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## OPARTA NA GRAFACH DETEKCJA STRUKTUR STEROWANIA W MODELACH WEWNĄTRZKOMÓRKOWYCH SZLAKÓW SYGNAŁOWYCH

**Streszczenie.** W pracy przedstawiono grafową metodę wykrywania struktur regulacyjnych w modelach wewnątrzkomórkowych szlaków sygnałowych. Modele matematyczne przekształcane są do grafów interakcji, co umożliwia wyszukiwanie struktur oraz weryfikację ich cech charakterystycznych. Działanie metody zilustrowano na przykładzie struktury feedback–feedforward. Zaproponowane podejście może wspomagać analizę biologicznych architektur regulacyjnych.

## GRAPH-BASED DETECTION OF CONTROL STRUCTURES IN MODELS OF INTRACELLULAR SIGNALLING PATHWAYS

**Summary.** A graph-based methodology for detecting control structures in intracellular signalling pathway models is presented. Mathematical models are transformed into interaction graphs, enabling structural matching with subsequent verification of structure-specific features. The approach is demonstrated using a feedback–feedforward control structure and can support the analysis of biological regulatory architectures.

### 1. Introduction

Intracellular signalling pathways play a fundamental role in transmitting information within cells, enabling responses to external stimuli and coordinating internal biological processes. These pathways consist of complex networks of molecular interactions in which signals initiated by extracellular factors are propagated through cascades of biochemical reactions, ultimately affecting gene expression, metabolism, and cellular behaviour [8]. Owing to their biological significance and their involvement in numerous diseases, including cancer, signalling pathways have become an important subject of mathematical modelling and systems-level analysis.

In parallel, control engineering has developed a wide range of well-established control structures designed to ensure stability, disturbance rejection, and desirable dynamic performance. Concepts originating from control theory have increasingly inspired synthetic biology, where regulatory circuits analogous to classical engineering controllers have been implemented in living cells. Examples include integral [1], proportional-integral [5], and PID-like controllers [4], as well as hierarchical and cascade control architectures [9, 7].

The growing interaction between control engineering and systems biology has also stimulated interest in identifying regulatory motifs within naturally occurring signal-

ling pathways. Feedforward motifs are among the most frequently reported examples [13]. However, from a control-theoretic perspective, many structures classified as feedforward motifs do not correspond to genuine feedback–feedforward control systems but rather to parallel arrangements of dynamic components [12, 6].

This observation motivates the development of systematic methods for detecting control structures in intracellular signalling pathways. Identifying pathway fragments that exhibit structural and functional similarities to classical engineering control architectures could provide new insights into biological regulation while facilitating the design of synthetic regulatory networks. Furthermore, such an approach would enable the transfer of established control-theoretic concepts to the analysis and interpretation of complex biological systems.

In this work, a graph-based approach to detecting control structures in intracellular signalling pathway models is proposed. The method transforms mathematical models into interaction graphs and identifies occurrences of predefined control architectures through structural matching and verification of their key properties.

## 2. Model transformation

The proposed approach for detecting control structures in intracellular signalling pathways relies on transforming a mathematical model into a graph representation suitable for structural analysis. Since signalling pathway models are commonly formulated as systems of ordinary differential equations (ODEs) [2], the transformation process converts equations describing pathway dynamics into directed graphs called interaction graphs (IG).

### 2.1. Interaction graph

The proposed method assumes that intracellular signalling pathways are represented as first-order state-space models of the form:

$$\frac{dx}{dt} = f(x, u) \quad (1)$$

where  $x = [x_1, x_2, \dots, x_n]^T$  denotes the vector of state variables and  $u$  represents external inputs or stimuli.

The model is transformed into a directed interaction graph, where vertices correspond to model variables and directed edges represent interactions between variables.

An edge from node  $x_i$  to node  $x_j$  exists whenever variable  $x_i$  appears on the right-hand side of the equation defining the dynamics of  $x_j$ . Thus, graph edges describe qualitative dependencies among model variables independently of the precise mathematical form of these interactions.

