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Current State-of-The-Art and Future Directions in Systems Biology

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Abstract—Systems Biology offers the promise of decoding genetic information, optimizing pharmaceutical design, and aiding in the development of precision medicine. These advances require the bimodal approach of deriving information from experimental data and integrating such information via computational modeling. However, choosing an appropriate experimental assay and computational model is paramount to the accuracy and relevancy of the output. Here, we delve into the fundamental concept of several commonly used modeling approaches, their advantages and limitations, as well as potential applications. We review and compare experimental assays used in systems biology, based on the throughput, simplicity and possibility for quantification. In addition, we review current experimental models used in conjunction with assays to provide parameters and/or validation for computational modeling. Lastly, we present applications of systems biology in medicine: case studies, clinical opportunities, and future directions of systems biology.

Keywords—computational modeling, high-throughput, deterministic, stochastic, agent-based, qFlow cytometry.

I. Introduction

Systems biology has positively impacted several translational and clinical research areas: diagnostics, drug discovery and personalized medicine [1]-[3]. Our bodies consist of many integrated biological and chemical components that communicate on multiple scales: from genomes to molecules to cells that make up the organs. Some fields of biology focus on probing and studying components of a system one at a time, and one scale at a time. However, such a linear approach is not enough when it comes to solving systemic problems, such as cancer. Systems biology, on the other hand, aims to understand biological functions by integrating experimental data across different scales with predictive computational modeling. This integrative approach is driven by three major fields of research and technology: 1) Physiologically-relevant experimental models that allow us to define biological systems; 2) High-throughput technologies that probe biological systems at molecular-level with single-cell resolution; 3) Multi-scale predictive modeling that can integrate such biological data to predict system mechanism and responses.

Experimental models offer a window to empirically define and probe a biological system, and test response from a given stimulation. Without experimental models, no definitive statements regarding the structure or function of biological systems could be made. However, experimental models are often difficult to choose from, due to the large variety and heterogeneity among those models. In addition, each model has their advantages and limitations depending on the experimental parameters one would like to probe. To alleviate confusion and provide guidance on which experimental model to use, we lay out the strengths and weakness of some commonly used experimental models, and the relevancy between those models and the physiological systems that they represent.

The complexity of the biological system and heterogeneity at different scales has posed great challenges in systems biology and hindered its application in biomarker discovery and drug development. In order to expand the approach of systems biology in those areas, it is crucial to develop technologies for genomic, transcriptomic and multiplex proteomic analyses at single-cell resolution [4][5]. In the past two decades, a variety of high-throughput technologies such as cDNA microarrays have greatly benefited the advancement of systems biology [6][7]. These technologies also generate “big data” or -omics data, providing insight into novel therapeutic targets or critical nodes. However, one challenge that remains is how to interpret and integrate these massive datasets in order to provide meaningful insights. Computational modeling is a useful tool to integrate big data and possibly overcome this challenge.

Computational modeling allows big datasets generated from experimental models and high-throughput assays to be analyzed in a physiological context in shorter times with lower material costs. Computational models allow probing of a biological system, using experimentally derived knowledge of the system, to provide new insights into the system function and predict system responses to various stimuli [8]. However, the biological system must first be experimentally defined to a certain extent before computational models can be developed, otherwise the computational models will fail to be physiological relevant. As knowledge and data of biological systems continues to grow, computational models will be critical for organizing, interpreting, and utilizing these data to improve decision making in pharmaceutical development.

Systems biology, the iteration between experimental models to define a system, high-throughput assays to map biomarkers within the system, and computational models to predict system

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function and response, will accelerate discovery in several fields of biomedical research. As such, this review will help contextualize this field by first presenting several types of computational models: describing metrics for selection of appropriate modeling paradigms. We will then outline four types of commonly used experimental models in systems biology and discuss quantitative high-throughput techniques, with a focus on angiogenesis assays and quantitative proteomics. Furthermore, we present selected examples of recent research contributions that applied systems biology to better understand and treat diseases such as breast cancer and Alzheimer's. We conclude by proposing two possible opportunities for incorporating systems biology approaches towards cancer treatment.

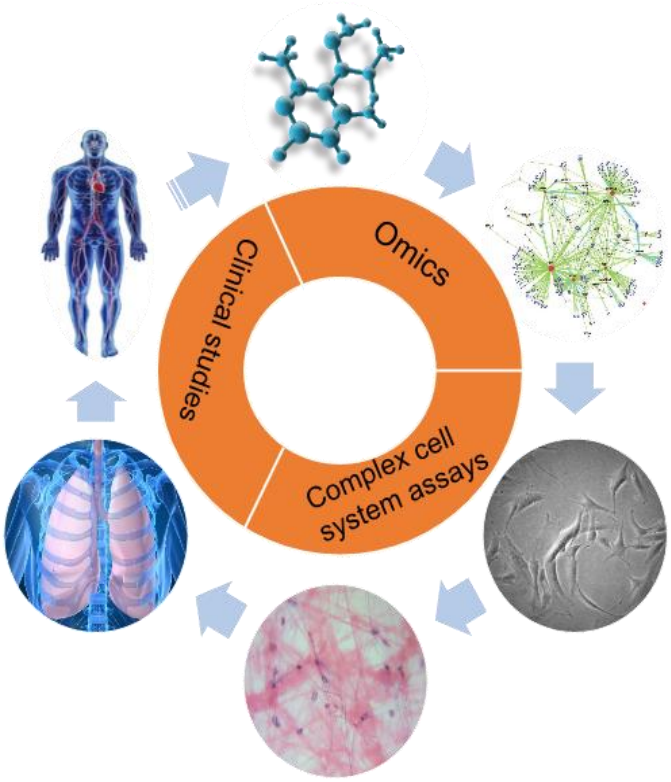

Figure 1 Experimental approaches to systems biology in the pharmaceutical industry.

II. Computational Model Selection

You et al described succinctly the major driving force behind the creation of computational models: "To be confident how the car works, we should be able to put the [individual] parts back together and demonstrate that the car works" [9]. One goal of modeling is to probe empirical data and derive the mechanisms and critical nodes of interaction, i.e., the 'parts', that comprise the system, the 'car' (Figure 2). A model of the system can be defined using data derived from literature, experiments, -omic databases, or clinical studies. Models are iteratively tested and improved by comparing model predictions with results, measured empirically. Experimentally validated models can provide predicted responses of systems, and provide insight into mechanisms and pathways of interacting system components.

The ideal computational model should be robust, computationally efficient, and faithful to empirical data. Not all models are created equally. It is important when building a model to choose the most efficient type of model for the given system. For example, one model type may better represent phenomena at a specific scale than another type. In this section, we will review some of the different modeling approaches that are commonly used in systems biology studies, focusing on their strengths and weaknesses for modeling a specific system.

