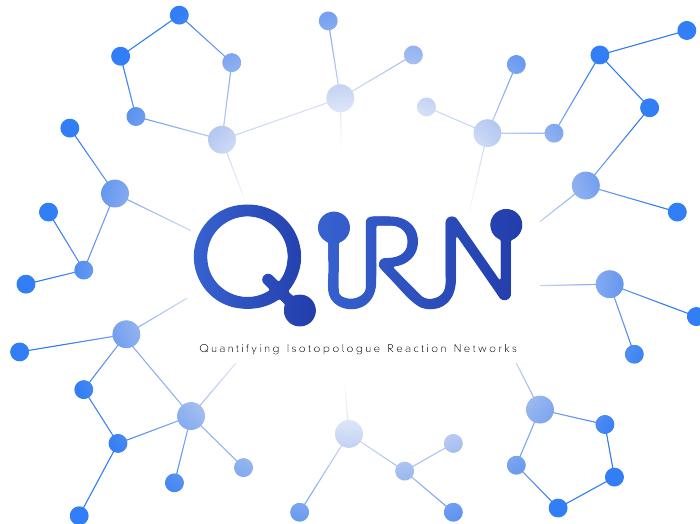


Quantifying Isotopologue Reaction Networks (QIRN) User Guide



Introduction

Quantifying Isotopologue Reaction Networks (QIRN) is a forward, numerical modelling tool that constructs reaction networks of any kind and reports isotopic compositions of substrates within that network. In this user guide, we will show you: 1.) How to generate the necessary input files for QIRN and 2.) How to use the associated graphic user interface (GUI).

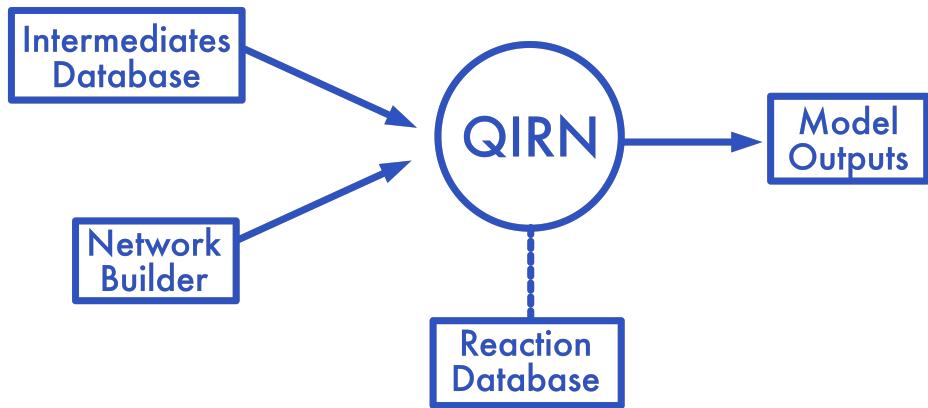


Figure 1: The structure of how QIRN builds user-defined reaction networks.

Input Files

QIRN has three comma-separated-value (csv) files that it pulls from to construct user-defined networks. Two of these ('Intermediates Database' and 'Network Builder') the user interacts with. The final input ('Reaction Database') is the same for all users and is not changed, only referenced, by the user.

Intermediates Database

The Intermediates Database holds information about all the substrates that have ever been modelled in QIRN for a given isotope system. Here, the example is for carbon. This information includes the number of carbon atoms in the molecule, its starting concentration, its intramolecular isotope composition, and its boundary conditions. The atomic number never changes. The other three terms are changed by the user to define the initial conditions and boundary conditions of the model. If the "Reservoir" column is changed to "1", QIRN will keep the molecule's concentration and isotope composition constant over time. In the "Intramolecular" column, a vector of in numbers in delta-values can be input in the order of the molecule's atomic ordering. For example, glucose, can have a -10‰,-5‰,-10‰,-5‰,-10‰,-5‰ intramolecular structure from C1 to C6, respectively. These would be input as "-10,-5,-10,-5,-10,-5". If you are editing the csv file in Excel, the cell must be a "text" format.

IntermediatesDatabase				
Intermediate	Number of Carbons	Initial Concentration	Intramolecular d13C	Reservoir
1,3-bisphosphoglycerate	3	0		
1,3-bisphosphoglycerate_cytoplasm	3	0		
2-isopropylmalate	7	0		
2-oxobutanoate	4	0		
2-phosphoglycerate	3	0		
3-4-hydroxyphenylpyruvate	9	0		

Figure 2: Example of part of the Reaction Database. "Product D" and "Reaction Barcode" columns are not shown here.

