Mathematical Analysis of Biological Interaction Networks (17w5099)

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1 Overview of the Field

Recent breakthroughs in experimental and high-throughput data analysis techniques have provided an unprecedented window into the complexity of the biochemical interactions in living cells. Fundamental metabolic and signaling pathways corresponding to basic cellular functions such as osmolarity, chemotaxis, the cell cycle, and apoptosis are now known to consist of dozens of metabolites interacting via hundreds of interdependent chemical reactions. New experimental techniques such as fluorescent proteins, meanwhile, have allowed real-time observations of such complex dynamical behavior as periodicity, hysteresis, and stochastic fluctuations.

Mathematics has emerged as a pivotal player in grappling with the complexity of these biochemical systems, and is a cornerstone of current systems biology research. Two common mathematical modeling frameworks for networks of biochemical interactions include systems of ordinary differential equations (ODEs) and stochastic continuous-time Markov chains (CTMCs). The classical mass-action form for the interaction rates reduces the ODE formulation to a polynomial dynamical system, which has led to wide-spread interest from researchers in algebraic geometry. When properly posed, these models have the ability to capture important properties of the physical system without the expense and time-consumption of laboratory experimentation and data collection. These models can also allow the generation of hypotheses which can then be tested in the laboratory.

Significant mathematical research has been conducted on relating structural properties of the underlying network of chemical interactions to admissible dynamical behaviors and properties. These approaches have attempted to identify motifs which contribute to biologically important behaviors such as biochemical switching, oscillatory behavior, concentration robustness, and persistence (i.e. non-exhaustion) of metabolites. Classical network-based approaches have included flux balance analysis, stoichiometric network analysis, chemical reaction network theory, and monotone systems theory. Remarkably, many results have been derived which guaranteed qualitative behavior which is robust to the system's parameters and initial conditions.

Research on network-based approaches to biochemical reaction models is now conducted by prominent researchers in a wide breadth of complementary mathematical disciplines, including differential equations and control theory, stochastic processes, algebraic geometry, optimization and computation, and analysis.

While work in these areas is often distinct in flavor, it has been the community's experience that the most successful approaches come from sharing ideas across lines of expertise. This workshop focused on establishing communication among the different disciplines and building new collaborations.

2 Workshop Structure

The workshop contained a mix of prepared talks and free time for collaboration and discussion. Since the workshop participants had areas of expertise spanning many distinct mathematical disciplines, the invited talks were chosen to reflect a diversity of mathematical approaches. Eight speakers were selected to give 40 minute talks on Monday and Tuesday which served as overviews of the notation and common approaches taken in their respective disciplines. On Monday morning, David F. Anderson and Gheorghe Craciun (both UW-Madison) introduced the notation relevant to two common dynamical modeling frameworks for biochemical interaction networks: deterministic ODE models and stochastic CTMC models. On Monday afternoon, Anne Shiu (Texas A&M) and Alicia Dickenstein (University of Buenos Aires) outlined algebraic approaches for the study of chemical interaction networks. Early Tuesday morning, Anne Condon (UBC) and Erik Winfree (Caltech) explained how reaction networks are used as computational tools, both in theory and in the laboratory. Later on Tuesday morning, Yiannis Kaznessis (University of Minnesota) and Eduardo Sontag (Rutgers University) discussed some engineering applications of reaction network theory. The remaining talks were 25 minutes in length and focused predominantly on current research. Speakers were also encouraged to present open problems for discussion. In total, 26 of the 42 participants gave talks, with many of the other participants leading breakout sessions.

The schedule was left deliberately more open in the later part of the week, so that as the week progressed it would allow for the open problems that were raised to have more time to be discussed in self-organized sessions. In total, two hours of breakout session / free time was offered during Monday (late afternoon), two and a half hours during Tuesday (early afternoon), all of Wednesday afternoon, two and a half hours on Thursday (early afternoon), and complete free time on Friday. Prior to the daily breakout sessions, participants were invited to propose open problems / discussion areas on a white board in the TCPL foyer. At the beginning of the breakout session time, the participants self-organized into groups and session rooms were assigned according to the size of the interested groups. Several of these groups continued discussing over multiple days, this included a group discussing extinction events in discrete reaction networks and one discussing the possibility of lifting oscillations from small networks to large ones.

