Limitation and averaging for deterministic and stochastic biochemical reaction networks

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***Abstract*— We discuss model reduction of multiscale networks of biochemical reactions used in systems biology as models for cell physiology and pathology. For linear kinetic models, which appear as "pseudo-monomolecular" subsystems of the nonlinear reaction networks, we obtain a general reduction algorithm based on cycle averaging and surgery. The same algorithm, when applied to stochastic networks, allows to reduce simulation time by many orders of magnitude.**

I. Introduction

Systems biology uses networks of biochemical reactions as paradigms for normal and pathologic cell functioning. In such models, the cell has several compartments (nucleus, cytosol, organelles, etc.) but each compartment is considered to be well stirred. Transport between compartments is possible. The models are usually medium size (tens to hundreds of reactions and species). Therefore, their simulation by ODE solvers is not really a problem even for stiff systems. However, biological models present specific difficulties. Thus, the reaction mechanism is most of the time hypothetic and finding parameter values is difficult. For such systems, we would like to have simple rules allowing us to understand why a model functions the way it does and which consequences have on dynamics the various modifications of the mechanism. Furthermore, one would like to known which aspects (for instance parameter values) of the model are essential and which are not. All these questions could be answered by computing reduced mechanisms. Another specificity of molecular systems in biology is their stochasticity. The law of large numbers does not apply in biology as it does in physics, where fluctuations existing at microscopic scale are wiped-out in the thermodynamic limit (except for critical and turbulent systems). Biological systems behave similarly to critical or turbulent physical systems: they have many fluctuating scales.Molecular species in small number are responsible for stochastic phenomena such as intermittence or bursting, occurring in protein production, random action potential firing, calcium signalling, etc.. In systems biology, stochastic modelling by Markov jump dynamics (Gillespie SSA algorithm [1]) represents a very time consuming industry. There are two solutions to this problem. The first one is similar to the one employed by stiff deterministic solvers: avoiding adaptatively, but blindly the individual simulation of reactions that repeat very frequently. The second solution, that we propose here is to pre-condition the system by simplifying it to a less stiff model.

In this paper we present such a pre-conditioning algorithm that works equally well for deterministic and for stochastic models. This algorithm is based on nontrivial generalizations of limitation (whose "naive" versions are well-known for chains and acyclic networks) to hierarchies of cycles and on averaging.

II. Algorithms

A. Linear submechanisms

There are two types of linear submechanisms: monomolecular networks and first order networks. The structure of monomolecular reaction networks can be completely defined by a simple digraph, in which vertices correspond to chemical species A_i, edges correspond to reactions $A_i \to A_j$ with rate constants $k_{ji} > 0$. For each vertex, A_i, a positive real variable c_i (concentration) is defined.

The deterministic kinetic equation is

$$\frac{dc_i}{dt} = \sum_j k_{ij} c_j - (\sum_j k_{ji}) c_i, \tag{1}$$

First order reaction networks include monomolecular networks as a particular case, and are characterized by a single substrate and by reaction rates that are proportional to the concentration of the substrate. First order reaction networks can contain reactions that are not monomolecular, such as $A \to A + B$, or $A \to B + C$. We shall restrict ourselves to pseudo-conservative first order reactions, ie reactions that do not change the total number of molecules in a given submechanism ($A \to A + B$ reactions are allowed, provided that B is external to the submechanism; similarly $A \to B + C$ reactions are allowed, provided that either B or C is external to the submechanism). With such constraints, the total number of molecules in the sub-mechanism is conserved and the kinetic equations are the same as (1). Degradation reactions can be studied in this framework by considering a special component (sink), that collects degraded molecules. Further release of the constraints is possible. For instance, the system can be opened by allowing constant (or slowly variable) production terms in Eq.(1). These terms will change the steady state, but will not influence the relaxation times of the system.

The algorithms described in the paper can be applied to linear sub-mechanisms of a non-linear network, given

fixed (or slowly changing) values of external inputs (boundaries). For instance, even in systems of binary reactions, one can define pseudo-monomolecular reactions when one of the substrates of the binary reaction is not changing (or changing slowly). This condition can be fulfilled if the substrate is in excess, for instance.

