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Formalization of Integrative Physiology

Marek Mateják

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Board chairperson: Prof. MUDr. Jaroslav Pokorný, DrSc.

Doctorand's institution: Institute of Pathological Physiology 1. LF UK

Supervisor: doc. MUDr. Jiří Kofránek, CSc.

ABSTRACT

New information technologies bring with them new possibilities for defining and simulating complex physical systems. A huge amount of progress was made in this field with the Modelica language standard, developed by the worldwide nonprofit Modelica Association. Using the Modelica language specification, new chemical, hydraulic, thermal and population components for human physiology were designed for the implementation of the physiological principles in this thesis. Similarly to the electrical circuits already implemented in the Modelica Standard Library, it is also possible to connect the components of these libraries to the diagrams and, in this way, define more complex components of physiological systems. Using this kind of implementation, this thesis presents an extension and improvement of the HumMod version 1.6 model, developed at the University of Mississippi Medical Center (Jackson, MS), which has more than 5,000 variables. As a result of the use of graphical diagrams, our implementation is more expandable and more modifiable at each point. The precise rules of connections lead to fewer implementation errors. In addition, the visual verification of the model is achieved, because the physiological connections of diagrams are self-describing, which allows them to be directly examined and presented in the form in which they are implemented.

A new acid-base model for blood gas transport was here designed and integrated. This extension of HumMod 1.6 was more appropriate for describing the status of blood during oxygen and carbon dioxide transport, even during respiratory or metabolic acid-base disorders. The presented theory of multiple ligands binding to hemoglobin A is used to describe the equilibrium of oxygenation, carboxylation and oxygen-linked (de)protonation. This integrative approach not only shifts the oxygen-hemoglobin dissociation curve, it can also be used to calculate the carbon dioxide saturation and changes of linked protonation, which are significant for maintaining the pH of blood during blood gas exchange.

As a language for this complex physiological integrations, Modelica can be used—with new proposed physiological libraries behind it—thanks to the already established commercial and noncommercial support.

ABSTRAKT

Nové informační technologie přinášejí možnosti jak exaktně popsat a simulovat komplexní fyzikální systémy. Pokrok v tomto směru umožnila standardizace jazyka Modelica neziskovou celosvětovou asociací firem, univerzit a jednotlivců Modelica Association. Standard jazyka umožnil v této disertaci vytvořit chemické, hydraulické, tepelné a populační komponenty pro základní principy fyziologie člověka. Tyto nové Modelikové knihovny byly nazvány PHYSIOLIBRARY a CHEMICAL. Jejich základní komponenty je možné v Modelice graficky propojovat a tak vytvářet komplexnější komponenty fyziologických systémů, obdobně jako se v Modelice vytvářejí modely elektronických obvodů ze základních prvků elektronických komponent. Disertace ukazuje, jak lze obdobným způsobem vytvořit i tak komplexní modely jakým je model integrativní fyziologie člověka HumMod 1.6 který má více než 5000 proměnných. A nejen to, tyto modely je potom možné velmi intuitivně modifikovat a rozšiřovat. Disertační práce tak model amerických autorů HumMod 1.6 (www.hummod.org) nejen implementovala, ale i rozšířila o vlastnosti krve a hemoglobinu, které původní model neměl. Při reimplementaci modelu bylo odhaleno (a americkým autorům reportováno) 30 logických, matematických a fyziologických chyb, na které se při důkladné analýze modelu narazilo.

Byl vytvořen a integrován nový model acidobazické rovnováhy a transportu krevních plynů. Toto rozšíření modelu HumMod mnohem věrohodněji popisuje stav acidobazické rovnováhy krve a přenosu krevních plynů i v respiračních a metabolických acidobazických poruchách. Díky integračnímu přístupu byl také navržen nový pohled na přenos krevních plynů pomocí hemoglobinu A. Tento integrační model dokáže popsat nejen disociační křivku hemoglobinu pro kyslík, ale i pro oxid uhličitý a dokonce i pro kyslíkem propojené vodíkové ionty, které se významně podílejí na udržování pH v krve při výměně krevních plynů.

Prakticky i teoreticky pomocí exaktních definic je v práci ukázáno, že integrace nových poznatků do jednoho komplexního modelu lidské fyziologie je možná a přínosná. Jeho jazykem by mohla být právě Modelica s novými, prací vytvořenými, knihovnami fyziologických komponent díky podpoře velkého množství komerčních i nekomerčních nástrojů.

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1 INTRODUCTION

Integrative physiology is a relatively young branch of physiology that describes the complex connected mechanisms and regulations of physiological systems at all levels (from molecular, cellular, tissue and organs to a level that encompasses the entire body). Physiological knowledge arises from examination and comparisons of the functionality of living organisms and nature; however, the basis of integrative physiology should also include experiments and data that can be used to generalize mathematical relations. Therefore, the fourth section of this dissertation thesis formalizes the reproducible experiment, which can be described using the physiological model (pg. 40-44). This model is useful for describing many real experiments. It is theoretically and practically shown that the model can be integrated in a way that the resulting model is at least as good as all the models available prior to integration. Typically, this integration means finding a new theory that can describe all the desired phenomena. Its identification with real experiments in particular gives rise to the new model that describes the desired experiment with the required precision.

