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A Versatile Platform for Multilevel Modeling of Physiological Systems: SBML-PHML Hybrid Modeling and Simulation

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Abstract Specialized languages used for describing computational models in the field of systems biology and physiology, such as Systems Biology Markup Language (SBML), CellML, and Physiological Hierarchy Markup Language (PHML), have been devised to enhance effective model reuse and sharing among researchers for developing large, multilevel models. Each language has its own specialty. By combining two of these languages, i. e. SBML for illustrating subcellular phenomena and PHML for expressing supracellular dynamics, a novel technology has been developed to describe models of multilevel biophysiological systems. For practical use of the aforementioned languages, consolidated software applications providing intuitive graphical user interfaces are necessary. Starting from 2011, a versatile platform called PhysioDesigner has been developed for multilevel modeling of physiological systems based on PHML. In this article, we focus on the newly developed distinguishing features of PhysioDesigner and PHML for the development of multilevel biophysiological models using SBML-PHML hybridization.

Keywords: multilevel modeling, SBML-PHML hybrid modeling, PhysioDesigner.

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1. Introduction

In order to integrate the massive amounts of data generated by clinical and laboratory studies with simulation results, computable mathematical models are themselves increasing in size and complexity [1]. For this reason, it is essential that pieces of models are able to be shared and re-used, in the same manner of building blocks. To promote effective collaboration for building large-scale models, fundamental tools that support these activities should be consolidated.

To enhance model sharing, several pioneering efforts have been undertaken. For example, XML-based descriptive language formats used to describe the dynamics of biological and physiological systems, such as SBML [2], CellML [3], NeuroML [4], and PHML, have been proposed. The main objective in developing these languages was to establish a common communication foundation for enhancing exchange of models among collaborators.

To use these languages effectively for multilevel modeling of physiological systems, application supports are essential. Many applications have been developed and published and listed on websites, such as software applications for SBML [5], NeuroML [6] and CellML [7]. For example, CellDesigner [8] is a versatile modeling tool of biochemical networks based on SBML. Although many applications employ single languages, some tools support more than one. For example, VCell, an environment for virtual cell modeling and simulation [9], and JSim, a simulation framework that natively uses a modeling language called Mathematical Modeling Language (MML) [10], can import SBML and CellML models. However, in general, these applications convert the imported models into their own native languages. Or, while they support multiple languages, each of the models parsed by such applications must be written in single language, which can be either SBML or others.

PHML, which is a successor to *insilico*ML [11], was developed relatively recently compared with the languages mentioned above. It was developed in parallel with PhysioDesigner [12] (**Fig. 1**), a platform on which users can build mathematical models of multilevel physiological systems with a graphical user interface. Models built with PhysioDesigner are written in PHML, which is effective at explicitly describing the hierarchical structure of physiological systems. The main elements in a PHML model are called modules. Modules form a tree structure to express the hierarchical structure of phys-

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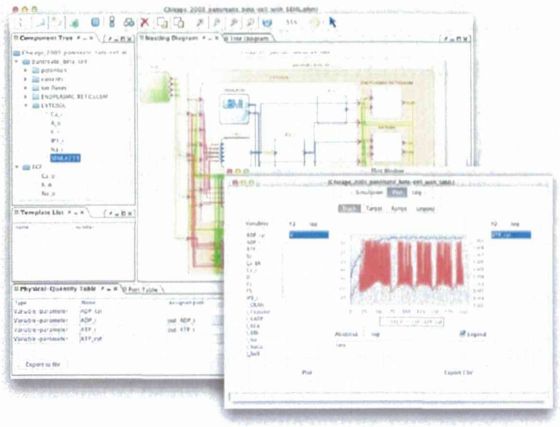

Fig. 1 Snapshots of PhysioDesigner and Flint.

iological systems, and they form a network to imitate the functional relationship among physiological components. Each module is quantitatively characterized by the defining physical quantities inside.

