

Getting started with rxncon – A step-by-step guide



First steps

Here, we show you how to get started with the translation of a signaling network. As an example, we show how the first part of the mammalian apoptosis network (adapted from [1]) is translated into the rxncon notation (see Figure 1).

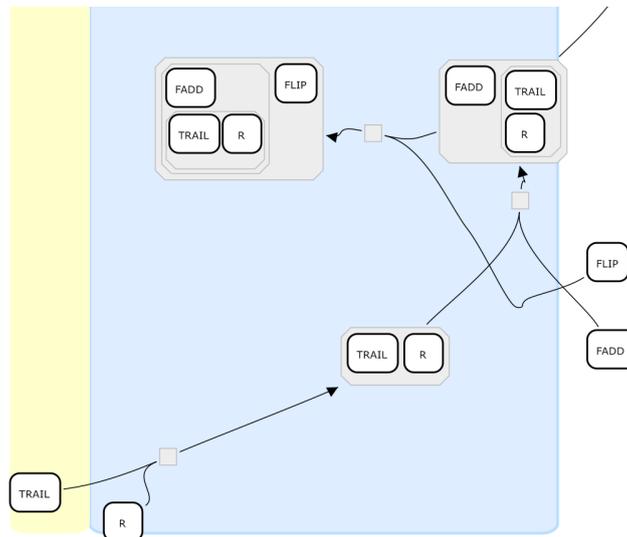


Figure 1: Detail of the apoptosis network.

To begin with, it is helpful to consider the following question: What is a *reaction*, and what is a *contingency*? With **reactions** one describes the change of a state of a molecule (or more general an entity). In the example here, you can see that the signaling cascade is initialised by the binding of the ligand (TRAIL) to a receptor (R). This is the first elemental reaction taking place in the apoptosis pathway. The newly formed complex (R--TRAIL) reacts with a new protein, FLIP, to form a bigger complex. At this point, the first **contingency**, or contextual constraint has to be formulated: FLIP can only bind to the receptor once the ligand TRAIL and receptor have formed the R--TRAIL complex. Thus, contingencies are regarded as the contextual constraints upon which entities react with each other.

The working sheet

On the rxncon website (www.rxncon.org), you find a template excel sheet. Download the file and open it. You will see that the excel file consists of different sheets and predefined cells. The excel file contains the following sheets:

- Instructions (optional)
- (I) Reaction list
- (II) Metabolic reaction list (Not yet functional, to be implemented)
- (III) Contingency list
- (IV) Reaction definition
- (V) Contingency definition
- (VI) ORF IDs *S. cerevisiae*
- (VII) Non ORF IDs

Familiarise yourself with the structure! You may have a look at the *Instructions* to have a brief overview. Most important for now will be the *(I) Reaction list* and *(III) Contingency list*. To help you with the network translation, you may take a look at *(IV) Reaction definition* and *(V) Contingency definition*. There you find the currently implemented notations (and explanations) in rxncon.

Your first reactions and contingencies

As mentioned earlier, the first elemental reaction (Reaction 1, see Figure 2) in the apoptosis network is the binding of the ligand TRAIL to the receptor R. As you also can see, the reaction takes place without any constraints, it is actually the initialisation of the pathway, and thus, no contingencies have to be specified, yet.

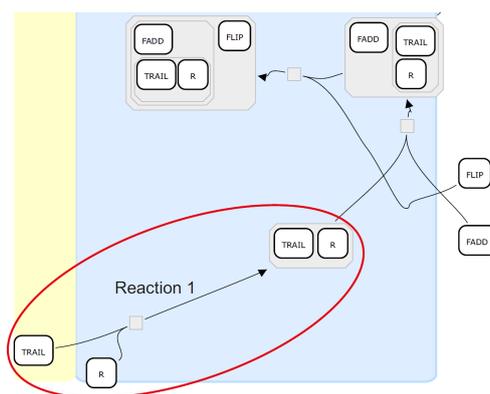


Figure 2: Reaction 1. Binding of the ligand TRAIL to the receptor R.

To define this reaction you need open the *(I) Reaction list*. Navigate to cell L2 where you may specify TRAIL as Component A and R in Q2 as Component B. In cell P2 you also have to specify the type of reaction. In this case, we deal with a simple interaction, which are represented with i. See Figure 4 for an example.

