

# Construction of executable *in silico* models of network dynamics based on experimental data

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## Abstract

*Keywords:*

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## 1. Introduction

## 2. Materials and Methods

Table 1 shows the measured effects of treatments with IL-1 $\beta$  and Wnt-3a on the expression of some proteins in chondrocyte donor cells. The table shows that both cytokines cause effects on the measured protein concentrations. Moreover, the combined action of the two treatments is not always the sum of the two isolated cases (see for example Sox9 and ColIIa). Finally, the transcriptional response to the treatments may vary over time (see the case of ColIIa under Wnt-3a treatment, and experimental data in Suppl. Mat.). From this we infer that the signaling pathways of IL-1 $\beta$  and Wnt-3a have some common parts, possibly upstream of their target transcription factors. As time is an important variable when considering crosstalk events, we propose to build a computational model of the dynamics of the two pathways. Such an *in silico* model should allow us to identify the key interactions in the crosstalk. Thanks to the model, we will be able to refine our current knowledge on the studied network by identifying specifically targeted laboratory experiments.

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	IL-1 $\beta$ 10 ng/ml	Wnt-3a 75 ng/ml	IL-1 $\beta$ + Wnt-3a 10 ng/ml 75 ng/ml
IL-1 $\beta$	+++	-	++
Axin	-	++	+
Sox9	+	-	-
ColIIa	-	-/+	-

Table 1: The effects of treatments with IL-1 $\beta$  and Wnt-3a on the expression of some proteins in human knee chondrocytes. Effects were measured as n-fold variations with respect to the initial concentrations, and are represented in the table as follows: + under 10 times increase; ++ more than 10 but less than 100 times increase; +++ more than 100 times increase. Similarly, - means less than 10 times decrease. We use -/+ in one case to indicate that treatment with Wnt-3a causes an initial decrease ( $\sim 0.5$ -fold) followed by an increase ( $\sim 2$ -fold) in the production of ColIIa.

### What is ANIMO?

ANIMO (Analysis of Networks with Interactive MOdeling) is a software tool that allows the biologists to virtually *play* with abstract network models. Such models are based on cause-and-effect relationships, such as “A activates B”, and their behavior is described in semi-quantitative terms. This approach requires less precise knowledge about kinetic parameters, while still enabling the user to easily investigate the dynamics of a modeled network.

The idea at the base of ANIMO is to provide experts with a means to visually represent their knowledge on biological interactions. Networks created in this way are then enriched with simplified kinetics, producing *dynamic models*. Thanks to the Cytoscape-based user interface, the visual representation of a pathway available in ANIMO closely resembles the networks commonly found in textbooks.

The network topology, equipped with informations on its dynamic behavior, is automatically translated to an underlying formal model based on Timed Automata. The formal model is used *behind the scenes* to simulate the evolution of the network. In a few seconds of computation, ANIMO presents the user with a graph plotting the levels of activation of all components in the network during the simulation period. A slider under the graph lets the user further explore the evolution of the model: moving the slider makes the coloration of the network instantly reflect the activity of all components along the simulation interval.

## **Analysis of Networks with Interactive MOdeling**

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### *2.1. Preparing experimental data for use with ANIMO*

The measurements obtained from the digital imaging analysis of the PP96 plates were stored in a spreadsheet. We will now illustrate the process through which this raw data was normalized and rescaled to make it suitable for working with ANIMO.

A spreadsheet with all the data generated by applying the following steps is available in the Supplementary Materials.

#### *2.1.1. Background subtraction*

In order to estimate the background noise in the data, three wells in each plate were kept empty (blank wells). A value of background noise was calculated for each spot as the average value of that spot in the blank wells. The background noise values were then subtracted from the data of the corresponding spots in all the other wells.

#### *2.1.2. Normalization*

As both the reference and the normalization HSP60 spots yielded inconsistent results, we decided to normalize the data grouping them by treatment condition and using one plate as reference. Plate 3 (C20A4 cell line) was chosen as reference, as it shared conditions with both the other plates (donor cells). Two normalization groups were then defined, separating the data for IL-1B treatment in plate 1 from the data for treatments with Wnt-3a and Wnt-3a

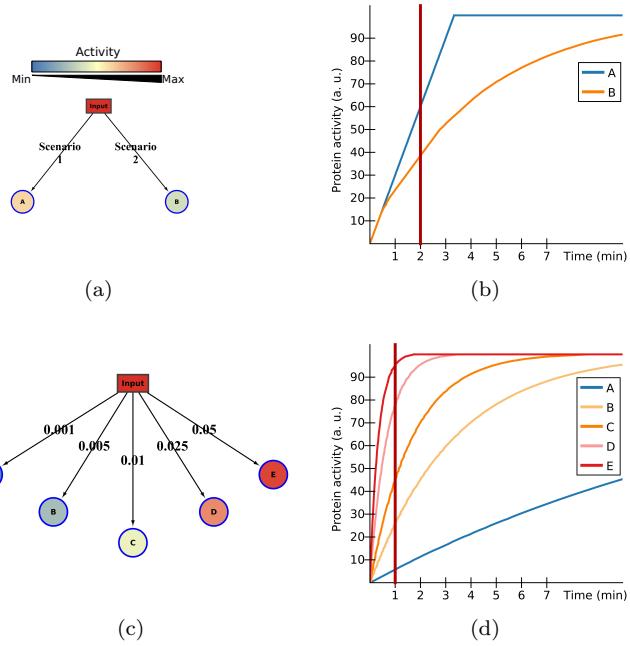


Figure 1: Example interaction settings for an ANIMO model. Each graph represents the time evolution of the network on its left. The vertical red lines in the graphs represent the point in time on which the coloration of the nodes in the corresponding network is based.

