MODELLING IN SYSTEMS BIOLOGY, NEUROLOGY, AND PHARMACY

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Abstract. In the last decade some significant changes occurred in biomedical area with introduction of systemic view to the area. In neurology, the systemic paradigm was already well accepted, while other areas of bio-medicine were more or less accepting the reductionism paradigm where systems were studied through the analysis of its isolated sub-systems. Genome project showed that it is not possible to explain the diversity of life only with combinations of genes, which resulted in a new science called systems biology. Pharmacokinetics introduced theory of dynamical systems into pharmacy long ago and enabled systemic studies of so called ADME process (Administration, Distribution, Metabolism, and Elimination) of drug fate in the body. Mathematical modelling, as one of the principal tools of control engineering, is now becoming necessary tool also in the analysis of biological systems.

1 Introduction

In the last decade some significant changes occurred in biomedical area with introduction of systemic view to the area. In neurology, the paradigm that contribution of systems that consist of simple elements can be more than just a sum of contributions of the elements was already well accepted, while other areas of bio-medicine were more or less accepting the reductionism paradigm where systems were studied through the analysis of its isolated sub-systems. Genome project showed that it is not possible to explain the diversity of life only with combinations of genes. The discovery strongly affected the way of thinking in the community which resulted in a new science called systems biology. Systems biology is, in contrast to reductionism, interested in characteristics that emerge from interactions of large groups of simple sub-systems that form a whole system. The ideas were adopted also in pharmacy, where the rate of success in discovery of new drugs is relatively poor in compare with the improved measuring methods [22].

To explore the characteristics of the system that originate from the cooperation of sub-systems, modelling of biological systems has become necessary procedure. The origins of idea that biological systems can be viewed as special sort of machines can already be found in Leonardo Da Vinci's anatomical studies, however, it took a few additional centuries for the idea to evolve to systems biology.

In the first half of the 20th century pharmacokinetics [18, 53, 50, 57, 12, 30, 11, 49] became widely accepted. Pharmacokinetics assumes that drugs are distributed throughout the body after administration and are metabolised and eliminated from the body. Dynamical mathematical models in the form of compartment models [19] are used to describe the relations. Compartments are defined as special regions of the body (blood and well perfused organs, less well perfused organs) or, as organs in physiological compartment models. In ideal case of pharmacokinetic study, two types of administration are used to identify the number of compartments and the effect of absorption through intestinal tract, most commonly bolus injection and oral administration. After the administration, drug levels are monitored in blood or plasma. The procedure can be considered as identification process that identifies most probable order of the model and model parameters. The order of the model equals the number of compartments, therefore, it indicates the quality of drug distribution within the body. If one-compartment model is identified, the drug is equally well distributed thru-out the body, whereas two-compartment model indicated two types of tissues with respect to drug distribution. Originally, the models were of linear character, however, later, some non-linearities (Michaelis-Menten, Langmuir, ...) with bio-chemical background (enzyme reactions, active transport, ...) were added. Pharmacokinetics introduced theory of dynamical systems into pharmacy and enabled systemic studies of so called ADME process (Administration, Distribution, Metabolism, and Elimination) of drug fate in the body. Most important was introduction of dynamic point of view in the pharmacy as an upgrade of statistical analysis of static properties. Pharmacokinetic modelling enabled drug dosage regimen adjustment with respect to specifics of treated persons, as well as some relatively limited studies of mechanisms involved in the ADME process. However, the pharmacokinetics was limited to concentration profiles of drug, while drug effect was mostly neglected. Pharmacodynamics introduced study of drug effects in the body, however, it was never as systematic as pharmacokinetics. The problem was that mechanisms of drug effect were not understood to the point where mechanistic mathematical models could be built, at the same time, measurements of necessary quantities were not always possible, thus simple models with sigmoidal characteristics and of mainly statical nature were used. The combination of pharmacokinetics and pharmacodynamics allowed better adjustment of dosage regimens since the optimisation goal was now drug effect and not drug concentration. However, pharmacodynamics was never

as popular as pharmacokinetics since the models were not so reliable. Nevertheless, the systems dynamics was introduced to the area with several benefits, such as systemic point of view, scientifically one of the most important ones. Pharmacokinetics and pharmacodynamics consider all processes in the body as a single complex process, and detailed mechanisms of sub-systems are not studied, thus they represent a macroscopic view of the system.