3 Presentation Highlights

David Anderson (University of Wisconsin-Madison): *Stochastic models of CRNs. An overview.*

The workshop opened with a survey talk on the field of reaction networks, highlighting one of the core questions in the field: *what are the connections between dynamical behavior and network structure in CRNs?* This presentation set the stage for many subsequent talks by reviewing CRN terminology (reaction networks, stoichiometric space, linkage classes, weak reversibility, complex-balancing, deficiency, etc.) and CRN mass-action models, both deterministic and stochastic. In particular, a number of ways to represent stochastic CRNs were surveyed, including Gillespie algorithms, Kurtz's random time change representation, and the chemical master equation. An overview of current research directions in stochastic CRNs followed. One of the important open questions is determining when well-behaved deterministic dynamics of a CRN (for example, uniqueness of positive equilibria, stability of equilibria, etc) guarantees that the stochastic dynamics is also well-behaved (for example, positive recurrent). This is known to be true for deficiency zero, weakly reversible networks, which are known to be positive recurrent [21]. The search for other classes of networks with this property is another area of current interest; it is conjectured that weak reversibility is a sufficient condition for positive recurrence. The list of open questions also included finding conditions for stochastic CRNs to undergo an extinction event, and studying when combining well-behaved stochastic CRNs results in a well-behaved CRN. These questions have reoccured in later talks, and have also been the topic of some of the breakout sessions.

Gheorghe Craciun (University of Wisconsin-Madison): *On possible future directions for the mathematical analysis of reaction networks.*

Prof. Craciun's talk comprised a historical overview of the developments leading to the current state-of-the-art in deterministic CRNs (including the proof of the global attractor Conjecture). Many of the ideas in the field (for example detail and complex balancing) go back to Boltzmann, and attempts at proving the stability of detailed (complex) balanced equilibria go back to work of Shear and Higgins in the 1960's and before the celebrated work of Horn and Jackson. A first possible research direction identified in this talk is applying the machinery of deterministic CRNs to the Boltzmann equation. There is an interesting connection between the convergence to equilibrium in complex-balanced CRNs and Boltzmann's H-theorem (the distribution of molecule velocities in an ideal gas converges to the Maxwell distribution). This connection has been studied with the simplifying assumption of spacial homogeneity and when finite number of velocities are possible [22], and an open question was proposed on extending this approach to an infinite number of velocities. A theory of detail balanced and complex-balanced *infinite* reaction networks would be a key ingredient in this direction. Another area of exploration is the study of piecewise constant differential inclusions, a far-reaching generalization of complex-balanced systems. These objects have a very rich algebraic and combinatorial structure, are connected to toric dynamical systems, and are a key ingredient in the recent proof of the global attractor conjecture [5]. An interesting open question proposed in the talk is whether piecewise linear differential inclusions or some related general object can give rise to a Lyapunov function the way toric dynamical systems do.

Daniele Cappeletti (University of Wisconsin-Madison): *Stochastically Modeled Reaction Networks with Absolute Concentration Robustness.*

It is known [20] that certain structural conditions of a deterministic mass-action CRN imply absolute concentration robustness (i.e. some of the species have the same same concentration at all positive steady states). Recent work [21] has however also shown that under the same conditions on the network the stochastic CRN undergoes an extinction event with probability 1. This talk discussed a new result resolving this discrepancy between the deterministic and stochastic models. A key observation is that while in general when rescaled stochastic trajectories converge to a deterministic trajectory, in complex systems with differences of orders of magnitude the hypothesis needed for such general rescaling and convergence is violated, so the result does not apply. The talk presented the idea to divide the dynamics into a slow and a fast subsystem, and hypothesize that the fast subsystem has a product form stationary distribution. Under certain conditions, one can then show that up to any fixed time the averages of the ACR species counts tend to their ACR equilibria. It is an open question how fast this convergence is, and whether the convergence rate depends on the initial state. Finally, a conjecture proposing that all ACR systems have extinction events was shown false by way of counterexample.

Badal Joshi (California State University San Marco): *Graphically balanced equilibria and stationary measures of reaction networks.*

Graph-related symmetries of a reaction network give rise to certain special equilibria (such as complex balanced equilibria) in deterministic models. Correspondingly, in stochastic models these symmetries give rise to certain special stationary measures. Some new balance measures were discussed in the stochastic setting: the reaction balanced measure, the complex balanced measure, the reaction vector balanced measure and the cycle balanced measure. The reaction vector balancing is the same notion as detailed balancing in Markov chain theory. Relations between these balance measures in the stochastic setting were discussed. For example, reaction balance implies complex balance, reaction vector balance, and cycle balance. The talk discussed the idea of decomposing both deterministic and stochastic systems into so-called "factor systems" and establishing the correspondence between factors of a complex balanced deterministic system and those of the corresponding stochastic system. It is known by work of Dickenstein and Perez Millan that complex balance and cycle balance implies reaction balance. In the stochastic setting complex balance and reaction vector balance implies cycle balance and reaction balance, but this is not true in the deterministic setting.

