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Narrative-based computational modelling of the Gp130/JAK/STAT signalling pathway

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Abstract

Background

Appropriately formulated quantitative computational models can support researchers in understanding the dynamic behaviour of biological pathways and support hypothesis formulation and selection by "in silico" experimentation. An obstacle to widespread adoption of this approach is the requirement to formulate a biological pathway as machine executable computer code. We have proposed a novel, biologically intuitive, narrative-style modelling language for biologists to formulate the pathway which is then automatically translated into an executable format.

Results

We introduce the approach by presenting a computational model of the gp130/JAK/STAT signalling pathway derived from a biological narrative and show that the model reproduces the dynamic behaviour of the pathway derived by biological observation. We then "experiment" on the model by simulation and sensitivity analysis to define those parameters which dominate the dynamic behaviour of the pathway. The model predicts that nuclear compartmentalisation and phosphorylation status of STAT are key determinants of the pathway and that alternative mechanisms of signal attenuation exert their influence on different timescales.

Conclusions

The described language allows researchers to model biological systems without explicitly dealing with formal notations and mathematical expressions, nevertheless making it possible to obtain simulation and analysis results. We present the model and the sensitivity analysis results we have obtained, that allow us to identify the

parameters which are most sensitive to perturbations. The results are shown to be in agreement with existing mathematical models of the gp130/JAK/STAT pathway.

Background

Biological signalling pathways of even modest complexity cannot be comprehensively analysed within a feasible timescale by currently available experimental tools. However appropriate pathway models can be used to generate, explore and refine hypothesises guiding the formulation and prioritisation of experimental interventions. This has conventionally been approached by the use of models inspired by chemical kinetics and articulated mathematically in the form of ordinary differential equations. Recently an alternative approach: "molecules as computation" has been proposed in which a pathway is formulated as an executable computer programme [1,2] which can be interrogated to determine the dynamic behaviour, robustness and parameter sensitivities of the model [3]. The outcomes of in silico experimentation on the computer model can then be used to inform the design of biological interventions in vitro.

One key challenge of this approach is the accurate description of biological pathways in the form of an executable computer language. From the biologist's perspective the formulation has to capture the biologically interesting features of the pathway and be readily understood by other biologists. From the computer science perspective the formulation has to conform to the rules of formal methods in computer science: it must be logically precise and unambiguous. There is therefore a potential language gap between what the biologist understands and what the computer model encodes. We have recently described a high level biologically-intuitive textual language in which the signalling pathway is articulated in the form of a narrative of events

concerning the interactions between components located in different compartments [4]. This articulation of the pathway is then translated into an executable computer programme for further analysis. In this paper we describe, develop and interrogate such an executable model of the gp130/JAK/STAT signalling pathway [5] using the Narrative Language approach and explore its predictions by in silico experimentation.

The gp130/JAK/STAT signalling pathway (see for example [5]) is the subject of significant clinical and biological interest, not least due to the key role it plays in human fertility, neuronal repair, haematological development and embryonic stem cell renewal [6]. Members of the gp130 cytokine family, such as LIF or OSM, bind to the common signal transducing receptor chain gp130 and a second signalling receptor LIFR or OSMR [7]. Homo- or hetero-dimerisation of gp130, LIFR and OSMR induces activation of the receptor associated kinase JAK which in turn phosphorylates the latent transcription factor STAT which, as a consequence, undergoes homo-dimerisation, translocates to the nucleus and activates the transcription of downstream gene targets (Figure 1). Several features of this pathway make it an attractive case study for a computer programme based modelling approach. A characteristic feature of the gp130/JAK/STAT system is the role of spatial confinement in which the transcription factor STAT undergoes nuclear/cytoplasmic shuttling which is regulated by JAK-mediated phosphorylation at the plasma membrane and T-Cell Protein Tyrosine Phosphatase (TC-PTP)-mediated de-phosphorylation in the nucleus [8,9]. Aside from gp130/JAK activation by ligand the dynamics of the pathway can be regulated by a variety of mechanisms, which include STAT-mediated induction of Suppressor of Cytokine Signalling (SOCS) family proteins, which suppress JAK activation [10] and the Protein Inhibitor of Activated STAT (PIAS), an E3 family

ubiquitin ligase which induces proteolytic degradation of phospho-STAT [11,12]. Chronic nuclear STAT activation and/or JAK activation [13,14] have been implicated as a predisposing event in a variety of tumour types indicating that pathway dynamics have significant impact on cell behaviour. Elucidating the relative influence of different pathway parameters on the activity of STAT will guide the evaluation of therapeutic interventions.

Much experimental data is available on the gp130/JAK/STAT pathway and several mathematical models have been developed based on ordinary differential equations (see for example [15-17]). We present a different approach to modelling this pathway based on formal computational methods. We use a novel textual language for modelling biochemical systems, and perform simulations through the BetaWB simulator, an existing tool [18] based on Gillespie's stochastic simulation algorithm [19]. A key feature of the formal computational approach is the ability to rigorously explore, by in silico experimentation, the dynamic behaviour of the model to determine both the role of signal modifiers such as SOCS and the importance of parameter values. We validate our computational model by showing that it produces outputs which conform to those produced by experiment. We then perform in silico experiments on the model to determine first and second order parameter sensitivities and the effects of various types of pathway modulators. We show that the dynamic behaviour of the pathway is dominated by the rate of STAT de-phosphorylation and nuclear export and that these two variables result in bistable pathway behaviour when combined together in the model.

Methods

Narrative Modelling Language

We use a novel modelling language expressly designed to describe dynamically regulated and spatially -confined biochemical pathways.

The basic entities of the language are molecules (*components*) and sub-cellular locations (*compartments*). In the language molecules can interact (e.g. bind/unbind), undergo biochemical modification (e.g. phosphorylation/de-phosphorylation) and re-locate between compartments. The time dependent behaviour of the pathway is described in the form of a narrative of events involving these basic entities and functions, which imposes a temporal sequence and defines inter-dependencies and contingencies. In the narrative approach each of the elements can denote 'real' (i.e. experimentally defined) or abstract (e.g. hypothetical) entities. In silico exploration of the pathway model is simply enabled by modifying the narrative description and/or changing parameter values.

We now describe the detailed implementation of the Narrative Language as illustrated in the gp130/JAK/STAT pathway model (Additional files 1-8).

A *compartment* represents a cellular or subcellular compartment (e.g. *nucleus*, *cytosol*, *cell membrane*) or an abstract location; it is described by an identifier, a name, the size, and the number of spatial dimensions (to distinguish between 2D compartments, i.e. membranes, and 3D ones).

A *component* represents a molecule involved in the system, and it is identified by a name, an informal description, a list of interaction sites, a list of states, a list of

locations, and the initial quantity of molecules present. Since a biochemical reaction may describe the modification of interaction sites, a component is seen as a list of interaction sites, each defined by a name and a state (e.g. *active*, *bound*, *phosphorylated*). In the even that interaction sites are not known, states can also be associated with the entity itself representing modifications of generic interaction sites. A label associated with each state and site specifies the status of the component at system initialisation. If the location of the protein is relevant to the model, the compartments in which it can be located during the evolution of the system are specified. A label is associated with each compartment which is set to "true" if the protein is in that compartment in the initial configuration. Finally, the initial quantity of the component is set.

A *reaction* represents a biochemical modification occurring among the components of the system; it is described by an identifier, an informal description, the reaction type (e.g. *binding*, *unbinding*, *dimerization*, *phosphorylation*, *relocation*), the reaction rate (i.e. the kinetic constant), and the reaction volume; a reliability value can be associated to reaction rate and reaction volume.

A feature of this implementation of the narrative approach is the use of reliability values associated with numerical parameters. This is a percentage value which describes the reliability of the associated numerical value, and it can be used to distinguish between values that are highly certain because obtained from high quality biological experiment, and others which are inferred as a result of un-verified assumptions or 'guesswork' (e.g. *100%* indicates high precision data, while *0%* indicates a value which has no experimental evidence). Reliability values do not

influence the behaviour of the programme but are annotations to inform use of the model. In particular modellers can employ reliability values to identify parameter dependencies to be explored during model refinement.

A *narrative of events* describes the evolution of the system; it is a sequence of basic events, each of which is a constrained textual description of a biochemical reaction involving at most two components. Events can be grouped into processes. An *event* is described by an identifier, an informal description, a semiformal description, the identifier of the reaction associated with the event, and the identifier of the alternative events (if present). The *semiformal description* specifies the occurring reaction (e.g. *phosphorylates*, *relocates to*, *binds*, *unbinds*) and the involved component(s), and it can be prefixed by conditions (on the state of components/sites, or on their position). We assume that each event involves an interaction or a modification of one site for each involved protein. If the site is known, it can be specified; otherwise, it is assumed that one of the component's defined states is involved; a list of sites can be specified as a shortcut for simultaneous steps involving different sites (e.g. simultaneous phosphorylation of two sites of one protein).

The last feature of the language concerns the order of events in the evolution of the system. Two events occurring in a generic system of interacting entities can be *concurrent* (independent events, e.g. events involving different proteins), *sequential* (one event can occur only after the other one has occurred, e.g. a phosphorylation of a site of a protein is allowed only after it is bound to another protein), or *alternative* (mutually exclusive events, i.e. if one event occurs, the other one cannot occur, e.g. competitive binding of different ligands to a receptor). We distinguish between

concurrent, sequential, and alternative events as follows. Conditions are used to enforce the ordering of sequential events; an additional parameter is specified to handle alternative events; events that are not explicitly declared either alternative or sequential, are considered independent and are treated as concurrent events.

Model Description & Simulation

We developed a model of the gp130/JAK/STAT signalling pathway in the proposed Narrative Language. The full model is supplied as Additional files (Tables 1-8). The entities we consider in the model are: two ligands (LIF and OSM), three membrane-bound receptors (gp130, LIFR and OSMR), one effector (STAT3), and two inhibitors (SOCS3 and PIAS3). The receptor associated kinase JAK and TC-PTP phosphatase are implicitly modelled.

Each receptor contains at least one ligand binding site (OSMR has only one site for OSM, while LIFR and gp130 also have one site for LIF), one binding site for SOCS3 inhibitor, and some phosphorylation sites. Moreover, receptors can be in dimeric state (an additional site in gp130 allows us to distinguish between the two types of OSM receptors). STAT3 has one phosphorylation and four binding sites (for receptors and PIAS3 inhibitor), and it can be monomeric or dimeric (STAT3 can form homodimers).

