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Systems modeling: a pathway to drug discovery

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Systems modeling is emerging as a valuable tool in therapeutics. This is seen by the increasing use of clinically relevant computational models and a rise in systems biology companies working with the pharmaceutical industry. Systems models have helped understand the effects of pharmacological intervention at receptor, intracellular and intercellular communication stages of cell signaling. For instance, angiogenesis models at the ligand–receptor interaction level have suggested explanations for the failure of therapies for cardiovascular disease. Intracellular models of myeloma signaling have been used to explore alternative drug targets and treatment schedules. Finally, modeling has suggested novel approaches to treating disorders of intercellular communication, such as diabetes. Systems modeling can thus fill an important niche in therapeutics by making drug discovery a faster and more systematic process.

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Introduction

The advent of combinatorial chemistry, structure-based design and genomics each marked shifts in the drug discovery paradigm. Can modeling and, specifically, the quantitative modeling of cell signaling pathways, be the next milestone [1–3]? There are some noteworthy challenges facing the pharmaceutical industry today where modeling might play a key role. Firstly, modeling enables systematic integration of the overwhelming amount of relevant information that has accumulated from high-throughput screening methods. Further, models help in establishing a mechanistic understanding of the disease and of drug action, which is a marked shift from the traditional 'black-box' approach to drug discovery. Finally, systems modeling helps in predicting probable side effects, and finding optimal dosages and treatment schedules.

The phrase 'quantitative models of cell signaling' refers to the creation of *in silico* representations of signal flow within and between cells. This signal flow occurs through a series of protein and small molecule interactions, enzymatic reactions, translocation steps and other elementary chemical and cellular processes. Each of these can be characterized quantitatively in the computer. Simple models consider a uniform cell and mass-action chemistry, represented as ordinary differential equations. More sophisticated models capture the spatial and structural attributes of the *in vivo* system. Ideally, these models allow one to explore the probable effects of perturbations on a system, and to identify areas that could be amenable to therapeutic intervention. Importantly, such hypothesis generation and testing is typically faster than *in vitro* or *in vivo* experiments.

In Figure 1 we detail the process of modeling a biological signaling system. This process begins with extensive data-mining from the literature to obtain quantitative parameters such as chemical rate constants. Other specific inputs to the model might be details about key regulatory pathways, known interactions with pharmacological agents, tissue specificity, and so on. Often, it is necessary to use indirect data or even educated guesses to set a parameter for which direct data do not exist. The model is considered valid if it can explain a substantial set of experimental observations. Finally, the model is used to generate hypotheses that can then be tested. Through this iterative process, one gains a better understanding of the biological system and insights into possible therapeutic interventions.

