What it takes to understand and cure a living system: computational systems biology and a systems biology-driven pharmacokinetics–pharmacodynamics platform

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The utility of model repositories is discussed in the context of systems biology (SB). It is shown how such repositories, and in particular their live versions, can be used for computational SB: we calculate the robustness of the yeast glycolytic network with respect to perturbations of one of its enzyme activities and one transport system. The robustness with respect to perturbations in the key enzyme phosphofructokinase is surprisingly large. We then note the upcoming convergence of pharmacokinetics–pharmacodynamics (PK–PD) and bottom-up SB. In PK alone, quite a few one-, two- or more compartment models provide valuable initial guesses and insights into the absorption, distribution, metabolism and excretion of particular drugs. These models are phenomenological however, forbidding implementation of molecule-based tools and network information. In order to facilitate a fruitful synergy between SB and PK–PD, and between PK and PD, we present a new platform that combines standard phenomenological models used in the PK/PD field with mechanism-based SB models and approaches.

Keywords: computational; systems; biology; pharmacokinetics; pharmacodynamics

1. INTRODUCTION

Genomics started from biochemistry and then molecular biology. It was paralleled by a development in physics and mathematics, which led to applications of non-equilibrium thermodynamics in biology, mathematical biology and ultimately to metabolic control analysis, flux balance analysis and dynamic network modelling. These two upward movements have since been combined into a scientific discipline called Systems Biology [1]. Systems Biology (SB) aims at understanding how biological function emerges in the interactions between components of biological systems [2]. Ultimately, SB should enable one to understand how improper networking of the macromolecules of living organisms leads to their diseases and how molecular interference may redirect those networks to their proper functioning. SB has progressed to new understanding of the organization

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and functioning of metabolic and signal transduction pathways in ways that had been impossible with molecular and cell biology, and indeed with functional genomics, alone [3]. Moreover, not even SB has delivered yet the understanding of the functioning of entire organisms, such as in an understanding of disease or in actual drug discovery.

An important issue here is the vastness of the networks of living organisms. An organism as 'simple' as Saccharomyces cerevisiae (baker's yeast) contains 6000 genes, with tens of thousands of interactions of its gene products [4]. In one view, every gene depends on all other genes [5], which would lead to a number of interactions exceeding the number of atoms in the universe, if one takes into account that the interactions between any two may be influenced by indirect interactions with any of the others. But even if the interactions are more ordered than this, the extreme bottom-up approach to whole organism SB that would describe the activity of every individual macromolecule, is not within the reach of the present computation methodologies, and, even worse, not within the reach of the necessary experimentation facilities.

Middle-out SB has been proposed as an alternative [6]. By starting at the physiological level, i.e. closer to where biological function actually resides, it should obtain more bearing on pathophysiology. Indeed, the SB of the heart has been able to help with the correlating QT characteristics of the electrocardiogram with experimental drug-toxicity markers [7]. On the other hand, it has not yet connected physiology with much of the molecular world, such as in genetics and molecular drug design. In the two types of SB, simulations play a somewhat different role [8].

In strictly bottom-up SB, such as the one defined by the silicon-cell approach [9], simulations of systems function are achieved by coupling the molecular interaction properties of system components in a computer programme. The simulations use known, experimentally determined interaction parameters, and do not fit these to experimentally observed system functioning. The disadvantage of this is that the simulation results may be distant from the observed system's function, e.g. owing to trivial experimental error in the determination of the parameter values. The advantage is that discoveries of hitherto unknown interactions become possible; these are not 'fitted away'. This approach has enabled the discovery of a phenomenon that can occur in catabolic pathways and is known as the ‘turbo explosion’ [10], the discovery of the function of hitherto un-understood regulatory mechanisms and cell anatomical features [11], and indeed the identification of two new types of drug target [12].