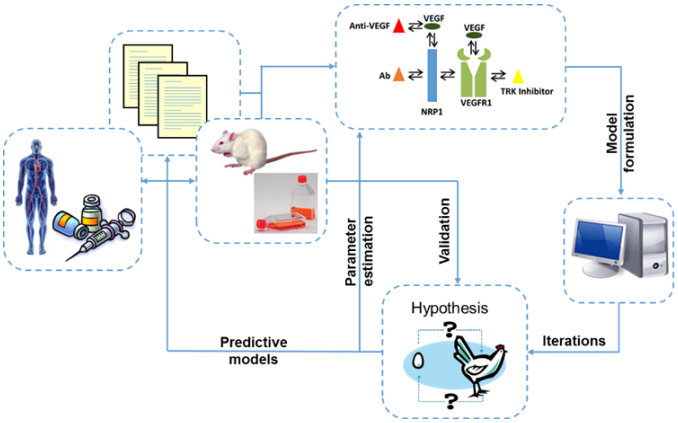

Figure 2 The continuous cycle that systems biology studies generally follow. Experimental studies derive the first information about the system components, which are utilized to develop a computational model. After performing model validation, models give additional insight into the system, which are then confirmed empirically. Additional experimental studies allow refinement of the model, and the cycle starts anew.

A. Kinetic Equation -based Modeling

Kinetic equations, possibly the most common form of representing biochemical interactions, can be derived from stoichiometric and empirical data. Kinetic equations are implemented through the use of ordinary differential equations (ODEs) and they can give information about the fluctuations in concentrations as a function of time (Table 1). Kinetic equations require explicit definition of every state, interaction, and rate constants, and are thusly described as "deterministic".

Table 1 ODE Modeling:
The interaction of two species with a forward rate constant KF, and reverse rate constant KR.

Reaction Equation	ODE
$A + B \underset{k_r}{\overset{k_f}{\rightleftarrows}} AB$	$\frac{d[AB]}{dt} = k_f[A][B] - k_r[AB]$
$A + X \cdot B \underset{k_r}{\overset{k_f}{\rightleftarrows}} AB_X$	$\frac{d[AB_X]}{dt} = k_f[A][B]^X - k_r[AB_X]$
$Y \cdot A + X \cdot B \underset{k_r}{\overset{k_f}{\rightleftarrows}} A_Y B_X$	$\frac{d[A_Y B_X]}{dt} = k_f[A]^Y[B]^X - k_r[A_Y B_X]$

Kinetic equation based modeling is useful as it allows hypothesized interactions to be explored and validated. If the hypothesized model is unable to match empirical results, the

model parameters and interaction networks can easily be redefined iteratively, through the use of parameter estimation, until the empirical results are reproduced. Several methods of estimating parameters [10][11] and measuring parameter uncertainty [12][13] have been described. One of the main drawbacks associated with kinetic based modeling is the amount of information that is necessary: reaction definitions, reaction rates, and initial protein concentrations [14]. A second drawback is when simulating several orders of magnitude of reactions, the computational strain becomes quite large. One way to reduce this computational intensity is to make the interaction network Boolean, meaning every state exhibits one of two cases- on or off, allowing great simplification of the system [9]. While the Boolean model is very robust for gene networks, cellular interactions or compartmental changes are not very amenable to the system. Thus, systems with more complex interaction networks are usually modeled with ODEs. Additionally, ODEs can only be accurately applied to a continuum. Thus in situations where reactions occur stochasticity, such as at low concentrations, kinetic based approaches can be highly inaccurate. Altogether, kinetic equation based modeling can provide straightforward insight into the hypothesized network.

B. Stochastic Modeling

Stochastic modeling is the modeling of random, "non-deterministic," processes. It can be applied to a variety of topics including metabolite diffusion and receptor coupling. One stochastic technique with widespread use for systems of chemical reactions is the Gillespie algorithm. It captures stochastic processes and predicts the next state of the system [15]. This allows for better prediction of highly random interactions compared to deterministic modeling, but it is not without challenges. The randomness introduced into the system may not necessarily correlate with inherent system stochasticity, and it may model noise. Thus, it is possible for the Gillespie algorithm to arrive at different simulation results, a phenomenon that does not occur in deterministic modeling. It is best used for few reactions and few species; otherwise, the calculation time necessary for a purely stochastic model can be significant. The Gillespie algorithm without kinetic equations can also be implemented, giving a homogeneously distributed system that contains only basic elementary reactions. Such conditions may not well represent intercellular signaling, and thus it is a very rare exception [9].

Another stochastic approach involves combining differential equations with to create a combinational model. One commonly used combinational model is that of Langevin. Each Langevin equation is comprised of two terms: (1) the differential equation, derived via deterministic modeling, and (2) a noise term. Introduction of both noise and dynamics provide accuracy to the model, without introducing significant computational time.

Stochastic modeling has also been explored to better reflect the randomness of small scale binding-unbinding behavior. Modeling techniques such as Monte Carlo methods allow for the creation of randomized data inputs through random sampling and/or probability distributions. This allows for the creation of "trials" in computational modeling for interrogating model robustness. However, it is debatable whether the increased computing power and number of trials necessary for a Monte Carlo simulation results in meaningful accuracy improvement. A study by Mac Gabhann et al compared a Mcell Monte Carlo simulation to a Gillespie model and to a purely deterministic model to determine if there were differences among these approaches [16]. Here, they examined the binding of the vascular endothelial growth factor (VEGF)-A variant, VEGF165, to its receptors, VEGFR1 and VEGFR2 on the cellular scale. They determined that there was not an appreciable difference in output amongst the three models, when examining a sample size representative of a cell surface. This suggests that for applications on the cellular scale, the more convenient deterministic models are sufficient. This study did have some notable limitations: it did not account for receptor clustering, receptor internalization, or ligand secretion. Furthermore, simulations over small surface areas revealed differences between stochastic and deterministic models, indicating that further studies might help identify the deterministic to stochastic relevancy threshold [16].

Stochastic systems can also be modeled using Bayesian methods to represent probabilistic noise [17]. Capturing probabilistic noise is advantageous as physiological processes are not purely deterministic in nature, and every process has some degree of stochasticity. One Bayesian method, Markov chain Monte Carlo simulation, is used to estimate initial parameters, discover motifs, or predict transcription factor binding sites [17]. Markov chain Monte Carlo simulations are able to include biological noise into their simulations, and have achieved 95% physiologically accurate results [18]. Markov chain Monte Carlo simulations have been applied to calculate mRNA transcript concentration in a series of spotted cDNA wells. Bayesian methods have also been applied to discriminate physical interactions across thousands of genes in cancer tumor models, such as gliomas. These statistical network models study the topology of cellular systems to find key genetic interactions regulating cancer development [19]. One major disadvantage of Bayesian methods is that it causes large computational loads, resulting from the large amount of calculations and processes; as such, a complete Bayesian analysis of models containing high physiological complexity is infeasible with current technology.

In stochastic models, it is important to note that noise introduced externally is typically assumed to be additive. This means that noise is not accounted for directly in the differential parameters, but as a constant, input separately. Additionally, fluctuations due to noise only affect the measurements, as the reality of the dynamics being affected by noise would require the differential equations factoring in that noise, which may make calculations more complex. This would involve the creation of a differential term that varied the noise on a temporal scale, which could complicate the model.

