

# Dynamic and static limitation in reaction networks, revisited

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## Abstract

The concept of limiting step gives the limit simplification: the whole network behaves as a single step. This is the most popular approach for model simplification in chemical kinetics. However, in its simplest form this idea is applicable only to the simplest linear cycles in steady states. For such the simplest cycles the nonstationary behaviour is also limited by a single step, but not the same step that limits the stationary rate. In this paper, we develop a general theory of static and dynamic limitation for all linear multiscale networks, not only for simple cycles. Our main mathematical tools are auxiliary discrete dynamical systems on finite sets and specially developed algorithms of “cycles surgery” for reaction graphs. New estimates of eigenvectors for diagonally dominant matrices are used.

Multiscale ensembles of reaction networks with well separated constants are introduced and typical properties of such systems are studied. For any given ordering of reaction rate constants the explicit approximation of steady state, relaxation spectrum and related eigenvectors (“modes”) is presented. In particular, we proved that for systems with well separated constants eigenvalues are real (damped oscillations are improbable). For systems with modular structure, we propose to select such modules that it is possible to solve the kinetic equation for every module in the explicit form. All such “solvable” networks are described. The obtained multiscale approximations that we call “dominant systems” are computationally cheap and robust. These dominant systems can be used for direct computation of steady states and relaxation dynamics, especially when kinetic information is incomplete, for design of experiments and mining of experimental data, and could serve as a robust first approximation in perturbation theory or for preconditioning.

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## 1 Introduction

Which approach to model reduction is the most important? Population is not the ultimate judge, and popularity is not a scientific criterion, but “Vox populi, vox Dei,” especially in the epoch of citation indexes, impact factors and bibliometrics. Let us ask Google. It gave on 31st December 2006:

- for “quasi-equilibrium” – 301000 links;
- for “quasi steady state” 347000 and for “pseudo steady state” 76200, 423000 together;
- for our favorite “slow manifold” [1,2] 29800 links only, and for “invariant manifold” slightly more, 98100;
- for such a framework topic as “singular perturbation” Google gave 361000 links;
- for “model reduction” even more, as we did expect, 373000;
- but for “limiting step” almost two times more – 714000!

Our goal is the general theory of static and dynamic limitation for multiscale networks. The concept of limiting step gives, in some sense, the limit simplification: the whole network behaves as a single step. As the first result of our paper we introduce further specification in this idea: the whole network behaves as a single step in statics, and as *another* single step in dynamics, the stationary rate and the relaxation time to this stationary rate are limited by different reaction steps, and we describe how to find these steps.

The concept of limitation is very attractive both for theorists and experimentalists. It is very useful to find conditions when a selected reaction step becomes the limiting step. We can change conditions and study the network experimentally, step by step. It is very convenient to model a system with limiting steps: the model is extremely simple and can serve as a very elementary building block for further study of more complex systems, a typical situation both in industry and in systems biology.

In IUPAC Compendium of Chemical Terminology one can find two articles with definition of limitation [3,4]. Rate-determining step (rate-limiting step): “These terms are best regarded as synonymous with rate-controlling step.” “A rate-controlling (rate-determining or rate-limiting) step in a reaction occurring by a composite reaction sequence is an elementary reaction the rate constant for which exerts a strong effect – stronger than that of any other rate constant – on the overall rate.”

It is not wise to object to a definition and here we do not object, but, rather, complement the definition by additional comments. The main comment is that usually when people are talking about limitation they expect significantly more: there exists an elementary reaction a rate constant for which exerts such a strong effect on the overall rate that the effect of all other rate constants together is significantly smaller. Of course, this is not yet a formal definition, and should be complemented by a definition of “effect”, for example, by “control function” identified by derivatives [3] of the overall rate of reaction, or by other overall rate “sensitivity parameters”.

For the IUPAC Compendium definition a rate-controlling step always exists, because among the control functions generically exists the biggest one. For the notion of limitation that is used in practice there exists a difference between systems with limitation and systems without limitation.

An additional problem arises: are systems without limitation rare or should they be treated equitably with limitation cases? The arguments in favor of limitation typicalness are as follows: the real chemical networks are very multiscale with very different constants and concentrations. For such systems it is improbable to meet a situation with compatible effects of different stages. Of course, these arguments are statistical and apply to generic systems from special ensembles.

During last century, the concept of limiting step was revised several times. First simple idea of a “narrow place” (a least conductive step) could be applied without adaptation only to a simple cycle of irreversible steps that are of the first order (see Chap. 16 of [5] or the paper of R.K. Boyd [6]). When researchers try to apply this idea in more general situations they meet various difficulties such as:

- Some reactions have to be “pseudomonomolecular.” Their constants depend on concentrations of outer components, and are constant only under condition that these outer components are present in constant concentrations, or change sufficiently slow. For example, the simplest Michaelis–Menten enzymatic reaction is  $E + S \rightarrow ES \rightarrow E + P$  ( $E$  here stands for enzyme,  $S$  for substrate, and  $P$  for product), and the linear catalytic cycle here is  $S \rightarrow ES \rightarrow S$ . Hence, in general we must consider nonlinear systems.
- Even under fixed outer components concentration, the simple “narrow place” behaviour could be spoiled by branching or by reverse reactions. For such reaction systems definition of a limiting step simply as a step with the smallest constant does not work. The simplest example is given by the cycle:  $A_1 \leftrightarrow A_2 \rightarrow A_3 \rightarrow A_1$ . Even if the constant of the last step  $A_3 \rightarrow A_1$  is the smallest one, the stationary rate may be much smaller than  $k_3b$  (where  $b$  is the overall balance of concentrations,  $b = c_1 + c_2 + c_3$ ), if the constant of the reverse reaction  $A_2 \rightarrow A_1$  is sufficiently big.

In a series of papers [7,8], D.B. Northrop clearly explained these difficulties with many examples based on the isotope effect analysis and suggested that the concept of rate-limiting step is “outmoded”. Nevertheless, the main idea of limiting is so attractive that Northrop’s arguments stimulated the search for modification and improvement of the main concept.

W.J. Ray (Jr.) [9] proposed to use the sensitivity analysis. He considered cycles of reversible reactions and suggested a definition: *The rate-limiting step in a reaction sequence is that forward step for which a change of its rate constant produces the largest effect on the overall rate.* In his formal definition of sensitivity functions the reciprocal reaction rate ( $1/W$ ) and rate constants ( $1/k_i$ ) were used (see [9] ) and the connection between forward and reverse step constants (the equilibrium constant) was kept fixed.

Ray’s approach was revised by G.C. Brown and C.E. Cooper [10] from the system control analysis point of view (see [11]). They stress again that there is no unique rate-limiting step specific for an enzyme, and this step, even if it exists, depends on substrate, product

and effector concentrations. They demonstrated also that the control coefficients

$$C_{k_i}^W = \left( \frac{k_i}{W} \frac{\partial W}{\partial k_i} \right)_{[S],[P],\dots},$$

where  $W$  is the stationary reaction rate and  $k_i$  are constants, are additive and obey the summation theorems (as concentrations do). Simple relation between control coefficients of rate constants and intermediate concentrations was reported in [12]. This relation connects two type of experiments: measurement of intermediate levels and steady-state rate measurements.

For the analysis of nonlinear cycles the new concept of *kinetic polynomial* was developed [13,14]. It was proven that the stationary state of the single-route reaction mechanism of catalytic reaction can be described by a single polynomial equation for the reaction rate. The roots of kinetic polynomial are the values of the reaction rate in the steady state. For a system with limiting step the kinetic polynomial can be approximately solved and the reaction rate found in the form of a series in powers of the limiting step constant [15].

In our approach, we analyze not only the steady state reaction rates, but also the relaxation dynamics of multiscale systems. We focused mostly on the case when all the elementary processes have significantly different time scales. In this case, we obtain “limit simplification” of the model: all stationary states and relaxation processes could be analyzed “to the very end”, by straightforward computations, mostly analytically. Chemical kinetics is an inexhaustible source of examples of multiscale systems for analysis. It is not surprising that many ideas and methods for such analysis were first invented for chemical systems.

In Sec. 2 we analyze a simple example and the source of most generalizations, the catalytic cycle, and demonstrate the main notions on this example. This analysis is quite elementary, but includes many ideas elaborated in full in subsequent sections.

There exist several estimates for relaxation time in chemical reactions (for example, [16]), but even for the simplest cycle with limitation the main property of relaxation time is not widely known. For a simple irreversible catalytic cycle with limiting step the stationary rate is controlled by the smallest constant, but the relaxation time is determined by the second in order constant. Hence, if in the stationary rate experiments for that cycle we mostly extract the smallest constant, in relaxation experiments another, the second in order constant will be observed.

It is also proven that for cycles with well separated constants damped oscillations are impossible, and spectrum of the matrix of kinetic coefficients is real. For general reaction networks with well separated constants this property is proven in Sec. 4.

Another general effect observed for a cycle is robustness of stationary rate and relaxation time. For multiscale systems with random constants, the standard deviation of constants that determine stationary rate (the smallest constant for a cycle) or relaxation time (the second in order constant) is approximately  $n$  times smaller than the standard deviation of the individual constant (where  $n$  is the cycle length). Here we deal with the so-called “order

statistics”. This decrease of the deviation as  $n^{-1}$  is much faster than for the standard error summation, where it decreases with increasing  $n$  as  $n^{-1/2}$ .

In more general settings, robustness of the relaxation time was studied in [17] for chemical kinetics models of genetic and signalling networks. We proved in [17] that for large multiscale systems with hierarchical distribution of time scales the variance of the inverse relaxation time (as well as the variance of the stationary rate) is much lower than the variance of the separate constants. Moreover, it can tend to 0 faster than  $1/n$ , where  $n$  is the number of reactions. It was demonstrated that similar phenomena are valid in the nonlinear case as well. As a numerical illustration we used a model of signalling network that can be applied to important transcription factors such as NFkB.

Each multiscale system is characterized by its structure (the system of elementary processes) and by the rate constants of these processes. To make any general statement about such systems when the structure is given but the constants are unknown it is useful to take the constant set as random and independent. But it is not obvious how to choose the random distribution. The usual idea to take normal or uniform distribution meets obvious difficulties, the time scales are not sufficiently well separated.

Statistical approach to chemical kinetics was developed in [18,19] and high-dimensional model representations (HDMR) were proposed as efficient tools to provide a fully global statistical analysis of a model. The work [20] was focused on how the network properties are affected by random rate constant changes. The rate constants were transformed to a logarithmic scale to ensure an even distribution over the large space.

The log-uniform distribution on sufficiently wide interval helps us to improve the situation, indeed, but a couple of extra parameters appears:  $\alpha = -\min \log k$  and  $\beta = \max \log k$ . We have to study the asymptotics  $\alpha \rightarrow -\infty$ ,  $\beta \rightarrow \infty$ . This approach could be formalized by means of the uniform invariant distributions of  $\log k$  on  $\mathbb{R}^n$ . These distributions are finite-additive, but not countable-additive (not  $\sigma$ -additive).

The probability and measure theory without countable additivity has a long history. In Euclid’s time only arguments based on finite-additive properties of volume were legal. Euclid meant by equal area the scissors congruent area. Two polyhedra are scissors-congruent if one of them can be cut into finitely many polyhedral pieces which can be re-assembled to yield the second. But all proofs of the formula for the volume of a pyramid involve some form of limiting process. Hilbert asks in his third problem: are two Euclidean polyhedra of the same volume scissors congruent? The answer is “no” (a review of old and recent results is presented in [46]). There is another invariant of cutting and gluing polyhedra.

Finite-additive invariant measures on non-compact groups were studied by G. Birkhoff [39] (see also [40], Chap. 4). The frequency-based Mises approach to probability foundations [41], as well as logical foundations of probability by R. Carnap [42] do not need the  $\sigma$ -additivity. Non-Kolmogorov probability theories are discussed now in the context of quantum physics [44], nonstandard analysis [45] and many other problems (and we do not pretend to provide here a full review of related works).

We answer the question: What does it mean “to pick a multiscale system at random”? We introduce and analyze a notion of multiscale ensemble of reaction systems. These ensembles with well separated variables are presented in Sec. 3.

The best geometric example that helps us to understand this problem is one of Lewis Carroll’s Pillow Problems (published in 1883) [21]: “Three points are taken at random on an infinite plane. Find the chance of their being the vertices of an obtuse-angled triangle.” (In an acute-angled triangle all angles are comparable, in an obtuse-angled triangle the obtuse angle is bigger than others and could be much bigger.) The solution of this problem depends significantly on the ensemble definition. What does it mean “points are taken at random on an infinite plane”? Our intuition requires translation invariance, but the normalized translation invariant measure on the plain could not be  $\sigma$ -additive. Nevertheless, there exist finite-additive invariant measures.

Lewis Carroll proposed a solution that did not satisfy modern scientists. There exists a lot of attempts to improve the problem statement [22–25]: reduction from infinite plane to a bounded set, to a compact symmetric space, etc. But the elimination of paradox destroys the essence of Carroll’s problem. If we follow the paradox and try to give a meaning to “points are taken at random on an infinite plane” then we replace  $\sigma$ -additivity of the probability measure by finite-additivity and come to the applied probability theory for finite-additive probabilities. Of course, this theory for abstract probability spaces would be too poor, and some additional geometric and algebraic structures are necessary to build rich enough theory.

This is not just a beautiful geometrical problem, but rather an applied question about proper definition of multiscale ensembles. We need such a definition to make any general statement about multiscale systems, and briefly analyze lessons of Carroll’s problem in Sec. 3.

In this section we use some mathematics to define the multiscale ensembles with well separated constants. This is necessary for background of the analysis of systems with limitation, but technical consequences are rather simple. We need only two properties of a typical system from multiscale ensemble with well separated constants:

- (1) Every two reaction rate constants  $k, k'$  are connected by relation  $k \gg k'$  or  $k \ll k'$  (with probability close to 1);
- (2) The first property persists, if we delete two constants  $k, k'$  from the list of constants, and add a number  $kk'$  or a number  $k/k'$  to that list (with probability close to 1).

If the reader can use these properties (when it is necessary) without additional clarification, it is possible to skip reading Sec. 3 and go directly to more applied sections. In Sec. 4 we study static and dynamic properties of linear multiscale reaction networks. An important instrument for that study is a hierarchy of auxiliary discrete dynamical system. Let  $A_i$  be nodes of the network (“components”),  $A_i \rightarrow A_j$  be edges (reactions), and  $k_{ji}$  be the constants of these reactions (please pay attention to the inverse order of subscripts). A discrete dynamical system  $\phi$  is a map that maps any node  $A_i$  in a node  $A_{\phi(i)}$ . To construct a first auxiliary dynamical system for a given network we find for each  $A_i$  the maximal constant of reactions  $A_i \rightarrow A_j$ :  $k_{\phi(i)i} \geq k_{ji}$  for all  $j$ , and  $\phi(i) = i$  if there are no reactions

$A_i \rightarrow A_j$ . Attractors in this discrete dynamical system are cycles and fixed points.

The fast stage of relaxation of a complex reaction network could be described as mass transfer from nodes to correspondent attractors of auxiliary dynamical system and mass distribution in the attractors. After that, a slower process of mass redistribution between attractors should play a more important role. To study the next stage of relaxation, we should glue cycles of the first auxiliary system (each cycle transforms into a point), define constants of the first derivative network on this new set of nodes, construct for this new network a (first) auxiliary discrete dynamical system, etc. The process terminates when we get a discrete dynamical system with one attractor. Then the inverse process of cycle restoration and cutting starts. As a result, we create an explicit description of the relaxation process in the reaction network, find estimates of eigenvalues and eigenvectors for the kinetic equation, and provide full analysis of steady states for systems with well separated constants.

The problem of multiscale asymptotics of eigenvalues of non-selfadjoint matrices was studied by Vishik, Ljusternik [27] and Lidskii [28]. Recently, some generalizations were obtained by idempotent (min-plus) algebra methods [29]. These methods provide natural language for discussion of some multiscale problems [30]. In the Vishik–Ljusternik–Lidskii theorem and its generalizations the asymptotics of eigenvalues and eigenvectors for the family of matrices  $\mathcal{A}_{ij}(\epsilon) = a_{ij}\epsilon^{A_{ij}} + o(\epsilon^{A_{ij}})$  is studied for  $\epsilon > 0$ ,  $\epsilon \rightarrow 0$ .

In the chemical reaction networks that we study, there is no small parameter  $\epsilon$  with a given distribution of the orders  $\epsilon^{A_{ij}}$  of the matrix nodes. Instead of these powers of  $\epsilon$  we have orderings of rate constants. On the other hand, the matrices of kinetic equations have some specific properties. The possibility to operate with the graph of reactions (cycles surgery) significantly helps in our constructions. Nevertheless, there exists some similarity between these problems and, even for general matrices, graphical representation is useful. The language of idempotent algebra [30], as well as nonstandard analysis with infinitesimals [31], can be used for description of the multiscale reaction networks, but now we postpone this for later use.

A multiscale system where every two constants have very different orders of magnitude is, of course, an idealization. In parametric families of multiscale systems there could appear systems with several constants of the same order. Hence, it is necessary to study effects that appear due to a group of constants of the same order in a multiscale network. The system can have modular structure, with different time scales in different modules, but without separation of times inside modules. We discuss systems with modular structure in Sec. 5. The full theory of such systems is a challenge for future work, and here we study structure of one module. The elementary modules have to be solvable. That means that the kinetic equations could be solved in explicit analytical form. We give the necessary and sufficient conditions for solvability of reaction networks. These conditions are presented constructively, by algorithm of analysis of the reaction graph.

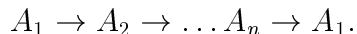
It is necessary to repeat our study for nonlinear networks. We discuss this problem and perspective of its solution in conclusion. Here we again use the experience summarized in the IUPAC Compendium [3] where the notion of controlling step is generalized onto nonlinear elementary reaction by inclusion of some concentration into “pseudo-first order

rate constant”.

## 2 Static and dynamic limitation in a simple catalytic cycle

### 2.1 General properties of a cycle

The catalytic cycle is one of the most important substructures that we study in reaction networks. In the reduced form the catalytic cycle is a set of linear reactions:



Reduced form means that in reality some of these reaction are not monomolecular and include some other components (not from the list  $A_1, \dots A_n$ ). But in the study of the isolated cycle dynamics, concentrations of these components are taken as constant and are included into kinetic constants of the cycle linear reactions.

For the constant of elementary reaction  $A_i \rightarrow$  we use the simplified notation  $k_i$  because the product of this elementary reaction is known, it is  $A_{i+1}$  for  $i < n$  and  $A_1$  for  $i = n$ . The elementary reaction rate is  $w_i = k_i c_i$ , where  $c_i$  is the concentration of  $A_i$ . The kinetic equation is:

$$\dot{c}_i = w_{i-1} - w_i, \quad (1)$$

where by definition  $w_0 = w_n$ . In the stationary state ( $\dot{c}_i = 0$ ), all the  $w_i$  are equal:  $w_i = w$ . This common rate  $w$  we call the cycle stationary rate, and

$$w = \frac{b}{\frac{1}{k_1} + \dots + \frac{1}{k_n}}; \quad c_i = \frac{w}{k_i}, \quad (2)$$

where  $b = \sum_i c_i$  is the conserved quantity for reactions in constant volume (for general case of chemical kinetic equations see elsewhere, for example, [26]). The stationary rate  $w$  (2) is a product of the arithmetic mean of concentrations,  $b/n$ , and the harmonic mean of constants (inverse mean of inverse  $k_i$ ).

### 2.2 Static limitation in a cycle

If one of the constants,  $k_{\min}$ , is much smaller than others (let it be  $k_{\min} = k_n$ ), then

$$\begin{aligned} c_n &= b \left( 1 - \sum_{i < n} \frac{k_n}{k_i} + o \left( \sum_{i < n} \frac{k_n}{k_i} \right) \right), \quad c_i = b \left( \frac{k_n}{k_i} + o \left( \sum_{i < n} \frac{k_n}{k_i} \right) \right), \\ w &= k_n b \left( 1 + O \left( \sum_{i < n} \frac{k_n}{k_i} \right) \right), \end{aligned} \quad (3)$$



or simply in linear approximation

$$c_n = b \left( 1 - \sum_{i < n} \frac{k_n}{k_i} \right), \quad c_i = b \frac{k_n}{k_i}, \quad w = k_n b, \quad (4)$$

where we should keep the first-order terms in  $c_n$  in order not to violate the conservation law.

The simplest zero order approximation for the steady state gives

$$c_n = b, \quad c_i = 0 \quad (i \neq n). \quad (5)$$

This is trivial: all the concentration is collected at the starting point of the “narrow place”, but may be useful as an origin point for various approximation procedures.

So, the stationary rate of a cycle is determined by the smallest constant,  $k_{\min}$ , if  $k_{\min}$  is sufficiently small:

$$w = k_{\min} b \quad \text{if} \quad \sum_{k_i \neq k_{\min}} \frac{k_{\min}}{k_i} \ll 1. \quad (6)$$

In that case we say that the cycle has a limiting step with constant  $k_{\min}$ .

