Petri Net Modeling via a Modular and Hierarchical Approach Applied to Nociception

Mary Ann Blätke[1], Sonja Meyer[1], Christoph Stein[2] and Wolfgang Marwan[1]

[1]Otto-von-Guericke Universität Magdeburg, Universitätsplatz 2, 39106 Magdeburg, Germany
[2]Dep. Anesthesiology, Charité - Freie Universität Berlin, Hindenburgdamm 30, 12200 Berlin, Germany
wolfgang.marwan@ovgu.de

Abstract. We describe signal transduction of nociceptive mechanisms involved in chronic pain by a qualitative Petri net model. More precisely, we investigate signaling in the peripheral terminals of dorsal root ganglion (DRG) neurons. It is a first approach to integrate the current neurobiological and clinical knowledge about nociception on the molecular level from literature in a model describing all the interactions between the involved molecules.

Due to the large expected total size of the model under development, we employed a hierarchical and modular approach. In our entire nociceptive network, each biological entity like a receptor, enzyme, macromolecular complex etc. is represented by a self-contained and functional autonomous Petri net, a module.

Analysis of the Petri net modules and simulation studies ensure the fulfillment of criteria important for biological Petri nets and the ability to represent the modeled biological function.

Key words: Petri net, qualititative approach, module, pain, nociception, G-protein-coupled receptor, large biological systems

1 Introduction

Clinical pain is a very complex phenomenon with behavioural, peripheral and central nervous system components. Often, pain can not be successfully treated due to the lack of knowledge about the molecular basis on which pain killers take effect. A mechanism-based pain therapy is largely missing, rendering undertreated pain a serious public health issue (see [7] and references therein).

At the molecular level, many extracellular stimuli and substances in the peripheral tissue are known that provoke nociceptive signaling in DRG neurons and subsequent pain (a complex sensation resulting from integration of peripheral and central messages). A variety of membrane components and intracellular signaling molecules have been identified that play key roles in pain sensation. Examples are G-protein-coupled receptors (GPCR), ion channels, receptor tyrosine kinases, cytokine and hormone receptors, which in turn activate a plethora
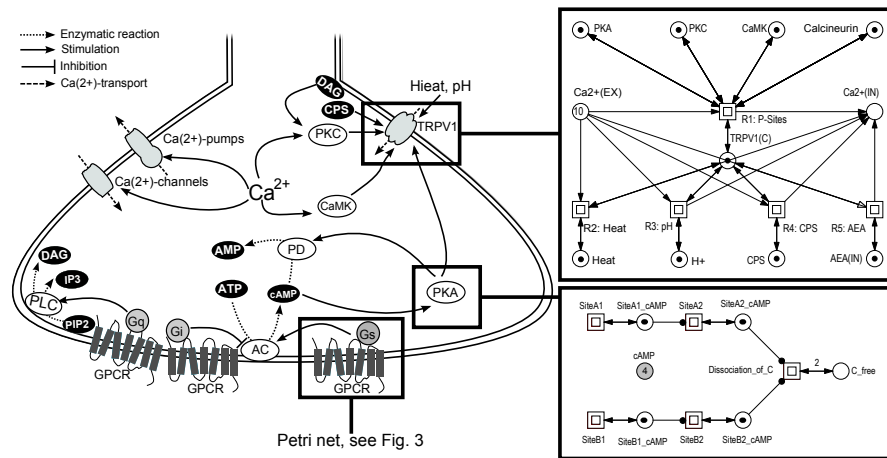
Fig. 1: *Left:* Signaling components in nociceptors. Nociception is triggered by a large number of extracellular signals acting through several receptor classes and initiating a plethora of intracellular signaling cascades. *Right:* Molecular entities like receptors, enzymes (and other biomolecules or macro-molecular complexes) are represented as functional units in the form of self-contained and functionally autonomous Petri nets. The subnets can be coupled by shared places representing identical, common components.

of signaling cascades like the cAMP pathway and calcium signaling [6, 7] (see Fig. 1). However, the quantitative and qualitative relationships between the different intracellular signaling mechanisms acting downstream of the receptor to which those substances bind are still poorly understood [7].

It seems straightforward to apply the Petri net framework to study pain signaling 'in silico', because Petri nets are designed for concurrent systems and also were shown to be ideally suited to model biological systems [9].

