

Computational Biology Modeling across Different Scales

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Abstract: One of the most formidable challenges in modern biology is to get a unified view of the various mechanisms governing the behavior and of the causal relationships among different parts of a living system. It is coming clearer nowadays that to get such comprehensive picture computational models embracing different observation levels in space and time have to be formulated to explain the enormous amount of data deriving from -omic high throughput measurements methods. In this article we aim at giving a meaning to the concept of multi-scale modeling in the framework of studies of biological systems with particular interest in understanding human physiology in disease conditions.

1 INTRODUCTION

Mathematical models of natural phenomena intend to describe reality. By means of the mathematical formalism allowing logical reasoning over designated variables we account for observations made through experimentation. Defining the variables of a mathematical model is a fundamental step actually setting up the range of logical deductions allowed by that model. For example, if we use a variable to describe the changes of a concentration of a protein in the blood we are definitely overlooking the dynamics of the atoms and the ions hence we cannot get any information about the folding of the protein itself. The origin of this oversight lays in a basic principle sometimes referred to as the *lex parsimoniae* most commonly known as the *Ockam's Razor*. "Pluralitas non est ponenda sine necessitate" in very simple words states that in the description of a phenomenon the most useful model is the most parsimonious one in terms of elements used. In this regard, following up the example above, it makes little sense to describe the laws governing the forces accounting for the folding of the protein if we are interested in the half-life of the protein and we can estimate its decay rate by fitting a curve to a set of experimental data about the concentration in the blood of that protein.

William of Ockham was a Franciscan monk and logician who lived in the 14th century in a village of the English county of Surrey. At that time the

principle of parsimony in describing and modeling a natural phenomena was well reasoned. However today, the situation is a "bit" different. The *lex parsimoniae* is still valid and indeed very much used when describing a phenomenon, but besides classical mathematical models allowing for an exact analytical approach, another modus operandi is now commonly employed. This is what we can call the *synthetic approach* consisting in constructing a *replica or toy* of the studied system in terms of the most important identified elements and the laws governing the relationship among them. Actually this approach is not new at all. The "engineer" Leonardo used to construct toy models of flight machines before attempting anything real-scale. What is new today is that we can use digital computers to construct toy models. We can instruct extremely powerful CPUs to execute algorithms representing entities and laws and we can then make all kinds of conceptual experiments on those entities and laws. This "digital synthetic" approach is commonly referred to as simulation. Today, when studying a certain natural phenomena, scientists first identify elements and basic laws governing the dynamics of the system, then they represent them as data structures and algorithms and finally execute the algorithms to observe how the system evolves. The Ockam's principle is still valid and used in the first phase of this process but after that, thanks to the fact that computers do the calculations, the parsimony is forsaken, and the complexity of the

initial toy model is augmented by simply adding new entities and laws. Indeed, with little difficulty we can detail processes incorporating hypothetical or experimentally derived knowledge. We can even *compose* pre-constructed models of different parts of the real system or arrange models describing reality at different scales of observation. This holistic approach is what in biology is called *systems biology* (Kitano, 2002). The class of systemic models therefore includes the one of multi-scale models.

Multi-scale modeling has been drawn a great deal of attention in biological modeling and is discussed in many recent articles and reviews (Qu et al., 2011; Dada and Mendes, 2011; Southern et al., 2008; Bassingthwaite et al., 2005; Coveney and Fowler, 2005; Engler et al., 2009; Grima, 2008). See for example the interesting attempt to provide a framework for multi-scale computational modeling that is given in (Sloot and Hoekstra, 2010) together with two examples showing how to bridge different single-scale models.

The present article aims at giving a meaning to the concept of multi-scale modeling in the framework of studies of biological systems in general with particular interest in understanding human physiology in disease conditions. This article provides a general introduction to the methodological issues of multi-scale modeling. For a more extensive reading including examples we recommend the above-cited reviews and also (Hunter and Nielsen, 2005; Meier-Schellersheim, et al., 2009; Murtola et al., 2009; Schnell et al., 2007; Bradley et al., 2011; Joshi et al., 2011).