It is worth emphasising that the interaction graph stores no information about the mathematical form or types of dependencies. An edge between variables can describe both linear and strongly non-linear relations, both inhibition and activation.

For further analysis it is necessary to transform both pathway model and control structure into their respective IGs.

### 2.2. Transformation algorithm

The transformation of a model into an interaction graph requires only the state equations, as their left-hand sides allow for extraction of model variables. Given that

input, the graph generation procedure can be performed as follows. First, extract the list of model variables and create respective graph nodes. Then, select the first variable to analyse and identify variables occurring on right-hand side of the state equation defining the variable's derivative. Create directed edges from detected variables to the currently analysed state variable. Repeat this procedure for all equations and record the final IG. Such an approach ensures that any kind of interaction, regardless of its mathematical form, is accounted for in the IG.

The control structure can be transformed in a similar manner if it is defined as a set of differential equations. However, control structures are often described as general block diagrams - e.g., Fig. 1a for feedback control - where the entire dynamics of particular elements like the controller or plant are represented as a single block.

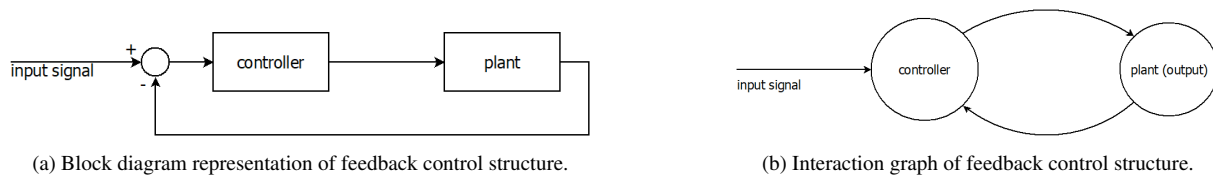


Fig. 1. Feedback control structure transformation: a) block diagram, b) interaction graph

In such a case, it is necessary to specify the variables representing key elements of the structure : controller, corrector, plant, etc. Such variables are connected with the output signal of their respective blocks, resulting in a graph shown in Fig. 1b.

### 3. Structure detection

The detection process consists of two stages: identifying general structural similarity and verifying structure-specific properties. The first stage defines the required condition for the existence of a control structure within a signalling pathway - the structure interaction graph must occur as a subgraph of the pathway interaction graph. The second stage takes into consideration the mathematical form of the interactions to prune false positive structure candidates.

#### 3.1. General structural similarity

The first stage searches for graph fragments whose topology matches the target control structure. In general, all graph nodes can be assumed to be potential inputs and outputs, and all possible combinations are evaluated. If additional information is available, biologically meaningful variables may be predefined as candidate inputs or outputs. Such prior information can significantly reduce computational complexity and improve the interpretability of the detected structures.

As a result, a set of model variables is obtained for every subgraph that is structurally identical to the control structure graph. In the set, each model variable can be assigned a role according to the node of the structure graph to which it corresponds. E.g., if variables A, B, and C of the pathway model are found to form connections corresponding to the feedback IG, then each of them will be assigned a role of input signal, controller, or plant depending on their location in the subgraph.

The resulting node sets are then treated as candidate structures. For those candidates, the second stage — verification of structure-specific properties — is performed.

### 3.2. Structure graph variants

Biological systems frequently contain chains of intermediate molecular interactions that perform functions analogous to a single control element. To account for such situations, a node-joining procedure is necessary, in which sequentially connected nodes can be treated as equivalent to a single functional element of a control structure. E.g., for feedback control structure from Fig. 1b the role of a plant can be performed by a chain of 3 sequentially connected nodes as shown in Fig. 2.

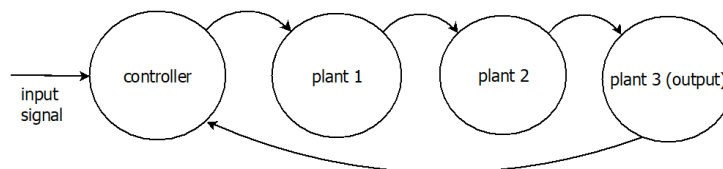


Fig. 2. IG of feedback control structure - plant formed from chained variables.