For the description of the nociceptive network we choose qualitative modeling as the preceding step for simulation studies which can be performed either stochastically or continuously. It has been shown that a continuous Petri net is equivalent to a structured description of ODEs [9]. However, it is known that many of the involved processes are inherently stochastic. Due to this reason, we prefer stochastic simulations studies to validate our model. The extension of the entire qualitative Petri net to a stochastic one with parameters from experimental data is not possible at the moment because kinetic information of nociceptive mechanisms is hitherto largely missing.

In our modular approach, a module represents a biological functional entity like a receptor, a channel, an enzyme or a macro-molecular complex in form of a self-contained and functional autonomous Petri net graph. The places of a module correspond to functional domains (binding domains, phosphorylation sites, autoinhibitoy domains etc.). These functional domains are regulated by other biological entities and second messengers or are responsible for the effector func-

tion. Thus, transitions stand for actions (dissociation, binding, phosphorylation etc.) occurring within a biological entity. There exist no input or output transitions (sources or sinks of a certain molecule). Due to mass conservation and the fact that a molecular entity is not used up by signaling, the corresponding Petri net graph must be covered with P-invariants [9]. Likewise, the Petri net graph of a module should be bounded to ensure that biological entities, second messengers, precursors, degradation products and energy equivalents do not accumulate. The coverage of T-Invariants of the whole module is not necessary due to the limitation of components which take part in the regulation of the module or which are substrates for the effector function. Therefore, the fulfillment of properties like liveness, reversibility and no dead states it is not mandatory. In contrast, substructures of the modules where reversible changes occur should be covered with T-invariants to assure that the initial state of the involved domains can be restored. Ideally, the computed T-invariants have to be covered by P-invariants [9]. Both, T- and P-invariants, correspond to important biological functions. The up and down regulation of molecular entities by others and second messengers should be reflected in the token flow of the module especially in the increase or decrease of its effector function.

2   Goal

Our goal is to represent nociceptive mechanisms in DRG neurons in a single, coherent Petri net and to establish relationships between signaling components. With the help of simulations we aim at reproducing effects of known nociceptive stimuli correctly and attempt to predict effects of specific perturbations (drugs for therapeutic interventions).
We also aim to establish a module repository. A major advantage is that the modules can be variably combined and reused in other systems according to the requirements of specific 'wet lab' or 'in silico' experiments.

3   Method

We collected literature about nociceptive signaling in DRG neurons, the most investigated cell type in pain-related studies at the molecular level. We extracted those nociceptive signaling components from the literature, whose molecular interaction with other pain-related components is well described and proven by experiments. Further, we searched in detail for the regulatory and effector functions of each of those molecules.
Subsequently, we translated each biological functional unit into a Petri net using the qualitative approach, see e.g. [1, 2, 9, 8]. We used time-free transitions and obtained a time-free Petri net accordingly [9]. Our nets were constructed with Snoopy, a tool to design and animate hierarchical graphs [13].
Each qualitative Petri net is finally subjected to a comprehensive analysis. Here, we apply all validation criteria for biochemical pathway models given in [9]. Therefore, we determine behavioural properties like liveness, reversibility and

boundness, as well as P- and T-invariants. The analyses have been performed using the software Charlie, a software tool to analyse place/transition nets [11]. Having successfully validated the qualitative model, we perform stochastic simulations by assigning stochastic rate functions to all reactions in the network to study the dynamic behaviour of the systems in terms of the flow of token in our model. In particular, we used the stochastic biomass action function, which is available in Snoopy, together with a simple test parameter sets. In these sets, the firing rates of transitions inactivating the effector function of a molecule are assumed to be lower compared to those of transitions activating the effector function (also see section 5).

4   Nociceptive Network

The entire nociceptive network is build by connection of the constructed modules. Here, places sharing the same molecules/molecular complexes (logical places) constitute the natural connections between the modules.

Currently, we have constructed approximately 40 modules on the basis of 251 scientific articles [8]. We expect that at least twice as many modules are required for a comprehensive description of the entire nociceptive network on the basis of the current knowledge.

