

## A Case Study Representing SIGNAL TRANSDUCTION IN LIVER CELLS As a Feedback Control Problem

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Systems research has undergone significant changes in recent years due to the inclusion of new applications for control—*e.g.*, microelectronics manufacturing or drug dosage adjustment for biomedical applications—and the use of systems concepts in new areas such as systems biology. As the results from research tend to influence what is taught in a classroom and vice versa, it is very important to have access to illustrative examples that can easily be presented to undergraduate students without requiring an advanced background in the systems area.

There are many applications in the field of drug infusion control<sup>[1-3]</sup> that have been developed and used in classroom example problems. The selection of examples from the field of systems biology, however, is much more limited. This is despite it being widely recognized that feedback loops are common to many cellular functions.<sup>[4-8]</sup> This paper addresses these points as it investigates a signal transduction pathway involved in the body's response to inflammation or injury, one of many areas of interest to systems biology. While the described system is of interest to the biomedical community, it is simple enough to be presented in an undergraduate class and contains feedback regulation of the signal transduction pathway. Additionally, the system can be appropriately described using block diagrams and transfer function models for perturbations around a steady state.

The outline of the paper is as follows: Section 1 is an introduction. Section 2 presents the biological significance of



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the system and describes the model representing the signal transduction pathway. A block diagram representation of the signal transduction pathway is developed in Section 3. Section 4 presents the transfer functions that describe the effect concentrations of some proteins in the pathway have on other proteins in the pathway and investigates the dynamic behavior of the signal transduction pathway. Furthermore, the dynamics of cells in which the regulatory mechanism does not function properly, as is often associated with certain types of cancer,<sup>[6]</sup> are investigated based upon the developed transfer function model and compared to the behavior of the original system. Section 5 presents how this model was used within an undergraduate process dynamics and control class taught at Texas A&M University, and Section 6 presents some conclusions.

## TARGET SYSTEM

Cell signaling refers to the process by which cells sense their environment, including communication with other cells. Signaling in cells is initiated by extra-cellular molecules that activate an intracellular signaling pathway, which ultimately leads to the formation of proteins involved in basic cellular processes like regulation of cell growth and division or expression of other, secreted proteins. This entire process in which biological information is transferred from extra-cellular signals into changes inside a cell is referred to as signal transduction. As malfunction of signaling pathways can be associated with some diseases, *e.g.*, certain types of cancer, cells usually have regulatory mechanisms built into signal transduction pathways.

The system under investigation in this paper deals with signaling pathways involved in a body's response to burn-injury-induced inflammation. The injured cells release cytokines, one of which is interleukin 6 (IL-6), to the bloodstream. These cytokines are sensed by hepatocytes in the liver, and they activate the acute phase response (APR). The acute phase response up- or down-regulates the expression of certain plasma proteins that take part in the body's response to the burn-injury-induced inflammation. Investigating cell signaling in hepatocytes stimulated by inflammatory agents is of crucial importance to understanding the mechanisms underlying the APR.

The specific topic of this paper is the development of a transfer function model of the JAK (Janus-Associated Kinases)/STAT (Signal Transducers and Activators of Transcription) signaling pathway in hepatocytes stimulated by IL-6.<sup>[9-10]</sup> Sig-