Alicia Dickenstein (University of Buenos Aires): *Algebraic tools in the study of reaction systems.*

This overview talk focused on questions of multistationarity in mass-action CRN models. Large classes of systems (especially enzymatic mechanisms) give rise to steady state varieties having particularly nice parametrizations (in work of Gunawardena, Thompson, Feliu, Wiuf, Dickenstein, Perez Millan, etc.). These

parametrizations rewrite the steady state algebraic equations using a minimal number of variables. For example, while the n site phosphorylation network involves $3n + 3$ variables, its steady state variety can be parametrized using only three variables. This reduction proves very useful in answering questions of existence and number of positive steady states, and finding regimes of parameters (reaction rate constants, total masses) for which multistationarity is possible. A recent paper of Conradi et al. uses degree theory and the parametrization of the steady state variety to devise explicit conditions on parameters that guarantee the existence of multiple equilibria. On the other hand, purely algebraic techniques (not relying on the Jacobian) are applicable for classes of enzymatic systems. The example of enzymatic cascades was presented: algebraic methods show that if the enzymes are different, only one positive steady state is possible; however, multistationarity is possible if the enzymes coincide.

Anne Shiu (Texas A& M): *Algebraic methods for analyzing bistability and oscillations in reaction systems.*

This overview talk reviewed the current state of the art in the dynamics of phosphorylation networks (where the phosphorylation mechanism is either processive, distributive, or mixed), as well as other important biological pathways, including the ERK network. The talk focused on the questions of multistationarity and oscillation, although results on stability of equilibria were also briefly mentioned. These networks exhibit an variety of combinations of multistationarity/oscillation behaviors. For example, processive networks are globally stable (Conradi and Shiu 2015); distributive networks are multistationary; mixed networks admit oscillations; the ERK network admits multistationarity. The proofs of these facts rely on parametrizations of the steady state varieties, and use of the Routh-Hurwitz conditions, or of Yang's Hopf-bifurcation criterion. It is an open question if the double phosphorylation mechanism can admit oscillations. One possible approach to attacking this question is lifting the periodic orbits of the corresponding mixed phosphorylation network (which are known to exist), using techniques from the recent work of M. Banaji. This became the topic of one of the breakout sessions.

Anne Condon (University of British Columbia): *An introduction to molecular programming with stochastic CRNs.*

CRNs can be viewed as a programming language. Prof. Condon introduced this idea by way of examples: the sum of two integers $n_1 + n_2$ can be computed by the network $X_1 \to Y, X_2 \to Y$ as the count of Y when all reactions stopped firing and the initial molecule counts for X_1 and X_2 are n_1 and n_2, respectively. Similarly, the network $X_1 + X_2 \to Y$ computes $\min(n_1, n_2)$, and network $X_1 \to T, X_2 + Y \to 0$ computes $n_1 - n_2$. Thinking of them as programs, it is desirable that networks are *output stable* (i.e. output does not change in any reachable state from the output state) and that they *stably compute* the output (i.e. output is always reachable from all possible inputs). A small discussion on conditions implying these properties followed, along with considerations on the speed of the computations. These have to do the propensity of the reactions in the stochastic model; it can be showed that computing the sum is $O(\log n)$, whereas computing the minimum is $o(n)$. CRNs can also be used to compute logical predicates, like "$n_1 > n_2$": a corresponding network is $L \to Y, X_2 + Y \to N, X_1 + N \to Y$, where the output is Y if $n_1 > n_2$ and N otherwise. It can be shown that union and intersections of predicates can be computed using CRNs. However, many of these predicates are semi-linear and are slow computing (they require linear time). In these cases once can construct faster programs (networks) that converge to the correct output with high probability.