This integrative approach based on physics is not entirely new. The idea that the entire complex human physiology can be integrated into one complex model has long since been in development at the University of Mississippi Medical Center, where Arthur C. Guyton et al. developed the idea of connecting physiological knowledge using exact mathematical notations. One of their first models, which began this integrative physiological development, was a model of the cardiovascular system with integrated volume, hormonal and neural regulations (Guyton, et al., 1972). The model was built from data of simplified cardiac functions (Guyton, 1965); perfusion of lungs, kidneys and skeletal muscles; neural and hormonal regulations, body fluid balance and the simplified transport of oxygen. The model simulates the relations between the regulation of blood circulation, blood pressure and volume, as well as the pathophysiological mechanisms that lead to chronic hypertension. For validation of the model's behavior,

Guyton et al. also proposed experiments based on nephrectomy in dogs, which showed good fit with their model's simulation (Guyton, et al., 1972). The model has continuously been extended and improved with ever more data and experiments. In this way, versions of the model called "Human" (Coleman and Randall, 1983), "Quantitative Circulation Physiology - QCP" (Abram, et al., 2007), "Digital Human", "Quantitative Human Physiology - QHP" (Hester, et al., 2008) and finally, the "HumMod" model (Hester, et al., 2010; Hester, et al., 2011) have been developed.

In the long-term, the integration of accessible physiological knowledge is one of the main goals of the Department of Physiology and Biophysics at the Arthur C. Guyton Research Center at the University of Mississippi Medical Centre. The team researching this theoretical physiology is composed of researchers with knowledge that overlap in mathematical, chemical, physical and physiological fields. The result of their years of work is a complex integrative model of human physiology. The model, called HumMod in version 1.6, is freely accessible for continued academic development under a GPL license.

In our opinion, the subsystem of ***acid-base homeostasis and blood gases transport*** is one of the weakest parts of the model HumMod 1.6. An extremely simplified calculation of blood acid-base, which does not use any form of non-bicarbonate acid-base buffer, needs to be replaced by a more complex calculation of pH regulations connected with the transport of oxygen (O₂) and carbon dioxide (CO₂). The Mississippi University model does not, for example, bind CO₂ to hemoglobin (Bauer and Schröder, 1972); it also does not release Bohr's protons (Bohr, et al., 1904) and does not calculate any titration properties for non-bicarbonate weak acids. It is known that the acid-base status of blood is determined by strong ion differences (Stewart, 1981), bicarbonate (i.e., HCO₃⁻ created by the hydration of CO₂: CO₂ + H₂O <-> HCO₃⁻ + H⁺) (Henderson, 1908), plasma buffers such as plasmatic proteins (Figge, et al., 1992), phosphates and significantly, also by hemoglobin (Antonini, et al., 1963) inside red blood cells. While most of these acid-base buffers bind hydrogen ions (H⁺) independently onto the

state of other substances, hemoglobin is different. Hemoglobin changes the quaternary form through the binding of oxygen (Monod, et al., 1965), which could also change other binding properties for H^+ and for CO_2 . There exist at least five different models of oxygen hemoglobin saturation: the allosteric model (Eaton, et al., 2007; Monod, et al., 1965), Adair's four-step oxygenation (Adair, 1925), Hill's model (Hill, 1913), approximation using hyperbolic tangents (Siggaard-Andersen and Siggaard-Andersen, 1990) and other polynomial approximations (Severinghaus, 1979). The oxygen dissociation curve (ODC) of each of these models describes oxygen saturation (sO_2) as dependent on partial oxygen pressure (pO_2) at a fixed and normal conditions in terms of temperature, pH, CO_2 , DPG and other factors. However, only the allosteric and Adair approach is based on the physical description of chemical processes. The other three models are only mathematical approximations of measured data, which does not matter until the model needs to be extended with more ligands. There are already published some of these complicated empirical extensions pertaining to shift of ODC to the left or to the right caused by effects of CO_2 and pH (Dash and Bassingthwaighe, 2010; Rees and Andreassen, 2005; Severinghaus, 1979; Siggaard-Andersen and Siggaard-Andersen, 1990). However, these extensions fail when the value of CO_2 and pH is outside the norm at the same time. The O_2 , CO_2 and H^+ equilibrium on hemoglobin exists in non-linear relations; therefore, the effect of CO_2 on sO_2 is significantly dependent on pH and vice versa (Siggaard-Andersen and Salling, 1971). In addition, none of the extended approximations can provide the state of the binding of each of these particular ligands. Therefore, the model will be better if it is built from physical and chemical theories that will provide answers to more questions, instead of relying only on the mathematical approximation of one variable.

Morrow et al. and Matthew et al. (Matthew, et al., 1977; Morrow, et al., 1976) showed that CO_2 carboxylates the amino-terminal of each subunits of hemoglobin tetramer. The different affinity for oxygenated and deoxygenated forms causes at first glance a competitive relation between CO_2 and O_2 . However, the chemical bonds are not competitive, because each ligand binds on different sides of the hemoglobin subunit. Thus, both CO_2

and O_2 can be bound at the same time on each subunit. Thanks to hemoglobin, blood is available to transfer 25% more CO_2 . Roughly 10-11% of this CO_2 is transported directly bonded as a carb-amino terminal of a hemoglobin subunit (Bauer and Schröder, 1972); the rest is the result of pH change caused by the binding of Bohr's protons (i.e., an increased blood capacity for bicarbonates).

In this way, hemoglobin can preserve the pH between arterial and venous blood. For example, in tissues, where the amount of H^+ is increased by CO_2 in the form of HCO_3^- , hemoglobin – during the release of oxygen – regulates pH by binding H^+ (the deoxygenated forms has higher affinity for Bohr's protons) (Bohr, et al., 1904; Siggaard-Andersen and Salling, 1971). There are more than ten bounding sites in a hemoglobin tetramer for Bohr protons. These sites change affinity for H^+ during a change in shape of the hemoglobin molecule, caused by the binding or releasing of oxygen. Most of these are amino acid side chains located in the beta-cleft (the place between beta subunits) (Perutz, et al., 1980; Zheng, et al., 2013). However, it is possible to simplify these Bohr's sides as having only two fictive sides (Antonini, et al., 1965).