C. Agent-based Modeling

Agent-based modeling involves the creation of a series of rules that constrain the model. Instead of a series of deterministic equations such as the kinetic ODE model, agent

based modeling is much more open-ended as the rules can be implemented to incorporate such aspects as orientation, proximity, velocity and even time. One example of this model type is the simulation of angiogenic sprouting. With this model, researchers were able to visualize the vessel filopodia as they grew, and were able to verify the presence of delta-notch mediated tip and stalk cell selection [20]. Recently, agent based modeling has been used to examine leukocyte rolling, adhesion, and extravasation in microvascular networks [21]; to understand angiogenesis by surveying the rules for cell behavior compared against experimental results [22]; and to examine breast cancer progression [23]. Agent based modeling is very useful as the implementation of rules introduces more noise than a kinetic ODE model. The open-endedness of the model comes at a cost however, as creating the rules for reactions are not trivial, and the equations can become computationally complex when modeling how each agent within the model responds to the rules. Doing five to ten equations for a single agent (molecule/gene/protein/cell/etc.) may be computationally inexpensive, but scale-up to hundreds or thousands of such agents would result in higher computational strain (Figure 3).

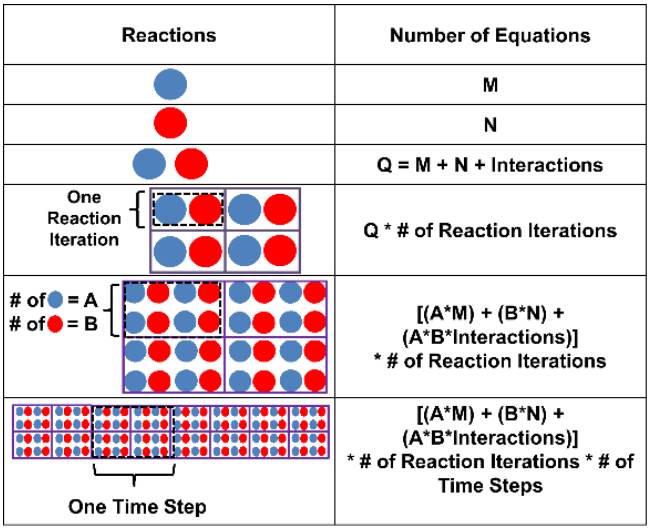

Figure 3 An example of the interaction between a blue particle and a red one and the increasing complexity of the model with experiment duplication, several time steps, and several particles (Figure is adapted from Janes et al 2005 [24]).

D. Selection of Model and Parameter

In order to create models with high physiological relevance, it is important to implement an effective model type, as incorrect model assignment can result in non-trivial deviations from empirical results. For example, if a kinetic ODE based model is utilized for a dilute, low volume system where stochastic movement is integral, the model will not be able to capture the random fluctuations in particle quantities. The random fluctuations are an essential feature of the system, and as such, its lack of integration is a non-trivial simplification in the model. Forcing the kinetic model to fit this stochastic process would be much more difficult than implementing it as a stochastic model. Relatedly, using a kinetic ODE based model, or even a Langevin stochastic model to simplify a complex interaction may result in lost information; whereas describing complexity via agent based model rules may better capture the system.

Deciding what type of model best represents the system of interest can also be subjective. One important aspect to note when comparing models is that a model or explanation cannot be proven, merely rejected. Meaning, even if a model is suggested and fits the empirical data, there is no proof that it is correct or that a "more accurate model" does not exist. Therefore, the success of a model can also be judged not simply by whether it best fits empirical data, but also on whether it provides an explanation for the outcome that was reached. Additionally, models that can predict system outcomes that can be further validated experimentally are often desired [25]. Models that help experimental design, predicting more effective experiments has been well examined [26][27].

III. Experimental Approaches

Experimental research offers new insight into fundamental biological processes. It can serve several purposes including providing insight into signals, providing parameters for computational modeling, identifying potential biomarkers for clinical applications (early-stage detection, progress, and predicted outcome), and providing potential targets for drug development (Figure 2). The complexity of cellular systems often necessitates high-throughput approaches, while the data requirements of computational models require quantification. Additionally, the development of quantitative and high-throughput technology can significantly improve the efficiency and accuracy of experimental measurements and allow us compare data between facilities, days.

A. Experimental Assays

Systems biology has catalyzed the development of quantitative and high-throughput biological tools. In turn, the development of these new technologies has revolutionized the way we practice biology. The majority of the current experimental tools used in systems biology can be classified into genomic technologies and high-throughput proteomics (Table 2). Genomic technologies are used to determine the sequence or abundance of individual gene to an entire genome at either DNA level or the transcriptional level. Whereas, post-translational modifications, protein abundance, and protein-protein interactions can be identified and quantified using high-throughput proteomics. We have detailed examples of these experimental tools in Table 2, detailing their respective throughput, simplicity, quantitative nature.

B. Genomic Technologies

There are three primary genomic technologies that are used in systems biology [28]-[31]: 1) DNA/RNA sequencing, 2) microarray, and 3) quantitative polymerase chain reaction (qPCR) (Table 2). Since the invention of the first automated DNA sequencer in 1986, the throughput of DNA sequencing

Table 2 Examples of experimental techniques used in systems biology research.
Within each assay subset, we evaluate and rank throughput, simplicity, and how quantitative their output is.

Genomic Technologies	**Throughput**	**Simplicity**	**Quantitative Output**
DNA/RNA Sequencing	High	Complex	Moderate
Microarray	High	Moderate	Low
qPCR	Moderate	Simple	High
High-throughput Proteomics			
Protein structure	**Throughput**	**Simplicity**	**Resolving power**
NMR	High	Simple	Low
Crystallography	Moderate	Moderate	Moderate
Mass spectrometry	High	Complex	High
Protein concentrations	**Throughput**	**Simplicity**	**Quantitative Output**
Mass spectrometry	High	Complex	High
qFlow cytometry	Moderate	Simple	High
Quantitative ELISA	Moderate	Simple	High
Protein interaction	**Throughput**	**Simplicity**	**Quantitative Output**
Yeast 2-hybrid	Moderate	Complex	Low
MS-based affinity purification	High	Moderate	Moderate
Quantitative SPR	Low	Simple	High

has increased more than 2000-fold [30][32]. The most powerful tool to decipher the complexity of genomes, next-generation sequencing (NGS), have been evolving over the past decade, leading to improvements such as longer read, higher throughput, and lower cost [29][33][34]. However, the relatively higher error rate and higher cost compared to other DNA platforms remains the limitation for NGS to be used clinically. Microarrays are widely used in genomic research due to its lower cost compared to NGS routines. Microarrays can be used to identify single-nucleotide polymorphisms (SNPs) [35][36] as well as measure expression levels of thousands of genes inexpensively [37]. Normalization for microarray measurement is considered to be challenging due to the variations in hybridizations; therefore, RNA sequencing is sometimes recommended in order to achieve more precise output [29][38][39]. qPCR is the gold standard for clinical gene detection due to its high sensitivity and specificity. With primers designed for targets of interest, qPCR can quickly and robustly detect specific targets, making it preferable for point-of-care applications [29][40][41].

C. High-throughput Proteomics

It is clear that proteins are the effectors of several critical cell behaviors. Biological systems cannot be decoded simply by studying the genes or RNAs due to the poor correlation between gene/RNA expression and protein expression [42]-[45]. Therefore, proteomics research applied to systems biology can enlighten on protein function, delineating how proteins regulate cell behavior on several levels. Towards this goal, proteomic technologies have been developed to characterize protein structure, protein-protein interactions, protein concentrations, and localization.