	K	L	M	N	O	P	Q	R
1	ComponentB[Species]	ComponentA[Name]	ComponentA[Domain]	ComponentA[Subdomain]	ComponentA[Residue]	Reaction	ComponentB[Name]	Component
2	charomyces cerevisiae	TRAIL				i	R	
3								
4								
5								
6								
7								
8								

Figure 3: Reaction list.

Once you have filled in the components and reaction type, you can check whether the reaction and resulting state have been automatically added in the cells B2 and D2.

Once your excel sheet is saved, close it and start rxncon by activating the *web2py* server. Your browser should automatically open and start the rxncon

environment. Upload your file in rxncon by pushing the button *Create New*, selecting the file and pushing *Submit*. Make sure you have saved your file in the *.xls* format. Whenever you apply changes to your file, you can update them by pushing *Update*.

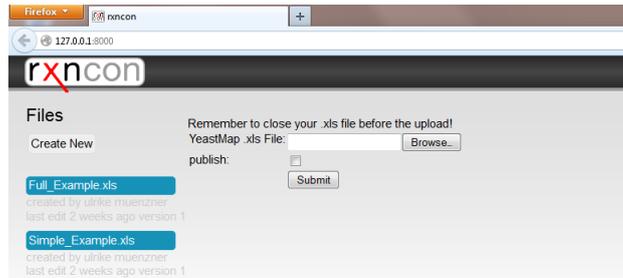


Figure 4: Upload page.

To have a first check and impression of the progress of your work you may navigate to the *Graph* menu. You can choose different layout formats there. Try SBGN–PD (the Systems Biology Graphical Notation standard for a process description, www.sbgng.org). If your input file is correct, a new tab (the Biographer editor) should open and show the network. Probably you should extend the cell compartment a little bit and then push the Grid-symbol. After editing, your network may look similar to the one in Figure 5.

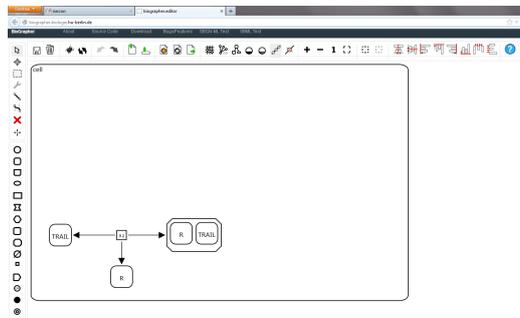


Figure 5: Reaction 1. Process description of the binding of the ligand TRAIL to the receptor R.

The next reaction (Reaction 2, see Figure 6) will be the protein–protein interaction between the protein FADD and the Receptor. Copy the first line in your reaction list to the next line (so you will keep the formatted cells), but now change Component A to FADD and Component B to R, specify the reaction type to ppi (as for protein—protein interaction) and press enter.

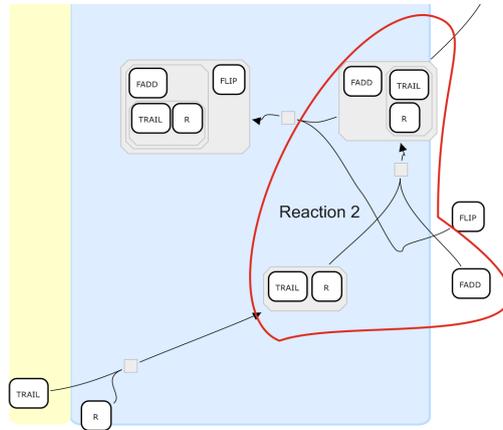


Figure 6: Reaction 2. Binding of the protein FADD to the receptor R while TRAIL is bound to R.

A new reaction (FADD_ppi_R) and a new state (FADD--R) have been created, but this time we need to specify a contingency: The reaction FADD_ppi_R only takes place once TRAIL has bound to the receptor.

Open the excel sheet *(III) Contingency list*. You may now specify a target and a modifier. Here, the target is the reaction in question. (In this case: FADD_ppi_R) The requirement would be the previous state of the receptor, TRAIL--R (which corresponds to the modifier). In our case, the modifier (TRAIL--R) is a *requirement*, and this is expressed with an exclamation mark (!) between modifier and target. Instead of typing the states manually, it is recommended that you link your target and modifier to the respective cells in the reaction list. By doing so, you will keep your network definition internally consistent, which will save you a lot of time in case you later change the name or reaction of a component.

