



A Guide to the Chemical Reaction Network Toolbox, Version 2.35

New in Version 2.35: Version 2.35 is the same as Version 2.3 with the addition of one module, accessible from the Reports menu (after running the Basic Report). When I was writing *Foundations of Chemical Reaction Network Theory* (Springer), it occurred to me that it would be great if I could enter a network in the Toolbox and then get LaTeX code for a network display along with LaTeX code for the corresponding system of differential equations. These could then be copied and pasted into LaTeX manuscripts for articles and books. After I finished *Foundations*, I decided to write such a module and add it to the Toolbox for the benefit of others. This should save you lots of work! I hope you like it. Please let me know if you find bugs: feinberg.14@osu.edu.

Marty

1. Preface

This is not a manual, but I hope you'll regard what follows to be a reasonably friendly introduction to the Chemical Reaction Network Toolbox. For me, the program is an indispensable tool. I hope that you'll learn as much from it as I have, either in a research setting or in the classroom. If you are a biologist, a chemist, or an engineer, there's not much you'll need to know about mathematics: The math takes place "behind the screen."

Of course, you should understand the questions the Toolbox is trying to answer. An important question (but not the only one) is this: Given a reaction network, is there any combination of rate constant values such that the corresponding mass action differential equations can admit two distinct positive steady state compositions that are stoichiometrically compatible? (The answer must be **yes** if, when taken with mass action kinetics, the network is to have the capacity for switch-like *bistability* involving positive steady states.) Similar questions can be asked and answered for reaction networks taken with kinetic rate functions that are not necessarily mass action but which satisfy certain weak constraints. (For mathematicians, there is an appendix to this Guide that will tell you how the mass action differential equations for a network come about.)

If you are just beginning in chemical reaction network theory, you can find an annotated bibliography — in particular articles relevant to the Toolbox — at the following site:

<http://www.crnt.osu.edu/annotated-bibliography>

(In some cases, pdfs are available there.) That bibliography is partitioned into three parts: *Early Reviews*, *Less Mathematical Articles*, and *More Mathematical Articles*. For users just getting started, I would recommend these early reviews:

[1] Feinberg, M., *Chemical oscillations, multiple equilibria and reaction network structure* in Dynamics and Modeling of Reactive Systems, edited by Warren E. Stewart, W. Harmon Ray and Charles C. Conley, Academic Press, New York, 1980. (pdf available www.crnt.osu.edu)

[2] Feinberg, M., *Chemical reaction network structure and the stability of complex isothermal reactors: I. The deficiency zero and deficiency one theorems*, Chemical Engineering Science, **42**, 2229-2268, 1987.

For mathematicians who want to delve deeper and see proofs, I'd recommend a download of some written lectures:

[3] Feinberg, M. *Lectures on Chemical Reaction Networks*, Written versions of lectures given at the Mathematics Research Center, University of Wisconsin, Autumn, 1979, available at

<http://www.crnt.osu.edu/LecturesOnReactionNetworks>

The book *Foundations of Chemical Reaction Network Theory* will be published by Springer in early 2019:

<https://www.springer.com/us/book/9783030038571>

This will be the most comprehensive guide to all that is in the Toolbox.

1. A. Some Toolbox History

The Chemical Reaction Network Toolbox was originally written as a program for the Microsoft DOS operating system. I wrote the original version — Version 1.0 — to implement theory developed for *mass action* networks of deficiency zero and deficiency one. (The “deficiency” is a non-negative integer index with which reaction networks can be classified. More on that later.) I got carried away and also provided a simulation module, ChemLab, with which the mass action differential equations for a network could be solved numerically and in which solutions could be represented graphically in a number of different formats, including phase portraits.. Version 1.0 would also write out the mass action differential equations for any network specified.

Somewhat later my Ph.D. student, Phillipp Ellison, developed theory that is quite good for large classes of mass action networks having deficiencies greater than one. Phillipp used my original code to extend the DOS version so that it could implement the results of his Ph.D. work. This became DOS Version 1.1, and it is still available at

<http://www.crnt.osu.edu/CRNTDOS>

You should know that the DOS versions (in particular the simulation module) can run quite well on advanced Windows machines, on Macs, and on Linux machines after installation of the DOSBox program on the respective machines. For a download of DOSBox and more information see

<http://www.dosbox.com/>.

