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Interferon-γ Stimulated STAT1 Signalling: from Experimental Data to a Predictive Mathematical Model

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Systems Biology

Systems biology aims to understand the complex dynamics of biochemical reaction networks by an interdisciplinary approach of mathematical modelling and quantitative cell biology [1 – 6]. Stimulating an intracellular signalling pathway by an extracellular ligand leads to temporal changes of intracellular protein concentrations. These temporal changes are caused by nonlinear processes, including protein complex formation, enzyme catalyzed reactions and feedback regulation. Thus simple measurements of experimental time courses for protein concentrations or measurements of reaction rate constants alone are not sufficient to understand the dynamics of a biochemical network. Mathematical models, describing the temporal response of network in response to systematic perturbations of other components are needed to unravel the nonlinear dynamics of biochemical networks [7, 8].

The properties of mathematical models can be investigated through formal analysis or numerical computer simulations. This approach allows us to explore relationships between structure and function of pathways by analysing mathematical models with methods from dynamical systems theory [9 – 11]. A model can only be as good as the data that were used to build it and hence the generation of quantitative, sufficiently rich time series is a major priority for systems biology. Towards this end, the development of error reduction strategies

for standardised quantitative data [12], optimisation methods to estimate reaction constants from experimental data [13 – 16], and design of experiments on the basis of model predictions has received attention [17, 18].

An important application of medical systems biology is to understand disease mechanisms and the response of cells to drugs in order to support the development of therapies. Computer simulations enable quantitative predictions of sRNAi experiments and the design of stimulus-profiles leading to a desired temporal response of a pathway target. But only recently, feedback to the wet lab in the form of quantitative predictions of pathway dynamics and supporting the design of experiments is taking place as we demonstrate in our work.

Workflow in Systems Biology

A fruitful systems biology project requires a close interaction between scientists from cell biology and those with experience in mathematical modelling. A typical workflow in systems biology follows a bottom-up strategy. It starts with a single signalling pathway, composed of few core proteins and thus allows a detailed analysis of its dynamical properties. The initial network will be refined and extended by an iterative cycle of quantitative cell biology, mathematical modelling, parameter estimation, model predictions and experimental validation of model predictions. A systems biology workflow is summarized in Figure 1. The objectives of a specific systems biology project determine the type of the experimental data needed and both determine the mathematical modelling approach. In the following, we describe the steps of the workflow in more detail. For each step a variety of approaches are possible but we restrict to selected examples.

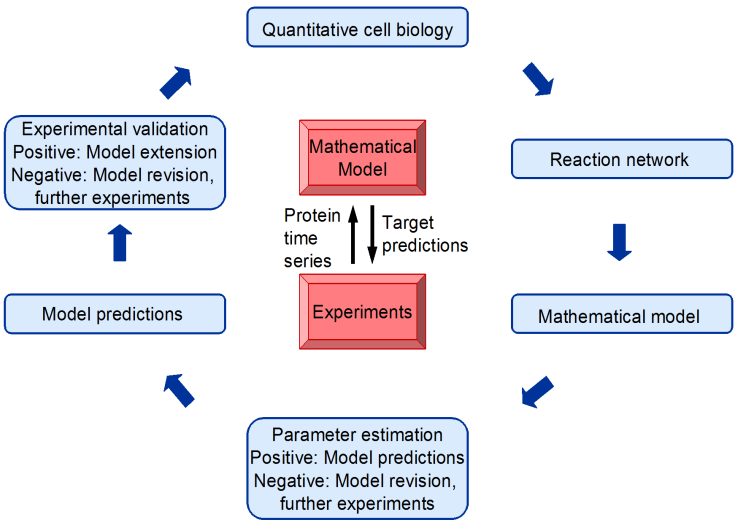

Figure 1. Workflow in systems biology.

Step 1: Quantitative cell biology

An appropriate approach to study the influence of a signalling pathway on cell proliferation is the *in vitro* stimulation of a pathway in a standardized cellular background. If cellular decisions are relevant (as in differentiation or apoptosis), then also flow cytometry data are needed which allow a sorting of cells with qualitatively different responses. In the above experiments temporal concentration changes are measured as an average over many cells. Protein concentration changes are quantified by immunoblots or by ELISA tests and gene expression changes of feedback target genes are quantified by Real-time PCR.

