Fuzzy Intervention in Biological Phenomena

Hazem N. Nounou*, Senior Member, IEEE, Mohamed N. Nounou, Senior Member, IEEE, Nader Meskin, Member, IEEE, Aniruddha Datta, Fellow, IEEE, and Edward R. Dougherty, Fellow, IEEE

I. ABSTRACT

An important objective of modeling biological phenomena is to develop therapeutic intervention strategies to move an undesirable state of a diseased network towards a more desirable one. Such transitions can be achieved by the use of drugs to act on some genes/metabolites that affect the undesirable behavior. Due to the fact that biological phenomena are complex processes with nonlinear dynamics that are impossible to perfectly represent with a mathematical model, the need for model-free nonlinear intervention strategies that are capable of guiding the target variables to their desired values often arises. In many applications, fuzzy systems have been found to be very useful for parameter estimation, model development and control design of nonlinear processes. In this paper, a model-free fuzzy intervention strategy (that does not require a mathematical model of the biological phenomenon) is proposed to guide the target variables of biological systems to their desired values. The proposed fuzzy intervention strategy is applied to three different biological models: a glycolytic-glycogenolytic pathway model, a purine metabolism pathway model, and a generic pathway model. The simulation results for all models demonstrate the effectiveness of the proposed scheme.

II. INTRODUCTION

Biological phenomena are complex processes with nonlinear dynamics that cannot be perfectly described by mathematical models. One main objective of these models is to design intervention strategies to drive the biological system from an undesirable (diseased) state into a more desirable one. Due to the challenges encountered to obtain these models and the uncertainty associated with them, the need for model-free nonlinear intervention strategies that are capable of guiding the target variables to their desired values often arises. Addressing such a need is main focus of this paper.

Different modeling frameworks have been proposed in the literature to represent the behavior of biological systems and include Probabilistic Boolean Networks (PBNs) [1], Bayesian networks [2], and S-systems [3]–[8] to name a few. With the availability of these models, the theoretical and analytical control studies of biochemical and metabolic systems have captured the interests of many researchers.

S-systems are proposed in [9], [10] as a canonical nonlinear model to capture the dynamical behavior of a large class of biological phenomena [11], [12]. They are characterized by a good trade-off between accuracy and mathematical flexibility [13]. In this modeling approach, nonlinear systems are approximated by products of power-law functions which are derived from multivariate linearization in logarithmic coordinates. It has been shown that this type of representation is a valid description of biological processes in a variety of settings. In [14], the authors studied the controllability of S-systems based on feedback linearization. Recently, the authors in [15] developed two different intervention strategies, namely indirect and direct, for biological phenomena modeled by S-systems.

All of the above methodologies assume the availability of mathematical models. Due to the fact that biological phenomena have complex nonlinear dynamics that are impossible to perfectly represent with a mathematical model, the need for model-free nonlinear intervention arises. Hence, in this paper we consider the problem of model-free fuzzy-based intervention for the control of target variables of biological phenomena modeled by S-systems.

Fuzzy systems, which can be viewed as nonlinear mappings between their inputs and outputs, have been found to be very useful for parameter estimation, model development and control design of nonlinear processes [16], [17]. In the context of bioinformatics and biological phenomena, fuzzy systems have been widely utilized in many applications, such as gene expression classification, clustering, and in gene regulatory networks as in [18], [19] and the references therein.

In this paper, fuzzy systems are utilized to develop fuzzy-based intervention strategies for the control of target variables of biological phenomena modeled by S-systems. There are many control schemes that can be used for the purpose of intervention. Biological systems are nonlinear processes that cannot be perfectly described by a mathematical model due to several challenges, such as the scarcity of biological data. Therefore, the advantage of the fuzzy controller is that it works as a model-free intervention scheme. In other words, the fuzzy controller does not require any knowledge of the biological system model. Only measurements of the target variables are needed for the fuzzy controller to decide the appropriate values of the manipulated variables to achieve the desired behavior. Moreover, fuzzy systems allow for the incorporation of prior knowledge about the biological system (whenever available) in the control design process. As case
studies, the proposed fuzzy intervention strategy is applied to three pathway models: a glycolytic-glycogenolytic pathway model, a purine metabolism pathway model, and a generic pathway model.

The paper is organized as follows. In Section III, the control problem for S-systems is formulated and the fuzzy-based intervention approach is proposed. In Section IV, simulation results for the three case studies are presented. Concluding remarks as well as possible future research directions are outlined in Section V.