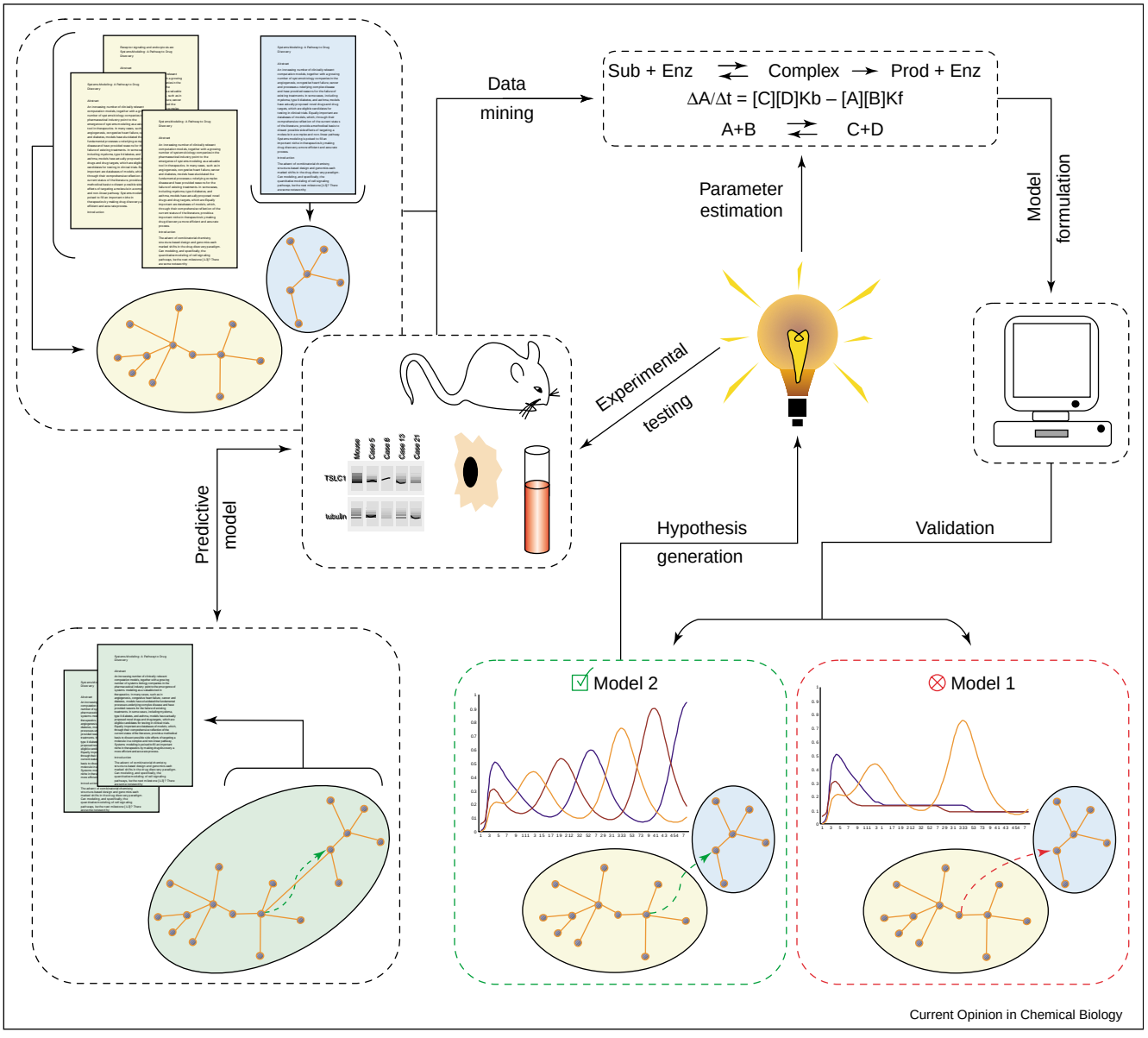
Where does signaling interface with therapeutics?

We discuss three levels of cellular responses, each of which can serve as possible nodes for drug targeting: signal reception, intracellular responses, and intercellular communication (Figure 2).

Signal reception

Cells receive inputs from different signaling molecules, which are often recognized by receptor proteins. At least 70% of drugs in development today target such receptors [4]. Quantitative cell-signal modeling can help elucidate the mechanisms of receptor responses to pharmacological interventions. For example, several models [5,6] have been developed to gain a better mechanistic understanding of basic fibroblast growth factor (FGF-2) receptor signaling, a pro-angiogenic pathway. Promoting angiogenesis is a promising therapeutic strategy for cardiovascular disease; modeling FGF-2 signaling, therefore, is a

