# Design and analysis of dynamical models of biochemical reaction networks

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## Introduction and objective

Molecular Cell Biology is undergoing a revolution, thanks to great improvements in data acquisition. High-throughput instrumentation enables us to observe in great details the inner machinery of the cell. Traditional biological approaches, trying to understand individual components basic properties prove themselves very limited to uncover these mechanisms. Quantitative systems biology is trying to tackle this challenge, in building dynamic models of biological systems, and exploiting them to uncover hidden dynamical mechanisms.
We developed SigNetSim, a framework to design and analyze biological dynamic models. This software uses the SBML standard to describe models, and allows the use of heterogeneous omics data into the modeling and validation processes. Moreover, we also provide tools to analyze the various dynamical properties of these models. This software will soon become part of the CeTICSdb platform, a collection of tools to store and analyse biological data produced in our laboratory.
As an example of application of this framework, we studied the SOS/Ras signaling pathway activation upon FGF2 treatment in mouse Y1 tumor cells.

## Materials and methods

To model, simulate and analyze the kinetics of biochemical reaction networks, we are developing the SigNetSim framework (Fig. 1). SigNetSim is a Python library, with both a command-line interface, a graphical interface, and a web interface (Fig. 2). It currently allows:

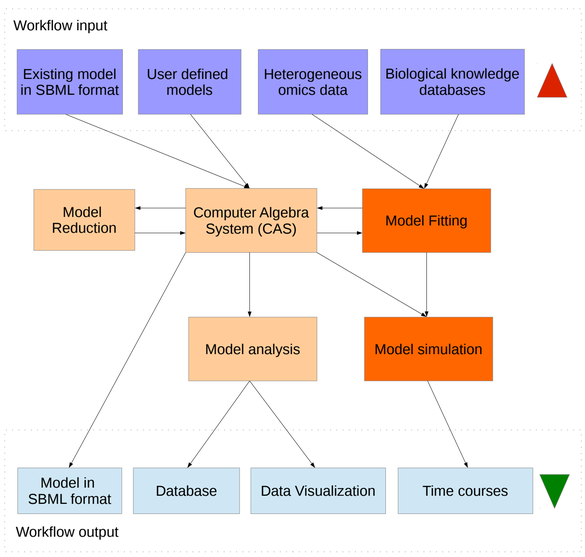



*Fig. 1: Workflow of the SigNetSim framework. Components in light orange are coded in Python, Components in dark orange are coded in C*

1. Description of the network through the standard SBML or through a list of reactions, including their respective parameters.
2. Mapping of the biological model to a system of differential algebraic equations, represented in a Computer Algebra System
3. Simulating the model using the SUNDIALS library
4. Fitting the model to experimental data, using parallel Simulated Annealing. It can notably use different experiments to fit the same model.
5. Model dynamical analysis, which might give predictions regarding untested experimental conditions (bifurcation analysis, parameter confidence estimation, ...).

This framework only uses free/open-source libraries, it will also be released under GPL license in the next future.

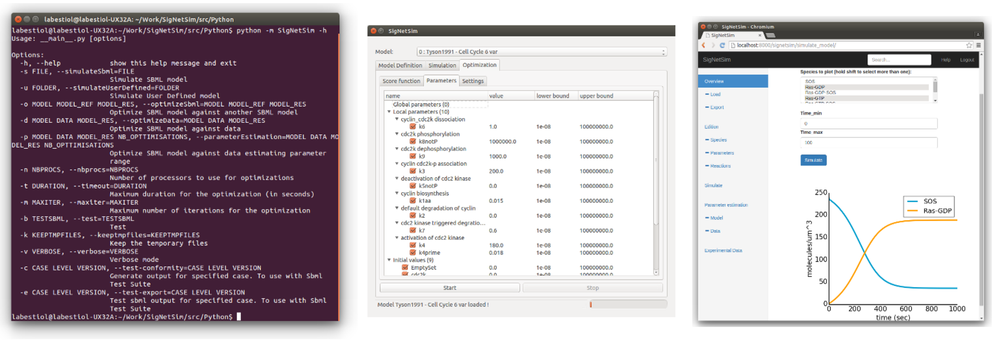


*Fig. 2: Interfaces for the SigNetSim library. Left: Command-line interface, Center: Graphical User interface (QT), Right: Web interface (Django)*