2 LEVELS OF BIOLOGICAL ORGANIZATION

Before we define what a multi-scale models is, it is first necessary to make clear what it is meant to formulate a model at a certain scale (Southern et al., 2008). In the natural sciences, to make an observation requires setting a temporal and a dimensional scale. For example, freely draw from disparate scientific fields, the phenomena of the continental drift is better described over a time span of million years, the evolution of a disease like multiple sclerosis in years or decades, the immune recognition of an infectious agent in days, the cell cycle and circadian rhythm in twenty-four hours and so on, to fast processes like the heart beat lasting about a second or the fold of a protein that takes place in microseconds and beyond. Likewise, certain phenomena are better seen over a length or space

scale of light years, as for example the formation of galaxies, or kilometers, like for the propagation of a tsunami wave, or micrometers to describe the duplication of a cell, and so on.

In general terms, while we can intuitively say if a determined process involves cells, molecules, or organs, it is not so simple to identify values for the lengths at which we switch from one level to the next (Southern et al., 2008). Major levels of biological organization are regulated at scales of many orders of magnitude in space and time (Figure), with space spanning from the molecular scale (10^{-10} m) to the living organism scale (1 m), and time from nanoseconds (10^{-9} s) to years (10^8 s).

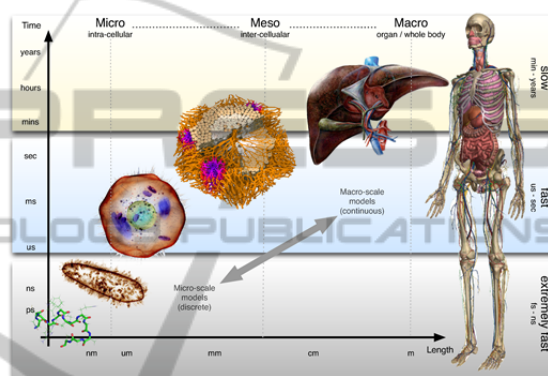


Figure 1: Multi-scale models of the human body targeting complex processes span many time and length scales of biological organization.

When combining models in a systemic way, the challenge remains in the manner the components are put together. Note that, in the study of complex phenomena as for instance human pathologies, a unified view is indeed necessary to reach a comprehension of the various mechanisms in action and of the causal relationships among different parts of that complex system that is the human body (Di Ventura et al., 2006). Complex diseases entail phenomena ranging all scales, from observations at the microscopic scales (from pico to micro meters) to microscopic tissue damage and embracing temporal events ranging from very fast processes lasting in the order of femto seconds (for example protein folding, protein docking, etc.) to slower microscopic events like DNA transcription, cellular mechanisms like meiosis or even lengthy ones like the embryogenesis or the evolution of a disease like diabetes or cancer (Hunter and Borg, 2003). In this regard, there is another important aspect that should not be left out from the whole picture. This is the contemporary data explosion deriving from genomic, transcriptomics, proteomics and

metabolomics studies consisting in high dimensional datasets produced by latest high throughput measurements methods (Deane et al., 2002). Also, other types of data coming from modern microscopy and biological imaging contribute to the detailed description of the constitutive parts and basic structures of living organisms (Southern et al., 2008). On that account, the current challenge expects to relate these datasets to higher level phenotypic characteristics and computational multi-scale modeling approaches are set to reveal quantitative mechanistic relationships between these various measurements (Di Ventura et al., 2006). For example, high throughput gene expression data can be used to infer knowledge of the intracellular activities that can be later ascribed to the behavior of cells in a higher-level description; e.g., the expression of the gene GATA3 in CD4 T lymphocytes in a certain experimental condition gives indication about the differentiation state of these cells, on the pattern of cytokine secreted and ultimately on the type of the immune response (Santoni et al., 2008); this is an information that is relevant to the construction of a computational model of the immune response.

For example, we have implemented a gene regulatory network (GRN) of the intracellular-level gene expression dynamics to characterize the Th1/Th2 cell differentiation, a phenomena that takes place at the cellular (mesoscopic) level. The GRN used represents the most extensive attempt to model the regulatory network controlling the differentiation of TH lymphocytes to date (Mendoza, 2006). Before integrating the minimalistic Boolean network dynamics in an agent-based model of the cell-cell interaction, we identified the genes coding for membrane receptors and those coding for soluble molecules to be secreted by the cell, with the idea of interpreting the former as the “input” and the latter as the “output” of the cell (left panel of Figure 2). Then we analysed the network Boolean dynamics using classical logical methods to identify the asymptotic regimens. In particular, three ‘attractors’ with relevant biological meaning were identified: two leading to TH1 (P1 and P2) and one to TH2 (P3) phenotype. For each time step of the simulation each undifferentiated T helper cell would individually transduce the input signals coming from the extracellular space through the cell receptors (right panel of Figure 2) into a micro-dynamics of the gene regulatory network eventually falling (or not) in one of the attractors. In the case one of the possible attractors is reached, then rule is fired and the cell becomes a Th1 or Th2, otherwise the cell remains in