When you look at the network (Figure 1), you will notice that FADD cannot bind to the receptor before the ligand has interacted with the receptor. This implicit piece of information needs to be clarified in a contingency. Go to the *(III) Contingency list*, copy the first contingency to the next line. Now the target reaction would be TRAIL_i_R and it should be prevented from happening by defining the FADD--R state as a modifier. The FADD--R state inhibits the receptor to interact with the ligand and is marked with x in the column for contingencies.

Your *(I) Reaction list* and *(III) Contingency list* should now look similar to these (Figure 7):

You may check your network again by visualising it as a PD. Follow the procedure as for reaction 1, but instead of uploading a new file, just update

L	M	N	O	P	Q
ComponentA[Name]	ComponentA[Domain]	ComponentA[Subdomain]	ComponentA[Residue]	Reaction	ComponentB[Name]
TRAIL				i	R
FADD				ppi	R
A	B	C	D		
Contingen	Target	Cont Modifier			
1	FADD_ppi_R	!	TRAIL--R		
2	TRAIL_i_R	x	FADD--R		

Figure 7: Top: Reaction list. Bottom: Contingency list.

your current one in rxncon. After editing, your network might look similar to the upper one in Figure 8. To show what would happen if the contingency was missing, have a look at the bottom panel in Figure 8. Compared to the correct network, you here have an additional state of R: FADD--R. The additional state is avoided by explicitly expressing (with a contingency) that FADD only binds to the receptor when it is bound to TRAIL, hence in the TRAIL--R state.

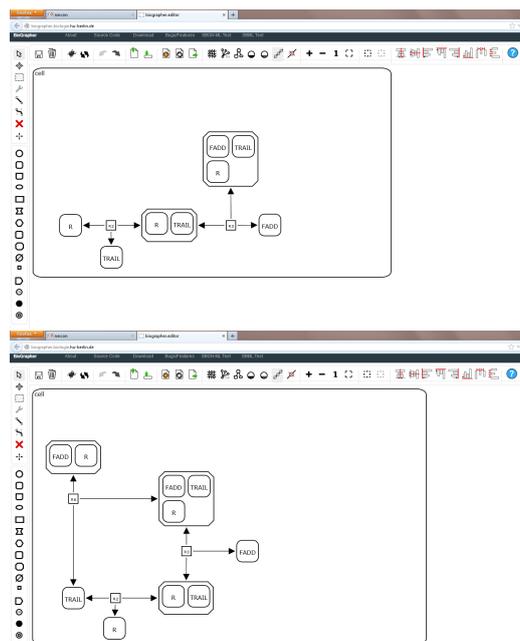


Figure 8: Top: Network *with* contingency prohibiting the reaction between FADD and the ligand-free receptor. Bottom: Network *without* contingency.

The next elemental reaction (Reaction 3, see Figure 9) according to the network would be the interaction between the protein FLIP and the receptor. Note that the reaction requires the receptor to be in a certain state, the state in which it is bound to the ligand TRAIL and the protein FADD.

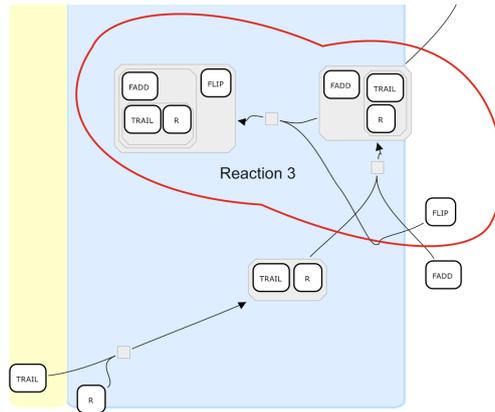


Figure 9: Reaction 3. Binding of the protein FLIP to the receptor R while TRAIL and FADD are bound to R.

Reaction 3 thus requires the state FADD--R and this requirement needs to be specified as a contingency. According to the network, FADD cannot react with the receptor while it is in the state FLIP--R. Hence, another contingency is needed. The reaction (target) FADD_ppi_R is inhibited by the state FLIP--R.

