Proceedings of the 2nd International Workshop on Biological Processes & Petri Nets (BioPPN2011)
online: http://ceur-ws.org/Vol-724 pp.117-134

Pain Signaling - A Case Study of the Modular Petri Net Modeling Concept with Prospect to a Protein-Oriented Modeling Platform

Mary Ann Blätke[1], Sonja Meyer[1] and Wolfgang Marwan[1]

[1]Otto-von-Guericke University Magdeburg, Universitätsplatz 2, 39106 Magdeburg, Germany
wolfgang.marwan@ovgu.de

Abstract. The construction of monolithic pathway models, as well as their coupling, curation and the integration of new data is arduous and inconvenient. The modular Petri net modeling concept we present here shows one way to manage these difficulties. In our concept, proteins are represented as functional units by Petri net submodels with a defined structure and connection interface, called modules. Each module integrates all publicly available information about its intramolecular changes and interactions with other molecules. Hence, a module corresponds to an interactive review written in a formalized language. This allows to intuitively understand the functionality of a protein. Modules of interacting proteins communicate through matching subnets, which renders the automatic generation of molecular networks possible. Here, we demonstrate the applicability and advantages of our concept on pain signaling. The molecular mechanisms involved in pain signaling are complex and poorly understood. To enhance our understanding of the mechanisms and to get an impression of the functional interactions among the involved pathways, we systematically build a model from modules of pain-relevant proteins. We also offer a prospect of a platform to organize approved curated modules in order to generate molecular networks. Hopefully, our concept helps bridging the gap between experimental bioscientists and theoretically oriented systems biologists.

Key words: Petri net, modular approach, pain signaling, molecular networks

1 Introduction

The modeling of large molecular networks in systems biology is a challenging process, as well as their steady curation and improvement. Our modular Petri net modeling concept described here, offers a way to handle these challenges by combing Petri net modeling with a modular approach. Modular approaches have already conquered the field of systems biology [1]. In the biological context, just monolithic pathways have been regarded as single entities up to now. In our concept, proteins are represented as functional units by a Petri net with a defined

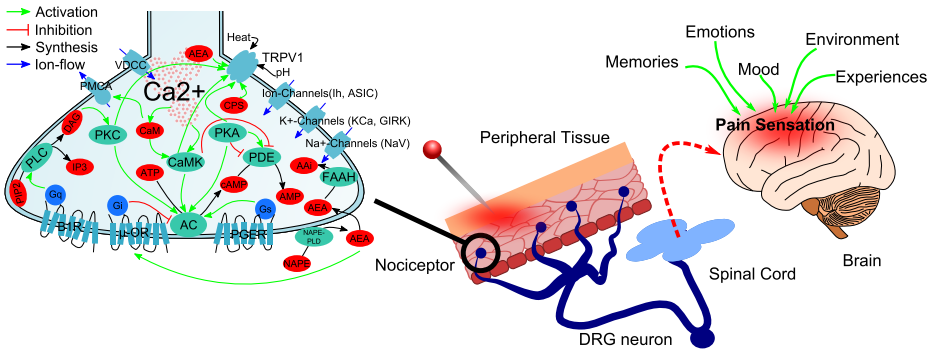


Figure 1: Nociceptor: Detector of noxious stimuli. The peripheral terminals of dorsal root ganglion (DRG) neurons, called nociceptors, detect noxious stimuli. Inside the nociceptor (left side) a plethora of signal cascades is responsible for the processing and the encoding of the noxious stimuli operating on membrane components. The action potential induced by sensitization runs along the DRG neuron to the spinal cord, where other nociceptive neurons receive the signal. The brain integrates the transmitted signal of the noxious stimuli and behavioral information (emotions, mood, memories etc.) and causes the painful sensation.

structure and connection interface, called module. Each module comprises and integrates scattered information about individual proteins, its intramolecular changes and interactions with other molecules. Therefore, a module is equivalent to an interactive review article written in a formalized language with the help of Petri nets. The graphical notation of the underlying mathematical model allows to intuitively understand the modeled protein. Modules of interacting proteins communicate through identical matching subnets, the connection interface. The coupling of monolithic pathway models is far from trivial in contrast to the combination of protein modules described here. The defined connection interface of each module allows to easily generate a comprehensive model of a molecular network from a set of modules. However, it has been proven that Petri nets are ideally suited to describe biological processes by their very nature. The Petri net formalism provides a mathematical language to describe parallel and concurrent processes of bipartite systems [2]. Therefore, we choose Petri nets to describe the molecular network of pain signaling (case study). Pain signaling comprises complex and diverse molecular mechanisms of parallel, convergent and concurrent processes. Up to now, a large variety of molecular pain mediators is known **Figure 1**. Nevertheless, especially the intracellular plethora of pain signaling cascades triggered by membrane components is underinvestigated and therefore partly unknown [3]. The challenge to represent pain signaling in terms of a comprehensive Petri net model has led to the development of our modular Petri net modeling concept and a modular network describing pain signaling in the peripheral terminals of dorsal root ganglion (DRG) neurons **Figure 1**. Our concept is not at all limited to pain signaling, the application to other molecular net-

works is straightforward. However, by studying pain signaling it turned out that our concept is well suited to handle large molecular networks. Since last year, we improved and extended our modular Petri net modeling concept presented here (compare [4]). In addition, we developed first ideas to manage the modules after curation by bioscientists in a database and thus, provide a platform to the scientific community. The platform also facilitates the automatic generation of a model of a molecular network from a collection of approved curated modules.

2 Petri Net Formalism

Petri nets offer a mathematical modeling language to describe concurrent and parallel processes, as well as communication and synchronization in bipartite systems. The graphical notation and construction of Petri nets allows to intuitively model such processes while being formally and mathematically consistent. Therefore, Petri nets are ideally suited to describe biological processes by their very nature [2]. The standard Petri net [2] consists of four elements: places, transitions, arcs and tokens. In biological systems places correspond to species (chemical compounds) and transitions describe the action occurring among the species ((bio-)chemical reactions). Arcs specify the relations between places and transitions. Tokens refer to the amount (discrete number, concentration) of a species. Further, transitions are allowed to fire (enabled) if all pre-places are sufficiently marked. By firing of transition it deletes tokens from its pre-places and produces tokens on its post-places.