Less-strict bottom-up SB examines if a proposed SB model could explain observed network behaviour. If system behaviour is fittable, the model is consistent with the observations if one foregoes experimental knowledge about the parameters. The model may or may not be correct then, because the fitted parameter values may or may not be equal to the actual ones that have or have not yet been determined. As many models of this type use abstract rate equations rather than true ones, this issue often becomes elusive. It is not helped either by the problem of unidentifiability of parameters in models.

Physiology has made ample use of simulations. These simulations started from the other end of the spectrum. They observed and quantified the functioning of living organisms, often in terms of phenomenological functions. Some of these, such as embodied by Monod's growth equation were very similar to molecular functions [13]. Others, such as Hodgkin-Huxley's equation were useful abstractions of the latter [4]. The equations used in pharmacokinetics (PK) constitute another example of the approach. They use abstractions of physiological processes to fit equations to observed dynamics of the concentrations of drugs in the patient. Parameters again refer to abstractions of real components of the systems; they include 'distribution volumes', which often much exceed realistic volumes, as they comprise the effects of partition coefficients. This is fine for quasi-steady states, but may not work well in dynamic situations, or when saturable kinetics determines distributions. Indeed, mechanistic PK is probably the most neglected field in the area of medically relevant biosimulations. It is often combined and considered together with mechanistic pharmacodynamics (PD). The full understanding of underlying phenomena such as physiological regulation and control, phenotypes, mutations and diseases should be important for future medicine. Semi-mechanistic models or even simple phenomenological formulae and models have been in use since the beginning of the twentieth century and have provided useful insights in, e.g. physiology- and PK-related issues [14]. Complete, mechanism-based but in most cases 'drug-free' models and simulations are more appreciated in the SB community, but they are not much employed by the PKPD community.

Combined application of SB and physiology-based PKPD may help SB reach its goal of addressing whole-body function and empower PKPD eventually to produce more effective molecular network-based treatment methods. Such an integrated approach has the potential of influencing and guiding the development of future drugs. It also places an expert in biosimulation in the position to talk to and advise medical and clinical researchers and practitioners, and vice versa. Over the years of medical or laboratory practice, the physicians and clinical scientists have gathered enormous expertise and ability to relate biochemical knowledge to disease symptoms. In most cases however, they have been deprived of the support of insights coming from mathematical models, and, conversely, their expertise has had little influence on the models developed by the SB community. A platform of PKPD and SB with readily usable modelling tools, could further empower the clinicians into making better therapy judgements and the systems biologists into making more realistic models.

A downside to the fascinating complexity of living organisms is that any of the areas within metabolism, absorption, distribution and excretion (ADME) is packed with open questions, the answering of which is essential for bottom-up PK. The intra- and
interindividual variability in every (patho-) physiological process makes it necessary to go through a painstaking process of data elicitation and collection. Database curation of the highest quality, indispensable for the purpose of systems analysis, is not a favourite task for modern academics, as crucial as it is. The lack of quantitative and standardized in vivo measurement techniques at the molecular level forces one to obtain in vitro data in artificial or cell line-derived constructs (e.g. Caco-2) or to interrogate animal models barely resembling the human. The accompanying hurdle is the in vitro-to-in vivo and/or inter-species extrapolation (often based on phenomenological and disputable allometric ‘laws’). Each of these steps is full of simplifications distorting the reality one thinks to observe.

Most of the research into applications of PK and PD to new and improved medicines and therapies, is carried out in medium and large biotechnology and pharmaceutical companies, each isolated from all others. Their limited resources concentrate on a few issues considered most relevant for a particular compound under development, and with the aim of satisfying requirements of a drug-regulatory body. This leaves other research areas insufficiently explored and mechanisms untested. This could be fair for one of the, rare, single-gene diseases, but since the majority of diseases are network diseases and the relevant networks tend to span the greater part of the genome’s image, such a cottage-industry approach is doomed to failure.