Step 2: Reaction network and structure of the mathematical model

The biochemical reaction network is translated into a nonlinear differential equation model, describing temporal changes of protein and mRNA concentrations. Ordinary differential equations (ODE) are an appropriate modelling approach if the experiments involve large numbers of proteins and mRNAs such that concentrations smoothly change over time. This is the case in the above introduced experiments. The mathematical models can include:

1. Receptor activation
2. Enzyme catalyzed reactions, for example phosphorylations of proteins
3. Protein complex formation
4. Intracellular transport and shuttling of proteins between subcellular compartments
5. Target gene expression and feedback regulation by expressed target genes.

Slow processes, for example gene transcription, should be appropriately modelled by delay differential equations [19]. In general, one applies mass action kinetics. Under certain assumptions models can be simplified, for example Michaelis-Menten kinetics.

Step 3: Parameter estimation

The parameters of a model, including reaction constants, delay times and total protein concentrations, are the remaining unknown components of the model. They can be estimated from ELISA time series, immunoblotting, microscopy and real-time PCR by global optimization approaches. Very fast scatter search algorithms with alternating global and local search have been developed for systems biology applications [16].

Step 4: Model validation and model-based predictions

Once model structure and parameter values are determined, the properties of a mathematical model are studied by formal analysis and computer simulations. However, a mathematical model is of benefit to the laboratory work only if it can predict the outcome of experiments,

which have not been used for model calibration. The design of refined experiments by model predictions will improve our understanding of biochemical networks. An important application of model driven experimental design is to achieve a deeper pathophysiological understanding of biochemical processes and to get knowledge about steps in the network which are difficult to measure. Mathematical models can be applied to predict an optimal range of stimulus concentrations, an optimal combination of different stimuli, as well as the kind and duration of stimulation to receive a desired temporal profile of the output signal.

If model predictions cannot be experimentally validated then one has to go back to *Step 2: Reaction network and structure of mathematical model.*

Modelling Strategy

A useful approach starts with a model which includes only those proteins for which experimental data are available, and interactions indirectly indicated by the time courses to be necessary in the biochemical process. An example is an oscillatory time series, but so far no negative feedback protein is known in the respective network. Different hypotheses about interactions, for example different feedback mechanisms through expressed target genes, or different transport mechanisms will be tested by different model structures.

If the mathematical model does not fit the experimental data then the following two reasons are possible: Important biochemical reactions are missing or reactions are not appropriately mathematically described. In the first case, a feedback loop or crosstalk with another signalling pathway may have not been considered and in the second case a delay differential equation could be more useful instead of an ordinary differential equation due to a time lag between the dynamics of the respective variables. Thus, one has to go back to *Step 2: Reaction network and structure of mathematical model.* Information about missing elements or inappropriate assumptions can be obtained also from the bad fit, as we will demonstrate in our example of the STAT1 signalling pathway.

An alternative strategy to the approach described above includes all known interactions of a biochemical network in a mathematical model. For example, given that there are 20 key proteins known, this leads to about 50 parameters in the model. Assuming that time series from only three proteins are available for parameter estimation. It is much easier to fit a large model with about 50 parameters to three experimental time series. Even many parameter sets will lead to a very good fit. Such a model is called unidentifiable. Predictions of an unidentifiable model can depend on the chosen parameter set of the best fits [18].

In the following sections we will apply our systems biology workflow to study the interferon-γ (IFNγ) stimulated STAT1 signalling pathway in pancreatic stellate cells (PSC); a fibroblast-like cell type in the pancreas.

Interferon-γ Stimulated STAT1 Signalling Pathway in PSC

Cells sense their environment through receptors. Signal transduction is the transfer of this information through a cell and between cells. Frequently, the immediate targets of signal transduction are transcription factors, which regulate the expression of specific target genes. The corresponding gene products mediate an appropriate physiological response to environmental changes. The family of STAT proteins (signal transducer and activator of transcription) exerts a dual role as signalling protein and transcription factor [20]. They transfer information directly from the receptor to the target genes. The STAT proteins can be activated by different receptors, and STAT signal transduction can influence different cellular functions, like proliferation, differentiation and immune response [20].

We studied the IFNγ stimulated STAT1 signalling pathway in PSC by a systems biology approach. STAT1 plays a key role in the mediation of antifibrotic effects of IFNγ effects in PSC [21, 22]. A detailed understanding of regulatory mechanisms controlling STAT1 activity in PSC may contribute to the development of a targeted antifibrotic therapy. In this work we present a summary of our results in [23] and few additional simulations. Details about the experimental protocol, quantified experimental data, mathematical model and simulation results are presented in [23].

Model of the IFNγ Stimulated STAT1 Signalling Pathway

Immunoblot time series for STAT1 activation, immunofluorescence time series for its nuclear translocation and RT-PCR time series for the expression of the target gene *SOCS1* measured in immortalised PSC formed the starting point for our systems biology project.