Here we focus on technologies used in systems biology that examine: protein structure, protein concentrations, and protein-protein interactions (Table 2).

1) Protein structure

There are three primary technologies used to identify protein structure in systems biology [42][46][47]: 1) Nuclear Magnetic Resonance (NMR), 2) crystallography and 3) mass spectrometry (MS). The advantage of NMR over the other two technologies listed here is that it allows us to identify the secondary structure, which means it can solve the 3D structure of a protein. However, the resolving power of NMR is lower compared to other technologies, and the molecular mass of protein complex is limited[48]. Crystallography allows high-resolution identification of protein structure, but requires the most prior knowledge of protein function of all the techniques outlined here. Relatively large amounts of protein need to be available for purification to form crystalline structures and the weight of the protein of interest needs to be approximately known.

Mass spectrometry is a means of peptide and protein identification by ionization and mass analysis. Biomarker identification by mass spectrometry allows for high resolution. However, mass spectrometry technology is limited by scalability as the large sample size requirement and pre-

processing limit single cell analyses. Additionally, the distinction of molecules by mass is complicated by extensive proteomes in which several molecules are similar in mass. One example of advances in resolving protein complexes is given by Ho et al., where they successfully applied mass spectrometry to systematically identify protein complexes in *Saccharomyces cerevisiae* [49].

2) Protein concentrations

There are three primary technologies that are used to quantify protein concentrations in systems biology [50]-[53]: 1) Mass spectroscopy, 2) quantitative flow (qFlow) cytometry, 3) quantitative ELISA, and 4) Immunohistochemistry. Here we start with several MS-based proteomic assays used in systems biology.

Protein phosphorylation, a post-translational modification that is critical to intracellular signaling, can be identified along with the sites of phosphorylation through the use of mass spectroscopy assays. While useful in assessing the amount and nature of phosphorylation, mass spectroscopy techniques cannot easily trace the act of phosphorylation itself, leaving the kinetics unknown. However, a powerful benefit of this technique is that these assays can be made in physiologically relevant environment and cellular conditions. These techniques are usually aided through the use of stable isotope labeling of amino acids in culture, such as with isotope tagged amine reactive agents or isotope-coded affinity tags [44], allowing for quantification of many different phosphorylation sites under different biological conditions [54]. A similar technique, selected reaction monitoring (SRM), is a form of double mass spectroscopy where the sample is ionized, fractionated, reionized, and then refractionated. Using heavy isotopes to quantitatively label certain ions allows for very accurate quantitation of preselected proteins or protein motifs. High-throughput techniques can increase the applicability of selected reaction monitoring [55] to new proteins. When SRM assay mixtures are available, they provide specific information about the concentrations of several proteins of interest simultaneously [56].

Multidimensional protein identification technology (MudPIT) is a mass spectroscopy technique that relies on data mining to identify and sort the protein fragments generated. Proteins are denatured and liquid chromatography is used to separate them by size, then tandem mass spectroscopy fragments the proteins and measures their distribution. Using computational techniques, the original protein mixture can be reconstructed [57]. MudPIT samples require several days of preparation, and the spectroscopy itself can take many hours. However, MudPIT allows quantitative analysis of thousands of proteins across a wide dynamic range.

Metabolic foot printing is a novel method that uses mass spectrometry in conjunction to measuring the metabolites that are excreted or not consumed by the cells from the media [58]. Instead of tracing what the cells have consumed through labelling, this technique finds the consumption through the analysis of cellular remains and excretion. This method is done through the stimulation of "overflow metabolism" which up-regulates the amount of excreted metabolites from the cells. This technique is very high-throughput, at the scale of 250 experiments in triplicate in three days. Additionally, this system has been proven to be very useful in detecting the metabolic patterns in single-gene knock-out strains in functional genome analyses [58]. These assays give mass/charge data which contain vital information about the metabolites remaining in the system. However, a purely direct injection system trades off some knowledge of the identity of the metabolites for speed. A method to remedy this tradeoff is to use time of flight mass spectrometry plus gas chromatography to give much more information about metabolite identity, while minimizing the run time to roughly 13-20 minutes per run [58].

Quantitative enzyme-linked immunosorbent assays (ELISAs) use enzyme-linked antibodies to tag proteins of interest. Proteins are detected by adding a substrate that reacts with the enzyme to produce a measureable signal. ELISAs offer protein quantification at the cellular scale, but also detect protein levels within fluid or dissociated tissue samples. Radiolabeling seeks to overcome the disadvantages to fluorescent tags by tagging proteins with radioactive isotopes. The emitted radiation is detected and combined with information about the isotope half-life and decay kinetics, which allows protein quantification at small scales. Despite these advances in receptor profiling, an inexpensive, high-throughput, and highly-sensitive experimental tool for in vivo measurements of protein profiles is still missing.

Quantitative flow (qFlow) cytometry allows measurement of several different fluorophores across hundreds of cells per second. Recently, qFlow cytometry has been used to profile angiogenic receptors on endothelial cells in vitro [59], and on endothelial cells from normal tissue [60], ischemic tissue [61], and breast cancer xenografts ex vivo [62]. Without compromising specificity, up to five different dyes can be used if spectral unmixing techniques and the near-infrared spectrum are used [63]. Recently, quantum dot-based nanosensors which exhibit narrow emission spectrums have been developed to target multiple molecules simultaneously [64]. Quantum dot-conjugated antibodies can be combined with qFlow cytometry to quantify membrane receptors in a multiplexed manner [65], [66]. Modern fluorophores and compatible antibodies can be used to stain for nearly any cellular protein, allowing intercellular diversity to be captured. In comparison to immunohistochemistry, qFlow cytometry images a greater number of cells but may sacrifice some subcellular resolution.

3) Protein interaction

Protein interaction assays seek to identify how proteins bind, and the function of protein binding. Here we introduce three quantitative methods used in systems biology to determine protein-protein interaction [67][68]: 1) Yeast two-hybrid assay, 2) MS-based affinity purification, and 3) surface plasma resonance (SPR).

Yeast two hybrid screening (Y2H) uses protein interactions to create a transcription factor for a reporter product, which tests for binary protein interactions [69]. The screening is simple and effective, but does not necessarily reflect in vivo conditions. MS-based affinity purification also probes the interaction of different proteins, though it is more technically complex than Y2H. In return for that complexity, the composition of macromolecular complexes can be tracked in more physiologically relevant conditions [70]. Surface plasmon resonance (SPR) quantifies the binding affinity of two types of molecules by detecting changes in the local refractive index upon protein-protein interaction on a biosensor. It has important applications in drug discovery and basic research because on-and-off kinetics can be measured [71].

IV. Experimental Models

Although large-scale gene, protein, and metabolite measurements (-omics) provide accurate identification of molecules and potential molecular interactions, it remains challenging to understand biological mechanisms with this information by itself. Therefore, experimental models that allow incorporation of biological complexity with knowledge of cellular and higher level system responses are beneficial. In all, an ideal experimental model for systems biology studies is reproducible, technically straightforward, quantitative, and has physiological meaning. The selection of appropriate experimental models for probing a biological system can be a challenging task. Here we describe some commonly used experimental models and discuss their strengths and limitations, mostly focusing on angiogenesis assays.

