SigNetSim: an e-Science framework to assist the design of kinetic models of molecular signaling network

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Introduction and aim

One of the current major challenge in Molecular Cell Biology is to properly analyse the big data yielded by modern high-throughput omics techniques (e.g. genomics, transcriptomics, proteomics). Classical biology intuition and qualitative, static interactome schemes are no longer sufficient to study underlying biological mechanisms from such huge amount of data. On the other hand, computational approaches are suitable to tackle this and other timely goals of nowadays biological research.

To this end, e-Science is an emergent discipline within Computational Sciences focusing on developing theories and tools to allow biological scientific investication on a computationally intensive environment. Therefore, the objective of this work is the development of SigNetSim, an e-Science framework to assist mathematical modeling and computational analysis of molecular signaling network kinetics.

This framework will allow the usage of heterogeneous omics data into modeling and validation processes. It will also allow advanced dynamical analysis, like model simplification, parameter confidence estimation, bifurcation analysis among others.

As an example of use of this framework we built a model of the SOS/Ras signalling pathway, and its crosstalk with MAPK and Akt/PI3K pathways, in the context of Y1 cells. We used data generated by our team, presented below (Fig. 1), to fit this model.

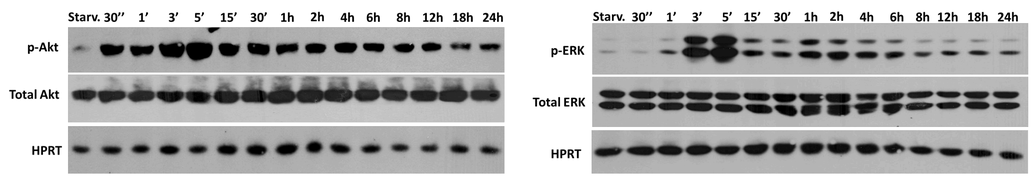


Fig. 1: Western blots of ERK1/2 and Akt1. Points were taken during a 24-hour period after stimulation of starved Y1 cells with FGF2 factor. These results are representative of two biological replicates. Fig. 1(a): phosphorylated Akt1 (top) and total Akt1 (middle). Fig. 1(b): phosphorylated ERK1/2 (top) and total ERK1/2 (middle). In both experiments, HPRT was used as loading control (bottom).

Materials and methods

To model, simulate and analyze the kinetics of enzymatic signaling networks, we developed the SigNetSim framework (Fig. 2). SigNetSim has a user-friendly graphical user interface and allows:

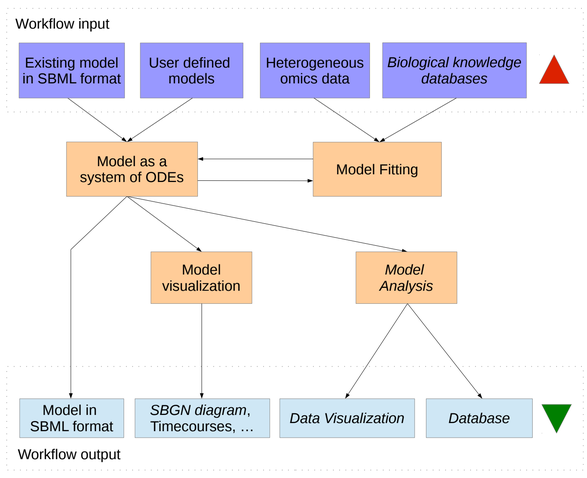


Fig. 2: workflow of the SigNetSim framework. Components under development are in italic.

1. Description of the network through the standard SBML or through a list of reactions, including their respective parameters.
2. Mapping of the reactions into system of ordinary differential equations.
3. Optimization (model-fitting analysis), which modifies the parameters to match experimental observations.
4. Model dynamical analysis, which gives predictions regarding untested experimental conditions and investigates potential therapeutic targets.

Conclusions and future work

- The results showed that this methodology might uncover novel mechanisms of signaling networks, particularly within the context of Ras/MAPK and PI3K/Akt pathways in the mouse Y1 adrenocortical tumor cell line.
- The SigNetSim framework will be improved with the inclusion of new components (e.g., computation of parameter confidence ranges) and also will be integrated with CeTICSdb, a platform under development, to use high-throughput omics data in the modeling process [4].
- Another goal is to study the cell cycle progress in Y1 cells. To this end, we will develop a hybrid model that encompasses the Ras/MAPK and PI3K/Akt pathways, gene regulatory networks, and transition signaling kinases.
- We also plan to integrate into SigNetSim access to biological knowledge databases, in order to import kinetic constants, modules, and so forth.