Including those variants of control structure graphs is necessary for the identification of more complex and extended realisations of the target structure while preserving its functional interpretation.

### 3.3. Key structure features verification

Structural similarity alone is insufficient for determining whether a candidate truly represents the desired control mechanism. Interaction graph representation, without differentiating the type of interaction, is just an approximation. Control structures are defined not only by information about the existence of interactions between elements but also by additional constraints associated with signal flow or the form of interactions.

Typically, those additional requirements are connected with specific summing junctions of the block diagram or limiting the signals that are allowed as input to a certain block. In the case of a feedback control structure, such an additional requirement is the summing junction forming the feedback loop. If we define  $x_{controller}$  as the controller's output,  $x_{plant}$  as the plant's output,  $x_{input}$  as the input signal, and  $x_1, \dots, x_n$  as other state variables in the model, the state equation for a potential controller must be defined as:

$$\begin{aligned} \frac{dx_{controller}}{dt} = & f(x_{controller}, x_{input}, x_1, \dots, x_n) + \\ & + g(x_{controller}, x_{plant}, x_1, \dots, x_n) + \\ & + h(x_{controller}, x_1, \dots, x_n) \end{aligned} \quad (2)$$

This form ensures the separation of the input signal and the feedback loop signal. It is especially important when analysing graph variants defined in section 3.2., as chaining variables can impact additional constraints while preserving structural similarity on the graph level.

The additional requirements are imposed on the specific formulation of the interactions, therefore this stage of verification is performed directly on the mathematical equations associated with candidate nodes.

## 4. Feedback-feedforward control structure example

Apart from the toy example of the feedback control structure, the proposed methodology is shown on a more complex but commonly used example: feedback-

feedforward control.

Feedback–feedforward (FB–FF) structures combine corrective action based on measured system output with anticipatory compensation of disturbances. Such architectures are widely used in engineering systems because they improve disturbance rejection and dynamic performance. In the particular variant of the FB–FF structure used for this example, the feedforward path is related to the input signal, not to disturbances (as shown in the block diagram in Fig. 3a) [10, 11].

Such a structure is characterized by a higher sensitivity of the output to changes in the feedforward path compared to changes in the feedback path [3]. This behaviour can be used as a preliminary filter of parameters when performing a sensitivity analysis of a model where FB–FF structures can be found.

#### 4.1. Detection of feedback-feedforward control structure

In order to detect FB–FF structures in any mathematical model, an interaction graph must be constructed according to the rules defined in section 2. Afterwards, key structure-specific characteristics must be defined for verification in procedure defined in section 3.3.

##### 4.1.1. Interaction graph

Taking into account block diagram from Fig. 3a we can define variables (nodes) as: input, controller, corrector and plant. Input is provided to both corrector and controller, and their outputs are then provided to the plant. Plant output is subsequently provided to the controller closing the feedback loop. The graph representation of those interaction is shown in Fig. 3b.

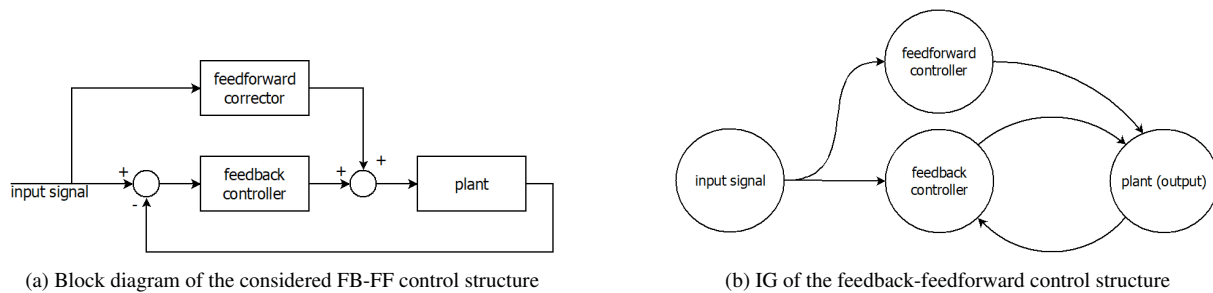


Fig. 3. FB–FF control structure: a) block diagram of structure under consideration, b) IG