In vitro models of angiogenesis (e.g. cell cultures on or in Matrigel, transwell, or scratch assays) typically serve to quantify cell migration, endothelial cell proliferation and tubule formation, or cell responses to pro- and anti-angiogenic factors. In vitro studies allow for precise control of cellular environments, cell isolation, and augmentation, providing a high level of reproducibility. However, in vitro cell lines may not accurately recapitulate the physiology present in vivo due to the lack of the complexity in cell environment. Environmental factors that cells have surrounding them such as vasculature, tensile stresses, stromal cells, and connective tissues contribute greatly to the overall cell response to stimuli [72]. Thus, monolayer endothelial cells cultured in static media may display different pathology to that observed in vivo. A recent study showed that proliferation rates in micro-channel assays continuously decreased, reaching 5% of the rate of cells cultured in flasks after 48 hours, maintaining this rate for 5 days [73]. This discrepancy may be due to different surface antigen levels or growth factors expression from proliferating cells in culture compared to normally quiescent cells in adult blood vessels [74]. In addition, differences have also been present between human umbilical vein endothelial cells (HUVECs) and human dermal microvascular endothelial cells (MECs) [75]. Imoukhuede et al quantified angiogenic receptor levels on HUVECs and MECs using high-throughput quantitative flow cytometry, revealing previously heterogeneities within each cell line [59]. Recently, experimental techniques of co-culturing multiple cell lines have been developed to more accurately capture the physiological environment. Bryan et al proposed a protocol for co-culturing endothelial cells and pericytes, revealing important interactions between these two cell types in angiogenesis [76]. Despite these recent advances, intrinsic heterogeneity across cell types and within cell lines needs to be better understood, in addition to the standardization of experimental models to provide more accurate measurements.

Beyond in vitro assays, there are a plethora of in vivo assays which allow the study of interactions between cells and the supporting environment. It is important to note that the cells and tissue that support in vivo environments are inherently heterogeneous, and can be observed even in the membrane proteins found on these cells [60]. As such, the characterization of such heterogeneities in vivo systems offers more insight into the biological system than in vitro assays. In the study of vasculature, it is critical to capture these interactions including those between endothelial cells and their supporting smooth muscle cells, fibroblasts, pericytes, basement membrane, and extracellular matrix. One widely performed in vivo angiogenesis study, which captures the vascular structure, is the chick chorioallantoic membrane (CAM) assay [77]. New formation of vessels can be observed and semi-quantified on the chorioallantoic membrane of chick embryos using image processing tools such as ImageJ.

Two limitations of many in vivo models is their inherent complexity, which fails to separate the mechanisms of each system component, and their difficulty in scaling up to high-throughput measurement. To overcome these limitations, multi-culture protocols have been developed for screening pro-angiogenic and anti-angiogenic compounds. For instance, Arnaoutova and Kleinman developed a high-throughput angiogenesis assay of endothelial cell tube formation that can be created in 3-6 hours [78], whereas CAM assays usually takes 15 days. Another approach is metabolic foot printing analyses, which primarily use yeast. Yeast experiments have the advantages of safety, ease of use, absence of ethical issues, large specificity of knock-out mutants are obtainable, and 42% of its genetic information has human homologues, so the data derived from this method is very similar to human data. This method allows for potential probing of parameter values without the use of complex computational analysis [58].

Beyond high-throughput in vitro and in vivo, there lies ex vivo models, which provide a convenient, higher throughput option than in vivo testing. The choroid sprouting assay involves removing choroid tissue from live test subjects, culturing on Matrigel, and observing subsequent angiogenesis [79]. Next, image analysis can be used to determine the extent of angiogenesis by observing vessel growth and formation, and much of the lab work can be automated. This allows for high-throughput testing of pharmacological compounds in a mostly life-like environment. This assay can be expensive, but has so far shown promise in accurately reflecting the effects of pharmacological compounds on a system. However, ex vivo samples can be difficult to maintain and thus may have lower survival rate compared to in vitro assays [80]. Additionally, certain cell phenotypes may be altered by the tissue extraction and cell isolation processes.

Lastly, in situ modeling involves the culturing of large tissue in order to study smaller cells within the tissue. For instance, liver cells can be cultured and studied within a large portion of the liver. The cells in the liver represents the native environment and thus will experience little environmental alternation. Additionally, this has the strengths of in vitro culturing; testing and controlling parameters is relatively easy. However, the removal of such a significant portion of tissue is invasive and cannot be performed on biopsies. Additionally, while the cell to cell environment is conserved, larger system to system interactions are lost, and thus potential signaling between systems may be sacrificed. Fluorescence in situ hybridization (FISH), one of the best-known in situ techniques, uses a labeled complementary DNA or RNA strand to probe and visualize a DNA or RNA sequence of interest within tissue sections. Although FISH is very useful for detecting and locating viral nucleic acids and distinguish infected cells, it is limited in providing quantitative data for sensitive computational modeling. However, recent advancement in microfluidic devices has made it possible for in situ high-throughput –omics characterization [81]. In the future, minimized microfluidic platforms that are integrated with sensitive microarrays, or "Lab on a chip", can be used for quantitative monitoring of gene expression or molecular interaction in vivo or in situ. This will provide systems biology with not only spatial, but also temporal information for advancing quantitative modeling.

While in vivo human models would be the ideal test bed when probing for system interactions or responses, cross-talk from other components may add unnecessary complexity to the study [72]. Thus, isolated systems such as in vitro modeling, ex vivo modeling or even in situ modeling can be preferential to in vivo modeling for probing individual interactions or mechanisms.

V. Applications of Bimodal Systems Biology: Experiment + Computation

Creating comprehensive computational models to identify and test new therapies is the goal of many systems biology researchers. Even though no models are completely accurate they still provide insight where knowledge gaps exist and can therefore direct further experimentation. For example, one model determined that direction and rate of endothelial cell migration are decoupled, thereby directing further modeling and experimentation [82]. More recent models have focused on the molecular interactions that drive endothelial cell migration, specifically the VEGF family of signaling molecules and their tyrosine kinase receptors with the goal of inhibiting these molecules to prevent tumor angiogenesis. In one example, a hypothesis that the selective binding of PlGF to VEGFR1 would increasesVEGFR2 signaling by displacing VEGF from VEGFR1, thus making VEGF more available for VEGFR2 was tested and described using a deterministic model [16]. This model demonstrates two primary bases for computational modeling; it elucidated signaling mechanisms as well as tested design assumptions by performing sensitivity analysis on assumed parameters.

All models make assumptions to derive their results. Most ODEs and other equation based modeling use parameter or concentration assumptions. However, inaccurate parameters can cause lead to inaccurate or physiologically irrelevant predictions [83]. One way to make these models more accurate would be to seed the models with distribution data rather than discrete values. Usually, a singular value is imputed for a certain parameter in the model. If a distribution of values was inputted into the model, the model would able to resolve the entire range of possible parameter values. The resulting distribution of solutions could be used to better understand the system. To reduce this dataset, a sensitivity analysis could be done to interrogate the sensitive parameters that would significantly affect model results, and then applying the distribution approach to these parameters to gauge response.