## Conclusions and future work

- This framework is still at its early stages, but already provide sufficient methods to build quantitative biological models. The results showed that this methodology might uncover novel mechanisms of biochemical reactions.
- We are presently adding new features to SigNetSim, including model reduction techniques, parameter analysis, access to biological databases, ...
- We are using SigNetSim to perform analysis in the biological data requirements, both in quantity and accuracy of data, in order to produce guidelines for experimental planning.
- We are also developing new methods to reduce analysis complexity, based on multi-resolution approximation methods.

## References

[1] J. Das, M. Ho, J. Zikherman, C. Govern, M. Yang, A. Weiss, A.K. Chakraborty, and J.P. Roose. Digital signaling and hysteresis characterize Ras activation in lymphoid cells. Cell 136.2 (2009): 337-351.
[2] E.T. Costa, F.L. Forti, T.G.F. Matos, A. Dermargos, F. Nakano, J. Salotti, K.M. Rocha, P.F. Asprino, C.K. Yoshihara, M.M. Koga, and H.A. Armelin. Fibroblast growth factor 2 restrains Ras-driven proliferation of malignant cells by triggering RhoA-mediated senescence. Cancer Research 68.15 (2008): 6215-6223.
[3] B. N. Kholodenko. Negative feedback and ultrasensitivity can bring about oscillations in the mitogenactivated protein kinase cascades. European Journal of Biochemistry, 267.6 (2000), 1583-1588.

## Example: Ras signalling pathway module in mouse Y1 tumor cells

To construct a model for FGF2 treatment for the Ras signaling pathway module in Y1 cells, we started with the SOS/Ras model of Das et al. [1] (Fig. 3(a)). At first, this model couldn't reproduce an observation from our laboratory, regarding the steady-state level of activated K-Ras (Ras-GTP) on starved cells, implying that the model of Fig. 3(a) misses important components of the Y1 system.

To fix the model, we included an additional GEF, whose presence was unknown in this cell line, and carried out new numerical simulations (Fig. 3(a)). This tentative hypothesis led to simulated results showing high steady state basal levels of Ras-GTP (Fig. 3(b)) in good agreement with published experimental data [2]. To experimentally validate our hypothesis, we probed Y1 cells for expression of additional GEFs by RT-PCR, confirming the expression of two additional GEFs.