Four compartments are involved in the system: the exosol (the extracellular space, where the ligands are located), the cell membrane (location of the receptors), the cytosol (initial location of the STAT3 effector), and the nucleus (to which the effector can translocate).

Tables 1 and 2 show the definition of compartments and components involved in the gp130/JAK/STAT pathway. The compartment volumes are calculated based on the average cell radius and ratio between intra-cellular compartment volumes stated in [20], and the initial amount of ligands are calculated based on the known extracellular concentration (500*pM*).

Table 3 shows the definition of reactions occurring in the pathway. Some of the reaction rates have been obtained from wet experiments, while others have been estimated based on information about similar reactions, or extracted from other models [15-17]; reliability values are assigned to reaction rates.

Finally, Tables 4-8 show the definition of the narrative of events, which describe the binding/unbinding of ligand/receptor pairs, the downstream LIF and OSM pathways (formation and activation of receptor complexes), the downstream STAT3 pathway (recruitment and activation of STAT3, and its nuclear/cytoplasmic shuttling), and the inhibition mechanisms.

Table 4 models the binding of ligands to receptors (reaction r1 in the graphical representation of the pathway shown in Figure 1 and events 1, 3, 5, 7 and 9 in the Narrative Language model), and the inverse unbinding reaction (r2, events 2, 4, 6, 8 and 10).

Table 5 models the dimerization of pairs of receptor subunits to form receptor complexes (gp130-LIFR or gp130-OSMR), which is triggered by the binding of a

ligand to one of the receptors (r3, events 11, 13, 15, 17, 19 and 21), and the dissociation of the receptor complexes (r4, events 12, 14, 16, 18, 20 and 22).

Table 6 models the activation (JAK-mediated phosphorylation) of the receptor complexes, the binding of STAT3 to a receptor complex, and the activation (phosphorylation) of STAT3. Once the receptor dimeric complex is formed, each receptor subunit (gp130, LIFR and OSMR) can phosphorylate on specific sites (r5, events 23, 25, 27 and 29). STAT3 can bind on receptors' phosphorylated sites (r7, events 31, 32 and 33), and the binding of STAT3 allows the phosphorylation of STAT3 on site Y705 (r8, events 37, 38 and 39).

Table 7 models the unbinding of STAT3 from receptor complexes, its homodimerization, and nuclear/cytoplasmic shuttling (relocation into the nucleus, de-phosphorylation by TC-PTP, dehomodimerization and relocation into the cytoplasm). Once phosphorylated, STAT3 can dissociate from the receptor complex (r10, events 41, 42 and 43); the phosphorylated site allows STAT3 to homodimerize (r11, event 44). When STAT3 is in dimeric form, it can translocate into the nucleus (r12 and r15, event 45) where it can carry out its specific functions (not modelled here): STAT3 binds to the DNA, activating the transcription of downstream gene targets. Nuclear STAT3 is inactivated through de-phosphorylation by TC-PTP (r13, event 46), which leads to its dedimerization (r14, event 47), and its export to the cytoplasm (r15, event 48), where STAT3 can undergo additional cycles of activation.

Table 8 models SOCS3 and PIAS3 inhibition mechanisms. SOCS3 is produced by active STAT3 (event 49) and degraded (event 50), and it acts by competing with

STAT3 in binding to receptors (events 51, 52 and 53). PIAS3 acts by binding to active nuclear STAT3 (event 57).

We developed a tool [21], *N2BB*, which implements an automatic translation of models described in the biologically-intuitive Narrative Language into executable computable models formulated in *BlenX* [22], a programming language inspired on the *Beta-binders* process calculus [23]. Process calculi, originally developed for modelling mobile communicating systems, have recently been proposed as appropriate for simulating biological processes [1], and they have proved themselves as powerful tools for dynamical modelling of complex biological systems [24-27]. Differently from differential equations, process calculi also allow for analysis of models (e.g. model-checking, equivalence, reachability, causality, and locality analysis).

The BlenX model derived from the Narrative Language model is compatible with the BetaWB [18], a collection of tools for modelling, simulating, and analysing BlenX models. Hence, the model can be imported into the BetaWB designer, or directly simulated by means of the BetaWB simulator; the time evolution of the simulation can be visualised by means of the BetaWB plotter or the Snazer tool [28]. For a detailed description of the BlenX language and of the implementation of the simulator, see [18,22].

Cells, Reagents & Cytokines

MCF-7 human breast cancer cells were obtained from American Type Culture Collection (Manassas, VA) and cultured as described [6]. The human oncostatin M

recombinant expression plasmid, pGEX-3C-OSM, was prepared, expressed and purified as described previously [6].

Western Blot, Immunofluorescence and Data Analysis

Serum starved MCF-7 cells were stimulated with 10ng/ml oncostatin M for increasing times (up to 480 minutes) at 37°C. For Western blot analysis, cell lysates were prepared and analysed as described [6] and monoclonal anti-phospho STAT3 (Tyr705) and STAT3 (Cell Signalling Technology) antibodies used for immunodetection. The density of the bands representing phospho-STAT3 and STAT3 were measured using ImageJ [29] and expressed as the ratio of phospho-STAT3 to STAT3. For immunofluorescence studies, MCF7 cells grown on coverslips were fixed with 4% paraformaldehyde (10 min, RT), permeabilised with 0.1% saponin solution (0.02M glycine, 0.1% saponin, 0.1M Tris/HCl pH8.5) for 20 min and blocked for 1 hour in 0.1% saponin solution (0.1% saponin, 0.1M Tris/HCl pH8.5) plus 2.5% foetal calf serum. Cells were immunostained with monoclonal anti-STAT3 antibody for 1 h at RT and incubated with Texas Red-conjugated secondary antibody containing Hoechst (Molecular Probes) for 45 min at RT. Coverslips were mounted with 5µl of Mowiol solution (10% Mowiol 4-88, 25% glycerol, 0.1M Tris/HCl pH8.5) on the slide and observed under confocal microscope. For localisation analysis, images captured were converted to greyscale and total STAT3 fluorescence calculated from the sum of pixel density values (ImageJ). Nuclear STAT3 fluorescence was calculated from selection of nuclear area (as determined by Hoechst staining) and cytoplasmic STAT3 fluorescence calculated by subtracting nuclear staining from total cellular staining. For each time-point analysis was performed on between 60 -100 cells (from multiple coverslips) and mean values ± 2SD were calculated for total nuclear and cytoplasmic STAT (expressed as percentage of total STAT).

Results and discussion

We used the N2BB tool to automatically translate the gp130/JAK/STAT pathway model into BlenX and we simulated the derived model using BetaWB. Our intention in the simulations that follow was to firstly define the behaviour of the full model and then to study the behaviour of the model in response to perturbations such as modifying numerical values and/or removing components from the model. A particular feature of the narrative approach is that it is very simple to change parameters or modify the narrative of events in the process of model exploration.

Our aim is to define those features, which exert the dominating influence on the dynamic behaviour of the pathway in the model for evaluation by biological experiment. This is an example of using computer models to explore a wide range of scenarios in silico to guide the formulation of the more laborious and expensive laboratory –based experiments.

The time evolution resulting from the simulation of the model is shown in the following pictures. Figure 2A reports the time evolution of the full model, while Figure 2B-2D show the evolution when different inhibitors are removed (in silico genetics). The amounts of different STAT3 forms are plotted: monomeric cytoplasmic, dimeric cytoplasmic, monomeric nuclear, and dimeric nuclear.
Figure 2A shows that in the initial configuration STAT3 is present in monomeric form in the cytoplasm. As the system undergoes dynamic evolution, STAT3 is rapidly phosphorylated, dimerized and translocated into the nucleus. At the same time cytoplasmic STAT3 is dramatically reduced. The system reaches a plateau for some

time and then slowly reverses as nuclear STAT3 levels fall and cytoplasmic levels rise. This behaviour conforms well to that obtained experimentally by observing the time-evolution of STAT3 phosphorylation (Additional file 9) and the re-location of STAT3 to the nuclear compartment in response to Gp130 activation (Additional file 10). However it is important to note that, whilst the model and experimental data are in quantitative agreement for early time points (0-200 minutes), the experimental data reveals a faster rate of recovery of cytoplasmic STAT3 than predicted from the model over longer time periods. We were unable to accelerate the rate of relocalisation of STAT3 in the model by parameter variation (data not shown) which we interpret as indicating that the current model does not include biological processes that influence the rate of nuclear/cytoplasmic shuttling at later time points. In this context we remark that the current model takes no account of the induction of new gene expression [6] by gp130 signalling over this time period which could include components that influence the rate of nuclear/cytoplasmic transfer.

We conclude that the computer model derived from the original narrative is able to capture the dynamic behaviour of the real pathway, demonstrating the validity of the approach.

In the next phase we explored the dependency of the model on the presence of various components which were ‘knocked out” by removal from the programme.

By comparing Figure 2A and 2B, in which we run the simulation in the absence of SOCS3, we observe that the effect of SOCS3 expression in response to STAT3 activation is to activate the slow attenuation process observed in the full model: removal of SOCS3 suppresses the delayed re-appearance of cytoplasmic STAT3 and the pathway exhibits prolonged and stable nuclear occupancy of STAT3.

The role PIAS3, which binds to phosphorylated nuclear STAT3 preventing it binding to DNA, is revealed by comparing Figure 2A and 2C. In this case removal of PIAS3 yields an initial increase of active STAT3, but the nuclear STAT3 signal attenuates with a faster time course than in the full model, leading to an increase in cytoplasmic (and therefore inactive) STAT3.

Figure 2D shows the outcomes of removing the nuclear phosphatase TC-PTP from the model. In this case there is a rapid accumulation of nuclear STAT3 which reaches steady state and fails to attenuate. In this case cytoplasmic STAT3 is rapidly eliminated and does not re-appear.

Comparing the consequences of removing three different types of inhibitor from the model it is clear that each has characteristic temporal effects. The consequences of TC-PTP inhibition are significantly more rapid than removal of SOCS3 or PIAS3. Inhibition of SOCS3 and TC-PTP lead to prolonged and stable activated STAT3, whereas inhibition of PIAS3 accelerates the rate of activated STAT3 decay by inducing accumulation of cytoplasmic STAT3.