As with each chemical reaction, the binding of ligands to hemoglobin are also dependent on temperature (Atha and Ackers, 1974; Weber and Campbell, 2011; Weber, et al., 2014). For example, the shift of ODC caused by different temperatures is a known factor. Using theories of physical chemistry, it should be possible to calculate the enthalpy of chemical reactions, not only to shift dissociation coefficients, but also to calculate the amount of heat consumed or released by a reaction.

Another weakness of the HumMod 1.6 model is its form of implementation. Although the source code separates "physiological definitions", they are not equations in a mathematical sense, but only assignments where the value, calculated from some expression, is stored to the selected variable. The entire HumMod model is implemented as "causal", which in the

prescribed sequence calculates the unknown variables from the expressions of known variables using an algorithm. This does not constitute a formulation of equations, but simply the implementation of an algorithm (i.e., causal implementation). However, in complicated models, situations often exist where the equations are not able to reach physical relations through only a simple sequence of assignments. Furthermore, for a numerical solution, it is necessary to select an iterative method; for these cases, the HumMod provides a construct of implicit equations.

The significant weakness of the HumMod 1.6 model's implementation is also the redundancy of the physical relations. The implementation of physical laws is repeated in the model as many times as they are used. The base rule in informatics is to define the functions and objects once and to use them by referencing selected settings, instead of copying the contents of functions and objects in many places. The language, in which HumMod 1.6 is implemented suffers a lack of modern computer language constructs that can simplify the readability and visual verification of the implementation. The original source code is divided into hundreds of files; as such, it is not surprising than the research and development teams of complex physiological models in the Physiome Project (Bassingthwaight, 2000; Hunter, et al., 2002) rather use and extend older and simpler models.

The ideal language for the implementation of complex physical systems is Modelica (www.modelica.org). Equations can be formulated in Modelica without manual algebraic manipulations ("acausal" implementation). This language also allows for representing the usage of objects by graphical icons, which refer to the definition with exact physical relations (<http://book.xogeny.com>). For example, the electrical resistor is defined according to Ohm's law and is represented by a typical rectangle icon inside electrical circuits. Once the object with its icon has been defined, it can be used with different settings and as many times as necessary. This object-oriented approach also allows for defining hierarchical components that are composed from many other connected components. Almost each

model can be implemented only by graphical diagrams that have a relatively small set of domain-specific physical laws (called first principles), implemented as Modelica libraries. The physical connections of Modelica components provide visual verification, which is readable also by non-Modelica users. This approach brings to the industry a very powerful tool for communication between programmer, developer and researcher.

As a result, Modelica is already being used in many industrial applications (from the automotive and aircraft industry to the construction of robots and the design of power plants). Nowadays, there exist a number of commercial and non-commercial (for example OpenModelica) Modelica environments that can support the building and simulation of models in this language. The libraries in Modelica today primarily represent electrical, mechanical or magnetic domains. However, the Modelica language is generally enough for also defining libraries in chemical, thermal, as well as in hydraulic or population domains of physiology, which prior to this work did not exist. For example, it should be possible from the components of these libraries to implement the component for microcirculation, which is hydraulic resistance within the cardiovascular system. These components can be affected by many inputs, causing local vasoconstriction or vasodilation. Using this object in different locations of cardiovascular diagrams for different tissues with different settings, local microcirculation can react to all known local factors with a prescribed sensitivity. The question that arises as a result is: is it possible to formalize the first principles in physiology to create a compact Modelica library, from which it should be possible to implement the HumMod 1.6 model, as well as the more detailed chemical processes during blood gases transport?

2 AIMS OF THE WORK

Hypothesis 1 (formalization):

Modelica[®], as the most recent generation of object-oriented equation-based computer language designed for the dynamic simulation of large complex physical systems and machines, is suitable for the exact formalization of integrative human physiology.

Hypothesis 2 (integrative):

Mathematical formalizations of physiological knowledge about one organism can be integrated using graphical hierarchical physical diagrams into one complex physiological model, which will simulate all integrated physiological experiments.

The formalization hypothesis arises from the observation that Modelica can elegantly describe even very complex physical machine models. The language of Modelica is so general that the user is able to define their own physical units, physical quantities, physical relations and components of physical diagrams, as well as their connections. However, robust and compact support for physiology requires formalizing the first principles, i.e., the physical laws behind almost all physiological processes. For these purposes, the HumMod 1.6 model from Mississippi University will be analyzed, as it is assumed to be the most complete and accessible model of human integrative physiology (<http://hummod.org>). Following on, it should be possible to re-implement and extend the HumMod 1.6 model using combinations of almost only these physical laws – the first principles of physiology. Modelica implementation of the model using graphical diagrams will provide visual verification of the model through feedback from the physiological community (non-Modelica users), thereby providing data for follow-up improvement and development of the model. The goal of the thesis is therefore to create new libraries as part of the Modelica

environment that will be usable for many other models. These new libraries will contain general, fixed, valid and verified sets of components for intuitive use in graphical diagrams.