In neurology, the detailed research of neurons began in the first half of the 20th century. It soon became clear that such simple elements must create a new quality, when inter-connected in great numbers. First mechanistic studies resulted in artificial neural networks (ANNs), however, the simulations never resulted in complex behaviour of its natural counterparts. Almost in parallel, electromagnetic signals of functional brain were recorded and functional analysis of brain functions began. Hans Berger was one of the inventors of method for measuring potentials on the scalp, called electroencephalography [42], that is still one of the most common methods for measuring brain activity. Almost a century after the beginning of systematic research of the brain, the functioning of the brain still eludes the researchers in spite of numerous new measuring methods (MRI, MEG, PET, ...). Interesting fact about neurology is that it adopted systemic view and understood the importance of systems dynamics almost from the beginning, which is rare occasion in bio-sciences.

Biochemistry [55] is a field of life-sciences with traditionally reductionistic approach to the analysis of systems. Considering the complexity of researched systems the approach is sound, however, it misses the additional complexity that is introduced with inter-connections of relatively simple elements (chemical reactions and involved molecules) into complex metabolic networks. Regulation mechanisms, based on equilibria of molecules introduce feedback mechanisms to the system, resulting in further complication of relations between involved molecules. Systems biology [29, 28] started to introduce the systemic view to the area, as well systems dynamics, being one of the most important causes for the complexity of the system. Feedback mechanisms are typical dynamical structures, thus, statical information is not sufficient for understanding the underlying mechanisms of metabolic processes.

Mathematical modelling as one of the principal tools of theoretical physics and control engineering is becoming essential tool for solving complex problems in life-sciences as well. Feedback mechanisms play important role in all biological systems thus it is important to understand the dynamical nature of feedback mechanisms. Two different approaches to systems analysis seem to be in a constant struggle, statics and dynamics. Many great results have been achieved by using statical approach, however, it becomes more and more clear also in life-sciences that statical analysis of dynamical systems can be sometimes misleading. There is also a big gap between life-sciences and engineering in the understanding of the term “understanding the mechanisms”. While life-sciences mostly define the process through symptoms, engineering struggles to create a working prototype of the system. Only when the prototype is operational in desired manner, the mechanisms have been correctly understood. Thus modelling and simulation, as integral tool of the systemic approach paradigm, is important addition to the standard analysis procedures for studying biological systems. The following examples are intended to elucidate the contributions of modelling, simulation and control engineering to the area of life-sciences.

2 Models

There is a large variety of models that is used in life-sciences, however, mathematical models are most flexible. As mentioned above, most common models are compartmental models of all kinds [19, 58]. However, the complexity of biological systems often requires more sophisticated modelling techniques, such as artificial intelligence methods: artificial neural networks [21], fuzzy modelling [3], Bayesian networks [47] splines [52, 10] genetic algorithms [16, 20], principal component analysis [24], wavelet analysis [36], and many combinations of the methods. Due to such complex mechanisms and dynamic properties of the systems, the behaviour of biological systems can often be characterised as deterministic chaos [1, 45].

3 Pharmacokinetic studies

3.1 Antibiotics in fever and defervescence conditions

Fever (febrile state) causes complex response of the organism to external stimuli and some changes in the internal environment (thermo-regulatory centre) that affects also the pharmacokinetics (PK) of drugs. Very few data about the influence of PK changes on pharmacodynamic (PD) data is available especially for the cases of human “in vivo” studies. The fever must be namely artificially induced or humans with acute febrile disease must be included in the study which is problematic.

The aim of the study was the comparison of three antibiotics Penicillin G (PG), Ciprofloxacin (CIP), and Cefazolin (CF) kinetics during fever and defervescence. All three drugs are actually and widely used in clinical practice.

Since they are used in febrile as well as in afebrile states of the patients, the evaluation of PD consequences of PK changes during fever and defervescence should be studied with modelling and simulation to evaluate higher dosage regimen designs for severely ill patients.

When studying drug pharmacokinetics the corresponding pharmacokinetic model must first be defined. The latter is simplified representation of the drug passage through the human body after application.