B. Dominant pathways by cycle surgery in deterministic networks

The idea of dominant subsystems in asymptotic analysis of dynamical systems is due to Newton and developed by Kruskal [6]. Complex regulatory networks in metabolism and signalling activate only a limited number of pathways in order to fulfill a given physiologic task. The set of active pathways can change for unusual stresses (such as exposure to a toxin) or in pathologic situations. The concept of dominant pathways could serve to explain such dynamic transitions. In [3] we have based the construction of dominant subsystems on a generalized limitation approach. This approach selects dominant pathways and produces simplified reaction mechanisms.

We consider total separation of the constants namely either $k_I << k'_I$ or $k'_I << k_I$ for all $I = ij$, $I' = i'j'$. In this case the dominant subsystem can be worked-out by cycle surgery [3]. The algorithm, justified by estimates for the eigenvalues and eigenvectors (inspired, but not fully covered by Gershgorin theorem) of the kinetic matrix [3], consists of three stages:

I. Constructing the auxiliary reaction network.

For each A_i, let us define κ_i as the maximal kinetic constant for reactions $A_i \to A_j$: $\kappa_i = \max_j\{k_{ji}\}$. For correspondent j we use the notation $\phi(i)$: $\phi(i) = \arg\max_j\{k_{ji}\}$.

An auxiliary reaction network $\mathcal{W}$ is the set of reactions $A_i \to A_{\phi(i)}$ with kinetic constants κ_i. The correspondent kinetic equation is

$$\dot{c}_i = -\kappa_i c_i + \sum_{\phi(j)=i} \kappa_j c_j, \qquad (2)$$

II Glueing cycles

In general, the auxiliary network $\mathcal{V}$ has several cycles $C_1, C_2, ...$ with periods $\tau_1, \tau_2, ... > 1$.

These cycles will be "glued" into points and all nodes in the cycle C_i, will be replaced by a single vertex A^i.

Reactions $A \to B$ exiting from cycles ($A \in C_i$, $B \in C_j, j \neq i$ or $B \in C_i$) are changed into $A^i \to B$ with the rate constant renormalization: let the cycle C^i be the following sequence of reactions $A_1 \to A_2 \to ...A_{\tau_i} \to A_1$, and the reaction rate constant for $A_i \to A_{i+1}$ is k_i (k_{τ_i} for $A_{\tau_i} \to A_1$). For the limiting reaction of the cycle C_i we use notation $k_{\lim\, i}$. If $A = A_j$ and k is the rate reaction for $A \to B$, then the new reaction $A^i \to B$ has the rate constant $kk_{\lim\, i}/k_j$. This rate is obtained using quasi-stationary distribution for the cycle.

The new auxiliary network $\mathcal{V}^1$ is computed for the network of glued cycles. Then we decompose it into cycles, glue them, iterate until a acyclic network is obtained $\mathcal{V}^n$.

III Restoration of cycles

The dynamics of species inside glued cycles is lost after the previous step. A full multi-scale approximation (including relaxation inside cycles) can be obtained by cycle restoration. This is done starting from the acyclic auxiliary network $\mathcal{V}^n$ back to $\mathcal{V}^1$ through the hierarchy of cycles. Each cycle is restored according to the following procedure:

For each glued cycle node A_i^m, node of $\mathcal{V}^m$,

- Recall its nodes $A_{i1}^{m-1} \to A_{i2}^{m-1} \to ...A_{i\tau_i}^{m-1} \to A_{i1}^{m-1}$; they form a cycle of length τ_i.
- Let us assume that the limiting step in A_i^m is $A_{i\tau_i}^{m-1} \to A_{i1}^{m-1}$
- Remove A_i^m from $\mathcal{V}^m$
- Add τ_i vertices $A_{i1}^{m-1}, A_{i2}^{m-1}, ...A_{i\tau_i}^{m-1}$ to $\mathcal{V}^m$
- Add to $\mathcal{V}^m$ reactions $A_{i1}^{m-1} \to A_{i2}^{m-1} \to ...A_{i\tau_i}^{m-1}$ (that are the cycle reactions without the limiting step) with correspondent constants from $\mathcal{V}^{m-1}$
- If there exists an outgoing reaction $A_i^m \to B$ in $\mathcal{V}^m$ then we substitute it by the reaction $A_{i\tau_i}^{m-1} \to B$ with the same constant, i.e. outgoing reactions $A_i^m \to ...$ are reattached to the heads of the limiting steps
- If there exists an incoming reaction in the form $B \to A_i^m$, find its prototype in $\mathcal{V}^{m-1}$ and restore it in $\mathcal{V}^m$
- If in the initial $\mathcal{V}^m$ there existed a "between-cycles" reaction $A_i^m \to A_j^m$ then we find the prototype in $\mathcal{V}^{m-1}$, $A \to B$, and substitute the reaction by $A_{i\tau_i}^{m-1} \to B$ with the same constant, as for $A_i^m \to A_j^m$ (again, the beginning of the arrow is reattached to the head of the limiting step in A_i^m)