There exist of course a number of excellent tools for physiologically based whole-body models like SMDYP, GASTROPLUS and PKSIM or ADAPT II, WinNonlin, NONMEM and KINETICA for compartmental (population) PK analysis. The tools in the first category suffer from their closed architecture making open source collaboration impossible. On the other hand, tools in the latter category are accessible as standalone applications, running to a large extent under Windows only. Their user-friendliness varies between very sophisticated but expensive, and disputable (e.g. Fortran syntax in NONMEM) but free or inexpensive. We consider the last point important, as mathematically and/or IT inexperienced users will not be encouraged to use them.

It is about time to start an open source collaboration. Many different academic parties and eventually the patients will benefit, as will the industry itself, provided it engages in the corresponding, new, expertise-based business models that are much closer to those of the software industry than imagined. In this paper we shall introduce SBPKPD, a platform for such an open-source collaboration.

2. LIVE MODEL REPOSITORIES FOR BIOLOGICAL SYSTEMS

Many mathematical models exist that are relevant to a variety of biological processes. Most of these remain buried in the literature. They have typically been written in any of a variety of rapidly outdating computer languages. Most often the source code does not accompany the publication. Although in principle, some of these models can be re-coded in current modeling languages, the practice is that the models have rarely been described in sufficient detail to do this. Year-on-year models are being lost and wheels are being re-invented.

One of the great contributions of the SB community to science has begun to halt this loss of impetus of pre-existing simulations and models. It constitutes a lingua franca for biomathematical models, which allows exchange of models between modelling platforms [15]. This facility has also led to a multiplication of the use of contemporary models, where scientists remote to the subject area of the models are now analysing them by a wide variety of methods, such as elementary mode analysis and flux balance analysis. This phenomenon is reminiscent of the surge in the utility of DNA sequences after they had been deposited in gene and later genome sequence databases, with bioinformatics as the resulting discipline.

The utility of databases has also penetrated the SB community, although less so. A number of open-source model repositories exist, with a broad spectrum of models and simulation facilities (Java Web Simulation (JWS); http://ijj.biochem.sun.ac.za/ [16,17] and Biomodels.net http://www.ebi.ac.uk/biomodels-main/ [18], or more specialized (e.g. CCDB; http://www.itb.cnr.it/cellcycle/, which contains cell cycle-related models only)). Together, JWS online and Biomodels store hundreds of kinetic models for metabolic, signal transduction and gene-expression pathways. Both initiatives curate the models before they are added to the respective model repositories. Focus of the curation in the Biomodels repository is on the model description in the scientific publication in which the model was published, while the JWS online curation uses the model as supplied by the authors and communicates with the authors to resolve any issues such as potential differences between model description in the manuscript and the actual model as supplied by the authors. The Biomodels and JWS online groups work closely together and actively exchange models. JWS online is linked to a number of scientific journals to aid in the reviewing of manuscripts that contain kinetic models. Reviewers have access via a password-protected site to the models, and if the manuscript is accepted for publication the models are moved to the public database. During the review process, the manuscript is checked for correctness of the model description to ensure that the models in JWS online correctly represent the models in the publications.

JWS online also offers the possibility to run simulations and multiple analysis options (e.g. steady-state and metabolic-control analysis) for any of its models online, i.e. without downloading of software tools. This is what defines it as a ‘live’ model repository, i.e. the models are alive through the web. Through the web, one can change parameter values in any of the models and calculate the implications for model behaviour. One can also determine which steps in a modelled pathway most determine a specific flux or concentration. The view is to make mathematical models produced by SB useful to scientists who are ignorant
of mathematics. The use of JWS online is close to experimentation. It may be important that quality control of models is disentangled from the application or validation of the models. If these important activities are mixed, internal inconsistency of modelling may cover up for lack of experimental validation.

JWS online also has the perspective of the silicon organism, also called the virtual biochemical organism (human) (http://vhhuman.org/). This means that it hopes that its models can be linked up with each other such that they grow, ultimately to cover significant parts of entire organisms. This may seem less efficient than the approach of genome-wide kinetic models for entire organisms, but it may not be. The automobile industry is using modular production lines to improve the robustness of the overall production flow to fluctuations in the activities in individual steps. Modularity also makes the quality control manageable. Checking the quality of a genome-wide model is impossible for any individual because of the great complexity. Scientific experts may still be able to check the quality of pathway models.