Single parameter sensitivity analysis

We next turned to analysis of the parameter sensitivities of the model. In this case we ran simulations in which individual parameters were systematically varied to observe the dependency of model behaviour on individual values. The aim here is to define those values, which have greatest impact on model behaviour.

We first examined the dependency of the model on the amount of STAT3 in the system. In this case we ran simulations containing different numbers of STAT3

molecules at initialisation (Figure 3). We observe that the duration of nuclear occupancy is relatively stable to STAT3 perturbation until it drops below a threshold value (in this case between 30-300 molecules) when it collapses into 'noise'. However the number of STAT3 molecules had a significant effect on peak amplitude where there was an approximately linear relationship between the maximum number of activated STAT3 molecules reached and the total number of molecules in the system.

We next examined the rate of TC-PTP de-phosphorylation, the amount of ligand, the rate of JAK kinase phosphorylation and the rate of nuclear export (Figure 4A-4D). The outcomes of these simulations show that the behaviour of the model is differentially dependent on particular values.

Slowing the rate of phospho-STAT3 dephosphorylation exhibited significant effects on activated STAT3 amplitude and duration noted over the complete range analysed (Figure 4C). De-phosphorylation rates impacted on both the peak amplitude and duration of activated STAT3 indicating, as reported by others [30], that nuclear de-phosphorylation of activated STAT3 is a an important determinant of signalling dynamics. Similar sensitivities were found on varying the rate of nuclear export (Figure 4D): although in this case nuclear export had no impact on peak signal amplitude and its main effect was on signal duration.

By contrast the model was relatively robust to variations in either the amount of ligand in the system or the rate of JAK activation (Figure 4A and 4B) where significant impacts on signal dynamics only become apparent at extreme values.

Indeed similar to the dependency on STAT3 numbers it appears as though behaviour of the model is relatively robust to parameter changes in these processes over several orders of magnitude.

Multi-dimensional parameter sensitivity

The foregoing in silico experiments revealed that the dynamic behaviour of the gp130/JAK/STAT pathway is most sensitive to two parameters: the rate of nuclear STAT3 de-phosphorylation and the rate of STAT3 nuclear export. We were interested to learn if the model exhibited higher order dependencies when parameters were varied in combinations. Exhaustive implementation of this approach is currently computationally expensive. For this study we therefore chose to study the interaction between nuclear de-phosphorylation of STAT3 and export of de-phosphorylated STAT3. To this end 35 simulations were run in which each parameter was changed simultaneously (Figure 5). The results of this experiment were surprising. Instead of exhibiting a graded response across the whole parameter landscape, as might be predicted from the behaviour of each individual value, the system exhibits a bistable response in which, for the majority of conditions, the system is either constitutively activated or constitutively repressed. The system only exhibits sensitivity to parameter variation in a narrow region of values towards the middle of the ranges chosen. In this region both peak amplitude and signal duration were dependant upon the interplay between nuclear export and STAT3 de-phosphorylation.

Conclusions

Our purpose in this study was to explore the practical utility of the biological narrative approach for in silico exploration of a complex signalling pathway. We show that the biologist-specified narrative yields outcomes which conform well to experimental data. By ‘experimenting’ on the model by parameter exploration and component

removal we were able to explore the influence of different elements on the dynamic behaviour of the pathway. These studies showed that the rate of STAT nuclear export and nuclear localised dephosphorylation were key determinants of signalling dynamics. This conclusion is supported by in vitro experimental data in which inhibition of nuclear export by either drug treatment [31,32] or mutation of the Nuclear Export Sequence [33] results in prolonged nuclear accumulation of phosphorylated STAT.

This outcome indicates that the model captures the dynamic behaviour of the real pathway well and encouraged further exploration of the model into issues which would be resource intensive – or technically challenging – to address by biological experimentation. In particular we were interested in exploring the potential for interactions between parameters which are not currently addressable by biological experiment. We found, combining nuclear export and nuclear dephosphorylation, that the two parameters interact strongly yielding a 'switch-like' behaviour. This type of modelling analysis may inform future considerations of multi-step mutagenesis or combination drug therapy scenarios in the gp130/JAK/STAT pathway.

We have demonstrated in this study that a language can be used to describe biological signalling pathways in a way, which is formal and unambiguous for computational execution but intuitive to the biologist. The approach exploits the particular advantages of the "molecule as computation" paradigm of Regev and Shapiro [1]: the resulting models are computable, relevant and understandable. There are also practical advantages to process calculus models in that they can be readily modified to explore different scenarios and interrogated using model checking tools [3] to formally verify

the model and explore its quantitative behaviour. Collectively the approach is therefore extensible in that, as new biological information on the pathway becomes available, it can be readily incorporated into the model.

The Narrative Language has a number of explicit features which capture some cardinal features of biological signalling pathways. It defines the temporal relationships between events (i.e. sequential, concurrent and competing events). It defines the location of proteins and the reaction volumes, and it deals with multi-compartmental models thereby making spatial location and confinement a central feature. Species in the model can exist in multiple states and locations. The Narrative Language can therefore be employed to model any biological process involving state transitions of different types; inter-molecular interactions; spatial confinement and temporal evolution. Moreover, the biochemical entities, interactions and information which can be modelled in the Narrative Language are very similar to the ones modelled in the representation used in the NCI-Nature Pathway Interaction Database (PID) [34], a curated collection of biomolecular pathways represented in a graphical language. Considered the analogies between the PID and our proposal, we are currently developing a mapping between the PID representation and the Narrative Language. This translation would provide us with a significant number of well-understood pathways, which can be directly simulated into the BetaWB.

Finally we present a translation into the BlenX language [22] in this paper but, in principle, translation of the Narrative Language into other current or future languages is possible. Thus the biological formulation of the pathway is separable from the computer method employed for simulation and analysis.

Authors' contributions

MLG designed the computational model, performed the simulation experiments, analysed the data and wrote the paper. AD performed the laboratory experiments and analysed the data. NUD participated in designing the model, performed the laboratory experiments and analysed the data. JKH participated in designing the model, analysed the data and wrote the paper. CP participated in analysing the data and writing the paper. All authors participated in conceiving the experiments, read and approved the final manuscript.

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Figures

Figure 1 - Graphical representation of the gp130/JAK/STAT pathway
Closed arrows represent biochemical reactions, dotted arrows represent transports.

Figure 2 - Role of inhibitors in the gp130/JAK/STAT pathway
(A) Full model. (B) SOCS3 knock out. (C) PIAS3 knock out. (D) TC-PTP knock out. For each experiment the amounts of different STAT3 forms are plotted: monomeric cytoplasmic (STAT3-C), dimeric cytoplasmic (STAT3-PDC), monomeric nuclear (STAT3-N), and dimeric nuclear (STAT3-PDN).

Figure 3 - Single parameter sensitivity analysis: variation of STAT3 initial amount
The evolution of the system has been observed varying the initial amount of STAT3. (A) 30 molecules. (B) 300 molecules. (C) 3000 molecules. (D) 30000 molecules. The amount of active nuclear STAT3 (STAT3-PDN) is plotted.

Figure 4 - Single parameter sensitivity analysis
The evolution of the system has been observed varying each parameter individually. (A) Ligands initial amount. (B) JAK-mediated phosphorylation. (C) TC-PTP-mediated dephosphorylation. (D) Nuclear export. The amount of active nuclear STAT3 (STAT3-PDN) is plotted.

Figure 5 - Multi-dimensional parameter sensitivity
The evolution of the system has been observed varying simultaneously the rate of nuclear STAT3 de-phosphorylation and the rate of STAT3 nuclear export. The considered values are as in Figure 4C and 4D. The rows (A-G) refer to the export rate, while the columns (i-v) refer to the dephosphorylation rate. The amounts of active nuclear STAT3 (STAT3-PDN) and monomeric nuclear STAT3 (STAT3-N) are plotted.

Additional files

Additional file 1 - Gp130/JAK/STAT pathway model: list of compartments
Format: PDF Filename: table1.pdf

Additional file 2 - Gp130/JAK/STAT pathway model: list of components
Format: PDF Filename: table2.pdf

Additional file 3 - Gp130/JAK/STAT pathway model: list of reactions
Format: PDF Filename: table3.pdf

Additional file 4 - Gp130/JAK/STAT pathway model: list of events (ligand-receptor bindings)
Format: PDF Filename: table4.pdf

Additional file 5 - Gp130/JAK/STAT pathway model: list of events (receptor complexes formation)
Format: PDF Filename: table5.pdf

Additional file 6 - Gp130/JAK/STAT pathway model: list of events (STAT3 activation)
Format: PDF Filename: table6.pdf

Additional file 7 - Gp130/JAK/STAT pathway model: list of events (STAT3 unbinding and shuttling)
Format: PDF Filename: table7.pdf

Additional file 8 - Gp130/JAK/STAT pathway model: list of events (SOCS3 and PIAS3 inhibition)
Format: PDF Filename: table8.pdf

Additional file 9 - Time-evolution of STAT3 phosphorylation
(A) Levels of phospho-STAT3 (upper panel) and total STAT3 (lower panel) after

stimulation with oncostatin M.

(B) Densitometric analysis of immunoblots showing the time-course of phospho-

STAT3/STAT3 ratios.

(C) Simulation time-course of phospho-STAT3/STAT3 ratios.

Format: PDF Filename: figure6.pdf

Additional file 10 - Nuclear localisation of STAT3
(A) Time-course of STAT3 nuclear/cytoplasmic localisation.

(B) Images representative of STAT3 localisation after stimulation with Oncostatin M:

(i) control; (ii) 20 minutes; (iii) 4 hours; (iv) 8 hours.

(C) Simulation time-course of STAT3 nuclear/cytoplasmic localisation.