Reaction Database

The Reaction Database serves as a list of all reactions that QIRN has ever modelled and all their relevant information. Reactions are catalogued based on their Enzyme Commision (EC) number. If the reaction is not enzymatic a new ID may be given to it (i.e. 'TSPsulfate' for the transport of sulfate into a cell). If there is only one reactant in the reaction, only fill out the column for Reactant A. Similarly, only fill out the column for Product C if only one product is formed. In the case of a condensation function, QIRN will condense Reactants A and B (or C and D in the reverse direction) so that the last atom on A is connected to the first atom on B. For cleavage reactions of Reactant A to Products C and D, QIRN will cleave Reactant A based on the carbon numbering of Product C, which is reads from the Intermediates Database. Thus if Reactant A and Product C are six and four atoms long, respectively, Product C will be composed of Reactant A's sites 1-4 and Product D will be composed of Reactant A's sites 5 and 6. This can be changed by assigning a "Reaction Barcode" to the reaction. These strings of integers tell QIRN how to transition atoms from reactant to product and are applied to any type of reaction where atomic rearrangements occur. The Reaction Database must be in the same directory as QIRN's python scripts. This is a default when you download the QIRN installation package.

ID	Enzyme	Reactant A	Reactant B	Product C	P
EC 5.3.1.1	Triose phosphate isomerase	DHAP		glyceraldehyde-3-phosphate	
EC 5.3.1.1 Cytoplasm	Triose phosphate isomerase cytoplasm	DHAP_cytoplasm		glyceraldehyde-3-phosphate_cytoplasm	
EC 5.3.1.6	Isomerase	ribulose-5-phosphate		ribose-5-phosphate	
EC 5.3.1.9	Phosphohexose isomerase	glucose-6-phosphate		fructose-6-phosphate	
EC 5.4.2.11	Phosphoglycerate mutase	3-phosphoglycerate		2-phosphoglycerate	
EC 6.2.1.4	Succinyl-CoA Synthetase	succinylCoA		succinate	
EC 6.4.1.1	Pyruvate carboxylase	pyruvate	HCO3	malate	
EC 6.4.1.2	acetylCoA carboxylase	acetylCoA	co2_ACCase	malonylCoA	
F6PS	F6P Aldolase Sink	fructose-6-phosphate_cytoplasm_aldolase		glucose-6-phosphate	
F6PSII	F6P Aldolase Sink II	fructose-6-phosphate_cytoplasm		glucose-6-phosphate	
Lipid synthesis	Lipid synthesis	acetylCoA		Lipids	

Figure 3: Example of part of the Reaction Database. "Product D" and "Reaction Barcode" columns are not shown here.

Network Builder

The Network Builder file is where users define the topology of their reaction network including, the reactions, their forward and reverse rate constants, and their isotope effects. Reactions are input as their EC number or other identifier defined by the Reaction Database. QIRN searches the Reaction Database for these reactions and will alert the user if any reactions do not match the database. QIRN then uses the Intermediates Database to select the appropriate substrates in the network, their starting concentrations and isotopic compositions. Forward and reverse reaction rates refer to the mass action rate constant for monoisotopic isotopologue. When defining rate constants, remember that steady state fluxes are a function of both the steady state concentration of the reactant(s) and the rate constant. Therefore, there is no simple relationship between the rate constant and absolute flux, unless all of the substrates are given a concentration of unity at the start of the simulation and rate constants at each substrate node are chosen so the inputs and outputs are equivalent. In this case, substrate concentrations are always unity and are constant and the system begins in steady state. Thus, the rate constants are equivalent to the steady state flux. This is the recommended strategy for choosing initial conditions in QIRN. For a discussion of exceptions to this, see the main text.

NetworkBuilder_glycolysis							
Reaction	Forward Rate Constant	Reverse Rate Constant	Carbon Site of Fractionations Forward Reaction	KIE Forward	Carbon Site of Fractionations Reverse Reaction	KIE Reverse	
EC 2.7.1.1	0.1	0					
EC 5.3.1.9	0.1	0					
EC 2.7.1.11	0.1	0					
EC 4.1.2.13	0.1	0					
EC 2.7.2.3	0.1	0					
EC 5.3.1.1	0.1	0					
EC 4.2.1.11	0.1	0					
EC 5.4.2.11	0.1	0					
EC 2.7.1.40	0.1	0					
EC 1.2.1.12	0.1	0					
EC 1.2.4.1	0.1	0,1,2,3		0.98,0.98,0.997			
EC 6.4.1.1	0.1	0					

Figure 4: Example of a Network Builder file for the glycolysis pathway.

Site-specific kinetic isotope effects (KIEs) for both forward and reverse are also defined in the Network Builder. They are assigned as fractionation factors, where values less than one represent normal isotope effects (fractionation against the rare isotope). Equilibrium isotope effects (EIEs) are equivalent to the ratio of the forward to reverse KIEs. For example, if a reaction has no KIE but a 0.995 EIE, the user would input 1.0 for the forward KIE and 1.005025 for the reverse KIE. Each one of these KIEs is site-specific and must be assigned to an atomic site on the reactant to be imposed. This is assigned in columns 4 and 6 of the Network Builder file. Here, a list of numbers indicates the atomic sites where isotope effects should be imposed. The string of KIEs in columns 5 and 7 should be the same lengths as those in columns 4 and 6. For example, if the "Carbon Site of Forward Reaction Foward Reaction" is "1,2,3" and "KIE Forward" for that reaction is "0.98,0.98,0.997", then QIRN will assign 20‰ isotope effects on the C-1 and C-2 sites of the reactant and a 3‰ isotope effect on the C-3 site. In condensation reactions, the atomic sites and their KIEs are assigned from the first atom of Reactant A to the last atom of Reactant B. For example, if Reactant A and B are both C₂ molecules with isotope effects on the C-1 site of each, the user would input "1,3" in column 4 for that reaction. For the reverse reaction, the same principle applies, but the atomic sites are now structured from the first atom of Product C to the last atom of Product D.