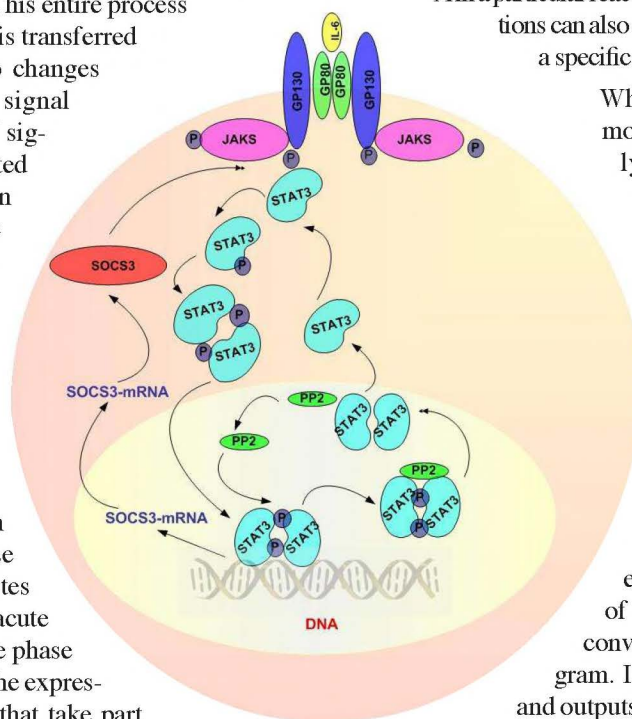
aling through the JAK/STAT pathway is regulated by SOCS3 (Suppressors Of Cytokine Signaling 3) proteins. These proteins are induced by the JAK/STAT signaling pathway once the signal emanating from the cell surface reaches the nucleus of the cell. SOCS3 regulates further signaling from the cell surface to the nucleus of the cell by inhibiting the activation of STAT3, a process that is usually taking place as a result of binding of IL-6 to the receptors on the cell surface.

## BLOCK DIAGRAM REPRESENTATION

The system under investigation is based upon the JAK/STAT pathway of the model presented in Singh, et al.,<sup>[11]</sup> and is shown in Figure 1. The model of the JAK/STAT pathway consists of 33 ordinary differential equations, in which each state corresponds to the concentration of a particular protein or protein complex in either the cytosol or the nucleus. It is assumed that the cytosol is "well-mixed" and, separately, that the nucleus can also be viewed as "well-mixed." The differential equation for a particular component (A) is written as:

$$\frac{dN_A}{dt} = \sum v_{A, \text{produced}} - \sum v_{A, \text{consumed}} \quad (1)$$

where  $v_A$  represents the rate of production/consumption of species A in a particular reaction. It should be noted that these reactions can also include formation and degradation of a specific protein/protein complex.



While the availability of the detailed model can have advantages for analyzing the dynamic concentration profiles of some specific components of the system, *e.g.*, dynamics of phosphorylated STAT3 outside of the nucleus, it is not always required, nor is it necessarily always feasible, to model every single component of the system. Instead, it is important to know the dynamic profiles of certain key components and the effect a change in the concentration of one component has on others present in the system. This type of cause-effect relationship can be conveniently represented in a block diagram. If the relationships between inputs and outputs can be appropriately described by

linear ordinary differential equations, then transfer functions can be derived that capture the input-output behavior of the individual components of the system.

These transfer functions are determined by investigating individual cause-effect relationships in which step inputs are

