, Cannon WR, *et al*. Integrating machine learning and multiscale modeling—perspectives, challenges, and opportunities in the biological, biomedical, and behavioral sciences. *npj Digital Med* 2019;**2**:115. doi: 10.1038/s41746-019-0193-y.

146. Hajishamsaei M, Pishevar A, Bavi O, *et al*. A novel in silico platform for a fully automatic personalized brain tumor growth. *Magn Reson Imaging* 2020;**68**:121–6. doi: 10.1016/j.mri.2019.12.012.

147. Sbalzarini IF, Koumoutsakos P. Feature point tracking and trajectory analysis for video imaging in cell biology. *J Struct Biol* 2005;**151**:182–95. doi: 10.1016/j.jsb.2005.06.002.

148. Sbalzarini IF. Modeling and simulation of biological systems from image data. *Bioessays* 2013;**35**:482–90. doi: 10.1002/bies.201200051.

149. Burrage K, Hancock J, Leier A, *et al*. Modelling and simulation techniques for membrane biology. *Brief Bioinform* 2007;**8**:234–44. doi: 10.1093/bib/bbm033.

150. Zeller-Plumhoff B, Roose T, Clough GF, *et al*. Image-based modelling of skeletal muscle oxygenation. *J Roy Soc Interface* 2017;**14**:20160992. doi: 10.1098/rsif.2016.0992.

151. Costes SV, Ponomarev A, Chen JL, *et al*. Image-based Modeling reveals dynamic redistribution of DNA damage into nuclear sub-domains. *PLoS Comput Biol* 2007;**3**:e155. doi: 10.1371/journal.pcbi.0030155.

152. Vadakkumpadan F, Arevalo H, Prassl AJ, *et al*. Image-based models of cardiac structure in health and disease. *Wiley Interdiscip Rev Syst Biol Med* 2010;**2**:489–506. doi: 10.1002/wsbm.76.

153. Mittal L, Kumari A, Suri C, *et al*. Insights into structural dynamics of allosteric binding sites inHCV RNA-dependent RNA polymerase. *J Biomol Struct Dynam* 2019;**6**:1–14. doi: 10.1080/07391102.2019.1614480.

154. He S, Li Q, Jiang X, *et al*. Design of small molecule autophagy modulators: a promising druggable strategy. *J Med Chem* 2018;**61**:4656–87. doi: 10.1021/acs.jmedchem.7b01019.

155. Wickham H, Francois R, Henry L, *et al*. Others. Dplyr: A Grammar of Data Manipulation. *R Package Version 04*, 2015, 3.

156. Wickham H. ggplot2. *WIREs Comp Stat* 2011;**3**:180–5. doi: 10.1002/wics.147.

157. Gentleman RC, Carey VJ, Bates DM, *et al*. Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol* 2004;**5**:R80.

158. Bischl B, Lang M, Kotthoff L, *et al*. Mlr: machine learning in R. *J Mach Learn Res* 2016;**17**:1–5.

159. Smyth GK. limma: linear models for microarray data. In: Gentleman R, Carey VJ, Huber W *et al*. (eds). *Bioinformatics and Computational Biology Solutions Using R and Bioconductor*. New York, NY: Springer New York, 2005, 397–420.

160. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. *BMC Bioinform* 2008;**9**:559.

161. Durinck S, Spellman PT, Birney E, *et al*. Mapping identifiers for the integration of genomic datasets with the R/bioconductor package biomaRt. *Nat Protoc* 2009;**4**:1184–91.

162. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 2014;**15**:550.

163. Palla G, Derényi I, Farkas I, *et al*. Uncovering the overlapping community structure of complex networks in nature and society. *Nature* 2005;**435**:814–8.

164. Chen EY, Tan CM, Kou Y, *et al*. Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinform* 2013;**14**:128. doi: 10.1186/1471-2105-14-128.

165. Xu Y, Luo X-C. PyPathway: python package for biological network analysis and visualization. *J Comput Biol* 2018;**25**:499–504. doi: 10.1089/cmb.2017.0199.

166. Maere S, Heymans K, Kuiper M. BiNGO: a Cytoscape plugin to assess overrepresentation of gene ontology categories in biological networks. *Bioinformatics* 2005;**21**:3448–9. doi: 10.1093/bioinformatics/bti551.

167. Zhang C, Hanspers K, Kuchinsky A, *et al*. Mosaic: making biological sense of complex networks. *Bioinformatics* 2012;**28**:1943–4.