Version 2.0 began, in part, as a port of the DOS version to Windows. It was begun by Philipp, using our code from the DOS version, but no attempt was made to reproduce the ChemLab simulation component. (Maybe someday!) Version 2.0 was extended by Haixia Ji. Version 2.1 implemented an expansion of deficiency-oriented theory in Version 2.0, in particular newer results in Haixia's Ph.D. thesis. For a network that you enter, the Toolbox's Higher Deficiency Report consolidates network analysis that derives from both Philipp's work and Haixia's.

Haixia also added code to produce, for an entered network, the *Mass Action Injectivity Report*. This contains network analysis having origins in separate work with an earlier Ph.D. student, Gheorghe Craciun:

[4] Craciun, G.: Systems of Nonlinear Differential Equations Deriving from Complex Chemical Reaction Networks. Ph.D. thesis, Department of Mathematics, The Ohio State University (2002).

[5] Craciun, G., Feinberg, M.: Multiple equilibria in complex chemical reaction networks. I. The injectivity property. *SIAM Journal on Applied Mathematics*, **65**, 1526–1546 (2005).

[6] Craciun, G., Tang, Y. and Feinberg, M. Understanding bistability in complex enzyme-driven reaction networks. *Proc. Natl Acad Sci USA*, **103**, 8697-8702, 2006.

*You should know that you will have no need to understand how any of the theory works. The various reports will tell you the results of the theory – for example, whether, for the entered network, there can be any assignment of rate constants such that the resulting **mass action** differential equations can admit two distinct positive steady-state compositions that are stoichiometrically compatible.*

Version 2.2 became significantly more powerful in that it gave information — in particular about the capacity of a network to give multiple steady states — even when the kinetics is not mass action, so long as the kinetics satisfies quite weak and natural conditions. In the references below, we show that a reaction network that has a structural property called *concordance* cannot admit two distinct stoichiometrically compatible equilibria, at least one of them positive, so long as the kinetics falls within a wide class called *weakly monotonic*. (Mass action kinetics is just a special case.) For still broader kinetics — e.g., *weakly two-way monotonic* or, more generally, *weakly monotonic with respect to an influence specification* — multiple steady states are similarly precluded provided that the network satisfies conditions that are stronger variants of concordance. In fact, *one can assert more than just the preclusion of multiple steady states*; this is discussed in [7]-[9] below:

[7] Shinar, G. and M. Feinberg, *Concordant chemical reaction networks*, *Mathematical Biosciences*, **240**, 92-113 (2012).

[8] Shinar, G. and M. Feinberg, *Concordant chemical reaction networks and the species-reaction graph*, *Mathematical Biosciences*, **241**, 1–23 (2013).

[9] Knight, D., Shinar, G., Feinberg, M., *Sharper graph-theoretical conditions for the stabilization of complex reaction networks*, Mathematical Biosciences, **262**, 10–27 (2015).

So how do you know if a network has the concordance property (or one of its stronger variants)? New modules, written by Haixia Ji and Daniel Knight, will tell you the answer, in response to a network that you supply. And those modules will also tell you some consequences of concordance. *Again, you don't have to understand how the theory works or even what concordance means — that's a little complicated.* It's the *consequences* of concordance that matter, and the Toolbox will tell you what some of those consequences are.

Version 2.3 (extended by Daniel Knight) is very similar to Version 2.2. The main differences reside in the way the primary concordance module is implemented and in the nature of information given by the Concordance Report. We think the newer reports carry much more information. Also new was a module that tells you whether a reaction network is “degenerate” or “nondegenerate,” an issue raised in references [7]–[9]. Here it suffices to say that degenerate networks are poor candidates for the description of real phenomena, for they lack robustness. Certain qualitative phenomena exhibited by a model derived from a degenerate network can disappear if the network is perturbed even slightly, say by making an irreversible reaction reversible, with a vanishingly small rate constant for the newly added reverse reaction.

Readers interested in the details of how some of the modules are implemented numerically might consult the PhD dissertations of Haixia Ji and Daniel Knight.

[10] Ji, Haixia, *Uniqueness of Equilibria for Complex Chemical Reaction Networks*, Ph.D. thesis, Department of Mathematics, The Ohio State University, June, 2011.

[11] Knight, D., *Reactor Behavior and its Relation to Chemical Reaction Network Structure*. Ph.D. thesis, Department of Chemical and Biomolecular Engineering, The Ohio State University August, 2015.

In Version 2.35 there is one additional module, accessible from the Reports menu, once the Basic Report has been written. This new entry on the Reports menu, *Write LaTeX ODEs*, is meant to help you write articles or books. For the network entered, it writes LaTeX code for a reaction network display and for the corresponding differential equations (both general and mass action). These can then be copied and pasted into your LaTeX manuscripts. The Toolbox output is a self-contained LaTeX document, so you can use it in a LaTeX viewer to see how the display will look.