Figure 1

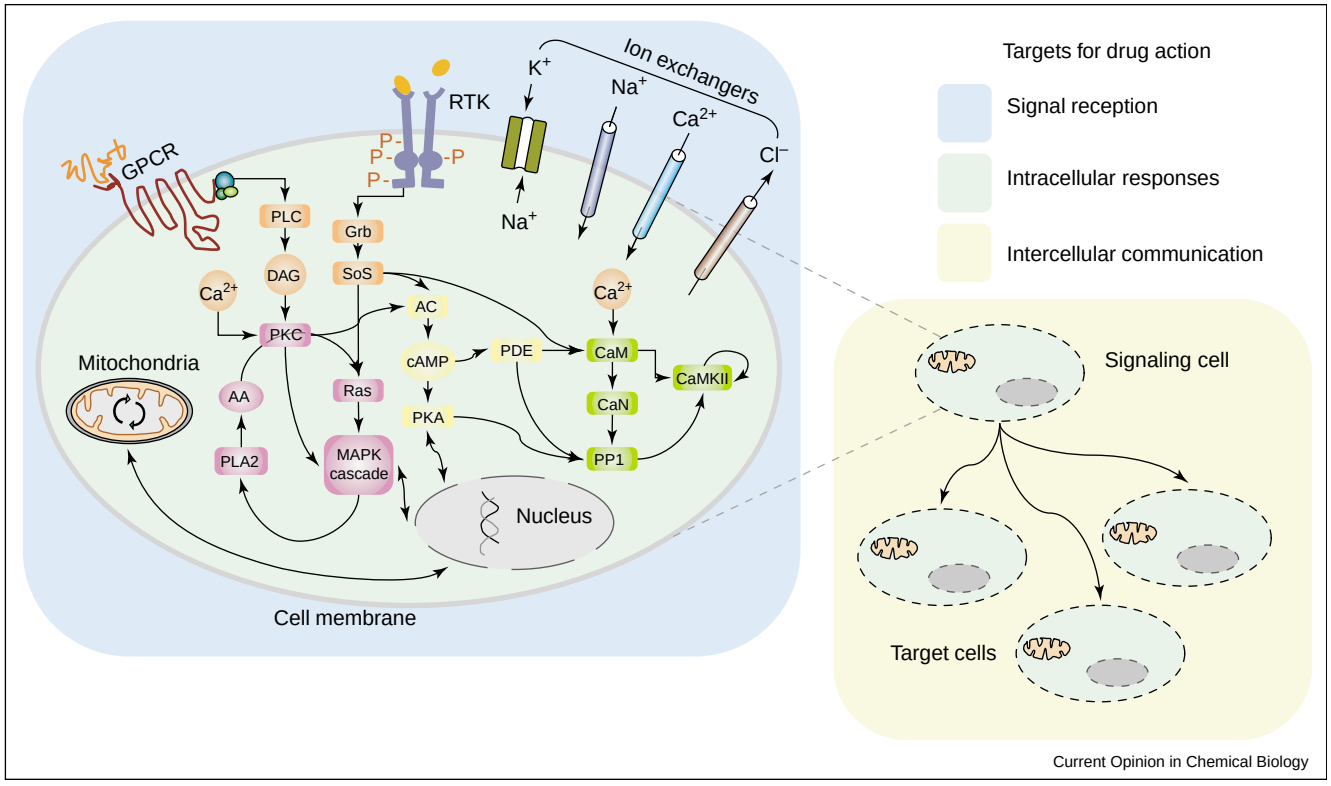


Iterative process of modeling a biological signaling system. Data are collated from literature and experiments, and parameters are estimated where necessary. Models are considered to be valid when simulation results match a significant set of experimental observations. Finally, the model is used to generate hypothesis, which can be experimentally tested.

much needed study. These computational studies were inspired, specifically, by the need to explain failed clinical trials involving intracoronary infusion of FGF-2 in humans [7]. The models suggest that the administered FGF-2 may only cause transient increases in intracellular FGF-2 because of unanticipated rate-limiting steps in the pathway. Such a discovery is made much easier through a detailed kinetic analysis of signaling pathways.

Another therapeutically important receptor target is the epidermal growth factor receptor (EGFR) family. This is one of the best understood signaling systems, and computational models have played a significant role in its elucidation [8]. EGFR inhibition is a promising anticancer treatment strategy [9]. The successful breast cancer drug Herceptin® (trastuzumab) is an example of a drug that targets human EGFR 2 (HER2). Models have since then been developed to better understand HER2 signaling and its role in various cancer types [10]. Despite the increasing use of EGFR inhibitory agents, much remains to be understood about their activity and preferred clinical applications. Computational models of EGFR seem poised to aid in filling in the gaps.

Figure 2



Interface of signaling with therapeutics. Three levels of a generic cellular response. Each level can serve as a possible node for drug targeting: signal reception (in blue), intracellular responses (in green), and intercellular responses (in light green).

Quantitative signaling models are being used to understand the mechanism of many other receptor–ligand interactions with therapeutic importance. For instance, the role of immune-receptor signaling in antigen recognition has been studied through mathematical models [11]. Similar modeling techniques have been used to explore how dimerization of G-protein-coupled receptors affect receptor localization, signaling and internalization in diseased states [12].

Intracellular responses

Once an external stimulus has been communicated to the cell interior, it is amplified and diversified through the activation of various signaling and metabolic pathways. Here, we see the beginnings of signaling complexity and the need to have a systems-level understanding of information processing and convergence. Cross-talk amongst the various pathways may yield switching, oscillations and other emergent properties that are not characteristic of the individual pathways themselves [13,14]. In a therapeutic context, modeling this cross-talk can reveal non-intuitive drug targets and show possible side effects, as we illustrate below.