This expected total size of the model under development precludes a flat representation. Thus, a modeling approach is applied, which yields immediately a hierarchically structured model. So far, the latest version of the entire network consists of 22 connected modules, the representation is distributed over 67 pages with a nesting depth up to 4, compare Fig. A.1 in the appendix. The model consists of about 300 places and 350 transitions.

5   Example for a Module : G-Protein-coupled Receptor

In this section, we representatively describe the construction and structural analysis of one functional unit of our entire net, the G-protein-coupled receptor (GPCR), a typical seven-helix-transmembrane receptor.

GPCRs relay external signals by activating heterotrimeric guanine-nucleotide-binding proteins (G-protein). Seven-helix receptors form the largest family of transmembrane receptors and are therefore crucial components in many signal cascades including nociceptive pathways. There are several GPCRs in nociception interacting specifically with endogenous and exogenous opioids, cannabinoids or substances released as a result of inflammation (e.g. bradykinin), thus having substantial modulating effects on pain sensation. A heterotrimeric G-protein consists of α^1, β and γ subunits (see also [3–5]). Fig. 2 shows the interaction of GPCR with coupled G-protein.

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[1] The $G\alpha$ subunit occurs in three main isoforms with distinct functions: $G\alpha_s$ (stimulation of adenylyl cyclases), $G\alpha_i$ (inhibition of adenylyl cyclases)and $G\alpha_q$ (stimulation
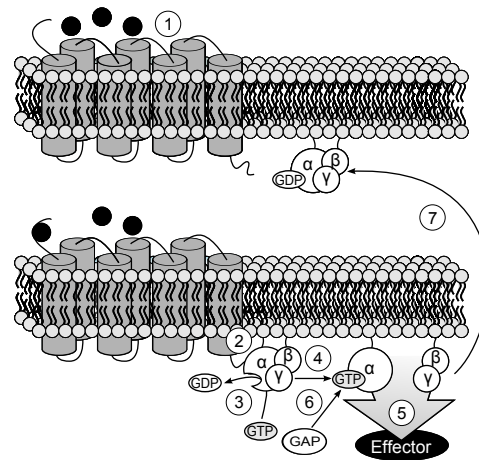
Fig. 2: Regulation of GPCR and its coupled G-protein (see also [3–5]): The activation of a GPCR occurs by binding of a specific ligand at the extracellular side *(step 1)* causing a conformational change *(step 2)*, which activates the recruited resting G-protein in its GDP-bound form. This causes the exchange of GDP by GTP in the specific binding pocket of the Gα subunit. *(step 3)*. Gα subunits with GTP bound dissociate from the G-protein complex *(step 4)* and act on further downstream signal molecules like adenylyl cyclase or phospholipase C β *(step 5)*. The remaining Gβ/γ subunit in addition causes multiple regulatory effects mostly on ion channels and on isoforms of adenylyl cyclases *(also step 5)*. The effector function of the Gα subunit is terminated by the binding of a GTPase activating protein (GAP) stimulating the intrinsic GTPase function of the Gα subunit. GTP is hydrolysed to GDP *(step 6)*. The GDP bound form of the Gα subunit then reassociates with the Gβ/γ subunit to assume its initial pre-stimulus state *(step 7)*.

The regulatory mechanisms and effector functions of GPCRs and the associated G-proteins are translated into a place/transition Petri net (see Fig. 3).

Places may either represent individual molecules or functional states of more complex molecules. Places that are connected by two opposite edges (in this example replaceable by read arcs) with a transition represent molecules or states, which are necessary for a signaling event to occur without being consumed by the reaction. Transitions describe biochemical reactions and molecular interactions.

To provide a neat arrangement of the Petri net, we used coarse transitions (double squares), integrated at the top level. The entire (flattened) place/transition Petri net of this submodel consists of 27 places and 17 transitions connected by 72 edges.