Though HumMod 1.6 contains thousands of physical relations, it still calculates the processes in a simplified manner. Almost every part of the model can be improved on and implemented in more detail. Since our laboratory has a long tradition in calculation acid-base homeostasis and blood gases transport (Kofránek, 1980; Kofránek, 2009; Mateják, et al., 2015; Matousek, et al., 2011), the work will focus on extension of the model for improving the calculation of blood pH, O₂ and CO₂ content in blood. As noted in the introduction, the simulation of blood in HumMod 1.6 cannot be calculated using non-bicarbonate acid-base buffers, and it does not calculate the binding of CO₂ or H⁺ to hemoglobin. These processes are so significant that the HumMod 1.6 experiences problems calculating the total content of CO₂ even in normal conditions. It is therefore not surprising that simulation results differ from observations of blood status in different conditions (Siggaard-Andersen, 1971; Siggaard-Andersen, et al., 1972; Siggaard-Andersen and Salling, 1971; Siggaard-Andersen, et al., 1972). To fix these discrepancies, the titration properties of plasmatic proteins (Figge, et al., 1992), phosphates and erythrocytes should be implemented. It has been found that erythrocytes play the most significant role, because they are connected to blood plasma via chloride shift (membrane channel “Band 3”, which passively exchanges one HCO₃⁻ for one Cl⁻ in both directions), by providing the carbonic anhydrase for the Henderson-Hasselbalch reaction ($\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{HCO}_3^- + \text{H}^+$), as well as through the sophisticated properties of hemoglobin. These properties includes binding of multiple ligands (O₂, CO₂ and H⁺) to hemoglobin as well as their dependences. This work should take into account all of these processes and should describe all the properties of hemoglobin A (HbA)¹, which have to

¹ Hemoglobin A makes up 97% of total hemoglobin in adults. It is a protein-tetramer composed of two alpha and two beta subunits coded by genes HbA1 and

date only been described separately (no integrated model has been developed that describes all of these importantly connected phenomena) and are as follows:

1. Oxygen dissociation curve for HbA (Severinghaus, 1979)
2. Carboxylation of oxygenated and deoxygenated HbA at different pH (Bauer and Schröder, 1972; Matthew, et al., 1977)
3. Releasing H^+ during oxygenation of HbA (Siggaard-Andersen, 1971)
4. The change in dissociations at different temperatures (Reeves, 1980; Weber, et al., 2014)

Thus, the goal is to propose a new multiple-ligands allosteric theory to describe all of these experiments that play a significant role in the acid-base status of blood during blood gases transport.

The building of a complex integrated model holds an implicit assumption that the resulting relations will be at least as good as the models that describe each of the phenomena separately. This is very important for integrative physiology, because a complex model must converge in each phase of development into better description of the simulated reality. This assumption is the primary factor of the second (integrative) hypothesis.

Improvement of the model by integration is not certain and great care must be taken during each phase of development to achieve a good fit with all the described experiments. For this reason, there remains some discussion on whether it is better to use a small, simple model or very large and complex models (Gavaghan, et al., 2006). However, if it is possible to create a better integrative theory that will describe all the selected experiments in detail, this work must present the methods for physiological

HbA2 in the 16th chromosome, and HBB in the 11th chromosome. In the middle of each four subunits is a hem with one iron atom.

knowledge integration that must be applied during each phase of the development of a complex integrative model.

Development can continue only by observing the above rules, which will prevent the loss of any integrated knowledge. From this point of view, a large and complex integrative physiological model may not be comparable with simple physiological model; however, when integrated, the result must be better than both of these separated models.

A goal of the thesis is to demonstrate the use of the formalized rules of integrative physiology alongside real observations and physiological experiments. The new integrated and validated theories should finally be implemented using hierarchical graphical diagrams to allow for visual verification of the connected physiological principles. The simulation results must always be in good agreement with accumulated real data sets.

3 MATERIALS AND METHODS

The work is based on exact definitions that must have unique meanings; only in this way can an integrative theory of human physiology be built that is representable through a computer simulation. Most of the exact formalization methods needed can be found in the domain of physics, where mathematical relations have a long history.

The meaning of variables can be exactly defined using ***physical quantities and physical units***. Physical quantities such as pressure, temperature, volume and mass are internationally accepted terms that are suitable for the description of the parameters and variables of physiological models. In general, the physical quantities are anatomically located within the body fluids, organs, tissues, fluids, cells or organelles. Unfortunately the values of the same physical quantity in the same location are not unique, as they may be represented in different physical units. This is critical for computer

simulations, where values can be shared between many integrated relations. However, in the field of physics, this problem has been solved by the definitions of the International System of Units (SI). This work fully accepts these SI-units without any exceptions, despite the fact that some values are extremely small (e.g., $1 \text{ ml} = 10^{-6} \text{ m}^3$) and some very unusual in physiology and medicine (e.g., Kelvin for temperature or charge of ions in Coulomb).

All ***physical definitions and relations*** between variables are always formulated for SI-units; this significantly simplifies the use of physics when dealing with physiological principles.

The complex model in ***Modelica*** is an input for the compiler, which is developed by independent teams of mathematicians and programmers to maximize the class of numerically resolvable mathematical problems (Engelson, et al., 1999; Mattson, et al., 1997). Thus, the user generally does not need to manually solve mathematical expressions in order to implement a calculation algorithm, which has to date been the most difficult aspect of integrative computational physiology. Therefore, the implementation of the integrative model relates closely to physiological theory, enabling the researcher to focus almost only on developing theory. However, the exact theory that is representable by a computer simulation must be mathematically well-defined using the correct rules for building base Modelica components and their connections for the specific first principles of physiology.

Elementary physical laws can in Modelica be represented as library components that can be used as building blocks for completing the entire, complex model. The primary principle of the implementation of the model as a hierarchical graphical diagram is represented in Modelica language as a construct called the ***physical connector***. This connector can be seen as an analogy for electrical circuit connections that have a pair of variables: flow and nonflow (e.g., electric current and electric potential). Each component can have as many connectors as needed, though this is typically one or

two. When flow is not accumulated in the component, the sum of all flow variables in each component's connector is zero at all times (i.e., what occurs as a flow inside some connectors also occurs as a flow outside other connectors). The connection between components using the physical connector also does not lose or add any flow to the system, supporting the idea that energy, mass, substance amount or elementary particles from/to nothing cannot be created or lost. The only places where new flow can be generated, accumulated or transformed are in the components. Therefore, the connections of physical connectors always generate only simple equalities of nonflow variables, in addition to the mentioned physical junction law for flows. This approach is so general that in Modelica, there already exist electrical components, e.g., resistor, capacitor and inductor (Mattsson, et al., 1998), thermal components such as thermal conductance and a thermal capacitor (Elmqvist, et al., 2003), as well as mechanical components such as a spring or damper (Engelson, et al., 1999).