### 2.3 Dynamical limitation in a cycle

If  $k_n/k_i$  is small for all  $i < n$ , then the kinetic behaviour of the cycle is extremely simple: the coefficients matrix on the right hand side of kinetic equation (1) has one simple zero eigenvalue that corresponds to the conservation law  $\sum c_i = b$  and  $n-1$  nonzero eigenvalues

$$\lambda_i = -k_i + \delta_i \quad (i < n). \quad (7)$$

where  $\delta_i \rightarrow 0$  when  $\sum_{i < n} \frac{k_n}{k_i} \rightarrow 0$ .

It is easy to demonstrate (7): let us exclude the conservation law (the zero eigenvalue)  $\sum c_i = b$  and use independent coordinates  $c_i$  ( $i = 1, \dots, n-1$ );  $c_n = b - \sum_{i < n} c_i$ . In these coordinates the kinetic equation (1) has the form

$$\dot{c} = Kc - k_n A c + k_n b e^1 \quad (8)$$

where  $c$  is the vector-column with components  $c_i$  ( $i < n$ ),  $K$  is the lower triangle matrix with nonzero elements only in two diagonals:  $(K)_{ii} = -k_i$  ( $i = 1, \dots, n-1$ ),  $(K)_{i+1,i} = k_i$  ( $i = 1, \dots, n-2$ ) (other elements are equal to zero);  $A$  is the matrix with nonzero elements only in the first row:  $(A)_{1i} \equiv 1$ ,  $e^1$  is the first basis vector ( $e_1^1 = 1$ ,  $e_i^1 = 0$  for  $1 < i < n$ ). After that, eq. (7) follows simply from continuous dependence of spectra on matrix.

The relaxation time of a stable linear system (8) is, by definition,  $\tau = [\min\{Re(-\lambda_i) \mid i = 1, \dots, n-1\}]^{-1}$ . For small  $k_n$ ,

$$\tau \approx 1/k_\tau, \quad k_\tau = \min\{k_i \mid i = 1, \dots, n-1\}. \quad (9)$$

In other words,  $k_\tau$  is the second slowest rate constant:  $k_{\min} \leq k_\tau \leq \dots$ .

## 2.4 Relaxation equation for a cycle rate

A definition of the cycle rate is clear for steady states because stationary rates of all elementary reactions in cycle coincide. There is no common definition of the cycle rate for nonstationary regimes. In practice, one of steps is the step of product release (the “final” step of the catalytic transformation), and we can consider its rate as the rate of the cycle. Formally, we can take any step and study relaxation of its rate to the common stationary rate. The single relaxation time approximation gives for rate  $w_i$  of any step:

$$\dot{w}_i = k_\tau(k_{\min}b - w_i); \quad w_i(t) = k_{\min}b + e^{-k_\tau t}(w_i(0) - k_{\min}b), \quad (10)$$

where  $k_{\min}$  is the limiting (the minimal) rate constant of the cycle,  $k_\tau$  is the second in order rate constant of the cycle.

So, for catalytic cycles with the limiting constant  $k_{\min}$ , the relaxation time is also determined by one constant, but another one. This is  $k_\tau$ , the second in order rate constant. It should be stressed that the only smallness condition is required,  $k_{\min}$  should be much smaller than other constants. The second constant,  $k_\tau$  should be just smaller than others (and bigger than  $k_{\min}$ ), but there is no  $\ll$  condition for  $k_\tau$  required.

One of the methods for measurement of chemical reaction constants is the relaxation spectroscopy [32]. Relaxation of a system after an impact gives us a relaxation time or even a spectrum of relaxation times. For catalytic cycle with limitation, the relaxation experiment gives us the second constant  $k_\tau$ , while the measurement of stationary rate gives the smallest constant,  $k_{\min}$ . This simple remark may be important for relaxation spectroscopy of open system.

## 2.5 Ensembles of cycles and robustness of stationary rate and relaxation time

Let us consider a catalytic cycle with random rate constants. For a given sample constants  $k_1, \dots, k_n$  the  $i$ th order statistics is equal its  $i$ th-smallest value. We are interested in the first order (the minimal) and the second order statistics.

For independent identically distributed constants the variance of  $k_{\min} = \min\{k_1, \dots, k_n\}$  is significantly smaller than the variance of each  $k_i$ ,  $\text{Var}(k)$ . The same is true for statistic of every order. For many important distributions (for example, for uniform distribution), the variance of  $i$ th order statistic is of order  $\sim \text{Var}(k)/n^2$ . For big  $n$  it goes to zero faster than variance of the mean that is of order  $\sim \text{Var}(k)/n$ . To illustrate this, let us consider  $n$  constants distributed in interval  $[a, b]$ . For each set of constants,  $k_1, \dots, k_n$  we introduce “symmetric coordinates”  $s_i$ : first, we order the constants,  $a \leq k_{i_1} \leq k_{i_2} \leq \dots \leq k_{i_n} \leq b$ , then calculate  $s_0 = k_{i_1} - a$ ,  $s_j = k_{i_{j+1}} - k_{i_j}$  ( $j = 1, \dots, n-1$ ),  $s_n = b - k_{i_n}$ . Transformation  $(k_1, \dots, k_n) \mapsto (s_0, \dots, s_n)$  maps a cube  $[a, b]^n$  onto  $n$ -dimensional simplex  $\Delta_n = \{(s_0, \dots, s_n) \mid \sum_i s_i = b - a\}$  and uniform distribution on a cube transforms into uniform distribution on a simplex.

For large  $n$ , almost all volume of the simplex is concentrated in a small neighborhood of its

center and this effect is an example of measure concentration effects that play important role in modern geometry and analysis [34]. All  $s_i$  are identically distributed, and for normalized variable  $s = s_i/(b-a)$  the first moments are:  $\mathbf{E}(s) = 1/(n+1) = 1/n + o(1/n)$ ,  $\mathbf{E}(s^2) = 2/[(n+1)(n+2)] = 2/n^2 + o(1/n^2)$ ,

$$\text{Var}(s) = \mathbf{E}(s^2) - (\mathbf{E}(s))^2 = \frac{n}{(n+1)^2(n+2)} = \frac{1}{n^2} + o\left(\frac{1}{n^2}\right).$$

Hence, for example,  $\text{Var}(k_{\min}) = (b-a)^2/n^2 + o(1/n^2)$ . The standard deviation of  $k_{\min}$  goes to zero as  $1/n$  when  $n$  increases. This is much faster than  $1/\sqrt{n}$  prescribed to the deviation of the mean value of independent observation (the “law of errors”). The same asymptotic  $\sim 1/n$  is true for the standard deviation of the second constant also. These parameters fluctuate much less than individual constants, and even less than mean constant (for more examples with applications to statistical physics we address to [35]).

It is impossible to use this observation for cycles with limitation directly, because the inequality of limitation (6) is not true for uniform distribution. According to this inequality, ratios  $k_i/k_{\min}$  should be sufficiently small (if  $k_i \neq k_{\min}$ ). To provide this inequality we need to use at least the log-uniform distribution:  $k_i = \exp \Delta_i$  and  $\Delta_i$  are independent variables uniformly distributed in interval  $[\alpha, \beta]$  with sufficiently big  $(\beta - \alpha)/n$ .

One can interpret the log-uniform distribution through the Arrhenius law:  $k = A \exp(-\Delta G/kT)$ , where  $\Delta G$  is the change of the Gibbs free energy in reaction (it includes both energetic and entropic terms:  $\Delta G = \Delta H - TS$ , where  $\Delta H$  is enthalpy change and  $\Delta S$  is entropy change in reaction,  $T$  is temperature). The log-uniform distribution of  $k$  corresponds to the uniform distribution of  $\Delta G$ .

For log-uniform distribution of constants  $k_1, \dots, k_n$ , if the interval of distribution is sufficiently big (i.e.  $(\beta - \alpha)/n \gg 1$ ), then the cycle with these constants has the limiting step with probability close to one. More precisely we can show that for any two constants  $k_i, k_j$  the probability  $\mathbb{P}[k_i/k_j > r \text{ or } k_j/k_i > r] = (1 - \log(r)/(\beta - \alpha))^2$  approaches one for any fixed  $r > 1$  when  $\beta - \alpha \rightarrow \infty$ . Relaxation time of this cycle is determined by the second constant  $k_\tau$  (also with probability close to one). Standard deviations of  $k_{\min}$  and  $k_\tau$  are much smaller than standard deviation of single constant  $k_i$  and even smaller than standard deviation of mean constant  $\sum_i k_i/n$ . This effect of stationary rate and relaxation time robustness seems to be important for understanding robustness in biochemical networks: behaviour of the entire system is much more stable than the parameters of its parts; even for large fluctuations of parameters, the system does not change significantly the stationary rate (statics) and the relaxation time (dynamics).

## 2.6 Systems with well separated constants and monotone relaxation

The log-uniform identical distribution of independent constants  $k_1, \dots, k_n$  with sufficiently big interval of distribution ( $(\beta - \alpha)/n \gg 1$ ) gives us the first example of ensembles with well separated constants: any two constants are connected by relation  $\gg$  or  $\ll$  with probability close to one. Such systems (not only cycles, but much more complex networks too) could be studied analytically “up to the end”.

Some of their properties are simpler than for general networks. For example, the damping oscillations are impossible, i.e. the eigenvalues of kinetic matrix are real (with probability close to one). If constants are not separated, damped oscillations could exist, for example, if all constants of the cycle are equal,  $k_1 = k_2 = \dots = k_n = k$ , then  $(1 + \lambda/k)^n = 1$  and  $\lambda_m = k(\exp(2\pi im/n) - 1)$  ( $m = 1, \dots, n-1$ ), the case  $m = 0$  corresponds to the linear conservation law. Relaxation time of this cycle may be relatively big:  $\tau = \frac{1}{k}(1 - \cos(2\pi/n))^{-1} \sim n^2/(2\pi k)$  (for big  $n$ ).

The catalytic cycle without limitation can have relaxation time much bigger than  $1/k_{\min}$ , where  $k_{\min}$  is the minimal reaction rate constant. For example, if all  $k$  are equal, then for  $n = 11$  we get  $\tau \approx 20/k$ . In more detail the possible relations between  $\tau$  and the slowest constant were discussed in [33]. In that paper, a variety of cases with different relationships between the steady-state reaction rate and relaxation was presented.

For catalytic cycle, if a matrix  $K - k_n A$  (8) has a pair of complex eigenvalues with nonzero imaginary part, then for some  $g \in [0, 1]$  the matrix  $K - gk_n A$  has a degenerate eigenvalue (we use a simple continuity argument). With probability close to one,  $k_{\min} \ll |k_i - k_j|$  for any two  $k_i, k_j$  that are not minimal. Hence, the  $k_{\min}$ -small perturbation cannot transform matrix  $K$  with eigenvalues  $k_i$  (7) and given structure into a matrix with a degenerate eigenvalue. For proof of this statement it is sufficient to refer to diagonal dominance of  $K$  (the absolute value of each diagonal element is greater than the sum of the absolute values of the other elements in its column) and classical inequalities.

Let us give a detailed proof based on the explicit form of  $K$  left and right eigenvectors. Let vector-column  $x^i$  and vector-row  $l^i$  be right and left eigenvectors of  $K$  for eigenvalue  $-k_i$ . For coordinates of these eigenvectors we use notation  $x_j^i$  and  $l_j^i$ . Let us choose a normalization condition  $x_i^i = l_i^i = 1$ . It is straightforward to check that  $x_j^i = 0$  ( $j < i$ ) and  $l_j^i = 0$  ( $j > i$ ),  $x_{j+1}^i = k_j x_j^i / (k_{j+1} - k_i)$  ( $j \geq i$ ) and  $l_{j-1}^i = k_{j-1} l_j^i / (k_{j-1} - k_j)$  ( $j \leq i$ ), and

$$x_{i+m}^i = \prod_{j=1}^m \frac{k_{i+j-1}}{k_{i+j} - k_i}; \quad l_{i-m}^i = \prod_{j=1}^m \frac{k_{i-j}}{k_{i-j} - k_i} \quad (11)$$

(when these coordinates have sense). Under selected normalization condition, the inner product of eigenvectors is:  $l^i x^j = \delta_{ij}$ , where  $\delta_{ij}$  is the Kronecker delta.

For ensembles with well separated constants, with probability close to one,

$$\frac{k_{i-j}}{k_{i-j} - k_i} \approx \begin{cases} 1, & \text{if } k_i \ll k_{i-j}; \\ 0, & \text{if } k_i \gg k_{i+j}, \end{cases} \quad (12)$$

Hence,  $|l_{i-m}^i| \approx 1$  or  $|l_{i-m}^i| \approx 0$ . To demonstrate that also  $|x_{i+m}^i| \approx 1$  or  $|x_{i+m}^i| \approx 0$ , we shift nominators in the product (11) on such a way:

$$x_{i+m}^i = \frac{k_i}{k_{i+m} - k_i} \prod_{j=1}^{m-1} \frac{k_{i+j}}{k_{i+j} - k_i}.$$

Exactly as in (12), each multiplier  $k_{i+j}/(k_{i+j} - k_i)$  here is either almost 1 or almost 0,

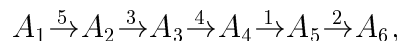
and  $k_i/(k_{i+m} - k_i)$  is either almost 0 or almost  $-1$ . In this 0-1 asymptotics

$$\begin{aligned} l_i^i &= 1, l_{i-m}^i = 1 \text{ if } k_{i-j} > k_i \text{ for all } j = 1, \dots, m, \text{ else } l_{i-m}^i = 0; \\ r_i^i &= 1, r_{i+m}^i = -1 \text{ if } k_{i+j} > k_i \text{ for all } j = 1, \dots, m-1 \text{ and } k_{i+m} < k_i, \\ &\text{else } r_{i+m}^i = 0. \end{aligned} \quad (13)$$

In this asymptotic, only two coordinates of right eigenvector  $r^i$  can have nonzero values,  $r_i^i = 1$  and  $r_{i+m}^i = -1$  where  $m$  is the first such positive integer that  $i+m < n$  and  $k_{i+m} < k_i$ . It is possible that such  $m$  does not exist. In that case only  $r_i^i = 1$ . (Let us remind that we consider vectors in the subspace  $\sum_i c_i = 1$  with coordinates  $c_1, \dots, c_{n-1}$ . In the full system of coordinates  $c_1, \dots, c_n$ , the last case corresponds to  $r_i^i = 1, r_n^i = -1$ .) For left eigenvector  $l^i$ ,  $l_i^i = \dots = l_{i-q}^i = 1$  and  $l_{i-q-j}^i = 0$  where  $j > 0$  and  $q$  is the first such positive integer that  $i-q-1 > 0$  and  $k_{i-q-1} < k_i$ . It is possible that such  $q$  does not exist. In that case all  $l_{i-j}^i = 1$ . It is straightforward to check that in this asymptotic  $l^i r^j = \delta_{ij}$ .

The simplest example gives the order  $k_1 > k_2 > \dots > k_n$ :  $l_{i-j}^i = 1$  for  $j \geq 0$ ,  $r_i^i = 1$ ,  $r_n^i = -1$  and all other coordinates of eigenvectors are zero.

For less trivial example, let us find the asymptotic of left and right eigenvectors for a chain of reactions:



where the upper index marks the order of rate constants:  $k_4 > k_5 > k_2 > k_3 > k_1$  ( $k_i$  is the rate constant of reaction  $A_i \rightarrow \dots$ ).

For left eigenvectors, rows  $l^i$ , we have the following asymptotics:

$$\begin{aligned} l^1 &\approx (1, 0, 0, 0, 0, 0), l^2 \approx (0, 1, 0, 0, 0, 0), l^3 \approx (0, 1, 1, 0, 0, 0), \\ l^4 &\approx (0, 0, 0, 1, 0, 0), l^5 \approx (0, 0, 0, 1, 1, 0). \end{aligned} \quad (14)$$

For right eigenvectors, columns  $r^i$ , we have the following asymptotics (we write vector-columns in rows):

$$\begin{aligned} r^1 &\approx (1, 0, 0, 0, 0, -1), r^2 \approx (0, 1, -1, 0, 0, 0), r^3 \approx (0, 0, 1, 0, 0, -1), \\ r^4 &\approx (0, 0, 0, 1, -1, 0), r^5 \approx (0, 0, 0, 0, 1, -1). \end{aligned} \quad (15)$$

For convenience, we use all six coordinates,  $c_{1-6}$ .

The matrix elements of  $A$  in the eigenbasis of  $K$  are  $(A)_{ij} = l^i A x^j$ . From obtained estimates for eigenvectors we get  $|(A)_{ij}| \lesssim 1$  (with probability close to one). This estimate does not depend on values of kinetic constants. Now, we can apply the Gershgorin theorem (see, for example, [36] and for more details [37]) to the matrix  $K - k_n A$  in the eigenbasis of  $K$ : the characteristic roots of  $K - k_n A$  belong to discs  $|z + k_i| \leq k_n R_i(A)$ , where  $R_i(A) = \sum_j |(A)_{ij}|$ . If the discs do not intersect, then each of them contains one and only one characteristic number. For ensembles with well separated constants these discs do not intersect (with probability close to one). Complex conjugate eigenvalues could not belong to different discs. In this case, the eigenvalues are real – there exist no damped oscillations.

## 2.7 Limitation by two steps with comparable constants

If we consider one-parametric families of systems, then appearance of systems with two comparable constants may be unavoidable. On a continuous way  $k_i(s)$  from one system with well separated constants to another such system constants may coincide: such a point  $s$  that  $k_i(s) = k_j(s)$  may exist, and this existence may be stable, that is, such a point persists under continuous perturbations.

For catalytic cycle, we are interested in the following intersection only:  $k_{\min}$  and the second constant are of the same order, and are much smaller than other constants. Let these constants be  $k_j$  and  $k_l$ ,  $j \neq l$ . The limitation condition is

$$\frac{1}{k_j} + \frac{1}{k_l} \gg \sum_{i \neq j, l} \frac{1}{k_i}. \quad (16)$$

The steady state reaction rate and relaxation time are determined by these two constants. In that case their effects are coupled. For the steady state we get in first order approximation instead of (4):

$$\begin{aligned} w &= \frac{k_j k_l}{k_j + k_l} b; \quad c_i = \frac{w}{k_i} = \frac{b}{k_i} \frac{k_j k_l}{k_j + k_l} \quad (i \neq j, l); \\ c_j &= \frac{b k_l}{k_j + k_l} \left( 1 - \sum_{i \neq j, l} \frac{1}{k_i} \frac{k_j k_l}{k_j + k_l} \right); \quad c_l = \frac{b k_j}{k_j + k_l} \left( 1 - \sum_{i \neq j, l} \frac{1}{k_i} \frac{k_j k_l}{k_j + k_l} \right). \end{aligned} \quad (17)$$

Elementary analysis shows that under the limitation condition (16) the relaxation time is

$$\tau = \frac{1}{k_j + k_l}. \quad (18)$$

The single relaxation time approximation for all elementary reaction rates in a cycle with two limiting reactions is

$$\dot{w}_i = k_j k_l b - (k_j + k_l) w_i; \quad w_i(t) = \frac{k_j k_l}{k_j + k_l} b + e^{-(k_j + k_l)t} \left( w_i(0) - \frac{k_j k_l}{k_j + k_l} b \right). \quad (19)$$

The catalytic cycle with two limiting reactions has the same stationary rate  $w$  (17) and relaxation time (18) as a reversible reaction  $A \leftrightarrow B$  with  $k^+ = k_j$ ,  $k^- = k_l$ .

In two-parametric families three constants can meet. If three smallest constants  $k_j, k_l, k_m$  have comparable values and are much smaller than others, then static and dynamic properties would be determined by these three constants. Stationary rate  $w$  and dynamic of relaxation for the whole cycle would be the same as for 3-reaction cycle  $A \rightarrow B \rightarrow C \rightarrow A$  with constants  $k_j, k_l, k_m$ . The damped oscillation here are possible, for example, if  $k_j = k_l = k_m = k$ , then there are complex eigenvalues  $\lambda = k(-\frac{3}{2} \pm i\frac{\sqrt{3}}{2})$ . Therefore, if a cycle manifests damped oscillation, then at least three slowest constants are of the same order. The same is true, of course, for more general reaction networks.

In  $N$ -parametric families of systems  $N + 1$  smallest constants can meet, and near such a “meeting point” a slow auxiliary cycle of  $N + 1$  reactions determines behaviour of the entire cycle.

### 3 Multiscale ensembles and finite-additive distributions

#### 3.1 Ensembles with well separated constants, formal approach

In previous section, ensembles with well separated constants appear. We represented them by a log-uniform distribution in a sufficiently big interval  $\log k \in [\alpha, \beta]$ , but we were not interested in most of probability distribution properties, and did not use them. The only property we really used is: if  $k_i > k_j$ , then  $k_i/k_j \gg 1$  (with probability close to one). It means that we can assume that  $k_i/k_j \gg a$  for any preassigned value of  $a$  that does not depend on  $k$  values. One can interpret this property as an asymptotic one for  $\alpha \rightarrow -\infty$ ,  $\beta \rightarrow \infty$ .