1. B. A Few Words about Installation

The *Chemical Reaction Network Toolbox* is a Windows program, but it does not need to be installed in a fancy way. That is, it is a stand-alone program that runs from its own directory, just as the DOS version does. It is probably a good idea to make a directory for the program, perhaps called *CRNT*, on any drive that you will use and then keep all of your associated files there — for example, network files but also the main program file. If you also use the DOS version, — there are good reasons to do so, in particular the simulation environment — it too should have its own directory with a different name because DOS network files are not compatible with the Windows version (and vice versa).

Mac users should know that the Windows version of the Toolbox will run quite nicely in the presence of *WineBottler*, a free Mac application for running Windows programs. It's available here:

<http://winebottler.kronenberg.org/>

1. C. Acknowledgments

The Chemical Reaction Network Toolbox owes a great intellectual debt to my late colleague Fritz Horn who, with Roy Jackson, made some of the major discoveries leading to early results in reaction network theory.

Work on reaction network theory has received a great deal of encouragement over the years, both from chemical engineers and mathematicians. Especially helpful among the engineers were Rutherford Aris, Roy Jackson and, in the earliest days, Fritz Horn, all great pioneers in the subject. On the mathematical side, the interest and support shown by Chris Jones, Mike Reed, Jim Serrin, Dave Terman and, especially, Charles Conley remain gratifying. Although I have mentioned some students whose work underlies the Toolbox in explicit ways, others have contributed to reaction network theory struggles that, in indirect ways, made the Toolbox possible. Thanks to Vivek Dahholkar, Paul Berner, Bob Carmola, Ming Yue, David Rumschitzki, Marcelo Korc and, especially, Paul Schlosser, whose remarkable work set in motion later development of the injectivity-oriented theory in Gheorghe Craciun's Ph.D. work (which is also remarkable). Recent work on concordant chemical reaction networks was with Guy Shinar and Daniel Knight. Guy and I are grateful to Uri Alon for his support and example.

Much of the theory underlying the Chemical Reaction Network Toolbox has been supported over many years by the United States National Science Foundation and, more recently, by the National Institutes of Health.

2. The Chemical Reaction Network Toolbox: An Overview

The basic object of study in *Chemical Reaction Network Theory* is a reaction network, *not* the network taken with a particular kinetics (or, when the kinetics is mass action, with a particular assignment of rate constants). A typical question that CRNT tries to answer is of the following kind: For a specified reaction network, does there exist *some* assignment of kinetics within a particular class (e.g., mass action, weakly monotonic) such that the corresponding differential equations have Property X? Property X might be the existence of multiple steady states, the existence of periodic composition oscillations, and so on.

Motivated by considerations of biology, the focus is entirely on isothermal systems. (This is often inappropriate in a more general chemical engineering context.) When the temperature is constant, complexity in the governing equations comes from the chemistry itself, uncomplicated by thermal effects. This is the situation that interests me most because then the relationship between dynamical behavior and reaction network structure shows itself most clearly.

Much — but certainly not all — of the emphasis in this version of the Toolbox is on implementation of deficiency-oriented parts of chemical reaction network theory, so now's the time for me to tell you about the deficiency stuff. Then I'll be in a better position to tell you what the Toolbox does.

It turns out that reaction networks can be classified by means of a non-negative integer index called the *deficiency*. There are networks of deficiency zero, of deficiency one, of deficiency two, and so on. The deficiency has little to do with the size of the network. (There are deficiency zero networks that contain thousands of species and thousands of reactions.) In any case, you won't have to know how to calculate the deficiency of a reaction network because the Toolbox does that for you.

Some time ago Fritz Horn, Roy Jackson and I did work that gave rise to the *Deficiency Zero Theorem*. This says that deficiency zero networks — even very complex ones — are, in some ways, *boring*. Taken with *mass action* kinetics, the corresponding (isothermal) differential equations *never* give rise to multiple positive steady states, they *never* give rise to an unstable positive steady state, and they *never* give rise to periodic composition oscillations. This is true *no matter how intricate the network might be and no matter what (positive) rate constant values are assigned to the various reactions*.