PHML itself was originally designed to present hierarchical structures of physiological systems, including even subcellular phenomena. However, because SBML is a language dedicated to describing subcellular phenomena such as signal transduction and protein-protein interactions, it is better to use SBML to describe subcellular phenomena. We can then benefit from existing SBML resources such as the BioModels database[13]. To edit the SBML part, we can also use CellDesigner or other applications that are dedicated to manipulating SBML, which is a considerably better method than re-implementing a function to edit SBML on PhysioDesigner.

For describing computable models of multilevel physiological systems including subcellular and supracellular phenomena, a novel technology that incorporates SBML and PHML has been developed to leverage the advantages from both languages. In the PHML framework, it is possible to integrate an SBML model into a functional network of modules of a PHML model by embedding the SBML model into one of the modules. Then, the module containing SBML represents a model of subcellular phenomena as modeled by the SBML model. This process is not merely simple embedment of SBML models into a PHML model at the model description level, but is also a computable hybridization accompanied by numerical simulation with Flint, a simulator developed concomitantly with PhysioDesigner.

In this article, we introduce a new technique developed on PhysioDesigner for SBML-PHML hybrid modeling and simulation.

2. PhysioDesigner Overview

PhysioDesigner is a versatile platform that supports modeling and simulation of physiological systems with multiple spatiotemporal levels. The current version as of October 2013 is 1.0 beta6, and it is available at http://physiodesigner.org. Development of PhysioDesigner began in 2011, with the inheritance of all features from *insilico*IDE [14-16], and the tool is posted at http://www.physiome.jp[17].

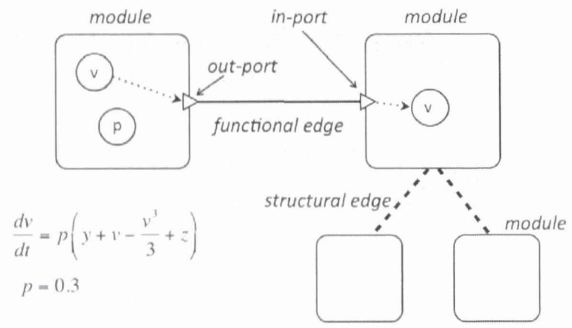

Fig. 2 Scheme of a PHML model. Modules are fundamental elements for constructing a model in PHML. Each module (shown as a rounded rectangle) is quantitatively characterized by physical quantities (shown as encircled letters). Relationships among modules are explicitly represented by edges (solid line: functional edge, dashed line: structural edge). A module can have offspring modules forming a tree-like structure representing the hierarchical structure seen in physiological systems. The value of a physical quantity can be exported from a module through an out-port (small triangle on the right edge of the module) to another module, which enters the module through an in-port (small triangle on the left edge of the module). The equation and parameter at the bottom left corner are examples of the definitions of physical quantities in the module on the left.

The main components in a PHML model are called modules, which represent biological and physiological elements (**Fig. 2**). Multiple modules can be defined as a single module at one level above. For example, many cells form a tissue. These modules at the lower level represent physiological entities that are more precise in spatial scale and more detailed in logical scale. By this nested representation of modules forming the module tree structure, hierarchical structures of physiological systems are explicitly expressed in a model.

Each module is quantitatively characterized by several physical quantities such as states defining system dynamics, and variable and static parameters. The dynamics such as ordinary/partial differential equations, and functions of physical quantities are defined by mathematical equations using physical quantities. To define physical quantities in a module, it is often necessary to refer to values of physical quantities defined in other modules. The value of a physical quantity can be exported from a module through an out-port. Then, the numerical information is carried from the out-port to an in-port of a destination module which is pointed by a functional edge linking them. The value arriving at the in-port can be used to define a value of a physical quantity in the destination module. In this sense, modules form a large functional network in a model.