III. FUZZY CONTROL

Consider the nonlinear system of the form
\[ \dot{x} = f(x) + g(x)u, \] (1)
\[ y = h(x), \]
where \( x \) is the vector of dependent variables (genes/metabolites), \( u \) is the vector of independent (control) variables, and \( y \) is the vector of target (output) variables. An example of the system (1), in the S-system form, is:
\[ \dot{x}_i = \alpha_i \prod_{j=1}^{N+m} x_{g_{ij}}^{\alpha_{ij}} - \beta_i \prod_{j=1}^{N+m} x_{h_{ij}}^{\beta_{ij}}, \quad i = 1, 2, ..., N \] (2)
where \( \alpha_i > 0 \) and \( \beta_i > 0 \) are rate coefficients and \( g_{ij} \) and \( h_{ij} \) are kinetic orders and there exist \( N + m \) variables (genes/metabolites) where the first \( N \) variables are dependent and the remaining \( m \) variables are independent variables. Assume that \( r \) out of the \( N \) dependent variables are target (or output) variables (i.e., genes/metabolites that need to be regulated to some desired final values), where these output variables are defined as
\[ y_j = x_i, \quad j = 1, ..., r, \] (3)
and \( i \in I \subset \{1, ..., N\} \), where \( I \) is the set of indices corresponding to the dependent variables that are selected as output variables. The steady-state analysis of the S-system model [15] shows that when the number of dependent variables with pre-specified desired values is equal to the number of independent variables (which means that we have enough degrees of freedom), the above S-system model equations will have a unique steady-state solution under a non-singularity assumption. Hence, in order to control the expressions/concentrations of the target variables, we consider an integral control approach where the following \( r \) equations are added to the above S-system
\[ \dot{x}_i = u_j, \quad j = 1, ..., r, \] (4)
where \( i \in U \subset \{N + 1, ..., N + m\} \), where \( U \) is the set of indices corresponding to the independent variables that are used as control variables. This means that \( r \) out of the \( m \) independent variables will be used as control variables, and the overall system will have \( r \) inputs and \( r \) outputs. It should be noted that the formulation above can be easily extended to deal with systems having more inputs than outputs. Let us denote by \( \mathcal{X} = \{1, ..., N\} + U \), hence \( \mathcal{X} \) corresponds to the indices of all variables except the independent variables that are not used as control variables. Here, it is assumed that the values of the independent variables that are not used as control variables are known constants (i.e., \( x_i = \delta_i, i \in \{N + 1, ..., N + m\} - U \), where \( \delta_i \) are known constants).

The S-system with integral control (2)-(4) can be written in the form (1), where \( x = [x_i]^T \in \mathbb{R}^{N+r}, i \in \mathcal{X}, u = [u_1, ..., u_r]^T \in \mathbb{R}^r, y = [y_1, ..., y_r]^T \in \mathbb{R}^r \) and
\[ f(x) = \left[ \begin{array}{c} \alpha_1 \prod_{j=1}^{N+m} x_{g_{1j}}^{\alpha_{1j}} - \beta_1 \prod_{j=1}^{N+m} x_{h_{1j}}^{\beta_{1j}} \\ \vdots \\ \alpha_N \prod_{j=1}^{N+m} x_{g_{Nj}}^{\alpha_{Nj}} - \beta_N \prod_{j=1}^{N+m} x_{h_{Nj}}^{\beta_{Nj}} \\ 0 \\ \vdots \\ 0 \end{array} \right] \]
\[ g(x) = \begin{bmatrix} 0_{N \times r} \\ I_{r \times r} \end{bmatrix}, h(x) = [x_i]^T, i \in \mathcal{Y}. \]
Using the Euler approximation, we can write the discrete time representation of equation (1) as
\[ x[k+1] = F(x[k]) + G(x[k])u[k], \] (5)
\[ y[k] = H(x[k]), \]
where \( F(x[k]) = x[k] + T_n f(x[k]), G(x[k]) = T_n g(x[k]), H(x[k]) = h(x[k]), T_n \) is the sampling time, \( x[k] \) is defined as the sampled continuous-time state \( x(kT_s) \), \( u[k] := u(kT_s) \), and \( y[k] := y(kT_s) \).

Problem Formulation:
Suppose that the outputs of the S-system (2) are initially at the steady state condition \( y_{ss}^j, j = 1, ..., r \). Let us denote by \( y_{ss}^j, j = 1, ..., r \), the desired final steady state values of the output (target) variables. Then, the fuzzy control problem is to find the control inputs \( u_j, j = 1, ..., r \), that can guide the target variables from the initial steady state condition to the final one.

A. Fuzzy Control Design
In this section, a description of the fuzzy controller is presented. The fuzzy controller is composed of the following four elements [16]: 1) a rule-base (a set of If-Then rules), which contains a fuzzy logic quantification of the experts linguistic description of how to achieve good control; 2) an inference mechanism, which emulates the experts decision making in interpreting and applying knowledge about how best to control the system; 3) a fuzzification interface, which converts controller inputs into information that the inference mechanism can easily use to activate and apply rules; and 4) a defuzzification interface, which converts the conclusions of the inference mechanism into actual inputs for the process. The fuzzy controller can be viewed as an artificial decision maker that operates in a closed-loop system in real time. It gathers process output data, compares it to the desired response, and then decides what the system input should be to ensure that the performance objectives will be met.