Computation of the invariants shows the coverage of the net by P- and partly by T-invariants (see Fig. 4). Furthermore, there are no invariants without biological

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of phospholipase d β). GPCR are mostly associated with one particular G-protein isoform.
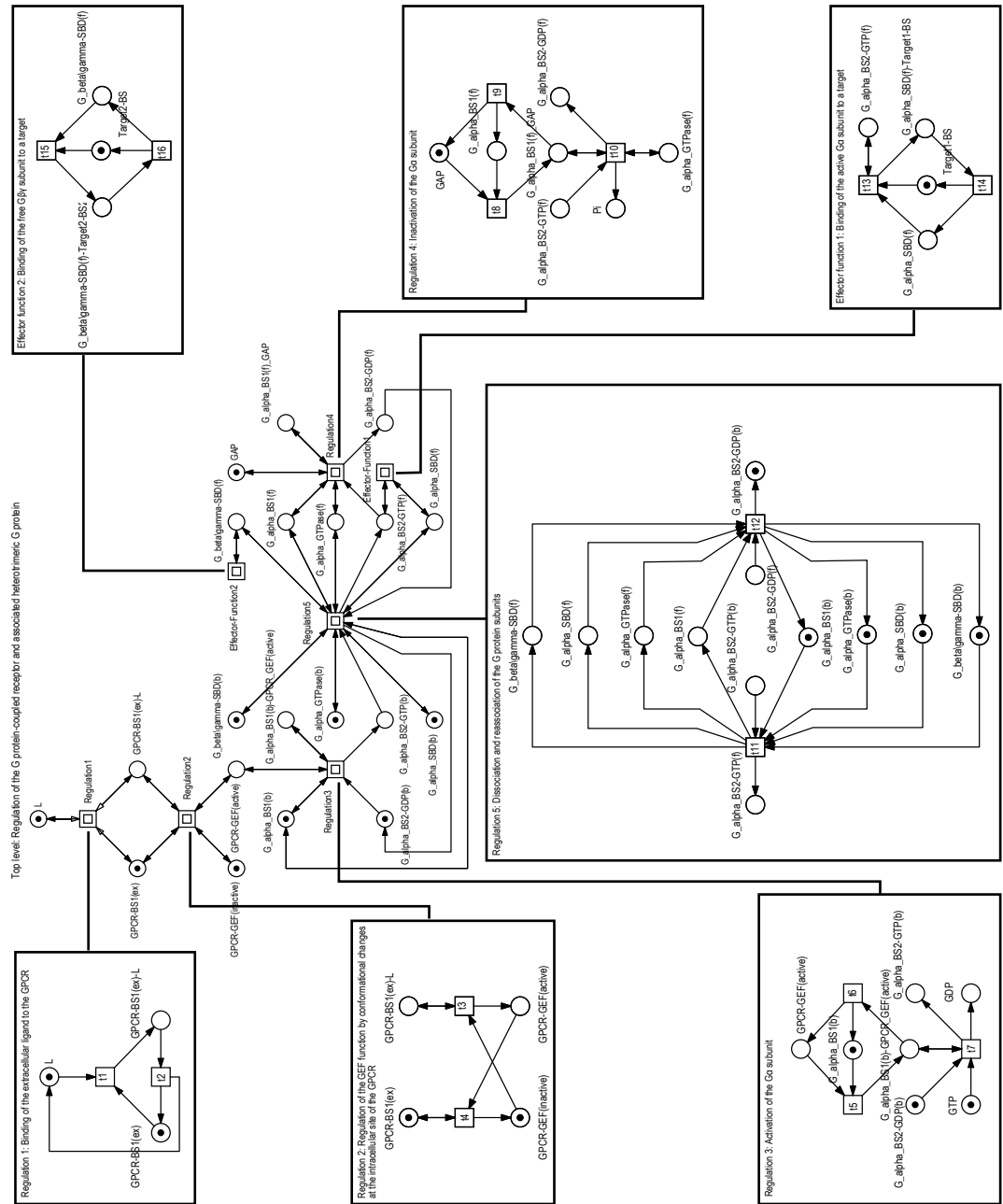
Fig. 3: Petri net module representing GPCR and G-protein regulation: The top level in the center represents all functional sites of GPCR and G-protein which take part in the regulation and effector function. The surroundig Petri nets show the respective coarse transitions in detail.
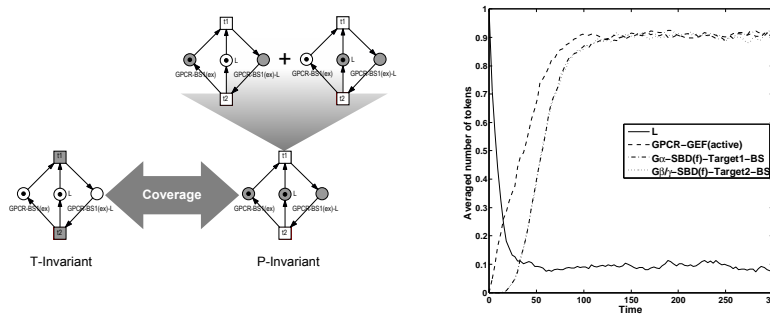
Fig. 4: *Left:* Result of the structural analysis. Shown are T-Invariant 1 (with transitions t1, t2) and P-Invariant 1 (with places GPCR-BS1(ex), GPCR-BS1(ex)-L), 2 (with places L, GPCR-BS1(ex)-L). For the biological meaning of these invariants and all others of the GPCR module see Tab. A.1 and Tab. A.2 in the appendix. *Right:* Result of a stochastic simulation run with test firing rates.