The concept of capsulation, or making a package of a

physiological function, was introduced to PHML to enhance sharing and reuse of models or their components. Capsulation is an operation that involves the encapsulation of an arbitrary number of modules acting together as a certain physiological function by a capsule module. All edge connections to (from) encapsulated modules from (to) the outside of the capsule must pass through the capsule module once in order to secure the independence of the encapsulated modules. Namely, the capsule module acts as an interface or gateway for all modules in the capsule. With this isolation of modules, it becomes easier to reuse the encapsulated modules in another part of the model or in other models.

Simulations of PHML models are conducted by the simulator Flint[12, 16], which is being developed concurrently with PhysioDesigner. Flint was rebranded from *insilico* Sim [18, 19], and is also available at http://physiodesigner.org. One of the features of Flint is that it can execute simulation of SBML models as well as PHML models, using the SBML ODE Solver Library (SOSlib) to parse SBML.

3. SBML and PHML Hybrid Modeling

3.1 Concept and Basic Usage

Because PHML is designed to represent the hierarchical structure of physiological phenomena, it is possible to describe a model that includes integration of subcellular and supracellular phenomena. However, instead of modeling subcellular phenomena with PHML, a novel modeling method of hybridizing SBML and PHML has been developed.

SBML-PHML hybrid modeling is achieved by embedding a whole SBML model into a PHML module (**Fig. 3**). The module then represents the biological system expressed by SBML. The SBML model is integrated into a tree structure expressing the hierarchical structure of multilevel biophysiological systems, and also in a functional network composed of modules.

A limitation is that one module can include only one SBML model. However, obviously one PHML model can have multiple modules that contain an SBML model each. Hence, this function can be utilized not only in subcellular-supracelluar multilevel modeling, but also to create a PHML-based network of multiple SBML models expressing phenomena in a single cell.

3.2 Value Exchange between SBML and PHML

To functionally integrate an SBML model into a PHML network, numerical information must be exchanged between them during a simulation. The main players in an SBML model carrying numerical information (or representing biological entities) are called species, which are used mainly to express, for example, the concentration of ions and molecules that take part in one or more reactions. To define a reaction, parameters such as velocity constant as well as species are used. In a module containing an SBML model, associations between physical quantities and species or parameters have to be defined to form a bridge between the SBML moiety and the PHML moiety.

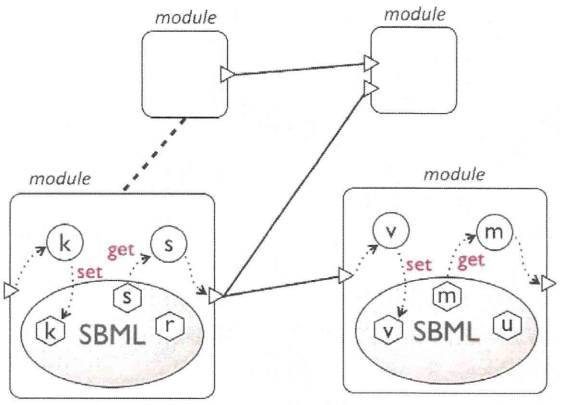

Fig. 3 Schematic diagram of an SBML-PHML hybrid model. A module of PHML may contain a whole SBML model represented by an oval in a module. By making associations between physical quantities in the module (letters in circles) and species/parameters in the SBML model (letters in hexagons), the SBML model is functionally integrated into the network of modules. There are two ways to accomplish this. One is a "get" action, which is used to retrieve the values from the SBML portion and carry them to the PHML portion. The other is a "set" action used to override the values or dynamics originally defined in the SBML portion by those defined in the PHML portion. Dotted arrows indicate the flow of values.

These are two-way actions. One is the "get" action, which converts the value defined in a species or parameter in the SBML model to a physical quantity. Then the other physical quantities in the module can utilize the numerical information defined in the SBML model via the physical quantities of the "get" action. This is similar to associating a physical quantity with an in-port to receive a value carried to it.