A schematic of the fuzzy controller for an S-system with the integral control architecture is shown in Figure 1, where
the S-system is assumed to have \( r \) inputs denoted by the \( r \)-dimensional vector \( u[k] = [u_1[k] \ldots u_r[k]]^T \), \( r \) outputs denoted by the \( r \)-dimensional vector \( y[k] = [y_1[k] \ldots y_r[k]]^T \), and \( r \) desired steady-state values of the outputs denoted by the \( r \)-dimensional vector \( y^{ss^t}[k] = [y_1^{ss^t}[k] \ldots y_r^{ss^t}[k]]^T \).

The inputs to the fuzzy controller are most often generated using some linear function of the system output \( y[k] \) and the desired steady-state values of the output \( y^{ss^t}[k] \). Here, a special case of such a linear map was found to be useful in many applications is used, where the inputs to the fuzzy controller are the error \( e[k] = [e_1[k] \ldots e_r[k]]^T \) and the derivative (change of error) \( c[k] = [c_1[k] \ldots c_r[k]]^T \), defined as

\[
e[k] = y^{ss^t}[k] - y[k], \quad c[k] = \frac{e[k] - e[k-1]}{T_s},
\]

respectively. The universes of discourse (the ranges of values) for all S-system signals are normalized to the interval \([-1, 1]\) by means of constant scaling factors. Here, the gains \( g_c \), \( g_e \), and \( g_u \) are employed in our fuzzy controller design to normalize the universe of discourse for the error \( e[k] \), derivative of error \( c[k] \), and controller output \( u[k] \), respectively (e.g., \( g_c = [g_c1 \ldots g_cr]^T \) so that \( g_c e_1[k] \) is a scaled input to the fuzzy controller). The membership functions for the two inputs (error and change of error) are shown in Figure 2. Note that the integers \(-5, -4, -3, -2, -1, 0, 1, 2, 3, 4 \) and \( 5 \) are used to represent the linguistic values "very large negative (VLN)" , "large negative (LN)" , "negative (N)" , "small negative (SN)" , "very small negative (VSN)" , "zero" , "very small positive (VSP)" , "small positive (SP)" , "positive (P)" , "large positive (LP)" , and "very large positive (VLP)" respectively. Such linguistic-numeric values constitute quite a convenient representation of the linguistic descriptions associated with the linguistic variables. The effect of scaling gains for the input membership functions is as follows: if, for example, \( g_{c1} = 1 \), the membership function for the error \( e_1 \) will be the one shown in Figure 2. However, if \( g_{c1} < 1 \) (or \( g_{c1} > 1 \)), the membership function is uniformly spread out (or contracted) by a factor of \( 1/g_{c1} \). On the other hand, the effect of scaling gain for the output membership functions (shown in Figure 3) is as follows: if, for example, \( g_{u1} = 1 \), the membership function for the controller output \( u_1 \) will be the one shown in Figure 3. However, if \( g_{u1} < 1 \) (or \( g_{u1} > 1 \)), the membership function is uniformly contracted (or spread out) by a factor of \( g_{u1} \). In our fuzzy controller design, \( r \) multi-input single-output (MISO) fuzzy controllers are utilized, one for each S-system input as it is equivalent to using one \( r \)-input \( r \)-output MIMO fuzzy controller. The knowledge-base for the fuzzy controller associated with the \( n^{th} \) S-system input is generated from the

\[
\text{IF } \tilde{e}_1 \text{ is } \tilde{E}_1^j \text{ and } \ldots \text{ and } \tilde{e}_r \text{ is } \tilde{E}_r^k \text{ and } \tilde{c}_1 \text{ is } \tilde{C}_1^i \text{ and } \ldots \text{ and } \tilde{c}_r \text{ is } \tilde{C}_r^m \text{ THEN } \hat{u}_n = \hat{U}_{j_1\ldots k_1\ldots i_1\ldots m_1},
\]

where \( \tilde{e}_i \) and \( \tilde{c}_i \) denote the linguistic variables associated with the controller inputs \( e_i \) and \( c_i \), respectively, \( \hat{u}_n \) denotes the linguistic variable associated with the controller output \( u_n \), \( \tilde{E}_i^j \) and \( \tilde{C}_i^j \) denote the \( b^{th} \) linguistic values associated with \( \tilde{e}_i \) and \( \tilde{c}_i \), respectively, and \( \hat{U}_{j_1\ldots k_1\ldots i_1\ldots m_1} \) denotes the consequent linguistic value associated with \( \hat{u}_n \) for the rule (7). This control rule may be quantified using fuzzy set theory to obtain a fuzzy implication of the form:

\[
\text{IF } E_1^k \text{ and } \ldots \text{ and } E_r^k \text{ and } C_1^i \text{ and } \ldots \text{ and } C_r^m \text{ THEN } \hat{u}_n = \hat{U}_{j_1\ldots k_1\ldots i_1\ldots m_1},
\]

where \( E_i^k \), \( C_i^k \), and \( U_{j_1\ldots k_1\ldots i_1\ldots m_1} \) denote the fuzzy sets that quantify the linguistic statements "\( \tilde{e}_i \) is \( \tilde{E}_i^k \)", "\( \tilde{c}_i \) is \( \tilde{C}_i^m \)" and \( \hat{u}_n = \hat{U}_{j_1\ldots k_1\ldots i_1\ldots m_1} \), respectively. The rule base of the fuzzy controller is shown in Table I.

**TABLE I**

| The rule base of the fuzzy controller. |

<table>
<thead>
<tr>
<th>( u )</th>
<th>( \text{Error} )</th>
<th>( \text{Change in error} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( 1 )</td>
<td>( 1 )</td>
<td>( 5 )</td>
</tr>
<tr>
<td>( -5 )</td>
<td>( -4 )</td>
<td>( -3 )</td>
</tr>
</tbody>
</table>
Hence, as an example, one fuzzy control rule based on the rule based shown in Table I could be

**IF** error is very large positive (VLP) and change-in-error is very large positive (VLP)

**Then** controller output is very large negative (VLN). (9)

The fuzzy implication of this controller can be represented by the fuzzy relation

\[ R_{n,k,l,...,m}^{j} = \left( E_{1}^{j} \times \ldots \times E_{r}^{j} \right) \times \left( C_{1}^{1} \times \ldots \times C_{m}^{k} \right) \times U_{n,k,...,m}^{j}. \]

The decision mechanism of the fuzzy controller for this control rule can be written as

\[ \hat{U}_{n,k,...,m}^{j}[k] = \left( \hat{E}_{1}[k] \times \hat{E}_{1}[k] \times \ldots \times \hat{E}_{r}[k] \right) \times \left( \hat{C}_{1}[k] \times \hat{C}_{2}[k] \times \ldots \times \hat{C}_{r}[k] \right) \circ R_{n,k,...,m}^{j}, \]

where \( \hat{E}_{j}[k] \) and \( \hat{C}_{j}[k] \) denote the fuzzified error and change of error, respectively, associated with the \( j \)th element of \( e[k] \) or \( c[k] \). \( \hat{U}_{n,k,...,m}^{j}[k] \) denote the implied fuzzy set, and \( \circ \) denotes the Zadeh’s composition. A more detailed explanation of equation (11) can be found in [20]. In fuzzy system design, a fuzzy implication exists for every possible combination of fuzzy sets describing the inputs to the fuzzy systems. Hence, the fuzzy controller is made up of many fuzzy implications whose overall control action may be computed by the “center of gravity” method expressed as

\[ u_{n}[k] = \frac{\sum_{j,k,...,m} A_{n,k,...,m}^{j} \hat{A}_{n,k,...,m}^{j}[k] \hat{C}_{n,k,...,m}^{j}[k]}{\sum_{j,k,...,m} A_{n,k,...,m}^{j} \hat{A}_{n,k,...,m}^{j}[k] \hat{C}_{n,k,...,m}^{j}[k]}, \]

where \( \hat{A}_{n,k,...,m}^{j}[k] \) and \( \hat{C}_{n,k,...,m}^{j}[k] \) are the area and center of area, respectively, of the membership function associated with \( \hat{U}_{n,k,...,m}^{j}[k] \).

Next, the fuzzy control scheme presented above will be applied to three biological pathways modeled by S-systems.

### IV. CASE STUDIES

In this section, we demonstrate the efficacy of the fuzzy intervention approach developed in this paper by applying it to three different biological models: the glycolytic-glycogenolytic pathway model (case study 1) [14], [21], the purine metabolism pathway model (case study 2) [22], and a generic pathway model (case study 3) [3]. In all case studies, fuzzy intervention is used to guide the target variables to their desired values. Also, for the three case studies, sensitivity analyses are conducted to study the robustness of the fuzzy intervention algorithm to different mode uncertainties, which include parameter variations (in the glycolytic-glycogenolytic pathway model), measurement noise (in the purine metabolism pathway model), and process noise (in the generic pathway model). The three case studies are presented next. The fuzzy controllers in all case studies shown below use minimum to represent the premise and implication, and center of gravity (COG) defuzzification.