##### 4.1.2. Structure-specific features

Similarly to the feedback control structure, the additional constraints that the equations must fulfil to represent the FB–FF structure are connected with the summing junctions: the feedback defining junction combines output and input, and the feedforward defining function combines outputs from both control paths.

Feedback junction constrains the equation representing the feedback controller, separating feedback control signal from feedforward path. Feedforward junction constrains the equation representing the plant ensuring both control paths act together and independently. Adopting notation from section 3.3. and defining  $x_{corrector}$  as the output of the feedforward corrector, the required structure of equations is as in Eq. 3 for the plant and in Eq. 4 for the controller.

$$\begin{aligned} \frac{dx_{plant}}{dt} = & f_p(x_{plant}, x_{controller}, x_1, \dots, x_n) + \\ & + g_p(x_{plant}, x_{corrector}, x_{input}, x_1, \dots, x_n) + \\ & + h_p(x_{plant}, x_1, \dots, x_n) \end{aligned} \quad (3)$$

$$\begin{aligned} \frac{dx_{controller}}{dt} = & f_c(x_{controller}, x_{plant}, x_1, \dots, x_n) + \\ & + g_c(x_{controller}, x_{input}, x_1, \dots, x_n) + \\ & + h_c(x_{controller}, x_1, \dots, x_n) \end{aligned} \quad (4)$$

## 4.2. Synthetic test models

The procedure described in section 4.1. was implemented in Python alongside a set of synthetic test models representing parallel connection, a simple feedback control structure, a parallel connection with a feedback loop, and different variants of the FB-FF structure. Block diagrams of the examples are provided in Fig. 4.

