User Manual

Absolute Concentration Robustness Exploration (ACRE)

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Contents

1 HOW TO INSTALL **3**
1.1 Requirements 3
1.2 Package Contents 3

2 SYSTEM SUMMARY **4**
2.1 Functionality 4
2.2 Workflow 4

3 USER INTERFACE GUIDE **6**
3.1 Example 6
3.2 Load Modules 6
3.3 Module IO Selection 7
3.4 Create/Delete/Move Nodes 7
3.5 Set Constraints 9
3.6 Save/Load Workspace 10
3.7 Set Report Options 11
3.8 Enumerate and Analyze 11
3.9 Restricted Search 11
3.10 Scalability 13

HOW TO INSTALL

1.1 Requirements

The program is packaged as an executable JAVA file, to run it, you need JAVA Runtime Environment downloaded. To download JAVA, please go to the following website: www.java.com/getjava/

Any Operating System is sufficient to run the software as long as it has a JAVA Runtime Environment available for it. Once you have JAVA 1.6 or above installed you should be able to run the application by typing the following command in Command of Windows or Terminal of Mac OS or Linux:

java -jar ACRE.jar

1.2 Package Contents

The Package contains the following:

1. Modules: a folder containing sample SBML files for frequently-seen modules.
2. Workspaces: a folder containing sample workspaces.
3. ACRE.jar: the executable application file.
4. UserManual_ACRE.pdf: this user manual.

SYSTEM SUMMARY

2.1 Functionality

The overall idea of ACRE is to enumerate all the biochemical reaction networks that consist of user-created nodes from user-selected modules under user-specified constraints; to apply Shinar and Feinberg's theorem [1]; and to determine which of the networks have the absolute concentration robustness (ACR) property.

2.2 Workflow

ACRE is a JAVA-based system that has been tested on Windows 7, Mac OS X 10.8, and Ubuntu 11.10. ACRE requires JAVA 1.6 or above. The workflow of the software is illustrated in Fig 2.1.

Step 1: ACRE reads modules in the System Biology Markup Language (SBML) format. ACRE extracts *reactions*, *reactants*, *products* and *stoichiometry* from SBML files for the analysis of the ACR property. ACRE provides a number of basic, frequently-seen modules. Alternatively, the user can create/import their own modules as long as their SBML files contain the required information.

Step 2: Once a module is loaded, the user specifies the input and output species of the module. An input species is a reactant or a product that can be replaced by an external output species, and an output species is a reactant or product that can replace an external input species. The input and output species cannot be the same for the same module. This restriction is to prevent invalid networks to be constructed. In the case that the user wishes to specify different inputs and outputs for a particular module, that module can be loaded multiple times. The user then creates as many realizations of modules as needed, each of which is referred to as a node in our tool.

Step 3: Two types of constraints can be used to restrict the composition of networks, i.e., *local constraints* and *global constraints*. Local constraints are set to each node, and these constrain the way in which nodes are connected to build networks. By default, the local constraints are set to "no connection", which means that the outputs of a node cannot be connected to any other node. Global constraints are defined at the network level. The user can specify the minimum and maximum number of connection edges that the network can possess. Global constraints can prevent the construction of over-sparse or over-dense networks, and also limit the search space of all possible networks in order to achieve improved computational efficiency.

Step 4: For analysis of the results, the user can select the information to be generated in the report. This can include runtime, the number of constructed

Figure 2.1: Workflow of ACRE

networks, and the number of networks with ACR. The tool can provide an output of the networks with ACR in SBML, and specify which of the species in each of these networks have the ACR property. The report can be displayed on screen and saved to a file. Furthermore, the workspace can be saved or loaded at any time for future use. ACRE also supports restricted search. The user can specify which species is desired to have ACR. The tool will thus report only those networks that have ACR on the specified species.

Step 5: ACRE enumerates all the possible networks with the created nodes under user-specified constraints. For each network, the tool applies Shinar and Feinberg's theorem to deterministically identify which species have ACR. Once all the networks are enumerated, the results are summarized and a report is generated.

USER INTERFACE GUIDE

3.1 Example

In this section, an overview of the user interface will be provided, with a step-by-step example illustration. The following example is a chemical reaction network presented in [1], which is known to have ACR on species C, Fig 3.1.

Suppose one has created four SBML files for this network. Those are:
$M1 : 2A \leftrightarrows B \to C$
$M2 : B + C \leftrightarrows D \to 2B$
$M3 : A + E \to F$
$M4 : 2B \to A + E, 2B \leftrightarrows F$

Note that the same letter in different modules does not necessarily denote the same species. We now demonstrate how to apply ACRE to further investigate the structural features of ACR with respect to these modules. Once ACRE is opened, the main window is shown as in Fig 3.2.