Definition 1 (Petri net). [1] A Petri net *is a quadruple* $N = (P, T, f, m_0)$, *where:*

- P, T *are finite, non empty, disjoint sets.* P *is the set of* places. T *is the set of* transitions.
- f: $((P \times T) \cup (T \times P) \rightarrow \mathbb{N}_0$ *defines the set of directed* arcs, *weighted by non-negative integer values*
- m_0: $P \rightarrow \mathbb{N}_0$ *gives the* initial marking.

One benefit of Petri nets is the formal analysis of the network structure. The topological properties of a Petri net are also meaningful in a biological context and give valuable hints to validate the network structure [2]. Important criteria to validate a biological Petri net are liveness, boundedness, reversibility, as well as T- and P-Invariants [2].

Definition 2 (Boundedness). [1]

- *A place* p *is* k-bounded *if there exists a positive integer number* k, *which represents an upper bound for the number of tokens on this place in all reachable markings of the Petri net:*
  $\exists k \in \mathbb{N}_0 : \forall m \in [m_0\rangle : m(p) \leq k.$

- *A Petri net is* k-bounded *if all its places are* k-bounded.
- *A Petri net is* structurally bounded *if it is bounded in any initial marking.*

Definition 3 (Liveness). [1]

- *A transition* t *is* dead in the marking m *if it is not enabled in any marking* m' *reachable from:* $\nexists m' \in [m\rangle : m'(t)$.
- *A transition* t *is* live *if it is not dead in any marking reachable from* m_0.
- *A marking* m *is* dead *if there is no transitions which is enabled in* m.
- *A Petri net is* deadlock-free *if there are no reachable dead markings.*
- *A Petri net is* live *if each transitions is live.*

Definition 4 (Reversibility). [1] *A Petri net is* reversible *if the initial marking can be reached again from each reachable marking:* $\forall m \in [m_0\rangle : m_0 \in [m\rangle$.

Definition 5 (P-invariants, T-invariants). [1]

- *The* incidence matrix *of* N *is a matrix* $\mathbb{C} : P \times T \to \mathbb{Z}$*, indexed by* P *and* T*, such that* $\mathbb{C}(p,t) = f(t,p) - f(p-t)$.
- *A* place vector *(transition vector) is a vector* $x : P \to \mathbb{Z}$*, indexed by* P *(*$y : T \to \mathbb{Z}$*, indexed by* T*)*
- *A place vector (transition vector) is called* P-invariant (T-invariant) *if it is a nontrivial nonnegative integer solution of the linear equation system* $x \cdot \mathbb{C} = 0$ *(*$\mathbb{C} \cdot y = 0$*).*
- *The set of nodes corresponding to an invariant's nonzero entries are called the* support *of this invariant* x*, written as* $supp(x)$.
- *An invariant* x *is called* minimal *if* $\nexists$ *invariant* z*:* $supp(z) \subset supp(x)$*, i.e. its support does not contain the support of any other invariant* z*, and the greatest common divisor of all nonzero entries of* x *is 1.*
- *A net is* covered by P-Invariants (T-invariants) *if every place (transition) belongs to a P-invariant (T-invariant).*

Thereby, we can determine if the model of the molecular network contains dead-states, is live or if it can reset its initial state (reversible). To ensure the mass conversation the coverage by P-invariants and the boundedness of the Petri net must be considered. P-invariants describe sets of related species or states of a species. Boundedness ascertains that no species infinitely accumulates in the network. T-invariants contain a set of actions/reactions to reset its initial state [2].

Several specialized Petri net classes like qualitative, stochastic, continuous, hybrid Petri nets and their colored counterparts are available to describe different scenarios and to consider different simulative approaches. All network classes are convertible into each other without changing the network structure. This allows the application of the same powerful analysis techniques to the underlying qualitative structure for all Petri net network classes [2].

In particular, we use stochastic Petri nets to describe the inherently stochastic nature of biological processes. In addition to the standard Petri net, firing rates are assigned to each transition, which are determined by random variables depending on the probability distribution. Therefore, we use stochastic simulation to investigate the dynamic behavior by the time-dependent token flow [2].

Definition 6 (Stochastic Petri Net). [1] *A biochemically interpreted stochastic Petri net is a quintuple SPN_{Bio} = (P, T, f, v, m_0), where:*

- *P, T are finite, non empty, disjoint sets. P is the set of* places. *T is the set of* transitions.
- *f: $((P \times T) \cup (T \times P) \rightarrow \mathbb{N}_0$ defines the set of directed* arcs, *weighted by non-negative integer values*
- *v: $T \rightarrow H$ is a function, which assigns a stochastic hazard function h_t to each transition t, whereby* $H := \bigcup_{t \in T} \left\{ h_t | \, h_t : \mathbb{N}_0^{|^\bullet t|} \rightarrow \mathbb{N}^+ \right\}$ *is the set of all stochastic hazard functions, and v(t) = h_t for all transitions $t \in T$.*
- *m_0: $P \rightarrow \mathbb{N}_0$ gives the* initial marking.

3 Modular Modeling Concept

The enormous amount of regulative events in pain signaling **Figure 1** results into the development of a modular modeling concept, which considers every protein as functional independent unit. The basic concept presented last year (see reference [4]) has now been improved and extended to a defined modeling concept. The suggested modular modeling concept uses Petri nets to allow the assembling of molecular networks from functional Petri net submodels of the involved proteins with a defined structure and connection interface, called modules. A module reflects all the intramolecular changes of a protein and its interactions with other molecules as reported in the literature. Therefore, a module comprises wide-spread information about each protein. **Figure 3 and 4** show exemplary the modules of two proteins and their regulation. Non-proteins (ions, second messenger, energy equivalents etc.) are contained in the modules as interactants and indirectly connect the proteins. The structure and the dynamic behavior of each module has to fulfill certain criteria to be valid to meet the requirements of our modular Petri net modeling concept **Table 1**. After positive validation, the modules can be easily linked to a modular network by the defined connection interface of each module. Additionally, we are now able to predict the properties of the complex modular network from the topological properties of the combined modules **Table 1**. In this section, we explain all steps needed for the construction of a single module from literature and the assembling of the modular network from the constructed modules.