That property allows us to simplify algebraic formulas. For example,  $k_i + k_j$  could be substituted by  $\max\{k_i, k_j\}$  (with small relative error), or

$$\frac{ak_i + bk_j}{ck_i + dk_j} \approx \begin{cases} a/c, & \text{if } k_i \gg k_j; \\ b/d, & \text{if } k_i \ll k_j, \end{cases}$$

for nonzero  $a, b, c, d$  (see, for example, (12)).

Of course, some ambiguity can be introduced, for example, what is it,  $(k_1 + k_2) - k_1$ , if  $k_1 \gg k_2$ ? If we first simplify the expression in brackets, it is zero, but if we open brackets without simplification, it is  $k_2$ . This is a standard difficulty in use of relative errors for round-off. If we estimate the error in the final answer, and then simplify, we shall avoid this difficulty. Use of  $o$  and  $\mathcal{O}$  symbols also helps to control the error qualitatively: if  $k_1 \gg k_2$ , then we can write  $(k_1 + k_2) = k_1(1 + o(1))$ , and  $k_1(1 + o(1)) - k_1 = k_1 o(1)$ . The last expression is neither zero, nor absolutely small – it is just relatively small with respect to  $k_1$ .

The formal approach is: for any ordering of rate constants, we use relations  $\gg$  and  $\ll$ , and assume that  $k_i/k_j \gg a$  for any preassigned value of  $a$  that does not depend on  $k$  values. This approach allows us to perform asymptotic analysis of reaction networks. A special version of this approach consists of group ordering: constants are separated on several groups, inside groups they are comparable, and between groups there are relations  $\gg$  or  $\ll$ . An example of such group ordering was discussed at the end of previous section (several limiting constants in a cycle).

### 3.2 Probability approach: finite additive measures

The asymptotic analysis of multiscale systems for log-uniform distribution of independent constants on an interval  $\log k \in [\alpha, \beta]$  ( $\alpha, \beta \rightarrow \infty$ ) is possible, but parameters  $\alpha, \beta$  do not present in any answer, they just should be sufficiently big. A natural question arises, what is the limit? It is a log-uniform distribution on a line, or, for  $n$  independent identically distributed constants, a log-uniform distribution on  $\mathbb{R}^n$ .

It is well known that the uniform distribution on  $\mathbb{R}^n$  is impossible: if a cube has positive probability  $\epsilon > 0$  (i.e. the distribution has positive density) then the union of  $N > 1/\epsilon$  such disjoint cubes has probability bigger than 1 (here we use the finite-additivity of probability). This is impossible. But if that cube has probability zero, then the whole space has also zero probability, because it can be covered by countable family of the cube translation. Hence, translation invariance and  $\sigma$ -additivity (countable additivity) are in contradiction (if we have no doubt about probability normalization).

Nevertheless, there exists finite-additive probability which is invariant with respect to Euclidean group  $E(n)$  (generated by rotations and translations). Its values are densities of sets.

Let  $D \subset \mathbb{R}^n$  be a Lebesgue measurable subset. Density of  $D$  is the limit (if it exists):

$$\rho(D) = \lim_{r \rightarrow \infty} \frac{\lambda(D \cap \mathbb{B}_r^n)}{\lambda(\mathbb{B}_r^n)}, \quad (20)$$

where  $\mathbb{B}_r^n$  is a ball with radius  $r$  and centre at origin. Density of  $\mathbb{R}^n$  is 1, density of every half-space is 1/2, density of bounded set is zero, density of a cone is its solid angle (measured as a sphere surface fractional area). Density (20) and translation and rotational invariant. It is finite-additive: if densities  $\rho(D)$  and  $\rho(H)$  (20) exist and  $D \cap H = \emptyset$  then  $\rho(D \cup H)$  exists and  $\rho(D \cup H) = \rho(D) + \rho(H)$ .

Every polyhedron has a density. A polyhedron could be defined as the union of a finite number of convex polyhedra. A convex polyhedron is the intersection of a finite number of half-spaces. It may be bounded or unbounded. The family of polyhedra is closed with respect to union, intersection and subtraction of sets. For our goals, polyhedra form sufficiently rich class. It is important that in definition of polyhedron *finite* intersections and unions are used. If one uses countable unions, he gets too many sets including all open sets, because open convex polyhedra (or just cubes with rational vertices) form a basis of standard topology.

Of course, not every measurable set has density. If it is necessary, we can use the Hahn-Banach theorem and study extensions  $\rho_{\text{Ex}}$  of  $\rho$  with the following property:

$$\underline{\rho}(D) \leq \rho_{\text{Ex}}(D) \leq \bar{\rho}(D),$$

where

$$\underline{\rho}(D) = \liminf_{r \rightarrow \infty} \frac{\lambda(D \cap \mathbb{B}_r^n)}{\lambda(\mathbb{B}_r^n)}, \quad \bar{\rho}(D) = \limsup_{r \rightarrow \infty} \frac{\lambda(D \cap \mathbb{B}_r^n)}{\lambda(\mathbb{B}_r^n)}.$$



Functionals  $\underline{\rho}(D)$  and  $\overline{\rho}(D)$  are defined for all measurable  $D$ . We should stress that such extensions are not unique. Extension of density (20) using the Hahn–Banach theorem for picking up a random integer was used in a very recent work [43].

One of the most important concepts of any probability theory is the conditional probability. In the density–based approach we can introduce the conditional density. If densities  $\rho(D)$  and  $\rho(H)$  (20) exist,  $\rho(H) \neq 0$  and the following limit  $\rho(D|H)$  exists, then we call it conditional density:

$$\rho(D|H) = \lim_{r \rightarrow \infty} \frac{\lambda(D \cap H \cap \mathbb{B}_r^n)}{\lambda(H \cap \mathbb{B}_r^n)}. \quad (21)$$

For polyhedra the situation is similar to usual probability theory: densities  $\rho(D)$  and  $\rho(H)$  always exist and if  $\rho(H) \neq 0$  then conditional density exists too. For general measurable sets the situation is not so simple, and existence of  $\rho(D)$  and  $\rho(H) \neq 0$  does not guarantee existence of  $\rho(D|H)$ .

On a line, convex polyhedra are just intervals, finite or infinite. The probability defined on polyhedra is: for finite intervals and their finite unions it is zero, for half–lines  $x > \alpha$  or  $x < \alpha$  it is 1/2, and for the whole line  $\mathbb{R}$  the probability is 1. If one takes a set of positive probability and adds or subtracts a zero–probability set, the probability does not change.

If independent random variables  $x$  and  $y$  are uniformly distributed on a line, then their linear combination  $z = \alpha x + \beta y$  is also uniformly distributed on a line. Indeed, vector  $(x, y)$  is uniformly distributed on a plane (by definition), a set  $z > \gamma$  is a half–plane, the correspondent probability is 1/2. This is a simple, but useful stability property. We shall use this result in the following form. If independent random variables  $k_1, \dots, k_n$  are log–uniformly distributed on a line, then the monomial  $\prod_{i=1}^n k_i^{\alpha_i}$  for real  $\alpha_i$  is also log–uniformly distributed on a line.

### 3.3 Carroll obtuse problem and paradoxes of conditioning

Lewis Carroll’s Pillow Problem #58 [21]: “Three points are taken at random on an infinite plane. Find the chance of their being the vertices of an obtuse–angled triangle.”

A random triangle on an infinite plane is presented by a point equidistributed in  $\mathbb{R}^6$ . Due to the density – based definition, we should take and calculate the density of the set of obtuse–angled triangles in  $\mathbb{R}^6$ . This is equivalent to the problem: find a fraction of the sphere  $\mathbb{S}^5 \subset \mathbb{R}^6$  that corresponds to obtuse–angled triangles. Just integrate... . But there remains a problem. Vertices of triangle are independent. Let us use the standard logic for discussion of independent trials: we take the first point  $A$  at random, then the second point  $B$ , and then the third point  $C$ . Let us draw the first side  $AB$ . Immediately we find that for almost all positions of the the third point  $C$  the triangle is obtuse–angled (see [22]). L. Carroll proposed to take another condition: let  $AB$  be the longest side and let  $C$  be uniformly distributed in the allowed area. The answer then is easy – just a ratio of areas of two simple figures. But there are absolutely no reasons for uniformity of  $C$  distribution. And it is more important that the absolutely standard reasoning for independently chosen points gives another answer than could be found on the base of joint distribution. Why

these approaches are in disagreement now? Because there is no classical Fubini theorem for our finite-additive probabilities, and we cannot easily transfer from a multiple integral to a repeated one.

There exists a much simpler example. Let  $x$  and  $y$  be independent positive real number. This means that vector  $(x, y)$  is uniformly and independently distributed in the first quadrant. What is probability that  $x \geq y$ ? Following the definition of probability based on the density of sets, we take the correspondent angle and find immediately that this probability is  $1/2$ . This meets our intuition well. But let us take the first number  $x$  and look for possible values of  $y$ . The result: for given  $x$  the second number  $y$  is uniformly distributed on  $[0, \infty)$ , and only a finite interval  $[0, x]$  corresponds to  $x \geq y$ . For the infinite rest we have  $x < y$ . Hence,  $x < y$  with probability 1. This is nonsense because of symmetry. So, for our finite-additive measure we cannot use repeated integrals (or, may be, should use them in a very peculiar manner).

### 3.4 Law of total probability and orderings

For polyhedra, there appear no conditioning problems. The law of total probabilities holds: if  $\mathbb{R}^n = \cup_{i=1}^m H_i$ ,  $H_i$  are polyhedra,  $\rho(H_i) > 0$ ,  $\rho(H_i \cap H_j) = 0$  for  $i \neq j$ , and  $D \subset \mathbb{R}^n$  is a polyhedron, then

$$\rho(D) = \sum_{i=1}^m \rho(D \cap H_i) = \sum_{i=1}^m \rho(D|H_i)\rho(H_i). \quad (22)$$

Our basic example of multiscale ensemble is log-uniform distribution of reaction constants in  $\mathbb{R}_+^n$  ( $\log k_i$  are independent and uniformly distributed on the line). For every ordering  $k_{j_1} > k_{j_2} > \dots > k_{j_n}$  a polyhedral cone  $H_{j_1 j_2 \dots j_n}$  in  $\mathbb{R}^n$  is defined. These cones have equal probabilities  $\rho(H_{j_1 j_2 \dots j_n}) = 1/n!$  and probability of intersection of cones for different orderings is zero. Hence, we can apply the law of total probability (22). This means that we can study every event  $D$  conditionally, for different orderings, and than combine the results of these studies in the final answer (22).

For example, if we study a simple cycle then formula (4) for steady state is valid with any given accuracy with unite probability for any ordering with the given minimal element  $k_n$ .

For cycle with given ordering of constants we can find 0-1 approximation of left and right eigenvectors (13). This approximation is valid with any given accuracy for this ordering with unite probability.

If we consider sufficiently wide log-uniform distribution of constants on a bounded interval instead of the infinite axis then these statements are true with probability close to 1.

For general system that we study below the situation is slightly more complicated: new terms, auxiliary reactions with monomial rate constants  $k_\zeta = \prod_i k_i^{\zeta_i}$  could appear with integer (but not necessary positive)  $\zeta_i$ , and we should include these  $k_\zeta$  in ordering. It follows from stability property that these monomials are log-uniform distributed on infinite interval, if  $k_i$  are. Therefore the situation seems to be similar to ordering of constants,

but there is a significant difference: monomials are not independent, they depend on  $k_i$  with  $\varsigma_i \neq 0$ .

Happily, in the forthcoming analysis when we include auxiliary reactions with constant  $k_\varsigma$ , we always exclude at least one of reactions with rate constant  $k_i$  and  $\varsigma_i \neq 0$ . Hence, for we always can use the following statement (for the new list of constants, or for the old one): if  $k_{j_1} > k_{j_2} > \dots > k_{j_n}$  then  $k_{j_1} \gg k_{j_2} \gg \dots \gg k_{j_n}$ , where  $a \gg b$  for positive  $a, b$  means: for any given  $\varepsilon > 0$  the inequality  $\varepsilon a > b$  holds with unite probability.

If we use sufficiently wide but finite log-uniform distribution then  $\varepsilon$  could not be arbitrarily small (this depends on the interval with), and probability is not unite but close to one. For given  $\varepsilon > 0$  probability tends to one when the interval width goes to infinity. It is important that we use only finite number of auxiliary reactions with monomial constants, and this number is bounded from above for given number of elementary reactions. For completeness, we should mention here general algebraic theory of orderings that is necessary in more sophisticated cases [47,48].

## 4 Relaxation of multiscale networks and hierarchy of auxiliary discrete dynamical systems

### 4.1 Definitions, notations and auxiliary results

#### 4.1.1 Notations

In this Sec., we consider a general network of linear (monomolecular) reactions. This network is represented as a directed graph (digraph): vertices correspond to components  $A_i$ , edges correspond to reactions  $A_i \rightarrow A_j$  with kinetic constants  $k_{ji} > 0$ . For each vertex,  $A_i$ , a positive real variable  $c_i$  (concentration) is defined. A basis vector  $e^i$  corresponds to  $A_i$  with components  $e_j^i = \delta_{ij}$ , where  $\delta_{ij}$  is the Kronecker delta. The kinetic equation for the system is

$$\frac{dc_i}{dt} = \sum_j (k_{ij}c_j - k_{ji}c_i), \quad (23)$$

or in vector form:  $\dot{c} = Kc$ .

To write another form of (23) we use stoichiometric vectors: for a reaction  $A_i \rightarrow A_j$  the stoichiometric vector  $\gamma_{ji}$  is a vector in concentration space with  $i$ th coordinate  $-1$ ,  $j$ th coordinate  $1$ , and zero other coordinates. The reaction rate  $w_{ji} = k_{ji}c_i$ . The kinetic equation has the form

$$\frac{dc}{dt} = \sum_{i,j} w_{ji} \gamma_{ji}, \quad (24)$$

where  $c$  is the concentration vector. One more form of (23) describes directly dynamics of reaction rates:

$$\frac{dw_{ji}}{dt} \left( = k_{ji} \frac{dc_i}{dt} \right) = k_{ji} \sum_l (w_{il} - w_{li}). \quad (25)$$

It is necessary to mention that, in general, system (25) is not equivalent to (24), because there are additional connections between variables  $w_{ji}$ . If there exists at least one  $A_i$  with two different outgoing reactions,  $A_i \rightarrow A_j$  and  $A_i \rightarrow A_l$  ( $j \neq l$ ), then  $w_{ji}/w_{li} \equiv k_{ji}/k_{li}$ . If the reaction network generates a discrete dynamical system  $A_i \rightarrow A_j$  on the set of  $A_i$  (see below), then the variables  $w_{ji}$  are independent, and (25) gives equivalent representation of kinetics.

A linear conservation law is a linear function defined on the concentrations  $q(c) = \sum_{i=1}^n q_i c_i$ , whose value is preserved by the dynamics (23). The set of all the conservation laws forms the left kernel of the matrix  $K$ . Equation (23) always has a linear conservation law:  $b(c) = \sum_i c_i = \text{const}$ . If there is no other independent linear conservation law, then the system is weakly ergodic.

Two vertices are called adjacent if they share a common edge. A path is a sequence of adjacent vertices. A graph is connected if any two of its vertices are linked by a path. A maximal connected subgraph of graph  $G$  is called a connected component of  $G$ . Every graph can be decomposed into connected components.

A directed path is a sequence of adjacent edges where each step goes in direction of an edge. A vertex  $A$  is reachable by a vertex  $B$ , if there exists an oriented path from  $B$  to  $A$ .

A nonempty set  $V$  of graph vertexes forms a *sink*, if there are no oriented edges from  $A_i \in V$  to any  $A_j \notin V$ . For example, in the reaction graph  $A_1 \leftarrow A_2 \rightarrow A_3$  the one-vertex sets  $\{A_1\}$  and  $\{A_3\}$  are sinks. A sink is minimal if it does not contain a strictly smaller sink. In the previous example,  $\{A_1\}$ ,  $\{A_3\}$  are minimal sinks. Minimal sinks are also called ergodic components.

A digraph is strongly connected, if every vertex  $A$  is reachable by any other vertex  $B$ . Ergodic components are maximal strongly connected subgraphs of the graph, but inverse is not true: there may exist maximal strongly connected subgraphs that have outgoing edges and, therefore, are not sinks.

We study ensembles of systems with a given graph and independent and well separated kinetic constants  $k_{ij}$ . This means that we study asymptotic behaviour of ensembles with independent identically distributed constants, log-uniform distributed in sufficiently big interval  $\log k \in [\alpha, \beta]$ , for  $\alpha \rightarrow -\infty$ ,  $\beta \rightarrow \infty$ , or just a log-uniform distribution on infinite axis,  $\log k \in \mathbb{R}$ .

#### 4.1.2 Sinks and ergodicity

If there is no other independent linear conservation law, then the system is weakly ergodic. The weak ergodicity of the network follows from its topological properties.

The following properties are equivalent and each one of them can be used as an alternative definition of weak ergodicity:

- (1) There exist the only independent linear conservation law for kinetic equations (23) (this is  $b(c) = \sum_i c_i = \text{const}$ ).

- (2) For any normalized initial state  $c(0)$  ( $b(c) = 1$ ) there exists a limit state  $c^* = \lim_{t \rightarrow \infty} \exp(Kt) c(0)$  that is the same for all normalized initial conditions. (For all  $c$ ,  $\lim_{t \rightarrow \infty} \exp(Kt) c = b(c)c^*$ .)
- (3) For each two vertices  $A_i, A_j$  ( $i \neq j$ ) we can find such a vertex  $A_k$  that oriented paths exist from  $A_i$  to  $A_k$  and from  $A_j$  to  $A_k$ . This means that the following structure exists:

$$A_i \rightarrow \dots \rightarrow A_k \leftarrow \dots \leftarrow A_j.$$

One of these paths can be degenerated: it might be  $i = k$  or  $j = k$ .

- (4) The network has only one minimal sink (one ergodic component).

For every monomolecular kinetic system, the Jordan cell for zero eigenvalue of matrix  $K$  is diagonal and the maximal number of independent linear conservation laws (i.e. the geometric multiplicity of the zero eigenvalue of the matrix  $K$ ) is equal to the maximal number of disjoint ergodic components (minimal sinks).

Let  $G = \{A_{i_1}, \dots, A_{i_l}\}$  be an ergodic component. Then there exists a unique vector (normalized invariant distribution)  $c^G$  with the following properties:  $c_i^G = 0$  for  $i \notin \{i_1, \dots, i_l\}$ ,  $c_i^G > 0$  for all  $i \in \{i_1, \dots, i_l\}$   $b(c^G) = 1$ ,  $Kc^G = 0$ .

If  $G_1, \dots, G_m$  are all ergodic components of the system, then there exist  $m$  independent positive linear functionals  $b_1(c), \dots, b_m(c)$  such that  $\sum_i b_i = b$  and for each  $c$

$$\lim_{t \rightarrow \infty} \exp(Kt)c = \sum_{i=1}^m b_i(c)c^{G_i}. \quad (26)$$

So, for any solution of kinetic equations (23),  $c(t)$ , the limit at  $t \rightarrow \infty$  is a linear combination of normalized invariant distributions  $c^{G_i}$  with coefficients  $b_i(c(0))$ . In the simplest example,  $A_1 \leftarrow A_2 \rightarrow A_3$ ,  $G_1 = \{A_1\}$ ,  $G_2 = \{A_3\}$ , components of vectors  $c^{G_1}, c^{G_2}$  are  $(1, 0, 0)$  and  $(0, 0, 1)$ , correspondingly. For functionals  $b_{1,2}$  we get:

$$b_1(c) = c_1 + \frac{k_1}{k_1 + k_2}c_2; \quad b_2(c) = \frac{k_2}{k_1 + k_2}c_2 + c_3, \quad (27)$$

where  $k_1, k_2$  are rate constants for reaction  $A_2 \rightarrow A_1$ , and  $A_2 \rightarrow A_3$ , correspondingly. We can mention that for well separated constants either  $k_1 \gg k_2$  or  $k_1 \ll k_2$ . Hence, one of the coefficients  $k_1/(k_1 + k_2)$ ,  $k_2/(k_1 + k_2)$  is close to 0, another is close to 1. This is an example of the general zero-one law for multiscale systems: for any  $l, i$ , the value of functional  $b_l$  (26) on basis vector  $e^i$ ,  $b_l(e^i)$ , is either close to one or close to zero (with probability close to 1).

We can understand better this asymptotics by using the Markov chain language. For non-separated constants a particle in  $A_2$  has nonzero probability to reach  $A_1$  and nonzero probability to reach  $A_3$ . The zero-one law in this simplest case means that the dynamics of the particle becomes deterministic: with probability one it chooses to go to one of vertices  $A_2, A_3$  and to avoid another. Instead of branching,  $A_2 \rightarrow A_1$  and  $A_2 \rightarrow A_3$ , we select only one way: either  $A_2 \rightarrow A_1$  or  $A_2 \rightarrow A_3$ . Graphs without branching represent discrete dynamical systems.