![Image of Case Study 1 - Glycolytic-glycogenolytic pathway](image)

**A. Case Study 1 - The glycolytic-glycogenolytic pathway model**

This well-studied biological pathway model representing the glycolytic-glycogenolytic pathway is shown in Figure 4 [14], [21]. Glycolysis is the process of breaking up a six-carbon glucose molecule into two molecules of a three-carbon compound (called pyruvate), and glycogenolysis is the process by which the stored glycogen in the body is broken up to meet the needs for glucose. In glycolysis, the phosphorylase enzyme acts on the polysaccharide glycogen to reduce its length by one glucose unit. The glucose unit is released as a glucose-1 phosphate molecule.

The glycolytic-glycogenolytic pathway can be mathematically represented by the following S-system model

\[
\begin{align*}
\dot{x}_1 &= 0.077884314z_4^{0.66}x_6 - 1.06270825x_1^{1.53}x_2^{0.59}x_7 \\
\dot{x}_2 &= 0.585012402x_1^{0.05}x_2^{0.41}x_3^{0.32}x_4^{0.62}x_5^{0.38} \\
&- 0.0007934561x_2^{3.97}x_3^{3.06}x_8 \\
\dot{x}_3 &= 0.0007934561x_2^{3.97}x_3^{3.06}x_8 - 1.05880847x_3^{3.3}x_9
\end{align*}
\]

where \( N = 3, m = 7 \) and the independent variables have the values \( x_4 = 10, x_5 = 5, x_6 = 3, x_7 = 40, x_8 = 136, x_9 = 2.86, \) and \( x_{10} = 4. \) We next consider two intervention scenarios: single-input single-output and two-input two-output intervention scenarios.

1) **Scenario #1: Single-Input Single-Output (SISO) Fuzzy Intervention:** In this case, we seek to control \( x_1 \) by manipulating \( x_4 \), i.e.

\[ y_1 = x_1, \quad \dot{x}_4 = u_1, \] (14)

and all other \( x_i \)'s \( i = 5, 6, 7, 8, 9, 10 \) are kept fixed. Physically, this corresponds to the problem of using the inorganic phosphate ion concentration to control the concentration of glucose-1-phosphate. In the absence of any additional glucose, the concentration of glucose-1-phosphate is what determines the input to glycolysis. The target value for \( y_1 \) is selected as \( y_1^{target} = 1 \).

Here, the fuzzy controller has two inputs: the error and change of error, defined as

\[ e_1[k] = y_1^{target} - y_1[k], \quad e_1[k] = \frac{e_1[k] - e_1[k-1]}{T_s}, \] (15)

respectively, where \( T_s = 1 \) minute. The scaling gains of the input and output membership functions of the fuzzy controller are selected as \( g_{e_1} = 10, g_{e_2} = 5, \) and \( g_{u_1} = 15. \) Figure 5 depicts the trajectory response and the control input, respectively, for the above system. It is clear from Figure 5 that the output
$y_1$ converges to its desired value. The closed-loop response of the system is slow due to the internal dynamics of the Glycolytic-glycogenolytic pathway model. The settling time can be reduced by changing the controller gains. However, this can affect the transient response of the closed-loop system.

Remark 1: The scaling gains of the fuzzy controller are tuned to achieve an acceptable performance. The process used to select these parameters can be described as follows. As a first guess, the scaling gains $g_{c1}$ and $g_{c2}$ are selected to place the centers of the membership functions over the desired ranges of error and change of error, respectively. Based on our discussion in section III-A, this can be achieved by selecting $g_{c1}$ (and $g_{c2}$) to place the centers of the leftmost and rightmost membership functions of the error (and change of error) at $-1/g_{c1}$ and $1/g_{c1}$ ($-1/g_{c2}$ and $1/g_{c2}$), respectively. On the other hand, the scaling gain of the output membership functions is initially selected to cover the desired range of fuzzy controller output. Based on our discussion in section III-A, this can be achieved by selecting $g_{u1}$ and $g_{u2}$ to place the centers of the leftmost and rightmost membership functions of the output at $-g_{u1}$ and $g_{u1}$, respectively. This process usually provides a good initial guess of the controller parameters. Then, these parameters are tuned further to achieve an acceptable performance. Starting with the initial guess of the controller parameters, the difficulty of the tuning process varies depending on the complexity of the application. For this particular application, it was not very challenging to select the controller parameters that provide an acceptable performance.