The other action is similar, but in the opposite direction, i.e. "set" action. A physical quantity originating in the PHML part with a "set" definition can affect the SBML part by overriding the original definition of species or parameters in the SBML model, without direct modification of the SBML model itself. Even if the species have dynamics originally defined in the SBML model, it is completely overridden by that defined in the physical quantity, replacing the value at every step during the course of a simulation. By this interpretation rule of the definition of the bridge between species/parameter and physical quantity, the SBML model can be effectively involved in the model.

The process of embedding an SBML model in a module is assisted by a PhysioDesigner interface shown in **Fig. 4**. PhysioDesigner employs a dialog box in which a list of the species involved in the SBML model is shown, so that users can define the interaction between SBML species and PHML physical quantities. The direction of the action ("get" or "set") and the associated physical quantity can be selected by combo-boxes.

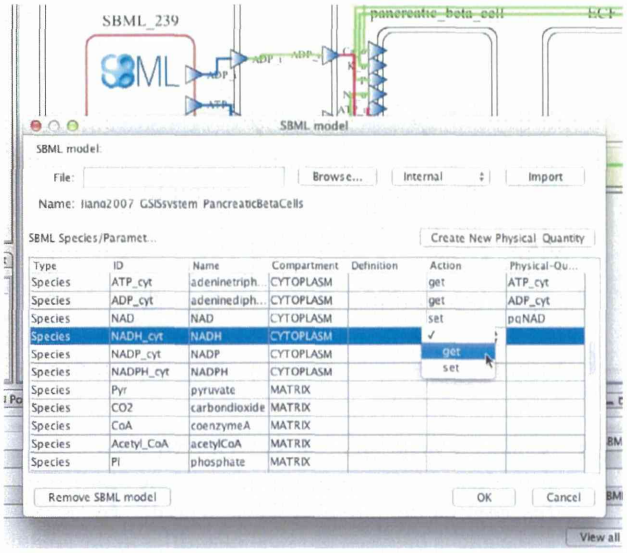

Fig. 4 Dialog of PhysioDesigner to support embedment of an SBML model into a module. It shows a list of species included in the SBML model. The two right-most columns provide an interactive interface to select the action (get or set) and a physical quantity to be associated.

3.3 Simulation of Hybrid Models

Flint is capable of parsing and simulating SBML models as well as PHML models. In particular, it is possible to take into account the SBML models embedded in a PHML model. Flint uses the SBML ODE Solver Library (SOSlib) [20] to extract formulas as abstract syntax trees (AST). SOSlib converts reaction rules or assignments in an SBML model to ODEs and algebraic equations, and events to conditional statements.

After extracting these ASTs, Flint detects species and parameters from which values should be transferred to physical quantities according to the bridge definition with a "get" action, or detects species and parameters which should be overridden by physical quantities according to the definition with a "set" action. Interpretation of a "get" action is simple. The value defined by a species or a parameter is transferred to a physical quantity. The value does not need to be constant. In the case of a "set" action, if the target is a parameter, the interpretation is still rather simple. The value of the parameter is replaced by that of the corresponding physical quantity. If the target is a species, we need to be careful not to introduce any inconsistency into the reaction network described in the SBML model. In our framework, the interpretation of the hybridization of SBML and PHML is carried out after extracting equations from the SBML side. At this stage, the dynamics of each species is described by a single ODE derived by interpreting the reaction formulae. The derivative of the species is replaced by the definition of a physical quantity without harming the consistency of the logic modeled in the other part of the SBML model.

Once the ASTs are merged into other formulae coming from the PHML part, they are sent together to the next stage to generate the bytecode for the execution of a simulation. Hence, Flint can handle elements defined in SBML Level 2, which is supported by the latest SOSlib. In other words, this is a limitation of Flint in supporting SBML. Note that to solve equations, Flint does not call the solver API of SOSlib. Instead, it creates a bytecode including the numerical integration algorithm implemented by Flint.