The same is not true for networks of higher deficiency. Among the deficiency *one* networks there is already a great deal of subtlety. For some deficiency one networks there are, for example, mass action rate constants that give rise to multiple positive steady states. For slightly different deficiency one networks no such rate constants exist. Deficiency one networks come up very often in the study of reactors involving catalysis, including enzyme catalysis, so it is important to know something about them.

There is now a fairly complete theory of multiple steady states for deficiency one networks. In effect, deficiency one theory translates questions about a complex system of nonlinear algebraic equations into questions about several simple systems of *linear inequalities*. Deficiency one theory

will answer the following question, either affirmatively or negatively: *Given a (regular) deficiency one network, is there an assignment of positive rate constants such that the resulting isothermal mass action differential equations admit multiple positive steady states?* When the answer is yes, deficiency one theory will construct an example – that is, a set of rate constants and two distinct steady states consistent with those rate constants. (The theory will also tell you whether there is a set of rate constants that gives rise to a degenerate steady state — that is, a steady state associated with an eigenvalue of zero.)

For mass action networks having deficiency higher than one, theory goes off in two directions. One of these, developed in the Ph.D. work of Phillipp Ellison and Haixia Ji, remains — to some extent — deficiency-oriented and for that reason I will, for the purpose of the Toolbox, call it *higher-deficiency theory*. The other direction has its roots in Ph.D. work of Gheorghe Craciun. There the focus is on a network property called *injectivity*. I'll discuss these separately.

Higher deficiency theory works very much like deficiency one theory, at least in spirit. It also translates questions about a complex system of non-linear algebraic equations into questions about several simple systems of inequalities and equalities. Very often, all of these inequality-equality systems that the theory deduces for a particular network are strictly *linear* (as in the deficiency one case).

However, for *some* networks of higher deficiency, these systems contain a few *nonlinear* inequalities. As with deficiency one theory, the higher-deficiency theory will try to answer the following question, either affirmatively or negatively: *Given a reaction network, is there an assignment of positive rate constants such that the resulting isothermal mass action differential equations admit two distinct positive steady states that are stoichiometrically compatible?* When it can answer the question affirmatively, higher deficiency theory will construct an example — that is, a set of rate constants and two distinct steady states consistent with those rate constants. When the network is such that the theory is unable to translate the problem into the study of systems of entirely *linear* inequalities, it *might* be unable to offer a definitive answer. (This situation does not arise for regular deficiency one networks, so for that reason deficiency one networks are especially pleasant.)

Mass action injectivity theory will sometimes give answers when the higher deficiency theory gives no answers at all. If a network has the *mass action injectivity property*, then there can be no assignment of rate constants such that the resulting mass action differential equations admit two distinct positive steady states that are stoichiometrically compatible, nor can the those equations admit a degenerate positive steady state. Determination of whether a network has the mass action injectivity property can be difficult, *but the Toolbox will do it for you*, and it will tell you the consequences of that determination — for example the impossibility of multiple steady states.

You don't even need to know what "the mass action injectivity property" means, but, for the record, the definition that the Toolbox uses is the one invoked in the following article:

[12] Craciun, G., and M. Feinberg, *Multiple equilibria in complex chemical reaction networks: semi-open mass action systems*. SIAM J. Appl. Math., **70**, 1859-1977, 2010.

So far, almost all that I’ve said — either about deficiency-oriented theory or about mass action injectivity theory — has been in relation to networks endowed with mass action kinetics. If the kinetics is (perhaps) *not* mass action but instead satisfies far weaker constraints (e.g., weakly monotonic) then there are still things that can be said — for example, the absence of multiple stoichiometrically-compatible positive steady states — if the network is *concordant* (or, more generally, *concordant with respect to an influence specification*). The *Toolbox* will determine for you whether the network is or is not concordant (or concordant with respect to an influence specification). And it will tell you some consequences of its findings.

So, in light of all that has been said, what does the Toolbox do? In short, for a specified network it writes *reports*. After you enter a network — more on that later — you should immediately go to the Reports Menu and select the **Basic Report**.

The Basic Report will, as the name suggests, tell you some basic features of the network — all discussed in reference [2] above — including its deficiency. If the deficiency is *zero*, the Basic Report will tell you some consequences of the Deficiency Zero Theorem. If the deficiency is *one* you should then go the Reports Menu and run the **Deficiency One Report**. (Sometimes consequences of the Deficiency One Theorem will also be discussed in the Basic Report.) If the Basic Report indicates that the network’s deficiency is greater than one, you can then select the **Higher Deficiency Report** from the Reports Menu. That report focuses on the network’s capacity to admit multiple steady states in the mass action context, and you can also run the **Zero Eigenvalue Report** to learn the network’s capacity for a degenerate positive steady state.