Format PDF Filename: figure7.pdf

Figure 1
extracellular space
LIF
gp130
LIFR
r1
r2
r3
r4
r5
r6
r7
r8
r9
r10
STAT3
P
cytoplasm
r11
r12
r13
r14
r15
nucleus

Figure 5

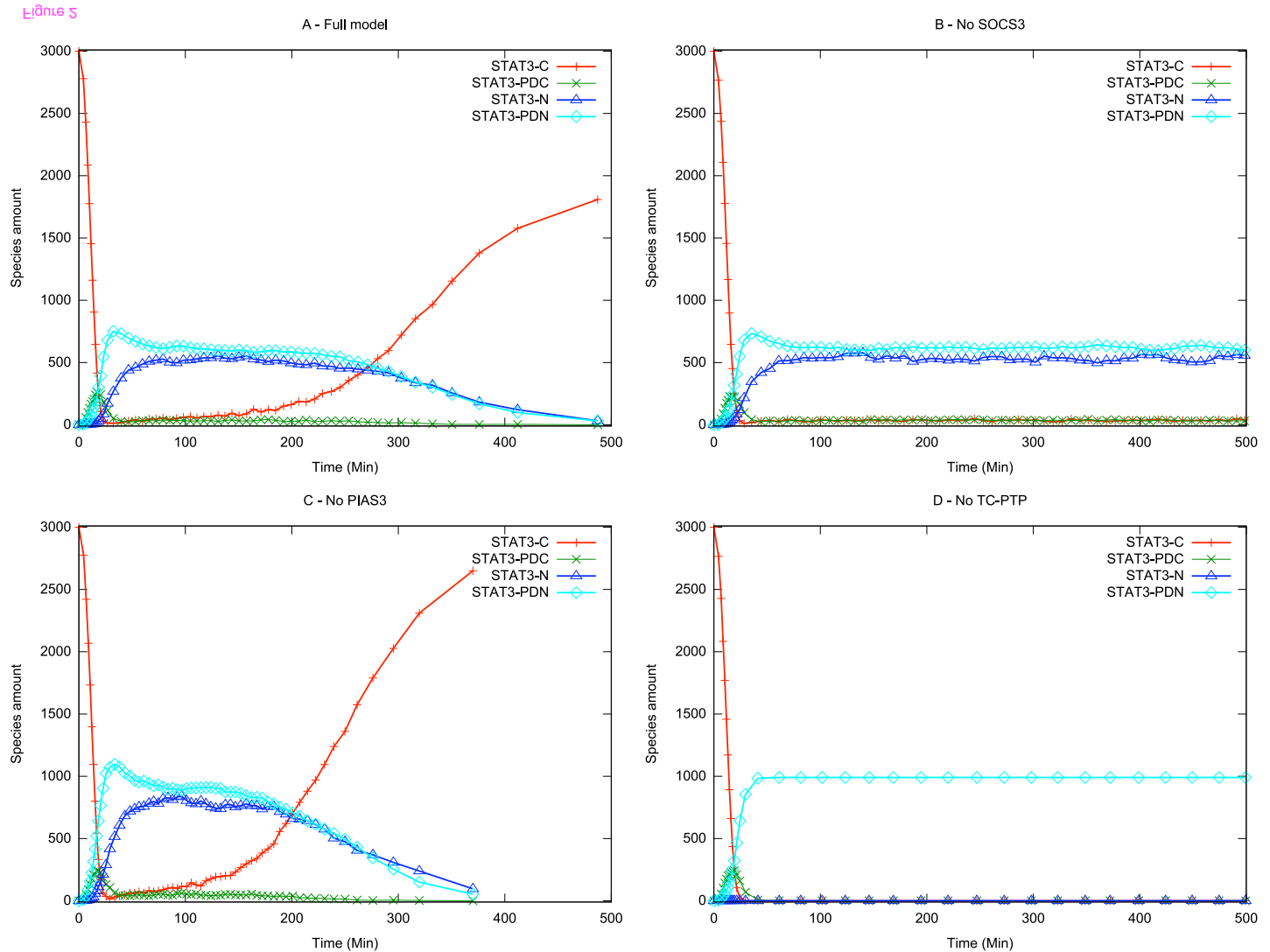

A - Full model
B - No SOCS3
C - No PIAS3
D - No TC-PTP
STAT3-C
STAT3-PDC
STAT3-N
STAT3-PDN
Species amount
Time (Min)
0
500
1000
1500
2000
2500
3000
100
200
300
400

Figure 3

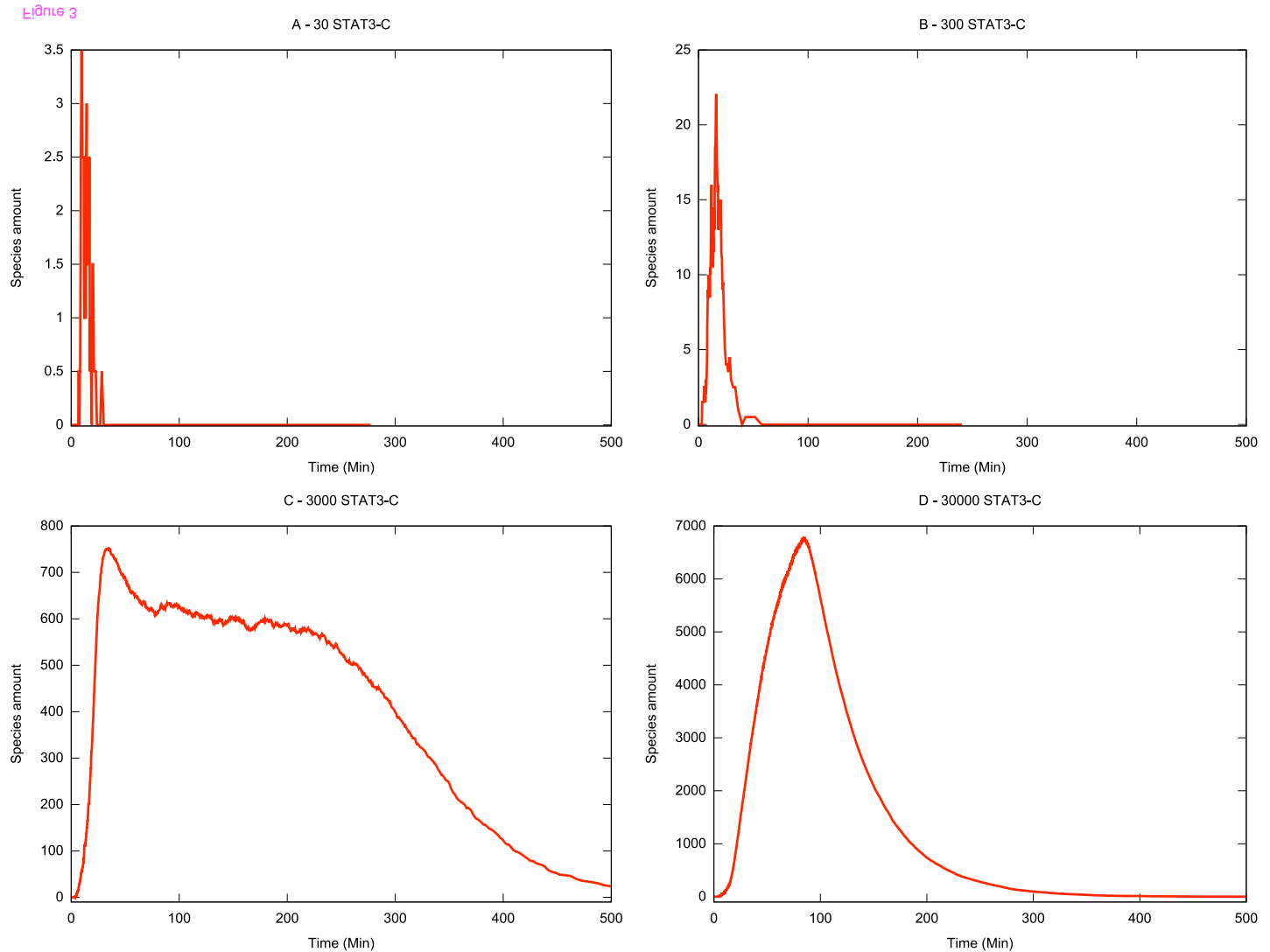
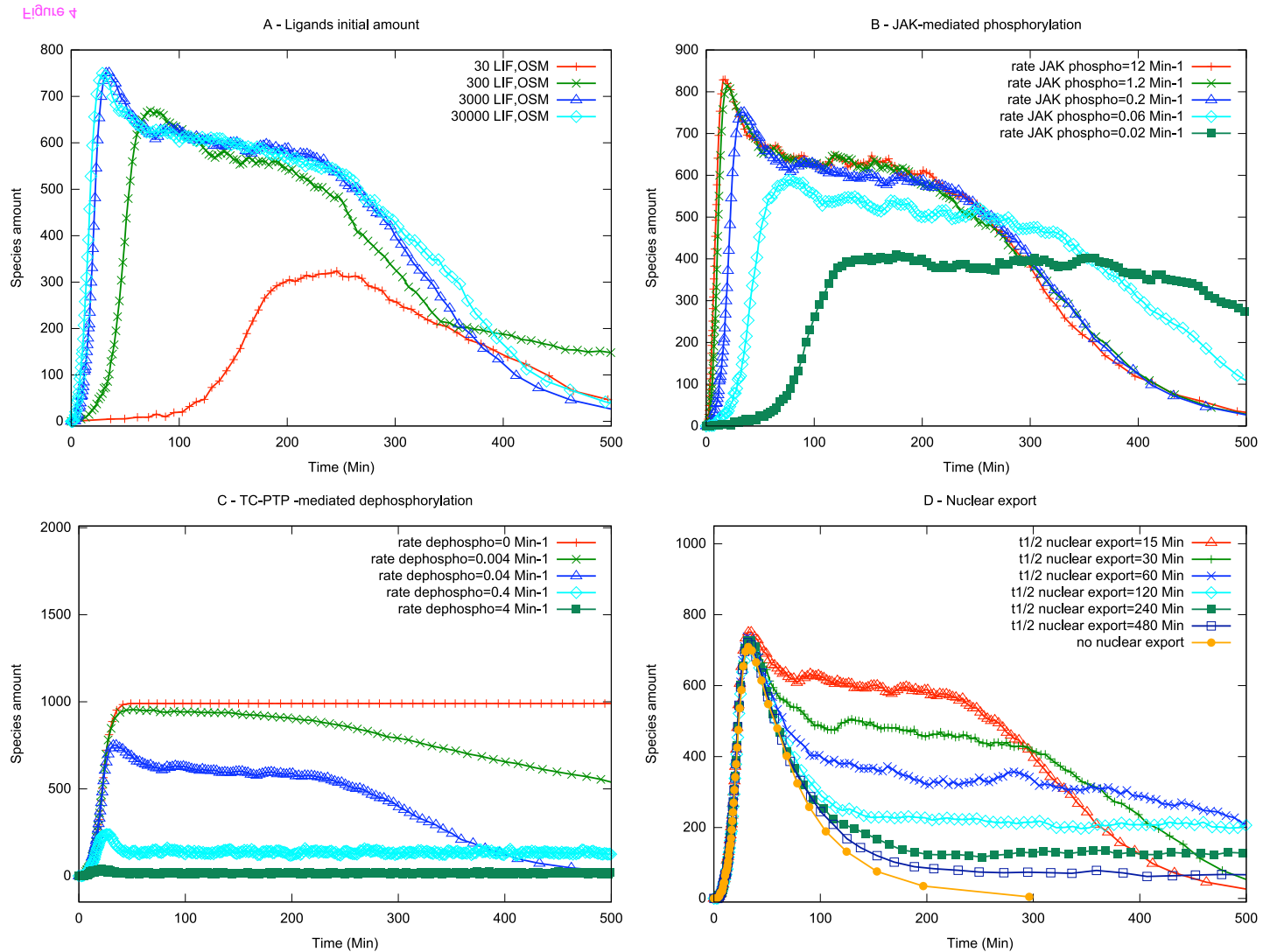