Erik Winfree (Caltech): *Computing with CRNs.*

Prof Winfree started his talk by discussing the similarities between programming molecules to perform a certain task in synthetic biology and programming electrical systems, but also pointing out that they are also vastly different. Our gap in the component-wise understanding of biological systems (smallest living thing is composed of thousands of interacting components) requires a distinct approach to bioengineering. The dynamics of CRNs are extremely varied, encompassing stable systems, oscillations, and chaos. He then proceeded to describe the implementation of the kinetic control of DNA strand displacement, and how it can be used to create desired CRN. He presented toehold-mediated strand displacement, where an incumbent strand is initially hybridized to a complementary displacement domain of a target strand by base pairing, and an invader strand is complementary both to the displacement domain of the target and to an adjacent toehold domain, allowing it to displace the incumbent to form a more stable duplex. The displacement is initiated by binding of the invader to the toehold, this is then followed by a branch migration process in which base pairs between target and incumbent break and are replaced by base pairs with the invader, and the overall

reaction rate for this displacement is strongly dependent on the toehold binding strength. Distinct species get distinct domains, and reactions in the system are then based on displacement. Experimental displacillator results were presented: the dynamics is slow but works reasonably well (no sustained oscillations, though). Probabilistic inference was discussed, as were chemical Boltzmann machines, and the complexity of robust CRN distributions.

Yiannis Kaznessis (University of Minnesota): *Closure scheme for chemical master equations.*

Probability distributions (in stochastic CRNs) can be described by moments, which encode the complete information on the distribution. Moments obey differential equations that govern the dynamic and steady state behavior of stochastic reaction networks. However, moment equations are generally not closed (derivatives of moments depend on higher order moments). The *zero-information closure scheme* for the moment equation starts with the premise that while higher order probability moments may not be numerically negligible, they contain little information to reconstruct the master probability. Higher order moments are then related to lower order ones by maximizing the information entropy $-\sum P(x)\ln(P(x)$ of the network; this is done by solving a system of non-linear algebraic equations coming from the corresponding Lagrange multipliers. Once the moments are computed, they are used to reconstruct the probability distribution, and the stability around steady states can be assessed by computing the eigenvalues of the moment Jacobian. The Schlogl model was used to illustrate the power of the approach, but also its limitations: for example, bimodality is lost when the system size is increased.

Eduardo Sontag (Rutgers): *Dynamic response phenotypes in systems biology: Scale-invariance and monotone I/O systems.*

This overview talk focused on output responses to external inputs. Some adaptive systems (ranging from bacterial chemotaxis pathways to signal transduction mechanisms) have an additional feature: *scale invariance*, i.e. the property that output depends not on the absolute change of the input, but rather on the scaling factor by which the input changes. A theoretical framework characterizing scale invariance was discussed, and typical structures for scale invariant mechanisms were presented (for example, incoherent feed-forward loops). The theory correctly predicts that the E. coli output behavior is unchanged under scaling of its ligand input signal, which has been verified experimentally. Chemosensing mechanisms, as well as cancer immunotherapy mechanisms have also been observed experimentally to exhibit scale invariance, with implications to model selection and validation.

Robert Brijder (Hasselt University): *Sufficient Conditions for the Eventual Dying of Reactions in Discrete Chemical Reaction Networks.*

In this talk the speaker presented conditions on CRNs for some of the reactions to stop firing in the long term, followed by a corollary that gives a sufficient condition for an extinction event to occur. The condition is based on the structure of the network alone (phrased in terms of Petri nets), and it produces a statement about the reachability of certain states, being therefore independent of the stochastic rates one may assign. A *subconservative* CRN of stoichiometric matrix Γ is defined by the existence of a a positive vector c such that $c\Gamma \leq 0$. A CRN is enlarged by adding edges from componentwise "larger" to "smaller" complexes, and in such a way that the reactions added do not end at complexes of a certain set $\mathcal{Y}$ (the enlarged network is called *dom-CRN Y-admissible*). It turns out that if the set $\mathcal{Y}$ of complexes has a certain structural property, if the CRN is subconservative, then any $\mathcal{Y}$-admissible dom-CRN is guaranteed an extinction event. Finding sufficient conditions for extinction in discrete CRNs that enlarge the class presented here is an open problem, as is the study of computational decidability of extinction questions. These issues have been the focus of one of the breakout sessions.

Robert Johnson (Caltech): *Formal Verification of Chemical Reaction Network Equivalence: A Bisimulation Approach*

This talk defined two CRNs to be equivalent if the have the same simulated behavior. This notion of equivalence is relevant in molecular programming, where the simplest mechanism for performing a task are often desirable. The setup is that of classical CRNs endowed with mass-action kinetics of rate constants set to one. The equivalence definition relies on syntactic Markovian bisimulation, which is also central to devising algorithms that check CRN equivalence. This area of research presents a series of open questions, for example can bisimulation prove some feature that a correct implementation must have, or can it give bounds bounds on the size of a correct implementation?