After running the Basic Report, you will always be able to select the **Mass Action Injectivity Report** from the Reports Menu. That report will tell you whether the network has the mass action injectivity property. In some instances — in particular for networks of deficiency three or more — it can be advantageous to run the Mass Action Injectivity Report *before* running the Higher Deficiency Report. Sometimes the Basic Report will offer that same advice.

The **Concordance Report**, the **Strong Concordance Report**, and the **Arbitrary Influences Concordance Report** will tell you whether or not a particular network is concordant, strongly concordant, or concordant with respect to species influences that you specify. These are the reports to run if you are dealing with a network for which the kinetics is perhaps not mass action. The **Network Degeneracy Test** will tell you whether a reaction network is “degenerate” or “nondegenerate,” an issue raised in references [7]-[9] above. For reasons indicated earlier, degenerate networks are poor models for real behavior.

As I indicated earlier, the **Write LaTeX ODEs** module (accessible on the Reports menu after writing the Basic Report) will deliver LaTeX code for display of the network, and also LaTeX code for the corresponding differential equations, in both the mass action and general kinetics cases

About the speed of generating the various reports: For moderately-sized networks all reports are usually written quite quickly. For larger networks, involving, say, more than 20 reactions, a particular report might be very fast but another might take a *very* long time. A good example is the network provided in the accompanying example file *Huang-Ferrell Model MAPK Signaling.NET*.

(More on that later.) It has 22 species and 30 reactions. The Huang-Ferrell model is a *mass action* system, and the Higher Deficiency Report will tell you quite quickly that there are indeed rate constants for which the resulting mass action differential equations give multiple steady states. Since the network is *intended* to be a mass action model, written at the level of elementary binding and unbinding steps, it makes sense to run the mass-action modules.

However, if you decide to run the Concordance Report, it will take *many hours* to tell you that this somewhat large network is *not* concordant. (Indeed, it can't be if, as the Higher Deficiency Report already told us, the network admits multiple steady states for some weakly monotonic kinetics — e.g., mass action kinetics.) In fact, you might think that the program crashed because it seems unresponsive. But it's just doing what you asked it to do. The program aims to please.

On the other hand, if you run the Concordance Report for the supplied file *Wnt Pathway Model Goentoro-Kirschner.NET*, you'll get a response almost immediately. In this case there are 13 species and 20 reactions. The network is concordant and, in fact, strongly concordant..

3. How to Enter a Reaction Network

Reaction entry is fairly intuitive. When the initial program title closes, you will see a blank screen with a menu bar at the top. From the File menu, you can choose New Network or else Open Network; the second choice permits you to open a network that you have saved from a previous session.

If you choose New Network you should first decide how you want to name the chemical species. The default is alphabetical — A, B, C, and so on — in which case you can have no more than 26 species. By clicking the appropriate button, you can also choose to name the species A1, A2, A3,..., A100. You can also devise names of your own choosing, such as H2O, ATP, or Brittany. Species names are case-sensitive. (Here again you can have as many as 100 species.) In the last case, the Toolbox will ask you to declare your species names before network entry begins in earnest.

Then you just add reactions one by one. Click the little “reversible” box if you want the reaction to be reversible. As you enter reactions you will see the list of all reactions grow in a nearby window. For reasons connected to reaction network theory, you will also see an evolving list of “complexes.” (The complexes are the objects that appear at the heads and tails of the reaction arrows. Thus, the complexes of the reaction $A + B \rightarrow C$ are $A + B$ and C .) If you want to re-use a complex for a new reaction entry (e.g., for $A + B \rightarrow D$, just double click on that complex in the complex display window.) If you decide that you want to delete or otherwise edit a reaction that you've entered, just double-click on that reaction in the reaction display box, and you'll be presented with handy choices.

There is a little reaction-entry trick you might find helpful. In various applications — see reference [1] or [2] — it is useful to include in a network reactions of the form $A \rightarrow 0$ (“A reacts to zero”) or $0 \rightarrow A$ (“zero reacts to A”). To add such reactions you can insert them in the normal way or you can just double-click on the name of the appropriate species (e.g., A), in which case you will

be given a choice of adding the *effluent reaction* $A \rightarrow 0$, the *feed reaction* $0 \rightarrow A$, or both. As of version 2.2 there are buttons that will permit immediate entry (or removal) of effluent or feed reactions for *all* species.