One of the most frequently used approaches to PK modelling is compartmental analysis, which is increasingly used in all areas of biomedicine but also in ecology and in chemical reactions kinetics description. In engineering, however it often occurs that some models are of compartmental type in spite of the fact that they are not explicitly recognised as such.

Compartmental model consists of finite number of homogeneous, well stirred, lumped subsystems, called compartments, which exchange within each other and with the environment so that the quantity or concentration of material in particular compartment is described by a first order differential equation.

Extensive "in vivo" study offered enough antibiotics concentrations measurements for the model development. The key question was which model, one or two compartment, fit the data better, and if model structure is the same for febrile and afebrile state. From the obtained results the following can be concluded [9, 8, 7]:

- PK of the studied antibiotics in the transition from febrile to afebrile stage of the patients is mostly changed (model structure is changed),
- volume of distribution in the mentioned transition is also changed in most cases,
- distribution of all substances under investigation is faster in febrile stage, therefore, attempts to decrease body temperature are not desired (unless necessary due to other medical reasons),
- the identified pharmacokinetic parameters exhibit great inter-patient variabilities,
- the results of the study do not support the adjustment of the studied drugs dosage during fever,
- discontinuous character and stiffness of the developed multiple dosing models may cause serious numerical problems in simulation if they are not correspondingly treated (hybrid discrete-event and dynamical models),
- results of the study have their direct application in clinical practice in spite of the fact that they were obtained from extremely simple linear pharmacokinetical models.

3.2 Paracetamol suppository study

When studying paracetamol availability after rectal administration, the differences between slower and faster release suppositories were discovered. Approach with modelling and simulation of compartment-based models was used to explore the differences.

A study of paracetamol from layered excipient suppositories [13, 48] shows that many different mechanisms are involved in the drug pharmacokinetics. There is also a large number of articles, each dealing with only one or with a few of the mechanisms that are evaluated separately. However, there is little information available on how the mechanisms interact in the organism and thus govern the pharmacokinetics of the drug, which means that systemic view in the expert knowledge is missing.

Statistically significant difference in absorption between the two studied formulations of suppositories is established in [13]. The methods used in [13], including classical compartmental and non-compartmental analysis enabled the postulation that higher content of mono-di-glycerides in slower release formulation augmented the extent of absorption by absorption enhancing effect and higher viscosity of mono-di-glycerides. However, no indications on the mechanism of absorption could be found. It is known that absorption from lower regions of rectum by-passes liver, while in higher regions of rectum the drug is absorbed mainly through hepatoportal system. The extent and the rate of absorption, therefore, depend on when and where the suppository melts and how it migrates.

The aim of this work was to develop a model to test the hypothesis that different pathways of absorption in rectum in combination with properties of suppositories are the main reason for the differences in rate and extent of absorption between faster and slower release suppositories. The compartmentation as shown in Figure 1 was used for modelling. Application of fuzzy modelling, as a representative of artificial intelligence methods has already shown a significant advantage over classical mathematical modelling methods in some cases of bio-medicine [31]. Important part of the area's knowledge base consists of "fuzzy" rules and observations that cannot be fully incorporated in classical mathematical models. Human-like reasoning of fuzzy models is, therefore, very suitable for

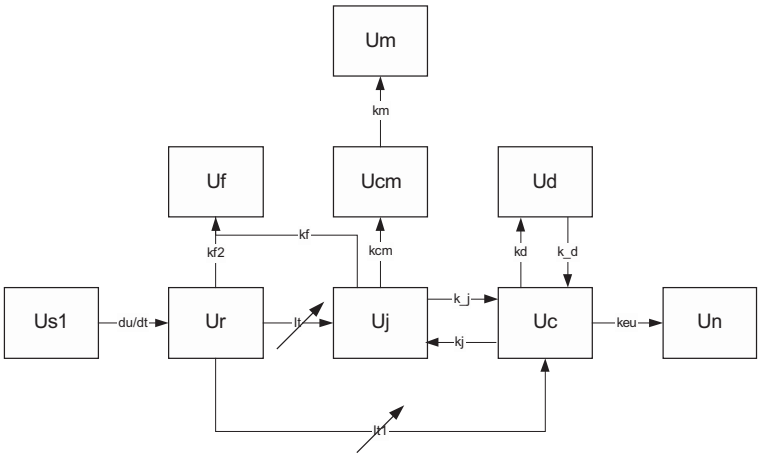

Figure 1: Compartmentation of human body with respect to paracetamol fate after suppository administration

the area. However, compartmental structure of the model is still of great importance. Its structural similarity to the observed system as well as established way of thinking in the area make it the most efficient model representation. The combination of fuzzy modelling and compartment based models, i.e. partial fuzzyfication of compartment model, can thus be considered as a tool for combining data analysis and expert knowledge.