4. Examples of Hybrid Modeling

4.1 A Simple Example

A simple example of SBML-PHML hybrid modeling, basically a caricature model of the disposition of carboxydichlorofluroscein in hepatocyte, is illustrated in this section [21]. Only two or three players are extracted from the complicated signal transduction pathways originally proposed for this model (**Fig. 5A**). The model contains carboxydichlorofluroscein diacetate (CDFDA) in a hepatocyte, which is hydrolyzed to carboxydichlorofluroscein (CDF). The initial concentrations for CDFDA and CDF are set at 10 and 0 μM, respectively. By the hydrolytic reaction, the concentration of CDFDA decreases and CDF increases (**Fig. 5B**), which is simulated by Flint.

Based on the above SBML model, we expand the model on PhysioDesigner by adding a component representing extracellular CDFDA that can diffuse passively into the cell. Extracellular CDFDA is added as a physical quantity and a module in PHML creating a hybrid model (**Fig. 5C**).

From the mathematical point of view, the original dynamics of the intracellular CDFDA/CDF concentration are described by the following ordinary differential equations (ODEs).

$$\frac{d}{dt}[\mathrm{CDFDA_{in}}] = -k_1[\mathrm{CDFDA_{in}}], \quad (1)$$

$$\frac{d}{dt}[\mathrm{CDF_{in}}] = k_1[\mathrm{CDFDA_{in}}]. \quad (2)$$

where $[\mathrm{CDFDA}_{in}]$ and $[\mathrm{CDF}_{in}]$ represent the concentrations of intracellular CDFDA and CDF, respectively. $[\mathrm{CDFDA}_{in}]$ decreases monotonically and $[\mathrm{CDF}_{in}]$ increases. Adding extracellular CDFDA that diffuses into the hepatocyte modifies the ODEs as follows:

$$\frac{d}{dt}[\mathrm{CDFDA_{ex}}] = -k_2[\mathrm{CDFDA_{ex}}], \quad (3)$$

$$\frac{d}{dt}[\mathrm{CDFDA_{in}}] = -k_1[\mathrm{CDFDA_{in}}] + k_2[\mathrm{CDFDA_{ex}}], \quad (4)$$

$$\frac{d}{dt}[\mathrm{CDF_{in}}] = k_1[\mathrm{CDFDA_{in}}]. \quad (5)$$

where [CDFDAex] represents the concentration of extracellular CDFDA. After modification, $[\mathrm{CDFDA}_{in}]$ no longer decreases monotonically, but shows a single peak depending on the reaction velocity constants k_1 and k_2 (**Fig. 5D**).

The first step of SBML-PHML hybrid modeling for this example is to create two modules on PhysioDesigner. One is a hepatocyte module consisting of the dynamics of intracellular CDFDA/CDF, in which the SBML model is

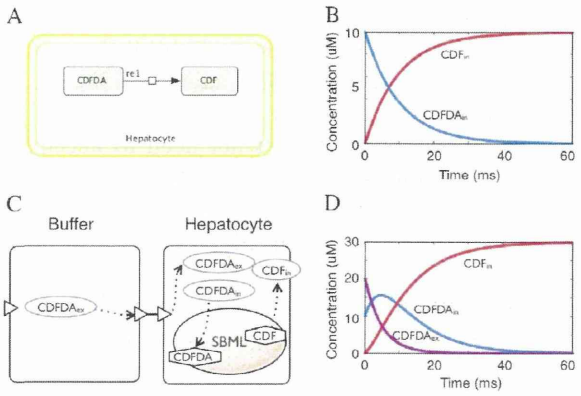

Fig. 5 A simple example of SBML-PHML hybrid modeling. A. Two-species model on CellDesigner. B. Simulation result of the simple model containing two species. The concentration of CDFDA decreases exponentially (blue curve), whereas that of CDF increases asymptotically (red curve). C. Schematic representation of the hybrid model of this simple example. D. Simulation results of the hybrid model from Flint. The concentration of external CDFDA starts from 20 μM and decreases (violet curve), and that of internal CDFDA has a unimodal peak (blue curve).

imported. The other is to implement the extracellular buffer, consisting of extracellular CDFDA. Once the SBML model is imported into the hepatocyte module, a variable-type physical quantity is created, and a bridge with a "get" action between the physical quantity and a species representing CDF in SBML are established to monitor its dynamics. In addition, the dynamics of intracellular CDFDA in the SBML model have to be overridden. For this, a state type physical quantity is created to implement an ODE shown in Eq. 4. The bridge between the state type physical quantity and the species with the "set" action should be defined.