Nicolette Meshkat (Santa Clara University): *Using algebraic matroids and avoiding differential algebra in identifiability, observability, and indistinguishability.*

The questions of structural identifiability (i.e. uniquely recovering parameters), structural observability (uniquely recovering trajectories), structural indistinguishability (uniquely recovering models) in CRN input/output systems can be attacked using algebraic tools. The example of a linear 2-compartment model was used to exemplify the techniques, but these are applicable to general systems. If one of the species is used as the output (and thus observed without error for all time, and for any input), can one then uniquely identify all the parameters? It turns out that the answer is "no" even in this simple case. An algorithm for identifying rational combinations of parameters is based on the input/output equations and using differential algebra. This information can be then used to extract individual parameters that are identifiable using Groebner basis and elimination ideals; however, choosing a monomial order that leads to the desired result is in general hard to do. In new work, the speaker used elements of matroid theory to devise methods of choosing the correct order that allows certain parameters to be uniquely identified. Moreover, similar techniques are applicable to questions of indistinguishability.

Atsushi Mochizuki (RIKEN, Japan): *Observation and Control of Complex Nonlinear Systems Based on Network Structures.*

Understanding the dynamics of CRNs is a difficult task even when measurements on all species are possible. For example, measuring responses to changes in total amounts of enzymes is very subtle, as some changes are counter-intuitive, while some metabolites do not change at all. This talk presents a theory based in part on sensitivity analysis that links qualitative responses of a CRNs to its structure alone. In particular, it is shown that a particular set of nodes in the CRNs *the feedback vertex set* determines the CRN dynamics. In other words, this implies that steady states, periodic oscillations, quasi-periodic oscillations and other long-term behaviors, can be identified by measurements of a subset of molecules in the network, and this subset is determined by the network structure alone. Moreover, change in the dynamics (for example switching from one stable steady state to another) can be achieved by controlling only the feedback vertex set. A concrete regulatory network of 90 genes was analyzed using these techniques, and the theory predicted five genes that can be used to control the overall dynamics, in agreement with experimental observations.

Ankit Gupta (ETH Zurich): *Numerical estimation of the stationary solution of the chemical master equation.*

This talk focused on numerical estimation of the stationary solution for the chemical master equation. Solving the (generally infinite) system of ODE corresponding to the chemical master equation is a daunting task, as is, in general, finding the stationary solution of the chemical master equation. If the state space is infinite, the latter is equivalent to solving an infinite dimensional linear system, which can't be done directly. Instead, a truncation of the state space is considered (e.g. by truncating at certain a order of magnitude, by cutting off absorbing states, and by sending reactions to absorbing space to a designated state in the truncated space). It is shown that the stationary distribution corresponding to the truncated system approximates the stationary distribution of the original system, if the infinite state-space is irreducible, and if the reaction dynamics is exponential ergodic (which can be checked by constructing a Foster-Lyapunov function). The algorithm is shown to be effective even for very large state spaces ($\sim 10^9$ states), and on par with SSA performance for concrete protein pathways examples.

Matthew Johnston (San Jose State University): *Network Translation and Absolute Concentration Robustness.*

A species is said to have absolute concentration robustness (ACR) if its attains the same value at all positive steady states. This suggests evolved structures in biochemical reaction networks which are capable of maintaining narrow ranges for certain reactants. This talk presented new results expanding the class of networks known to have ACR (e.g., from work of Feinberg and Shinar), and conditions that ensure that the ACR values can be determined. The technique uses tools from generalized CRNs and translated CRNs, and show that (1) if translation is deficiency zero and weakly reversible, all complexes on common linkage class are robust; (2) if translation is deficiency one, then all pairs of nonterminal complexes are robust; and (3) the ACR value can be determined if deficiency is zero and under a few additional conditions. These results are illustrated by way of examples, including the futile cycle and the EnvZ-OmpR signaling pathway.

Heinz Koeppl (Technische Universität Darmstadt): *Biochemical networks in random environments: modeling and inference.*

Many cellular processes on the single-cell level show significant cell-to-cell variability even when the cells are genetically identical and share the same growth conditions. An additional source of variation in intracellular systems may be needed to account for this, as the noise from standard stochastic CRN models is insufficient to explain the magnitude of these differences. This talk focused on adding a source of variability from the random environment in which the cellular process is embedded. A generative joint model was presented in which the reactions had additional static random variables factoring multiplicatively into the kinetic rate. The main question of interest was whether one could represent this enhanced model with a simpler model which behaves as if embedded in a random environment. Various approaches, based on the chemical master equation, the Fokker-Planck equation, and the Liouville equation were discussed. In an example of a first order decay reaction filtering theory was used to make a successful simplification, in which conditional information on the additional static random variable factors was obtained from observations of the species counts over time.