2) Scenario #2: Two-Input Two-Output Fuzzy Intervention: In this case, we seek to control $x_1$ by manipulating $x_4$ and control $x_2$ by manipulating $x_5$, i.e.

$$y_1 = x_1, \quad y_2 = x_2, \quad \dot{x}_4 = u_1, \quad \dot{x}_5 = u_2,$$

and all other $x_i$'s $i = 6, 7, 8, 9, 10$ are kept fixed. Physically, this corresponds to the problem of using the glucose concentration to control the concentration of glucose-1-phosphate, and using the inorganic phosphate ion to control the concentration of glucose-6-phosphate. The main difference here is that the infusion of glucose can also be used to increase the concentration of glucose-6-phosphate instead of relying just on the output from glycogenolysis. The target values for $y_1$ and $y_2$ are selected as $y_1^{sd} = 1$ and $y_2^{sd} = 8$, respectively.

Here, two SISO fuzzy controllers are used, and each of them has two inputs: the error and change of error, which are defined as

$$e_i[k] = y_i^{sd}[k] - y_i[k], \quad c_i[k] = c_i[k] - c_i[k-1],$$

respectively, for $i = 1, 2$, where $T_s = 1$ minute. The scaling gains of the input and output membership functions of the first fuzzy controller are selected as $g_{c1} = 10$, $g_{u1} = 50$, and $g_{u2} = 4$, and the scaling gains of the input and output membership functions of the second fuzzy controller are selected as $g_{c2} = 1$, $g_{u1} = 5$, and $g_{u2} = 0.2$. Figure 6 shows the responses of $y_1$ and $y_2$ compared with their corresponding desired values, where it is clear that $y_1$ and $y_2$ follow their corresponding desired values.

3) Sensitivity Analysis - Case Study 1: Due to the fact that mathematical models are not a perfect representation of the biological phenomena, it is important to study the robustness of the fuzzy intervention strategies in the presence of model uncertainties. Hence, here we conduct a sensitivity analysis to study the ability of the fuzzy intervention strategies to handle variations in the S-system model parameters. To this end, a Monte Carlo analysis is performed, where the closed-loop system is simulated 500 times, where in each simulation all the parameters of the S-system model (13) are perturbed within $\pm 3\%$ of their nominal values. The simulation results of the sensitivity analysis are shown in Figures 7 and 8 for scenarios #1 and #2, respectively. The closed-loop responses in blue are the responses for the S-system model with nominal parameters (i.e., the original model parameters), compared with the desired target values in red. The bars in green represent intervals of $3\sigma$ of the system response at different time instances due to variations in the model parameters, where $\sigma$ is the standard deviation of the system responses from all 500 realizations. It is clear that the fuzzy intervention strategies are able to guide the target variables to their desired values in the presence of uncertainties in the S-system model parameters.
B. Case Study 2 - The purine metabolism pathway model

Purine metabolism provides the organism with building blocks for the synthesis of DNA and RNA, and is intimately connected with the dynamics of other key compounds, such as ATP [22]. Several diseases are related to purine metabolism, some causing elevated concentrations of uric acid in blood, which may result in gout, or may lead to mental retardation, and self-mutilation. Some of these diseases result from partial or total enzyme deficiencies or by the overexpression of key enzymes. The purine metabolism pathway model can be found in [22] (Figure 10.4, page 349). As shown in [22], the purine pathway can be mathematically represented by the following S-system model