Next, the buffer module has to be implemented with a state type physical quantity defined by Eq. 3. Additionally, a relationship between two modules has to be defined by linking an edge between them to transport the value of extracellular CDFDA, since the value is used in Eq. 4 in the hepatocyte module (**Fig. 5C**). **Figure 5D** presents the simulation result of the extended model, showing a monotonic decrease in extracellular CDFDA, a monotonic increase in intracellular CDF, and a unimodal increase followed by decrease in intracellular CDFDA.

4.2 Realistic Example

Let us observe another more realistic example involving insulin secretion from pancreatic β-cells. It is known that pancreatic β-cells exhibit complex and periodic spike-burst activity in response to an elevated concentration of extracellular glucose. There is a model that reproduces membrane-potential-level dynamics, called the Chicago model [22], which includes membrane potential, ATP/ADP concentrations, and various ionic currents such as sodium, potassium, and calcium. One of the components is an ATP-sensitive K^+ current, the channel of which is inhibited by high ATP concentration, resulting in membrane depolarization followed by an influx of Ca^{2+} and exocytosis of insulin granules. The model mainly focuses on the electrical mechanism of burst generation, in which oscillation of Ca^{2+} concentration, in particular, plays an important role. However, it does not pay much attention to the biochemical mechanism of glucose metabolism and ATP generation by the TCA cycle in mitochondria, although ATP concentration plays an important role in triggering insulin secretion. There is another model written in SBML that represents the glucose-stimulated insulin secretion network of pancreatic β-cells [23], which includes many entities relating to glycolysis, the TCA cycle, the respiratory chain, NADH shuttles, and the pyruvate cycle. This model, however, does not include membrane potential and ionic currents.

Using the above two models, we can create SBML-PHML hybrid multilevel models. Because the dynamics of ATP and ADP in the network model written in SBML (**Fig. 6A**) are described more carefully than those in the Chicago model (written in PHML), it is worthwhile to spool them up in the Chicago model. For this, the physical quantities representing ATP and ADP in the Chicago model are removed, and a module importing the network model is introduced (**Fig. 6B**). Then, two-variable parameter type physical quantities representing ATP and ADP concentrations are created in the module, and bridges from ATP and ADP species in the network model to those new physical quantities are established to get the values from the SBML portion to the PHML portion. By using these two physical quantities instead of the original in the Chicago model, the network model is effectively integrated through ATP and ADP dynamics, and the hybrid model using Flint exhibits a periodic burst of the membrane potential (**Fig. 6C**).

5. Linkage with Other Software

PhysioDesigner cannot edit SBML models embedded in modules. Because several kinds of software dedicated to SBML, such as CellDesigner, are available, it is not worthwhile to re-implement the same function in PhysioDesigner. To communicate with other software, we leverage the Garuda platform (http://www.garuda-alliance.org/), which is a new software developed to provide a means for systems biology tools to interoperate seamlessly. PhysioDesigner and Flint comply with the Garuda Alliance [24]. PhysioDesigner can extract an embedded SBML model from the PHML model, and send it to CellDesigner to browse or edit via the Garuda platform. Of course, it is also possible to receive an SBML model and import it into a module not only from CellDesigner but also from other software that can send out an SBML model via the Garuda platform.