Mercedes Perez Millan (University of Buenos Aires): *Checking multistationarity in MESSI systems.*

Many enzyme mechanisms fall under the class of MESSI (Modifications of type Enzyme-Substrate or Swap with Intermediates) systems. Here swaps includes transfer reactions, e.g. $X_p + Y \rightleftharpoons X_pY \to X + Y_p$. This talk presented new results on the study of steady states of MESSI systems; in particular, the focus was on toric MESSI systems, i.e. systems whose steady state variety can be described by binomial equations. A theorem characterizing multistationarity (and based on elimination of intermediates) for these systems is presented. It is shown that there exists a unique positive steady state if and only if there is a certain sign compatibility between minors of two matrices (one of which is the stoichiometric matrix, and the other comes from exponents of binomials in the steady state variety). A number of common examples can be studies using this results: for example, it follows that enzymatic cascades with common phosphotase for both layers is multistationary. The sign conditions can be rephrased in terms of certain subspaces intersecting certain orthants. The talk concluded with a discussion of algorithms checking these sign conditions and their connection with finding circuits in oriented matroids.

Lea Popovic (Concordia University): *Rare event calculations for chemical reaction systems.*

Theory of large deviations for random processes and rate calculations for frequencies of rare events was applied to stochastic models of CRNs. In order to define "rare" events one first needs to establish a concentration of measure property for the sequence of variables / sequence of processes based on some scaling parameter N (typical examples include the sample average of i.i.d variables and the empirical measure of i.i.d variables or of sequential outcomes of a Markov chain). In the chemical reaction network context the standard scaling would be to consider large amounts of all species and fast rates of all reactions. Rare events can then play a key role in switching between stable states in CRNs whose mean dynamics exhibits bistability. This talk showed how to determine the time scale of switching based on calculations from large deviation theory, and also compared the calculations for the rate of switching based on a scaled Markov chain model for the CRN to the calculations for this rate based on a small diffusion model for the same CRN. A framework for large deviation theory and results for multi-scale CRNs was also presented.

Greg Rempala (The Ohio State University): *Law of large numbers for the SIR process on a random graph.*

Motivated by the recent Ebola outbreak, and informed by epidemiological data coming from this outbreak, the speaker developed an SIR model evolving on a multilayer random graph with given degree distribution, where edges in different layers correspond to potentially infectious contacts of different types. The evolution of the graph follows the assumption that infectious individuals drop each of their contacts according to an exponential distribution, and that the dropped contacts, which could account for behavioral changes due to disease such as isolation or decreased mobility, cannot be recovered. This setup allows the derivation of a law of large numbers for a large graph that results in a system of ODEs which drive the evolution of various quantities of interest, such as the proportions of infected and susceptible vertices, as the number of nodes tends to infinity. The result is shown to be in concordance with data collected during the recent Ebola outbreak in Africa.

Alan Rendall (Johannes Gutenberg-Universitt Mainz): *Multiple steady states in models for the Calvin cycle of photosynthesis.*

A number of models have been proposed in the literature for the Calvin cycle, leading to a number of statements (many of which are based on numerical simulations) on the number and stability of steady states

(see Prof. Rendall's recent paper "A Calvin Bestiary"). However, many of these results are not very careful, and sometime overlook physical requirements on the models (e.g. that the steady states must be positive). The focus of the talk is the rigorous analysis of a simple model with five unknowns: it is proved that there are parameters for which there are two positive steady states, one of them stable, and a special case where there is a continuum of steady states. The analysis is made possible by identifying a weak fold bifurcation with 1D center manifold. More detailed mechanisms were also discussed. Using elementary flux modes, the Pettersson model (with fifteen unknowns and expressed as a system of differential-algebraic equations) is shown to have two positive equilibria for certain choices of parameters. The Poolman model is also discussed, and it can give rise to three positive steady states.