A - 30 STAT3-C
B - 300 STAT3-C
C - 3000 STAT3-C
D - 30000 STAT3-C
Species amount
Time (Min)
0
100
200
300
400
500
0.5
1
1.5
2
2.5
3
3.5
5
10
15
20
25
600
700
800
1000
2000
3000
4000
5000
6000
7000

Figure 4


A - Ligands initial amount
Species amount
Time (Min)
30 LIF,OSM
300 LIF,OSM
3000 LIF,OSM
30000 LIF,OSM
B - JAK-mediated phosphorylation
rate JAK phospho=12 Min-1
rate JAK phospho=1.2 Min-1
rate JAK phospho=0.2 Min-1
rate JAK phospho=0.06 Min-1
rate JAK phospho=0.02 Min-1
C - TC-PTP -mediated dephosphorylation
rate dephospho=0 Min-1
rate dephospho=0.004 Min-1
rate dephospho=0.04 Min-1
rate dephospho=0.4 Min-1
rate dephospho=4 Min-1
D - Nuclear export
t1/2 nuclear export=15 Min
t1/2 nuclear export=30 Min
t1/2 nuclear export=60 Min
t1/2 nuclear export=120 Min
t1/2 nuclear export=240 Min
t1/2 nuclear export=480 Min
no nuclear export

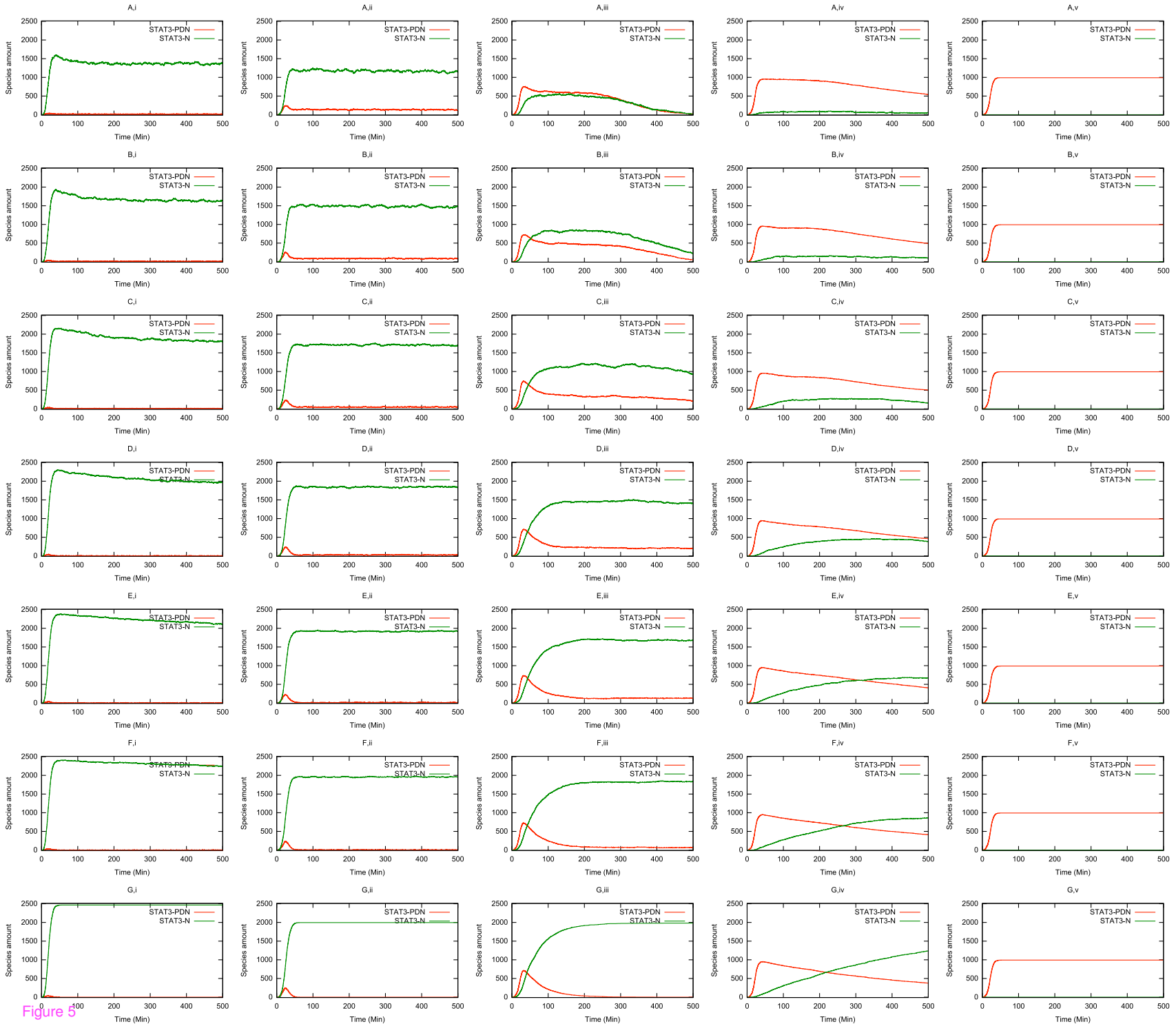

A,i
A,ii
A,iii
A,iv
A,v
B,i
B,ii
B,iii
B,iv
B,v
C,i
C,ii
C,iii
C,iv
C,v
D,i
D,ii
D,iii
D,iv
D,v
E,i
E,ii
E,iii
E,iv
E,v
F,i
F,ii
F,iii
F,iv
F,v
G,i
G,ii
G,iii
G,iv
G,v
STAT3-PDN
STAT3-N
Species amount
Time (Min)
0
500
1000
1500
2000
2500
0
100
200
300
400
500

Figure 5

Table 1 - Gp130/JAK/STAT pathway: list of compartments

id	name	size	unit of measure	dimensions
1	**exosol**	9.91×10^{-12}	L	3
2	**cellMembrane**	1.26×10^{-7}	dm^2	2
3	**cytosol**	2.09×10^{-12}	L	3
4	**nucleus**	0.25×10^{-12}	L	3

Table 2 - Gp130/JAK/STAT pathway: list of components

name	descr	site	site_state	site_act	state	state_act	comp	comp_act	init_amount	rel
LIF	ligand				**bound**	**false**	1	**true**	3000	100%
OSM	ligand				**bound**	**false**	1	**true**	3000	100%
gp130	receptor	LIF	**bound**	**false**	**bound**	**false**	2	**true**	1000	50%
		OSM	**bound**	**false**						
		Y767	**phospho**	**false**						
		Y814	**phospho**	**false**						
		Y905	**phospho**	**false**						
		Y915	**phospho**	**false**						
		SOCS3	**bound**	**false**						
		typeI	**dimer**	**false**						
		typeII	**dimer**	**false**						
LIFR	receptor	LIF	**bound**	**false**	**bound**	**false**	2	**true**	1000	50%
		OSM	**bound**	**false**	**dimer**	**false**				
		Y981	**phospho**	**false**						
		Y1001	**phospho**	**false**						
		Y1028	**phospho**	**false**						
		SOCS3	**bound**	**false**						
OSMR	receptor	OSM	**bound**	**false**	**bound**	**false**	2	**true**	1000	50%
		Y917	**phospho**	**false**	**dimer**	**false**				
		Y945	**phospho**	**false**						
		SOCS3	**bound**	**false**						
STAT3	effector	Y705	**phospho**	**false**	**dimer**	**false**	3	**true**	3000	30%
		gp130	**bound**	**false**			4	**false**		
		LIFR	**bound**	**false**						
		OSMR	**bound**	**false**						
		PIAS3	**bound**	**false**						
SOCS3	inhibitor				**bound**	**false**	3	**true**	0	100%
PIAS3	inhibitor				**bound**	**false**	4	**true**	1000	20%