(a) The two basic scenarios. Scenario 1 makes the speed of the interaction depend only on the activity level of the upstream node `Input`. In this case the upstream node is constantly active, so the activity of A increases linearly with time. Scenario 2 depends on the activity level of the `Input` node, and on the *inactivity* of B. This makes the rate of activation of B decrease proportionally to the current activity level of B: the more B is active, the slower the activation process will occur.

(c) Choosing a value for the scaling factor  $k$ . While all interactions here are based on scenario 2, the value of their parameter  $k$  (written on the edges) determines the speed at which the downstream node is activated: a larger value of  $k$  means a faster reaction.

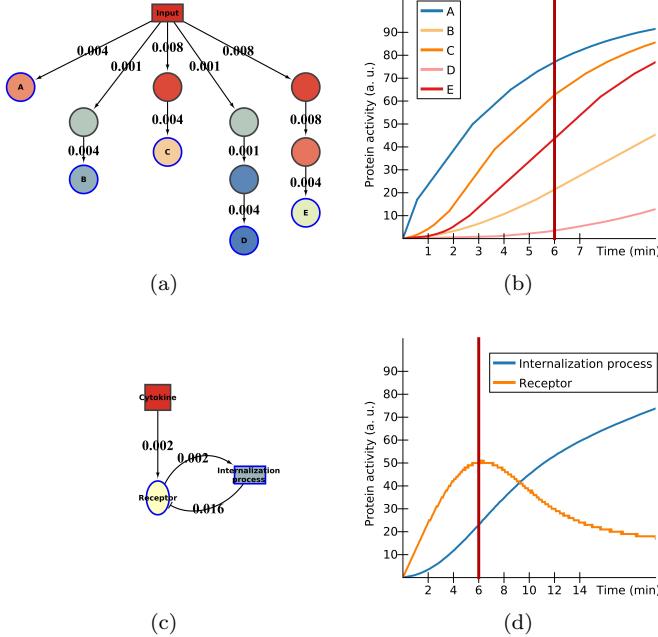


Figure 2: Example interaction settings for an ANIMO model. Each graph represents the time evolution of the network on its left. The vertical red lines in the graphs represent the point in time on which the coloration of the nodes in the corresponding network is based.

(a) Relation between network topology and delays. In order to delay the activation process ensuing an external signal (node **Input**), we can reduce the reaction speed by changing  $k$  or adding an intermediate node. Introducing a slowly activated ( $k = 0.001$ ) node between **Input** and **B** is enough to activate **B** at a much slower rate than **A**, even if the value of  $k$  for the last step is left unchanged at  $k = 0.004$ . Increasing the parameter of the newly introduced reaction lets us fine tune the speed of the response, making the activation of **C** faster than **B**, but still slower than **A**. We repeat the process of introducing a delay with nodes **D** and **E**: an additional intermediate node further reduces the response time.

(c) Peak dynamics are often observed in experimental data. In order to have the activity level of a node increase and successively decrease, the simplest way is to model it with a feed-back loop. In the example, we model the internalization of a receptor following its activation. Note that the interaction **Internalization process**  $\rightarrow$  **Receptor** is an inhibition: being based on scenario 2, it will reduce the activity of the **Receptor** node with a rate proportional to the current activity of both involved nodes. Scenario 1 dynamics are sufficient to model the two activating reactions **Cytokine**  $\rightarrow$  **Receptor** and **Receptor**  $\rightarrow$  **Internalization Process**. Key to the peak dynamics is the fact that the inactivation of the **Receptor** node is much faster (higher  $k$  value) than its activation.

+ IL-1B in plate 2. The two groups were associated to the corresponding two groups in plate 3.

For each of the five considered spots (Akt, ERK, GSK-3B, JNK1, p38), the average of the data was computed over all time points in each group. These values were then divided by the average of the corresponding data in plate 3, obtaining the normalization factor. In two cases (JNK1 and p38) the treatment with Wnt-3a was not considered for the computation of the normalization factor, as the response in that case was considerably lower than with the other two treatments.

At the end of this process, each of the five spots in plate 1 and plate 2 had their own normalization factor, while all factors for plate 3 were equal to 1. Each data point was then divided by the corresponding normalization factor.

#### *2.1.3. Data scaling*

As ANIMO models are semi-quantitative, it is not required to precisely estimate the molecular concentrations in experimental data. For this reason, we rescaled all time series on a 0-100 interval, dividing all the values by their maximum over a normalization group. For instance, the scaled value for ERK in plate 2 is obtained dividing each normalized value by the maximum normalized value of ERK over the two treatment conditions Wnt-3a and Wnt-3a + IL-1B in plate 2.

#### *2.1.4. Average series*

As the experiments were done in triplicate, three time series are available for each treatment and spot. The time series we use as reference in our ANIMO models contain average and standard deviation values over the triplicate values.

### **3. Results**

### **4. Discussion**

### **5. Conclusions**