meaning (see Tab. A.1 and Tab. A.2 in the appendix). Thus, essential validation criteria for a Petri net model of a signal transduction network are fulfilled.

Stochastic simulations with test rates show the expected effector function of the module. The dissociation of the ligand from GPCR (t2) and the dissociation of the targets from the substrate binding sites of both G-protein subunits (t16, t18) are assumed to proceed slower *(BioMassAction(0.01))* than all other reactions *(BioMassAction(0.1))*. Upon ligand binding to the receptor (decrease of free ligand), we first observe an increase in the activated GPCR GEF function, followed by an increase of the dissociated G-protein subunits, which can subsequently trigger downstream signaling events.

6   Conclusion

Models allow to perform experiments 'in silico', to study the systems properties and behaviour, to make predictions and thus to contribute to a further understanding of the involved processes. As the body of biological data is steadily increasing, it becomes more and more important to find a way to integrate huge amounts of available information in the form of a model. We are currently working on a method consisting of a modular design principle that allows to check and validate each functional subunit thoroughly due to its managable size. Step by step connection and combination of subunits (in the form of submodels) and validation of the connected parts ensures that the resulting composed net is coherent as well. Depending on specific 'wet lab' experiments, which are performed to validate the model in turn, different modules can be combined in order to study the behaviour of subsystems or of the entire system that has been modeled. As many biological functional units (like enzymes, receptors) play a role in different signaling pathways, the respective modules can be reused and recombined in different ways. The modules can be applied to other Petri net

classes; they can be easily converted into a colored Petri net [12], or a stochastic Petri net, see intoduction. In a next step we intend to color our low-level Petri net [12] in cooperation with the group of Prof. Heiner. This more compact description will enable us to depict and study the behavior of populations of nociceptive DRG neurons as well as multiple copies of biological entities.

As far as pain and the contribution of nociceptors is concerned, we hope to contribute with our net to a mechanism-based pain therapy by identifying possible targets for the development of new therapeutic intervention strategies.

The modular design together with the Petri net framework seems to be a promising tool to handle large biological systems even when exact quantitative parameter values are missing.

7   Acknowledgements

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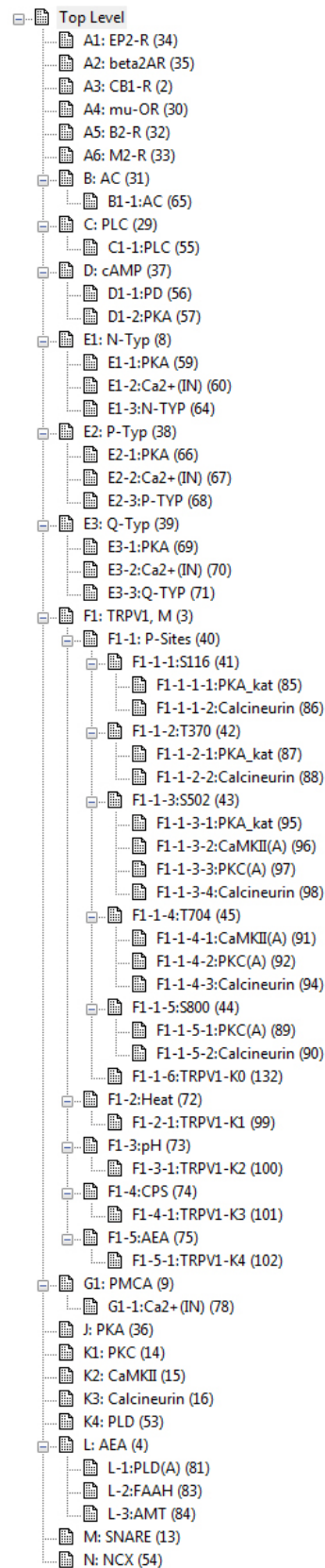
Tab. 1: List of P-invariants and their interpretation