3.1 Network Structure and Properties of a Module

We construct modules based on the information about the structure of a protein, interactions with other components and intramolecular changes during its regulation given in the literature. Each place of a module corresponds to a specific state of a functional protein domain (phosphorylation site, catalytic and

[1] Mathematical definitions are taken from [2].

inhibitory domain etc.) or a specific state of a non-protein (free or bound, substrate or product etc.). In this context, a transition describes a shift between two different states of a protein domain or non-protein by a molecular action (binding/dissociation, (de-)phosphorylation, conformational changes, substrate processing etc.). Each module is constructed in such a way, that it obeys criteria important for biological networks, which are given in detail in [2] and summarized in **Table 1**. All states of a protein domain and all states of a non-protein constitute a P-invariant (see also **Section 2**). Therefore, the whole module must be covered by P-invariants. In this context, P-invariants ensure mass conservation. Sequential state shifts that restore an initial state of a protein domain or a non-protein form T-invariants (see also **Section 2**). Consequently, all T-invariants are covered by P-invariants. Places connected with a transition by a double arc (see **Figure 3 and 4**) indicate molecular states responsible for other state shifts without changing itself. To prohibit external sinks and sources of protein domains, the modules are not bounded by transitions. However, a module might well be bounded by places. Boundary places have their origin in modules of other proteins or represent non-proteins, which are consumed or produced. The principle of double entry-bookkeeping is a part of the modular modeling concept, since every module has to contain all interactions with other molecules. Thus, modules of two interacting proteins share identical matching subnets describing the interaction mechanism.

Table 1: Topological properties of the modules and their biological interpretation linked with their transferability to the modular network.

Properties	Module	Modular network
A Properties that must be fulfilled for each module		
Ordinary	Just natural elementary regulation steps are considered. Therefore, the arc weights are uniformly set to "1".	All properties are direct transferable to the modular network, because they are fulfilled by all modules.
Homogeneous	Due to ordinary: state-shifts produce (consume) the same amount of tokens on each place.	
Connected	Among all different states of protein domains and non-proteins exist at least one indirect path to represent the interrelated structure of a protein.	
Covered with P-invariants	A set of related states of a protein domain or of a non-protein must form a P-invariant. In consequence, the module must be covered with P-invariants.	
Boundedness	The coverage with P-invariants causes boundedness of each module and avoids the infinite accumulation of tokens in a module.	
B Properties that must not be fulfilled for each module		
Pure	Every module contains states of a protein domain responsible for other state shifts without changing itself. Therefore, each module contains double arcs.	

Boundary Transitions	The modules are not bounded by transitions to avoid external sinks and sources of protein domains and non-proteins.	All properties are direct transferable to the modular network, because they are fulfilled by all modules.
Conservative	The formation of protein complexes results in a non-token-preservingly firing of transitions.	
Static conflict free	A module contains at least one state of a protein domain or a non-protein attending on multiple state shifts.	
Strongly covered with T-Invariants	A module contains two sequential actions reproducing the initial state of the involved protein domain or non-protein.	
C Properties that are variable among all modules		
Dead Transition	Depending on the initial marking.	Depending on the initial marking.
Dead states	Depending on the specific regulation of a protein, the respective module contains at least one set of sequential state shifts acting independent of all other actions. In this case, the module has no dead state.	The modular network has no dead state if a least one module has no dead state.
Dynamic conflict free	Depending on the specific regulation of a protein, certain state shifts in the respective module do not inhibit other actions in the same module. Consequently, the module has no dynamic conflicts.	The modular network is not dynamic conflict free, if one module contains a dynamic conflict.
Boundary places	Depending on the specific regulation of a protein, the respective module contains places corresponding to protein domains of other interacting proteins or non-proteins that are irreversibly changed in the respective module. In this case, the module has boundary places. The following properties cannot be fulfilled if a module has at least one boundary place: – Strongly connected – Non-blocking multiplicity – Covered with T-Invariants – Siphon-Trap Property – Liveness	All of these properties can be transfered to the modular network, if at least one module in the modular network has at least one boundary place that does not gain a pretransition after module coupling.

3.2 Validation of a Module

The topological properties can be used for the validation of each module and locating inconsistencies in the module structure [2]. Each topological property has a significant biological interpretation. A set of those properties must be fulfilled, another set must not be fulfilled and a third set is variable depending on the unique function and structure of each module **Table 1** (see [2] for further explanations). After the construction of a module, its structure is checked whether it obeys the given criteria given in **Table 1**. Each module is also subjected to stochastic simulation studies. The kinetic function of each transition

can be defined by known kinetic parameters (binding, dissociations, and affinity constants) or more complex kinetic equations (michaelis menten, hill kinetic). If kinetic information are not available, the kinetic parameters can be determined by trial and error or by more sophisticated parameter estimation methods. The observed dynamic behavior, i.e. the time-dependent token-flow (see also references [4,5,6]), must in principle reflect the modeled effector function. A module is valid if its structure confirms the given topological properties and the dynamic behavior reflects the experimental obtained time curves.

3.3 Generation of a Modular Network

Next, the modules are connected by identical places of non-proteins and identical matching subnets among the modules. All shared elements have to be indicated as such in each module by declaring the included transitions and places as logical nodes (connection interface). Afterwards, the modules can be combined to one comprehensive simulative model. No further interventions are required. The coupling procedure does not affect the structure and properties of each module. Even the kinetic of the modules are kept and inherited to the resulting modular network. Hence, simulation with the modular network can be performed right after its generation.