Non-intuitive drug targets in signaling networks

Haugh *et al.* [15] have developed a model of the phospholipase C (PLC) pathway, a key signaling pathway shown to be essential for cell motility and directionality [16]. It has been implicated in cancer growth and metastasis and is a prospective target for cancer therapeutics. While one intuitively expects that direct inhibition of PLC would be the best therapeutic, their model argues otherwise. The authors show that aminosteroid U73122, a putative inhibitor of PLC, is relatively inefficient because it would require adversely high drug concentrations. The substrate of PLC, phosphatidylinositol (4,5)-bisphosphate, is predicted to be a more effective target since it has rapid turnover and needs to be constantly resupplied to the plasma membrane.

The nuclear factor-κB (NF-κB) pathway is another system that has been well studied through modeling, especially for cancer therapy [17••,18]. The NF-κB family of proteins are transcriptional regulators that play important roles in cell-survival and apoptosis. Because persistent NF-κB activity is linked to tumor formation and metastasis, this pathway has emerged as an attractive drug target. Bortezomib, a recently approved drug for multiple myeloma, acts by inhibiting upstream targets of NF-κB nuclear translocation. Surprisingly, Sung *et al.* [18] showed through their model that for effective dampening of nuclear NF-κB levels, bortezomib must inhibit its targets by at least 95%. In reality, achieving even 65% activity is a challenge

because of limits on maximum dosage. As an alternative, the authors showed that a hypothetical drug directly inhibiting cytoplasmic NF-κB was more effective at low dosages. This study not only brings to light a novel drug target, but also discusses the effects of drug dosages and alternative treatment schedules.

The components of the β-adrenergic signaling network have received considerable attention as possible therapeutic targets in congestive heart failure due to reduced myocyte contractility. Saucerman *et al*. [19•] analyzed the effects of adenylyl cyclase (AC) overexpression and showed that it has a beneficial effect on myocyte contractility through an increase in cAMP levels. However, gene therapy is required to attain therapeutic levels of AC. As an alternative, their model shows that a hypothetical drug designed to increase the affinity of Gs-α to AC would have equivalent therapeutic value.

A particularly interesting and ambitious project is the computer simulation of a human cancer cell by Christopher *et al*. [20•]. This model integrates over 1000 genes and proteins that are central to cell survival and homeostasis, and cites over 3650 literature references, with the focus of addressing clinically relevant questions. The authors have conducted a series of *in silico* knockdown experiments, which were validated by siRNA methods. The model is now being used to explore possible drug targets and to provide a mechanistic understanding of drug action.

Drug targets in metabolic pathways

Like signaling networks, metabolic pathways are complex networks for which modeling has provided therapeutic insights. This is especially the case when the metabolic pathways of infectious agents contain unique enzymes that do not have a human homologue.

Trypanosomes are protozoa that infect humans and cause Chagas' disease and sleeping sickness. Helfert *et al*. have suggested, through a combination of genetics and computer modeling, that the glycolytic enzyme triosephosphate isomerase is essential for trypanosome survival [21]. The conventional view held that targeting this enzyme might not be effective, because the parasites could still produce ATP through alternative pathways.

Mitochondrial metabolism has been suggested as a target for therapeutic manipulation in cardiovascular disease, provided we understand the underlying signaling [22]. Mitochondrial models range from ones that explore only energy metabolism [23,24] to those that explore broader ranges of physiological functions [25]. These models integrate cellular signaling with metabolic and regulatory pathways. Such studies address mitochondrial signaling complexity as a first step to exploring pharmacological intervention.

Side effects as a result of cross-talk

A qualitative characterization of signaling networks can be very useful in addressing toxicity issues and side effects. For instance, it has been suggested that the toxic effects of pyrazinamide, a drug used for treating tuberculosis, could have been predicted using pathway analysis based on the literature available at the time of approval [26]. Further, the blue vision side effect seen in patients treated with Viagra® (sildenafil citrate) might have been predicted using pathway information [1]. In this case, the drug binds to both its intended target, phosphodiesterase-5 in smooth muscle, as well as a homologous protein phosphodiesterase-6 in the eye, giving rise to blue vision.