Table 3 - Gp130/JAK/STAT pathway: list of reactions

id	type	rate	unit	rel	react_vol	unit	rel
1	**binding**	4.8×10^{7}	$M^{-1}Min^{-1}$	80%	$9.91\cdot10^{-12}$	l	50%
2	**unbinding**	3.6×10^{-1}	Min^{-1}	80%	$9.91\cdot10^{-12}$	l	50%
3	**binding**	4.8×10^{7}	$M^{-1}Min^{-1}$	80%	$9.91\cdot10^{-12}$	l	50%
4	**unbinding**	3.6×10^{-2}	Min^{-1}	80%	$9.91\cdot10^{-12}$	l	50%
5	**binding**	4.8×10^{7}	$M^{-1}Min^{-1}$	80%	$9.91\cdot10^{-12}$	l	50%
6	**unbinding**	3.6×10^{-2}	Min^{-1}	80%	$9.91\cdot10^{-12}$	l	50%
7	**binding**	4.8×10^{7}	$M^{-1}Min^{-1}$	80%	$9.91\cdot10^{-12}$	l	50%
8	**unbinding**	3.6×10^{-1}	Min^{-1}	80%	$9.91\cdot10^{-12}$	l	50%
9	**binding**	4.8×10^{7}	$M^{-1}Min^{-1}$	80%	$9.91\cdot10^{-12}$	l	50%
10	**unbinding**	3.6×10^{-2}	Min^{-1}	80%	$9.91\cdot10^{-12}$	l	50%
11	**dimerization**	**inf**	$M^{-1}Min^{-1}$	50%	$9.91\cdot10^{-12}$	l	50%
12	**dedimerization**	0	$M^{-1}Min^{-1}$	0%	$9.91\cdot10^{-12}$	l	50%
13	**phosphorylation**	0.2	Min^{-1}	80%	$9.91\cdot10^{-12}$	l	50%
14	**dephosphorylation**	0	Min^{-1}	0%	$9.91\cdot10^{-12}$	l	50%
15	**binding**	4.8×10^{8}	$M^{-1}Min^{-1}$	20%	$2.09\cdot10^{-12}$	l	50%
16	**unbinding**	0.06	Min^{-1}	30%	$2.09\cdot10^{-12}$	l	50%
17	**phosphorylation**	0.2	Min^{-1}	80%	$2.09\cdot10^{-12}$	l	50%
18	**dephosphorylation**	0	Min^{-1}	0%	$2.09\cdot10^{-12}$	l	50%
19	**unbinding**	**inf**	Min^{-1}	10%	$2.09\cdot10^{-12}$	l	50%
20	**homodimerization**	**inf**	Min^{-1}	50%	$2.09\cdot10^{-12}$	l	50%
21	**relocation**	1	Min ($t_{1/2}$)	10%	$2.09\cdot10^{-12}$	l	50%
22	**dephosphorylation**	0.04	Min^{-1}	20%	$0.25\cdot10^{-12}$	l	50%
23	**dehomodimerization**	**inf**	Min^{-1}	20%	$0.25\cdot10^{-12}$	l	50%
24	**relocation**	15	Min ($t_{1/2}$)	10%	$0.25\cdot10^{-12}$	l	50%
25	**synthesis**	0.01	Min^{-1}	50%	$0.25\cdot10^{-12}$	l	50%
26	**binding**	6.0×10^{7}	$M^{-1}Min^{-1}$	20%	$2.09\cdot10^{-12}$	l	50%
27	**unbinding**	0.006	Min^{-1}	30%	$2.09\cdot10^{-12}$	l	50%
28	**binding**	1.0×10^{8}	$M^{-1}Min^{-1}$	20%	$0.25\cdot10^{-12}$	l	50%
29	**unbinding**	0.06	Min^{-1}	30%	$0.25\cdot10^{-12}$	l	50%
30	**degradation**	0.01	Min^{-1}	50%	$0.25\cdot10^{-12}$	l	50%

Table 4 - Gp130/JAK/STAT pathway: list of events (ligand-receptor bindings)

id	description	react	alt
	LIF-gp130 binding		
1	**if** gp130.LIF **is not bound and** LIF **is not bound and** gp130.typeI **is not dimer and** gp130.typeII **is not dimer then** LIF **binds** gp130 **on** LIF	1	
2	**if** gp130.LIF **is bound and** LIF **is bound and** gp130.typeI **is not dimer and** gp130.typeII **is not dimer and** gp130.Y767 **is not phospho then** LIF **unbinds** gp130 **on** LIF	2	
	LIF-LIFR binding		
3	**if** LIFR.LIF **is not bound and** LIF **is not bound and** LIFR **is not dimer then** LIF **binds** LIFR **on** LIF	3	
4	**if** LIFR.LIF **is bound and** LIF **is bound and** LIFR **is not dimer and** LIFR.Y981 **is not phospho then** LIF **unbinds** LIFR **on** LIF	4	
	OSM-gp130 binding		
5	**if** gp130.OSM **is not bound and** OSM **is not bound and** gp130.typeI **is not dimer and** gp130.typeII **is not dimer then** OSM **binds** gp130 **on** OSM	5	1
6	**if** gp130.OSM **is bound and** OSM **is bound and** gp130.typeI **is not dimer and** gp130.typeII **is not dimer and** gp130.Y767 **is not phospho then** OSM **unbinds** gp130 **on** OSM	6	
	OSM-LIFR binding		
7	**if** LIFR.OSM **is not bound and** OSM **is not bound and** LIFR **is not dimer then** OSM **binds** LIFR **on** OSM	7	3
8	**if** LIFR.OSM **is bound and** OSM **is bound and** LIFR **is not dimer and** LIFR.Y981 **is not phospho then** OSM **unbinds** LIFR **on** OSM	8	
	OSM-OSMR binding		
9	**if** OSMR.OSM **is not bound and** OSM **is not bound and** OSMR **is not dimer then** OSM **binds** OSMR **on** OSM	9	
10	**if** OSMR.OSM **is bound and** OSM **is bound and** OSMR **is not dimer and** OSMR.Y917 **is not phospho then** OSM **unbinds** OSMR **on** OSM	10	

Table 5 - Gp130/JAK/STAT pathway: list of events (receptor complexes formation)

id	description	react	alt
	LIF pathway → type I receptor gp130:LIFR		
11	**if** gp130.LIF **is bound and** LIFR.LIF **is not bound and** gp130.OSM **is not bound and** LIFR.OSM **is not bound and** LIFR **is not dimer and** gp130.typeI **is not dimer and** gp130.typeII **is not dimer then** LIFR **dimerizes with** gp130 **on** typeI	11	2
12	**if** gp130.LIF **is bound and** LIFR.LIF **is not bound and** gp130.OSM **is not bound and** LIFR.OSM **is not bound and** LIFR **is dimer and** gp130.typeI **is dimer and** gp130.typeII **is not dimer and** LIFR.Y981 **is not phospho and** gp130.Y767 **is not phospho then** LIFR **dedimerizes from** gp130 **on** typeI	12	
13	**if** LIFR.LIF **is bound and** gp130.LIF **is not bound and** LIFR.OSM **is not bound and** gp130.OSM **is not bound and** LIFR **is not dimer and** gp130.typeI **is not dimer and** gp130.typeII **is not dimer then** LIFR **dimerizes with** gp130 **on** typeI	11	4
14	**if** LIFR.LIF **is bound and** gp130.LIF **is not bound and** LIFR.OSM **is not bound and** gp130.OSM **is not bound and** LIFR **is dimer and** gp130.typeI **is dimer and** gp130.typeII **is not dimer and** LIFR.Y981 **is not phospho and** gp130.Y767 **is not phospho then** LIFR **dedimerizes from** gp130 **on** typeI	12	
	OSM pathway → type I receptor gp130:LIFR		
15	**if** gp130.OSM **is bound and** LIFR.OSM **is not bound and** gp130.LIF **is not bound and** LIFR.LIF **is not bound and** LIFR **is not dimer and** gp130.typeI **is not dimer and** gp130.typeII **is not dimer then** LIFR **dimerizes with** gp130 **on** typeI	11	6
16	**if** gp130.OSM **is bound and** LIFR.OSM **is not bound and** gp130.LIF **is not bound and** LIFR.LIF **is not bound and** LIFR **is dimer and** gp130.typeI **is dimer and** gp130.typeII **is not dimer and** LIFR.Y981 **is not phospho and** gp130.Y767 **is not phospho then** LIFR **dedimerizes from** gp130 **on** typeI	12	
17	**if** LIFR.OSM **is bound and** gp130.OSM **is not bound and** LIFR.LIF **is not bound and** gp130.LIF **is not bound and** LIFR **is not dimer and** gp130.typeI **is not dimer and** gp130.typeII **is not dimer then** LIFR **dimerizes with** gp130 **on** typeI	11	8
18	**if** LIFR.OSM **is bound and** gp130.OSM **is not bound and** LIFR.LIF **is not bound and** gp130.LIF **is not bound and** LIFR **is dimer and** gp130.typeI **is dimer and** gp130.typeII **is not dimer and** LIFR.Y981 **is not phospho and** gp130.Y767 **is not phospho then** LIFR **dedimerizes from** gp130 **on** typeI	12	

OSM pathway → type II receptor gp130:OSMR			
19	**if** gp130.OSM **is bound and** OSMR.OSM **is not bound and** gp130.LIF **is not bound and** OSMR.LIF **is not bound and** OSMR **is not dimer and** gp130.typeI **is not dimer and** gp130.typeII **is not dimer then** OSMR **dimerizes with** gp130 **on** typeII	11	6
20	**if** gp130.OSM **is bound and** OSMR.OSM **is not bound and** gp130.LIF **is not bound and** OSMR.LIF **is not bound and** OSMR **is dimer and** gp130.typeI **is not dimer and** gp130.typeII **is dimer and** OSMR.Y917 **is not phospho and** gp130.Y767 **is not phospho then** OSMR **dedimerizes from** gp130 **on** typeII	12	
21	**if** OSMR.OSM **is bound and** gp130.OSM **is not bound and** OSMR.LIF **is not bound and** gp130.LIF **is not bound and** OSMR **is not dimer and** gp130.typeI **is not dimer and** gp130.typeII **is not dimer then** OSMR **dimerizes with** gp130 **on** typeII	11	10
22	**if** OSMR.OSM **is bound and** gp130.OSM **is not bound and** OSMR.LIF **is not bound and** gp130.LIF **is not bound and** OSMR **is dimer and** gp130.typeI **is not dimer and** gp130.typeII **is dimer and** OSMR.Y917 **is not phospho and** gp130.Y767 **is not phospho then** OSMR **dedimerizes from** gp130 **on** typeII	12	

Table 6 - Gp130/JAK/STAT pathway: list of events (STAT3 activation)

id	description	react	alt
Receptors phosphorylation			
23	**if** gp130.typeI **is dimer and** gp130 **is not bound then** gp130 **phospho on** Y767;Y814;Y905;Y915	13	
24	**if** gp130.typeI **is dimer and** gp130 **is not bound then** gp130 **dephospho on** Y767;Y814;Y905;Y915	14	
25	**if** LIFR **is dimer and** LIFR **is not bound then** LIFR **phospho on** Y981;Y1001;Y1028	13	
26	**if** LIFR **is dimer and** LIFR **is not bound then** LIFR **dephospho on** Y981;Y1001;Y1028	14	
27	**if** gp130.typeII **is dimer and** gp130 **is not bound then** gp130 **phospho on** Y767;Y814;Y905;Y915	13	
28	**if** gp130.typeII **is dimer and** gp130 **is not bound then** gp130 **dephospho on** Y767;Y814;Y905;Y915	14	
29	**if** OSMR **is dimer and** OSMR **is not bound then** OSMR **phospho on** Y917;Y945	13	
30	**if** OSMR **is dimer and** OSMR **is not bound then** OSMR **dephospho on** Y917;Y945	14	