\[
\begin{align*}
    \dot{x}_1 &= 0.898 x_1 - 0.03 x_1 x_5 - 0.45 x_5 x_7 - 0.04 x_5 x_6 - 0.65 x_7 x_8 - 31.333 x_1^{1.27} x_2 - 0.15 x_1 x_2 x_3 - 0.06 x_5 x_2 x_4 - 0.323 x_5 x_2 x_6 - 0.085 x_5 x_2 x_7 - 0.075 x_5 x_2 x_8 - 0.0092 x_5 x_2 x_9, \\
    \dot{x}_2 &= 1.629 x_1 x_2 - 0.28 x_1 x_3 - 0.319 x_1 x_4 - 0.03 x_3 x_4 x_5 - 0.04 x_4 x_6 - 0.06 x_5 x_6 - 5.584 x_2^{1.16} x_4 - 0.012 x_5^{1.01} x_6 - 0.11 x_7 x_1 x_6 - 0.11 x_7 x_2 x_6, \\
    \dot{x}_3 &= 3.5932 x_2 x_4 - 0.24 x_2 x_5 - 0.05 - 66544 x_3 x_4 - 0.95 x_4 x_5, \\
    \dot{x}_4 &= 0.08208 x_1 x_4 - 0.00025 x_5^{0.004} x_4 - 0.00418 x_4 x_5 - 0.0023 x_5 - 0.00037 x_4 x_5, \\
    \dot{x}_5 &= 7.2067 x_4 - 0.06 - 9.006 x_5, \\
    \dot{x}_6 &= 0.29 x_5^{0.9} - 220.14 x_4 x_5 - 0.79 x_6 - 0.748, \\
    \dot{x}_7 &= 1.283 x_2^{1.16} x_3 - 0.09 x_5 - 0.3738 x_4^{0.12} x_2, \\
    \dot{x}_8 &= 0.04884 x_1 x_2 x_3 - 0.0013 x_4 x_5 x_4 x_5 - 0.00019 x_4 x_5 x_7 - 0.00004 x_4 x_5 x_8 - 0.00029 x_4 x_5 x_9 - 0.00009 x_4 x_5 x_9 x_9, \\
    \dot{x}_9 &= 0.00229 x_4 x_9^{0.0058} x_9 - 0.0058 x_1 x_9 - 0.0017 x_1 x_9 - 0.0058 x_1 x_9 - 0.0086 x_1 x_9, \\
    \dot{x}_{10} &= 0.00151 x_4 x_9^{0.0058} x_9 - 0.0017 x_1 x_9 - 0.0058 x_1 x_9 - 0.0086 x_1 x_9 - 2.2655 x_9^{0.13} x_9, \\
    \dot{x}_{11} &= 1024.195 x_2 x_7 - 0.05 x_8 x_7 - 0.115389 x_1^{1.1}, \\
    \dot{x}_{12} &= 5.5062 x_4 x_7 - 0.005254 x_1^{1.12}, \\
    \dot{x}_{13} &= 0.103 x_4 x_9^{0.4} x_9 - 0.04 x_1^{0.19} x_1^{0.19} - 0.0817 x_2 x_5 x_1^{0.18} - 0.0562 x_2 x_5 x_1^{0.18}, \\
    \dot{x}_{14} &= 0.7234 x_1^{0.34} x_5 x_5 - 0.9325 x_1^{0.34}, \\
    \dot{x}_{15} &= 0.2615 x_8 x_1^{0.13}, \\
    \dot{x}_{16} &= 0.949 x_1^{0.55} - 0.003874 x_4 x_4 x_5^{0.21}. \\
\end{align*}
\]

where \( N = 16, m = 2 \) and the independent variables have the values \( x_{17} = 18 \) and \( x_{18} = 1400 \). The variables of the above S-system model are defined in [22]. A key metabolite in purine biosynthesis is the PRPP \( (x_1) \), which is affected by variations in RSP \( (x_{17}) \), and hence \( x_{17} \) will be used as an input variable to control \( x_1 \). Hence, in this case we have

\[
y_1 = x_1, \quad \dot{x}_{17} = u_1, \quad (18)
\]

and \( x_{18} \) is kept fixed. The target value of \( y_1 \) is selected as \( y_1^{\text{ref}} = 10 \). Here, the fuzzy controller has two inputs: the error and change of error, as defined in (15), where \( T_s = 1 \) minute. The scaling gains of the input and output membership functions of the fuzzy controller are selected as \( g_{c1} = 10, g_{c2} = 0.5 \), and \( g_{c3} = 1 \). Figure 9 shows the output response and the control input, respectively, where the output \( y_1 \) approaches its desired value.

1) Sensitivity Analysis - Case Study 2: It is well known that measurement noise can have a profound impact on the closed-loop performance, and hence it is important to study the robustness of the fuzzy intervention strategy in the presence of measurement noise. Hence, here we conduct a sensitivity analysis to study the ability of the fuzzy intervention strategy to handle different levels of measurement noise. To this end, several Monte Carlo simulation studies are conducted, where the closed-loop system is simulated 500 times, where in each
simulation the output is contaminated with white noise which corresponds to a specific signal-to-noise ratio (SNR). The SNR is the ratio of the variance of the noise-free output to the variance of the added noise. Here, a Monte Carlo simulation analysis is conducted for each of the following SNRs: 5, 10, 20, 30, 40, and 50.

When SNR is equal to 10, the simulation results of the sensitivity analysis is shown in Figure 10. The closed-loop response in blue is the response when no noise is added, compared with the desired target value in red. The bars in green represent intervals of $\sigma$ of the system response at different time instances due to measurement noise, where $\sigma$ is the standard deviation of the system responses from all 500 realizations. It has been found that the mean of the responses during the last 50 minutes of simulation of all 500 realizations is 9.9881 and the mean of $3\sigma$ of the responses during the last 50 minutes of simulation of all 500 realizations is 0.4409. A summary of the simulation results for different Monte Carlo simulations of 500 realizations at various SNRs is shown in Table II. It can be seen from Table II that as the SNR increases, the mean of responses approaches the desired reference value of 10 and the mean of $3\sigma$ decreases, indicating less variability around the desired output. The above simulation results show that the fuzzy intervention strategy is able to guide the target variables to their desired values with acceptable degree of accuracy in the presence of measurement noise.