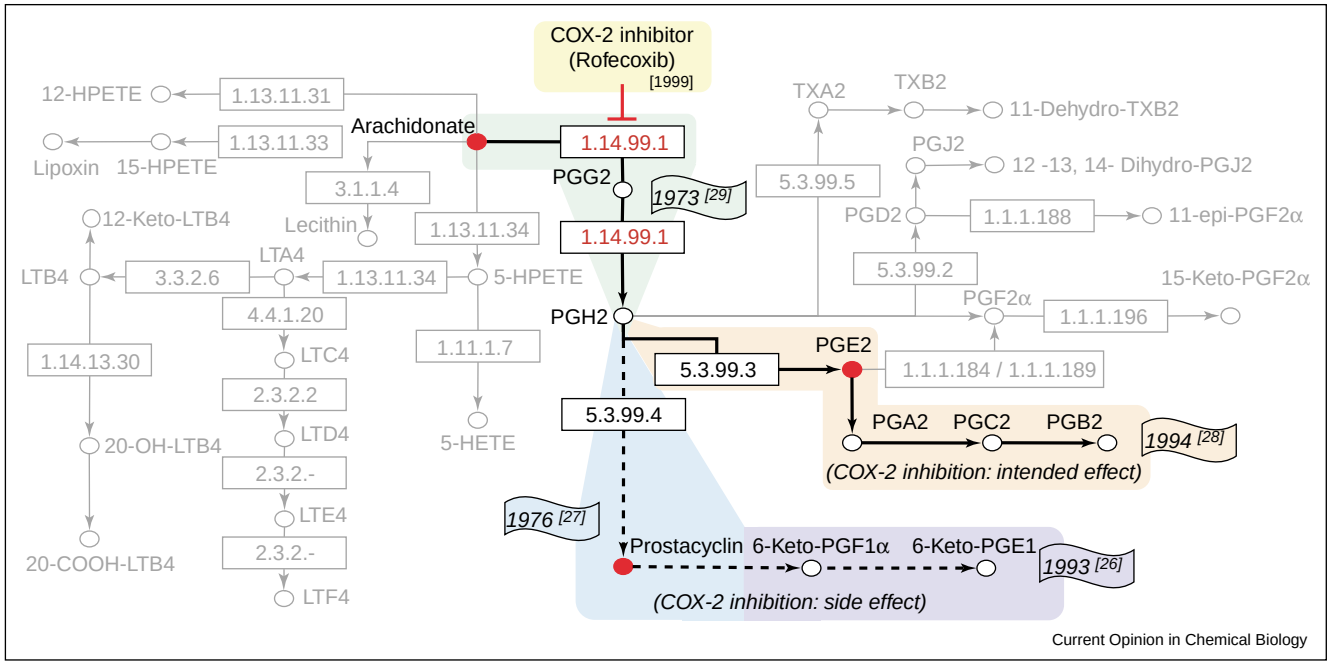
Finally, we consider the recent Vioxx® (rofecoxib) recall made by Merck in September 2004; perhaps the largest recall yet made by the pharmaceutical industry. The recall was made when patients using rofecoxib, a cyclooxygenase-2 (COX-2) inhibitor, for arthritis treatment were observed to be more prone to heart attacks. In retrospect we see that there were a few reports of increased heart problems amongst the volunteers even during the clinical trials. Rofecoxib exerts its anti-inflammatory properties by inhibiting PGE2 activation downstream to COX-2. Observed side-effects arose because COX-2 also inhibited prostacyclin, a platelet inhibitor (Figure 3). This significantly increased the risk of clot formation in arteries. Interestingly, much of the relevant pathway information regarding this COX-2 inhibitor side-effect was available at the time of clinical trials in 1999, albeit in a scattered form [27–30]. This is where modern pathway databases can help to organize the scattered but essential pieces of information to facilitate efficient look-up of drug targets involved in multiple critical pathways. Figure 3 shows the prostaglandin pathway (KEGG map ID:00590) adapted from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database [31], which strikingly points to both the desired and harmful effects of COX-2 inhibition.

Intercellular communication

Cells do not function in isolation. Many physiological effects arise from synergistic interactions between multiple cells. Several disease conditions, such as diabetes, asthma and heart disease, are due in part to faulty intercellular communication.

Kansal [32] reviews some of the modeling approaches in type-2 diabetes. Specifically, Kitano *et al*. [33••] formulated a model to address this metabolic disorder. They used the model to describe interactions between adipocytes, hepatocytes, skeletal muscle cells and pancreatic β cells. The authors have discussed the possibility of targeting multiple molecules in a pathway, which might synergistically attenuate the progress of the disease. The model specifically explored the use of infliximab, an antibody for tumor necrosis factor-α (TNF-α), in combination with other anti-TNF-α strategies to treat type-2 diabetes.