the undifferentiated state. More formally, we obtained a partition of the space of all possible configurations $\Omega = \{0,1\}^{17}$ (17 are the genes of the GRN) considering hyper spheres of radius two centred in P1, P2 and P3, that is, $B_{P_i} = \{x \in \Omega: d(P_i, x) \leq 2\}$, $i = 1,2,3$, where $\forall a, b \in \Omega, d(a, b) = \sum_{j=1}^{17} |a_j - b_j|$ and $B_{P_0} = \Omega - (B_{P_1} \cup B_{P_2} \cup B_{P_3})$ is the remaining space. Note that $B_{P_1} \cap B_{P_2} \neq \emptyset$ while $(B_{P_1} \cup B_{P_2}) \cap B_{P_3} = \emptyset$. The rule states that, at time $t + 1$, undifferentiated Th cells at time t , whose internal network state belongs to $B_{P_1} \cup B_{P_2}$, are marked as Th1; those with internal state in B_{P_3} are marked Th2 and all the rest do not differentiate.

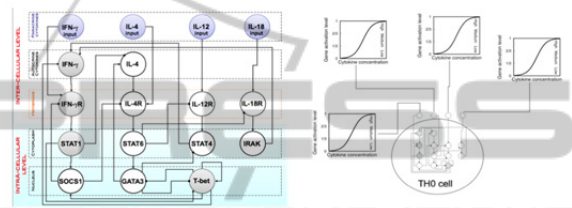


Figure 1: Left panel: The GRN used to control the differentiation of Th cells. Nodes correspond to genes/molecules involved in the Th1/2 switch. Connectors ending with an arrow indicate activation while those ending with a dot indicate inhibition. Right panel: The differentiation of each uncommitted Th cell depends on the concentration of input cytokines surrounding it. These cytokines determine the activation level of the corresponding input nodes, i.e., if c_x denotes the concentration of an input cytokine, then the activation level of the corresponding input node is given by $[m \cdot c^2 / (\theta^2 + c^2)]$ where $[x]$ denotes the smallest integer greater than x , m are the activation levels and θ is a constant.

The resulting automaton was able to reproduce a dynamics that was consistent with macroscopic observable phenomena at the cell population level still remaining compatible with a realistic gene expression profile at the microscopic level (Santoni et al., 2008). This example shows that the two levels of description (intracellular and extracellular) can realistically be integrated supposed that (i) the intracellular gene regulatory network is biologically sound and allows for relevant asymptotic regimens and (ii) the stable dynamics at the lower level can be rationally translated into an action (the rule) at the upper level.

As already mentioned, mathematical models that try to describe such mechanisms, usually fix a spatial and the temporal scale and describe the system with a mathematical or computational (i.e., algorithmic) formalism (Dada and Mendes, 2011; Engler et al., 2009; Qu et al., 2011). Computers do

the rest as they provide the dynamics by executing (resolving) the rules just described in mathematical formalism. The dynamics is dependent on parameters and initial conditions so that one generally tries hypothetical scenarios modifying those initial conditions to get a feeling of the systems behavior (Meier-Schellersheim et al., 2009; Schenell, 2007). This process leads itself in discovering new knowledge. However, the problem is that the real system is in general not isolated hence a local description is not sufficient to disclose crucial mechanisms. It comes quite clear that one of the reasons why biological phenomena are intrinsically complex is because they are influenced by variables that are outside a single level of space/temporal description.

If we take into consideration the space, a good way to define a scale is to selectively assign processes to their position within a biological hierarchy i.e., whether they represent interactions between organs, within a tissue, between cells, and so on. We can refer to these hierarchical positions as levels of a biological organization. A relevant note to this question is expressed in (Southern et al., 2008), namely, when comparing different organisms with each other, the specific spatial-temporal scales in standard international units may be quite different, even when looking at the same level of biological organization and it would therefore be beneficial for multi-scale modeling in bio-medicine to refer to these levels of organization.