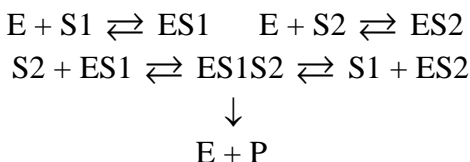
Once you have completed your reaction list, you can give the network a title (recommended) and you can add a comment. If you choose to do so, you can then save the network by choosing that option on the File menu. Networks are saved in files of the form *.NET. It is probably best to save networks in the same directory that you use for the Toolbox program.

The Network entry on the main menu will allow you to either clear an entered network (and its completed reports) or else edit the current network. Recall that when you edit a network, double clicking an existing reaction in the current reactions list will provide useful editing options for that reaction.

4. Some Examples

We are including with the program distribution three *.NET files for you to play with:

Simple Two-Substrate Enzyme Mech. NET: This example is motivated by a very simple “toy” reactor considered in reference [6] of Section 1. The chemistry is quite elementary, involving a classical (mass action) elementary-step model for two-substrate enzyme catalysis — the random (or unordered) binding model — whereby two substrates, S1 and S2, react to form a product P in the presence of an enzyme E:



In [6], the substrates are fed to a stirred-flow-reactor, within which the enzyme is entrapped by means of membranes at the inlet and outlet ports. The substrates and products are presumed to pass freely through the membrane in the effluent stream. (As explained in [1] or [2], a reaction such as $S1 \rightarrow 0$ is added to the true chemistry in order to model the outflow of S1, while $0 \rightarrow S1$ is added to account for the presence of the feed stream.)

If you run the Basic Report, you will see that the network has a deficiency of *two*. The Higher Deficiency Report will then tell you that there are parameter values for which the corresponding mass action differential equations for the reactor exhibit multiple steady states, and you will be shown a concrete example. The Zero Eigenvalue Report will give an example of parameter values for which there is a degenerate positive steady state. The Mass Action Injectivity Report will tell you that the network is *not* injective. Indeed, it *cannot* be injective: Recall that if the network has the injectivity property, the mass action differential equations are qualitatively incapable of multiple positive steady states.

Similarly, the network cannot be concordant, as the Concordance Report will tell you.

Huang-Ferrell Model MAPK Signaling.NET. This is the somewhat complicated cascade model for MAPK signaling that appears in

Huang, C.-Y. and J. E. Ferrell, Jr., Ultrasensitivity in the mitogen-activated protein kinase cascade, *Proc. Nat. Acad. Sci. USA*, **93**, 10078-10083, 1996.

In this case the Basic Report will tell you that the network's deficiency is *five*. Here again, the Higher Deficiency Report and the Zero Eigenvalue Report will tell you that the mass action differential equations have the *qualitative* capacity to engender multiple steady states or a degenerate positive steady state. The reports give examples of rate constant values for which these phenomena might be realized. (The Toolbox is smart in its own way, but it knows nothing about MAPK signaling, so the rate constant examples that it generates are not at all cognizant of the actual chemistry. The Toolbox only seeks to determine whether the network has the *qualitative* capacity to engender phenomena of a particular kind. When the answer is *yes*, it provides an example.) The Mass Action Injectivity Report will tell you, as it should, that the network is *not* injective.

As I've already said, the Concordance Report will tell you, as it should, that the network is *not* concordant, but this takes a very long time.

Wnt Pathway Model Goentoro-Kirschner.NET: This network was motivated by a paper, authored by Lea Goentoro and Marc Kirschner, about interesting properties of the WNT pathway:

Goentoro, L. and Kirschner, M., Evidence that fold-change, and not absolute level, of β -catenin dictates Wnt signaling, *Mol. Cell*, **36**, 872-884 (2009).

For this example, the Basic Report will tell you that the network's deficiency is two. The Higher Deficiency Report will tell you that, despite the complexity of the network, there is no assignment of rate constants such that the resulting mass action differential equations admit two distinct positive steady states that are stoichiometrically compatible. (So the network's qualitative capacity for positive-steady-state bistability is precluded.) The Mass Action Injectivity Report indicates that the network is injective — so, again, multiple positive steady states are precluded.

In this case, preclusion of multiple steady states with *mass action* kinetics came from two different sources, the Higher Deficiency Report and the Mass Action Injectivity Report. For other networks it can happen that the Mass Action Injectivity Report is indecisive because it merely determines that the network is not injective, but the Higher Deficiency Report nevertheless tells you that multiple positive steady states are impossible. (Injectivity is sufficient to preclude multiplicity but not necessary.) For still other networks it can happen that the Higher Deficiency Report is indecisive, while the Mass Action Injectivity Report indicates that the network is injective) so multiple positive steady states are impossible). Alas, it can also happen that the network is not injective and the Higher Deficiency Report is indecisive.