**Figure 1. (above)** JAK/STAT signaling pathway induced by IL-6 in hepatocytes.

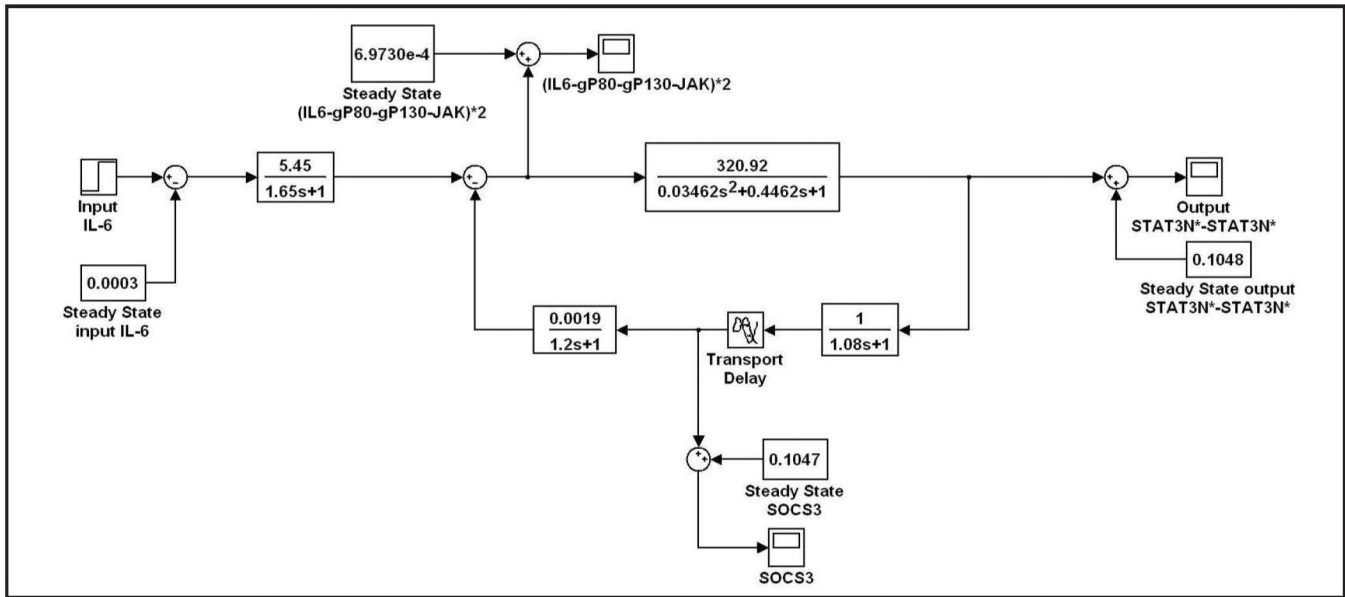


Figure 2. Block diagram representation of signaling pathway implemented in Simulink.

used to excite the system. It is then possible to derive the transfer function by numerically determining parameters, such that the difference between the response of the nonlinear model and the transfer function model is minimized.

The following dynamic relationships were identified as important for describing signaling through the JAK/STAT pathway:

- ▶ Effect of IL-6 concentration on the receptor complex concentration
- ▶ Effect of changes in the concentration of the receptor complex on concentration of STAT3 in the nucleus
- ▶ Effect of concentration of nuclear STAT3 on concentration of formed SOCS3
- ▶ Effect of concentration of SOCS3 on the receptor complex concentration that can participate in cell signaling

The last of these four dynamic relationships is responsible for the feedback effect in the pathway. An illustration of the block diagram can be found in Figure 2.

It should be noted that an increase in IL-6 concentration will lead to an increase in receptor complexes that participate in signaling, and an increase in the number of receptor complexes will also lead to more signaling and a larger amount of nuclear STAT3. More nuclear STAT3 will lead to increased transcription and translation of the plasma proteins involved in the APR, while at the same time it leads to the formation of higher levels of SOCS3. SOCS3, on the other hand, has a negative effect on the activity in the pathway as it prevents phosphorylation of STAT3 by binding to the receptor complexes.

The concentration of IL-6 is used as the input for the system and the concentration of nuclear STAT3 is used as the output of the model.

## SIMULATION STUDIES

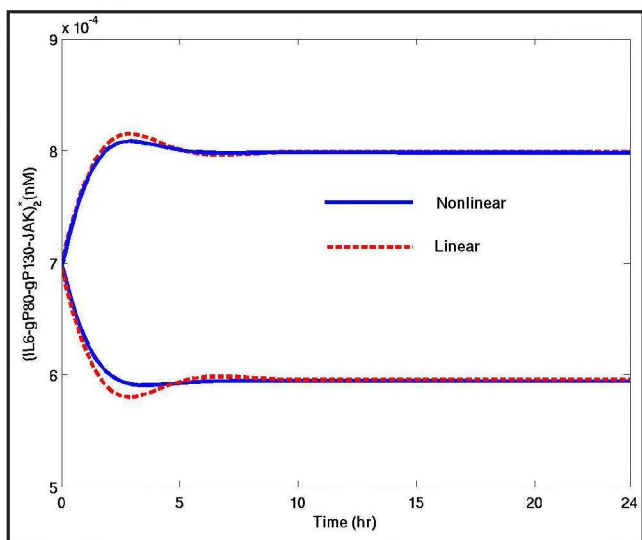
In order to identify the transfer functions, the cell is assumed to be at steady state with a constant input of  $3.0 \times 10^{-4}$  nM of IL-6, resulting in a concentration of the phosphorylated receptor complex  $(\text{IL6-gp80-gp130-JAK}^*)_2$  of  $6.973 \times 10^{-4}$  nM, a concentration of SOCS3 of 0.1047 nM, and a concentration of the nuclear STAT3 dimer  $(\text{STAT3N}^*-\text{STAT3N}^*)$  of 0.1048 nM. The cell is perturbed from the steady-state by a step change of  $\pm 10\%$  in the concentration of IL-6, which serves as the input to the system. The obtained output trajectories are used for identification of the following transfer functions:

$$G_1 = \frac{(\text{IL6-gP80-gP130-JAK}^*)_2}{\text{IL-6}} = \frac{5.45}{1.65s+1}$$