In the case of paracetamol rectal availability the use of partially fuzzyfied model allowed systemic combination of all described mechanisms found in the literature and measured data. In spite of non-identifiability, the model showed that patterns that explained differences in bioavailabilities of the two formulations of suppositories could be found [5].

3.3 Histamine and methylhistamine equilibrium

Histamine is an endogenous amine, present in humans, animals and plants. It is known as a mediator in physiological processes, and is involved in many pathological processes. During inflammatory or allergic reactions it is released from stores in mast cells and has an important role in adverse reactions. When released, it is rapidly in-activated by metabolic processes and therefore is difficult to measure the rapid changes of histamine concentrations in body fluids.

Efforts are made to find a marker for histamine appearance in the body. It is found in the literature that its metabolite, methylhistamine (tele-methylhistamine, N-methylhistamine) may be one of them. It has been reported that histamine-N-methyltransferase, one of the two important enzymes (the second is diamine oxidase) involved in transformation of histamine, was shown to be stimulated in the cerebral cortex of adult rats by chronic stress. The elimination half-life of methylhistamine (M) in plasma of the rat is about ten times longer than of histamine (Hi) ($t_{\frac{1}{2}}(M) = 43\ min$, $t_{\frac{1}{2}}(Hi) = 3.5\ min$). There is also a threefold methylhistamine plasma to whole blood concentration ratio compared to histamine. Since metabolic and transport pathways of histamine and methylhistamine are complex and not very well known, the relationship between levels of the two substances in plasma may be elucidated by mathematical modelling, to support a hypothesis that methylhistamine can be a marker of histamine appearance in plasma.

Therefore, a four compartment model was composed to describe the relation between the two amines. At the same time, two experiments were performed on the rats. In the first experiment, histamine was injected, and in the second experiment, methylhistamine was injected. Whereas the first experiment resembled the natural behaviour of the system, the second experiment was somewhat unusual, as were the measured profiles. The injection of methylhistamine caused extensive raise of histamine levels in blood. The simplest modelling solution to the problem was that at high blood levels, methylhistamine is metabolised back into histamine. However, no indication of the mechanism could be found in the literature that would support the idea. Therefore, the model structure was changed such that control loop was added that regulated histamine to methylhistamine levels (see Figure 2). The model was able to simulate the two situations sucessfully (see Figure 3 The physiological reasons for that were relatively easy to find in the literature. Therefore, the model was successfully validated and since the model involved only the two substances, it can be concluded, that methylhistamine can be used as a marker for histamine release [6].