However, the implementation of theory into Modelica is simply the second step of the complex integrative physiological model's development. The first step is to integrate the theories that describe the experiments. The methods for the integration were formalized in section 4. The reproducible **real experiment** is defined as a sampled function that transforms the settings to the outputs. This transformation is done by the real measurement of data following the experiment conducted at the defined settings. In physiology, it is typical that the mean values of the concentrations, temperatures, flows, pH, etc. remain constant at normal optimal conditions; this is called homeostasis. These typical values of conditions and physiological variables are referred to as the **default setting**. Using the default setting rapidly simplifies the use of the model, because there is not needed to set all parameters prior to each experiment. There are needed as the setting of the model or experiment only the selected significant parameters that are not in normal values. The model is a function defined as a set of hybrid ordinary differential equations. If all measured data of the experiment closely resemble the simulated results, it can be said that the

model ***describes*** the experiment. Having exactly defined this relation, comparison of the model can be easily formalized as model A is ***at least as good as*** model B if and only if all experiments described by model B can be described by model A. However, not all models are comparable, because an experiment E can exist that is only described by A, while experiment F may be described only by B. This is a typical situation in the repositories of physiological models, for example, in project Physiome (www.physiome.org), where each model can simulate different processes. However, the current work wishes to present new horizons of physiological modeling and therefore formalizes ***integration***, which claims that for each pair of incomparable models, a model must always exist that is at least as good as the individual models being integrated. Using this approach, the development of the large and complex integrative model of human physiology is always able to converge to more precise descriptions of the real processes within the human body.











4 RESULTS

The results of this thesis can be divided into two groups: (1) the new Modelica libraries (www.physiolibrary.org) implementing the carefully selected first principles of physiological processes for building physiological models; (2) the complex model of human physiology (www.physiomodel.org), which extends the HumMod 1.6 model with the new model, which includes acid-base and blood gases transport.

The new Modelica libraries for physiology are mentioned first in the thesis, because the complex model is built on them; however, practically, the process of development initially implemented the complex HumMod 1.6 model in Modelica. Following on, the analysis of elementary processes led to general patterns that were repeated in many instances. After this, robust decomposition was observed for the elementary physical laws, which are in original model entirely hidden after flattened list of assignments of

expression composed from particular variables. These carefully extracted *first principles* from hydraulic, thermal and population domains (Matejak, 2014a) were extended in this thesis with new chemical principles, which are much more usable than the chemical principles of HumMod 1.6; one reason for this is, for example, because concentration or osmolarity is not always the same in chemical equilibrium in contrast with electrochemical potential. The relatively small compact set of components can be used for a large set of physiological models, which makes these free open-source libraries – called “*Physiolibrary*” and the “*Chemical*” library – an extremely useful tool for academic or commercial development of physiological models in Modelica. The components in the libraries can be connected using the connectors shown in Table 1.

Table 1: *Applied physical connectors; new connectors proposed in thesis are shown in italics.*

Connector	Nonflow	Flow ²
  Electrical	Electric potential	Electrical current
  Thermal	Temperature	Flow of heat
  <i>Chemical</i>	<i>Electrochemical potential</i>	<i>Molar flow</i>
  <i>Hydraulic</i>	<i>Pressure</i>	<i>Volumetric flow</i>
  <i>Population</i>	<i>Size of population</i>	<i>Change of population</i>

The physical connectors are the base of the components of Physiolibrary (Matejak, 2014; Matejak, et al., 2014), where are implemented the first principles of physiology. From these first principles (library components), almost any physiological process can be derived. Some of these are presented as analogies with components of electrical circuits. For example, resistance is defined as Ohm’s law (e.g. electrical resistance, thermal conductance, diffusion permeability, hydraulic resistance), accumulation is a simple differential equation of the integration of flow (e.g. electrical ca-

² The flow incoming to the component has positive value. The flow outgoing from component is negative.

capitance, heating of mass, accumulation of chemical substance, accumulation of volume) and inertia is an effort to answer changes in flow (e.g. electrical inductance, hydraulic inertia). However, most components such as chemical reactions, ideal radiator or change of population per member are extremely domain specific and as a result, there may not exist any analogies between electrical, chemical, hydraulic, thermal or population domains (Mateják, et al., 2014). Each of the components has a graphical icon that represents its usage in the model and is defined by graphical diagrams called circuits. Once the user translates the model, defined as a diagram of these components, the mathematical equations, which are hidden behind the connections and the components, are automatically generated. The main purpose of using graphical representations of the mathematical relations is to achieve visual verification, readability, reusability, reduce the number of errors and to allow for the intuitive reorganization or extension of the model (Mateják, et al., 2008).

As already noted, these libraries were separated from the complex physiological model in the final phase of development. However, this work began with the reimplementing of the original Guyton model from 1972 into Modelica (Mateják, et al., 2009). One year later, the QHP model was also implemented into Modelica (Mateják and Kofránek, 2010). This model was one of the primary results of our successful national project, “E-Golem: medical learning simulator of human physiological functions as a background of e-learning teaching of critical care medicine” (2006-2009, MSM/2C, 2C06031).