### 4.1.3 Decomposition of discrete dynamical systems

Discrete dynamical system on a finite set  $V = \{A_1, A_2, \dots, A_n\}$  is a semigroup  $1, \phi, \phi^2, \dots$ , where  $\phi$  is a map  $\phi : V \rightarrow V$ .  $A_i \in V$  is a periodic point, if  $\phi^l(A_i) = A_i$  for some  $l > 0$ ; else  $A_i$  is a transient point. A cycle of period  $l$  is a sequence of  $l$  distinct periodic points  $A, \phi(A), \phi^2(A), \dots, \phi^{l-1}(A)$  with  $\phi^l(A) = A$ . A cycle of period one consists of one fixed point,  $\phi(A) = A$ . Two cycles,  $C, C'$  either coincide or have empty intersection.

The set of periodic points,  $V^p$ , is always nonempty. It is a union of cycles:  $V^p = \cup_j C_j$ . For each point  $A \in V$  there exist such a positive integer  $\tau(A)$  and a cycle  $C(A) = C_j$  that  $\phi^q(A) \in C_j$  for  $q \geq \tau(A)$ . In that case we say that  $A$  belongs to basin of attraction of cycle  $C_j$  and use notation  $Att(C_j) = \{A \mid C(A) = C_j\}$ . Of course,  $C_j \subset Att(C_j)$ . For different cycles,  $Att(C_j) \cap Att(C_l) = \emptyset$ . If  $A$  is periodic point then  $\tau(A) = 0$ . For transient points  $\tau(A) > 0$ .

So, the phase space  $V$  is divided onto subsets  $Att(C_j)$ . Each of these subsets includes one cycle (or a fixed point, that is a cycle of length 1). Sets  $Att(C_j)$  are  $\phi$ -invariant:  $\phi(Att(C_j)) \subset Att(C_j)$ . The set  $Att(C_j) \setminus C_j$  consist of transient points and there exists such positive integer  $\tau$  that  $\phi^q(Att(C_j)) = C_j$  if  $q \geq \tau$ .

## 4.2 Auxiliary discrete dynamical systems and relaxation analysis

### 4.2.1 Auxiliary discrete dynamical system

For each  $A_i$ , we define  $\kappa_i$  as the maximal kinetic constant for reactions  $A_i \rightarrow A_j$ :  $\kappa_i = \max_j \{k_{ji}\}$ . For correspondent  $j$  we use notation  $\phi(i)$ :  $\phi(i) = \arg \max_j \{k_{ji}\}$ . The function  $\phi(i)$  is defined under condition that for  $A_i$  outgoing reactions  $A_i \rightarrow A_j$  exist. Let us extend the definition:  $\phi(i) = i$  if there exist no such outgoing reactions.

The map  $\phi$  determines discrete dynamical system on a set of components  $V = \{A_i\}$ . We call it the auxiliary discrete dynamical system for a given network of monomolecular reactions. Let us decompose this system and find the cycles  $C_j$  and their basins of attraction,  $Att(C_j)$ .

Notice that for the graph that represents a discrete dynamic system, attractors are ergodic components, while basins are connected components.

An auxiliary reaction network is associated with the auxiliary discrete dynamical system. This is the set of reactions  $A_i \rightarrow A_{\phi(i)}$  with kinetic constants  $\kappa_i$ . The correspondent kinetic equation is

$$\dot{c}_i = -\kappa_i c_i + \sum_{\phi(j)=i} \kappa_j c_j, \quad (28)$$

or in vector notations (24)

$$\frac{dc}{dt} = \tilde{K}c = \sum_i \kappa_i c_i \gamma_{\phi(i)} i; \quad \tilde{K}_{ij} = -\kappa_j \delta_{ij} + \kappa_j \delta_{i \phi(j)}. \quad (29)$$

For deriving of the auxiliary discrete dynamical system we do not need the values of rate constants. Only the ordering is important. Below we consider multiscale ensembles of kinetic systems with given ordering and with well separated kinetic constants ( $k_{\sigma(1)} \gg k_{\sigma(2)} \gg \dots$  for some permutation  $\sigma$ ).

In the following, we analyze first the situation when the system is connected and has only one attractor. This can be a point or a cycle. Then, we discuss the general situation with any number of attractors.

#### 4.2.2 Acyclic auxiliary system with one attractor: structure and eigenvectors

In the simplest case, the auxiliary discrete dynamical system is acyclic and has only one attractor, a fixed point. Let this point be  $A_n$  ( $n$  is the number of vertices). For such a system, it is easy to find explicit analytic solution of kinetic equation (28). First of all, these systems have a characteristic property among all auxiliary dynamical systems: the stoichiometric vectors of reactions  $A_i \rightarrow A_{\phi(i)}$  form a basis in the subspace of concentration space with  $\sum_i c_i = 0$ : there exist  $n - 1$  reaction, and their stoichiometric vectors are independent. On the other hand, existence of cycles implies linear connections between stoichiometric vectors, and existence of two attractors in acyclic system implies that the number of reactions is less  $n - 1$ , and their stoichiometric vectors could not form a basis in  $n - 1$ -dimensional space.

For ensembles with well separate constants, relaxation of the whole network is approximated by solution of auxiliary kinetic equation (28) with high accuracy, with probability close to 1. To prove this statement, let us first find left and right eigenvectors of matrix  $\tilde{K}$  of auxiliary kinetic system (28) for acyclic auxiliary dynamics. In this case, for any vertex different from the attractor there is an eigenvector. Right eigenvectors will be constructed by recurrence starting from the vertex and moving in the direction of the flow. The construction is in opposite direction for left eigenvectors.

For zero eigenvalue, the right eigenvector  $r^0$  has only one nonzero coordinate,  $c_n = 1$ , the left eigenvector is a row  $l^0 = (1, 1, \dots, 1)$  (this corresponds to the linear conservation law  $l^0 c = \sum_i c_i = \text{const}$ ), the normalization condition holds:  $l^0 r^0 = 1$ . Of course,  $l^0$  is left eigenvector with zero eigenvalue for any network of monomolecular reactions. If for such a network the auxiliary discrete dynamical system is acyclic and has only one attractor, a fixed point, then vector  $r^0$  is right eigenvector with zero eigenvalue, because in this case there is no outgoing reaction of the form  $A_n \rightarrow A_i$  ( $i = 1, \dots, n - 1$ ).

Nonzero eigenvalues of  $\tilde{K}$  (28) are  $-\kappa_i$  ( $i = 1, \dots, n - 1$ ). For left and right eigenvectors of  $\tilde{K}$  with eigenvalue  $-\kappa_i$  we use notations  $l^i$  (vector-row) and  $r^i$  (vector-column), correspondingly, and apply normalization condition  $r_i^i = l_i^i = 1$ . For given  $i$ ,  $\tau_i$  is the minimal integer such that  $\phi^{\tau_i}(i) = n$  (this is a “relaxation time” i.e. the number of steps to reach attractor). All indices  $\{\phi^k(i) \mid k = 0, 1, \dots, \tau_i\}$  are different. For right eigenvector  $r^i$  only coordinates  $r_{\phi^k(i)}^i$  ( $k = 0, 1, \dots, \tau_i$ ) could have nonzero values, and

$$(\tilde{K}r^i)_{\phi^{k+1}(i)} = -\kappa_{\phi^{k+1}(i)}r_{\phi^{k+1}(i)}^i + \kappa_{\phi^k(i)}r_{\phi^k(i)}^i = -\kappa_i r_{\phi^{k+1}(i)}^i.$$

Hence,

$$\begin{aligned}
r_{\phi^{k+1}(i)}^i &= \frac{\kappa_{\phi^k(i)}}{\kappa_{\phi^{k+1}(i)} - \kappa_i} r_{\phi^k(i)}^i = \prod_{j=0}^k \frac{\kappa_{\phi^j(i)}}{\kappa_{\phi^{j+1}(i)} - \kappa_i} \\
&= \frac{\kappa_i}{\kappa_{\phi^{k+1}(i)} - \kappa_i} \prod_{j=0}^{k-1} \frac{\kappa_{\phi^{j+1}(i)}}{\kappa_{\phi^{j+1}(i)} - \kappa_i}.
\end{aligned} \tag{30}$$

The last transformation is convenient for estimation of the product for well separated constants (compare to (12)):

$$\frac{\kappa_{\phi^{j+1}(i)}}{\kappa_{\phi^{j+1}(i)} - \kappa_i} \approx \begin{cases} 1, & \text{if } \kappa_{\phi^{j+1}(i)} \gg \kappa_i, \\ 0, & \text{if } \kappa_{\phi^{j+1}(i)} \ll \kappa_i; \end{cases} \quad \frac{\kappa_i}{\kappa_{\phi^{k+1}(i)} - \kappa_i} \approx \begin{cases} -1, & \text{if } \kappa_i \gg \kappa_{\phi^{k+1}(i)}, \\ 0, & \text{if } \kappa_i \ll \kappa_{\phi^{k+1}(i)}. \end{cases} \tag{31}$$

For left eigenvector  $l^i$  coordinate  $l_j^i$  could have nonzero value only if there exists such  $q \geq 0$  that  $\phi^q(j) = i$  (this  $q$  is unique because the auxiliary dynamical system has no cycles). In that case (for  $q > 0$ ),

$$(l^i \tilde{K})_j = -\kappa_j l_j^i + \kappa_j l_{\phi(j)}^i = -\kappa_i l_j^i.$$

Hence,

$$l_j^i = \frac{\kappa_j}{\kappa_j - \kappa_i} l_{\phi(j)}^i = \prod_{k=0}^{q-1} \frac{\kappa_{\phi^k(j)}}{\kappa_{\phi^k(j)} - \kappa_i}. \tag{32}$$

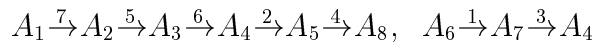
For every fraction in (32) the following estimate holds:

$$\frac{\kappa_{\phi^k(j)}}{\kappa_{\phi^k(j)} - \kappa_i} \approx \begin{cases} 1, & \text{if } \kappa_{\phi^k(j)} \gg \kappa_i, \\ 0, & \text{if } \kappa_{\phi^k(j)} \ll \kappa_i. \end{cases} \tag{33}$$

As we can see, every coordinate of left and right eigenvectors of  $\tilde{K}$  (30), (32) is either 0 or  $\pm 1$ , or close to 0 or to 1 (with probability close to 1). We can write this asymptotic representation explicitly (analogously to (13)). For left eigenvectors,  $l_i^i = 1$  and  $l_j^i = 1$  (for  $i \neq j$ ) if there exists such  $q$  that  $\phi^q(j) = i$ , and  $\kappa_{\phi^d(j)} > \kappa_i$  for all  $d = 0, \dots, q-1$ , else  $l_j^i = 0$ . For right eigenvectors,  $r_i^i = 1$  and  $r_{\phi^k(j)}^i = -1$  if  $\kappa_{\phi^k(j)} < \kappa_i$  and for all positive  $m < k$  inequality  $\kappa_{\phi^m(j)} > \kappa_i$  holds, i.e.  $k$  is first such positive integer that  $\kappa_{\phi^k(j)} < \kappa_i$ . Vector  $r^i$  has not more than two nonzero coordinates. It is straightforward to check that in this asymptotic  $l^i r^j = \delta_{ij}$ .

In general, coordinates of eigenvectors  $l_j^i, r_j^i$  are simultaneously nonzero only for one value  $j = i$  because the auxiliary system is acyclic. On the other hand,  $l^i r^j = 0$  if  $i \neq j$ , just because that are eigenvectors for different eigenvalues,  $\kappa_i$  and  $\kappa_j$ . Hence,  $l^i r^j = \delta_{ij}$ .

For example, let us find the asymptotic of left and right eigenvectors for a branched acyclic system of reactions:





where the upper index marks the order of rate constants:  $\kappa_6 > \kappa_4 > \kappa_7 > \kappa_5 > \kappa_2 > \kappa_3 > \kappa_1$  ( $\kappa_i$  is the rate constant of reaction  $A_i \rightarrow \dots$ ).

For left eigenvectors, rows  $l^i$ , we have the following asymptotics:

$$\begin{aligned} l^1 &\approx (1, 0, 0, 0, 0, 0, 0, 0), & l^2 &\approx (0, 1, 0, 0, 0, 0, 0, 0), & l^3 &\approx (0, 1, 1, 0, 0, 0, 0, 0), \\ l^4 &\approx (0, 0, 0, 1, 0, 0, 0, 0), & l^5 &\approx (0, 0, 0, 1, 1, 1, 1, 0), & l^6 &\approx (0, 0, 0, 0, 0, 1, 0, 0), \\ l^7 &\approx (0, 0, 0, 0, 0, 1, 1, 0) \end{aligned} \quad (34)$$

For right eigenvectors, columns  $r^i$ , we have the following asymptotics (we write vector-columns in rows):

$$\begin{aligned} r^1 &\approx (1, 0, 0, 0, 0, 0, 0, -1), & r^2 &\approx (0, 1, -1, 0, 0, 0, 0, 0), & r^3 &\approx (0, 0, 1, 0, 0, 0, 0, -1), \\ r^4 &\approx (0, 0, 0, 1, -1, 0, 0, 0), & r^5 &\approx (0, 0, 0, 0, 1, 0, 0, -1), & r^6 &\approx (0, 0, 0, 0, 0, 1, -1, 0), \\ r^7 &\approx (0, 0, 0, 0, -1, 0, 1, 0). \end{aligned} \quad (35)$$

For convenience, we use all eight coordinates,  $c_{1-8}$ .

#### 4.2.3 Relaxation of a system with acyclic auxiliary dynamical system

Let us assume that the auxiliary dynamical system is acyclic and has only one attractor, a fixed point. This means that stoichiometric vectors  $\gamma_{\phi(i)i}$  form a basis in a subspace of concentration space with  $\sum_i c_i = 0$ . For every reaction  $A_i \rightarrow A_l$  the following linear operators  $Q_{il}$  can be defined:

$$Q_{il}(\gamma_{\phi(i)i}) = \gamma_{li}, \quad Q_{il}(\gamma_{\phi(p)p}) = 0 \text{ for } p \neq i. \quad (36)$$

The kinetic equation for the whole reaction network (24) could be transformed in the form

$$\begin{aligned} \frac{dc}{dt} &= \sum_i \left( 1 + \sum_{l \neq \phi(i)} \frac{k_{li}}{\kappa_i} Q_{il} \right) \gamma_{\phi(i)i} \kappa_i c_i \\ &= \left( 1 + \sum_{j, l (l \neq \phi(j))} \frac{k_{lj}}{\kappa_j} Q_{jl} \right) \sum_i \gamma_{\phi(i)i} \kappa_i c_i \\ &= \left( 1 + \sum_{j, l (l \neq \phi(j))} \frac{k_{lj}}{\kappa_j} Q_{jl} \right) \tilde{K} c, \end{aligned} \quad (37)$$

where  $\tilde{K}$  is kinetic matrix of auxiliary kinetic equation (29). By construction of auxiliary dynamical system,  $k_{li} \ll \kappa_i$  if  $l \neq \phi(i)$ . Notice also that  $|Q_{jl}|$  does not depend on rate constants.

Let us represent system (37) in eigenbasis of  $\tilde{K}$  obtained in previous subsection. Any matrix  $B$  in this eigenbasis has the form  $B = (\tilde{b}_{ij})$ ,  $\tilde{b}_{ij} = l^i B r^j = \sum_{qs} l_q^i b_{qs} r_s^j$ , where  $(b_{qs})$  is matrix  $B$  in the initial basis,  $l^i$  and  $r^j$  are left and right eigenvectors of  $\tilde{K}$  (30), (32). In eigenbasis of  $\tilde{K}$  the Gershgorin estimates of eigenvalues and estimates of eigenvectors are much more efficient than in original coordinates: the system is stronger diagonally

dominant. Transformation to this basis is an effective preconditioning for perturbation theory that uses auxiliary kinetics as a first approximation to the kinetics of the whole system.

First of all, we can exclude the conservation law. Any solution of (37) has the form  $c(t) = br^0 + \tilde{c}(t)$ , where  $b = l^0 \tilde{c}(t) = \sum_i \tilde{c}_i(t) = 0$ . On the subspace of concentration space with  $\sum_i c_i = 0$  we get

$$\frac{dc}{dt} = (1 + \mathcal{E}) \text{diag}\{-\kappa_1, \dots, -\kappa_{n-1}\}c, \quad (38)$$

where  $\mathcal{E} = (\varepsilon_{ij})$ ,  $|\varepsilon_{ij}| \ll 1$ , and  $\text{diag}\{-\kappa_1, \dots, -\kappa_{n-1}\}$  is diagonal matrix with  $-\kappa_1, \dots, -\kappa_{n-1}$  on the main diagonal. If  $|\varepsilon_{ij}| \ll 1$  then we can use the Gershgorin theorem and state that eigenvalues of matrix  $(1 + \mathcal{E}) \text{diag}\{-\kappa_1, \dots, -\kappa_{n-1}\}$  are real and have the form  $\lambda_i = -\kappa_i + \theta_i$  with  $|\theta_i| \ll \kappa_i$ .

To prove inequality  $|\varepsilon_{ij}| \ll 1$  (for ensembles with well separated constants, with probability close to 1) we use that the left and right eigenvectors of  $\tilde{K}$  (30), (32) are uniformly bounded under some non-degeneration conditions and those conditions are true for well separated constants. For ensembles with well separated constants, for any given positive  $g < 1$  and all  $i, j$  ( $i \neq j$ ) the following inequality is true with probability close to 1:  $|\kappa_i - \kappa_j| > g\kappa_i$ . Let us select a value of  $g$  and assume that this *diagonal gap condition* is always true. In this case, for every fraction in (30), (32) we have estimate

$$\frac{\kappa_i}{|\kappa_j - \kappa_i|} < \frac{1}{g}.$$

Therefore, for coordinates of right and left eigenvectors of  $\tilde{K}$  (30), (32) we get

$$|r_{\phi^{k+1}(i)}^i| < \frac{1}{g^k} < \frac{1}{g^n}, \quad |l_j^i| < \frac{1}{g^q} < \frac{1}{g^n}. \quad (39)$$

We can estimate  $|\varepsilon_{ij}|$  and  $|\theta_i|/\kappa_i$  from above as  $\text{const} \times \max_{l \neq \phi(s)} \{k_{ls}/\kappa_s\}$ . So, the eigenvalues for kinetic matrix of the whole system (37) are real and close to eigenvalues of auxiliary kinetic matrix  $\tilde{K}$  (29). For eigenvectors, the Gershgorin theorem gives no result, and additionally to diagonal dominance we must assume the diagonal gap condition. Based on this assumption, we proved the Gershgorin type estimate of eigenvectors in Appendix 1. In particular, according to this estimate, eigenvectors for the whole reaction network are arbitrarily close to eigenvectors of  $\tilde{K}$  (with probability close to 1).

So, if the auxiliary discrete dynamical system is acyclic and has only one attractor (a fixed point), then the relaxation of the whole reaction network could be approximated by the auxiliary kinetics (28):

$$c(t) = (l^0 c(0))r^0 + \sum_{i=1}^{n-1} (l^i c(0))r^i \exp(-\kappa_i t), \quad (40)$$

where  $l^0$  and  $r^0$  are left (vector-row) and right (vector-column) eigenvectors of  $\tilde{K}$  correspondent to zero eigenvalue ( $l^0 = (1, 1, \dots, 1)$ ,  $r_i^0 = \delta_{in}$ , and  $\delta_{in}$  is Kronecker delta),  $l^i$  and  $r^i$  are left and right eigenvectors of  $\tilde{K}$  correspondent to eigenvalue  $-\kappa_i$ . For  $l^i$  and  $r^i$

one can use exact formulas (30) and (32) or 0-1 asymptotic representations based on (33) and (31) for multiscale systems. This approximation (40) could be improved by iterative methods, if necessary.

#### 4.2.4 Auxiliary system with one cyclic attractor

The second simple particular case on the way to general case is a reaction network with components  $A_1, \dots, A_n$  whose auxiliary discrete dynamical system has one attractor, a cycle with period  $\tau > 1$ :  $A_{n-\tau+1} \rightarrow A_{n-\tau+2} \rightarrow \dots \rightarrow A_n \rightarrow A_{n-\tau+1}$  (after some change of enumeration). We assume that the limiting step in this cycle (reaction with minimal constant) is  $A_n \rightarrow A_{n-\tau+1}$ . If auxiliary discrete dynamical system has only one attractor then the whole network is weakly ergodic. But the attractor of the auxiliary system may not coincide with a sink of the reaction network.

There are two possibilities:

- (1) In the whole network, all the outgoing reactions from the cycle have the form  $A_{n-\tau+i} \rightarrow A_{n-\tau+j}$  ( $i, j > 0$ ). This means that the cycle vertices  $A_{n-\tau+1}, A_{n-\tau+2}, \dots, A_n$  form a sink for the whole network.
- (2) There exists a reaction from a cycle vertex  $A_{n-\tau+i}$  to  $A_m$ ,  $m \leq n - \tau$ . This means that the set  $\{A_{n-\tau+1}, A_{n-\tau+2}, \dots, A_n\}$  is not a sink for the whole network.