A final use of the two model repositories is computational SB. Here, network properties can be calculated for any of the models. Particularly, JWS online can be useful here as it enables through web experimentation. We shall now exemplify this.

3. COMPUTATIONAL SB: ROBUSTNESS

Metabolic pathways not only serve to produce commodities that are necessary for survival, they do this in a robust way, i.e. independently of the many fluctuations that occur in living organisms and their environment. Life is intricately dependent on fluctuations as the progression of processes with time depends on them. The progression is little more than the effect of a bias in fluctuations and in the response to them [13,19]. In isolated biochemical reaction pathways, the inherent fluctuations tend to be relatively small, as the number of molecules is large. However, when such pathways are subject to gene-expression control, there may be relatively long-lasting fluctuations in enzyme levels. These may then cause variations in steady-state fluxes and metabolite concentrations between individual cells [20].

Here we shall consider the robustness of a particular metabolic pathway, i.e. yeast glycolysis to perturbations in the concentrations of enzymes involved in it. We shall use a precise definition of robustness [21]. The robustness of pathway flux with respect to the variation of the concentration of any enzyme $e_i$ is defined as the percentage by which one can reduce the concentration of the enzyme $e_i$ such that the pathway flux goes down by only 1 per cent. We were interested in the robustness of the rate at which yeast cells consume sugar when they produce alcohol. We guessed that the pathway flux should be least robust to perturbations of the most heavily regulated enzyme in the pathway, i.e. phosphofructokinase (PFK); i.e. the enzyme at a regulatory hub. To examine this issue, we consulted our dynamic model for yeast glycolysis [22], which is conveniently stored in the JWS repository. We calculated the glucose uptake flux ($88.1505 \text{ mM min}^{-1}$) in the standard state. Then we varied the $V_{\text{max}}$ of PFK away from its standard value of 182.903 and clicked ‘state’ and ‘evaluate model’ to obtain the flux through the glucose uptake system. We found that a reduction of the $V_{\text{max}}$ of PFK to 85 led to a glucose uptake flux of 87.3311, which is close to 99 per cent of the initial value. We then divided the percentage reduction in $V_{\text{max}}$ by the percentage reduction in flux to find a robustness coefficient of 58.

Is a robustness of 58 high? We compared this robustness with the robustness of the same flux to perturbations of the glucose transport $V_{\text{max}}$. A reduction of the $V_{\text{max}}$ from 97.264 to 96.26 reduced the pathway flux to 87.256. This corresponded to a robustness of 1.1. This result was in contrast to our initial guess, where we expected the pathway to be least robust with respect to perturbation of the PFK activity.

4. SB VERSUS PK AND PD

An important deliverable of the JWS online and Biomodels facilities will become the connecting of adjacent models into larger models of part of the whole cell. Such an activity could greatly reduce the total complexity of the modelling of whole organisms. Success is not guaranteed however; it will depend on whether the biological function is indeed modular and on advances in multi-scale modelling approaches. The organization of whole organisms into tissues, of tissues into cells, and of cells into organelles, as well as the separation between transcription, translation and metabolism [23], suggests that biology is indeed modular, perhaps because of the same robustness requirements as the automobile industry. At the same time, where such obvious modules are absent this may signal a functional reason, and the approach might not work.

For PKPD, we conjecture that an effective way of dealing with the vital problems of biomedical simulations and modelling is an integral analysis combining the approaches from the poles of the mathematical repertoire, i.e. traditional, phenomenological dose–response curves and mechanistic models. As limited as it may appear, the phenomenological approach tends to be correct for the dataset to which it has been fitted: once the best-fitting dose–response functional form has been found for a particular perturbation of a particular organism in a particular state, it should be independent of the current and future knowledge about the biological system. If the dose–response relationship happens to be unique also mechanistically, then it should remain valid forever. This may be rare however. Biology is renowned for its adaptive capabilities.