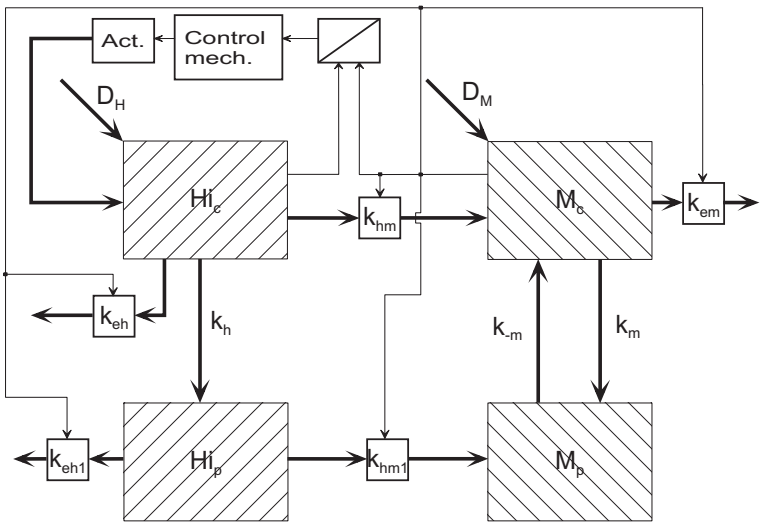

Figure 2: Compartmental model of histamine kinetics

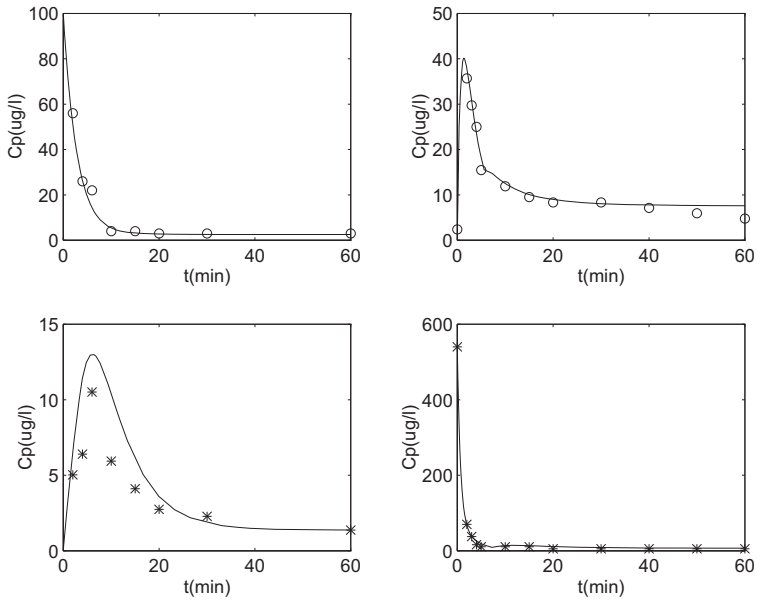

Figure 3: Simulated vs. measured kinetics of histamine and methylhistamine

4 Brain code during motoric activity and working memory tasks

Macroscopically, brain can be considered as a huge system of highly interconnected oscillators - large groups of neurons, who repeatedly polarise and depolarise their membranes, thus causing electrical currents to flow and conduct the information through their network. Due to highly interconnected network of neurons and their complex mechanisms for signal propagation, it is possible that their activity never stops as long as sufficient number of neurons is still alive. However, when idle, groups of neurons can synchronise themselves in well known brain rhythms that represent natural (resonance) rhythms (alpha, beta, etc.) of groups of neurons and is defined by their physico-chemical characteristics as well as the structure of the network. Cumulative effects of neuronal activity of the brain can be detected on the scalp in the form of potentials and currents, as well as electric and magnetic fields by means of electroencephalography (EEG) and magnetoencephalography (MEG) [42].

Brain is divided into brain regions that control specific body functions, however, the regions also have to join their efforts to solve more complex tasks. The theory of binding tries to explain how different aspects of perception and action functionally integrate in the brain to form a unitary experience and reaction [54, 37, 38, 15, 51, 46, 39, 23, 4]. The theory presumes that there is no specific center in the brain that would gather the information from the brain regions, governing senses, motion, etc., and then make the decision about the appropriate reaction. It is very likely that the regions bind themselves, when necessary, through the activity of their long-range neuronal connections. Any activity desynchronises currently active groups of neurons from their natural rhythm which can be detected also in cumulative electrical activity on the scalp, if the active groups are large enough and are close enough to the surface. When analysing EEG/MEG measurements, fMRI scans, etc., raised coherence between the brain regions

involved in specific task can be observed in certain brain rhythms, phase-locking analysis, and power spectra of the brain activity also suggest that the brain regions somehow synchronise and desynchronise themselves when performing tasks together [51, 15, 25]. If the produced EM fields also help to activate neurons or synchronise neuronal assemblies is yet unclear.

One of the important question for brain functioning research is the coding of the information on brain activity in the EM signals. Literature [25] suggests that the information might be coded in the phase characteristics of the signals. Since EEG signals represent a superposition of signals of all active neurons in the brain, desynchronisations of neuronal groups can be considered as phase distortions of the natural (idle) rhythms. The concept is similar to phase modulation of signals, where phase-shifts of carrier signal code the information. Although, it is not reasonable to expect, that in completely idle state the brain would produce single-frequency sine wave oscillations, using the concept, one should be able to identify most dominant tasks that are performed in the brain at the time of observation. To test the concept, EEG data from five healthy volunteers, performing different hand-gripping tasks [33, 34] and of three healthy volunteers performing working memory tests [32] was used. The EEG signals were phase-demodulated and feed into artificial neural network, to predict corresponding activity of the persons.