In the first case, the limit (for  $t \rightarrow \infty$ ) distribution for the auxiliary kinetics is the well-studied stationary distribution of the cycle  $A_{n-\tau+1}, A_{n-\tau+2}, \dots, A_n$  described in Sec. 2 (2), (3) (4), (6). The set  $\{A_{n-\tau+1}, A_{n-\tau+2}, \dots, A_n\}$  is the only ergodic component for the whole network too, and the limit distribution for that system is nonzero on vertices only. The stationary distribution for the cycle  $A_{n-\tau+1} \rightarrow A_{n-\tau+2} \rightarrow \dots \rightarrow A_n \rightarrow A_{n-\tau+1}$  approximates the stationary distribution for the whole system. To approximate the relaxation process, let us delete the limiting step  $A_n \rightarrow A_{n-\tau+1}$  from this cycle. By this deletion we produce an acyclic system with one fixed point,  $A_n$ , and auxiliary kinetic equation (29) transforms into

$$\frac{dc}{dt} = \tilde{K}_0 c = \sum_{i=1}^{n-1} \kappa_i c_i \gamma_{\phi(i) i}. \quad (41)$$

As it is demonstrated, dynamics of this system approximates relaxation of the whole network in subspace  $\sum_i c_i = 0$ . Eigenvalues for (41) are  $-\kappa_i$  ( $i < n$ ), the corresponded eigenvectors are represented by (30), (32) and 0-1 multiscale asymptotic representation is based on (33) and (31).

In the second case, the set  $\{A_{n-\tau+1}, A_{n-\tau+2}, \dots, A_n\}$  is not a sink for the whole network. This means that there exist outgoing reactions from the cycle,  $A_{n-\tau+i} \rightarrow A_j$  with  $A_j \notin \{A_{n-\tau+1}, A_{n-\tau+2}, \dots, A_n\}$ . For every cycle vertex  $A_{n-\tau+i}$  the rate constant  $\kappa_{n-\tau+i}$  that corresponds to the cycle reaction  $A_{n-\tau+i} \rightarrow A_{n-\tau+i+1}$  is much bigger than any other constant  $k_{jn-\tau+i}$  that corresponds to a ‘‘side’’ reaction  $A_{n-\tau+i} \rightarrow A_j$  ( $j \neq n - \tau + i + 1$ ):  $\kappa_{n-\tau+i} \gg k_{jn-\tau+i}$ . This is because definition of auxiliary discrete dynamical system and assumption of ensemble with well separated constants (multiscale asymptotics). This inequality allows us to separate motion and to use for computation of the rates of outgoing reaction  $A_{n-\tau+i} \rightarrow A_j$  the quasi steady state distribution in the cycle. This means

that we can glue the cycle into one vertex  $A_{n-\tau+1}^1$  with the correspondent concentration  $c_{n-\tau+1}^1 = \sum_{1 \leq i \leq \tau} c_{n-\tau+i}$  and substitute the reaction  $A_{n-\tau+i} \rightarrow A_j$  by  $A_{n-\tau+1}^1 \rightarrow A_j$  with the rate constant renormalization:  $k_{jn-\tau+1}^1 = k_{jn-\tau+i} c_{n-\tau+i}^{\text{QS}} / c_{n-\tau+1}^1$ . By the superscript QS we mark here the quasistationary concentrations for given total cycle concentration  $c_{n-\tau+1}^1$ . Another possibility is to recharge the link  $A_{n-\tau+i} \rightarrow A_j$  to another vertex of the cycle (usually to  $A_n$ ): we can substitute the reaction  $A_{n-\tau+i} \rightarrow A_j$  by the reaction  $A_{n-\tau+q} \rightarrow A_j$  with the rate constant renormalization:

$$k_{jn-\tau+q} = k_{jn-\tau+i} c_{n-\tau+i}^{\text{QS}} / c_{n-\tau+q}^{\text{QS}}. \quad (42)$$

We apply this approach now and demonstrate its applicability in more details later in this section.

For the quasi-stationary distribution on the cycle we get  $c_{n-\tau+i} = c_n \kappa_n / \kappa_{n-\tau+i}$  ( $1 \leq i < \tau$ ). The original reaction network is transformed by gluing the cycle  $\{A_{n-\tau+1}, A_{n-\tau+2}, \dots, A_n\}$  into a point  $A_{n-\tau+1}^1$ . We say that components  $A_{n-\tau+1}, A_{n-\tau+2}, \dots, A_n$  of the original system belong to the component  $A_{n-\tau+1}^1$  of the new system. All the reactions  $A_i \rightarrow A_j$  with  $i, j \leq n - \tau$  remain the same with rate constant  $k_{ji}$ . Reactions of the form  $A_i \rightarrow A_j$  with  $i \leq n - \tau, j > n - \tau$  (incoming reactions of the cycle  $\{A_{n-\tau+1}, A_{n-\tau+2}, \dots, A_n\}$ ) transform into  $A_i \rightarrow A_{n-\tau+1}^1$  with the same rate constant  $k_{ji}$ . Reactions of the form  $A_i \rightarrow A_j$  with  $i > n - \tau, j \leq n - \tau$  (outgoing reactions of the cycle  $\{A_{n-\tau+1}, A_{n-\tau+2}, \dots, A_n\}$ ) transform into reactions  $A_{n-\tau+1}^1 \rightarrow A_j$  with the “quasistationary” rate constant  $k_{ji}^{\text{QS}} = k_{ji} \kappa_n / \kappa_{n-\tau+i}$ . After that, we select the maximal  $k_{ji}^{\text{QS}}$  for given  $j$ :  $k_{jn-\tau+1}^{(1)} = \max_{i > n-\tau} k_{ji}^{\text{QS}}$ . This  $k_{jn-\tau+1}^{(1)}$  is the rate constant for reaction  $A_{n-\tau+1}^1 \rightarrow A_j$  in the new system. Reactions  $A_i \rightarrow A_j$  with  $i, j > n - \tau$  (internal reactions of the site) vanish.

Among reactions of the form  $A_{n-\tau+i} \rightarrow A_m$  ( $m \geq n - \tau$ ) we find

$$\kappa_{n-\tau+i}^{(1)} = \max_{i,m} \{k_{mn-\tau+i} \kappa_n / \kappa_{n-\tau+i}\}. \quad (43)$$

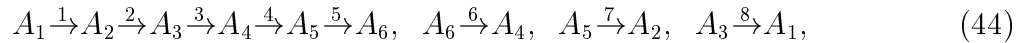
Let the correspondent  $i, m$  be  $i_1, m_1$ .

After that, we create a new auxiliary discrete dynamical system for the new reaction network on the set  $\{A_1, \dots, A_{n-\tau}, A_{n-\tau+1}^1\}$ . We can describe this new auxiliary system as a result of transformation of the first auxiliary discrete dynamical system of initial reaction network. All the reaction from this first auxiliary system of the form  $A_i \rightarrow A_j$  with  $i, j \leq n - \tau$  remain the same with rate constant  $\kappa_i$ . Reactions of the form  $A_i \rightarrow A_j$  with  $i \leq n - \tau, j > n - \tau$  transform into  $A_i \rightarrow A_{n-\tau+1}^1$  with the same rate constant  $\kappa_i$ . One more reaction is to be added:  $A_{n-\tau+1}^1 \rightarrow A_{m_1}$  with rate constant  $\kappa_{n-\tau+i}^{(1)}$ . We “glued” the cycle into one vertex,  $A_{n-\tau+1}^1$ , and added new reaction from this vertex to  $A_{m_1}$  with maximal possible constant (43). Without this reaction the new auxiliary dynamical system has only one attractor, the fixed point  $A_{n-\tau+1}^1$ . With this additional reaction that point is not fixed, and a new cycle appears:  $A_{m_1} \rightarrow \dots \rightarrow A_{n-\tau+1}^1 \rightarrow A_{m_1}$ .

Again we should analyze, whether this new cycle is a sink in the new reaction network, etc. Finally, after a chain of transformations, we should come to an auxiliary discrete dynamical system with one attractor, a cycle, that is the sink of the transformed whole reaction network. After that, we can find stationary distribution by restoring of glued

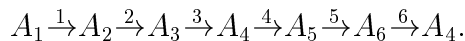
cycles in auxiliary kinetic system and applying formulas (2), (3) (4), (6) from Sec. 2. First, we find the stationary state of the cycle constructed on the last iteration, after that for each vertex  $A_j^k$  that is a glued cycle we know its concentration (the sum of all concentrations) and can find the stationary distribution, then if there remain some vertices that are glued cycles we find distribution of concentrations in these cycles, etc. At the end of this process we find all stationary concentrations with high accuracy, with probability close to one.

As a simple example we use the following system, a chain supplemented by three reactions:



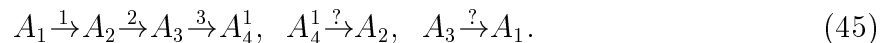
where the upper index marks the order of rate constants.

Auxiliary discrete dynamical system for the network (44) includes the chain and one reaction:



It has one attractor, the cycle  $A_4 \xrightarrow{4} A_5 \xrightarrow{5} A_6 \xrightarrow{6} A_4$ . This cycle is not a sink for the whole system, because there exists an outgoing reaction  $A_5 \xrightarrow{7} A_2$ .

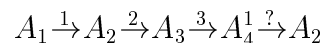
By gluing the cycle  $A_4 \xrightarrow{4} A_5 \xrightarrow{5} A_6 \xrightarrow{6} A_4$  into a vertex  $A_4^1$  we get new network with a chain supplemented by two reactions:



Here the new rate constant is  $k_{24}^{(1)} = k_{25}\kappa_6/\kappa_5$  ( $\kappa_6 = k_{46}$  is the limiting step of the cycle  $A_4 \xrightarrow{4} A_5 \xrightarrow{5} A_6 \xrightarrow{6} A_4$ ,  $\kappa_5 = k_{65}$ ).

Here we can make a simple but important observation: the new constant  $k_{24}^1 = k_{25}\kappa_6/\kappa_5$  has the same log-uniform distribution on the whole axis as constants  $k_{25}$ ,  $\kappa_6$  and  $\kappa_5$  have. The new constant  $k_{24}^1$  depends on  $k_{25}$  and the internal cycle constants  $\kappa_6$  and  $\kappa_5$ , and is independent from other constants.

Of course,  $k_{24}^{(1)} < \kappa_5$ , but relations between  $k_{24}^{(1)}$  and  $k_{13}$  are a priori unknown. Both orderings,  $k_{24}^{(1)} > k_{13}$  and  $k_{24}^{(1)} < k_{13}$ , are possible, and should be considered separately, if necessary. But for both orderings the auxiliary dynamical system for network (45) is



(of course,  $\kappa_4^{(1)} < \kappa_3 < \kappa_2 < \kappa_1$ ). It has one attractor, the cycle  $A_2 \xrightarrow{2} A_3 \xrightarrow{3} A_4^1 \xrightarrow{2} A_2$ . This cycle is not a sink for the whole system, because there exists an outgoing reaction  $A_3 \xrightarrow{7} A_1$ . The limiting constant for this cycle is  $\kappa_4^{(1)} = k_{24}^{(1)} = k_{25}k_{46}/k_{65}$ . We glue this cycle into one point,  $A_2^2$ . The new transformed system is very simple, it is just a two step cycle:  $A_1 \xrightarrow{1} A_2^2 \xrightarrow{7} A_1$ . The new reaction constant is  $k_{12}^{(2)} = k_{13}\kappa_4^{(1)}/\kappa_3 = k_{13}k_{25}k_{46}/(k_{65}k_{43})$ . The auxiliary discrete dynamical system is the same graph  $A_1 \xrightarrow{1} A_2^2 \xrightarrow{7} A_1$ , this is a cycle, and we do not need further transformations.

Let us find the steady state on the way back, from this final auxiliary system to the original one. For steady state of each cycle we use formula (4).

The steady state for the final system is  $c_1 = bk_{12}^{(2)}/k_{21}$ ,  $c_2 = b(1 - k_{12}^{(2)}/k_{21})$ . The component  $A_2^2$  includes the cycle  $A_2 \xrightarrow{2} A_3 \xrightarrow{3} A_4^1 \xrightarrow{1} A_2$ . The steady state of this cycle is  $c_2 = c_2^{(2)} k_{24}^{(1)}/k_{32}$ ,  $c_3 = c_2^{(2)} k_{24}^{(1)}/k_{43}$ ,  $c_4^{(1)} = c_2^{(2)} (1 - k_{24}^{(1)}/k_{32} - k_{24}^{(1)}/k_{43})$ . The component  $A_4^1$  includes the cycle  $A_4 \xrightarrow{4} A_5 \xrightarrow{5} A_6 \xrightarrow{6} A_4$ . The steady state of this cycle is  $c_4 = c_4^{(1)} k_{46}/k_{54}$ ,  $c_5 = c_4^{(1)} k_{46}/k_{65}$ ,  $c_6 = c_4^{(1)} (1 - k_{46}/k_{54} - k_{46}/k_{65})$ .

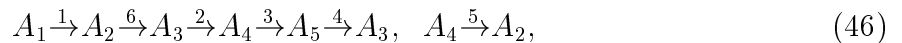
For one catalytic cycle, relaxation in the subspace  $\sum_i c_i = 0$  is approximated by relaxation of a chain that is produced from the cycle by cutting the limiting step (Sec. 2). For reaction networks under consideration (with one cyclic attractor in auxiliary discrete dynamical system) the direct generalization works: for approximation of relaxation in the subspace  $\sum_i c_i = 0$  it is sufficient to perform the following procedures:

- To glue iteratively attractors (cycles) of the auxiliary system that are not sinks of the whole system;
- To restore these cycles from the end of the first procedure to its beginning. For each of cycles (including the last one that is a sink) the limiting step should be deleted, and the outgoing reaction should be recharged to the head of the limiting steps (with the proper normalization), if it was not deleted before as a limiting step of one of the cycles.

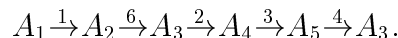
The heads of outgoing reactions of that cycles should be recharged to the heads of the limiting steps. Let for a cycle this limiting step be  $A_m \rightarrow A_q$ . If for a glued cycle  $A^k$  there exists an outgoing reaction  $A^k \rightarrow A_j$  with the constant  $\kappa$  (43), then after restoration we add the outgoing reaction  $A_m \rightarrow A_j$  with the rate constant  $\kappa$ . Kinetic of the resulting acyclic system approximates relaxation of the initial networks (under assumption of well separated constants, for given ordering, with probability close to 1).

Let us construct this acyclic network for the same example (44). The final cycle is  $A_1 \xrightarrow{1} A_2^2 \xrightarrow{2} A_1$ . The limiting step in this cycle is  $A_2^2 \xrightarrow{2} A_1$ . After cutting we get  $A_1 \xrightarrow{1} A_2^2$ . The component  $A_2^2$  is glued cycle  $A_2 \xrightarrow{2} A_3 \xrightarrow{3} A_4^1 \xrightarrow{1} A_2$ . The reaction  $A_1 \xrightarrow{1} A_2^2$  corresponds to the reaction  $A_1 \xrightarrow{1} A_2$  (in this case, this is the only reaction from  $A_1$  to cycle; in other case one should take the reaction from  $A_1$  to cycle with maximal constant). The limiting step in the cycle is  $A_4^1 \xrightarrow{1} A_2$ . After cutting, we get a system  $A_1 \xrightarrow{1} A_2 \xrightarrow{2} A_3 \xrightarrow{3} A_4^1$ . The component  $A_4^1$  is the glued cycle  $A_4 \xrightarrow{4} A_5 \xrightarrow{5} A_6 \xrightarrow{6} A_4$  from the previous step. The limiting step in this cycle is  $A_6 \xrightarrow{6} A_4$ . After restoring this cycle and cutting the limiting step, we get an acyclic system  $A_1 \xrightarrow{1} A_2 \xrightarrow{2} A_3 \xrightarrow{3} A_4 \xrightarrow{4} A_5 \xrightarrow{5} A_6$  (as one can guess from the beginning: this coincidence is provided by the simple constant ordering selected in (44)). Relaxation of this system approximates relaxation of the whole initial network.

To demonstrate possible branching of described algorithm for cycles surgery (gluing, restoring and cutting) with necessity of additional orderings, let us consider the following system:



The auxiliary discrete dynamical system for reaction network (46) is



It has only one attractor, a cycle  $A_3 \xrightarrow{2} A_4 \xrightarrow{3} A_5 \xrightarrow{4} A_3$ . This cycle is not a sink for the whole network (46) because reaction  $A_4 \xrightarrow{5} A_2$  leads from that cycle. After gluing the cycle into a vertex  $A_3^1$  we get the new network  $A_1 \xrightarrow{1} A_2 \xrightarrow{6} A_3^1 \xrightarrow{2} A_2$ . The rate constant for the reaction  $A_3^1 \rightarrow A_2$  is  $k_{23}^1 = k_{24}k_{35}/k_{54}$ , where  $k_{ij}$  is the rate constant for the reaction  $A_j \rightarrow A_i$  in the initial network ( $k_{35}$  is the cycle limiting reaction). The new network coincides with its auxiliary system and has one cycle,  $A_2 \xrightarrow{6} A_3^1 \xrightarrow{2} A_2$ . This cycle is a sink, hence, we can start the back process of cycles restoring and cutting. One question arises immediately: which constant is smaller,  $k_{32}$  or  $k_{23}^1$ . The smallest of them is the limiting constant, and the answer depends on this choice. Let us consider two possibilities separately: (1)  $k_{32} > k_{23}^1$  and (2)  $k_{32} < k_{23}^1$ . Of course, for any choice the stationary concentration of the source component  $A_1$  vanishes:  $c_1 = 0$ .

(1) Let us assume that  $k_{32} > k_{23}^1$ . In this case, the steady state of the cycle  $A_2 \xrightarrow{6} A_3^1 \xrightarrow{2} A_2$  is (according to (4))  $c_2 = bk_{23}^1/k_{32}$ ,  $c_3 = b(1 - k_{23}^1/k_{32})$ , where  $b = \sum c_i$ . The component  $A_3^1$  is a glued cycle  $A_3 \xrightarrow{2} A_4 \xrightarrow{3} A_5 \xrightarrow{4} A_3$ . Its steady state is  $c_3 = c_3^1 k_{35}/k_{43}$ ,  $c_4 = c_3^1 k_{35}/k_{54}$ ,  $c_5 = c_3^1(1 - k_{35}/k_{43} - k_{35}/k_{54})$ .

Let us construct an acyclic system that approximates relaxation of (46) under the same assumption (1)  $k_{32} > k_{23}^1$ . The final auxiliary system after gluing cycles is  $A_1 \xrightarrow{1} A_2 \xrightarrow{6} A_3^1 \xrightarrow{2} A_2$ . Let us delete the limiting reaction  $A_3^1 \xrightarrow{2} A_2$  from the cycle. We get an acyclic system  $A_1 \xrightarrow{1} A_2 \xrightarrow{6} A_3^1$ . The component  $A_3^1$  is the glued cycle  $A_3 \xrightarrow{2} A_4 \xrightarrow{3} A_5 \xrightarrow{4} A_3$ . Let us restore this cycle and delete the limiting reaction  $A_5 \xrightarrow{4} A_3$ . We get an acyclic system  $A_1 \xrightarrow{1} A_2 \xrightarrow{6} A_3 \xrightarrow{2} A_4 \xrightarrow{3} A_5$ . Relaxation of this system approximates relaxation of the initial network (46) under additional condition  $k_{32} > k_{23}^1$ .

(2) Let us assume now that  $k_{32} < k_{23}^1$ . In this case, the steady state of the cycle  $A_2 \xrightarrow{6} A_3^1 \xrightarrow{2} A_2$  is (according to (4))  $c_2 = b(1 - k_{32}/k_{23}^1)$ ,  $c_3 = bk_{32}/k_{23}^1$ . The further analysis is the same as it was above:  $c_3 = c_3^1 k_{35}/k_{43}$ ,  $c_4 = c_3^1 k_{35}/k_{54}$ ,  $c_5 = c_3^1(1 - k_{35}/k_{43} - k_{35}/k_{54})$  (with another  $c_3^1$ ).

Let us construct an acyclic system that approximates relaxation of (46) under assumption (2)  $k_{32} < k_{23}^1$ . The final auxiliary system after gluing cycles is the same,  $A_1 \xrightarrow{1} A_2 \xrightarrow{6} A_3^1 \xrightarrow{2} A_2$ , but the limiting step in the cycle is different,  $A_2 \xrightarrow{6} A_3^1$ . After cutting this step, we get acyclic system  $A_1 \xrightarrow{1} A_2 \xrightarrow{6} A_3^1$ , where the last reaction has rate constant  $k_{23}^1$ .