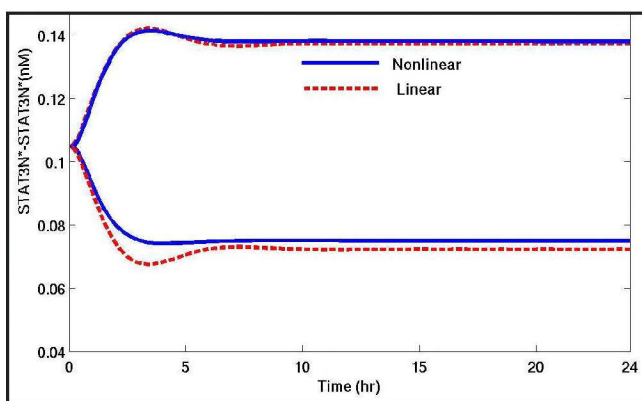
$$G_2 = \frac{\text{STAT3N}^*-\text{STAT3N}^*}{(\text{IL6-gP80-gP130-JAK}^*)_2} = \frac{320.92}{0.03462s^2 + 0.4462s + 1}$$

$$G_3 = \frac{\text{SOCS3}}{\text{STAT3N}^*-\text{STAT3N}^*} = \frac{e^{-0.6s}}{1.08s+1}$$

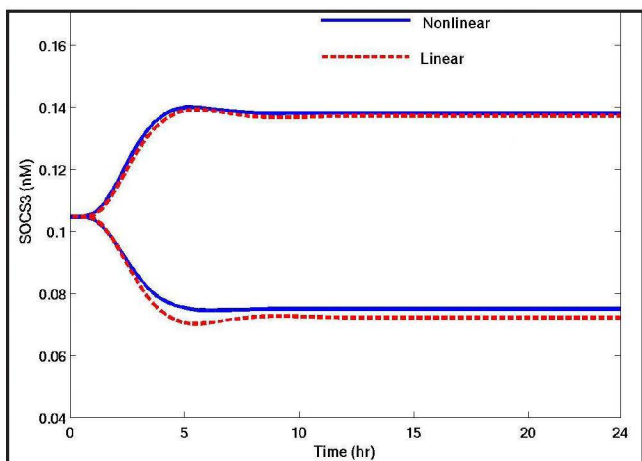
$$G_4 = \frac{(\text{IL6-gP80-gP130-JAK}^*)_2}{\text{SOCS3}} = \frac{0.0019}{1.2s+1}$$



**Figure 3.** Dynamic response of  $(IL6-gp80-gp130-JAK^*)_2$  complex for  $\pm 10\%$  step change in the IL-6 concentration around the steady state ( $0.5 \text{ pM}$  IL-6 concentration).