Although the quality of the prediction is not very high (approx. 75% of correct predictions of answers and quality of force prediction as shown in Figure 4), we must consider that brain is constantly adopting system and, therefore,

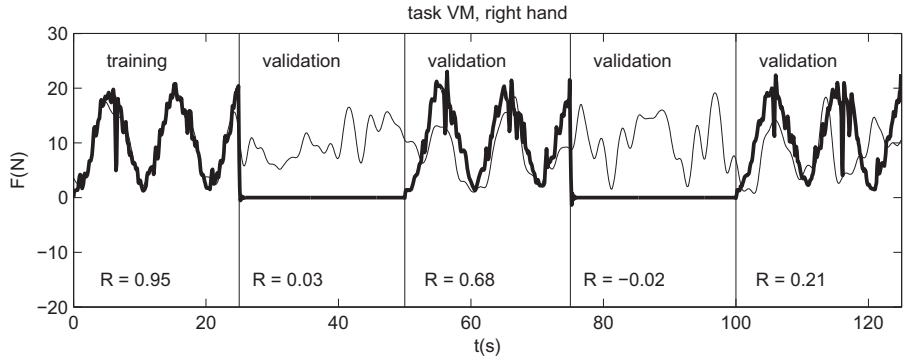

Figure 4: Training and prediction of gripping-force. Thin line - simulation, thick line measurements

occurring desynchronisations may vary with time as task is repeated several times. Next, the concept of phase demodulation is a very rough approximation that assumes idle brain rhythms to be single frequency periodic oscillations, which is certainly not true. In fact, brain rhythms are known to change quite a lot with time [42]. Brain is also never really idle. Computational problems can also be expected due to the fact, that carrier frequencies and the frequencies of signals that carry information are very similar. Therefore, information signals are distorted by phase-demodulation and further information is lost. Considering all that, the procedures will have to be adapted, to get better prediction, however, the concept is promising.

5 Modelling of cholesterol biosynthesis

Analysis and identification of metabolic networks is an important issue of biochemistry and biophysics. While regulatory networks, based on gene expression regulatory mechanisms, are getting a lot of attention lately [35], the underlying networks of metabolites are not in the centre of the current research focus. Some approaches on different methods of metabolic networks identification have been reported, mostly designed to identify the networks from experimental data [44, 41], and some approaches to reconstruct the networks on the basics of physical laws [40]. The goal of metabolic networks is to produce one or more target metabolites that are important for functioning of an organism. As target metabolites are formed in many consecutive steps, in many metabolic pathways situations occur where in certain step single pathway becomes branched into a network of pathways due to non-specificity of catalysing enzymes with respect to metabolites. Due to different affinity of enzymes with respect to substrate molecules, a primary pathway is formed; however, when any of the enzymes in the network is disturbed (by xenobiotics or as a result of mutations), the order of reactions can get mixed and alternative pathways are formed in order to compensate for the disturbance. In normal situation, alternative pathway conveys a negligible portion of the whole metabolic flow; however, when they are many, their cumulative flow can sometimes match the metabolic flow of the primary pathway, especially, when primary pathway is disturbed. When one or more enzymes are disturbed, some intermediates can be accumulated and some novel-ones can be formed as a result of alternative pathways. In some cases alternative pathways represent escape routes for metabolic flow. Accumulation of standard metabolites, occurrence of novel-ones, and escape routes can trigger regulatory mechanisms in the studied metabolic network as well as other metabolic networks, thus causing system-wide disturbance. Due to such complex behaviour of metabolic networks, identifying all possible metabolites in the network is very important

for the development of safer and more effective drugs [22]. Simulation studies have been performed for identification of most suitable drug targets, however, usually only a single pathway networks are considered [17], while the alternative pathways are mostly neglected. Generation of complex mathematical models of cell metabolism in simple organisms is now well under way [56, 2, 43], however, not all the details in metabolic processes are yet known. Thus, simulation of such models can reproduce a realistic picture of global metabolism, but the models are not detailed enough for all the purposes of drug development.