The future direction of deterministic modeling in systems biology includes the move from single-scale, spatially isolated systems into larger, more comprehensive, multi-scale models (Figure 4). For example, a multi-scale model would incorporate stochasticity into ligand-receptor interactions, whole-cell responses from that binding action, and properties of the tissues made up of such cells. While elaborate, complex models of specific subsystems are an important focus of many modeling endeavors. Incorporation of multiple scales allows for integration of data at other scales, which will improve model robustness.

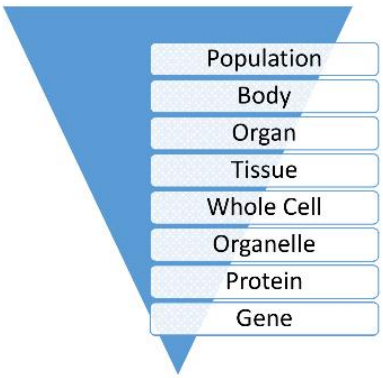

Figure 4 Modeling potential at different scales. Scale increases up the list. Each successively larger scale would be built using the smaller scale information below it.

There are significant computational challenges that accompany the development of multi-scale models. These challenges arise primarily from the fact that models at different scales use different data and modeling techniques. For example, signal transduction pathways are commonly modeled using ODEs, whereas ion channel opening is commonly modeled with stochastic techniques [84]. Agent-based techniques have been used for modeling the cellular level, such as in modeling endothelial cell migration [21]. These modeling techniques are carefully chosen as the best approach to representing the dynamics, spatial scale, and temporal scale of a particular system. When systems at multiple scales are combined, a more robust set of information can be captured, but integrating these different techniques is a significant computational challenge.

One recent technique for multi-scale modeling is the rule-based approach [85]. Rule-based modeling allows the integration of processes at different scales, as well as providing an iterative approach to model development. For example, Chen and colleagues used rule-based modeling to simplify the EGFR system from 400 interacting proteins to 21 proteins [86]. Rule-based modeling reduces ODE models to simplified rules based on the necessary inputs and outputs. Rule-based methods have been increasingly used in systems biology and are available with OLGIO, StochSim, and BioNetGen software.

Some techniques have been developed to alleviate the challenges of integrating model techniques at multiple scales. For example, Covert et al used flux-balance analysis and ODEs to model large-scale metabolic networks of carbohydrate uptake in *E. coli* [87]. The specific regulatory flux-balance analysis model was verified to show predictive power in different environmental conditions, while ODE modeling of transcriptional regulation from catabolites have been able to simulate observation in a wide range of experimental conditions [88]. The results of this integrated paradigm provide a framework to combining different modeling techniques and shows information gain that would not be possible when limited to a single modeling approach (Figure 5). However, the complexity of many systems would not allow for this approach. This approach is also computationally expensive: every iteration of model development involves recalculating the protein activity, gene expression, protein expression, flux distribution, and solving for new concentrations.

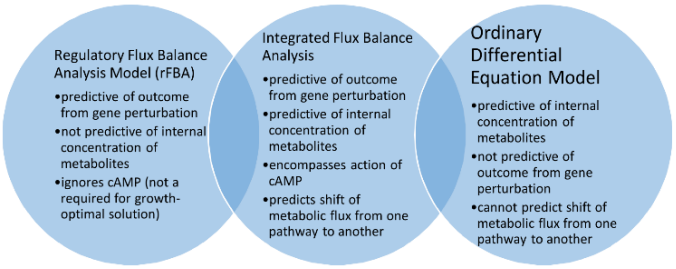

Figure 5 Illustration of the advantages of a combinatory model from different scales and modeling techniques based on information provided by Covert [87].

The integrated flux balance analysis approach provides a good example of the information gained from incorporating different model types. However, further approaches must be developed to integrate other techniques (i.e. agent-based models (ABM) and partial differential equations) and different scales. Another approach integrates an agent-based model of cell-level processes with a constrained mixture model (accounting for energy and mass balances) to model tissue-level processes (Figure 6) [89]. The agent-based model was based on data obtained in vitro while the constrained mixture model was based on tissue data. Both models were individually validated for the respective scales. However, disagreement between the two scales needed to be alleviated to create an integrated model. Specifically, the outputs from models, collagen and smooth muscle cell masses, had to be converged through the use of a heuristic genetic algorithm. While computational tools such as this genetic heuristic algorithm provide potential for systems biology, further algorithms and computational tools relevant to biological systems must be developed and refined for future models.

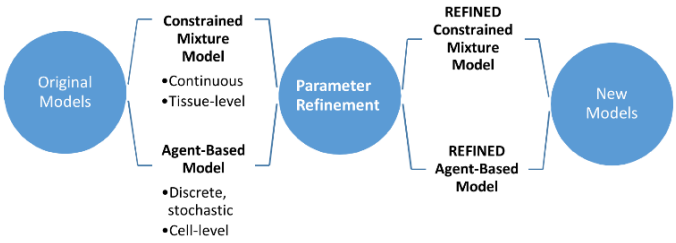

Figure 6 Illustration of the process and results of integration of different models from different scales based on information provided by Hayenga [89].

Towards these multi-scale modeling goals, a problem that must be overcome is that many different models exist to model distinct processes, while a unified whole-body model that incorporates the models of several researchers fails to exist. Reactome.org offers a good movement towards incorporating several cell processes into a qualitative framework. However, the challenges in unifying models is due to two major deficiencies: first, there is a lack of model uniformity; second, there is a lack of transparency in the dissemination of models. The lack of uniformity is partially due to a lack of knowledge about the implementation and design of different model types, and so as the field develops and more educated models are designed, greater uniformity will enable greater sharing and compatibility across models. Systems Biology Markup Language (SBML) is a modeling language that is gaining traction and may help overcome this limitation. However, transparency is a challenge that must be tackled side-by-side with uniformity. The presence of curated databases that accept well-notated, standardized models will enable greater sharing and implementation of current models.

VI. Application of Systems Biology in Personalized Medicine

A grand challenge in interfacing engineering with the life sciences, is understanding the complexity and heterogeneity in disease [90]. Previous decades have seen the collection of "big data" [91] in the genetic [92] and proteomic fronts [93]. Mapping complex interactions between genetics and proteomics requires the application of systems biology principles. By modeling the physiological pathways and processes the cause of complex diseases can be identified and efficient treatments can be developed.

The progression of systems biology applications to disease can follow a standard process as described in Figure 7. First, the genetic variation contributing to the disease can be identified using high-throughput gene sequencing across large patient populations. Next, pathways regulating this genetic variation can be computationally modeled to understand the proteomic role in the disease. The cell and tissue behavior can also be computationally modeled, as necessary. As computational models advance, new insights are discovered into the disease mechanisms, leading to more efficient drug development and personalized medicine. Here we describe three diseases that can benefit from this systemic approach: Metabolic Syndrome,

Alzheimer's Disease, and Cancer. We then present cancer in greater detail, highlighting modeling of ErB signaling, and anti-angiogenic approaches in cancer.

Figure 7 Schematic of systems biology approach to disease treatment.