Mechanistic models are in the focus of bottom-up SB. Yet, they tend to vary with time, as new data are being provided by the wet-laboratories. This is particularly true for bottom-up models for which the parameter values are obtained by refitting all parameters to an ever increasing dataset (e.g. cell cycle models for which not even the ultimate number of
molecular species is known [24]). Silicon cell-type bottom-up models are based on ‘hard’ parameter values, i.e. parameter values determined experimentally for the identified model components. These models tend to be constant, although they may only be valid for certain tissues with a defined set of expressed isoenzymes.

PD deals with the relationship between the drug concentration at the target and the effect on the functioning of the target organisms in terms of, e.g. a simple Hill equation with fitted $E_{\text{max}}$ and EC50 values and the Hill coefficient. More mechanistic PD modelling acknowledges that the relationship it is after, is not just a property of the molecular target, but in fact a property of the entire intracellular and sometimes even organismal metabolic and regulatory networks around the target. In such cases, SB is involved, either implicitly, or, more rarely, explicitly. Examples of such models are published predominantly in PKPD-related literature (for excellent reviews see the publications from the Jusko and Danhof groups [25,26]). From the SB side, models have been made that deal with the network effects radiating from drug action (e.g. [27,28]). Although there is a gap in the aim and description between these PD and SB models, the latter may be considered as complements to the former.

In standard PK, the relationship between the drug dosage and the dynamics of the concentration of the drug at the target site is described as the resultant of four processes (ADME). These processes are assumed to be linearly superimposed onto one another and to depend linearly on the concentrations of the drug in the various relevant compartments. There are SB models that relax these linear assumptions, including models where mechanistic properties of multi-drug resistance proteins and their bearing on the concentrations of drug in drug-resistant tumour cells are calculated [29]. We shall now try to produce synergies between SB and PKPD by providing a common platform.

5. SB PK PD: A SYSTEMS BIOLOGY-DRIVEN PK PD PLATFORM

In order to fill the two gaps between PK and SB and between PD and SB, we here establish a framework for collecting PK and PD models, which could be linked to SB models from, e.g. the JWS online database, and formatted in such a way that they can all be implemented in common, wider contexts (http://www.sbpkpd.org). The framework will create a nucleation point for PD and SB models from many research groups and may ultimately produce an exhaustive model collection. It should also create an open platform, i.e. a simulation/optimization framework with a library of textbook and frontline-research models, but also usable with user-provided models. As in JWS online, the models should be peer-reviewed and/or curated in a systematic way by a team of experienced modellers and domain experts. In addition, they should be updated regularly, but with version control and retention. According to this strategy, we here begin by implementing well-known PK models, compartment-based and compartment-free, phenomenological PD models, as well as effect and transit compartment link models. The latter link models are in fact a simplified substitute for complex signal transduction pathway models and it is only too obvious that the next step will be incorporating mechanistic nonlinear and feedback-driven SB models (e.g. epidermal growth factor receptor-related pathways like MAPK, Akt or JNK).

Although mechanistic models are an obvious choice and the main object of ongoing interest of theoretical research, in the context of clinical research, the phenomenological compartment-based models in PK and dose–response models in PD are practised much more: the majority of published PK/PD studies is based on simple one-, two- or three-compartment PK and phenomenological PD dose–response models, while few others contain an additional link model. Most of these combined models are relatively easy to implement for a skilled systems biologist, but require some advanced knowledge of simulation and optimization tools or even profound programming skills, something that most clinical/wet-laboratory researchers and physicians do not have.