Biological systems can be thought as hierarchical structures, i.e., genes that encode proteins; proteins that are building blocks of organelles and cells; cells that form tissue and organ; organs that form organisms; and organisms that give origin to individuals and populations. Different levels communicate each other in the sense that lower levels affect the higher ones and vice versa. For example proteins regulate gene expression. Therefore, in a biological system, interactions can occur both at the same scale (such as interactions between different cells) as well as between scales. This originates a very complex system in which one has to deal with multiple spatial and temporal scales and feedback loops.

In theory, one can develop a model of a biological system (such as a cardiac cell or the heart) consisting of the genes and proteins, or even the atoms. In practice however, existing computational tools are yet insufficient for this task. It should be noted that experiments are done at many scales, ranging from single molecules or proteins to whole organs and organisms, and therefore, experimental

information exists at different scales. Therefore, relying on different experimental data, a model can be formulated using two main approaches, i.e., top-down or bottom-up (Alberghina and Westerhoof, 2008).

If one chooses to take into account the individual elements and their interactions, studying the resulting biological system as a consequence of the emergent behavior of its single components, then the bottom-up approach takes place. The advantage of this type of approach is that it is adaptive and robust, in the sense that if the available biological knowledge varies, one can adapt the new knowledge to the specific components of the model, in a very selective way. Moreover this kind of approach is suitable for studying the emergent properties of systems consisting of a large number of interacting elements. The intensive computer power required is the main disadvantage for the bottom-up approach and can be sometimes even prohibitive. Moreover, the model itself can become too complicated to control.

Instead, one can decide not to look straight into the details of the individual elements, but to consider the system at the macroscopic level, using experimental observations as guidelines during the formulation of the model. The clear advantage of this approach is that it is relatively simple. On the other hand, the adaptability and the robustness of the model are less evident compared with the bottom-up approach. Moreover, it should be highlighted that the variables and parameters in these models are largely phenomenological without direct connection with detailed physiological parameters. Due to this reason, it may sometimes happen that the top-down approach does not correctly reveal the actual responsible mechanism, e.g., when there are multiple mechanisms for the same behavior or a single mechanism resulting in multiple phenomena. When existing components have to be integrated with some new part a third design principle, named "middle-out", is used (Hunter and Viceconti, 2009).

3 MODELING ACROSS DIFFERENT SCALES – FILLING THE GAP

Going from the lowest scale to higher levels one can choose among different modeling choices. Intracellular modeling approaches aim at a detailed, mechanistic description of molecular processes occurring inside single cells. These models usually

adopt the differential equation description to predict the molecular kinetics of specific cellular pathways starting from experimentally determined parameters. These models consist of mass action or Michaelis-Menten kinetic rate-law equations describing the changes of molecular concentrations. An example of a bi-domain model describing a phenomenon at a level that originates from the microscopic dynamics at a smaller space scale is the wave propagation in reactive media belonging to the class of the so-called Belousov–Zhabotinsky reaction. In a simple form (called the “oregonator” model) it may be understood in terms of the following schema (Tyson, 1994) including an autocatalytic reaction $A+Y \rightarrow X+P$, $X+Y \rightarrow 2P$, $A+X \rightarrow 2X+2Z$, $2X \rightarrow A+P$, $B+Z \rightarrow hY+Q$, where the variables represents concentrations of specific molecules (e.g., bromomalonacid, carbon dioxide, etc.) and h is a constant. Translated to ordinary differential equations the system is $dX/dt = AY - XY + AX - 2X^2$, $dY/dt = AY - XY + hBZ$, $dZ/dt = 2AX - BZ$, where A , B and P are held constant. The solution of this system has an oscillatory dynamics that, transposed to two spatial dimensions, describes a propagating wave. The bi-domain “nature” of the model in this example lays in the emergence of the wave at a level that is above the one chosen to describe the phenomena, that is the molecular level of the reactants (Murray, 2003).

An alternative to differential equations for intracellular models is the microsimulation of reactions within cells where the number of reagents is a small number (due to current computational limitations). The method developed many decades ago and known as the Gillespie algorithm (Gillespie, 1976; Gillespie, 1977) allows to accurately simulating chemical or biochemical systems of reactions generating a statistically correct trajectories as possible solutions of a stochastic equation as for example the differential equations corresponding to the time-evolution of stochastic processes that proceed by jumps (e.g., Markov jump process (Bailey, 1990)). A simplified version of this equation is the master equation describing the time evolution of the probability \bar{P} of a system to be in a set of states with regard to a continuous time variable t . The most familiar form of a master equation is a matrix form