Carsten Conradi (HTW Berlin): *Multistationarity in biochemical reaction networks.*

There is an extensive literature on necessary conditions for multistationarity for mass-action CRNs. Most of those results depend on the structure of the network alone, are based on injectivity of the vector field, and go back to the work of Craciun and Feinberg. Roughly speaking, if the Jacobian of the system doesn't change sign on the positive orthant for all values of rate constants, multistationarity is ruled out. On the other hand, finding sufficient conditions for multistationarity, and parameter regimes that allow it, is still, by and large, an open question. This talk presented new results in this direction (Conradi, Mincheva, Feliu, Wiuf). Using degree theory, one shows that if the vector field is dissipative, does not admit boundary steady states, its Brouwer degree restricted to compatibility classes has a predetermined sign, and if the Jacobian does not change sign, then multistationarity is not possible. On the other hand (and perhaps most importantly), if the determinant changes sign and a certain parametrization of the steady state variety is possible, then the system admits at least two positive steady states.

Stefan Mueller (University of Vienna): *Sign conditions in chemical reaction network theory.*

Many results on existence and uniqueness of equilibria in mass-action, or generalized mass-action systems are expressed in terms of sign conditions on certain subspaces. This talk is an overview of these results. In the classical theory of complex-balanced mass-action networks, there is a very useful parametrization of the steady states. This extends to generalized mass action (where the monomial rates of reactions are not necessarily linked to reactant complexes). Two notions of deficiency can be defined in this setting, and the kinetic deficiency is intimately linked to complex-balancing: zero kinetic deficiency implies existence of complex balanced equilibria for all rate constants, while kinetic deficiency equal to $\delta > 0$ implies the existence of δ conditions on the rate constants for complex balancing. The existence and uniqueness of steady states for generalized mass-action systems corresponds to surjectivity and injectivity of generalized polynomial maps, which can be translated in terms of sign conditions on vectors of the stoichiometric subspace and its orthogonal complement. The talk concluded with a complete characterization of long-term dynamics of generalized Lotka systems: depending on the kinetic monomial, the system exhibits various behaviors, from center oscillations to global stability.

Maya Mincheva (Northern Illinois University): *Interplay between diffusion and delay.*

Diffusion and delay play a common role in biological pattern formation, and have mathematical similarities as well: they both destabilize a stable equilibrium of a CRN system of differential equations. The question considered here is whether, when both delay and diffusion are present, the destabilizing can be achieved by diffusion or by the delay alone. It is known since Turing that diffusion alone can destabilize an equilibrium if the diffusion constants are not equal; but does that happen if the delay cannot destabilize the system equilibrium? The analysis presented in this talk shows that the answer is no: *If the nonsingular matrix A is strongly stable with respect to delay (i.e. linearly stable for any choice of delays), then $A - D$ is strongly stable with respect to delay for any diagonal diffusion matrix D. (Mincheva, Hinow, 2016)*. The analysis makes use of a matrix-theoretic characterization of strong delay stability due to Hopfbauer, and involves calculations on the (quasi-linear) Jacobian of the delay system.

Casian Pantea (West Virginia University): *Inheritance of multistationarity in CRNs: lifting nondegenerate (stable) steady states)*. This talk presented a series of modifications on a CRN that preserves its capacity for multistability/multistationarity. Some of these modifications have already appeared in the literature (in work of Craciun, Feinberg, and Joshi, Shiu); however, the talk discusses a new common framework for all these results using the implicit function theorem on local coordinates on stoichiometric classes. The notion of "reduced Jacobian", i.e. the Jacobian of the vector field restricted to stoichiometric classes) is a

central object of the analysis. Examples of modifications that preserve multistability are (1) adding dependent reactions (e.g. reverse reactions); (2) adding inflow and outflow; (3) adding new species and inflows and outflows of that species; (4) adding new reversible reactions (with a nondegenerancy condition: full column rank of new species/reaction submatrix is required); (5) adding intermediate complexes involving new species (with a certain rank nondegeneracy condition); (6) adding enzymatic mechanism, i.e. replacing $y \to y'$ by $E + y \leftrightharpoons I \to E + y'$.

Jinsu Kim (University of Wisconsin-Madison): *Lyapunov functions and Tiers for ergodicity and mixing times of stochastic reaction networks.*

In this talk it was shown how the standard Lyapunov function for ODE models of mass-action CRN can be used to verify ergodicity of the stochastic CRN model. More precisely, it was shown that under certain conditions the Lyapunov-Foster criteria are satisfied. The approach is based on splitting monomials in tiers (following Anderson's proof of the global attractor conjecture for the one linkage class case). A sufficient condition based on the network alone is presented: if (1) the network is *double full* (i.e. it contains complexes $2A$ for all species A) and (2) every double complex has a path to a single or empty complex, then the Markov chain is positive recurrent. The talk also included a discussion on mixing time (time until distance between probability profile and stationary distribution is epsilon small). It was shown that the conditions above give rise to a "super Lyapunov function" (in which case the expected time to extinction has a uniform upper bound regardless of initial condition, and convergence to the stationary distribution is in fact exponential). It is conjectured that detailed balance also implies exponential convergence to the stationary distribution.