Pathway of cholesterol biosynthesis [27, 14] begins with acetyl co-enzyme A. In many consecutive steps lanosterol is formed. Lanosterol is the first cyclic metabolite in the pathway Cholesterol is formed from lanosterol in nine consecutive steps. Most commonly two parallel pathways are described in literature [27]. Primary pathway begins with step catalysed by CYP51, while the rest of the enzymes are sorted as follows: TM7SF2, SC4MOL, NSDHL, HSD17B7, EBP, SC5DL, DHCR7, and DHCR24. Secondary pathway begins with DHCR24 and the rest of the enzymes are ordered in the same order as for primary pathway. In lanosterol, besides suitable domains for CYP51 and DHCR24, suitable domains for enzymes SC4MOL, EBP, and SC5DL can also be found, suggesting poor specificity of the enzymes. Considering the fact that skeletal structure of lanosterol is the same as of cholesterol, theoretically, the involved enzymes may have poor selectivity with respect to all intermediates of the pathway and may in principle form a complex network of pathways between lanosterol and cholesterol.

If we combine such complex structure with regulation mechanisms through gene expression [26] and activation and deactivation of enzymes, this becomes very flexible dynamical system with single intention, to regulate levels of cholesterol in the cell. However, broader view shows that cholesterol metabolism is not isolated in the cell and there are many control mechanisms that interconnect all the metabolic pathways in the cell. Furthermore, a cell is not isolated from the body and levels of cholesterol in plasma are regulated differently as levels in the cell. Hence, elevated cholesterol levels may have nothing to do with problems in intra-cellular cholesterol regulation. Thus it is not surprising that all current hypercholesteremia treatment strategies exhibit serious side effects.

6 Conclusions

While bioinformatics is relatively well established in all areas where large data-sets are found, dynamical modelling and simulation is less well accepted. In the area of life-sciences any procedure that requires mathematics is usually understood as bioinformatics, however, there are substatntial differences between the approaches. While bioinformatics looks for patterns in large databases with some inclusion of expert-knowledge, modelling and simulation tries to recreate observed systems in mathematical form, with maximum possible inclusion of expert-knowledge and validates the model against the database. Considering the complexity of the observed systems, development lead to several modelling techniques that are in between the data-driven and knowledge driven approaches. In reality, bio-systems are too complex to be analysed with only a single approach; measurements are problematic and structural knowledge of bio-systems is incomplete.

With increasing importance of systems biology to all areas of bio-sciences the models are becoming more and more complex that leads towards several problems. First, the problem of identifiability of such complex models cannot be neglected. Since the models are mostly of non-linear type (enzyme reactions) the optimisation methods must be used to identify the model parameter values, thus introducing all well known problems of optimisation in high-dimensional spaces. Next, a serious problem with simulation methods of complex non-linear, high-order dynamical systems cannot be neglected. The problem has two consequences, questionable precision of results and very long simulation times (or necessary use of supercomputers). The experimenting with and understanding of the behaviour of such complex models is of central interest, when trying to understand the modelled system. However, if model becomes too complex to understand, it also becomes obsolete. Simplification of such models is thus becoming a necessity. Simplification can be viewed as selective scale of different parts of the model. While it is not possible to neglect the holistic nature of the model, details that are not studied in detail can be simplified, however, not omitted.

There is also a significant problem with modelling goals. In order to use modelling and simulation efficiently, modelling goals must be set at the beginning, since modeller must decide which part of the system should be modelled in detail and which parts can be simplified. The idea is however somewhat strange to bio-scientists, since their model organisms are completely formed per se prior to experiments, while mathematical model must first be designed through sensitive design cycles, before it can be used for experiments. Since the system is extremely complex, not all the details should be included in the model in detail. In this sense, standardised descriptions of models, such as SBML (Systems Biology Markup Language) might result false understanding of modelling and simulation and lead to the goals of the type: “life, universe, and everything”.

The major problem in interdisciplinary research in life-sciences in reality represents the communication between scientists of different backgrounds. It takes a few years before communication becomes effective even in cases of highest motivation of the involved people.

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