3.4 Properties of the Modular Network

A new important achievement of the modular modeling concept is the determination of properties of the complex network from submodels, which might also be interesting for other Petri net applications. Due to the defined structure, the resulting uniform topological properties and defined connection interface of the modules, we are able to predict the properties of the modular network. Obviously, the respective non-variable properties among the modules can be transferred one-on-one to the modular network **Table 1**. From the comparison of the fulfillment of each variable property among the modules it can be deduced, whether the respective property holds for the modular network (see also **Table 1** for more details). Therefore, all properties of the modular network are derivable from the respective set of modules that assemble the modular network. Computational analyses with the place/transition analyzer Charlie [7] confirm the predicted properties (not shown here, see [6] for more details).

4 A Model Relevant for Pain Signaling

Pain signaling comprises complex and diverse processes. Therefore, a plethora of proteins and other components participate in the molecular regulation of painful sensations (see also **Figure 1**). Several members of the G-protein-coupled receptor family (GPCRs) are involved in pain signaling like opioid, cannabinoid, muscarinic, prostaglandine and β-2-adrenergic receptors. The GPCRs act through their G-proteins on adenylyl cyclases (Type VIII, V, I), phospholipase Cβ and

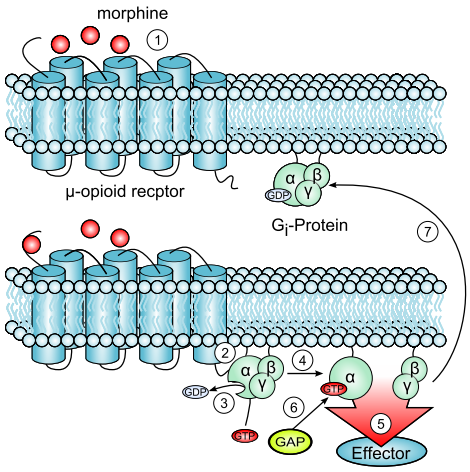


Figure 2: Activation of the Gi-protein by μ-opioid receptor. Morphine binds to the extracellular site of the μ-opioid receptor (muOR) **1**. Therefore, muOR changes its conformation and binds the Gi-protein **2**. GDP is exchanged by GTP at the Giα-subunit which in turn activates the Gi-protein **3**. The active Gi-protein dissociates into the Giα and Giβ/γ-subunit **4**. The Gi-protein subunits can now interact with their downstream targets **5**. GTP is hydrolyzed by the intrinsic GTPase of the Giα-subunit due to stimulation by a GTPase activating protein (GAP) **6**. The G-protein subunits reassociate if the Giα-subunit is inactive **7**. **Figure 3 and 4** show these mechanisms translated into two respective modules of muOR and the Gi-Protein.

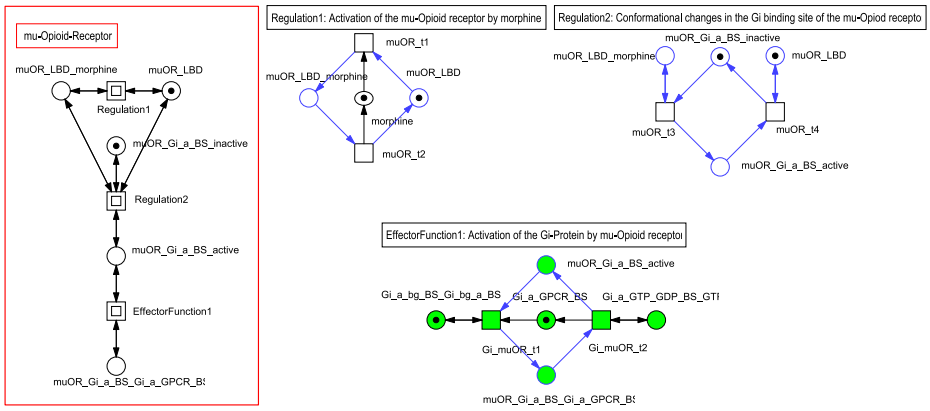


Figure 3: Module of the μ-opioid receptor. The module represents the regulation of the μ-opioid receptor as shown in **Figure 2**. The top-level of the μ-opioid receptor module is shown in the red rectangle on the left site. The macro transitions (black boxed squares) contain subnets on a deeper level. The corresponding subnets are shown on the right site and below. The blue arcs and blue framed places mark inputs from places shown on the top level. Green filled nodes indicate the connections interface that is used to connect the module of the μ-opioid receptor with the module of the Gi-protein **Figure 4**. Actions of the respective transitions are given in **Table 2**.

