Modular and Hierarchical Modelling Concept for Large Biological Petri Nets Applied to Nociception

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Abstract Here, we introduce a modular and hierarchical modeling concept for large biological Petri nets. This modeling concept suggests representing every functional system component of a molecular network by an autonomous and self-contained Petri net, so-called module. Due to the specific architecture of the modules, they need to fulfill certain properties important for biological Petri nets to be valid. The entire network is build-up by connecting the modules via common places corresponding to shared molecular components. The individual modules are coupled in a way that the structural properties that are common to all modules apply to the composed network as well. We applied this modeling concept on nociceptive signaling in DRG-neurons to compose a model describing pain on a molecular level for the first time. We verified the applicability of our modeling concept for very complex components and confirmed preservation of the properties after module coupling.

1 Introduction

A major issue in systems biology is the construction and validation of large biological networks, especially if the involved mechanisms should be considered in depth. This is the case for the nociceptive network in the peripheral endings of DRG-neurons (nociceptors) that are responsible for pain signaling (see Figure 1). Pain is a very complex phenomenon with behavioral, peripheral and central nervous system components, wherein nociception comprises the underlying molecular mechanisms [2]. (Chronic) pain is certainly one of the most serious public health issues (see [3,6] and references therein).Hitherto, there exists no coherent computational model for pain due to the complexity and lack of knowledge on the underlying molecular mechanisms. A complete and validated pain model would be an important progress to develop a mechanism-based pain therapy to successfully treat pain suffering.

In general, modular approaches have always been useful to manage large networks. So far, in systems biology just single pathways have been regarded as modules [1]. Our modular and hierarchical modeling concept is beyond this scope. It is a promising approach to handle large biological systems by treating

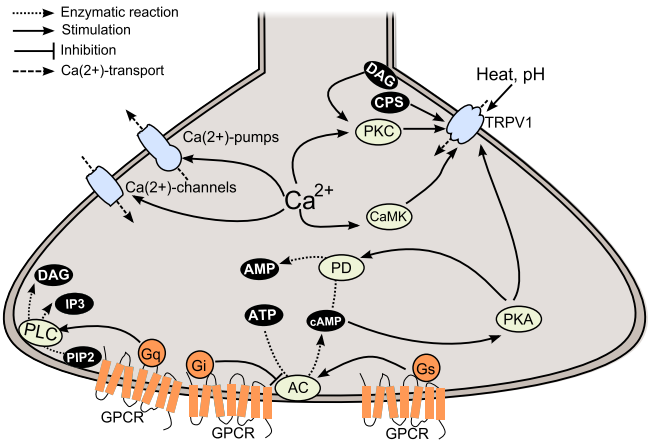

Figure 1. Illustration of a nociceptor. Primary subunits (modules) of the nociceptive network are enzymes (green), receptors (orange) and channels (blue). The secondary subunits like cAMP, Ca2+, DAG etc. are colored in black.

functional molecular components as single independent entities. In this respect, Petri nets are an appropriate tool. They are designed for concurrent systems. Thus, Petri nets are ideally suited to describe biological systems [5], like the nociceptive system. Also, they allow for a hierarchical arrangement of large and complex networks in the form of a neat graphical representation. Single functional proteins (receptors, channels, enzymes etc.) are represented by hierarchical, autonomous and self-contained Petri nets, called modules, which have to fulfill certain properties important for biological Petri nets [5]. Those firstly qualitative modules are validated by a comprehensive analysis and are subsequently subjected to stochastic simulation studies. The modular and hierarchical modeling concept implies a special coupling procedure of the modules to an entire network of communicating components. Advantageously, the properties of the entire network can be predicted due to the adhered properties of the single modules and the special module coupling.

The constructed pain model is a first approach to integrate the currently published neurobiological and clinical knowledge about nociception in one coherent and validated model describing all the interactions between the involved components. Hitherto, it contains 31 modules that have been constructed and connected by the modular and hierarchical modeling concept (see also section "'Nociceptive Network"'). For the construction and validation of the modules and the entire network we used the Petri net editor *Snoopy* [9] and the place/transition analysis tool *Charlie* [7].