3.2 Load Modules

The first step is to import SBML files that represent the modules of the desired chemical reaction network. To find out more about SBML representation and how to create SBML files, please refer to the website: http://sbml.org/.

In this example, we load the pre-prepared SBML files provided within the package. To open these files:

1. Click File → Load
2. Navigate to SBML/EX2a.xml
3. Open
4. Open EX2b.xml, EX2c.xml, and EX2d.xml in the similar way

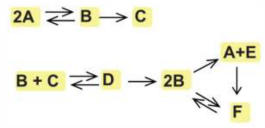

Figure 3.1: Example chemical reaction network with ACR on C (figure taken from [1])

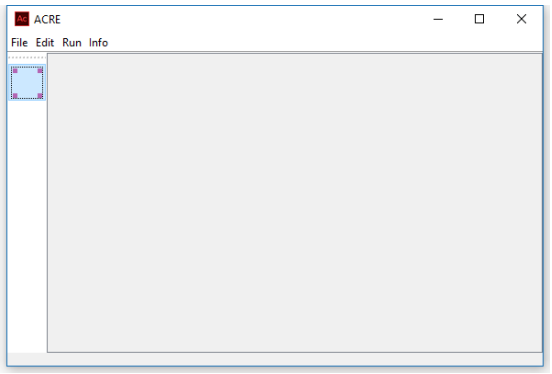

Figure 3.2: The main window of ACRE. The top panel is the menu bar, the left panel is the module bar, and the right grey panel is the node space for created nodes.

	Inputs	Outputs
M1	A, B	C
M2	C	B
M3	A, E	F
M4	B, F	A, E

3.3 Module IO Selection

After opening the SBML file the Module IO Selection window will appear. See Fig 3.3.

Note that in the white area at the bottom of this window, the reactions are listed, which correspond to *module1 (M1)*.

In this window, we define which of the species in the reactions should be inputs and outputs. To do that, choose the species from the drop down menu, then click the corresponding button (either Add Input, or Add Output).

In this example, we set the following inputs and outputs for the four modules:

Then click on "Done". We will see that four modules appear in the module bar as shown in Fig 3.4.

Note that ACRE will auto-generate a name for the module if none was given. Once a module is created, one can delete the module, by selecting the icon representing the module in the module bar, then choose Edit → Delete Module.

3.4 Create/Delete/Move Nodes

After creating modules, we need to create nodes. A node is a realization of a module. One can create as many nodes as needed for any module.

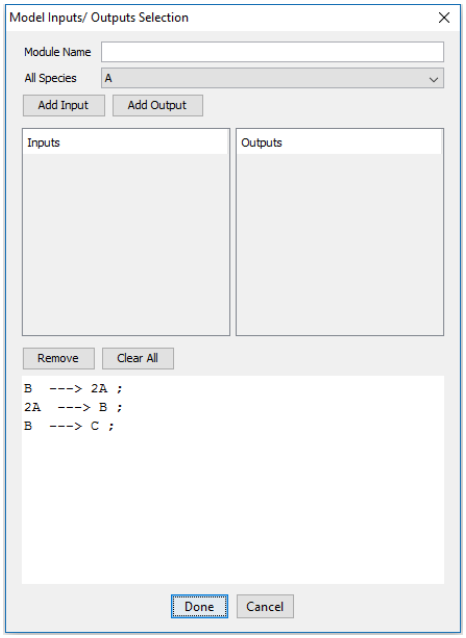

Figure 3.3: Module IO selection window.

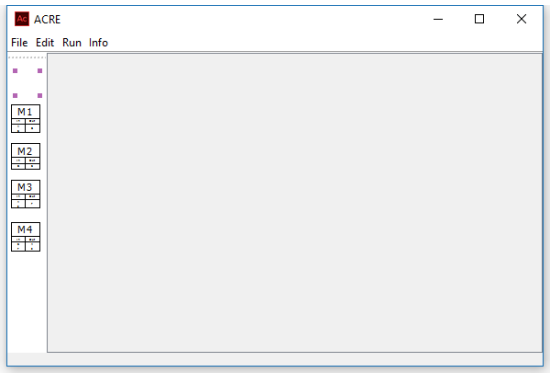

Figure 3.4: ACRE window with the four modules loaded.