$$\frac{d\bar{P}}{dt} = M \cdot \bar{P} \quad (1)$$

where M is the matrix specifying the connections. At a higher scale level of description, tissues or whole organs are modeled in two different ways: either as functional compartments or system units or as a

collection of microscopic components (e.g., cells). These two modeling paradigm use a completely different point of view in describing a functional unit as a tissue or organ. In the former case the organ is seen as a black box with known input-output relationship. This relation is typically derived from known facts and ultimately realized by differential equations linking stimulus with response or input to output or causes to effects. These kind of phenomenological models do not attempt to give an explanation of the observed behavior whereas they aim merely at reproducing it. They are quite useful when combined together to offer a bigger picture. The latter modeling paradigm proposes to represent a tissue as an array of individual units (i.e., cells) exchanging signals with the environment. A noticeable example of these *multicellular* systems has been originally developed to study the growth of solid tumors (Drasdo et al., 1995; Drasdo, 2000), and has later on been applied to simulate the function (the regeneration) of complex organs like the liver (Hoehme et al., 2010).

There are a number of ongoing projects whose aim is to simulate a whole cell (e.g., virtual cell (Schaff et al., 1977), e-cell (Normile, 1999; Takahashi et al., 2004)), whereas efforts aiming at simulating whole systems or organs are, for example, models of the heart (Hunter and Nielsen, 2005), of the liver (Holzhütter et al., 2012), and of the skeletal system (Viceconti, 2012). Other efforts aim at creating computational platforms suite to integrating various physiological processes (Eissing et al., 2011). These are integrative systems biology challenges that target the simulation of complex biological systems through multi scale integration of different mathematical and computational models. The approach is the so-called middle-out strategy proposed by Brenner, (1998) and Noble (2002; 2006), based on the principle that, in biology, there is no privileged level for the description of a certain phenomenon and that the inter-level causal relationships are driven by interactions between multiple levels. An application of the same modeling principle to nutritional sciences can be found in de Graaf et al., (2009) where the authors describe how multi-scale models integrating processes from the cellular up to the physiological levels are indeed necessary in answering important nutritional questions.

The use of different modeling paradigms however, introduces gaps between scales. Multi-scale modeling, besides modeling the individual system components, needs to address the issue of how to bridge the gaps between different

methodologies and between models at different scales. Unfortunately, there is not a specific or simple way to achieve this goal, but there are quite a number of empirical principles and methods that can provide some hint. For instance, adaptive mesh refinement in lattice models (Plewa et al., 2005) is used to scale down the details of a certain process, the Hidden Markov Models (Baum and Petrie, 1966) are used to deduce higher scale logics from the observation of lower scale patterns, equation free methods (Kevrekidis et al., 2003) based on the execution of microscopic simulation models allowing for computing the evolution equation of a system at a higher (e.g., coarse) level, etc.

Systems biology is the main area in which one can find this help. The goal of systems biology is to consider a biological system from a holistic perspective, and use both experiments and modeling and the interactions between experiments and modeling to reveal how the system behaves (Kitano, 2002; Kohl et al., 2010).

Specific modeling choices at a lower length scale favor the integration of information at higher scales and vice versa. For example, the individual- or agent-based modeling approach at the mesoscopic level (Castiglione et al., 2007) can be integrated to the microscopic intracellular description for which we can adopt either the continuous approach (as in Ribba et al., 2006, that integrates cell cycle regulation and macroscopic tumor dynamics with the aim with the aim of mathematically investigating this therapeutic failure the anti-metastatic agents called inhibitors of metalloproteinases), or Boolean networks to model intracellular events (like the regulation of gene activation as in the differentiation of T lymphocytes (Santoni et al., 2008)). In other words, taken out the necessary approximation, a fruitful approach in constructing large-scale mechanistic models is given by combining mechanistically detailed kinetic models (either continuous - equations based - or discrete - boolean networks) and coarse-grained (i.e., individual- or agent-based) models (Smallbone et al., 2007). Interestingly, it has been shown recently that complex system behavior is often largely defined by the interaction topology among the various model components (Brown et al., 2004; Gutenkunst et al., 2007). This finding further supports the expectation that in order to obtain meaningful predictions most likely only a few molecular processes need to be described in great detail with precise parameters estimates, while the rest of the system can be described using the coarse-grained interaction

topology (de Graaf et al., 2009).