The next model implemented into Modelica was HumMod 1.6 (Mateják and Kofránek, 2011). Having achieved this implementation, it became easy to extend the model with new acid-base theories, or new blood gas transport and cardiovascular details. Thus, in 2012, I implemented into the complex model Siggaard-Andersen’s new blood oxygen status model (Siggaard-Andersen and Siggaard-Andersen, 1990). The complex model was able to simulate the support of artificial ventilation, for example, as

well as extravascular oxygenation (Mateják, et al., 2012). These and many other inputs, such as infusions, dialyses, transfusions and hemorrhages, were designed for educational simulations, as part of the project “Virtual patient – simulator for medical education” (2011-2014, MPO/FR, FR-TI3/869). Using the same approach of educational simulation, different scenarios of acid-base and respiratory disorders, for example, ketoacidosis (Mateják, 2013), were also tested in the model into which the new acid-base calculations had already been implemented as a result of electroneutrality, along with calculations for each significant chemical substance. Furthermore, the general principle of ***allosteric equilibria*** was designed, which could be used, for example, to calculate the hemoglobin model with three ligands: oxygen, carbon dioxide and hydrogen ions (Mateják, 2015; Mateják, et al., 2015). As shown in this thesis, all these physiological descriptions can be easily integrated into a single model, which was named “***Physiomodel***”. The detailed structure of this model is described in the thesis in section 5.

The development of the ***multiple-ligands allosteric hemoglobin model*** also circumvents different obstacles. The praxis shows that it is not a good idea to extend models that are based on mathematical approximations, such as Hill’s, Siggaard’s or Severinghaus’ models. These extensions fail in their description of more reconnected phenomena of the binding dependences, which needed to be integrated together. In our case, these phenomena were composed from the oxygen saturation of hemoglobin (Severinghaus, 1979), carboxylation of hemoglobin (Bauer and Schröder, 1972) and Bohr’s protons binding to hemoglobin (Siggaard-Andersen and Salling, 1971) at variable values of pH and levels of O₂ a CO₂. However, if the model has a physical basis consisting of elementary chemical reactions with particular ligands (Mateják, 2015), it can be extended with other ligands such as chloride, 2,3-diphosphoglycerate or other organic phosphates. Moreover, our model describes chemical bounds and the accumulation and releasing of particular molecules (Figure 1), which enables its simple integration with complex physiological models.

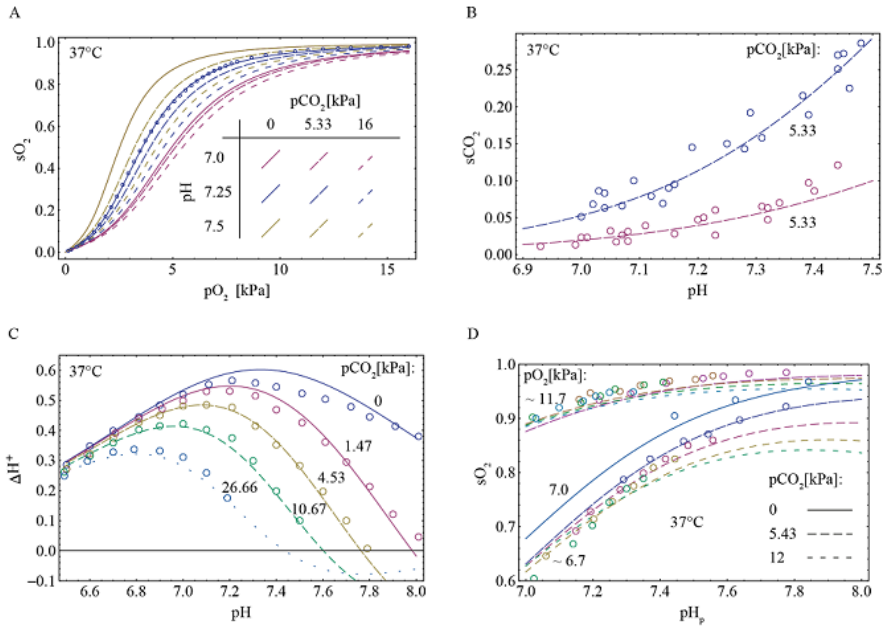


Figure 1: Comparison of measured data (circles) of hemoglobin oxygenation (Severinghaus, 1979), carboxylation (Bauer and Schröder, 1972), Bohr's titration (Siggaard-Andersen and Salling, 1971) and Bohr's effect (Naeraa, et al., 1963) with simulation outputs (lines) of the presented integrative hemoglobin model (Mateják, et al., 2015).

In Modelica, this model of hemoglobin binding can be represented using only four library components: *chemical substance* (representing specific forms of whole tetramer and also of independent sides in macromolecules at selected conformation); *chemical reaction* (representing each particular reaction of binding ligands); *chemical speciation* (for calculation of the whole tetramer concentration from the concentrations of its independent selected sides); *chemical solution* (representing extensive and intensive properties of the chemical solution in which all processes take place). These components from the proposed Chemical library make up a small amount of the building blocks of the chemical domain, which can be connected using chemical connectors (Table 1).

Similar to the chemical domain being based on physical chemistry, the hydraulic domain is composed of small numbers of components from which it is possible to create various models of the cardiovascular system (Kulhánek, et al., 2014). These models are represented using the Physiobrary and describes in detail the pulsating of blood circulation. Unfortunately, none of these pulsatile models were fully integrated with the Physiomodel, because the Physiomodel has many regulations that are primarily designed for non-pulsatile long-term simulations. However, their integration must nonetheless be possible in some form.

Regarding complex physiological models, the question is whether it makes sense to build “monster” models with thousands of equations and variables. Though this thesis theoretically proves that the integration of models must be possible (section 4, ***integration theorem***), their formalization in the level of first principles can be extremely difficult. However, having a complex model of accumulated physiological knowledge formalized in a computer language may present many benefits, as well as new approaches to integrative physiology.