	STAT3 binding		
31	**if** gp130.Y767 **is phospho and** STAT3 **is in** 3 **and** STAT3 **is not dimer and** gp130 **is not bound and** gp130.SOCS3 **is not bound and** STAT3.gp130 **is not bound and** STAT3.LIFR **is not bound and** STAT3.OSMR **is not bound and** STAT3.Y705 **is not phospho then** gp130 **binds** STAT3 **on** gp130	15	
32	**if** LIFR.Y981 **is phospho and** STAT3 **is in** 3 **and** STAT3 **is not dimer and** LIFR **is not bound and** LIFR.SOCS3 **is not bound and** STAT3.LIFR **is not bound and** STAT3.gp130 **is not bound and** STAT3.OSMR **is not bound and** STAT3.Y705 **is not phospho then** LIFR **binds** STAT3 **on** LIFR	15	
33	**if** OSMR.Y917 **is phospho and** STAT3 **is in** 3 **and** STAT3 **is not dimer and** OSMR **is not bound and** OSMR.SOCS3 **is not bound and** STAT3.OSMR **is not bound and** STAT3.gp130 **is not bound and** STAT3.LIFR **is not bound and** STAT3.Y705 **is not phospho then** OSMR **binds** STAT3 **on** OSMR	15	
34	**if** gp130.Y767 **is phospho and** STAT3 **is in** 3 **and** STAT3 **is not dimer and** gp130 **is bound and** gp130.SOCS3 **is not bound and** STAT3.gp130 **is bound and** STAT3.LIFR **is not bound and** STAT3.OSMR **is not bound and** STAT3.Y705 **is not phospho then** gp130 **unbinds** STAT3 **on** gp130	16	
35	**if** LIFR.Y981 **is phospho and** STAT3 **is in** 3 **and** STAT3 **is not dimer and** LIFR **is bound and** LIFR.SOCS3 **is not bound and** STAT3.LIFR **is bound and** STAT3.gp130 **is not bound and** STAT3.OSMR **is not bound and** STAT3.Y705 **is not phospho then** LIFR **unbinds** STAT3 **on** LIFR	16	
36	**if** OSMR.Y917 **is phospho and** STAT3 **is in** 3 **and** STAT3 **is not dimer and** OSMR **is bound and** OSMR.SOCS3 **is not bound and** STAT3.OSMR **is bound and** STAT3.gp130 **is not bound and** STAT3.LIFR **is not bound and** STAT3.Y705 **is not phospho then** OSMR **unbinds** STAT3 **on** OSMR	16	
	STAT3 phosphorylation		
37	**if** STAT3.gp130 **is bound then** STAT3 **phosphorylates on** Y705	17	
38	**if** STAT3.LIFR **is bound then** STAT3 **phosphorylates on** Y705	17	
39	**if** STAT3.OSMR **is bound then** STAT3 **phosphorylates on** Y705	17	
40	**if** STAT3 **is in** 3 **then** STAT3 **dephosphorylates on** Y705	18	

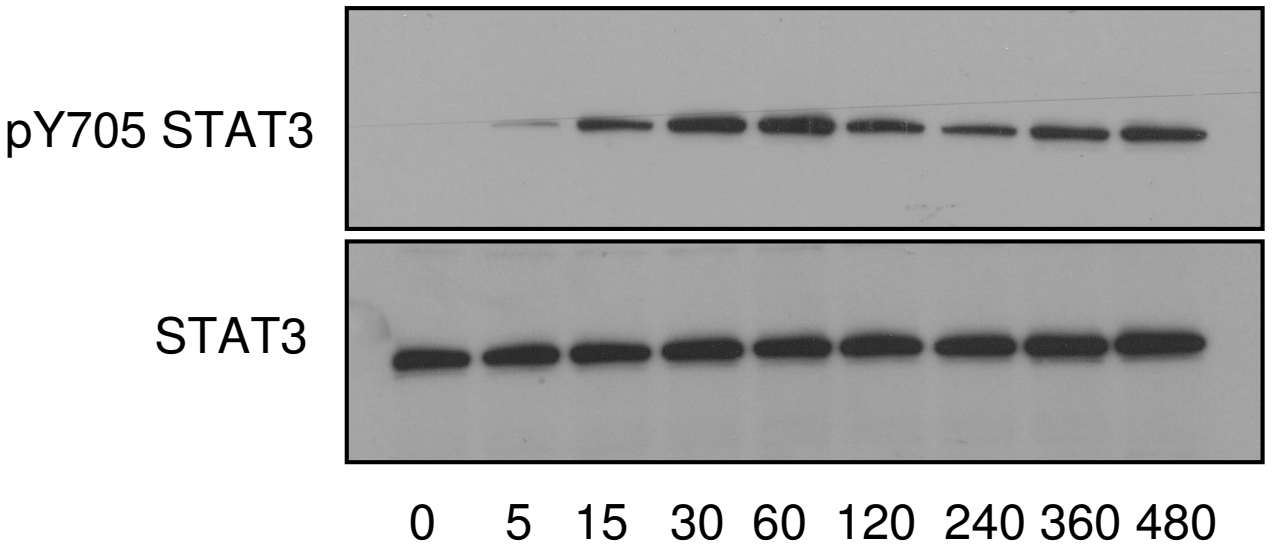
Table 7 - Gp130/JAK/STAT pathway: list of events (STAT3 unbinding and shuttling)

id	description	react	alt
	STAT3 unbinding and homodimerization		
41	**if** gp130.Y767 **is phospho and** STAT3 **is in** 3 **and** STAT3 **is not dimer and** gp130 **is bound and** gp130.SOCS3 **is not bound and** STAT3.gp130 **is bound and** STAT3.LIFR **is not bound and** STAT3.OSMR **is not bound and** STAT3.Y705 **is phosphorylated then** gp130 **unbinds** STAT3 **on** gp130	19	
42	**if** LIFR.Y981 **is phospho and** STAT3 **is in** 3 **and** STAT3 **is not dimer and** LIFR **is bound and** LIFR.SOCS3 **is not bound and** STAT3.LIFR **is bound and** STAT3.gp130 **is not bound and** STAT3.OSMR **is not bound and** STAT3.Y705 **is phosphorylated then** LIFR **unbinds** STAT3 **on** LIFR	19	
43	**if** OSMR.Y917 **is phospho and** STAT3 **is in** 3 **and** STAT3 **is not dimer and** OSMR **is bound and** OSMR.SOCS3 **is not bound and** STAT3.OSMR **is bound and** STAT3.gp130 **is not bound and** STAT3.LIFR **is not bound and** STAT3.Y705 **is phosphorylated then** OSMR **unbinds** STAT3 **on** OSMR	19	
44	**if** STAT3.Y705 **is phospho and** STAT3 **is not dimer and** STAT3.gp130 **is not bound and** STAT3.LIFR **is not bound and** STAT3.OSMR **is not bound then** STAT3 **homodimerizes**	20	
	STAT3 shuttling		
45	**if** STAT3 **is in** 3 **and** STAT3 **is dimer and** STAT3.gp130 **is not bound and** STAT3.LIFR **is not bound and** STAT3.OSMR **is not bound then** STAT3 **relocates to** 4	21	
46	**if** STAT3 **is in** 4 **and** STAT3 **is dimer and** STAT3.PIAS3 **is not bound then** STAT3 **dephospho on** Y705	22	
47	**if** STAT3 **is in** 4 **and** STAT3 **is dimer and** STAT3.Y705 **is not phospho then** STAT3 **dehomodimerizes**	23	
48	**if** STAT3 **is in** 4 **and** STAT3 **is not dimer and** STAT3.Y705 **is not phospho then** STAT3 **relocates to** 3	24	

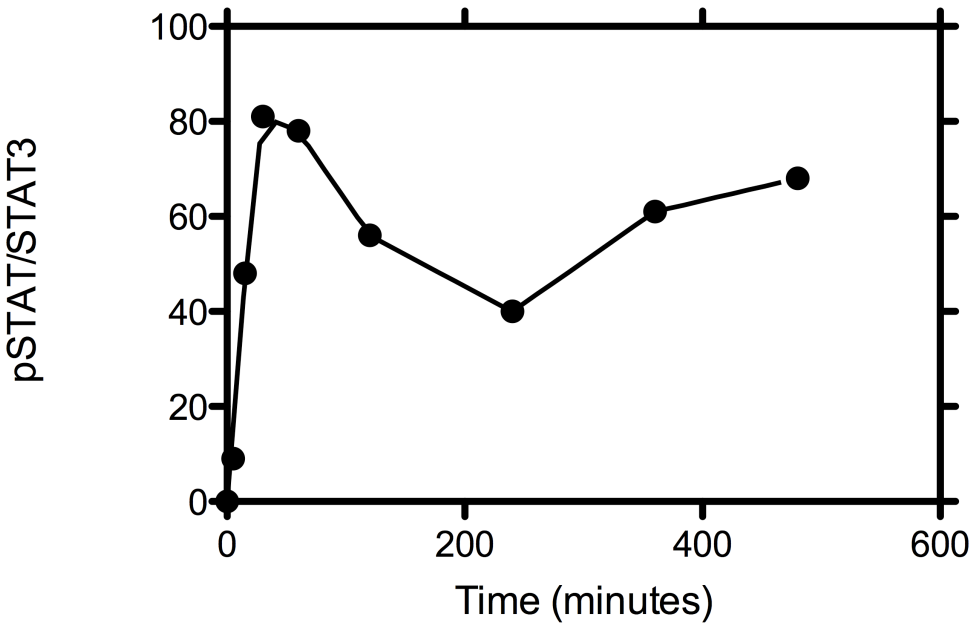
Table 8 - Gp130/JAK/STAT pathway: list of events (SOCS3 and PIAS3 inhibition)