**Figure 4.** Dynamic response of  $STAT3N^*-STAT3N^*$  complex for  $\pm 10\%$  step change in the IL-6 concentration

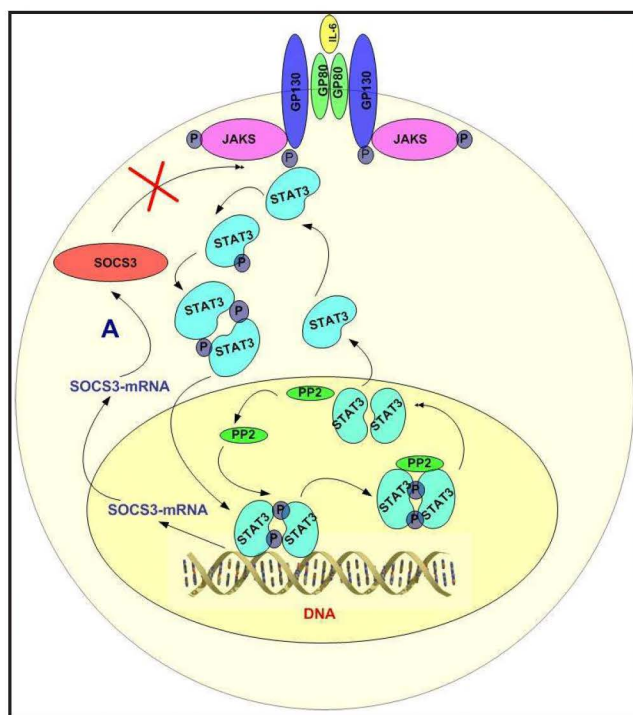


**Figure 5.** Dynamic response of SOCS3 for  $\pm 10\%$  step change in the IL-6 concentration about the steady state ( $0.5 \text{ pM}$  IL-6 concentration).

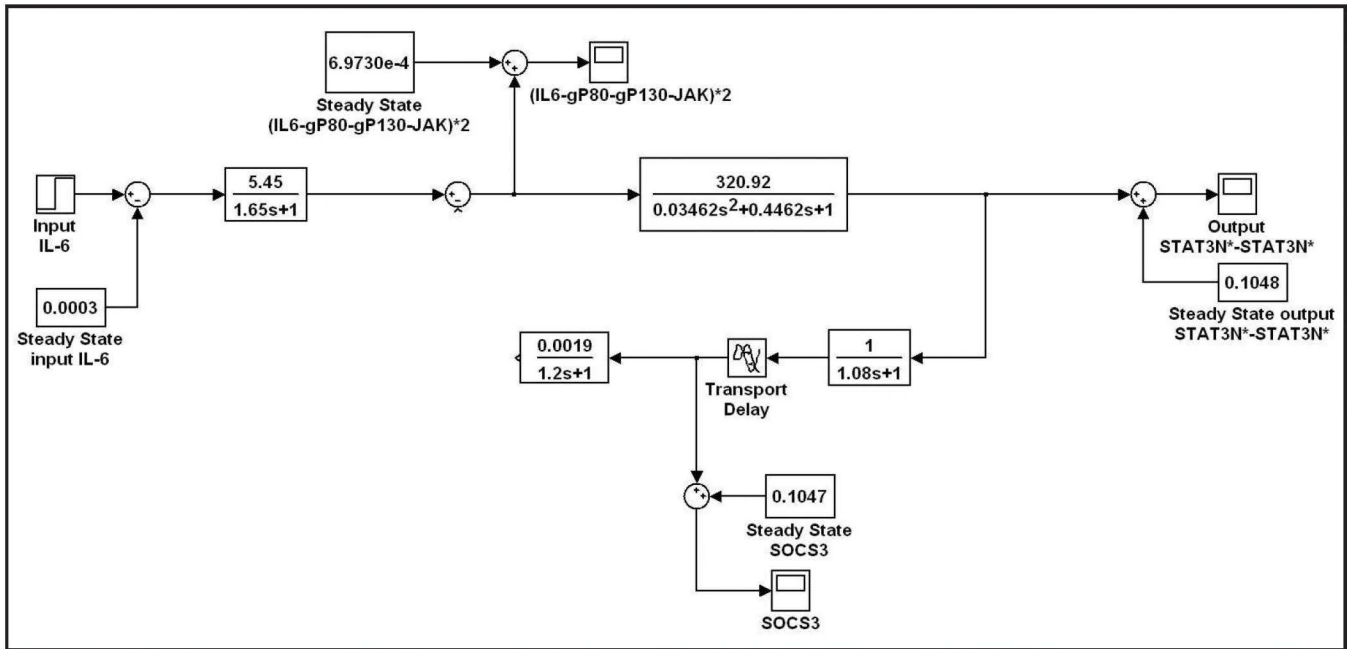
A comparison of the response of the original nonlinear system and the one obtained from the transfer function model is shown in Figures 3, 4, and 5. It can be concluded that the linear transfer function model can adequately represent the behavior of the original (nonlinear) system. It should be noted that this first set of simulation experiments was performed for the sole reason of determining the quality of the fit of the transfer function models to the response generated by the nonlinear system. It is also important to keep in mind that the linear approximation, resulting from the use of transfer functions, will only be able to represent the original nonlinear system for excitations near the conditions for which the linear model was derived.