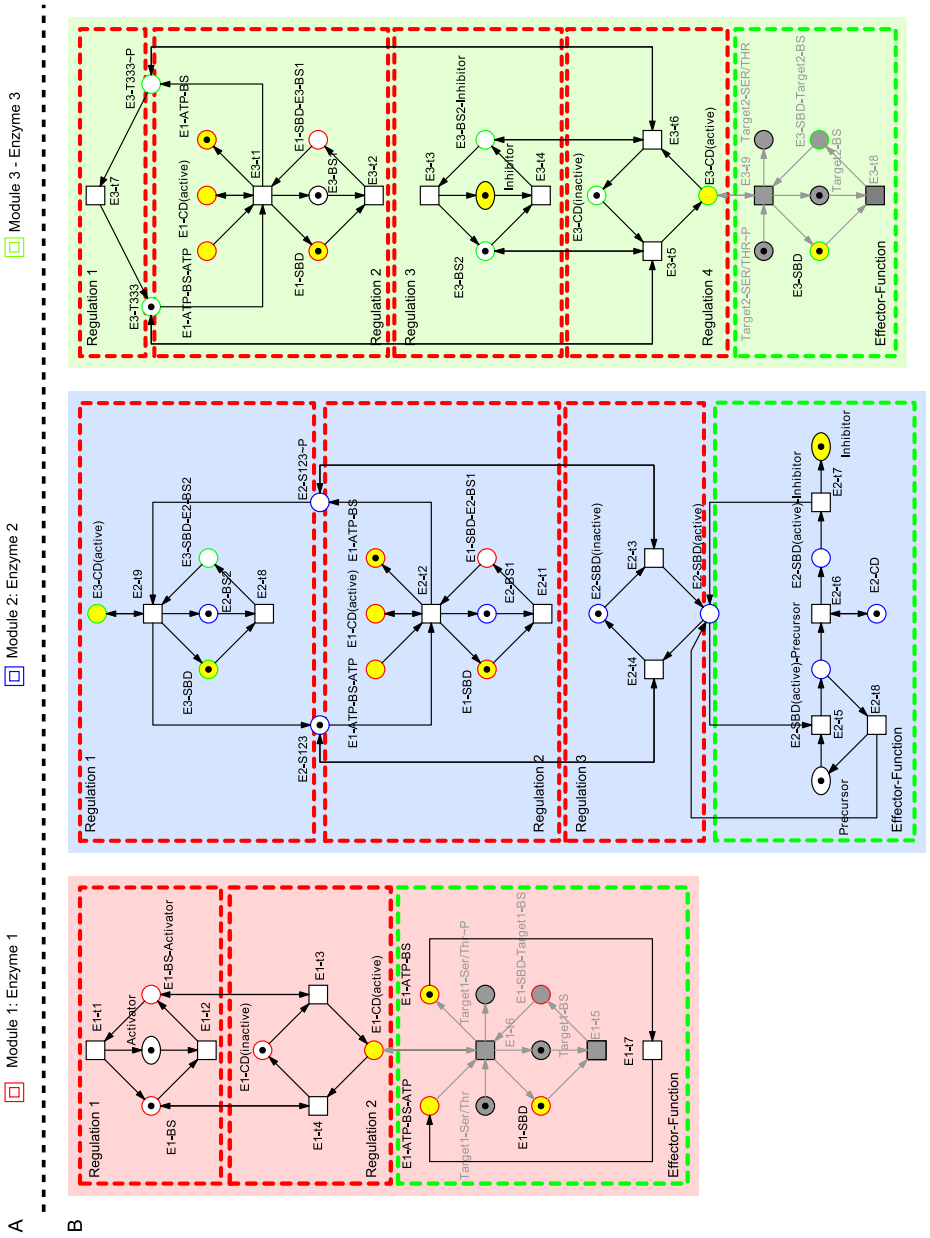

Figure 2. Example of a small toy network composed of three different enzymes to explain the method: enzyme 1 (Kinase, stimulated by the activator), enzyme 2 (Synthase for the inhibitor, regulated by phosphorylation), enzyme 3 (Phosphatase, regulated by the inhibitor and phosphorylation). (A) Top-level of the entire network containing three modules wrapped in coarse transition. (B) Flat representation of the network graphs of the modules showing enzyme 1 (red), enzyme 2 (blue) and enzyme 3 (green) and places are framed with the corresponding color. Circles indicate places belonging to the primary entities and oval places indicate secondary entities (activator, inhibitor and precursor). The modules consist of regulative subnets (red dashed rectangles') and subnets of effector function (green dashed rectangles). Logical places (yellow) connect the modules at deeper levels of the hierarchy tree. The grey places, transitions and arcs have been deleted after coupling.

2 Method

First, the identified components in the regarded system have to be categorized in primary and secondary entities. Primary entities are proteins or protein complexes (enzymes, receptors, ion channels, adaptor proteins etc.), whose function and activity can be regulated due to modification by other components. Secondary entities cannot undergo modifications of their activity and function. This group contains ligands, second messengers, precursor molecules, ions and energy equivalents. Secondary entities are regulators or substrates of primary entities or they are transported by those. Primary entities can be further differentiated by their function, whether they regulate other primary entities or process secondary entities. Every primary entity constitutes a module that contains a hierarchical arranged, autonomous and self-contained Petri net. Detailed information about the introduced modeling concept can be found in reference [4].