id	description	react	alt
	SOCS3 synthesis, degradation and inhibition		
49	**if** STAT3 **is in** 4 **and** STAT3 **is dimer and** STAT3.Y705 **is phospho and** STAT3.PIAS3 **is not bound then** STAT3 **synthesises** SOCS3	25	
50	SOCS3 **degrades**	30	
51	**if** gp130.Y767 **is phospho and** gp130.SOCS3 **is not bound and** SOCS3 **is not bound and** gp130.STAT3 **is not bound then** SOCS3 **binds** gp130 **on** SOCS3	26	
52	**if** LIFR.Y981 **is phospho and** LIFR.SOCS3 **is not bound and** SOCS3 **is not bound and** LIFR.STAT3 **is not bound then** SOCS3 **binds** LIFR **on** SOCS3	26	
53	**if** OSMR.Y917 **is phospho and** OSMR.SOCS3 **is not bound and** SOCS3 **is not bound and** OSMR.STAT3 **is not bound then** SOCS3 **binds** OSMR **on** SOCS3	26	
54	**if** gp130.Y767 **is phospho and** gp130.SOCS3 **is bound and** SOCS3 **is bound and** gp130.STAT3 **is not bound then** SOCS3 **unbinds** gp130 **on** SOCS3	27	
55	**if** LIFR.Y981 **is phospho and** LIFR.SOCS3 **is bound and** SOCS3 **is bound and** LIFR.STAT3 **is not bound then** SOCS3 **unbinds** LIFR **on** SOCS3	27	
56	**if** OSMR.Y917 **is phospho and** OSMR.SOCS3 **is bound and** SOCS3 **is bound and** OSMR.STAT3 **is not bound then** SOCS3 **unbinds** OSMR **on** SOCS3	27	
	PIAS3 inhibition		
57	**if** STAT3 **is in** 4 **and** STAT3 **is dimer and** STAT3.Y705 **is phospho and** STAT3.PIAS3 **is not bound and** PIAS3 **is not bound then** PIAS3 **binds** STAT3 **on** PIAS3	28	
58	**if** STAT3 **is in** 4 **and** STAT3 **is dimer and** STAT3.Y705 **is phospho and** STAT3.PIAS3 **is bound and** PIAS3 **is bound then** PIAS3 **unbinds** STAT3 **on** PIAS3	29	

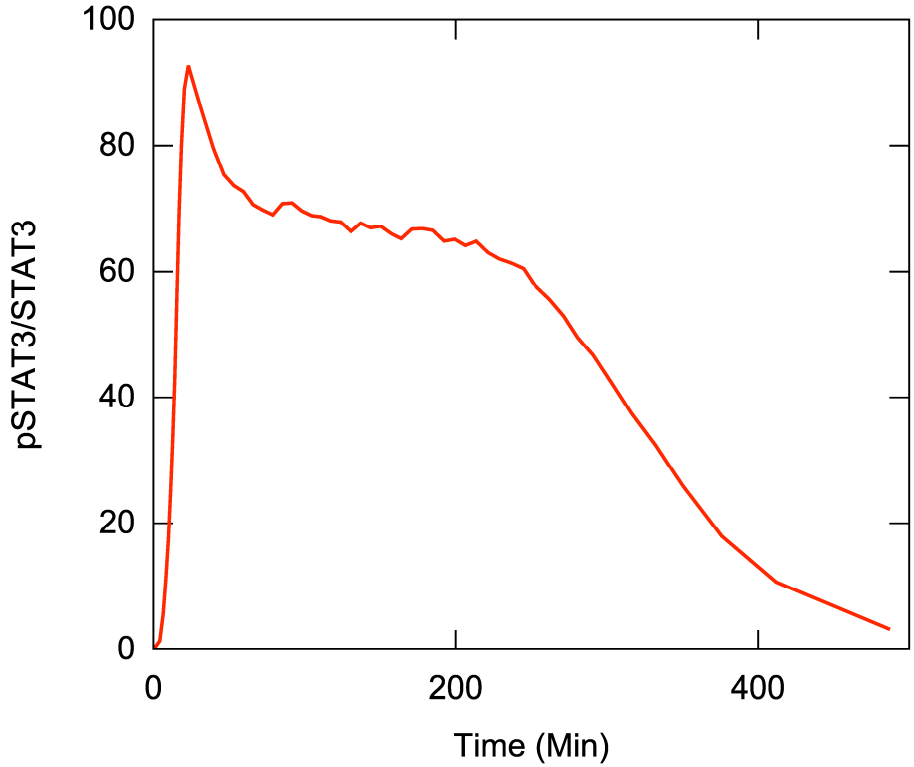
A


pY705 STAT3
STAT3
0 5 15 30 60 120 240 360 480

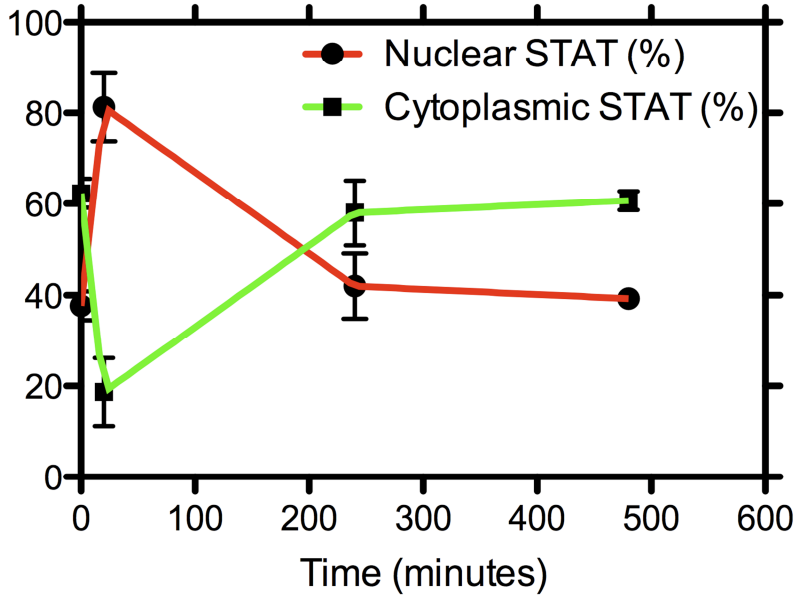
B


pSTAT/STAT3
100
80
60
40
20
0
0
200
400
600
Time (minutes)

C


pSTAT3/STAT3
100
80
60
40
20
0
0
200
400
Time (Min)

A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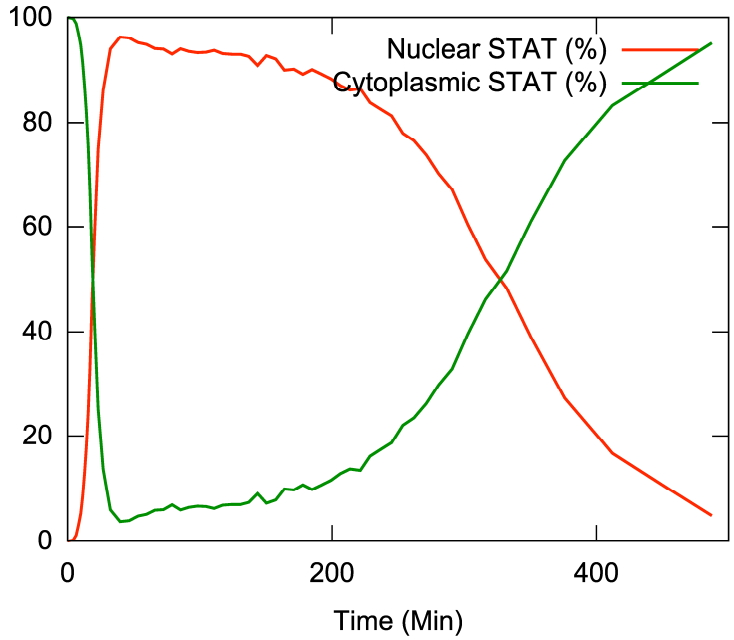
80
60
40
20
0
0
100
200
300
400
500
600
Nuclear STAT (%)
Cytoplasmic STAT (%)
Time (minutes)

C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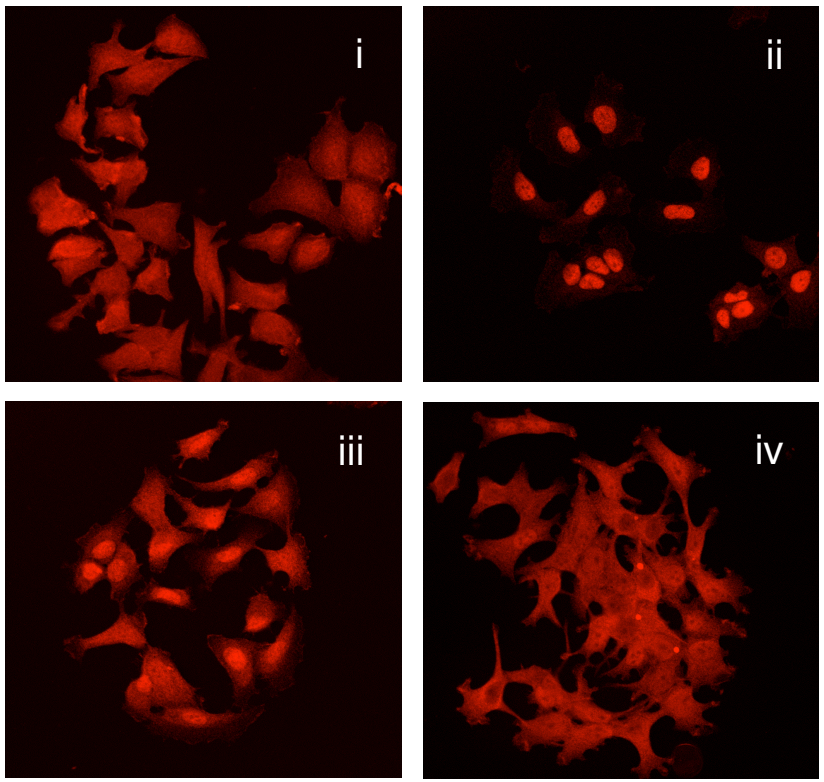
80
60
40
20
0
0
200
400
Nuclear STAT (%)
Cytoplasmic STAT (%)
Time (Min)

B


i
ii
iii
iv