Graphic User Interface

Running a Simulation

The graphic user interface (GUI) for QIRN gives users the ability to rapidly construct and analyze isotopologue networks. The GUI requires two inputs from the user, the Network Builder and Intermediate Database csv files. After loading the GUI, upload these files into QIRN.



Figure 5: Opening page for the QIRN GUI where you can select the necessary input files from your computer.

They can be stored anywhere on your computer. To run a simulation, click "Run Forward Model". QIRN will ask you to input a length of time to run the model. This input is in seconds with timesteps occurring every 0.1 seconds. Thus, if you input 300 seconds, QIRN will run the model for 3000 timesteps. Each network will require different amounts of time to reach steady state, as discussed in the main text. We recommend starting with 100 seconds and adjusting from there based on the outputs. While the model is running, QIRN will display a progress bar with an estimated time to completion in the command window.

```
100%|██████████| 1001/1001 [00:00<00:00, 11460.85it/s]
Number of reactions: 12
Number of substrates: 15
Number of isotopologues: 336
```

Figure 6: Screenshot of the progress bar from a QIRN simulation

Output Visualization

Once the model has completed running, QIRN will provide four modules for visualizing the outputs. The first two are the concentration of substrates and fluxes of reactions. These can modules report each of these parameters in time over the course of the entire simulation. They are useful for ensuring that QIRN has created the correct network topology and for assessing whether the simulation reached steady state conditions. The other two modules are for site-specific and compound-specific isotope compositions of each substrate. In the "Isotope Distributions" module, you can select individual substrates and QIRN will report their site-specific isotope composition. In the compound-specific module, QIRN will report the molecular average isotope composition of each substrate over the course of the entire simulation. This is a useful exercise for determining whether the model has reached isotopic steady state.

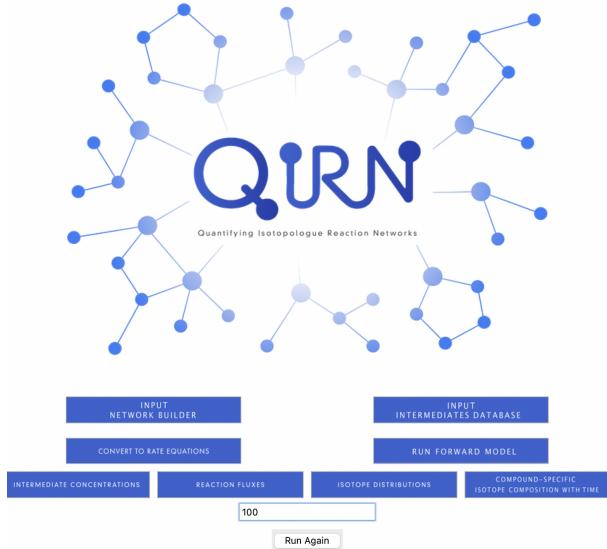


Figure 7: Options for data visualization after a simulation has completed running.

Implementing Network Changes

After running a simulation, changes to network topology, reaction rate constants, isotope effects, boundary conditions or can be made easily. Simply make the necessary changes to the Network Builder or Intermediate Database which is already uploaded into QIRN, save them, and hit the "Run Again" button. There is no need to re-upload them into QIRN again. You can also replace the Network Builder without replacing the Intermediates Database, or vice versa. Only when changes are made to the Reaction Database does the GUI need to be reloaded.

Converting to Rate Equations

In simulations where substrates concentrations do not begin at steady state, the steady state fluxes of reactions do not have a simple relationship to the reaction rate constants. Therefore, we developed an optimization function that inverts rate constants for reactions based on input steady state fluxes. To run this inversion, input a Network Builder and Intermediates Database file for the network. In the rate constants columns of the Network Builder file input the requested steady state fluxes for each reaction. Put in a specified time in which you would like to run the network to steady state. The rate constants inverted will depend on this time. If QIRN finds a set of rate constants that reaches the steady state fluxes, it will create a new csv file labelled with the name of your Network Builder file with an attached label of "FLUXINVERTED". This file is identical to the input Network Builder file with the inverted rate constants filled into the forward and reverse rate constant columns. In the command window, QIRN will report the residual between the requested steady state fluxes of reactions and those which are produced from the optimized reaction rate constants.