The specified reactions and contingencies in your excel sheet should now look like this (Figure 10):

L	M	N	O	P	Q
ComponentA[Name]	ComponentA[Domain]	ComponentA[Subdomain]	ComponentA[Residue]	Reaction	ComponentB[Name]
TRAIL				i	R
FADD				ppi	R
FLIP				ppi	R
A	B	C	D		
Contingen	Target	Cont	Modifier		
1	FADD_ppi_R	!	TRAIL--R		
2	TRAIL_i_R	x	FADD--R		
3	FLIP_ppi_R	!	FADD--R		
4	FADD_ppi_R	x	FLIP--R		

Figure 10: Top: All reactions. Bottom: All contingencies.

Update your network again and select a visualisation format. By moving around the entities from your network with the Biographer editor, you can arrange an output according to your ideas. An example output could be the one in Figure 11:

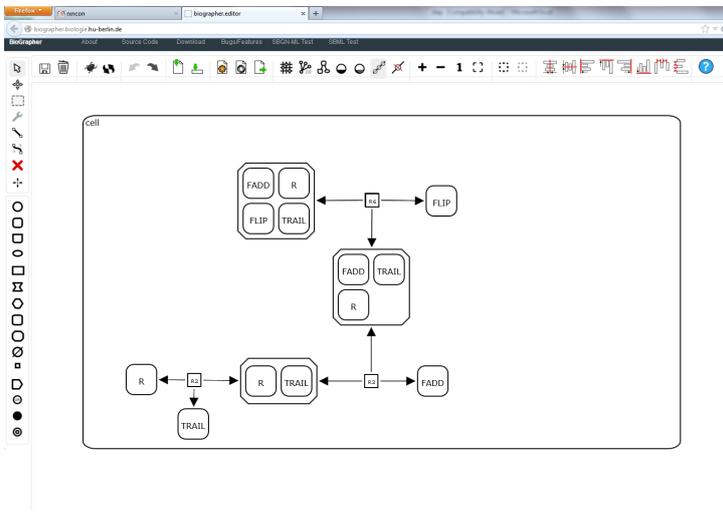


Figure 11: Example of a SBGN Process Description within the Biographer editor.

If you would have translated the network without specifying any contingencies, your output would look similar to the one in Figure 12. As you can see, without contingencies all possibilities will be applied, thus, every component appears in all possible states.

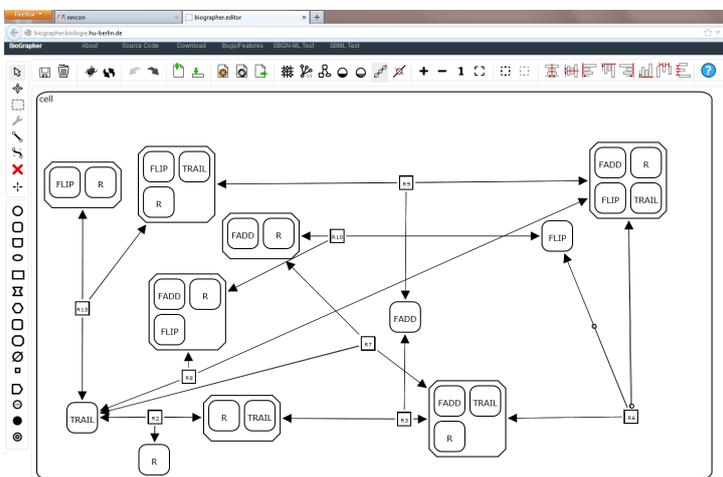


Figure 12: Process description of initial reactions in an apoptosis network lacking contingencies.

Working with rxncon

The SBGN-PD is only one example of output. Your network can be exported to other visualisation formats, too (*e.g.* SBGN Entity Relationship). You may also try the contingency matrix or Regulatory graph or export your network into the Systems Biology Markup Language (SBML) modeling standard.

Remarks

You have so far familiarised yourself with the rxncon framework, how to distinguish between reactions and contingencies, as well as how they are written down. To keep extending the network, you would need to extend the excel sheet that you have been working on.

We also recommend that you test to visualise your network at different levels of progress before finishing it completely. This applies especially to PD diagrams, as they can become large without contingencies. You will detect errors at an early stage and can correct them accordingly. Only a one-time translation of your network is needed, but you have multiple choices to generate a visualisation! Furthermore, your network can be automatically translated into a mathematical model for further analysis.

References

- [1] Thomas Handorf et al. Unpublished.