James Brunner (University of Wisconsin-Madison): *Robust permanence of deterministic reaction network models.*

In this talk the notion of *tropically endotactic* differential inclusions is presented. This is a far-reaching generalization of toric differential inclusions, and it emphasizes the rich combinatorial structure of vector fields corresponding to, for example, weakly-reversible mass-action networks. Tropically endotactic networks are defined starting with a certain polyhedral fan, whose exponentiation cuts out regions in the positive orthant on each of which the differential inclusion is defined by a constant cone. These regions correspond to areas of dominance of specific source monomials, which drive the dynamics in the direction of small cones around their respective reactions. A theorem is presented for 2D tropically endotactic differential inclusions, which are shown to be permanent – a continuum of trapping regions (much like level sets of a Lyapunov function) is defined, which drive the trajectory towards a compact set in the interior of the positive orthant. As an example, it is shown that if the Lotka-Volterra network is perturbed by pushing reaction vectors inwards, then the resulting system gives rise to a tropically endotactic differential inclusion, and the system is therefore persistent.

Germán Enciso (University of California-Irvine): Gave a tutorial on the stochastic models of reaction networks on Wednesday evening.

4 Scientific Progress Made

4.1 Breakout sessions summaries

Tuesday June 6

Cycle balance I (reported by G. Craciun). This group focused on determining whether cycle balance could replace complex balance in the construction of a Lyapunov function, along the lines of the Horn and Jackson theorem. The group established that, as stated, the question can be answered in the negative by way of counterexample: they constructed a cycle balanced network with three positive equilibria, which therefore can not have a global Lyapunov function.

Constructing Lyapunov functions for stochastic CRNs (reported by J. Kim). The standard Lyapunov function from the ODE CRN model does not readily work in the stochastic case. This group started considering alternative constructions, including piecewise linear Lyapunov functions. For bimolecular networks, the

group also considered defining Lyapunov functions as solutions of a certain PDE, using the classical Zubov's method.

Stochastic CRNs with extinction events I (reported by R. Brijder). The group considered the question of whether a given CRN has an extinction event, and what conditions on the network characterize this property. Issues of decidability and complexity of this question were also considered.

Oscillations in ODE phosphorylation models I (reported by A. Shiu). This group considered the existence of oscillations in the double phosphorylation system. One approach discussed here was "lifting" the oscillation from the mixed mechanism phosphorylation (shown to exist in a recent paper by Suwanmajo and Krishnan). Using a recent result of Banaji, a new model of mixed mechanism network was shown to exhibit oscillation.

General mass-action I (reported by M. Johnston). The group discussed results from the paper of Mueller and Regensburger, focussing on how existence of steady states and complex-balanced steady states relate to zero network deficiency and zero kinetic deficiency. Connections with toric dynamical systems were also discussed.

Thursday June 8

Combining networks in stochastic CRN models (reported by D. Anderson). The group considered the question of combining two "well-behaved" (for example, positive recurrent) stochastic CRNs: under what conditions is the resulting network also well-behaved? The group proved a series of preliminary results for small cases.

Stochastic CRNs with extinction events II (reported by R. Johnson). The use of Petri nets was considered in relation to the question: what are the states starting at which all reactions are guaranteed to fire for all times with nonzero probability? Complexity of this and related questions have been discussed, as well as decidability issues: the group identified four quantifiers relevant to these questions.

General mass-action and cyclic balance II (reported by D. Siegel). Relevant results due to Volpert were discussed, in particular the invariance of the positive orthant without monotonicity hypotheses, the connection between balance equalities and convergence to equilibrium, and the role of the Petri net cycles.

Oscillations in ODE phosphorylation models II (reported by A. Shiu). The group performed numerical searches of parameters for Hopf bifurcations in the double phosphorylation system, and also discussed the gaps in a recent paper claiming to have found such parameters. Moreover, candidates for minimal models of oscillations were considered.

Identifiability in stochastic CRNs models (reported by G. Rempala). The group fixed the gap in a long-standing proof about using MLE methods to consistently estimate parameters in stochastic models of CRNs, when incomplete observations of trajectories are observed.

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