Based on the experimental data, we first established a reduced reaction network of the IFNγ-stimulated STAT1 signalling pathway (Fig. 2).

The reaction network includes phosphorylation of unphosphorylated STAT1 (STAT1U) followed by rapid homodimer formation (STAT1D). STAT1D translocate into the nucleus and induce the transcription of specific target genes. When STAT1D is not bound to the DNA, dimers may dissociate, followed by protein dephosphorylation and nuclear export of the resulting STAT1U [24]. In the network it is also included that STAT1U can shuttle into the nucleus, where it induces the transcription of target genes [25]. The network considers *SOCS1* and *STAT1* itself as target genes of IFNγ-activated signalling through STAT1. The annotation *time delay* in Figure 2 refers to temporal differences between IFNγ action at the receptor level (including STAT1 activation) and consecutive steps, such as expression of target genes.

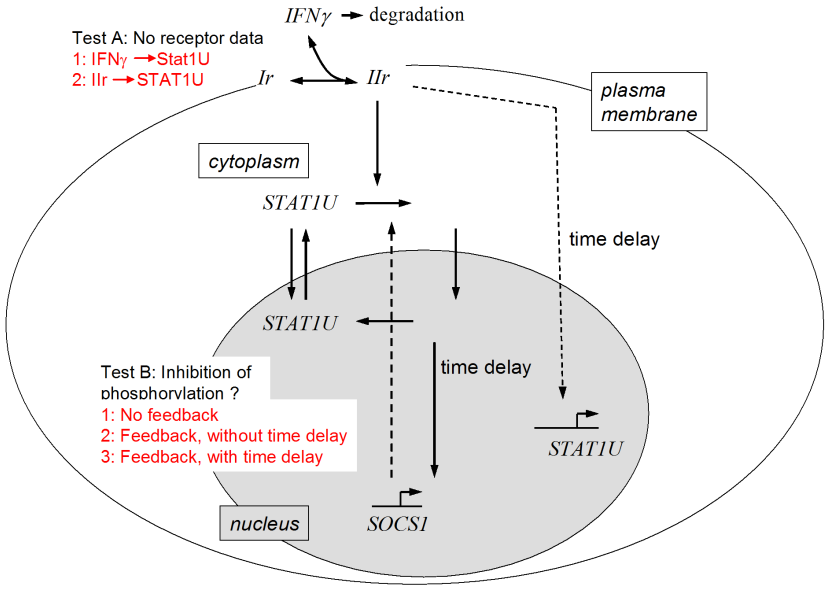

Figure 2. Reaction network of the IFNγ stimulated STAT1 signalling pathway. Modified reprint from [23] with permission from Elsevier.

So far, we have no experimental data for receptor activation. Therefore, we tested two models (Test A):

1. IFNγ mediates the phosphorylation of STAT1U
2. IFNγ reversibly binds to the interferon receptor (Ir) leading to receptor activation. The active complex is denoted by IIr.

SOCS 1 is a potential negative feed-back regulator inhibiting the phosphorylation of STAT1U [26, 27]. Our data show that levels of phosphorylated STAT1 (STAT1P) did not decrease for IFNγ = 100 ng/ml (Fig. 3B, right) despite induction of *SOCS 1* expression. This observation is compatible with two scenarios:

(1) The SOCS 1 concentration is low compared to the number of receptors. In this case, the effect of SOCS 1 on the reduction of STAT1P phosphorylation would be negligible.

(2) The negative feedback induced by SOCS 1 could be effective at late times (≥ 180 min) only, where it could reduce the slope of the late increase of STAT1P.

To further study the role of SOCS 1, we tested the following three hypotheses by mathematical modelling (Test B):

1. No feedback inhibition by SOCS 1
2. Feedback inhibition without time delay
3. Feedback inhibition with time delay

The reaction network was translated into a system of ordinary differential equations (ODE), which describe temporal changes of the network components as a function of interactions and transport processes. The delayed processes of increased STAT1U expression, *SOCS 1* transcription and hypothetical negative feedback by SOCS 1 were described by a distributed time delay. All parameters were estimated on the basis of time series for STAT1, STAT1P and *SOCS 1* mRNA by global optimisation using the fSSmb algorithm [14].

Model Simulations and Quantitative Predictions

Figure 3 shows a comparison between the experimental time series and the simulation results of the mathematical models (using optimised parameters) based on different hypotheses.