Acknowledgements

References

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Results

To construct a model for the Ras cycle module in Y1 cells, we started with the SOS/Ras model of Das et al. [2] (Fig. 3(a)). Rate constants and initial concentrations were adjusted using additional data from unpublished results of our laboratory. Our initial simulations showed that changes in the parameters of K-Ras, GTPase-activating proteins (GAPs) and SOS1/2 (a guanine exchange factor – GEF) could not simulate the [K-Ras-GTP] high basal levels underlying the malignant phenotype of Y1 cells (Fig. 3(b)–3(c)), implying that the model of Fig. 3(a) misses important components of the Y1 system.

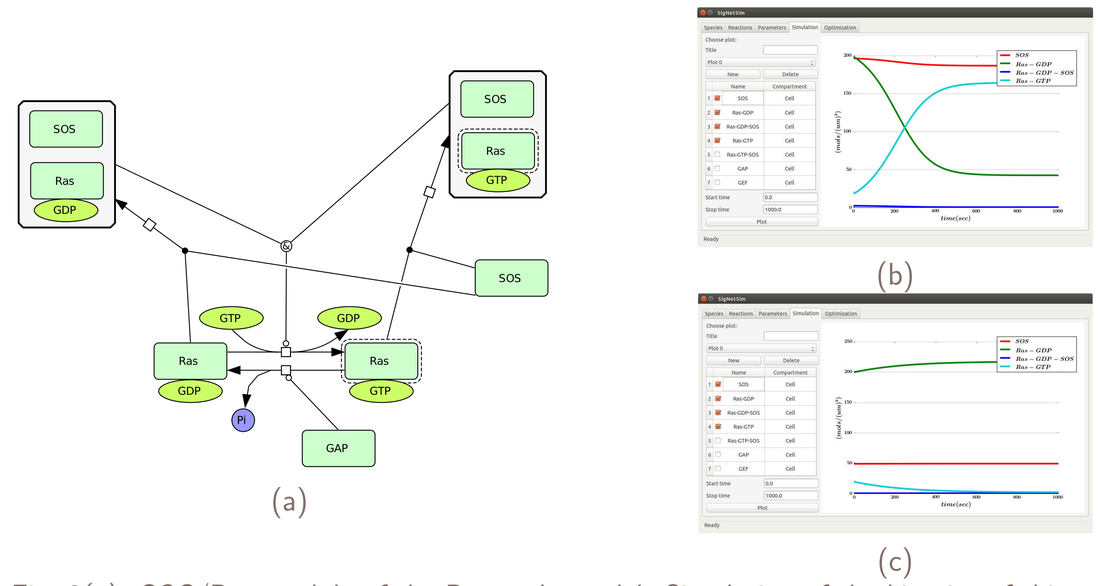


Fig. 3(a): SOS/Ras module of the Ras cycle model. Simulation of the kinetics of this model showed that the relatively high [K-Ras-GTP] basal levels could not be reproduced, since during the simulation it either decreased to levels close to zero (Fig. 3(b)) or went to an activated state which should not occur without FGF2 stimulation (Fig. 3(c)).

To fix the Fig. 3(a) model, we included an additional GEF, whose presence was unknown in this cell line, and carried out new numerical simulations (Fig. 4(a)). This tentative hypothesis led to simulated results showing high steady state basal levels of [K-Ras-GTP] (Fig. 4(b)) in good agreement with published experimental data [3]. 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. 4(c)).