In the initial phase of the platform development, the physicians and clinical scientists are especially our target users. As in JWS online, an intuitive web-based interface, compatible with virtually every web-browser and operating system, should make an easy and efficient use of our SBPKPD platform. Figure 1 shows a typical PK input panel of the platform we have now put in
PK models can be fitted to the user’s own data or to a selected dataset from our drug library, containing scanned published experimental data for a variety of diseases, dosing regimes and administration categories. An appropriate optimization algorithm can be selected from a list of both local and global algorithms with ‘nls’ as the default algorithm for nonlinear (weighted) least-squares from the R-package ‘stats’. The platform will be available to everyone free of charge, upon simple registration and through the web at: www sbpkpd org, obviating laborious installation and licensing problems. The web-laboratory scientist will be able to run the check of his/her data at the bench via a web browser.

For example, fitting dose/concentration-effect data to a Hill model will often result in a typical sigmoid curve in a semi-logarithmic plot (figure 2). A residuals plot helps to analyse the results. The tool provides parameter estimates (with their errors and goodness-of-fit measures), which are available upon downloading the log-file that is generated automatically with each run. Here the parameter of major interest—the EC50 value (green circle), is plotted directly next to the fitted Hill model curve (solid line).

Different dosing and administration regimes and various modes for single or repeated dosing at user-defined times are simple to set. A typical PK profile for a hypothetical drug with first dose at 6.00 h, administered orally seven times every 3 h is shown in figure 3. This can also be done for one-, two- or three-compartment, as well as for compartment-free PK models, for phenomenological PD models (e.g. Linear, E max, Hill, Gompertz) and for a number of indirect response models, for link models and for low-level SB-driven mechanistic models. Virtually, every combination of the PK-link-PD/SB modules contained in it, is enabled by our platform.

We designed a ModelScanner to be an intuitive tool that enables the quick set-up and comparison of different models, both in the simulation and in the fitting mode. An automated statistical analysis provides parameter estimates with their standard errors, covariance matrix, residual plots and goodness-of-fit measures, such as the Akaike Information and Schwartz criteria. For the more advanced user, calculation of typical PK/PD parameters (clearance, area under the curve, t 1/2, C max, t max, etc.) is performed automatically where appropriate.

6. TECHNICAL DETAILS AND OUTLOOK

To avoid typical problems of accessibility (owing to restriction to one platform or browser type), we based our SBPKPD on the platform-free Java-based Google Widget Toolkit technology. All models are implemented and run in R, a programming language for statistical computing (http://www.r-project.org/). The open software ‘base R plus recommended packages’ is managed by a group of recognized experts [30] and widely used in academic research as well as in conducting and filing clinical reports to regulatory bodies. Our platform makes the underused potential of R accessible to users that will not even know about this, because it all runs under the hood of our platform engine. To our knowledge, no tool in this area has been designed so far for execution on an R-based cluster, and we would like to use this exciting possibility for computationally expensive tasks.

The open source structure of SBPKPD means that all scientifically essential elements of the code will be accessible to the user. In contrast to other tools, we offer a continuously expanding library of experimental records for PK, PD and PKPD, offering first simulation and fitting experience within the PK and PD area on validated data.

The graphical-user interface collects user input and sends it to the SBPKPD server, where the data are processed and the results are fed back to the web-browser. The user has the option to project the results in multiple graphical formats. Alternatively, she/he may wish to download raw or interpreted data for further
analysis using as a tool of his/her choice. Web-service functionality will be added in a future version, which will make it accessible to other (web-based) tools and platforms. The connection to a commercial or public cluster or ‘cloud-computing’ facility (e.g. Amazon cloud) should allow computationally more demanding tasks like whole-body-scale simulations.

With its solid conceptual base and its mathematical background, our SBPKPD platform is suitable for further development into more specialized facilities. In a (semi)automatic in vitro—in vivo correlation system, existing models and approaches such as PK fitting supported by new processes like numerical deconvolution, could establish mathematical relations between the in vitro drug dissolution and its in vivo behaviour. Such an ‘IVIVC’ system could be quite useful for clinical and pharmaceutical research in the process of new drug admission, for which few tools exist, and which are all commercial: it is our goal to stimulate cross-institutional cooperation in this area by providing an open-source simulation and modelling platform, the development of which will also be guided by clinical users informed best about current needs in daily medical practice.

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