### C. Case Study 3 - The Generic branched pathway model

In this case study, the generic branched pathway model presented in [3] is considered. As shown in [3], the generic branched pathway can be represented by the following S-system model:

$$
\begin{align*}
\dot{x}_1 &= 20x_3^{-0.8}x_5^{1} - 10x_1^{0.5}, \\
\dot{x}_2 &= 8x_1^{0.5} - 3x_2^{0.75}, \\
\dot{x}_3 &= 3x_2^{0.75} - 5x_3^{0.5}x_4^{0.2}, \\
\dot{x}_4 &= 2x_1^{0.5} - 6x_4^{0.8},
\end{align*}
$$

(19)

where the system has four dependent variables $x_1, \ldots, x_4$ ($N = 4$) and one independent variable $x_5$ ($m = 1$), which is used as a control variable to control the concentration level of $x_3$. Hence, we have

$$y_1 = x_5, \quad \dot{x}_5 = u_1. \quad (20)$$

The target value of $y_1$ is selected as $y_1^{\text{ref}} = 10$. Here, the fuzzy controller has two inputs: the error and change of error, as defined in (15), where $T_s = 1$ minute. The scaling gains of the input and output membership functions of the fuzzy controller are selected as $g_{x1} = 2$, $g_{x2} = 0.5$, and $g_{u1} = 0.1$. The trajectory response and the control input are shown in Figure 11, where it is clear that the output $y_1$ converges to its desired value.

1) Sensitivity Analysis - Case Study 3: For this case study, a sensitivity analysis is conducted to study the robustness of the fuzzy intervention strategy in the presence of process noise. The process noise is defined as a white noise added to the state equations of the S-system model, such that $\dot{x}_i = x_i + \epsilon_i$, where for $i = 1, \ldots, 4$, $x_i$ is the $i^{th}$ state in the presence of process noise and $\epsilon_i$ is white noise with a certain variance. Several Monte Carlo simulation studies are conducted, where the closed-loop system is simulated 500 times, where in each simulation process noise of a certain variance is added. Here, Monte Carlo simulation studies are conducted for the following process noise variances: 0.1, 0.5, 1, 1.5 and 2.

When the process noise variance is equal to 1, the simulation results of the sensitivity analysis are shown in Figure 12. The closed-loop response in blue is the response when no process noise is used, compared with the desired target value in red.
Again, the bars in green represent intervals of $3\sigma$ of the system response at different time instances due to process noise, where $\sigma$ is the standard deviation of the system responses from all 500 realizations. It has been found that the mean of the responses during the last 50 minutes of simulation of all 500 realizations is 10.0002 and the mean of $3\sigma$ of the responses during the last 50 minutes of simulation of all 500 realizations is 0.4317.

A summary of the simulation results for different Monte Carlo simulations of 500 realizations at several process noise variances is shown in Table III. It can be seen from Table III that as the variance of process noise decreases, the mean of responses approaches the desired reference value of 10 and the mean of $3\sigma$ decreases. The above simulation results show that the fuzzy intervention strategy is able to guide the target variables to their desired values with acceptable degree of accuracy in the presence of process noise.

**V. Conclusions**

Biological systems are complex nonlinear processes that are impossible to perfectly represent with a mathematical model due to several challenges, such as the scarcity of biological data. Hence the development of model-free intervention strategies that are capable of guiding the target variables to their desired values is of utmost importance. In this paper, model-free fuzzy intervention strategies have been developed and applied to three pathway models: the glycolytic-glycogenolytic pathway model, the purine metabolism pathway model, and a generic pathway model. The glycolytic-glycogenolytic pathway is selected as it plays an important role in cellular energy generation when the level of glucose in the blood is low (fasting state) and glycogen has to be broken down to provide the substrate to run glycolysis. By controlling the glycolytic reaction, one can exert control over whether glycolysis will run or not under low glucose conditions. In the second case study, the purine pathway is selected due to its importance in providing the organism with the building blocks for the synthesis of DNA and RNA, and is intimately connected with the dynamics of key compounds, such as ATP. By controlling the concentration of Phosphoribosylpyrophosphate (PRPP), one can have control over the dynamics of purine biosynthesis. In the three case studies considered in this paper, simulation results show that the fuzzy controllers were able to guide the target variables to their desired values. Moreover, sensitivity analyses have been conducted to study the robustness of the fuzzy intervention algorithm to variations in model parameters, contamination due to measurement noise, and presence of process noise, in the three case studies, respectively. However, in the presence of time-varying dynamics of the biological system model, the fuzzy intervention scheme may not provide a good closed-loop performance. Hence, as a future research direction, we will consider the biological phenomena with time-varying dynamics, where adaptive intervention strategies will be utilized to cope with such biological system variations.

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**References**