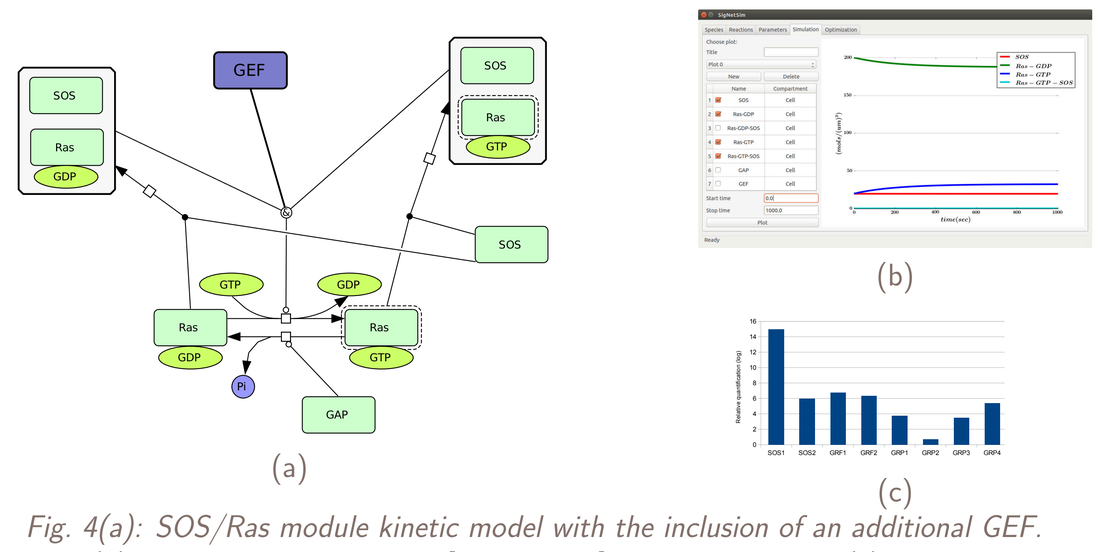


Fig. 4(a): SOS/Ras module kinetic model with the inclusion of an additional GEF. Fig. 4(b): numerical simulation of [K-Ras-GTP] basal levels. Fig. 4(c): histogram of relative abundance of GEFs in Y1 cells. Apart from SOS1/2, two additional GEFs are expressed in Y1 cells, namely RasGRF1/2 and RasGRP1/2/3/4.

Finally, we employed the adjusted SOS/Ras module in a more comprehensive model, in which the crosstalk between SOS/Ras, MAPK and PI3K/Akt pathways is described (Fig. 5(a)). Optimization of the parameters and curve-fitting analysis are in progress: so far, they yielded results that are gradually converging to the observed kinetic data of Fig. 1 (Fig. 5(b)–5(c)).

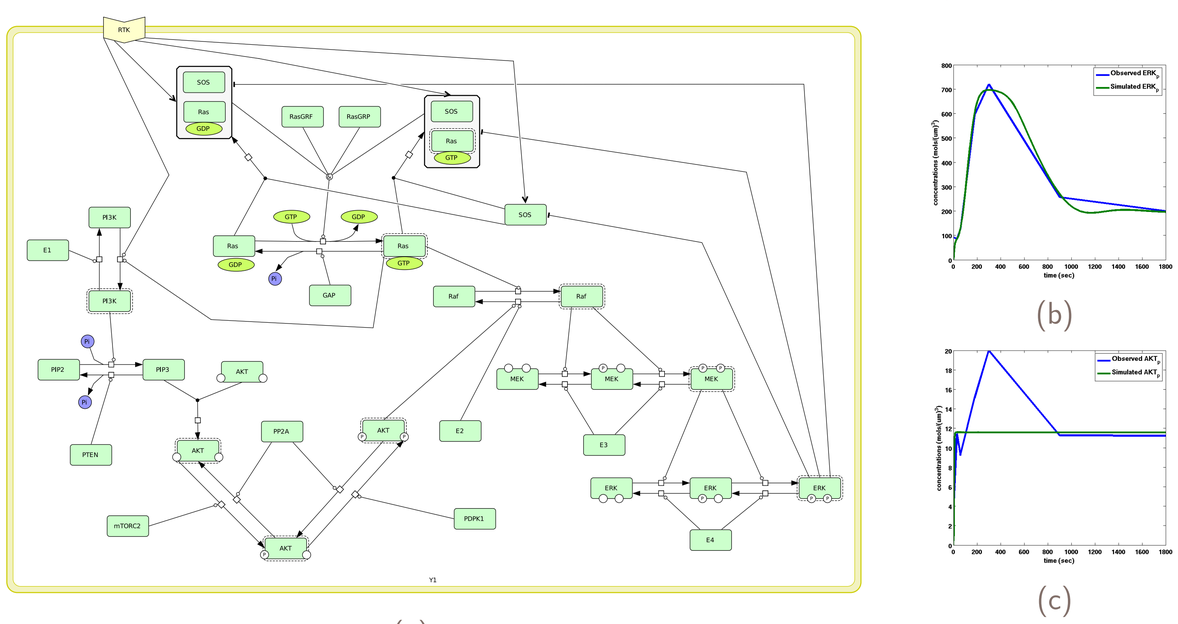


Fig. 5(a): crosstalk between Ras/MAPK and PI3K/Akt pathways. Fig. 5(b)–5(c): half-hour simulation of this model with the optimization performed so far; levels of pERK (Fig. 5(b), green line) approach the quantification of the Western blot data showed in Fig. 1(b) (Fig.5(b), blue line). However, the simulated pAkt levels (Fig.5(c), green line) only reproduces the high constitutive levels depicted in Fig. 1(a) (Fig.5(c), blue line).

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