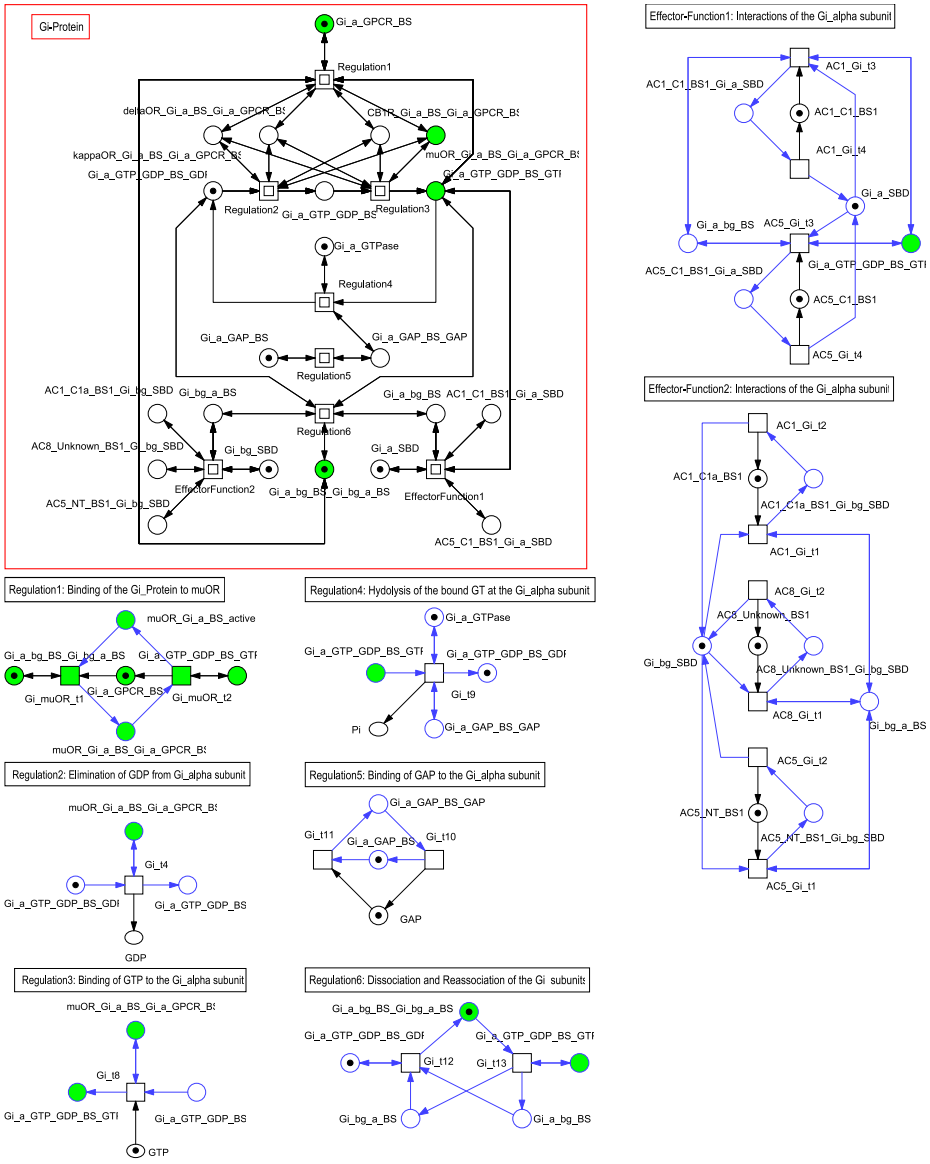


Figure 4: Module of the Gi-protein. The module represents the regulation of the Gi-protein as shown in **Figure 2**. The top-level of the Gi-protein is shown in the red rectangle on the left site. The macro transitions (black boxed squares) contain subnets on a deeper level. The corresponding subnets are shown on the right site and below. The blue arcs and blue framed places mark inputs from places shown on the top level. Green filled nodes indicate the connections interface that is used to connect the module of Gi-protein with the module of the μ-opioid receptor **Figure 3**. Actions of the respective transitions are given in **Table 2**.

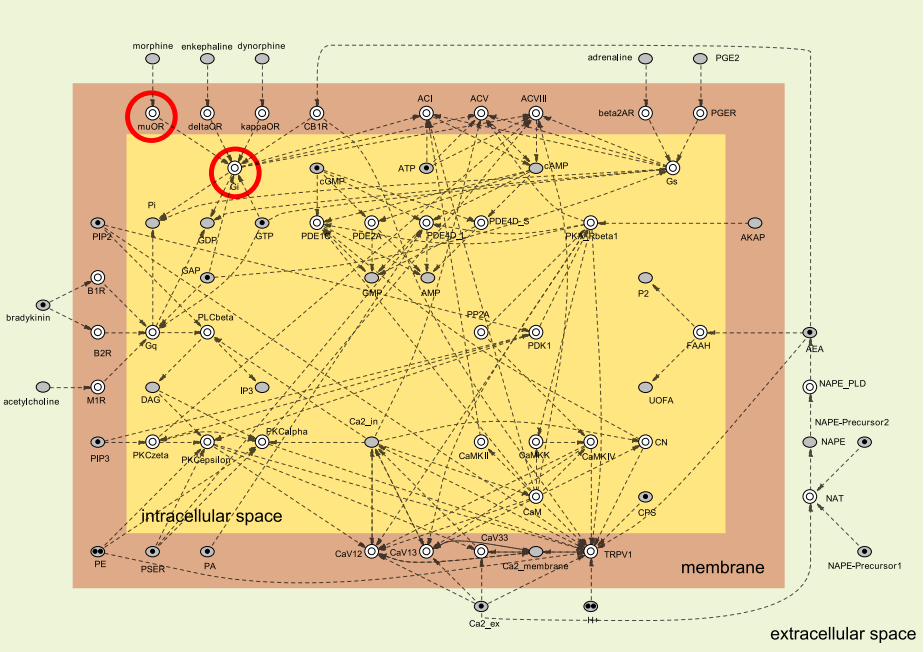


Figure 5: Modular Network Explaining Molecular Pain Mechanisms. Each macro place (boxed circles) contains a module of a pain-related protein. **Figure 3 and 4** (red circles) show two examples in detail. The grey shaded (logical) places represent the involved non-proteins (exceptions AKAP and GAP). The modules are arranged according to their localization (intracellular, membrane components, extracellular). The dashed arcs (no Petri net elements) indicate the interactions among all pain-related molecules.

ion-channels. Numerous protein kinases regulate pain signaling among them are different isoforms of PKA (RIIβ), PKC (α, ϵ, ζ), CaMK (II, IV). Opponents of the protein kinases are protein phosphatase 2A and calcineurin. Calmodulin is an important calcium-binding protein interacting with other pain-related protein like the voltage dependent calcium channels (CaV1.2, CaV1.3, CaV3.3) and the capsaicin receptor (TRPV1). Second messenger like DAG, Ca^{2+} and cAMP are also important components in the context of pain signaling and indirectly link proteins [3].

Each of those pain-relevant proteins is represented by its respective module. In total, 38 modules of pain-relevant proteins have been derived from clinical pain literature (all references can be found in [6]). New modules have been added and old modules have been updated by formulating the mechanisms in more detail since last year (e.g. compare the modules in **Figure 3 and 4** with the respective module shown in [4]). Exemplary, we show the mechanisms of the Gi-protein activation by the μ-opioid receptor **Figure 2**. Both, the μ-opioid receptor and the Gi-protein are represented by functional connectable modules (see **Figure**

3 and 4). The biological meaning of each transition refering to the steps shown in **Figure 2** are given in **Table 2**.