Number	Place	Interpretation
1	GPCR-BS1(ex) GPCR-BS1(ex)-L	Extracellular binding site of the GPCR is unbound or bound to the ligand.
2	GPCR-BS1(ex)-L L	The ligand is free in the extracellular space or bound to the GPCR.
3	GPCR-GEF(active) GPCR-GEF(inactive) Gα-BS1(b)-GPCR-GEF(active)	GEF part of the GPCR can be inactive or active or active and bound to the G-protein.
4	GAP Gα-BS1(f)-GAP	GAP is free in cytoplasma or bound to the G-Protein.
5	Gα-SBD(f)-Target1-BS Target1-BS	The target for the Gα subunit is free or bound to Gα substrate binding domain.
6	Gβ/γ-SBD-Target2-BS2 Target2-BS	The target for the Gβ/γ subunit is free or bound to Gβ/γ substrate binding domain.
7	Gα-GTPase(b) Gα-GTPase(f)	The confromation of the GTPase domain corresponds to that of the whole Gα subunit .
8	Gα-BS2-GDP(b) Gα-BS2-GDP(f) Gα-BS2-GTP(b) Gα-BS2-GTP(f)	The same as above goes for binding site 2 of the Gα subunit. In both cases GTP or GDP is bound.
9	Gα-BS1(b) Gα-BS1(b)-GPCR-GEF(active) Gα-BS1(f) Gα-BS1(f)-GAP	The same as above goes for binding site 1 of the Gα subunit. In both cases it can be unbound or bound to GAP respectively the GEF part of the GPCR.
10	Gβ/γ-SBD(b) Gβ/γ-SBD(f) Gβ/γ-SBD(f)-Target2-BS2	The Gβ/γ subunit can be associated to the G-protein complex (no substrate binding) or free (substrate binding possible).
11	Gα-BS1(b) Gα-BS1(b)-GPCR-GEF(active) Gα-SBD(f) Gα-SBD(f)-Target1-BS	If one domain is in the conformation where the Gα subunit is associated to the G-protein complex another domain can not be in the comformation where the Gα subunit is free (vice versa).
12	Gα-BS1(f) Gα-BS1(f)-GAP Gα-BS2-GDP(b) Gα-BS2-GTP(b)	see no. 11
13	Gα-BS2-GDP(b) Gα-BS2-GTP(b) Gα-SBD(f) Gα-SBD(f)-Target1-BS	see no. 11
14	Gα-SBD(b) Gα-GTPase(f)	see no. 11
15	Gα-BS1(f) Gα-BS1(f)-GAP	see no. 11

	Gα-SBD(b)	
16	Gα-BS2-GDP(f) Gα-BS2-GTP(f) Gα-SBD(b)	see no. 11
17	Gα-SBD(b) Gα-SBD(f) Gα-SBD(f)-Target1-BS	see no. 11
18	Gα-BS1(f) Gα-BS1(f)-GAP Gα-GTPase(b)	see no. 11
19	Gα-BS2-GDP(f) Gα-BS2-GTP(f) Gα-GTPase(b)	see no. 11
20	Gα-SBD(f) Gα-SBD(f)-Target1-BS Gα-GTPase(b)	see no. 11
21	Gα-BS1(b) Gα-BS1(b)-GPCR-GEF(active) Gα-GTPase(f)	see no. 11
22	Gα-BS2-GDP(b) Gα-BS2-GTP(b) Gα-GTPase(f)	see no. 11
23	Gα-BS1(b) Gα-BS1(b)-GPCR-GEF(active) Gα-BS2-GDP(f) Gα-BS2-GTP(f)	see no. 11
24	Gα-BS1(b) Gα-BS1(b)-GPCR-GEF(active) Gβ/γ-SBD(f) Gβ/γ-SBD-Target2-BS2	If the substrate binding domain is in the conformation where the Gβ/γ subunit is associated to the G-protein complex, another domain can not be in the comformation where Gα subunit is free (vice versa).
25	Gα-SBD(f) Gα-SBD(f)-Target1-BS Gβ/γ-SBD(b)	see no. 24
26	Gα-BS1(f) Gα-BS1(f)-GAP Gβ/γ-SBD(b)	see no. 24
27	Gα-BS2-GDP(f) Gα-BS2-GTP(f) Gβ/γ-SBD(b)	see no. 24
28	Gα-BS2-GDP(b) Gα-BS2-GTP(b) Gβ/γ-SBD(f) Gβ/γ-SBD-Target2-BS2	see no. 24
29	Gα-GTPase(f) Gβ/γ-SBD(b)	see no. 24
30	Gα-GTPase(b) Gβ/γ-SBD(f)	see no. 24