The Concordance Report tells you — quickly in this case — that the network *is* concordant. This implies that even if the kinetics is *not* mass action there is *still* no possibility of stoichiometrically-compatible positive steady states, so long as the kinetics is weakly monotonic. The Strong Concordance Report tells you— again quickly— that the network *is* strongly concordant. This im-

plies that there is no possibility of multiple stoichiometrically-compatible positive steady states, even if the kinetics falls within the still broader two-way weakly monotonic class.

5. About the Concordance Report and Options in Version 2.35

As of Version 2.3 the Concordance Report module gives more information than it did in Version 2.2, in particular about the nature of eigenvalues associated with equilibria. For a network of interest, the concordance module (in its default mode) determines not only whether the network is concordant but also whether its “fully open extension” is concordant. The fully open extension of a network is the original network taken with a reactions of the form $A \rightarrow 0$ (A reacts to zero), one for each species. This is because concordance or discordance of a network’s fully open extension gives very valuable information about the behavior of the original network — information that is included in the Concordance Report. In most cases, there is no serious time penalty for examining the concordance of both the network and its fully open extension. For very large networks, however, the time penalty could be distressing. For this reason the main menu now contains an Options tab that will allow the user to simply determine the concordance of the original network without also examining the concordance of the network’s fully open extension.

6. A Concluding Remark

I hope this little guide has been helpful. The *Toolbox* is a work in progress. We expect to add more theoretical capabilities to the program as Chemical Reaction Network Theory continues to evolve. Meanwhile, we know that the program itself has quirks — not, we hope, in the answers it gives but, rather, in its user experience. My students and I would be grateful for any comments or bug reports you would care to send. (Sample *.net files with instructions about how to provoke aberrant behavior are always helpful.) These can be addressed to feinberg.14@osu.edu.

Best wishes,



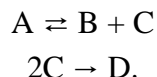
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Appendix. A Primer for Mathematicians

My purpose here is to tell mathematicians a little bit about how chemists and engineers formulate the (mass action) differential equations for a reaction network.

I'll proceed by way of an example. Imagine a closed vessel containing a liquid solution in which there are four chemical species, say A, B, C and D. The solution is stirred constantly and maintained at a fixed temperature. The stirring is so vigorous that the molar concentrations of the four species — I'll call these c_A , c_B , c_C and c_D — remain spatially uniform at all times. You can think of c_A as the number of molecules of A per unit mixture volume. (Actually, it's the number of molecules of A per unit volume divided by Avogadro's number, which is roughly 6 followed by 23 zeros. This scaling helps keep molar concentration reasonably close to 1.)

I'm going to suppose that the chemistry in the vessel is well described by the little reaction network



The symbol \rightarrow indicates a chemical reaction, and \rightleftharpoons indicates that reactions go both ways. Thus, A decomposes to form a molecule of B and a molecule of C. Conversely, a molecule of B combines with a molecule of C to form a molecule of A. Finally, two molecules of C combine to form a molecule of D. It's easy to see that the three reactions can cause the molar concentrations to change with time.

If we are to write differential equations that govern the mixture's evolution, we'll need to say something about how quickly reactions occur. For the situation under consideration, chemists and engineers generally suppose that the instantaneous occurrence rate of each reaction depends in its own way on the instantaneous molar concentrations: Associated with each reaction is a *rate function* that assigns to the mixture state (c_A, c_B, c_C, c_D) a non-negative molar *occurrence rate* per unit volume. (More precisely, the function's value is the number of occurrences per unit time per unit volume divided by Avogadro's number.) The collection of such functions — one for each reaction — is a *kinetics* for the reaction network.

More often than not, chemists invoke *mass action kinetics*, at least when the reactions are elementary. With mass action kinetics, the form of the rate function can be read off from the reaction itself:

To begin, let's consider the first reaction in our network, $A \rightleftharpoons B + C$. It's natural to suppose that the instantaneous occurrence rate per unit volume is simply proportional to the instantaneous number of molecules of A per unit volume. Thus, we take the molar occurrence rate per unit volume of $A \rightarrow B + C$ to be $k_1 c_A$, where k_1 is a (positive) *rate constant* for the reaction. (Rate constants are measured experimentally or are estimated from molecular theory.)