A. Metabolic Syndrome

Metabolic Syndrome (MetSyn), which contributes to diabetes and cardiovascular diseases, has been of great interest to systems biology research [94]. Risk factors for MetSyn include obesity, high triglyceride levels, low high density lipoprotein (HDL) or high low density lipoprotein (LDL) levels, high blood pressure, and high blood glucose levels [94]. The chance of inheriting MetSyn is as high as 70%, but only a small fraction of syndrome occurrence is explained by known genetic variations [94]. Thus, scientists are still applying high-throughput genetic sequencing to try and identify specific genes regulating MetSyn. This genetic sequencing relies on single nucleotide polymorphisms (SNPs), which are locations in the genome where a single nucleotide varies between members of the population. By comparing disease-associated SNPs to eSNPs (SNPs at experimental trait loci) using causality analysis, we can determine the source of damaging mutations. Using microarrays, SNPs present in diseased subjects are compared with eSNP databases to specifically identify candidate genes. This approach has previously yielded results, such as allowing researchers to identify that the SORT1 and neighboring genes are responsible for elevated LDL expression leading to cardiovascular disease. This was determined by analyzing correlation between three candidate genes (SORT1, PSRC1, and CELSR2) and an SNP known to correlate to LDL elevation. This insight was then validated experimentally in mice where SORT1 was blocked [95]. The complexity of MetSyn makes treatment progress difficult: hundreds of genes and proteins are involved but their identity and relationships remain undefined. As these proteins and relationships are defined, computer models can be created to map the interactome behind MetSyn and identify effective treatments.

B. Alzheimer's Disease

Alzheimer's disease (AD) provides an example of a disease in the next stage of systems analysis. AD is a neurogenerative disorder common in aging patients characterized by loss of memory, judgment, and communications skills. The neurological causes are commonly understood to be Amyloid Plaques (a buildup of protein fragments called Aβ) and neurofibrillary tangles (strands of a protein called 'tau') [96]. Currently, models of the molecular mechanisms are being designed to probe further into the functionality of these proteins. In one such model, the synapse is treated as a complex machine made up of thousands of proteins and lipids on both sides of the synapse. Their interaction is tuned by modifying their relative geometry to create more or less transmission of action potential across the synapse. This tuning can increase or decrease the "weight" that the outgoing signals have on the incoming signal. This model revealed that plaque buildup inhibits synaptic tuning and causes the memory loss and other cognitive dysfunction seen in AD [97]. By further probing protein interactions and validating experimentally, the exact mechanism of disruption that leads to AD can be elucidated and treated. To this end, efforts are being made to computationally model neural systems. One such model uses mass-action to understand the interaction of calcium with NDMA receptors and calmodulin-dependent protein kinase II in regulating synaptic strength [98]. This type of synaptic modeling can be applied further to understand the precise causes of AD symptoms. The same principles could be applied to other CNS disorders such as PTSD, Bipolar Disorder, ALS, and others to improve understanding and treatment.

C. Cancer

Another prominent application of systems biology has been in cancer research. Genetic analysis allowed identification of numerous oncogenes and tumor-suppressor genes such as p53, ErbB receptors, and RAS [99]. The systems that these genes regulate were modeled computationally. For example, the ErbB signaling pathway was modeled using mass-action kinetics. This model investigated the effects of ErbB overexpression, and determined that high ErbB expression leads to sustained signaling. This response was verified through testing in MCF-7 cells to complete the feedback loop characteristic of systems biology [100].

One example of a proposed cancer model uses glioma cell imaging data to generate a model. Initial parameters for the glioma cells in brain tumors can be found from patient specific data such as location, structure, and vasculature through the use of contrast-enhanced MRI. More parameters such as cell density and microvasculature could be obtained from histopathology, gene arrays, tissue cultures, and proteomic profiling. This proposed model would allow for the creation of a temporally evolving and relevant model that could allow for further insight in the mechanisms of brain cancer, which is a particularly lethal cancer variant [101].

An eventual goal of systems biology is to produce personalized treatment plans that are based on an intricate understanding of cancer function. [102]. One way in which the advancement of personalized medicine will be accomplished is through a more sophisticated understanding of tumor make-up derived from lineage tracing. Tumors can grow from one cell or from a number of mutated cells, with most mature tumors being derived from one cell. Tracing epigenetic variations in the tumor by taking advantage of silencing one X chromosome in females allowed verification of this monoclonality [103]. With the knowledge that most tumor cells derive from a single source, computational models mapping the tumor genotype could provide personalized understanding of tumor phenotype and patient reaction to various treatment options.

Another application of computational modeling is in the integration of smaller scale models into a larger system. Many system models are individual cells or units inside an organ. Integrating these models into a larger "organ" or "organ system" model could be done by making these "cells" into small compartments from which several can be tied together into one larger unit. This can help with modeling larger scale interactions of diseases, as several diseases do not target one specific area. Modeling the initial tumor, as well as the organs at which metastasis is most to occur could give insight into tumor growth and metastasis prevention.

D. Applications of Modeling Targeted Regimes in Cancer Treatment

VEGF and its receptors are regularly over-expressed in a wide variety of human cancers, including breast cancer. VEGF overexpression is an early step in breast cancer progression, and frequently occurs before tumor invasion [104]. Furthermore, some breast cancers that overexpress VEGF show resistance to chemotherapy and hormonal therapy. Therefore, targeting VEGF and its signaling axis becomes a potential approach of inhibiting breast cancer progression and metastasis.

As Rebecca et al put succinctly in their 2014 paper, "Despite excitement about the development of targeted therapy strategies of cancer, few cures have been achieved." [105] Clinical studies have shown that tumors may develop resistance to drugs that inhibit VEGF signaling. This is believed to be because endothelial cells, cancer-associated fibroblasts, and pericytes within individual tumor display strong heterogeneity. Thus, therapies among different patients, different tumor types, and different cancer stages exhibit contrasting responses [106]. Another explanation is that tumors may develop an alternative signaling pathway, such as PDGF signaling, to recruit pericytes and/or tumor-associated fibroblasts to support angiogenesis after VEGF inhibition [107].

A good case-study for the challenge of anti-angiogenic drug resistance is bevacizumab. Bevacizumab is the first FDA-approved drug to inhibit VEGF by binding to all the isoforms of the parent VEGF-A molecule. Bevacizumab has activity in multiple tumor types, and clinical trials have indicated benefits of Bevacizumab when combined with chemotherapy for the treatment of non-small-cell lung cancer [108] and metastatic colorectal cancer [109]. However, phase III breast cancer trials showed the addition of Bevacizumab failed to prolong time to progression or overall survival due to unexplained heterogeneous responses, in addition to severe side effects and development of drug resistance [110].

More thorough studies of the balance of angiogenic receptor levels and crosstalk between angiogenic signaling axes under pathological and physiological conditions are required to understand the development of drug resistance and develop more efficient therapeutics. To this end, the balance between VEGFR1 and VEGFR2 on HUVECs and their response to VEGF-A treatment in vitro has been profiled [59]. More recently, heterogeneity in MDA-MB-231 breast tumors was discovered by quantifying receptor levels of VEGFR1 and VEGFR2 on tumor endothelial cells in mouse xenografts [62]. Erber et al. showed resistance of tumor blood vessels to VEGFR2 targeting, by the tyrosine kinase inhibitor SU5416, is conferred by recruitment of pericytes, which provide endothelial cell survival signals through PDGF and Ang-Tie signaling [111].