Table 2: Biological interpretation of transitions contained in the modules of the μ-opioid receptor and Gi-Protein (compare **Figure 3 and 4**)

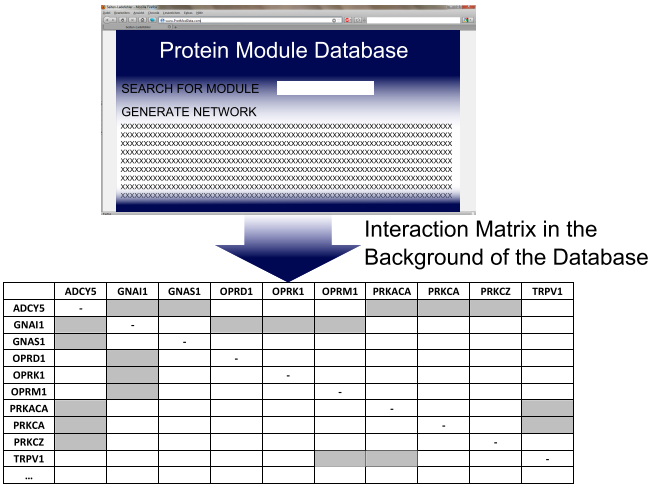
muOR_t1	- Binding of morphine to the ligand binding site of muOR
muOR_t2	- Dissociation of morphine from the ligand binding site of muOR
muOR_t3	- Conformational changes in the $G_i\alpha$ binding site of muOR due to the bound morphine (= activation)
muOR_t4	- Reverse conformational changes of the $G_i\alpha$ binding site of muOR if morphine is not bound to muOR (= inactivation)
G_i_muOR_t1	- Binding of the $G_i\alpha$ subunit (in complex with $G_i\beta/\gamma$) to the active $G_i\alpha$ binding site of muOR
G_i_muOR_t2	- Dissociation of the $G_i\alpha$ subunit from the active $G_i\alpha$ binding site of muOR if GDP is exchanged by GTP at the $G_i\alpha$ subunit
G_i_t1	- Dissociation of GDP from the GTP/GDP binding site of $G_i\alpha$ if $G_i\alpha$ is bound to muOR
G_i_t2	- Binding of GTP to the GTP/GDP binding domain of $G_i\alpha$ if $G_i\alpha$ is still bound to muOR
G_i_t3	- Hydrolysis of the bound GTP to GDP by the GPTase domain of $G_i\alpha$ if GAP is bound to $G_i\alpha$
G_i_t4	- Dissociation of the $G_i\alpha$-$G_i\beta/\gamma$ complex if GTP if bound to $G_i\alpha$
G_i_t5	- Reassociation of the $G_i\alpha$ and $G_i\beta/\gamma$ subunits if the $G_i\alpha$ subunit is loaded with GDP
G_i_t6	- Binding of GAP to the GAP binding site of $G_i\alpha$
G_i_t7	- Dissociation of GAP from the GAP binding site of $G_i\alpha$
AC8_G_i_t1	- Binding of $G_i\beta/\gamma$ subunit to AC8 at an unknown binding domian
AC8_G_i_t2	- Dissociation of $G_i\beta/\gamma$ subunit from AC8
AC8_G_i_t3	- Binding of $G_i\beta/\gamma$ subunit to AC1 at the C1a domain
AC8_G_i_t4	- Dissociation of $G_i\beta/\gamma$ subunit from AC1
AC8_G_i_t5	- Binding of $G_i\alpha$ subunit to AC1 at the C1 domain
AC8_G_i_t6	- Dissociation of $G_i\alpha$ subunit from AC1
AC8_G_i_t7	- Binding of $G_i\alpha$ subunit to AC5 at the C1 domain
AC8_G_i_t8	- Dissociation of $G_i\alpha$ subunit from AC5

The resulting modular network **Figure 5** generated from the set of modules of pain-relevant proteins consists of 713 places and 775 transitions spread over 325 pages with a nesting depth of 4. The top level of the modular network shown in **Figure 5** contains all non-proteins (logic places in grey) and modules of pain-relevant proteins represented by single macro places (boxed circles). **Figure 3 and 4** depict two of these modules exemplarily. All components are arranged by their localization in the nociceptor. Due to the complexity of the regulation events, the modules are hierarchically designed to conserve the neat-arrangement offered by Petri nets. The modules communicate through identical matching subnets among them on lower levels and non-proteins. Therefore, the interaction among the displayed components are not visible on the top level of the modular network in **Figure 5**.

Here, we added arcs (no Petri net element) to the top-level shown in **Figure 5** to indicate the interactions among the components involved in pain signaling. **Figure 5** illustrates the high degree of interactivity among the components which was not obvious from the literature. The authors of reference [3] discuss whether the pathways involved in pain signaling are parallel or convergent. The interaction scheme in **Figure 5** clearly indicates that the involved pathways are highly convergent and influence each other. Several feedback loops are contained (not shown here) to regulate the cAMP- and Ca^{2+}- level and the membrane voltage, which are important for the sensitization of the nociceptor and the initiation of action potentials resulting into painful sensations. Therefore, the regulation of pain signaling is very complex and the resulting dynamic behavior is not trivial at all. The modules of the pain-relevant proteins are still in the curation process by the pain community. Since kinetic data is still rare in the pain signaling context, we have not been yet able to parameterize the modules and therefore to perform reliable simulation studies. The topological properties of the achieved modular network confirm the predicted properties of a common modular network given in **Table 2**.