Architecture of a Module. Places of a module correspond to different states of functional domains of primary entities (phosphorylation sites, binding domains, inhibitory sequences etc.) or different states of secondary entities (free or bound, precursor or proceeded molecule etc.). In this context, transitions of a module describe inter-/intramolecular actions that occur within the corresponding primary entity (like binding/dissociation, (de-)phosphorylation, conformational changes or processing of substrates etc) and change the states of the involved entities. Every module contains two classes of subnets indicating the regulation or the effector function of a primary entity. The effector function subnets of those primary entities that might regulate a variety of other primary entities are generalized. The possible targets are fused to one abstract target. Such subnets can be reused for the construction of the regulatory subnets of discrete targets. An illustrative example of a regulative network consisting of three different enzymes is shown in Figure 2.

Validation of a Module. The constructed modules have to fulfill certain properties important for biological networks [5] to be valid which are considered by a comprehensive analysis. Table 1 gives an overview about the properties that every module must fulfill (see also Figure 3A). Having successfully validated the qualitative modules, they are subjected to a stochastic simulation, even if experimental parameters are not available so far. Simulation studies are carried out to analyze whether the dynamic behavior of the modules can in principle reflect the assigned effector function as indicated by the time-dependent token-flow. A stochastic mass action function is assigned to every transition that can be modulated by a parameter according to biological needs. The parameters are determined by 'in silico' experiments.

Assembling of the Modules to an Entire Network. The single modules can easily be connected to a larger network. The prerequisites for the direct and indirect coupling of the modules have been established separately. The subnets of the modules already consider all possible interactions. Thus, the modules are 'naturally' connected by places that are equivalent to complexes between the different entities (indicated as logical places) and actions on which the different entities participate. At the top-level of the entire network the modules are just

visible as coarse transitions. Thus, the connection of the modules is not immediately obvious and the network seems to be very compact. Due to logical places the complex branching of the modules is only visible on lower levels. The effector function subnets of primary entities showing the regulation of a variety of other primary entities are not needed anymore. Therefore, all places corresponding to abstract targets and transitions connected with abstract targets have to be be abolished. The entire network already contains all specified targets of those primary entities. Figure 2 shows also the coupling of the enzymes to an entire network.

Deducing Properties for the Entire Network from the Modules. Due to the way of coupling, it is possible to transfer the structural properties of the modules on the entire network (see Table 1A). We show that they do not change after the coupling procedure. The entire network still contains no boundary transitions but boundary places of secondary entities. Therefore, it cannot be covered with T-invariants. We observe that all T-invariants of the coupled modules are conserved in the entire network. Furthermore, the coverage of the entire network with P-invariants is achieved. Due to the special module coupling just certain actions can occur to the P-invariants. The P-invariants of each module can be retained or deleted without changing the coverage with P-invariants of the entire network. The retention of P-invariants can be divided in five cases: *(1)* Retention of unique P-invariants, *(2)* Melting of identical P-invariants, *(3)* Combination of overlapping P-invariants, *(4)* Deletion of states of abstract targets in a P-invariant and replacement by all possible specified targets, *(5)* Integration of P-invariants in retained P-invariants. A P-invariant that contains only states of an abstract target is deleted in the entire network, because the equivalent places have been deleted before. Due to the coverage of the entire network with P-invariants it is bounded. By virtue of boundness and the non-coverage with T-invariants the entire network cannot be live and reversible (see also Table 1B). After validating the entire network by its properties, the dynamic behavior must be investigated by simulation studies (see Figure 2B).

Table 1: Properties of the modules and the entire network

Properties	Fulfilled	Explanation
A - Structural Properties		
Pure	No	Every module contains actions that process just under certain intra-/intermolecular circumstances like a special state of a domain. The corresponding places of such domains are connected with the transition of an action by an double arc.
Ordinary	Yes	The arc weigth is "1" because just elementary actions are considered. Meaning just one element of every secondary entity and one state of every domain can attend on the educte side as well as on the product side.
Homogenous	Yes	Due to Ordinary.
Input transition	No	There are no boundary transitions (sinks or sources) that add or withdraw any tokens.
Output transition	No	