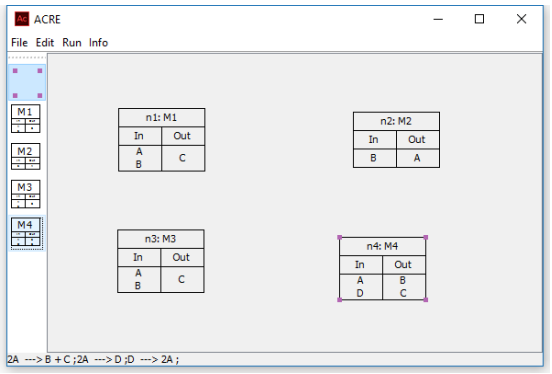

Figure 3.5: ACRE window with four nodes created.

To create a node, click on the module icon from the tool bar, then click anywhere in the node space, a node will appear, with the following information shown:

1. An auto-generated node name;
2. The module from which the node is created;
3. The lists of input and output species of this node.

In this example, we create one node for each module, as shown in Fig 3.5.

One can always move each node by first selecting the move tool from the module bar, which is the first tool showing 4 squares in the corners. To move a node:

1. Select the move tool from the module bar;
2. Drag and drop the required node.

To delete a node:

1. Select the move tool from the module bar;
2. Click on the required node, you will notice 4 small squares appearing on the corners;
3. Choose Edit → Delete Node.

3.5 Set Constraints

Each Node is associated with a set of local constraints regarding its inputs. To view/edit these constraints, select the node and then choose Edit → Constraints, the "Node Constraints Selection" window will appear as shown in Fig 3.6.

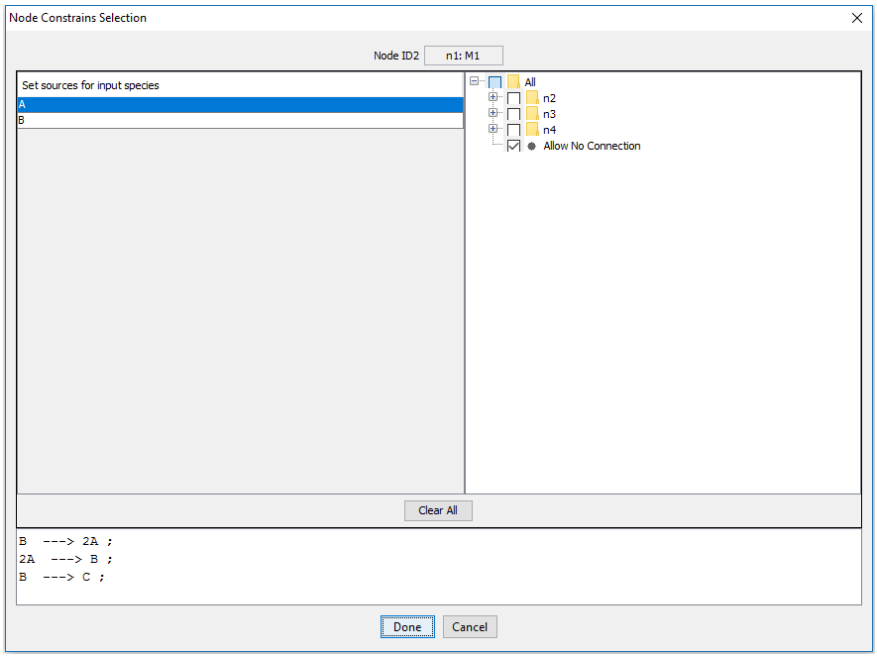

Figure 3.6: Local constraint selection window.

From this window one can choose for each input of the node which outputs of other nodes can be connected to it, and whether it can be left unconnected. A list of reactions will also appear in the text box at the bottom. Note that the user has to set the constraints for all the inputs species of all the nodes. Otherwise, the constraint will be set to no connection by default.

In this example, we require each input of each node must be connected by at least one output from other nodes. Thus, "Allow No Connection" option is disabled.

Global constraints are optional to prevent the tool from generating over-sparse or over-dense networks (Fig 3.7). By default, the minimum number of edges is 0 and the maximum is 2,147,483,647.

3.6 Save/Load Workspace

At any point one can save the workspace which constitutes of:

1. The modules created;
2. The nodes created;
3. The constraints.

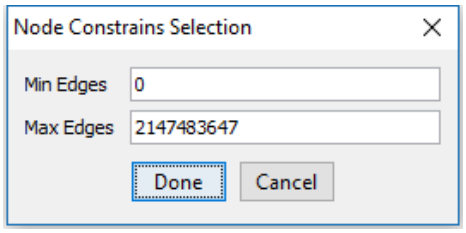

Figure 3.7: Global constraint selection window.