5 DISCUSSION

With exception of the HumMod model (Hester, et al., 2011) from the University of Mississippi Medical Centre, there exist entire repositories of physiological models based on hybrid ordinary differential equations. Most of these models have been supported by the international Physiome Project (Bassingthwaight, 2000; Hunter, et al., 2002; Hunter, et al., 2006).

The models are divided into specific areas of physiology. For example, for the model of the heart, a separate project called Cardiome (Bassingthwaight, 1997) was established. In Europe, the Physiome branch is titled Virtual Physiology Human (VPH) (Díaz-Zuccarini, et al., 2014; Hunter, et al., 2006) or EuroPhysiome (Fernandez de Canete, et al., 2014). The goals of the projects are uniform: to formalize physiology at the

level of computer simulation. For this formalization, special computer languages were developed such as System Biology Markup Language (SBML) and Cellular Markup Language (CellML) (Smith, et al., 2013). However, Modelica as a language for modeling complex physical systems is much better supported in terms of usage (International Modelica Conferences, 2000-to date; Modelica association of universities, companies and individuals; standardization of the Modelica computer language) and foundation (e.g., research projects within Europe spent € 75 million from 2007-2015 to further improve Modelica and Modelica-related technology).

Practically, Modelica language is not only better than SBML or CellML, but is also better than commercial products. The Mathwork (Inc., USA) grounded system Simscape in the MATLAB environment is very similar to Modelica and has almost the same possibilities of hierarchical graphical diagram modeling. However, this notation is not standardized and is therefore not supported in different environments or by other tools.

A much better commercial strategy was established by companies such as Dassault Systemes (Inc., France), Wolfram (Inc., USA) and Maplesoft (Inc., USA), whose commercial products already support the Modelica language in connection to additional software possibilities such as CAD³ systems, visualization tools and optimization or mathematical tools. The use of the Modelica language in these environments is, firstly, seen in the automotive and energy industries.

From an academic point of view, it is important that access is also available to noncommercial and free Modelica environments, for example, OpenModelica, in which the Physiobrary has already been tested and supported. Our implementation of the HumMod Golem Edition was also used as a benchmark for the testing and optimization of OpenModelica compilers and solvers (Kofránek, et al., 2011).

Maintaining a large amount of specific and separated physiological models in repositories is comparably easier than integrating them all into one

³ CAD – computer aided drafting.

complex model. The reverse option, i.e., generating specific small models for yielding a small number of inputs and outputs from one large complex model should be, at least theoretically, fully automatable. However, the significant potential of complex physiological models is not usable until the models have been effectively integrated. The Modelica language provides for the effective integration and implementation of a complex system at high levels of reusability, readability and error-proofness. Modelica is truly the latest generation of computer language that combines an object-oriented approach, code generation from hierarchical graphical diagrams, equation-based notation and many other useful properties. For this reason, Modelica is a perfect computer language candidate for integrative physiology. However, the most important aspect of integration is not implementation, but developing new theories that can describe the observed phenomena. In this context, the thesis integrates the HumMod 1.6 with a new model that includes acid-base calculations and blood gases transport. The new, integrative acid-base theory was built on the titration properties of carbonic acid, inorganic phosphates, albumin, globulins and hemoglobin; the electroneutrality of each body fluid is reached at the calculated pH. The most complex part of this process was observed for hemoglobin, the acid-base buffering properties of which are dependent on the state of other ligands such as O_2 or CO_2 . Reversely, the dissociation of these blood gases is dependent on pH. Furthermore, the bindings of O_2 and CO_2 are also cross-dependent. Processes such as oxygen dissociation, hemoglobin carboxylation and Bohr's proton binding have been known for some time. However, our model is the first to interconnect these aspects into one integrative model that can successfully describe all of these observations (Figure 1). The significant advantage of this is that the model uses only the formulated chemical first principles, which opens the door for follow-up improvements and extensions.

Today, the most common models of hemoglobin oxygen dissociation are allosteric models (Eaton, et al., 2007), which originates from the original

Monod-Wyman-Changeux (MWC) approach (Monod, et al., 1965). However, these models describe only the binding of oxygen at fixed conditions. They do not integrate accompanied binding or the release of CO_2 or H^+ . Our model is based on Adair's four-step approach of gradually loading O_2 for each subunit of hemoglobin tetramer molecule. These steps divide the state of hemoglobin molecules into five groups (with 0,1,2,3 or 4 bound O_2). For each of these groups, the CO_2 and H^+ equilibrium can be calculated at each of the four subunits. This new approach is called macromolecule equilibria, or chemical speciation (pg. 39-40 in the thesis) and is so general that it can also be applied to the above-mentioned allosteric models, which calculates only oxygen binding. From the results of this approach, is possible to express any concentration of any macromolecule form defined by the state of the modeled chemical bounds on its independent sites. This is possible because a chemical equilibrium always reaches a detailed balance (Shiryaeva, 2010) as a result of the thermodynamic and energetic properties of chemical reactions. This detailed balance is the result of chemical first principles formulated from the electrochemical potentials of a substance.

Implementation using graphical diagrams can potentially hide other information that can be directly and exactly derived and generated from the connections existing between the used components. For example, the heat energy of the chemical system, i.e., enthalpy, can be directly derived from the properties and amounts of substances. In this way, the implemented system reflects real behavior, e.g., when during an exothermic chemical reaction the temperature of the solution increases. Therefore, there is no need to make the assumption of a fixed temperature or fixed concentrations, because all variables are calculated at the same time in order to fulfill the dynamic, physical and differential equations of the simulated system. Our model shows that binding of O_2 to hemoglobin is an exothermic reaction (i.e., it releases heat) and that reversely, the released O_2 in metabolically active tissue consumes the same amount of heat (endothermic direction of the reaction). In this way, heat is smartly exchanged

between tissues and the core of the body (lungs) (Mateják, et al., 2015; Weber and Campbell, 2011; Weber, et al., 2014). Though this accounts only for 4-5% of produced heat, these processes can be integrated with the calculation of thermoregulation in the model. This and many other possibilities for relatively simple extensions of the complex physiological model would never have been established if the model had not been constructed above the formalized first principles of integrative physiology as based on physics and physical chemistry.