The very multi-scale nature of novel models in computational biology makes their development particularly challenging, not just from a biological point of view but also from a mathematical and computational perspective. Moreover, given the availability of already published models targeting a single scale, the sharing and reusing of such models has become an issue. A prominent attempt at solving this problem is provided by the Physiome Project (Bradley et al., 2011; Hunter and Borg, 2003), which aims at developing a framework for the modeling of the “whole” human body. As part of that initiative, the mark-up language CellML was introduced with the aim of establishing a world-wide-adopted standard in the development of cellular level that are modeled as sets of ODEs (Garny et al., 2008). Similarly, FieldML has been defined to model processes on the tissue and organ level that are represented as sets of PDEs (Christie et al., 2009). Along with CellML, another standard called Systems Biology Markup Language (SBML) (Hucka et al., 2003) has been proposed and is now beginning to make a significant impact on the modeling community as a means to exchange models. However, neither CellML nor SBML include explicit directives to deal with the problem of implementing a multi-scale computational model, although there are attempts to address this issue (Baylei, 1990).

Regardless the integration framework one decides to use there are few aspects that need to be taken into account when developing a multi-scale model. In general, the time scales on which the lower-level processes occur are much faster than those on which the higher-level processes occur. Usually this means that the lower-level processes can be assumed to occur instantaneously and can therefore be included as a representation of some kind of field at the higher level. The switch to a model at a higher level of organization is usually determined by the need to ensure that the calculations can be performed in reasonable time (Southern et al, 2008). When coupling together independent models of processes that occur on different scales or as part of different physical systems (as is in multi-organ systems) it is enticing to simply couple existing components (i.e., software) for the separate models to one another. However, this does not take into account how inaccuracies in the values of the variables that are passed between the two models may affect the combined model - one variable may be accurate enough in one model but when these models are coupled may first

introduce errors into the solution of the other model, and in turn the solution of the combined model. In order to prevent these inaccuracies from occurring one should consider the whole as a single model rather than the combination of two simpler ones. For instance, we can consider a microscopic simulator at the cellular level can be coupled with the description of the intracellular signaling activating a specific cellular pathway. In this example the differentiation of T lymphocytes into the phenotypes Th1, Th2, Treg and Th17 is described at a cellular level by means of individual entities (e.g., agent-based) whereas the gene regulation is described (at variance with the example above which use a Boolean network) by a system of differential equations describing activation level of each gene of the gene network represented with the following equation

$$\frac{dx_i}{dt} = \frac{-e^{Ch} + e^{-h(\omega_i - C)}}{(1 - e^{Ch})(1 + e^{-h(\omega_i - C)})} - \gamma_i x_i \quad (2)$$

where x_i is the activation level of the i^{th} gene, ω_i and γ_i are parameters relative to the network topology and C is a constant (Mendoza and Pardo, 2010). Here the lower level description of gene activation is determined at each upper-level time step by solving the system of ODEs, and the cell differentiation is executed at the upper level on the basis of the information coming from the gene expression levels. This procedure is iteratively executed at each time step and for each lymphocyte.

4 CONCLUSIONS

In the study of complex biological phenomena it is necessary to develop a unified view of the various mechanisms in action and of the causal relationships among different parts of that complex system (Di Ventura et al., 2006; Kitano, 2002). In this article we have briefly described the problems faced when one wants to link mathematical or computational models across different time and length scales.

In many areas of biology and physiology, multi-scale and multi-physics models are very much acclaimed, Although there exist an abundant literature for multi-scale models in science and engineering domains (Fish, 2010; Weinan, 2011), a lot remains to be done in terms of translating these mathematical theories and methodologies to the domains of biology and physiology (Evans et al., 2008; Caiazzo et al., 2011; Tahir et al., 2011).

A key unsolved issue is how to represent appropriately the dynamical behaviors of a high-dimensional model of a lower scale by a low-

dimensional model of a higher scale, so that it can be used to investigate complex dynamical behaviors at even higher scales of integration (Qu et al., 2011).

The use of different modeling techniques, introduces gaps between scales. Multi-scale modeling, besides modeling the system, needs to address the issue of how to bridge the gaps between different methodologies and between models at different scales. Unfortunately, there is no specific or simple way to tell how to achieve this objective, but there are empirical principles and methods that can be of help. The goal of computational systems biology to consider a biological system from a holistic perspective, and use both experiments and modeling to reveal how the system behaves (Kitano, 2002; Kohl et al., 2010). It is certainly one of the main research fields that can benefit from the use of multi-scale models and, at the same time, provide methodologies for their development.

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