168. Barsky A, Gardy JL, Hancock REW, *et al*. Cerebral: a Cytoscape plugin for layout of and interaction with biological networks using subcellular localization annotation. *Bioinformatics* 2007;**23**:1040–2. doi: 10.1093/bioinformatics/btm057.

169. Gil DP, Law JN, Murali TM. The PathLinker app: connect the dots in protein interaction networks. *F1000Research* 2017;**6**:58. doi: 10.12688/f1000research.9909.1.

170. Tang Y, Li M, Wang J, *et al*. CytoNCA: a cytoscape plugin for centrality analysis and evaluation of protein interaction networks. *Biosystems* 2015;**127**:67–72. doi: 10.1016/j.biosystems.2014.11.005.

171. Bindea G, Mlecnik B, Hackl H, *et al*. ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics* 2009;**25**:1091–3.

172. Montojo J, Zuberi K, Rodriguez H, *et al*. GeneMANIA Cytoscape plugin: fast gene function predictions on the desktop. *Bioinformatics* 2010;**26**:2927–8. doi: 10.1093/bioinformatics/btq562.

173. Zinovyev A, Viara E, Calzone L, *et al*. BiNoM: a Cytoscape plugin for manipulating and analyzing biological networks. *Bioinformatics* 2008;**24**:876–7.

174. Smoot M, Ono K, Ideker T, *et al*. PiNGO: a Cytoscape plugin to find candidate genes in biological networks. *Bioinformatics* 2011;**27**:1030–1. doi: 10.1093/bioinformatics/btr045.

175. Bader GD, Hogue CWV. An automated method for finding molecular complexes in large protein interaction networks. *BMC Bioinformatics* 2003;**4**:2.

176. Kamburov A, Pentchev K, Galicka H, *et al*. Consensus-PathDB: toward a more complete picture of cell biology. *Nucleic Acids Res* 2011;**39**:D712–7. doi: 10.1093/nar/gkq1156.

177. Vailaya A, Bluvas P, Kincaid R, *et al*. An architecture for biological information extraction and representation. *Bioinformatics* 2005;**21**:430–8. doi: 10.1093/bioinformatics/bti187.

178. Ideker T, Ozier O, Schwikowski B, *et al*. Discovering regulatory and signalling circuits in molecular interaction networks. *Bioinformatics* 2002;**18**:S233–40. doi: 10.1093/bioinformatics/18.suppl_1.s233.

179. Chin C-H, Chen S-H, Wu H-H, *et al*. cytoHubba: identifying hub objects and sub-networks from complex interactome. *BMC Syst Biol* 2014;**8**:S11.

180. Wang N-N, Dong J, Zhang L, *et al*. HAMdb: a database of human autophagy modulators with specific pathway and disease information. *J Chem* 2018;**10**:34. doi: 10.1186/s13321-018-0289-4.

181. Wu D, Huang Y, Kang J, *et al*. ncRDeathDB: a comprehensive bioinformatics resource for deciphering network organization of the ncRNA-mediated cell death system. *Autophagy* 2015;**11**:1917–26. doi: 10.1080/15548627.2015.1089375.

182. Deng Y, Zhu L, Cai H, *et al*. Autophagic compound database: a resource connecting autophagy-modulating compounds, their potential targets and relevant diseases. *Cell Prolif* 2018;**51**:e12403. doi: 10.1111/cpr.12403.

183. Deng W, Ma L, Zhang Y, *et al*. THANATOS: an integrative data resource of proteins and post-translational modifications in the regulation of autophagy. *Autophagy* 2018;**14**:296–310. doi: 10.1080/15548627.2017.1402990.

184. Nanduri R, Kalra R, Bhagyaraj E, *et al*. AutophagySMDB: a curated database of small molecules that modulate protein targets regulating autophagy. *Autophagy* 2019;**15**:1280–95.

185. Wang W, Zhang P, Li L, *et al*. ATD: a comprehensive bioinformatics resource for deciphering the association of autophagy and diseases. *Database* 2018, 2018;bay093. doi: 10.1093/database/bay093.

186. Jacomin A-C, Samavedam S, Promponas V, *et al*. iLIR database: a web resource for LIR motif-containing proteins in eukaryotes. *Autophagy* 2016;**12**:1945–53.

187. Chen K, Yang D, Zhao F, *et al*. Autophagy and tumor database: ATdb, a novel database connecting autophagy and tumor. *Database* 2020;**2020**:baaa052. doi: 10.1093/database/baaa052.