The component  $A_3^1$  is the glued cycle  $A_3 \xrightarrow{2} A_4 \xrightarrow{3} A_5 \xrightarrow{4} A_3$ . Let us restore this cycle and delete the limiting reaction  $A_5 \xrightarrow{4} A_3$ . The connection from glued cycle  $A_3^1 \xrightarrow{2} A_2$  with constant  $k_{23}^1$  transforms into connection  $A_5 \xrightarrow{2} A_2$  with the same constant  $k_{23}^1$ .

We get the acyclic system:  $A_1 \xrightarrow{1} A_2$ ,  $A_3 \xrightarrow{2} A_4 \xrightarrow{3} A_5 \xrightarrow{2} A_2$ . The order of constants is now known:  $k_{21} > k_{43} > k_{54} > k_{23}^1$ , and we can substitute the sign “?” by “4”:  $A_3 \xrightarrow{2} A_4 \xrightarrow{3} A_5 \xrightarrow{4} A_2$ .

For both cases,  $k_{32} > k_{23}^1$  ( $k_{23}^1 = k_{24}k_{35}/k_{54}$ ) and  $k_{32} < k_{23}^1$  it is easy to find the eigenvectors explicitly and to write the solution to the kinetic equations in explicit form.

### 4.3 Cycles surgery for auxiliary discrete dynamical system with arbitrary family of attractors

In this subsection, we summarize results of relaxation analysis and describe the algorithm of approximation of steady state and relaxation process for arbitrary reaction network with well separated constants.

#### 4.3.1 Hierarchy of cycles gluing

Let us consider a reaction network  $\mathcal{W}$  with a given structure and fixed ordering of constants. The set of vertices of  $\mathcal{W}$  is  $\mathcal{A}$  and the set of elementary reactions is  $\mathcal{R}$ . Each reaction from  $\mathcal{R}$  has the form  $A_i \rightarrow A_j$ ,  $A_i, A_j \in \mathcal{A}$ . The correspondent constant is  $k_{ji}$ . For each  $A_i \in \mathcal{A}$  we define  $\kappa_i = \max_j \{k_{ji}\}$  and  $\phi(i) = \arg \max_j \{k_{ji}\}$ . In addition,  $\phi(i) = i$  if  $k_{ji} = 0$  for all  $j$ .

The auxiliary discrete dynamical system for the reaction network  $\mathcal{W}$  is the dynamical system  $\Phi = \Phi_{\mathcal{W}}$  defined by the map  $\phi$  on the set  $\mathcal{A}$ . Auxiliary reaction network  $\mathcal{V} = \mathcal{V}_{\mathcal{W}}$  has the same set of vertices  $\mathcal{A}$  and the set of reactions  $A_i \rightarrow A_{\phi(i)}$  with reaction constants  $\kappa_i$ . Auxiliary kinetics is described by  $\dot{c} = \tilde{K}c$ , where  $\tilde{K}_{ij} = -\kappa_j \delta_{ij} + \kappa_j \delta_{i\phi(j)}$ .

Every fixed point of  $\Phi_{\mathcal{W}}$  is also a sink for the reaction network  $\mathcal{W}$ . If all attractors of the system  $\Phi_{\mathcal{W}}$  are fixed points  $A_{f1}, A_{f2}, \dots \in \mathcal{A}$  then the set of stationary distributions for the initial kinetics as well as for the auxiliary kinetics is the set of distributions concentrated the set of fixed points  $\{A_{f1}, A_{f2}, \dots\}$ . In this case, the auxiliary reaction network is acyclic, and the auxiliary kinetics approximates relaxation of the whole network  $\mathcal{W}$ .

In general case, let the system  $\Phi_{\mathcal{W}}$  have several attractors that are not fixed points, but cycles  $C_1, C_2, \dots$  with periods  $\tau_1, \tau_2, \dots > 1$ . By gluing these cycles in points, we transform the reaction network  $\mathcal{W}$  into  $\mathcal{W}^1$ . The dynamical system  $\Phi_{\mathcal{W}}$  is transformed into  $\Phi^1$ . For these new system and network, the connection  $\Phi^1 = \Phi_{\mathcal{W}^1}$  persists:  $\Phi^1$  is the auxiliary discrete dynamical system for  $\mathcal{W}^1$ .

For each cycle,  $C_i$ , we introduce a new vertex  $A_i$ . The new set of vertices,  $\mathcal{A}^1 = \mathcal{A} \cup \{A^1, A^2, \dots\} \setminus (\cup_i C_i)$  (we delete cycles  $C_i$  and add vertices  $A_i$ ).

All the reaction between  $A \rightarrow B$  ( $A, B \in \mathcal{A}$ ) can be separated into 5 groups:

- (1) both  $A, B \notin \cup_i C_i$ ;
- (2)  $A \notin \cup_i C_i$ , but  $B \in C_i$ ;
- (3)  $A \in C_i$ , but  $B \notin \cup_i C_i$ ;
- (4)  $A \in C_i, B \in C_j, i \neq j$ ;
- (5)  $A, B \in C_i$ .

Reactions from the first group do not change. Reaction from the second group transforms into  $A \rightarrow A^i$  (to the whole glued cycle) with the same constant. Reaction of the third type changes into  $A^i \rightarrow B$  with the rate constant renormalization (42): let the cycle  $C^i$  be the following sequence of reactions  $A_1 \rightarrow A_2 \rightarrow \dots \rightarrow A_{\tau_i} \rightarrow A_1$ , and the reaction rate



constant for  $A_i \rightarrow A_{i+1}$  is  $k_i$  ( $k_{\tau_i}$  for  $A_{\tau_i} \rightarrow A_1$ ). For the limiting reaction of the cycle  $C_i$  we use notation  $k_{\text{lim } i}$ . If  $A = A_j$  and  $k$  is the rate reaction for  $A \rightarrow B$ , then the new reaction  $A^i \rightarrow B$  has the rate constant  $kk_{\text{lim } i}/k_j$ . This corresponds to a quasistationary distribution on the cycle (4). The same constant renormalization is necessary for reactions of the fourth type. These reactions transform into  $A^i \rightarrow A^j$ . Finally, reactions of the fifth type vanish.

After we glue all the cycles of auxiliary dynamical system in the reaction network  $\mathcal{W}$ , we get  $\mathcal{W}^1$ . Strictly speaking, the whole network  $\mathcal{W}^1$  is not necessary, and in efficient realization of the algorithm for large networks the computation could be significantly reduced. What we need, is the correspondent auxiliary dynamical system  $\Phi^1 = \Phi_{\mathcal{W}^1}$  with auxiliary kinetics.

To find the auxiliary kinetic system, we should glue all cycles in the first auxiliary system, and then add several reactions: for each  $A^i$  it is necessary to find in  $\mathcal{W}^1$  the reaction of the form  $A^i \rightarrow B$  with maximal constant and add this reaction to the auxiliary network. If there is no reaction of the form  $A^i \rightarrow B$  for given  $i$  then the point  $A^i$  is the fixed point for  $\mathcal{W}^1$  and vertices of the cycle  $C_i$  form a sink for the initial network.

After that, we decompose the new auxiliary dynamical system, find cycles and repeat gluing. Terminate when all attractors of the auxiliary dynamical system  $\Phi^m$  become fixed points.

#### 4.3.2 Reconstruction of steady states

After this termination, we can find all steady state distributions by restoring cycles in the auxiliary reaction network  $\mathcal{V}^m$ . Let  $A_{f_1}^m, A_{f_2}^m, \dots$  be fixed points of  $\Phi^m$ . The set of steady states for  $\mathcal{V}^m$  is the set of all distributions on the set of fixed points  $\{A_{f_1}^m, A_{f_2}^m, \dots\}$ . Let us take one of these distributions,  $c = (c_{f_1}^m, c_{f_2}^m, \dots)$  (we mark the concentrations by the same indexes as the vertex has; other  $c_i = 0$ ).

To make a step of cycle restoration we select those vertexes  $A_{f_i}^m$  that are glued cycles and substitute them in the list  $A_{f_1}^m, A_{f_2}^m, \dots$  by all the vertices of these cycles. For each of those cycles we find the limiting rate constant and redistribute the concentration  $c_{f_i}^m$  between the vertices of the correspondent cycle by the rule (4) (with  $b = c_{f_i}^m$ ). As a result, we get a set of vertices and a distribution on this set of vertices. If among these vertices there are glued cycles, then we repeat the procedure of cycle restoration. Terminate when there is no glued cycles in the support of the distribution. The resulting distribution is the approximation to a steady state of  $\mathcal{W}$ , and all steady states for  $\mathcal{W}$  can be approximated by this method.

In order to construct the approximation to the basis of stationary distributions of  $\mathcal{W}$ , it is sufficient to apply the described algorithm to distributions concentrated on a single fixed point  $A_{f_i}^m, c_{f_j}^m = \delta_{ij}$ , for every  $i$ .

The steady state approximation on the base of the rule (4) has first order in the limiting constants. The zero order approximation also makes sense. For one cycle is gives (5):

all the concentration is collected at the start of the limiting step. The algorithm for the zero order approximation is even simpler than for the first order. Let us start from the distributions concentrated on a single fixed point  $A_{f_i}^m$ ,  $c_{f_j}^m = \delta_{ij}$  for some  $i$ . If this point is a glued cycle then restore that cycle, and find the limiting step. The new distribution is concentrated at the starting vertex of that step. If this vertex is a glued cycle, then repeat. If it is not then terminate. As a result we get a distribution concentrated in one vertex of  $\mathcal{A}$ .

### 4.3.3 Dominant kinetic system for approximation of relaxation

To construct an approximation to the relaxation process in the reaction network  $\mathcal{W}$ , we also need to restore cycles, but for this purpose we should start from the whole glued network network  $\mathcal{V}^m$  on  $\mathcal{A}^m$  (not only from fixed points as we did for the steady state approximation). On a step back, from the set  $\mathcal{A}^m$  to  $\mathcal{A}^{m-1}$  and so on some of glued cycles should be restored and cut. On each step we build an acyclic reaction network, the final network is defined on the initial vertex set and approximates relaxation of  $\mathcal{W}$ .

To make one step back from  $\mathcal{V}^m$  let us select the vertices of  $\mathcal{A}^m$  that are glued cycles from  $\mathcal{V}^{m-1}$ . Let these vertices be  $A_1^m, A_2^m, \dots$ . Each  $A_i^m$  corresponds to a glued cycle from  $\mathcal{V}^{m-1}$ ,  $A_{i1}^{m-1} \rightarrow A_{i2}^{m-1} \rightarrow \dots A_{i\tau_i}^{m-1} \rightarrow A_{i1}^{m-1}$ , of the length  $\tau_i$ . We assume that the limiting steps in these cycles are  $A_{i\tau_i}^{m-1} \rightarrow A_{i1}^{m-1}$ . Let us substitute each vertex  $A_i^m$  in  $\mathcal{V}^m$  by  $d_i$  vertices  $A_{i1}^{m-1}, A_{i2}^{m-1}, \dots A_{i\tau_i}^{m-1}$  and add to  $\mathcal{V}^m$  reactions  $A_{i1}^{m-1} \rightarrow A_{i2}^{m-1} \rightarrow \dots A_{i\tau_i}^{m-1}$  (that are the cycle reactions without the limiting step) with correspondent constants from  $\mathcal{V}^{m-1}$ .

If there exists an outgoing reaction  $A_i^m \rightarrow B$  in  $\mathcal{V}^m$  then we substitute it by the reaction  $A_{i\tau_i}^{m-1} \rightarrow B$  with the same constant, i.e. outgoing reactions  $A_i^m \rightarrow \dots$  are reattached to the heads of the limiting steps. Let us rearrange reactions from  $\mathcal{V}^m$  of the form  $B \rightarrow A_i^m$ . These reactions have prototypes in  $\mathcal{V}^{m-1}$  (before the last gluing). We simply restore these reactions. If there exists a reaction  $A_i^m \rightarrow A_j^m$  then we find the prototype in  $\mathcal{V}^{m-1}$ ,  $A \rightarrow B$ , and substitute the reaction by  $A_{i\tau_i}^{m-1} \rightarrow B$  with the same constant, as for  $A_i^m \rightarrow A_j^m$ .

After that step is performed, the vertices set is  $\mathcal{A}^{m-1}$ , but the reaction set differs from the reactions of the network  $\mathcal{V}^{m-1}$ : the limiting steps of cycles are excluded and the outgoing reactions of glued cycles are included (reattached to the heads of the limiting steps). To make the next step, we select vertices of  $\mathcal{A}^{m-1}$  that are glued cycles from  $\mathcal{V}^{m-2}$ , substitute these vertices by vertices of cycles, delete the limiting steps, attach outgoing reactions to the heads of the limiting steps, and for incoming reactions restore their prototypes from  $\mathcal{V}^{m-2}$ , and so on.

After all, we restore all the glued cycles, and construct an acyclic reaction network on the set  $\mathcal{A}$ . This acyclic network approximates relaxation of the network  $\mathcal{W}$ . We call this system the dominant system of  $\mathcal{W}$  and use notation  $\text{dom mod}(\mathcal{W})$ .

#### 4.3.4 Zero-one law for nonergodic multiscale networks

The fixed points  $A_{fi}^m$  of the discrete dynamical system  $\Phi^m$  are the glued ergodic components  $G_i \subset \mathcal{A}$  of the initial network  $\mathcal{W}$ . At the same time, these points are attractors of  $\Phi^m$ . Let us consider the correspondent decomposition of this system with partition  $\mathcal{A}^m = \cup_i Att(A_{fi}^m)$ . In the cycle restoration during construction of dominant system  $\text{dom mod}(\mathcal{W})$  this partition transforms into partition of  $\mathcal{A}$ :  $\mathcal{A} = \cup_i U_i$ ,  $Att(A_{fi}^m)$  transforms into  $U_i$  and  $G_i \subset U_i$  (and  $U_i$  transforms into  $Att(A_{fi}^m)$  in hierarchical gluing of cycles).

It is straightforward to see that during construction of dominant systems for  $\mathcal{W}$  from the network  $\mathcal{V}^m$  no connection between  $U_i$  are created. Therefore, the reaction network  $\text{dom mod}(\mathcal{W})$  is a union of networks on sets  $U_i$  without any link between sets.

If  $G_1, \dots, G_m$  are all ergodic components of the system, then there exist  $m$  independent positive linear functionals  $b_1(c), \dots, b_m(c)$  that describe asymptotical behaviour of kinetic system when  $t \rightarrow \infty$  (26). For  $\text{dom mod}(\mathcal{W})$  these functionals are:  $b_l(c) = \sum_{A \in U_i} c_A$  where  $c_A$  is concentration of  $A$ . Hence, for the initial reaction network  $\mathcal{W}$  with well separated constants

$$b_l(c) \approx \sum_{A \in U_i} c_A. \quad (47)$$

This is the zero-one law for multiscale networks: for any  $l, i$ , the value of functional  $b_l$  (26) on basis vector  $e^i$ ,  $b_l(e^i)$ , is either close to one or close to zero (with probability close to 1). We already mentioned this law in discussion of a simple example (27). The approximate equality (47) means that for each reagent  $A \in \mathcal{A}$  there exists such an ergodic component  $G$  of  $\mathcal{W}$  that  $A$  transforms when  $t \rightarrow \infty$  preferably into elements of  $G$  even if there exist paths from  $A$  to other ergodic components of  $\mathcal{W}$ .

#### 4.4 Example: a prism of reactions

Let us demonstrate work of the algorithm on a typical example, a prism of reaction that consists of two connected cycles (Fig. 1,2). Such systems appear in many areas of biophysics and biochemistry (see, for example, [49]).

For the first example we use the reaction rate constants ordering presented in Fig. 1a. For this ordering, the auxiliary dynamical system consists of two cycles (Fig. 1b) with the limiting constants  $k_{54}$  and  $k_{32}$ , correspondingly. These cycles are connected by four reaction (Fig. 1c). We glue the cycles into new components  $A_1^1$  and  $A_2^1$  (Fig. 1d), and the reaction network is transformed into  $A_1^1 \leftrightarrow A_2^1$ . Following the general rule ( $k^1 = k k_{\text{lim}}/k_j$ ), we determine the rate constants: for reaction  $A_1^1 \rightarrow A_2^1$

$$k_{21}^1 = \max\{k_{41}k_{32}/k_{21}, k_{52}, k_{63}k_{32}/k_{13}\},$$

and for reaction  $A_2^1 \rightarrow A_1^1$

$$k_{12}^1 = k_{36}k_{54}/k_{46}.$$

There are six possible orderings of the constant combinations: three possibilities for the choice of  $k_{21}^1$  and for each such a choice there exist two possibilities:  $k_{21}^1 > k_{12}^1$  or  $k_{21}^1 < k_{12}^1$ .

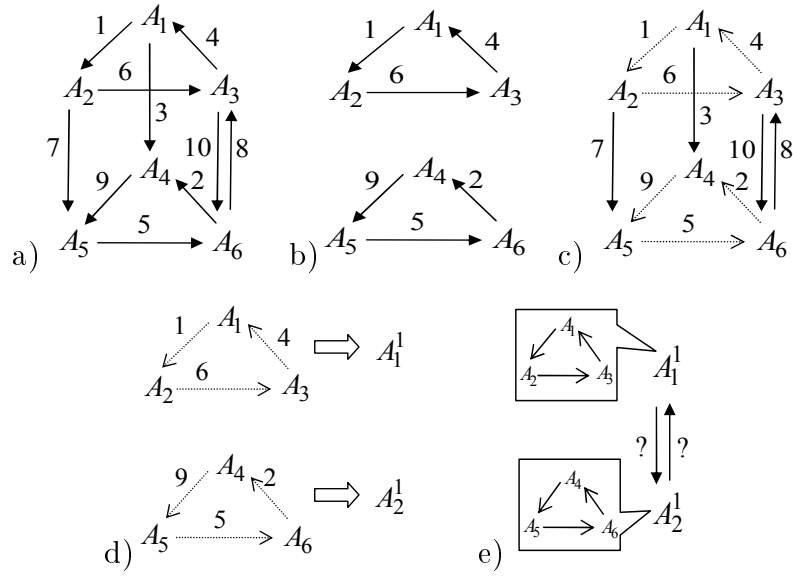


Fig. 1. Gluing of cycles for the prism of reactions with a given ordering of rate constants in the case of two attractors in the auxiliary dynamical system: (a) initial reaction network, (b) auxiliary dynamical system that consists of two cycles, (c) connection between cycles, (d) gluing cycles into new components, (e) network  $\mathcal{W}^1$  with glued vertices.

The zero order approximation of the steady state depends only on the sign of inequality between  $k_{21}^1$  and  $k_{12}^1$ . If  $k_{21}^1 \gg k_{12}^1$  then almost all concentration in the steady state is accumulated inside  $A_2^1$ . After restoring the cycle  $A_4 \rightarrow A_5 \rightarrow A_6 \rightarrow A_4$  we find that in the steady state almost all concentration is accumulated in  $A_4$  (the component at the beginning of the limiting step of this cycle,  $A_4 \rightarrow A_5$ ). Finally, the eigenvector for zero eigenvalue is estimated as the vector column with coordinates  $(0, 0, 0, 1, 0, 0)$ .

If, inverse,  $k_{21}^1 \ll k_{12}^1$  then almost all concentration in the steady state is accumulated inside  $A_1^1$ . After restoring the cycle  $A_1 \rightarrow A_2 \rightarrow A_3 \rightarrow A_1$  we find that in the steady state almost all concentration is accumulated in  $A_2$  (the component at the beginning of the limiting step of this cycle,  $A_2 \rightarrow A_3$ ). Finally, the eigenvector for zero eigenvalue is estimated as the vector column with coordinates  $(0, 1, 0, 0, 0, 0)$ .

For analysis of relaxation, let us analyze one of the six particular cases separately.

1.  $k_{21}^1 = k_{41}k_{32}/k_{21}$  and  $k_{21}^1 > k_{12}^1$ .

In this case, the finite acyclic auxiliary dynamical system,  $\Phi^m = \Phi^1$ , is  $A_1^1 \rightarrow A_2^1$ , and  $\mathcal{W}^1$  is  $A_1^1 \leftrightarrow A_2^1$ . We restore both cycles and delete the limiting reactions  $A_2 \rightarrow A_3$  and  $A_4 \rightarrow A_5$ . This is the common step for all cases. Following the general procedure, we substitute the reaction  $A_1^1 \rightarrow A_2^1$  by  $A_2 \rightarrow A_4$  with the rate constant  $k_{21}^1 = k_{41}k_{32}/k_{21}$  (because  $A_2$  is the head of the limiting step for the cycle  $A_1 \rightarrow A_2 \rightarrow A_3 \rightarrow A_1$ , and the prototype of the reaction  $A_1^1 \rightarrow A_2^1$  is in that case  $A_1 \rightarrow A_4$ ).

We find the approximate system for relaxation description: reactions  $A_3 \rightarrow A_1 \rightarrow A_2$  and  $A_5 \rightarrow A_6 \rightarrow A_4$  with original constants, and reaction  $A_2 \rightarrow A_4$  with the rate constant  $k_{21}^1 = k_{41}k_{32}/k_{21}$ .