First, we compared the parameter estimates of the two models of Test A in Figure 3A: Computer simulations of the model without receptor lead to good fit for all observables (data not shown) except of a poor fit for STAT1. The poor fit indicates that the reaction rate describing STAT1 transcription is too small. Therefore we included reversible receptor activation in a revised model to get a longer persisting signal for STAT1 transcription. The parameter estimates for this model lead to a good fit for all observables including STAT1U as shown in Figure 3A.

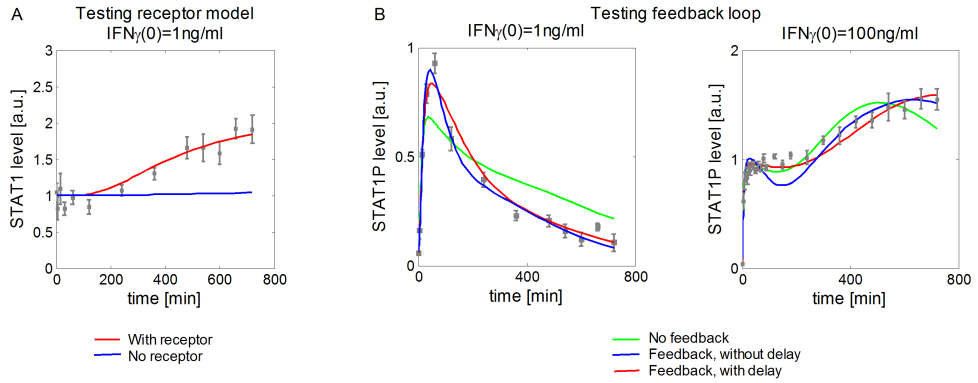

Figure 3. Comparison between (A) experimental time series and (B) computer simulations of the different models with estimated parameters. Modified reprint from [23] with permission from Elsevier.

Next, we compared the parameter estimates of the three models of Test B in Figure 3B: Our simulation results show that the model with the delayed negative feedback by Socs1 leads to the best fit of the experimental data for STAT1P compared with the models without a feedback and a non-delayed feedback, respectively.

Note, that the differences between the ‘‘best fit’’ model and the other two models of test B are small. A SOCS 1 siRNA knockdown experiment can reveal whether SOCS 1 negatively regulates STAT1 phosphorylation. If the outcome of this experiment will be negative then the identification of other interactions, for example other feedback loops or crosstalk with other signalling pathways can help to improve the model.

To validate the mathematical model with the IFNγ receptor and with the delayed negative feedback by SOCS 1, we tested its ability to predict the results of experiments not previously used for model calibration. We studied by computer simulations the influence of the stimulation scenario on the network dynamics. The simulation results for a stimulation with a single dose of IFNγ (1 ng/ml) and for multiple stimulations with smaller doses (4 x 0.25 ng/ml; applied at intervals of three hours) are shown in Figure 4. The model predictions have been experimentally validated. The experimental data are also shown in Figure 4. Even the complex staircase structure in the experimental time series of STAT1P (split-dose mode) was correctly indicated by the mathematical model. Furthermore predicted and measured slopes of increases were similar for most time points.

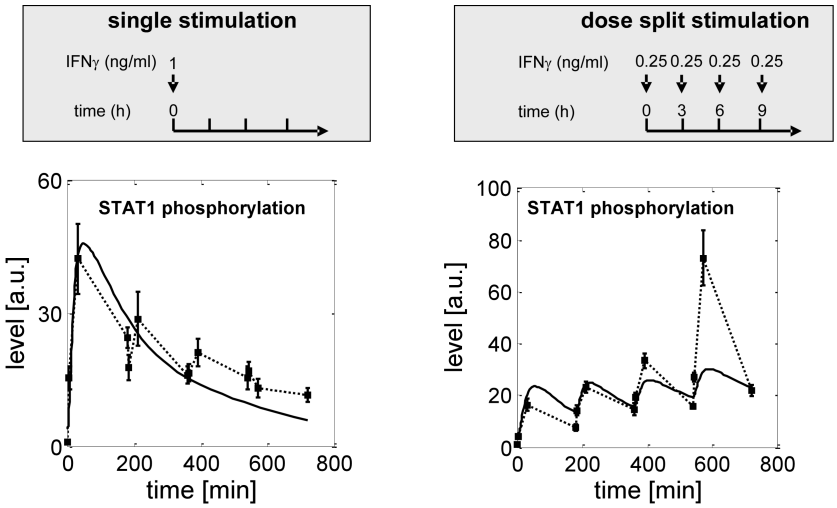

Figure 4. Model predictions and experimental validation. Reprint from [23] with permission from Elsevier.

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