6. Discussion

We have demonstrated a new function for creating SBML-PHML hybrid models developed on the existing versatile platform, PhysioDesigner. SBML is suitable for describing

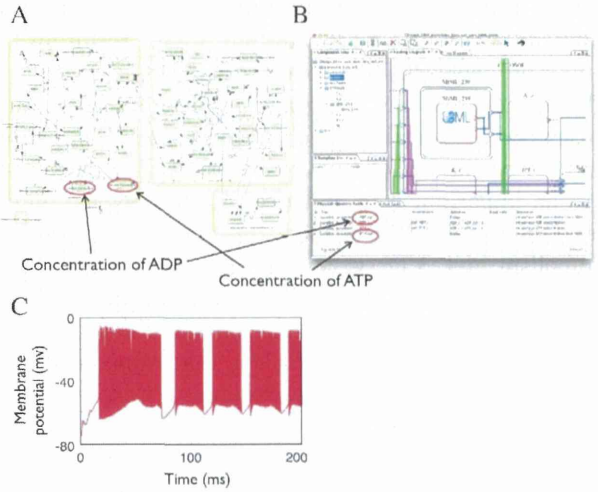

Fig. 6 SBML-PHML hybrid model of pancreatic β-cells. A. The network model on CellDesigner. B. Hybrid model on PhysioDesigner. In the hybrid model, ADP and ATP concentrations (indicated by red circles in A and B) defined in the SBML model are transferred to physical quantities in the PHML side with a "get" action. C. Simulation results of the hybrid model with Flint. The time course of the membrane potential showed a periodic burst.

subcellular biochemical phenomena such as signal transductions, while PHML was originally designed to describe modularity, hierarchical structures, and physiological system networks. The function of PhysioDesigner to embed SBML into another language without conversion is an unprecedented and effective method to achieve multilevel modeling of physiological systems.

Two methods can be considered to embed an SBML model into a PHML model. One is to convert the SMBL model into PHML, and import it as a module. The other is to retain the SBML model and define the relationship between the SBML species/parameters and the physical quantities. The former method was implemented, for example, for SBML and CellML exchange[25]. The latter method is adopted in our proposed modeling method, although the former is also technically feasible in our case.

The latter method has three advantages over the former method. First, targets modeled by SBML can be described by SBML most effectively, and many dedicated applications such as CellDesigner are available for working with SBML. With the aid of the Garuda platform, now PhysioDesigner can seamlessly consign an embedded SBML model in a module to CellDesigner for display and editing, and can receive the modified SBML model back. There is no need to reinvent the wheel, and to use such applications is the simplest and most direct way to work with SBML. However, direct modification of the embedded SBML model can lead to a collapse in the consistency of integration with PHML. Users must be careful to maintain the consistent bridges between the SBML and PHML models.

Second, since there is an entire SBML model in a PHML module, it is easy to know which version of the SBML model is used for the targeted phenomenon in the PHML model. If an updated SBML model exists, it is also simple to replace it.

Third, PhysioDesigner does not need to account for the SBML version to import a SBML model. When executing a simulation in Flint, Flint needs to extract equations and parameters from the SBML portion, hence it needs to parse the SBML model. But, this can be performed by the widely used libraries for SBML, which is much easier than maintaining our handcrafted converter from SBML to PHML.

On the other hand, the former method has an advantage in the process of integrating mathematical logic. The reason is as follows. In the case of the latter method, as explained in Section 4.1, users need to know Eq. 1 to obtain the expanded form (Eq. 4) to develop the SBML-PHML hybrid model. However, in some cases, it can be difficult to determine the original differential equation, because the equations are not explicitly described in SBML. If the SBML model is converted into PHML, integration in the mathematical logic may be simplified.

As described in Section 3.2, PhysioDesigner includes a dialog box to support the SBML model embedment process. However, users still need to maintain the consistency in the units of quantities and the time scales used in both SBML and PHML models. Support for this issue should be addressed in future development of PhysioDesigner. Physical quantities in PHML and species/parameters in SBML can be defined with unit information. As far as the units are defined and available in both models, PhysioDesigner has to, at least, raise a warning when it detects inconsistencies in the units.