5 Work in Progress: Establishing a Protein-Module Platform

We developed first basic ideas of a new protein-orientated modeling platform to open our modular Petri net modeling concept and the modules to the scientific community. This platform and the modular Petri net modeling concept provide the framework to organize the connectable modules in a database and to generate computational models of molecular networks from a central collection of approved curated modules.
Additionally to the Petri net of each module, a dataset will be provided in our database to characterize each module and the represented protein. The dataset contains information about the author and curator, the names of all places and transitions and their meaning, references to relevant literature used for the construction of the module, a list of open issues and information about the protein (accession number, gene symbol, synonyms, taxonomic classification, involved pathways etc.) extracted from the UniProt database [8].
The strict obedience of a naming convention for each node is the most important prerequisite to correctly link the modules by identical matching subnets and logical places of non-proteins. The identity of each module is determined by the unique accession number for proteins provided by UniProt [8]. The accession number will be used as prefix for all nodes but is not shown in the graph. More readable gene symbols for each protein, which are also provided from UniProt [8], are used as nicknames combined with common abbreviations of domains and their states to define a unique name for each place. The unique name of a transition is created from the gene symbols of the involved proteins and a counter. As a consequence, the module coupling is insensitive against author and version of a module, but sensitive against the organism. Also, the name of each node can



	ADCY5	GNAI1	GNAS1	OPRD1	OPRK1	OPRM1	PRKACA	PRKCA	PRKCZ	TRPV1
ADCY5	-									
GNAI1		-								
GNAS1			-							
OPRD1				-						
OPRK1					-					
OPRM1						-				
PRKACA							-			
PRKCA								-		
PRKCZ									-	
TRPV1										-
...										

Figure 6: Public Protein Module Platform. The protein-module platform allows to organize the modules and opens our modular Petri net modeling concept as well as the constructed modules to the scientific community. Based on shared nodes among the modules, an interaction matrix is created to control the module coupling (shown here on pain signaling).

be automatically generated. Checks will be integrated to prove the correctness of the names and thereby the connectivity of the module to its interactants.
To control the generation of a network from modules an interaction matrix is provided **Figure 6**. An interaction matrix, derived from the places of all modules, indicates which modules can be coupled by a common set of nodes. Thus, it controls the module coupling. First of all, the user adjusts the stringency at the organism level. Based on the interaction matrix, the generation of a modular network from modules might now occur in two ways: (a) pathway-oriented suggestion of a set interacting proteins, (b) iterative search of interactants from a chosen start protein. The pathway-oriented generation of modular networks will be achieved by tags that are added to the dataset of each module referring to involved pathways (e.g. pain signaling) and localization (e.g. DRG neuron, nociceptor). In a next step the connectivity of the modules has to be proven by the interaction matrix. **Figure 7** illustrates the application of the iterative search algorithm to generate a submodel of the model shown in **Figure 5**. Both possibilities lead to a list of interacting proteins showing all suitable modules. The user chooses for every protein the preferred module, if different versions of a module for one protein exist. Based on the module selection a comprehensive modular network is generated. The user can now execute simulations with the model and apply advanced structural analysis methods to investigate the model of the molecular network.

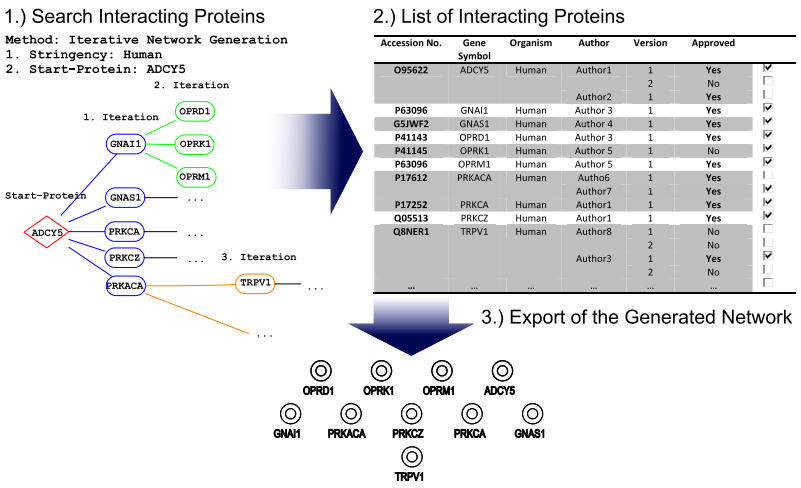


Figure 7: Iterative Generation of a Modular Network Before generating a modular network, the stringency is set by the user. A Start-protein must be chosen if the iterative search is applied to generate a network. Based on the interaction matrix **Figure 6**, the algorithm suggests new interaction partners step by step. After stopping the search, a list of all chosen proteins is created. Due to different opinions, there might exist different modules for one protein. In those cases, the user can choose the preferred module. Next, the modular network is generated from the chosen set of modules and exported.

6 Conclusion

The model relevant for pain signaling integrates the knowledge of approximately 320 scientific articles within 38 valid modules of important molecular pain actors in the nociceptor. The application of the modular modeling concept to the complex network of pain signaling proves its ability to cope with the specific demands of large and complex molecular networks. Our experience with biologists confirm the need of a molecule-oriented modeling concept. So far, the explained modular modeling concept is appreciated by our cooperation partners. It supports their work and improves the modeling of molecular networks. The concept is suited to test different hypothesis by exchanging different versions of a module in the modular network. Thus, the approach shed new light on molecular mechanisms. In a next straightforward step, the modules and therewith the complete modular network can be parameterized by experimental data, which are at the moment unfortunately still rare. After parameterization the model will be investigated by *in silico* experiments. The analysis of effects of systematic perturbation on the dynamic behavior of the model will help to pinpoint promising targets for the pain therapy. The extension of the model to colored Petri nets [9] enables the consideration of multiple copies of the pain-related proteins and of DRG neuron populations. The application of hybrid Petri nets [10] allows the combination of the current model with continuous models describing the generation of action

potentials in neurons. Sophisticated structural analysis methods will be applied to the model to screen for non-obvious properties that are defined by the Petri net structure. By this, we expect new information about multiple steady states, bifurcations and feedback loops that mainly determine the dynamic behavior of a network. At least, the validated and parameterized model and the mentioned investigations should contribute to the development of a mechanism-based pain therapy.