The concept of phenotypic resistance to VEGFR2 blockade is a well-discussed idea in the field of angiogenesis. Cassanovas et al. in 2005 [112] show that resistance to VEGFR2 blockade was associated with induction of proangiogenic factors other than VEGF, including members of FGF family, in a pancreatic islet carcinogenesis mouse model. To reduce the drug resistance due to alternative signaling, dual-targeting drugs have been studied. For example, Erber et al showed SU6668, which targets both VEGFR2 and platelet-derived growth factor receptor (PDGFR)-β caused 40% regression of tumor blood vessels in a C6 tumor mice model [111]. Other inhibitor cocktails include TAK-593 targeting VEGFR2 and PDGFRβ [113], sorafenib [114], a single-chain antibody targeting VEGF-A and PDGFRβ [115] and combination of AZD6244 (targeting MEK) and corenolanib (targeting PDGFR) [116].

Systems biology has brought valuable insights to understand the multi-step process of angiogenesis [117]. The complexity of signaling pathways involved makes it an excellent candidate for systems biology; and the combination of experimental profiling and computational modeling could offer new hypothesis. As Logsdon et al stated in their review article in 2014, "A model investigating the efficacy of targeting the VEGF co-receptor neuropilin predicted that inhibiting NRP-VEGFR coupling is a more effective strategy than blocking NRP1 expression or preventing VEGF-NRP binding" [117][118]. Another whole-body model of VEGF signaling investigated the level of free VEGF in the tumor affected by tumor microenvironment and drug characteristics such as the clearance rate and binding affinity [119]. The model further predicted that targeting $VEGF_{121}$ can reduce free VEGF in the tumor and yield effective reduction in VEGF signaling [120].

These findings indicate that the balance of VEGFR, PDGFR, and other surface receptors could provide insight into the behavior of cancer cells. With this in mind, it would be useful to profile the surface concentrations of VEGFR and PDGFR in multiple cancers and correlate these concentration profiles to cancer behavior, specifically response to anti-angiogenic therapies. This data could elucidate a series of classifications with distinct treatment profiles. Towards these goals, Imoukhuede and Popel have optimized qFlow cytometry, using it to characterize receptor concentrations on individual cells in a sample [59]. In concert with such new experimental techniques, computational models that involve multiple signaling pathways are being developed [121]. These models will provide insight into the significance of the receptor concentration profiles determined by the flow cytometry. By applying this to determine concentrations of VEGF and PDGF receptors on a variety of cancer samples, the necessary database could be produced and mined for insight.

E. Multi-Targeted Approaches to Anti-Angiogenesis

Endothelial cells in tumor vasculature exhibit a large amount of heterogeneity in expression as well as in vasculature structure. This heterogeneity is an important aspect to consider in treatment regimes, as differences in vascularity profiles can be the difference between an anti-angiogenic drug succeeding or failing in a patient [122]. There are many hypotheses for what causes these different expressions and modalities, one of which is that the extracellular matrix (ECM) contributes to the heterogeneity through molecular expression as well as physical parameters such as stiffness [123]. It is even believed that certain types of collagen in the extracellular matrix, type I and type IV, can induce chemo-resistance through interactions with certain integrin [124]. Cancer ECM is also much more stiffened than typical ECM, which results in a potentially drastic difference in the chemical environment of the cancer endothelial cells as stiffness correlates to a difference in growth factors and modifiers such as Yes-associated protein [125]. Further studies have showed that small-molecule inhibitors of collagen synthesis prevent angiogenesis and tumor growth [126]. As a result, a large amount of research has gone into the development of the "matrisome" a comprehensive library of proteins that complement the extracellular matrix [127]. This extensive library is the first step in developing a mechanism in differentiating tumor extracellular matrix from healthy matrix, which in turn can provide vital information on how endothelial cells are affected by these changes. These effects could be the exact criterion on which to discriminate cancerous endothelial cells from healthy endothelial cells.

Drugs targeted to the extracellular matrix can transport more easily, as it is not encapsulated by a plasma membrane. Extracellular matrix targets have been pursued in many applications already. One such medicine, Lysyl oxide, has been tested in murine models to inhibit tumor growth. Lysyl oxide is up-regulated in tumor ECM, and is an enzyme that interacts with the ECM, which makes it a viable target. Using nanoparticles rather than antibodies conjugated to the lysyl oxide resulted in definitive decreases in tumor sizes in the mouse models [128]. There are more drugs currently in clinical trials that target extracellular matrix binding. Cilengitide (EMD 121974) targets αvβ3 and αvβ5 integrins and inhibits them. It is currently in phase one and two clinical trials. Intetumumab (CNTO 95) is a monoclonal antibody that targets the αv integrin. It has shown some promise as it slightly increases survivability in cancer treatments. It is currently in clinical trials [129].

These examples suggest that the ECM is a viable target. Considering the past research suggesting correlations between cancer ECM and tumor angiogenesis, a robust model of ECM proteins, structure, and angiogenic signals could potentially lead to drug targets for anti-angiogenesis. For example, a factor that is commonly targeted for angiogenesis is VEGF. Tumor ECM can alter the uptake of VEGF through additional modification. Basement membrane matrix protein 9 (MMP9) is necessary for transport of angiogenic growth factor VEGFR, which means an increase in the MMP activity will increase the uptake of VEGF [130]. Tumors typically do increase MMP activity to facilitate growth and establish more vascularity. This upregulation of glycolytic activity increases the amount of lactic acid produced, which changes the local pH of the tumor to be more acidic. The local acidity further effects the endothelial cells inside the tumor as pH of the environment definitely affects the expression of the cells [131]. These changes in expression can be used to differential the healthy endothelial cells from the tumor endothelial cells.

A comprehensive model of ECM proteins, tumor endothelial cell growth pathways, and angiogenic factors may be a potential new arena for cancer drug target discovery. Moreover, combining not only kinetic reactions but also the effects of physical structure within the ECM and tumor vasculature growth —both which differ from that of normal cells– may result in a multi-scale model with robust predictability. These modeling techniques would allow for the probing of physical ECM effects on endothelial cells and could be an engine to develop new treatments and diagnostics for cancer. Since the amount of data pertaining to cancer and ECM interactions are so large, some models strive to simplify the overall model. This results in drastic decreases in computational processing time, while remaining faithful to the empirical data [132]. By elucidating the mechanisms by which the ECM affects the expression of tumorous cells, drugs that restore normal cell function or target irregular function could be developed.

VII. Concluding Remarks

Systems biology has developed substantially with recent advances in both computational modeling and sophistication of experimentation. As experimental models have begun to reach the limits of realism allowed by biology, the focus of experimentation has shifted to the advancement of high-throughput, quantitative technologies that allow us to screen organisms at multi-scales. The data obtained with such technologies in turn allows complex in silico testing and provides feedback to improve existing experimental models and predict stimuli responses. This iteration between experimentation and modeling has been applied to the treatment of diseases such as Metabolic Syndrome, Alzheimer's disease, and cancer. As the field moves forward, better understanding of the mechanisms behind these complex diseases will yield new treatment strategies and precision medicine.

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