6 CONCLUSIONS

This thesis has theoretically proven that all physiological models (as a hybrid-ordinary-differential equation) can be integrated into one complex model. However, the integration of these models often requires developing new theories, which can be extremely difficult. Integration is much easier if the models are based on first principles, as these principles have already been prepared for development. These first principles of physiology are in this instance implemented as a small compact set of components (as the free and open-source Modelica libraries PhysiLibrary and Chemical library) containing only a few types of physical connectors, and where all connectors of the same type can be connected together in diagrams. Using these types of connections and components, the hierarchical graphical development of a complex integrative physiological model was demonstrated with the reimplementing of the HumMod 1.6 model from the University of Mississippi, which was extended with new acid-base homeostasis and detailed blood gases transport. This resulting complex model is called the Physiomodel.

A new approach of integrative acid-base modeling was developed. Until now, there has been no need for calculating each charged particle in different body fluids (such as blood plasma, erythrocytes, intracellular fluid,

interstitium, the intracellular fluid of specific tissues), because for the estimation of acid-base disorders, only rough markers such as pH, strong ion difference (SID), bicarbonate (HCO_3^-) or alternatively, anion gap (AG) were selected. These markers are also included in the results of our model, but they are outputs, not inputs of the model. For example, HumMod 1.6 can calculate the SID of significant electrolytes, the balance of which is in detail modeled by absorption, storage and regulated kidney excretions. The transport of CO_2 was improved in the model presented in the thesis, so that the calculation of bicarbonate better reflects real processes such as the Henderson-Hasselbalch equation, binding CO_2 to hemoglobin and the Haldane effect in the case of hemoglobin. The biggest improvement, however, is the inclusion of the role of acid-base buffers to the model; for example, non-bicarbonate buffers such as albumin, globulins, phosphates and hemoglobin, because the level of these “weak acids” or “weak bases” significantly shift pH levels, even in cases where the conditions of SID and pCO_2 are the same. The pH is calculated from all these charged substances to find the total electroneutrality in each body fluid. The most complicated part of the process is calculating the charge of hemoglobin, because the state of H^+ binding is dependent on both CO_2 and O_2 .

As a result, the new integrative model of O_2 , CO_2 , H^+ and hemoglobin equilibrium was developed (Mateják, et al., 2015). This model is able to calculate the binding of O_2 , CO_2 and H^+ to hemoglobin in such detail that it is possible to express from the model a concentration of any selected form of hemoglobin tetramer, as defined by the number of bound O_2 , CO_2 and H^+ at defined conditions. This is possible because the formalized first principles, from which the model is exclusively composed, are based on physical chemistry. The thermodynamic equilibrium of a closed chemical system in physical chemistry always achieves a detailed chemical balance, where the dissociation coefficients of each reaction are estimated from a complex set of measurable data. Moreover, enthalpies can be estimated from temperature dependences that render some chemical reactions exothermic and others endothermic. Simulations using these data can show

the consumed heat during deoxygenation, carboxylation and during the loading of Bohr's protons, as well as the releasing of heat during oxygenation, decarboxylation and during releasing of Bohr's protons. Connecting this system to the thermoregulation of the HumMod 1.6, it was shown that in this way, 4-5% of the produced heat from metabolically active tissue can be transported to the body core (lungs).

In physiology, it is typical to have one term for complex process, regulation or even for an entire physiological system. Thanks to hierarchical decomposition, these terms can be implemented as components of an entire model. With graphical diagrams, there is almost no need to write down the source code; everything can be "drawn" using almost only the already defined components. This is because at the lowest level of hierarchical graphical diagrams, the selected first principles can be found, e.g., the components of Physiobrary. Inside these components, the physical laws that create the relations between the variables of the connectors are manually written. For example, in the chemical domain, this approach is strongly connected with the physical chemistry that allows for using all formalizations of the physical laws of physical chemistry. Having chemical processes defined through Gibbs energies and enthalpies, most parameters can also be found at the level where the formation energies of substances occur, which are well-known and tabulated values for inorganic and also for some organic molecules. However, for unknown types of molecules, these parameters can also be recalculated from dissociation coefficients, solubility coefficients and other measured data. Independent of the complex model built above the libraries, these libraries can also be improved. For example, if a theory for calculating the molecular energies from the structure of the molecule will be available, the components can be improved using this calculation, but the interface can remain the same; thus, the already implemented models will still be runnable even with new hypothetical versions of the libraries.

The finally-tested and verified versions of the Modelica libraries Physioli-
brary 2.3.0 and Chemical 1.1.0-beta have been published on the Modelica
libraries web pages (www.modelica.org/libraries) as a result of this work,
using a [Modelica License 2](#) under Charles University; this means that any-
one can use them at their own risk for commercial and noncommercial
purposes.

In contrast with the libraries, which have achieved their final look, the
complex Physiomodel (www.physiomodel.org) represents only the initial
demonstration of a complex and integrative model of human physiology.
It does not describe in detail all known physiological processes. It will re-
quire significant effort to integrate ever more molecular processes and
pathophysiological disorders into the model. However, as was shown, de-
velopment in this direction is possible and will be beneficial. As a result,
the model will be able to provide answers to complex and connected prob-
lems.

The accumulation of physiological knowledge into a readable yet complex
theory (in the form of hierarchical graphical diagrams that generate code)
at the computer simulation level has not been possible until now.

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