Input place	Yes[1]	The modules are bordered by places corresponding to domains of other primary entities or secondary entities.
Output place	Yes[1]	
Non-blocking multiplicity	No[1]	Due to boundary places this property cannot be fulfilled.
Conservative	No	Modules contain certain domains of primary substances that can build complexes with domains of the same or another primary substance as well as with secondary substances.
Static conflict free	No	Modules contain certain domains of primary entities and secondary entities that can attend on more than one action on the reactant side.
Connected	Yes	Every module must be connected, as well as the entire network.
Strongly Connected	No[1]	The boundery nodes preclude strong connectedness.
Covered with P-invariants	Yes	Every Module has to be covered with P-Invariants, because: – Every domain of a primary entity and every secondary entity must exist in one of the possible state. – There can just exist one of the possible states of a domain of a primary entity or a secondary entity at the same time. – There can just exist certain combinations of those states at the same time. Every P-Invariant has an important biological interpretation that contributes to the function of the module.
Covered with T-invariants	No[1]	Due to boundary places this property cannot be fulfilled. The same is valid for the entire network. But every T-Invariant has also an important biological interpretation that describes reversible processes like binding/dissociation, phosphorylation/dephosphorylation, activation/inactivation etc.)
Deadlock trap property	No[1]	Due to boundary places this property cannot be fulfilled. The same is valid for the entire network.
B - Behavioral Properties		
Structurally/ k-bounded	Yes	Due to the coverage with P-invariants the modules are bounded.
Strongly covered with T-invariants	No	Due to boundary places this property cannot be fulfilled. Also exist transitions describing two reverse actions.
Dead Transitions	No	The initial marking must assure that every action can proceed.
Dynamically conflict free	Yes[1]	Modules can contain actions that inhibit the feasibility of other actions.
Dead States	No[1]	Modules can contain actions that can act independent of the limitations by secondary entities.
Liveness	No[1]	Cannot be fulfilled because boundness and non-coverage with T-invariants.
Reversibility	No[1]	Due to boundary places this property cannot be fulfilled.

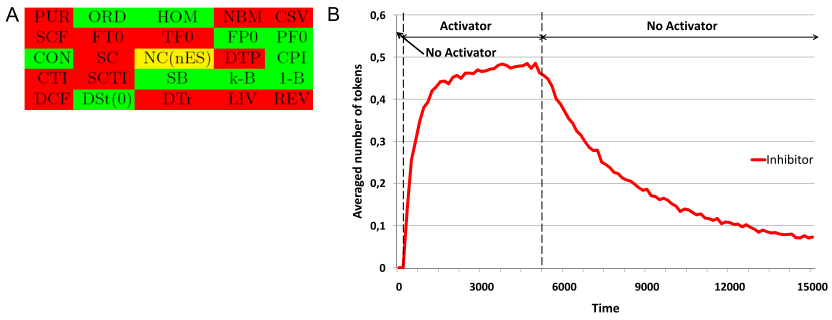

Figure 3. Validation of the modules and the entire network shown in Figure 1. (A) Identical properties of the modules and the entire network (exception: the module of enzyme 2 contains two dead states) determined with Charlie (red = no, green = yes). (B) Stochastic simulation study with the entire network showing the dependence of the inhibitor synthesis on the activator. The simulation result is conforming to the expected behavior, the inhibitor is mainly produced if the activator for enzyme 1 is available. The high amount of the inhibitor inactivates the antithetic enzyme 3.

3 Nociceptive Network

Currently, we have constructed 31 modules (see also figure 1) with the help of modular and hierarchical modeling concept on the basis of 320 scientific articles. All modules have been connected to an entire nociceptive network with a total size of 709 places, 800 transitions and 4391 arcs that are spread over 291 pages with a nesting depth of up to 4. The modules of nociceptive signaling components as well as the resulting nociceptive network have been validated. They adhere the given properties of the modular and hierarchical modeling concept. All modules and the entire nociceptive network as well as detailed results of the analysis and simulations studies can be found in reference [4].

4 Conclusion

With the help of the modular and hierarchical modeling concept we were able to construct and validate a number of modules of important nociceptive signaling components and assemble them to an entire nociceptive network [4]. Hitherto, the nociceptive network is not complete. Twice as many modules will be needed to describe all known interactions.
Nevertheless, we verified the applicability of our modeling concept even for very complex components and the preservation of the properties after module coupling.

[1] Exception for single modules are possible due to their functionality.

All constructed modules are well documented and organized in a library for reuse in other systems. The modules can be connected according to the specific demands of any 'wet lab' or 'in silico' experiments.
To investigate the whole nociceptive system with 'in silico' experiments, we first need to modularize the missing nociceptive components and parameterize the modules. We plan to establish a possible parameter set by trial and error. This parameter set can then be challenged by error analysis and model checking. With an initially parameterized nociceptive network we will presumably be able to: *(1)* investigate changes in network behavior on perturbations of the network, *(2)* predict experiments, *(3)* suggest possible targets for new intervention strategies in pain therapy based on sensitivity analysis. To investigate multiple copies of signaling components as well as diverse DRG-neuron population we also intend to color our low level net [8]. Further we want to extend reconstructed networks [10] out of experimental data by module mapping. We are still searching for new methods to screen the modules and the nociceptive network for non-obvious properties that are defined by their structure.
In summary, our modular and hierarchical modeling concept seems to be a promising way to handle and investigate large biological system, to develop new analysis approaches and Petri net applications.

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