This system graph is acyclic and, moreover, represents a discrete dynamical system, as it should be (not more than one outgoing reaction for any component). Therefore, we can estimate the eigenvalues and eigenvectors on the base of formulas (31), (33). It is easy to determine the order of constants because  $k_{21}^1 = k_{41}k_{32}/k_{21}$ : this constant is the smallest nonzero constant in the obtained acyclic system. Finally, we have the following ordering of constants:  $A_3 \xrightarrow{3} A_1 \xrightarrow{1} A_2 \xrightarrow{5} A_4$ ,  $A_5 \xrightarrow{4} A_6 \xrightarrow{2} A_4$ .

So, the eigenvalues of the prism of reaction for the given ordering are (with high accuracy, with probability close to one)  $-k_{21} < -k_{46} < -k_{13} < -k_{65} < -k_{41}k_{32}/k_{21}$ . The relaxation time is  $\tau = k_{21}/(k_{41}k_{32})$ .

We use the same notations as in previous sections: eigenvectors  $l^i$  and  $r^i$  correspond to the eigenvalue  $-\kappa_i$ , where  $\kappa_i$  is the reaction rate constant for the reaction  $A_i \rightarrow \dots$ . The left eigenvectors  $l^i$  are:

$$\begin{aligned} l^1 &\approx (1, 0, 0, 0, 0, 0), & l^2 &\approx (1, 1, 1, 0, 0, 0), & l^3 &\approx (0, 0, 1, 0, 0, 0), \\ l^4 &\approx (1, 1, 1, 1, 1, 1), & l^5 &\approx (0, 0, 0, 0, 1, 0), & l^6 &\approx (0, 0, 0, 0, 0, 1). \end{aligned} \quad (48)$$

The right eigenvectors  $r^i$  are (we represent vector columns as rows):

$$\begin{aligned} r^1 &\approx (1, -1, 0, 0, 0, 0), & r^2 &\approx (0, 1, 0, -1, 0, 0), & r^3 &\approx (0, -1, 1, 0, 0, 0), \\ r^4 &\approx (0, 0, 0, 1, 0, 0), & r^5 &\approx (0, 0, 0, -1, 1, 0), & r^6 &\approx (0, 0, 0, -1, 0, 1) \end{aligned} \quad (49)$$

The vertex  $A_4$  is the fixed point for the discrete dynamical system. There is no reaction  $A_4 \rightarrow \dots$ . For convenience, we include the eigenvectors  $l^4$  and  $r^4$  for zero eigenvalue,  $\kappa_4 = 0$ . These vectors correspond to the steady state:  $r^4$  is the steady state vector, and the functional  $l^4$  is the conservation law.

The correspondent approximation to the general solution of the kinetic equation for the prism of reaction (Fig. 1a) is:

$$c(t) = \sum_{i=1}^6 r^i(l^i, c(0)) \exp(-\kappa_i t). \quad (50)$$

Analysis of other five particular cases is similar. Of course, some of the eigenvectors and eigenvalues can differ.

The first order in constants ratios approximation for the steady state is described above as application of the rule (4) for restoring cycles. The first order approximation for eigenvectors is presented in Appendix 1.

Of course, different ordering can lead to very different approximations. For example, let us consider the same prism of reactions, but with the ordering of constants presented in Fig. 2a. The auxiliary dynamical system has one cycle (Fig. 2b) with the limiting constant  $k_{36}$ . This cycle is not a sink to the initial network, there are outgoing reactions from its vertices (Fig. 2c). After gluing, this cycles transforms into a vertex  $A_1^1$  (Fig. 2d). The glued network,  $\mathcal{W}^1$  (Fig. 2e), has two vertices,  $A_4$  and  $A_1^1$  the rate constant for the reaction  $A_4 \rightarrow A_1^1$  is  $k_{54}$ , and the rate constant for the reaction  $A_1^1 \rightarrow A_4$  is  $k^1 = \max\{k_{41}k_{36}/k_{21}, k_{46}\}$ . Hence, there are not more than four possible versions: two possibilities for the choice of

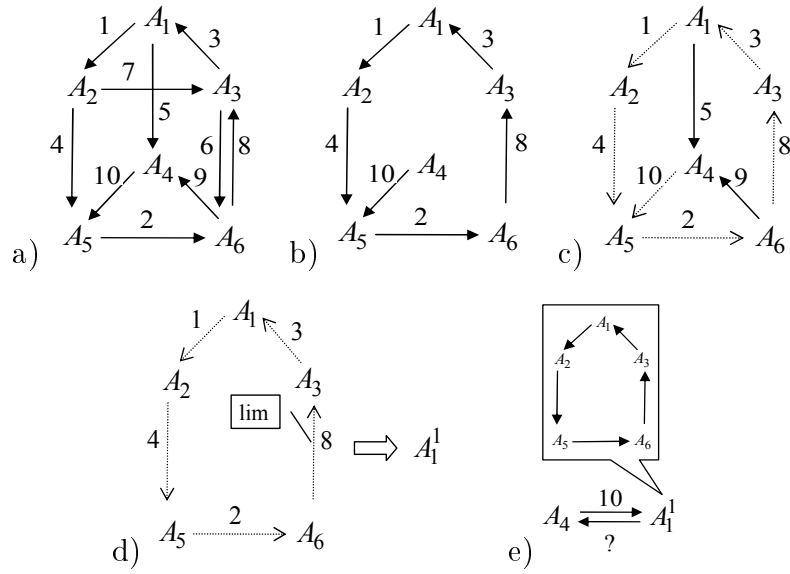


Fig. 2. Gluing of a cycle for the prism of reactions with a given ordering of rate constants in the case of one attractors in the auxiliary dynamical system: (a) initial reaction network, (b) auxiliary dynamical system that has one attractor, (c) outgoing reactions from a cycle, (d) gluing of a cycle into new component, (e) network  $\mathcal{W}^1$  with glued vertices.

$k^1$  and for each such a choice there exist two possibilities:  $k^1 > k_{54}$  or  $k^1 < k_{54}$  (one of them is impossible, because  $k_{46} > k_{54}$ ).

Exactly as it was in the previous example, the zero order approximation of the steady state depends only on the sign of inequality between  $k^1$  and  $k_{54}$ . If  $k^1 \gg k_{54}$  then almost all concentration in the steady state is accumulated inside  $A^1$ . After restoring the cycle  $A_3 \rightarrow A_1 \rightarrow A_2 \rightarrow A_5 \rightarrow A_6 \rightarrow A_3$  we find that in the steady state almost all concentration is accumulated in  $A_6$  (the component at the beginning of the limiting step of this cycle,  $A_6 \rightarrow A_3$ ). The eigenvector for zero eigenvalue is estimated as the vector column with coordinates  $(0, 0, 0, 0, 0, 1)$ .

If  $k^1 \gg k_{54}$  then almost all concentration in the steady state is accumulated inside  $A^4$ . This vertex is not a glued cycle, and immediately we find the approximate eigenvector for zero eigenvalue, the vector column with coordinates  $(0, 0, 0, 1, 0, 0)$ .

Let us analyze the relaxation process for one of the possibilities:  $k^1 = k_{46}$ , and, therefore  $k^1 > k_{54}$ . We restore the cycle, delete the limiting step, transform the reaction  $A_1^1 \rightarrow A_4$  into reaction  $A_6 \rightarrow A_4$  with the same constant  $k^1 = k_{46}$  and get the chain with ordered constants:  $A_3 \xrightarrow{3} A_1 \xrightarrow{1} A_2 \xrightarrow{4} A_5 \xrightarrow{2} A_6 \xrightarrow{5} A_4$ . Here the nonzero rate constants  $k_{ij}$  have the same value as for the initial system (Fig. 2a). Left eigenvectors are (including  $l^4$  for the zero eigenvalue):

$$\begin{aligned}
 l^1 &\approx (1, 0, 0, 0, 0, 0), \quad l^2 \approx (1, 1, 1, 0, 0, 0), \quad l^3 \approx (0, 0, 1, 0, 0, 0); \\
 l^4 &\approx (1, 1, 1, 1, 1, 1), \quad l^5 \approx (0, 0, 0, 0, 1, 0), \quad l^6 \approx (1, 1, 1, 0, 1, 1).
 \end{aligned} \tag{51}$$

Right eigenvectors are (including  $r^4$  for the zero eigenvalue):

$$\begin{aligned} r^1 &\approx (1, -1, 0, 0, 0, 0), & r^2 &\approx (0, 1, 0, 0, 0, -1), & r^3 &\approx (0, -1, 1, 0, 0, 0); \\ r^4 &\approx (0, 0, 0, 1, 0, 0), & r^5 &\approx (0, 0, 0, 0, 1, -1), & r^6 &\approx (0, 0, 0, -1, 0, 1), \end{aligned} \quad (52)$$

Here we represent vector columns as rows.

For the approximation of relaxation in that order we can use (50).

## 5 Limitation in modular structure and solvable modules

### 5.1 Modular limitation

The simplest one-constant limitation concept cannot be applied to all systems. There is another very simple case based on exclusion of “fast equilibria”  $A_i \rightleftharpoons A_j$ . In this limit, the ratio of reaction constants  $K_{ij} = k_{ij}/k_{ji}$  is bounded,  $0 < a < K_{ij} < b < \infty$ , but for different pairs  $(i, j)$ ,  $(l, s)$  one of the inequalities  $k_{ij} \ll k_{ls}$  or  $k_{ij} \gg k_{ls}$  holds. (One usually calls these  $K$  “equilibrium constant”, even if there is no relevant thermodynamics.) W.J. Ray (Jr.) [9] discussed that case systematically for some real examples. Of course, it is possible to create the theory for that case very similarly to the theory presented above. This should be done, but it is worth to mention now that the limitation concept can be applied to *any* modular structure of reaction network. Let for the reaction network  $\mathcal{W}$  the set of elementary reactions  $\mathcal{R}$  is partitioned on some modules:  $\mathcal{R} = \cup_i \mathcal{R}_i$ . We can consider the related multiscale ensemble of reaction constants: let the ratio of any two rate constants inside each module be bounded (and separated from zero, of course), but the ratios between modules form a well separated ensemble. This can be formalized by multiplication of rate constants of each module  $\mathcal{R}_i$  on a time scale coefficient  $k_i$ . If we assume that  $\ln k_i$  are uniformly and independently distributed on a real line (or  $k_i$  are independently and log-uniformly distributed on a sufficiently large interval) then we come to the problem of modular limitation. The problem is quite general: describe the typical behavior of multiscale ensembles for systems with given modular structure: each module has its own time scale and these time scales are well separated.

Development of such a general theory is outside the scope of our paper, and here we just find building blocks for the future theory, *solvable reaction modules*. There may be many various criteria of selection the reaction modules. Here are several possible choices: individual reactions (we developed the theory of multiscale ensembles of individual reactions in this paper), couples of mutually inverse reactions, as we mentioned above, acyclic reaction networks, ... .

Among the possible reasons for selection the class of reaction mechanisms for this purpose, there is one formal, but important: the possibility to solve the kinetic equation for every module in explicit analytical (algebraic) form with quadratures. We call these systems “solvable”.

## 5.2 Solvable reaction mechanisms

Let us describe all solvable reaction systems (with mass action law), linear and nonlinear.

Formally, we call the set of reaction solvable, if there exists a linear transformation of coordinates  $c \mapsto a$  such that kinetic equation in new coordinates for all values of reaction constants has the triangle form:

$$\frac{da_i}{dt} = f_i(a_1, a_2, \dots, a_i). \quad (53)$$

This system has the lower triangle Jacobian matrix  $\partial \dot{a}_i / \partial a_j$ .

To construct the general mass action law system we need: the list of components,  $\mathcal{A} = \{A_1, \dots, A_n\}$  and the list of reactions (the reaction mechanism):



where  $r$  is the reaction number,  $\alpha_{ri}$ ,  $\beta_{rk}$  are nonnegative integers (stoichiometric coefficients). Formally, it is possible that all  $\beta_k = 0$  or all  $\alpha_i = 0$ . We allow such reactions. They can appear in reduced models or in auxiliary systems.

A real variable  $c_i$  is assigned to every component  $A_i$ ,  $c_i$  is the concentration of  $A_i$ ,  $c$  is the concentration vector with coordinates  $c_i$ . The reaction kinetic equations are

$$\frac{dc}{dt} = \sum_r \gamma_r w_r(c), \quad (55)$$

where  $\gamma_r$  is the reaction stoichiometric vector with coordinates  $\gamma_{ri} = \beta_{ri} - \alpha_{ri}$ ,  $w_r(c)$  is the reaction rate. For mass action law,

$$w_r(c) = k_r \prod_i c_i^{\alpha_{ri}}, \quad (56)$$

where  $k_r$  is the reaction constant.

Physically, equations (55) correspond to reactions in fixed volume, and in more general case a multiplier  $V$  (volume) is necessary:

$$\frac{d(Vc)}{dt} = V \sum_r \gamma_r w_r(c).$$

Here we study the systems (55) and postpone any further generalization.

The first example of solvable systems give the sets of reactions of the form





(components  $A_k$  on the right hand side have higher numbers  $k$  than the component  $A_i$  on the left hand side,  $i < k$ ). For these systems, kinetic equations (55) have the triangle form from the very beginning.

The second standard example gives the couple of mutually inverse reactions:

$$\sum_i \alpha_i A_i \rightleftharpoons \sum_k \beta_k A_k, \quad (58)$$

these reactions have stoichiometric vectors  $\pm\gamma$ ,  $\gamma_i = \beta_i - \alpha_i$ . The kinetic equation  $\dot{c} = (w^+ - w^-)\gamma$  has the triangle form (53) in any orthogonal coordinate system with the last coordinate  $a_n = (\gamma, c) = \sum_i \gamma_i c_i$ . Of course, if there are several reactions with proportional stoichiometric vectors, the kinetic equations have the triangle form in the same coordinate systems.

The general case of solvable systems is essentially a combination of that two (57), (58), with some generalization. Here we follow [50] and present an algorithm for analysis of reaction network solvability. First, we introduce a relation between reactions “ $r$ th reaction directly affects the rate of  $s$ th reaction” with notation  $r \rightarrow s$ :  $r \rightarrow s$  if there exists such  $A_i$  that  $\gamma_{ri}\alpha_{si} \neq 0$ . This means that concentration of  $A_i$  changes in the  $r$ th reaction ( $\gamma_{ri} \neq 0$ ) and the rate of the  $s$ th reaction depends on  $A_i$  concentration ( $\alpha_{si} \neq 0$ ). For that relation we use  $r \rightarrow s$ . For transitive closure of this relation we use notation  $r \succeq s$  (“ $r$ th reaction affects the rate of  $s$ th reaction”):  $r \succeq s$  if there exists such a sequence  $s_1, s_2, \dots, s_q$  that  $r \rightarrow s_1 \rightarrow s_2 \rightarrow \dots \rightarrow s_q \rightarrow s$ .

The *hanging component* of the reaction network  $\mathcal{W}$  is such  $A_i \in \mathcal{A}$  that for all reactions  $\alpha_{ri} = 0$ . This means that all reaction rates do not depend on concentration of  $A_i$ . The *hanging reaction* is such element of  $\mathcal{R}$  with number  $r$  that  $r \succeq s$  only if  $\gamma_s = \lambda\gamma_r$  for some number  $\lambda$ . An example of hanging components gives the last component  $A_n$  for the triangle network (57). An example of hanging reactions gives a couple of reactions (58) if they do not affect any other reaction.

In order to check solvability of the reaction network  $\mathcal{W}$  we should find all hanging components and reactions and delete them from  $\mathcal{A}$  and  $\mathcal{R}$ , correspondingly. After that, we get a new system,  $\mathcal{W}_1$  with the component set  $\mathcal{A}_1$  and the reaction set  $\mathcal{R}_1$ . Next, we should find all hanging components and reactions for  $\mathcal{W}_1$  and delete them from  $\mathcal{A}_1$  and  $\mathcal{R}_1$ . Iterate until no hanging components or hanging reactions could be found. If the final set of components is empty, then the reaction network  $\mathcal{W}$  is solvable. If it is not empty, then  $\mathcal{W}$  is not solvable.

For example, let us consider the reaction mechanism with  $\mathcal{A} = \{A_1, A_2, A_3, A_4\}$  and reactions  $A_1 + A_2 \rightarrow 2A_3$ ,  $A_1 + A_2 \rightarrow A_3 + A_4$ ,  $A_3 \rightarrow A_4$ ,  $A_4 \rightarrow A_3$ . There are no hanging components, but two hanging reactions,  $A_3 \rightarrow A_4$  and  $A_4 \rightarrow A_3$ . After deletion of these two reactions, two hanging components appear,  $A_3$  and  $A_4$ . After deletion these two components, we get two hanging reactions,  $A_1 + A_2 \rightarrow 0$ ,  $A_1 + A_2 \rightarrow 0$  (they coincide). We delete these reactions and get two components  $A_1, A_2$  without reactions. After deletion these hanging components we obtain the empty system. The reaction network is solvable.

An oriented cycle of the length more than two is not solvable. For each number of ver-

tices one can calculate the set of all maximal solvable mechanisms. For example, for five components there are two maximal solvable mechanisms of monomolecular reactions:

- (1)  $A_1 \rightarrow A_2 \rightarrow A_4, A_1 \rightarrow A_4, A_1 \rightarrow A_3 \rightarrow A_5, A_1 \rightarrow A_5, A_4 \leftrightarrow A_5;$
- (2)  $A_1 \rightarrow A_2, A_1 \rightarrow A_3, A_1 \rightarrow A_4, A_1 \rightarrow A_5, A_2 \leftrightarrow A_3, A_4 \leftrightarrow A_5.$

It is straightforward to check solvability of these mechanism. The first mechanism has a couple of hanging reactions,  $A_4 \leftrightarrow A_5$ . After deletion of these reaction, the system becomes acyclic, of the form (57). The second mechanism has two couples of hanging reactions,  $A_2 \leftrightarrow A_3$  and  $A_4 \leftrightarrow A_5$ . After deletion of these reactions, the system also transforms into form (57). It is impossible to add any new monomolecular reactions between  $\{A_1, A_2, A_3, A_4, A_5\}$  to these mechanisms with preservation of solvability.

Finally, we should mention connections between solvable reaction networks and solvable Lie algebras [51,52]. Let us remind that matrices  $M_1, \dots, M_q$  generate a solvable Lie algebras if and only if they could be transformed simultaneously into a triangle form by a change of basis.

The Jacobian matrix for the mass action law kinetic equation (55) is:

$$J = \left( \frac{\partial c_i}{\partial c_j} \right) = \sum_r w_r J_r = \sum_{rj} \frac{w_r}{c_j} M_{rj}, \quad (59)$$

where

$$J_r = \gamma_r \alpha_r^\top \text{diag} \left\{ \frac{1}{c_1}, \frac{1}{c_2}, \dots, \frac{1}{c_n} \right\} = \sum_j \frac{1}{c_j} M_{rj}, \quad (60)$$

$$M_{rj} = \alpha_{rj} \gamma_r e^{j\top},$$

$\alpha_r^\top$  is the vector row  $(\alpha_{r1}, \dots, \alpha_{rn})$ ,  $e^{j\top}$  is the  $j$ th basis vector row with coordinates  $e_k^{j\top} = \delta_{jk}$ .

The Jacobian matrix (59) should have the lower triangle form in coordinates  $a_i$  (53) for all nonnegative values of rate constants and concentrations. This is equivalent to the lower triangle form of all matrices  $M_{rj}$  in these coordinates. Because usually there are many zero matrices among  $M_{rj}$ , it is convenient to describe the set of nonzero matrices.

For the  $r$ th reaction  $I_r = \{i \mid \alpha_{ri} \neq 0\}$ . The reaction rate  $w_r$  depends on  $c_i$  if and only if  $i \in I_r$ . For each  $i = 1, \dots, n$  we define a matrix

$$m_{ri} = \left[ 0, 0, \dots, \underbrace{\gamma_r}_i, \dots, 0 \right].$$

The  $i$ th column of this matrix coincides with the vector column  $\gamma_r$ . Other columns are equal to zero. For each  $r$  we define a set of matrices  $\mathcal{M}_r = \{m_{ri} \mid i \in I_r\}$ , and  $\mathcal{M} = \cup_r \mathcal{M}_r$ . The reaction network  $\mathcal{W}$  is solvable if and only if the finite set of matrices  $\mathcal{M}$  generates a solvable Lie algebra.

Classification of finite dimensional solvable Lie algebras remains a difficult problem [52].

It seems plausible that the classification of solvable algebras associated with reaction networks can bring new ideas into this field of algebra.

## 6 Conclusion: Concept of limit simplification in multiscale systems

In this paper, we study networks of linear reactions. For any ordering of reaction rate constants we look for the dominant kinetic system. The dominant system is, by definition, the system that gives us the main asymptotic terms of the stationary state and relaxation in the limit for well separated rate constants. In this limit any two constants are connected by relation  $\gg$  or  $\ll$ .