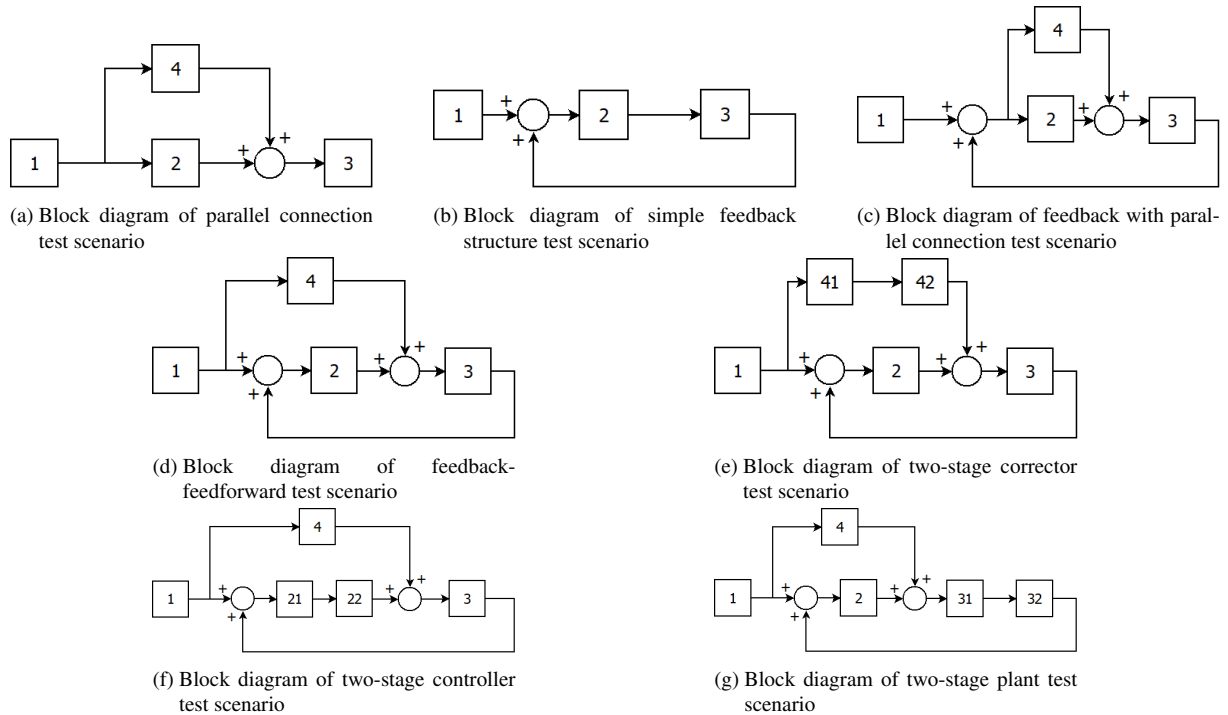


Fig. 4. Block diagrams used for tests: a) parallel connection, b) simple feedback control, c) feedback with parallel connection, d) feedback-feedforward control, e) two-stage corrector, f) two-stage controller, g) two-stage plant

Test examples were constructed so that for block diagrams 4a to 4c no FB-FF structure should be found, and for block diagrams 4d to 4g exactly 1 FB-FF structure should be found.

Table 1 shows the result of applying FB-FF structure search to test block diagrams. Numbers of candidates before the second stage of verification are shown to highlight the importance of the equation-level stage. Each set of variables representing a verified structure candidate is sorted according to the assigned roles of input, controller, plant, corrector. Chained variables are connected with a "\_\_\_".

Table 1

## Synthetic block diagrams tests results

| Test | Number of candidates<br>after stage 1 | Verified candidates<br>after stage 2 |
|------|---------------------------------------|--------------------------------------|
| A    | 0                                     | $\emptyset$                          |
| B    | 0                                     | $\emptyset$                          |
| C    | 6                                     | $\emptyset$                          |
| D    | 3                                     | {1, 2, 3, 4}                         |
| E    | 4                                     | {1, 2, 3, 41__42}                    |
| F    | 8                                     | {1, 21__22, 3, 4}                    |
| G    | 6                                     | {1, 2, 31__32, 4}                    |

As expected, no structures were found for tests A to C. Basing only on graph representation for test C - feedback loop with parallelly connected blocks 2 and 3 - 6 candidates were found. This result highlights that interaction graph is not sufficient to distinguish particular control structure. For tests D to G, some false candidates were initially found but were then filtered out in the second stage verification.

## 5. Conclusions and discussion

This paper presented a graph-based methodology for detecting control structures in intracellular signalling pathway models. The proposed approach transforms mathematical models into directed interaction graphs and identifies occurrences of pre-defined control architectures through structural matching followed by verification of structure-specific properties. The method was demonstrated using the example of the feedback-feedforward control structure, showing its ability to detect both direct and extended realisations of the target architecture. The examples chosen for the paper were deliberately kept simple to emphasise the idea of the proposed method. In internal tests the algorithm maintain its effectiveness for models containing over a dozen variables. The proposed framework provides a systematic link between control engineering concepts and systems biology, offering a tool for the analysis of natural signalling pathways.

Although the ideas underlying the proposed framework are independent of the size of the signalling pathway to be analysed, the computational cost of searching through large graphs can become a significant limitation. Moreover, in pathways with many variables and connections, the definition of the control structure may not cover additional connections with variables not forming the structure. Such connections should not necessarily be considered forbidden; however, it must be noted that they can influence the behaviour of the structure, resulting in the search for an approximate structure rather than a strict one. Finally, there may exist non-linear relations in the model that provide appropriate signalling only for a specific operating range. Should the system work in a different value range, the structure of candidates found using the procedure described in this paper may exhibit behaviour different from what is desired.

Future studies will investigate the application of the proposed methodology to large collections of signalling pathway models and assess its usefulness in identifying biologically relevant regulatory architectures.

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