A second experiment was run using the identified transfer function model. For these simulations, it was assumed that the effect of SOCS3 on the phosphorylation of STAT3 had been removed from the cell, as shown in Figure 6, and in the block diagram, shown in Figure 7. This effect is similar to a SOCS3 knockout cell where SOCS3 is not produced, which has medical significance associated with certain types of cancers. The only difference between a SOCS3 knockout cell and the behavior simulated here is that the feedback part is cut open after the formation of SOCS3 instead of before.

It can be observed from Figure 8 and Figure 9 that the signal is not down-regulated due to the absence of the effect of SOCS3 on the system. The receptor complex  $(IL6-gp80-gp130-JAK^*)_2$  (Figure 8) and the nuclear STAT3 dimer



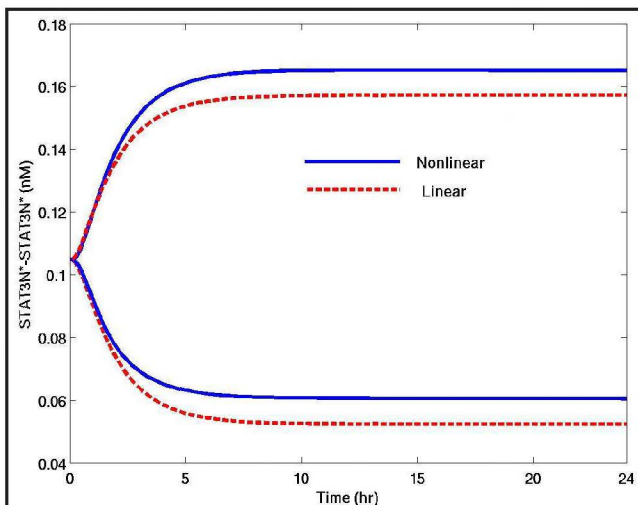
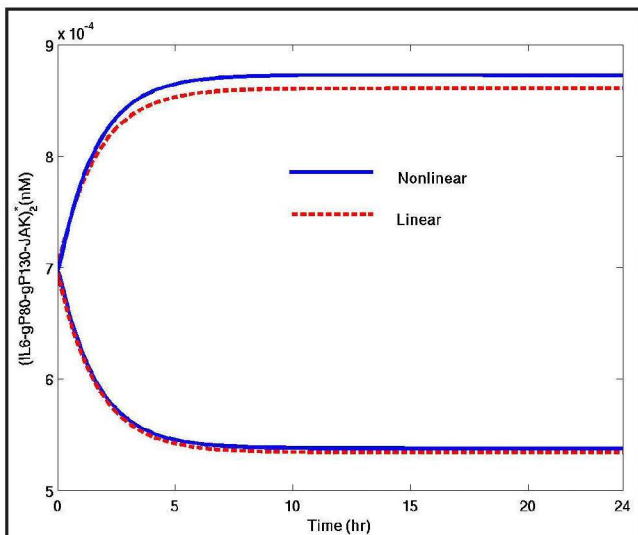
**Figure 6.** JAK/STAT signaling pathway induced by IL-6 in cells where the effect of SOCS3 on phosphorylation of STAT3 has been removed.



▲ **Figure 7. (above)** Block diagram of the “open-loop” signaling pathway implemented in Simulink.

◀ **Figure 8. (left)** Dynamic response of  $(IL6-gp80-gp130-JAK^*)_2$  complex for  $\pm 10\%$  step change in the IL-6 concentration around the steady state (0.5 pM IL-6 concentration) in SOCS3 knockout cells.

▼ **Figure 9. (below, left)** Dynamic response of  $STAT3N^*-STAT3N^*$  for  $\pm 10\%$  step change in the IL-6 concentration around the steady state (0.5 pM IL-6 concentration) in SOCS3 knockout cells.



( $STAT3N^*-STAT3N^*$ ) (Figure 9) show a larger deviation from the steady-state value when compared to the closed-loop responses shown in Figures 3 and 4. Moreover, the comparable open-loop response from the nonlinear and the transfer functions indicate that cell behavior can, locally, be adequately described by the transfer function model.