The modular modeling concept as such offers a lot of promising advantages and opportunities. All constructed modules can be easily reused in any other biological systems. Hence, the extension of the modular modeling concept to other biological systems is worthwhile. Every module by itself pools the currently spread knowledge about a protein and its interactants. The process of translating information about a protein into a module reveals missing interrelations. The modular modeling concept avoids inconsistencies in the entire complex modular network by constructing and validating first small independent submodels. The coupling procedure of the modules to an entire modular network by natural matching nodes is effortless. For different reasons, monolithic pathway models organized for example in the BioModels database [11] cannot be easily updated and combined with each other to give a more comprehensive model. Advantageously, the properties of the modular network can be deduced from the defined properties of the modules. The modeler and the curator just need to concentrate on one protein and its interactants. Also, the user has not inevitably to deal with the whole pathway and the theoretical concept itself.

The Petri net formalism itself offers quite few advantages against ODE models. As mentioned before, qualitative, continuous, stochastic and hybrid Petri nets as well as their colored counterparts are convertible in to each other without changing the qualitative structure. ODE systems do not offer the possibility to consider a model from such a range of corresponding sites without reconstructing the set of equations. Due to the graphical visualization of molecular networks by Petri nets, a bioscientist can intuitively understand the modeled mechanisms. This does not count for the mathematical representation of ODE systems. In case of ODE systems, the user has to deal with three different representations of a molecular network which do not obviously correspond to each other: (a) structure of the biological network, (b) the mathematical equations and (c) the implementation of those. Besides, the transformation of ODE systems into Petri nets is not unique. Various Petri nets can be constructed based on an ODE system [12]. The compact mathematical structure of an ODE might hide important biological information. Structural analysis techniques are sensitive to the respective structure of a Petri net. Therefore, the application of those techniques to Petri nets obtained from the variable transformation of ODEs leads also to variable results, which have to be treated with care [12].

The modular principle of the modeling concept offers some more possibilities to apply and extend the concept. The generated modular core network can be extended by gene expression, degradation and translocation modules. Even homo- and hetero-multimeric protein complexes can be modeled in detail with an ex-

tension modular modeling concept. Further, we plan to couple the network reconstruction for Petri nets [13] with the modular modeling concept. Here, nodes of the reconstructed network can be matched with corresponding modules. In addition, the minimal causal Petri nets reconstructed from experimental time series are extended with modules of the involved proteins.
The establishment of a platform for protein-modules and the opportunity to generate models of molecular networks form approved curated modules supports the switch from monolithic modeling to modular modeling of biological systems. Such a platform simplifies the exchange of data and knowledge among bioscientists by concentrating biological information about proteins and their interactants in the structure of the modules. Thereby, easing the access to systems biology for wetlab bioscientist.

7 Acknowledgement

This work is supported by the "Modeling Pain Switches" (MOPS) program of Federal Ministry of Education and Research (Funding Number: 0315449F). We thank Monika Heiner and co-workers for the outstanding cooperation on Petri nets and the software supply of Snoopy [14] and Charlie [7]. Further we thank the members of the MOPS consortia for supporting collaborations.

8 References

1. Alberghina, L, et al. A Modular Systems Biology Analysis of Cell Cycle Entrance into S-Phase. Systems Biology - Topic in Current Genetics. 2005, Vol. 13.
2. Heiner, M, Gilbert, D and Donaldson, R. Petri Nets for Systems and Synthetic Biology. [book auth.] M Bernardo, P Degano and G Zavattaro. SFM 2008. s.l. : Springer LNCS 5016. 2008.
3. Hucho, T and Levine, J. Signaling Pathway in Sensitization: Toward a Nociceptor Cell Biology. Neuron. 2007, Vol. 55.
4. Blätke, M.-A. et al. Petri Net Modeling via a Modular and Hierarchical Approach Applied to Nociception. Int.Workshop on Biological Processes & Petri Nets (BioPPN), satellite event of Petri Nets 2010. Braga, Portugal, 2010.
5. Blätke, M.-A., Marwan W. Modular and Hierarchical Modeling Concept for Large Biological Petri Nets Applied to Nociception.German Workshop on Algorithms and Tools for Petri Nets. Cottbus, Germany, 2010.
6. Blätke, M.-A. Petri-Netz Modellierung mittels eines modularen and hierarchischen Ansatzes mit Anwendung auf nozizeptive Signalkomponenten. Otto von Guericke University Magdeburg. 2010 (Diploma thesis).
7. Franzke, A. Charlie 2.0 - A Multi-Threaded Petri Net Analyzer. Brandenburg University of Technology Cottbus. 2009 (Diploma thesis).
8. Jain, E et al. Infrastructure for the Life Sciences: Design and Implementation of the UniProt Website. BMC Bioinformatics. 2009, Vol. 10.

9. Liu, F., Heiner, M. Colored Petri Nets to Model and Simulate Biological Systems. Int.Workshop on Biological Processes & Petri Nets (BioPPN), satellite event of Petri Nets 2010. Braga, Portugal, 2010.
10. Herajy, M., Heiner M. Hybrid Peri Nets for Modeling Hybrid Biochemical Interactions. German Workshop on Algorithms and Tools for Petri Nets. Cottbus, Germany, 2010.
11. Li, C. et al. BioModels Database: An Enhanced, Curated and Annotated Resource for Published Quantitative Kinetic Models. BMC Systems Biology. 2010, Vol. 4.
12. Soliman, S and Heiner, M. A Unique Transformation from Ordinary Differential Equations to Reaction Networks. PLoS ONE. 5, 2010.
13. Marwan, W., Wagler, A., Weismantel, R. Petri Nets as a Framework for the Reconstruction and Analysis of Signal Transduction Pathways and Regulatory Networks. Natural Computing. 2009.
14. Rohr, C, Marwan, W and Heiner, M. Snoopy - A Unifying Petri Net Framework to Investigate Biomolecular Networks. Bioinformatics. 2010, Vol. 26.