SBML models are archived, for example, in the BioModels Database[13]. There is also a PHML model database at http://physiome.jp. PhysioDesigner, with the aid of the Garuda platform and CellDesigner, provides a wider activity arena for users by utilizing resources in databases for SBML and PHML together to build multilevel models. In PhysioDesigner, it is also possible to integrate the morphometric information as a skeletal structure to create a computable model with a template/instance framework[26] for large-scale modeling. Integration of SBML and morphology into a PHML model provides a novel way to create large-scale multilevel models.

One of the problems that could arise when the model size becomes huge is a shortage of computing power for simulations. To solve this problem, development is underway to render the simulator Flint executable on the K supercomputer and computing cloud. The version of Flint for cloud computing is called Flint K3, and the 1.0 alpha version is posted at http://flintk3.org. The enhancement of Flint will facilitate scaling-up of models in terms of computational power.

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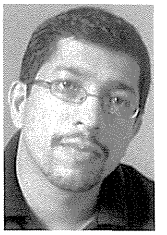

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Cell Host & Microbe
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Influenza Virus-Host Interactome Screen as a Platform for Antiviral Drug Development

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SUMMARY

Host factors required for viral replication are ideal drug targets because they are less likely than viral proteins to mutate under drug-mediated selective pressure. Although genome-wide screens have identified host proteins involved in influenza virus replication, limited mechanistic understanding of how these factors affect influenza has hindered potential drug development. We conducted a systematic analysis to identify and validate host factors that associate with influenza virus proteins and affect viral replication. After identifying over 1,000 host factors that coimmunoprecipitate with specific viral proteins, we generated a network of virus-host protein interactions based on the stage of the viral life cycle affected upon host factor downregulation. Using compounds that inhibit these host factors, we validated several proteins, notably Golgi-specific brefeldin A-resistant guanine nucleotide exchange factor 1 (GBF1) and JAK1, as potential antiviral drug targets. Thus, virus-host interactome screens are powerful strategies to identify targetable host factors and guide antiviral drug development.

INTRODUCTION

Viruses, which rely on host cellular functions to replicate, hijack the host cell machinery and rewire it for their own needs. A comprehensive understanding of host-virus interactions would greatly improve our understanding of the viral life cycle and be invaluable in identifying strategies to prevent or treat potentially deadly virus infections.

Influenza viruses cause annual epidemics and recurring pandemics, which have claimed millions of lives and had a considerable impact on public health and the global economy. Recent sporadic human infections with avian viruses of the H5N1 and H7N9 subtypes have raised concerns about the pandemic potential of these viruses (Gao et al., 2013; Li et al., 2014; Webster and Govorkova, 2006; Yen and Webster, 2009). Two antiviral drugs (that inhibit the ion channel [M2] or neuraminidase [NA] proteins) are available (Davies et al., 1964; Hayden, 2001), but the emergence of drug-resistant viruses has become a serious problem (Bright et al., 2005, 2006; Dawood et al., 2009; Nicoll et al., 2008). Therefore, there is an urgent need to identify targets for antiviral drugs.

In recent years, six genome-wide screens have identified a total of 1,449 human genes (including 110 human orthologs of *Drosophila* genes) with potential roles in the life cycle of influenza virus (Brass et al., 2009; Hao et al., 2008; Karlas et al., 2010; König et al., 2010; Shapira et al., 2009; Sui et al., 2009). Meta-analyses revealed limited overlap among these studies (de Chassey et al., 2012; Mehle and Doudna, 2010; Watanabe et al., 2010). This limited overlap may be caused by differences in the experimental conditions of the screens. Also, the experimental methods used in the screens might be suboptimal to investigate the whole life cycle of influenza viruses (e.g., using nonpermissive cells for influenza virus infection and/or nonauthentic influenza virus [i.e., recombinant viruses possessing reporter genes]). Moreover, the criteria used to determine the candidate host factors likely differed among the screens, and each screen might include a number of false positives. More importantly, most of these studies validated only subsets of potential host interaction