## MODEL USE IN THE PROCESS DYNAMICS AND CONTROL COURSE AT TAMU

The presented model has been used at several points throughout the Process Dynamics and Control course taught in the chemical engineering department at Texas A&M University:

- 1) It is used during the first week of the semesters when different systems that include feedback control are introduced to make the students aware of how often they come in contact with such systems.
- 2) The model is revisited when the material about deriving linear transfer functions from data is covered. In this specific case the data is generated by the original

nonlinear model whereas the linear transfer functions represent the model to be fit to this data.

- 3) Since the model contains negative feedback regulation, it is also used when the effect of negative feedback control on a system is discussed.

Using the same example throughout the semester allows students to participate in several steps of modeling and model validation, rather than just performing individual tasks. Also, this model describing a signal transduction pathway is used alongside models teaching traditional chemical engineering processes.

## CONCLUSIONS

This paper presented a case study in which a signal transduction pathway was represented as a block diagram, and linear transfer function models were identified in individual blocks for perturbations of the model around a steady state.

The system behavior was broken up into four components, and each part represented the effect a change in the concentration of one component has on others present in the signal transduction pathway. This was illustrated in how SOCS3 serves as an inhibitor of the signal transduction pathway, and how the effect SOCS3 has on the signaling activity can be appropriately described by negative feedback in the block diagram representation of the system.

Also shown was how the identified model correctly represented the behavior of the original system for the three key components chosen. Simulation studies have been performed on SOCS3 knockout cells, which can be compared to the "open-loop" behavior of the system, as there is no effect of SOCS3 on the signal transduction pathway. It was found that our identified model appropriately described the behavior of the SOCS3 knockout cell in this way.

The presented case study can serve as an example for illustrating feedback regulation in cell signaling for process control education.

## ACKNOWLEDGMENT

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## REFERENCES

1. Parker, R.S., and F.J. Doyle, "Control-Relevant Modeling in Drug Delivery," *Advances in Drug Delivery Reviews*, **48**, 211 (2001)
2. Rao, R.R., C.C. Palerm, B. Aufderheide, and B.W. Bequette, "Automated Regulation of Hemodynamic Variables," *IEEE Engineering in Medicine and Biology Magazine*, **20**(1), 24 (2001)
3. Hahn, J., T. Edison, and T. F. Edgar, "Adaptive IMC Control for Drug Infusion for Biological Systems," *Control Engineering Practice*, **10**(1), 45 (2002)
4. Asthagiri, A.R., and D.A. Lauffenburger, "A Computational Study of Feedback Effects on Signal Dynamics in a Mitogen Activated Protein Kinase (MAPK) Pathway Model," *Biotechnology Progress*, **17**, 227 (2001)
5. Bhalla, U.S., and R. Iyengar, "Emergent Properties of Networks of Biological Signaling Pathways," *Science*, **283**, 381 (2001)
6. Freeman, M., "Feedback Control of Intracellular Signaling in Development," *Nature*, **408**, 313 (2000)
7. Kholodenko, B.N. "Negative Feedback and Ultrasensitivity Can Bring about Oscillations in the Mitogen-Activated Protein Kinase Cascades," *Eur. J. Biochem.*, **267**, 1583 (2000)
8. Sontag, E.D., "Some New Directions in Control Theory Inspired by Systems Biology," *IEE Proceedings Systems Biology*, **1**, 9 (2004)
9. Heinrich, P.C., I. Behrmann, G. Muller-Newen, F. Schaper, and L. Graeve, "Interleukin-6-type Cytokine Signaling Through the gp130/JAK/STAT Pathway," *Biochemical Journal*, **334**, 297 (1998).
10. Heinrich, P.C., I. Behrmann, S. Haan, H.M. Hermanns, G. Muller-Newen, and F. Schaper, "Principles of Interleukin (IL)-6-type Cytokine Signaling and its Regulation," *Biochemical Journal*, **374**, 1 (2003)
11. Singh, A.K., A. Jayaraman, and J. Hahn, "Modeling Regulatory Mechanisms in IL-6 Signal Transduction in Hepatocytes," *Biotechnology and Bioengineering*, **95**, 850 (2006) □