On the other hand, an occurrence of the second reaction, $B + C \rightarrow A$, requires that a molecule of B collide with a molecule of C. The likelihood of such a collision is presumably reflected in

the product $c_B c_C$. Thus, the molar occurrence rate is taken to be $k_2 c_B c_C$, where k_2 is a rate for the reaction $B + C \rightarrow A$. Similarly, an occurrence of the third reaction, $2C \rightarrow D$, requires that two molecules of C collide, so the occurrence rate is taken to be $k_3 (c_C)^2$, where k_3 is again a rate constant.

Now we are in a position to formulate differential equations that govern the species concentrations. We'll begin by considering species A: Whenever the reaction $A \rightarrow B + C$ occurs, we *lose* a molecule of A, and that reaction occurs at rate $k_1 c_A$. When the reaction $B + C \rightarrow A$ occurs we *gain* a molecule of A; that reaction occurs at rate $k_2 c_B c_C$. The reaction $2C \rightarrow D$, on the other hand, has no direct effect on the population of A molecules. Thus, with a prime denoting time differentiation, we write

$$(c_A)' = -k_1 c_A + k_2 c_B c_C.$$

Similarly, for species B we write

$$(c_B)' = k_1 c_A - k_2 c_B c_C.$$

The situation for C is a little more complicated, so we need to be careful. When $A \rightarrow B + C$ occurs, we gain *one* molecule of C, and that reaction proceeds at rate $k_1 c_A$. When $B + C \rightarrow A$ occurs, we lose *one* molecule of C, and that reaction proceeds at rate $k_2 c_B c_C$. When reaction $2C \rightarrow D$ occurs, we lose *two* molecules of C; that reaction proceeds at rate $k_3 (c_C)^2$. Thus, we write

$$(c_C)' = k_1 c_A - k_2 c_B c_C - 2k_3 (c_C)^2.$$

(Note the multiplier 2 in the last term.) After writing the appropriate equation for species D, we finally obtain the full system

$$\begin{aligned}(c_A)' &= -k_1 c_A + k_2 c_B c_C \\(c_B)' &= k_1 c_A - k_2 c_B c_C \\(c_C)' &= k_1 c_A - k_2 c_B c_C - 2k_3 (c_C)^2 \\(c_D)' &= k_3 (c_C)^2.\end{aligned}$$

This will give you some idea of how the mass action differential equations for a reaction network are formulated. Recall that the *Write LaTeX ODEs* module in Version 2.35 will, for an entered network, actually write out the differential equations for you (in LaTeX code). If you want to put your newly acquired chemical skills to a test, just make up a reaction network of your own and write the corresponding differential equations. Then enter your network in the Toolbox, and see if LaTeX code, displayed in your favorite LaTeX viewer, agrees.

Before you get overly confident, you should know that I've only told you some basics. For example, I haven't told you what to do if there's a feed stream entering the vessel and an effluent stream leaving it. These will affect the species populations within the vessel, so the streams must be taken into account when differential equations are formulated. This is accomplished by invoking pseudo-reactions of the form $A \rightarrow 0$ or $0 \rightarrow A$. (See references [1] or [2].)

The simple example we've just considered already suggests why there can even *be* such a subject as chemical reaction network theory. Despite the casual presentation, it is apparent that passage from a reaction network to the corresponding mass action differential equations proceeds in a precise way. In fact, *the network itself determines those equations up to values of the rate constants*. Thus, it makes sense to ask, for example, about the relationship between reaction network structure and the variety of qualitatively distinct phase portraits the corresponding equations might generate (as the rate constants take on all positive values).

The late mathematician Charles Conley told me that, apart from Newton's laws of motion, he had come to regard the differential equations deriving from reaction networks as the class he'd most like to study. Mathematicians have, of course, worked on reaction network *examples* in great detail, but I don't think that's what Conley had in mind. The interest for him was in the larger picture, in the class as a whole.

There is real magic in the relationship between reaction network structure and qualitative properties of the induced differential equations, and there's more theory available than is implemented the Toolbox, much of it very beautiful. I'm confident that there's still more — a lot more — that remains to be uncovered. Mathematicians will find fertile ground here.

As I mentioned earlier, mathematicians will find helpful the written lectures I mentioned at the beginning. Here's the link again:

<http://www.crnt.osu.edu/LecturesOnReactionNetworks>

Also, the book *Foundations of Chemical Reaction Network Theory* will be published by Springer in early 2019:

<https://www.springer.com/us/book/9783030038571>