The topology of dominant systems is rather simple; they are those networks which are graphs of discrete dynamical systems on the set of vertices. In such graphs each vertex has no more than one outgoing reaction. This allows us to construct the explicit asymptotics of eigenvectors and eigenvalues. In the limit of well separated constants, the coordinates of eigenvectors for dominant systems can take only three values:  $\pm 1$  or  $0$ . All algorithms are represented topologically by transformation of the graph of reaction (labeled by reaction rate constants). We call these transformations “cycles surgery”, because the main operations are gluing cycles and cutting cycles in graphs of auxiliary discrete dynamical systems.

In the simplest case, the dominant system is determined by the ordering of constants. But for sufficiently complex systems we need to introduce auxiliary elementary reactions. They appear after cycle gluing and have monomial rate constants of the form  $k_\zeta = \prod_i k_i^{S_i}$ . The dominant system depends on the place of these monomial values among the ordered constants.

Construction of the dominant system clarifies the notion of limiting steps for relaxation. There is an exponential relaxation process that lasts much more than the others in (40), (50). This is the slowest relaxation and it is controlled by one reaction in the dominant system, the limiting step. The limiting step for relaxation is not the slowest reaction, or the second slowest reaction of the whole network, but the slowest reaction of the dominant system. That limiting step constant is not necessarily a reaction rate constant for the initial system, but can be represented by a monomial of such constants as well.

The idea of dominant subsystems in asymptotic analysis was proposed by Newton and developed by Kruskal [54]. A modern introduction with some historical review is presented in [55]. In our analysis we do not use the degrees of small parameters (as it was done in [27–29,54,55]), but operate directly with the rate constants orderings.

To develop the idea of systems with well separated constants to the state of a mathematical notion, we introduce multiscale ensembles of constant tuples. This notion allows us to discuss rigorously uniform distributions on infinite space and gives the answers to a question: what does it mean “to pick a multiscale system at random”.

Now we have the complete theory and the exhaustive construction of algorithms for linear

reaction networks with well separated rate constants. There are several ways of using the developed theory and algorithms:

- (1) For direct computation of steady states and relaxation dynamics; this may be useful for complex systems because of the simplicity of the algorithm and resulting formulas and because often we do not know the rate constants for complex networks, and kinetics that is ruled by orderings rather than by exact values of rate constants may be very useful;
- (2) For planning of experiments and mining the experimental data – the observable kinetics is more sensitive to reactions from the dominant network, and much less sensitive to other reactions, the relaxation spectrum of the dominant network is explicitly connected with the correspondent reaction rate constants, and the eigenvectors (“modes”) are sensitive to the constant ordering, but not to exact values;
- (3) The steady states and dynamics of the dominant system could serve as a robust first approximation in perturbation theory or as a preconditioning in numerical methods.

From a theoretical point of view the outlook is more important. Let us answer the question: what has to be done, but is not done yet? Three directions for further development are clear now:

- (1) Construction of dominant systems for the reaction network that has a group of constants with comparable values (without relations  $\gg$  between them). We considered cycles with several comparable constants in Sec. 2, but the general theory still has to be developed.
- (2) Construction of dominant systems for reaction networks with modular structure. We can assume that the ratio of any two rate constants inside each module be bounded and separated from zero, but the ratios between modules form a well separated ensemble. A reaction network that has a group of constants with comparable values gives us an example of the simplest modular structure: one module includes several reactions and other modules arise from one reaction. In Sec. 5 we describe all solvable modules such that it is possible to solve the kinetic equation for every module in explicit analytical (algebraic) form with quadratures (even for nonconstant in time reaction rate constants).
- (3) Construction of dominant systems for nonlinear reaction networks. The first idea here is the representation of a nonlinear reaction as a pseudomonomolecular reaction: if for reaction  $A + B \rightarrow \dots$  concentrations  $c_A$  and  $c_B$  are well separated, say,  $c_A \gg c_B$ , then we can consider this reaction as  $B \rightarrow \dots$  with rate constant dependent on  $c_A$ . The relative change of  $c_A$  is slow, and we can consider this reaction as pseudomonomolecular until the relation  $c_A \gg c_B$  changes to  $c_A \sim c_B$ . We can assume that in the general case only for small fraction of nonlinear reactions the pseudomonomolecular approach is not applicable, and this set of genuinely nonlinear reactions changes in time, but remains small. For nonlinear systems, even the realization of the limiting step idea for steady states of a one-route mechanism of a catalytic reaction is nontrivial and was developed through the concept of kinetic polynomial [15].

Finally, the concept of “limit simplification” will be developed. For multiscale nonlinear reaction networks the expected dynamical behaviour is to be approximated by the system

of dominant networks. These networks may change in time but remain small enough. The corresponding structure of fast–slow time separation in phase space is not necessarily a smooth slow invariant manifold, but may be similar to a “crazy quilt” and could consist of fragments of various dimensions that do not join continuously.

## Appendix 1: Estimates of eigenvectors for diagonally dominant matrices with diagonal gap condition

The famous Gershgorin theorem gives estimates of eigenvalues. The estimates of correspondent eigenvectors are not so well known. In the paper we use some estimates of eigenvectors of kinetic matrices. Here we formulate and prove these estimates for general matrices. Below  $A = (a_{ij})$  is a complex  $n \times n$  matrix,  $P_i = \sum_{j,j \neq i} |a_{ij}|$  (sums of non-diagonal elements in rows),  $Q_i = \sum_{j,j \neq i} |a_{ji}|$  (sums of non-diagonal elements in columns).

Gershgorin theorem ([36], p. 146): The characteristic roots of  $A$  lie in the closed region  $G^P$  of the  $z$ -plane

$$G^P = \bigcup_i G_i^P \quad (G_i^P = \{z \mid |z - a_{ii}| \leq P_i\}). \quad (61)$$

Analogously, the characteristic roots of  $A$  lie in the closed region  $G^Q$  of the  $z$ -plane

$$G^Q = \bigcup_i G_i^Q \quad (G_i^Q = \{z \mid |z - a_{ii}| \leq Q_i\}). \quad (62)$$

Areas  $G_i^P$  and  $G_i^Q$  are the Gershgorin discs.

Gershgorin discs  $G_i^P$  ( $i = 1, \dots, n$ ) are isolated, if  $G_i^P \cap G_j^P = \emptyset$  for  $i \neq j$ . If discs  $G_i^P$  ( $i = 1, \dots, n$ ) are isolated, then the spectrum of  $A$  is simple, and each Gershgorin disc  $G_i^P$  contains one and only one eigenvalue of  $A$  ([36], p. 147). The same is true for discs  $G_i^Q$ .

Below we assume that Gershgorin discs  $G_i^Q$  ( $i = 1, \dots, n$ ) are isolated, this means that for all  $i, j$

$$|a_{ii} - a_{jj}| > Q_i + Q_j. \quad (63)$$

Let us introduce the following notations:

$$\frac{Q_i}{|a_{ii}|} = \varepsilon_i, \quad \frac{|a_{ij}|}{|a_{jj}|} = \chi_{ij} \quad \left( \varepsilon_i = \sum_l \delta_{li} \right), \quad \min_j \frac{|a_{ii} - a_{jj}|}{|a_{ii}|} = g_i. \quad (64)$$

Usually, we consider  $\varepsilon_i$  and  $\chi_{ij}$  as sufficiently small numbers. In contrary,  $g_i$  should not be small, (this is the *gap condition*). For example, if for any two diagonal elements  $a_{ii}, a_{jj}$  either  $a_{ii} \gg a_{jj}$  or  $a_{ii} \ll a_{jj}$ , then  $g_i \gtrsim 1$  for all  $i$ .

Let  $\lambda_1 \in G_1^Q$  be the eigenvalue of  $A$  ( $|\lambda_1 - a_{11}| < Q_1$ ). Let us estimate the correspondent right eigenvector  $x^{(1)} = (x_i)$ :  $Ax^{(1)} = \lambda_1 x^{(1)}$ . We take  $x_1 = 1$  and write equations for  $x_i$  ( $i \neq 1$ ):

$$(a_{ii} - a_{11} - \theta_1)x_i + \sum_{j,j \neq 1,i} a_{ij}x_j = -a_{i1}, \quad (65)$$

where  $\theta_1 = \lambda_1 - a_{11}$ ,  $|\theta_1| < Q_1$ .

Let us introduce new variables

$$\tilde{x} = (\tilde{x}_i), \quad \tilde{x}_i = x_i(a_{ii} - a_{11}) \quad (i = 2, \dots, n).$$

In these variables,

$$\left(1 - \frac{\theta_1}{a_{ii} - a_{11}}\right) \tilde{x}_i + \sum_{j, j \neq 1, i} \frac{a_{ij}}{a_{jj} - a_{11}} \tilde{x}_j = -a_{i1}, \quad (66)$$

or in matrix notations:  $(1 - B)\tilde{x} = -\tilde{a}_1$ , where  $\tilde{a}_1$  is a vector column with coordinates  $a_{i1}$ . because of gap condition and smallness of  $\varepsilon_i$  and  $\chi_{ij}$  we can consider matrix  $B$  as a small matrix, for assume that  $\|B\| < 1$  and  $(1 - B)$  is reversible (for detailed estimate of  $\|B\|$  see below).

For  $\tilde{x}$  we obtain:

$$\tilde{x} = -\tilde{a}_1 - B(1 - B)^{-1}\tilde{a}_1, \quad (67)$$

and for residual estimate

$$\|B(1 - B)^{-1}\tilde{a}_1\| \leq \frac{\|B\|}{1 - \|B\|} \|\tilde{a}_1\|. \quad (68)$$

For eigenvector coordinates we get from (67):

$$x_i = -\frac{a_{i1}}{a_{ii} - a_{11}} - \frac{(B(1 - B)^{-1}\tilde{a}_1)_i}{a_{ii} - a_{11}} \quad (69)$$

and for residual estimate

$$\frac{|(B(1 - B)^{-1}\tilde{a}_1)_i|}{|a_{ii} - a_{11}|} \leq \frac{\|B\|}{1 - \|B\|} \frac{\|\tilde{a}_1\|}{|a_{ii} - a_{11}|}. \quad (70)$$

Let us give more detailed estimate of residual. For vectors we use  $l_1$  norm:  $\|x\| = \sum |x_i|$ . The correspondent operator norm of matrix  $B$  is

$$\|B\| = \max_{\|x\|=1} \|Bx\| \leq \sum_i \max_j |b_{ij}|.$$

With the last estimate for matrix  $B$  (66) we find:

$$|b_{ii}| \leq \frac{Q_1}{|a_{ii} - a_{11}|} \leq \frac{\varepsilon_1}{g_1} \leq \frac{\varepsilon}{g}, \quad |b_{ij}| = \frac{|a_{ij}|}{|a_{jj} - a_{11}|} \leq \frac{\chi_{ij}}{g_j} \leq \frac{\chi}{g} \quad (i \neq j), \quad (71)$$

where  $\varepsilon = \max_i \varepsilon_i$ ,  $\chi = \max_{i,j} \chi_{ij}$ ,  $g = \min_i g_i$ . By definition,  $\varepsilon \geq \chi$ , and for all  $i, j$  the simple estimate holds:  $|b_{ij}| \leq \varepsilon/g$ . Therefore,  $\|Bx\| \leq n\varepsilon/g$  and,  $\|B\|/(1 - \|B\|) \leq n\varepsilon/(g - n\varepsilon)$  (under condition  $g > n\varepsilon$ ). Finally,  $\|\tilde{a}_1\| = Q_1$  and for residual estimate we get:

$$\left|x_i + \frac{a_{i1}}{a_{ii} - a_{11}}\right| \leq \frac{n\varepsilon^2}{g(g - n\varepsilon)} \quad (i \neq 1). \quad (72)$$

More accurate estimate can be produced from inequalities (71), if it is necessary. For our goals it is sufficient to use the following consequence of (72):

$$|x_i| \leq \frac{\chi}{g} + \frac{n\varepsilon^2}{g(g-n\varepsilon)} \quad (i \neq 1). \quad (73)$$

With this accuracy, eigenvectors of  $A$  coincide with standard basis vectors, i.e. with eigenvectors of diagonal part of  $A$ ,  $\text{diag}\{a_{11}, \dots, a_{nn}\}$ .

## Appendix 2: Time separation and averaging in cycles

In Sec. 2 we analyzed relaxation of a simple cycle with limitation as a perturbation of the linear chain relaxation by one more step that closes the chain into the cycle. The reaction rate constant for this perturbation is the smallest one. For this analysis we used explicit estimates (13) of the chain eigenvectors for reactions with well separated constants.

Of course, one can use estimates (30), (31) (32) and (33) to obtain a similar perturbation analysis for more general acyclic systems (instead of a linear chain). If we add a reaction to an acyclic system (after that a cycle may appear) and assume that the reaction rate constant for additional reaction is smaller than all other reaction constants, then the generalization is easy.

This smallness with respect to all constants is required only in a very special case when the additional reaction has a form  $A_i \rightarrow A_j$  (with the rate constant  $k_{ji}$ ) and there is no reaction of the form  $A_i \rightarrow \dots$  in the non-perturbed system. In Sec. 5 and Appendix 1 we demonstrated that if in a non-perturbed acyclic system there exists another reaction of the form  $A_i \rightarrow \dots$  with rate constant  $\kappa_i$ , then we need inequality  $k_{ji} \ll \kappa_i$  only. This inequality allows us to get the uniform estimates of eigenvectors for all possible values of other rate constants (under the diagonally gap condition in the non-perturbed system).

For substantiation of cycles surgery we need additional perturbation analysis for zero eigenvalues. Let us consider a simple cycle  $A_1 \rightarrow A_2 \rightarrow \dots \rightarrow A_n \rightarrow A_1$  with reaction  $A_i \rightarrow \dots$  rate constants  $\kappa_i$ . We add a perturbation  $A_1 \rightarrow 0$  (from  $A_1$  to nothing) with rate constant  $\epsilon\kappa_1$ . Our goal is to demonstrate that the zero eigenvalue moves under this perturbation to  $\lambda_0 = -\epsilon w^*(1 + \chi_w)$ , the correspondent left and right eigenvectors  $r^0$  and  $l^0$  are  $r_i^0 = c_i^*(1 + \chi_{ri})$  and  $l_i^0 = 1 + \chi_{li}$ , and  $\chi_w$ ,  $\chi_{ri}$  and  $\chi_{li}$  are uniformly small for a given sufficiently small  $\epsilon$  under all variations of rate constants. Here,  $w^*$  is the stationary cycle reaction rate and  $c_i^*$  are stationary concentrations for a cycle (2) normalized by condition  $\sum_i c_i^* = 1$ . The estimate  $\epsilon w^*$  for  $-\lambda_0$  is  $\epsilon$ -small with respect to any reaction of the cycle:  $w^* = \kappa_i c_i^* < \kappa_i$  for all  $i$  (because  $c_i^* < 1$ ), and  $\epsilon w^* \ll \kappa_i$  for all  $i$ .

The kinetic equation for the perturbed system is:

$$\dot{c}_1 = -(1 + \epsilon)\kappa_1 c_1 + \kappa_n c_n, \quad \dot{c}_i = -\kappa_i c_i + \kappa_{i-1} c_{i-1} \quad (\text{for } i \neq 1). \quad (74)$$

In the matrix form we can write

$$\dot{c} = Kc = (K_0 - \epsilon k_1 e^1 e^{1\top})c, \quad (75)$$

where  $K_0$  is the kinetic matrix for non-perturbed cycle. To estimate the right perturbed eigenvector  $r^0$  and eigenvalue  $\lambda_0$  we are looking for transformation of matrix  $K$  into the form  $K = K_r - \theta r e^{1\top}$ , where  $K_r$  is a kinetic matrix for extended reaction system with components  $A_1, \dots, A_n$ ,  $K_r r = 0$  and  $\sum_i r_i = 1$ . In that case,  $r$  is the eigenvector, and  $\lambda = -\theta r_1$  is the correspondent eigenvalue.

To find vector  $r$ , we add to the cycle new reactions  $A_1 \rightarrow A_i$  with rate constants  $\epsilon k_1 r_i$  and subtract the correspondent kinetic terms from the perturbation term  $\epsilon e^1 e^{1\top} c$ . After that, we get  $K = K_r - \theta r e^{1\top}$  with  $\theta = \epsilon k_1$  and

$$\begin{aligned} (K_r c)_1 &= -k_1 c_1 - \epsilon k_1 (1 - r_1) c_1 + k_n c_n, \\ (K_r c)_i &= -k_i c_i + \epsilon k_1 r_i c_1 + k_{i-1} c_{i-1} \quad \text{for } i > 1 \end{aligned} \quad (76)$$

We have to find a positive normalized solution  $r_i > 0$ ,  $\sum_i r_i = 1$  to equation  $K_r r = 0$ . This is the fixed point equation: for every positive normalized  $r$  there exists unique positive normalized steady state  $c^*(r)$ :  $K_r c^*(r) = 0$ ,  $c_i^* > 0$ ,  $\sum_i c_i^*(r) = 1$ . We have to solve the equation  $r = c^*(r)$ . The solution exists because the Brauer fixed point theorem.

If  $r = c^*(r)$  then  $k_i r_i - \epsilon k_1 r_i r_1 = k_{i-1} r_{i-1}$ . We use notation  $w_i^*(r)$  for the correspondent stationary reaction rate along the ‘‘non-perturbed route’’:  $w_i^*(r) = k_i r_i$ . In this notation,  $w_i^*(r) - \epsilon r_i w_1^*(r) = w_{i-1}^*(r)$ . Hence,  $|w_i^*(r) - w_1^*(r)| < \epsilon w_1^*(r)$  (or  $|k_i r_i - k_1 r_1| < \epsilon k_1 r_1$ ). Assume  $\epsilon < 1/4$  (to provide  $1 - 2\epsilon < 1/(1 \pm \epsilon) < 1 + 2\epsilon$ ). Finally,

$$r_i = \frac{1}{k_i} \frac{1 + \chi_i}{\sum_j \frac{1}{k_j}} = (1 + \chi_i) c_i^* \quad (77)$$

where the relative errors  $|\chi_i| < 3\epsilon$  and  $c_i^* = c_i^*(0)$  is the normalized steady state for the non-perturbed system. For cycles with limitation,  $r_i \approx (1 + \chi_i) k_{\text{lim}}/k_i$  with  $|\chi_i| < 3\epsilon$ . For the eigenvalue we obtain

$$\begin{aligned} \lambda_0 &= -\epsilon w_1^*(r) = -\epsilon w_i^*(r) (1 + \varsigma_i) \\ &= -\epsilon w^*(1 + \chi) = -\epsilon k_i c_i^*(0) (1 + \chi) \end{aligned} \quad (78)$$

for all  $i$ , with  $|\varsigma_i| < \epsilon$  and  $|\chi| < 3\epsilon$ . Therefore,  $\lambda_0$  is  $\epsilon$ -small rate constant  $k_i$  of the non-perturbed cycle. This implies that  $\lambda_0$  is  $\epsilon$ -small with respect to the real part of every non-zero eigenvalue of the non-perturbed kinetic matrix  $K_0$  (for given number of components  $n$ ). For the cycles from multiscale ensembles these eigenvalues are typically real and close to  $-k_i$  for non-limiting rate constants, hence we proved for  $\lambda_0$  even more than we need.

Let us estimate the correspondent left eigenvector  $l^0$  (a vector row). The eigenvalue is known, hence it is easy to do just by solution of linear equations. This system of  $n - 1$  equations is:

$$-l_1(1 + \epsilon)k_1 + l_2 k_1 = \lambda_0 l_1, \quad -l_i k_i + l_{i+1} k_i = \lambda_0 l_i, \quad i = 2, \dots, n - 1. \quad (79)$$



For normalization, we take  $l_1 = 1$  and find:

$$l_2 = \left( \frac{\lambda_0}{k_1} + 1 + \epsilon \right) l_1, \quad l_{i+1} = \left( \frac{\lambda_0}{k_i} + 1 \right) l_i \quad i > 2. \quad (80)$$

Formulas (77), (78) and (80) give the backgrounds for surgery of cycles with outgoing reactions. The left eigenvector gives the slow variable: if there are some incomes to the cycle, then

$$\dot{c}_1 = -(1 + \epsilon)\kappa_1 c_1 + \kappa_n c_n + \phi_1(t), \quad \dot{c}_i = -\kappa_i c_i + \kappa_{i-1} c_{i-1} + \phi_i(t) \quad (\text{for } i \neq 1) \quad (81)$$

and for slow variable  $\tilde{c} = \sum l_i c_i$  we get

$$\frac{d\tilde{c}}{dt} = \lambda_0 \tilde{c} + \sum_i l_i \phi_i(t). \quad (82)$$

This is the kinetic equation for a glued cycle. In the leading term, all the outgoing reactions  $A_i \rightarrow 0$  with rate constants  $k = \epsilon k_i$  give the same eigenvalue  $-\epsilon w^*$  (78).

Of course, similar results for perturbations of zero eigenvalue are valid for more general ergodic chemical reaction network with positive steady state, and not only for simple cycles, but for cycles we get simple explicit estimates, and this is enough for our goals.

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