Figure 3



COX-2 inhibitor effects in the prostaglandin pathway. The prostaglandin signaling pathway adapted from the KEGG database. In 1999 Merck introduced the drug Rofecoxib, to treat arthritis. Rofecoxib (shown in light green) inhibits the COX-2 enzyme (1.14.99.1). COX-2's catalytic activity is important for two downstream pathways with distinct physiological consequences. Solid arrows point out the pro-inflammatory pathway, the inhibition of which is the intended path of drug action. Dotted arrows show the atheroprotective pathway, the inhibition of which leads to the observed side effects. The colors indicate the date by which each component of the pathway was discovered. Both the beneficial and harmful downstream components to COX-2 were known well before 1999 when the drug was introduced.

Asthma is an inflammatory disease that typically involves difficulty in breathing due to narrowed airways. The Asthma PhysioLab model developed by Stokes *et al.* [34] detailed the complex interactions of airway physiology with the inflammatory response. This model simulated asthmatic symptoms in response to exposure to an allergen. Contrary to what animal studies had shown, the model predicted that anti-interleukin-5 (IL-5) treatment would be ineffective in treating airway obstruction observed in asthma. Results of anti-IL-5 clinical trials lent credence to the model [35]. This initial success encouraged Pfizer to use the Asthma Physiolab for alternative drug target identification and evaluation.

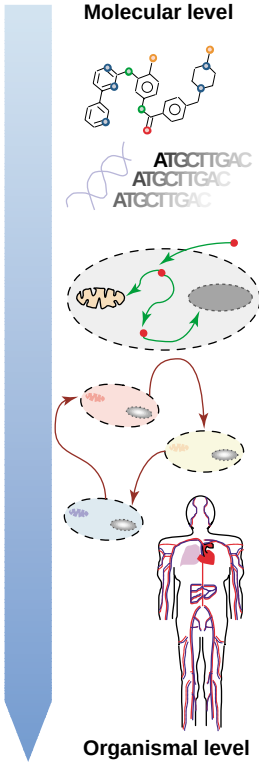
There is a rich history of cellular myocyte modeling in understanding heart physiology. These models link both inter- and intra- myocyte biochemistry to the electrical and mechanical properties of the whole heart. Winslow *et al.* [36] have discussed case studies where modeling has helped understand the consequences of altered protein dynamics at the myocyte level in causing a multicellular phenomenon such as arrhythmia. Similar efforts to link cellular biochemistry with whole organ function are being pursued by the internationally collaborative Physiome Project [37,38].

Conclusion

We have discussed models that describe cell responses to pharmacological intervention. These models span varying levels of quantitative detail. The more qualitative models help trace non-linear flow of information, and in so doing, can predict counter-intuitive effects of perturbing a system. Detailed quantitative models can additionally provide a mechanistic basis for the failure of certain treatment paradigms, and explore alternative drug targets. The extent to which a model can be predictive increases as one tightens the kinetic constraints on a system. A lack of quantitative detail may perhaps explain the surprisingly few models that attempt to identify drug targets at the level of gene transcription.

Although we have restricted our discussion to pathway modeling, other computational methods have also been important in understanding and treating disease (Figure 4). Pathway modeling is of particular promise, as its specificity and predictive power will only increase with the influx of more complete and accurate data. There are strong moves to enhance collaboration between researchers, drug developers and modelers [47], including the development of powerful modeling tools and common platforms for model interchange [38,48]. Together with clinically oriented

Figure 4



Computational methods	Description	Reference
Structure based design	Using the molecular structure of the target to identify lead compounds.	[39, 40]
Genomics	Mining the human genome to find additional members of gene families that contain known drug targets.	[41, 42]
Interaction maps	Using qualitative models of cell signaling networks to trace chemical interactions.	[43]
Metabolic control analysis	Studying the kinetics of multi-enzyme metabolic systems by analyzing flux control and distribution of intermediates.	[21, 44]
Quantitative intracellular signaling models	Using quantitative representation of signal transduction within a cell to find rate-limiting reactions and critical molecules.	[15, 18]
Probability weighted models	Statistical models used to understand cell population dynamics indiseases.	[45]
Intercellular signaling models	Using multi-cellular pathway models to understand and attenuate robustness of diseased states.	[33••]
Pharmacokinetic models	Mathematical models used to study absorption, distribution, metabolism and excretion of a drug.	[46]
Whole body simulations	Using integrative models of cells, tissues, and organs to quantitatively describe physiology and pathophysiology of the intact organism.	[38]

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Computational Methods in Therapeutics. A listing of *in silico* methods used to complement and advance conventional drug discovery. Further details can be found in [15,18,21,33••,38–46].

curation of model databases, these developments promise to take modeling from a primarily academic exercise to an integral part of the drug development process.

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