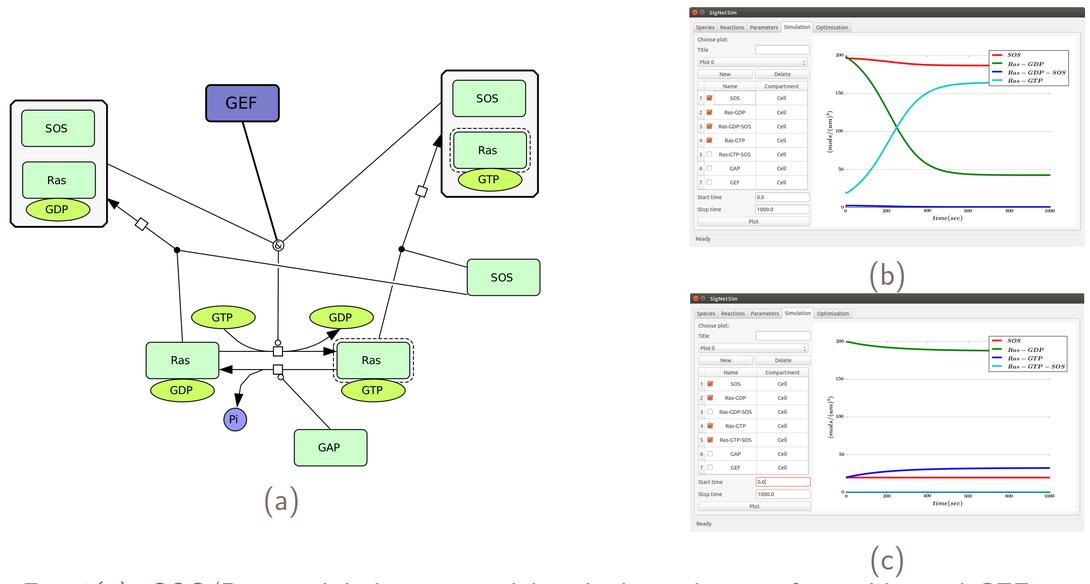



*Fig. 3(a): SOS/Ras module kinetic model with the inclusion of an additional GEF. Fig. 3(b): numerical simulation of Ras-GTP levels upon FGF2 treatment. Fig. 3(c): numerical simulation of Ras-GTP basal levels.*

Our team then performed time series measurements for Ras-GTP using enzyme-linked immunosorbent assay (ELISA), in order to better understand the dynamics and improve our model. These time series were performed on the same two conditions, starved and upon FGF2 treatment. The curve fitting is performed using the multiples scoring function algorithm. While we mostly succeeded in fitting the model (Fig. 4), we have to improve the mathematical description of SOS deactivation to explain the observed data.

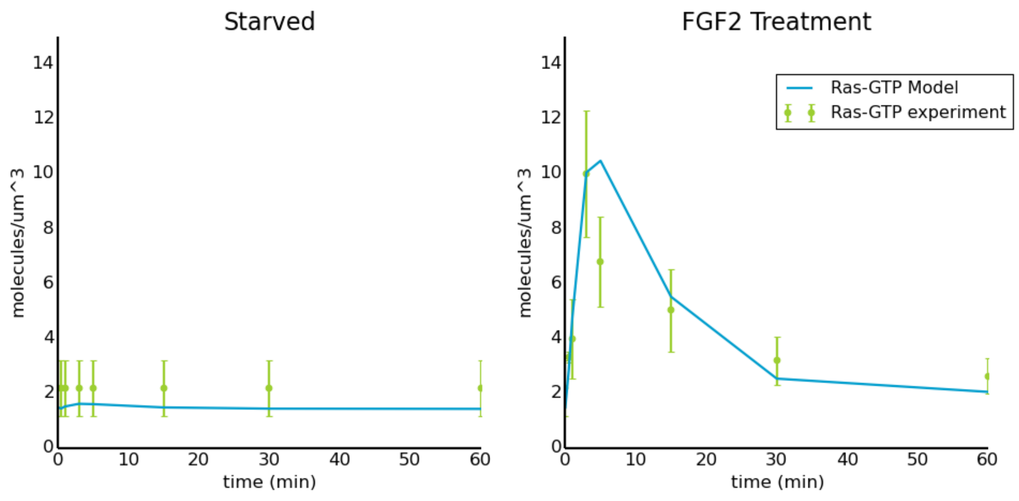



*Fig. 4: Ras-GTP fitting result for SOS/Ras model in Y1 tumor cells. Left: Starved cells, Right: Upon FGF2 treatment*

This work shows a classic use of SigNetSim as a modeling framework. The multiples conditions fitting enables us here to test various conditions, and to include them easily in a single model.

- Our prediction about the action of other GEFs to maintain a basal activity of Ras will be tested further using CRISPR.
- Our next step in developing the model will be including the MAPK model from Kholodenko [3], using data from a time series of ERK activity. This extended model will contain an improved SOS deactivation control through ERK.
- We will also be investigating in more detail the dynamics of the SOS activation by FGF2.
- We are also verifying the implications of the addition to the model of RasN17, a dominant negative mutant.
- Another goal is to study the cell cycle progress in Y1 and other cell lines (e.g., in E6E7-immortalized keratinocytes) through the development of hybrid models that encompass the Ras/MAPK and PI3K/Akt pathways, gene regulatory networks, and transition signaling kinases.

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