Saving/Loading the workspace comes in handy in case the user needs to modify the network later. To save/load network, use the corresponding menu item from the file menu.

3.7 Set Report Options

The final step is to enumerate and analyze all the networks that satisfy the specified local and global constraints. To do that, choose Run → Enumerate. Before the enumeration starts, the "Enumeration Options" window will appear as in Fig 3.8.

This window enables the user to select what should be included in the output. Make sure not to include the details of the enumeration in this step in the cases where a huge number of networks are anticipated.

In this example, we select to display the runtime, the total number of networks enumerated and analyzed, the number of networks that have ACR. We select to show such information on screen.

3.8 Enumerate and Analyze

Once the report options are set, we click OK button, which will start the enumeration and analysis process. After the whole process is finished, the desired report is displayed on screen as shown in Fig 3.9.

Interestingly, by allowing different ways to connect these four nodes, there are 9,216 networks in total, 8,537 of which have ACR species. ACRE is able to finish the enumeration and analysis process for this large number of networks in just 2 seconds.

3.9 Restricted Search

The user can specify which species is desired to have ACR. The tool will thus report only those networks that have ACR on the specified species.

In this example, if we restrict species A of *node1* to have ACR, only 289 networks are found to satisfy such requirement, as shown in Fig 3.10.

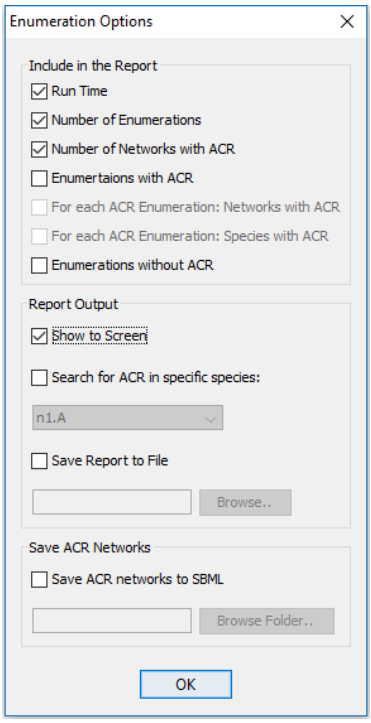

Figure 3.8: Enumeration options window.

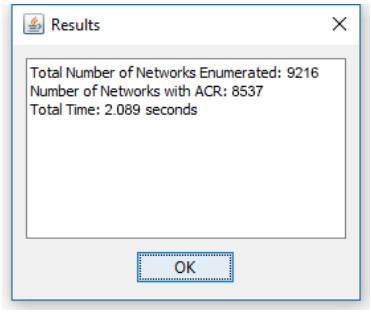

Figure 3.9: Output report.

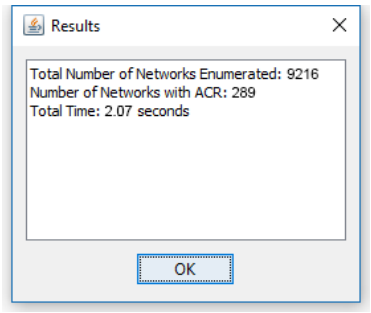

Figure 3.10: Output report for restricted search.

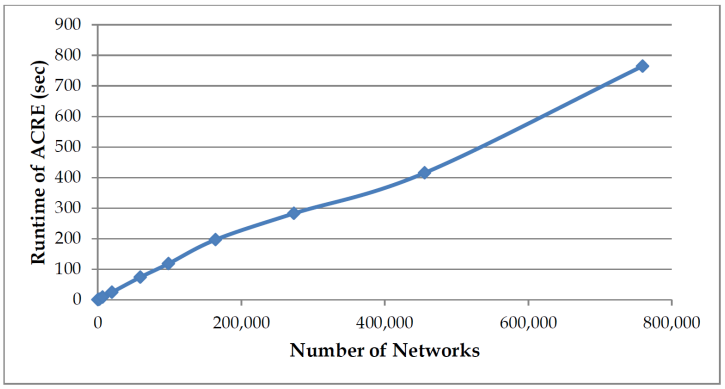

Figure 3.11: Runtime v.s. number of networks.

3.10 Scalability

Fig 3.11 shows the scalability of ACRE in terms of runtime and number of networks, from which an approximately linear relationship can be observed.

Reference

[1] Guy Shinar and Martin Feinberg. Structural sources of robustness in biochemical reaction networks. *Science*, 327(5971):1389–1391, 2010.