	Gβ/γ-SBD(f)-Target2-BS2	
31	Gα-SBD(b)	
	Gβ/γ-SBD(f)	see no. 24
	Gβ/γ-SBD-Target2-BS2	
32	GDP	Free GTP can just be in a high or low
	GTP	engergy state
33	GTP	The high energy state of GTP can just be
	Gα-BS2-GTP(b)	free, bound at the free Gα subunit or at
	Gα-BS2-GTP(f)	Gα subunit in the G Protein complex. If
	Pi	GTP is in one of those states there
		cannot be free Pi (vice versa)

- Top Level
  - A1: EP2-R (34)
  - A2: beta2AR (35)
  - A3: CB1-R (2)
  - A4: mu-OR (30)
  - A5: B2-R (32)
  - A6: M2-R (33)
  - B: AC (31)
    - B1-1:AC (65)
  - C: PLC (29)
    - C1-1:PLC (55)
  - D: cAMP (37)
    - D1-1:PD (56)
    - D1-2:PKA (57)
  - E1: N-Typ (8)
    - E1-1:PKA (59)
    - E1-2:Ca2+(IN) (60)
    - E1-3:N-TYP (64)
  - E2: P-Typ (38)
    - E2-1:PKA (66)
    - E2-2:Ca2+(IN) (67)
    - E2-3:P-TYP (68)
  - E3: Q-Typ (39)
    - E3-1:PKA (69)
    - E3-2:Ca2+(IN) (70)
    - E3-3:Q-TYP (71)
  - F1: TRPV1, M (3)
    - F1-1: P-Sites (40)
      - F1-1-1:S116 (41)
        - F1-1-1-1:PKA_kat (85)
        - F1-1-1-2:Calcineurin (86)
      - F1-1-2:T370 (42)
        - F1-1-2-1:PKA_kat (87)
        - F1-1-2-2:Calcineurin (88)
      - F1-1-3:S502 (43)
        - F1-1-3-1:PKA_kat (95)
        - F1-1-3-2:CaMKII(A) (96)
        - F1-1-3-3:PKC(A) (97)
        - F1-1-3-4:Calcineurin (98)
      - F1-1-4:T704 (45)
        - F1-1-4-1:CaMKII(A) (91)
        - F1-1-4-2:PKC(A) (92)
        - F1-1-4-3:Calcineurin (94)
      - F1-1-5:S800 (44)
        - F1-1-5-1:PKC(A) (89)
        - F1-1-5-2:Calcineurin (90)
      - F1-1-6:TRPV1-K0 (132)
    - F1-2:Heat (72)
      - F1-2-1:TRPV1-K1 (99)
    - F1-3:pH (73)
      - F1-3-1:TRPV1-K2 (100)
    - F1-4:CPS (74)
      - F1-4-1:TRPV1-K3 (101)
    - F1-5:AEA (75)
      - F1-5-1:TRPV1-K4 (102)
  - G1: PMCA (9)
    - G1-1:Ca2+(IN) (78)
  - J: PKA (36)
  - K1: PKC (14)
  - K2: CaMKII (15)
  - K3: Calcineurin (16)
  - K4: PLD (53)
  - L: AEA (4)
    - L-1:PLD(A) (81)
    - L-2:FAAH (83)
    - L-3:AMT (84)
  - M: SNARE (13)
  - N: NCX (54)

Fig. 5: Hierarchy graph of the entire nociceptive network.