C. Cycle averaging in stochastic linear chemical networks

The Markovian stochastic dynamics of a single molecule in a linear reaction network is given by the probability $p(j,t)$ that the molecule is in A_j at the time t. We can easily show that the master equation for $p(j,t)$ is the same as the deterministic kinetic equation (1). Considering only one molecule does not restrict generality because when several molecules are present in a linear network, these behave independently. Thus, the simplification method proposed for deterministic networks [3], [2] can be also applied to stochastic networks.

Simplified stochastic models will represent preconditioned models used in order to reduce simulation time. Instead of searching for a multiscale approximation, our purpose here is to find a reduced model that is computationally effective and which captures dynamics on time scales or order τ or slower. τ could be for instance the experimental time resolution.

In order to present the simplification algorithm let us use two simple examples.

First, let us consider a chain of molecular reactions $A_1 \to A_2 \to ...A_m$. The reaction rate constant for $A_i \to A_{i+1}$ is k_i. All rate constants are considered well separated, i.e. either $k_i << k_j$ or $k_i >> k_j$ for any $i \neq j$. The smallest rate constant in the chain is denoted by $k_{\lim}$. If $k_{\lim} >> 1/\tau$ (rapid chain), then within the timescale τ all molecules A_1 are transformed into molecules A_m. We

can thus ignore the chain reactions and consider that the entire initial mass of the chain is in A_m. This is equivalent to considering the chain at quasi-stationarity because the steady state probability distribution of a chain is a Dirac delta measure localized at the end of the chain. However, if we do not simplify chains, then simulating them by Gillespie's SSA [1] will not be computationally expensive because the mass of the chain is transferred to the end of the chain A_m in a number of steps that is relatively small.

As a second example, let us consider the cycle C be the following sequence of mono-molecular reactions $A_1 \to A_2 \to ...A_m \to A_1$. Let all rate constants be well separated and the smallest one be k_{lim} like before. We add to the cycle one branching reaction; this transforms A_j a component of the cycle into B a component exterior to the cycle. We consider the following distinct situations: (I) the branching reaction is $A_j \to B$ of rate constant k and $k << k_j$, (II) the branching reaction is $A_j \to B$ and $k >> k_j$, (III) the branching reaction is $A_j \to A_j + B$, or (IV) the branching reaction is $A_j \to A_{j+1} + B$ of rate constant k_j. In the situation (I) the exit reaction is faster and dominates the cycling reaction $A_j \to A_{j+1}$. According to the rule for auxiliary networks in this case (that we call "broken" cycle) the cycle can be opened (by eliminating the cycling reaction $A_j \to A_{j+1}$) and the resulting multiscale dynamics is the one of a chain; we recover the previous example and in this case we can safely decide to do nothing. In the situation (II) the exit reaction is much slower than the cycling reaction. In this case the molecules inside the cycle have rapid transformations and the mass distribution inside the cycle can be considered to reach quasi-stationary distribution. As discussed in [4], [3], [2], the relaxation time of a cycle with separated constants is the inverse of the second slowest rate constant $k^{(2)} >> k^{(1)} = k_{lim}$. To understand this, one should consider the two possible paths to equilibrate a cycle, one passing through the slowest step and the quicker one passing through the second slowest step: the quicker short-cuts the first one. Thus, a cycle can be considered quasi-stationary if $k^{(2)} >> 1/\tau$. A non-averaged fast cycle could be computationally expensive in SSA, because a molecule can perform a huge number of steps along the cycle on the timescale τ. The corresponding condition involves the quasi-stationary flux (not the relaxation time) and reads $k^{(1)} >> 1/\tau$.

From a quasi-stationary cycle, the mass is lost stochastically, but slowly by the branching reaction. The intensity of the loss process can be calculated by replacing X_j by its average with respect to the quasi-stationary distribution of the cycle. The average of X_j is $\bar{X}_j = N(t)k_{\lim}/k_j$, where $N(t)$ is the total mass inside the cycle $N = \sum_{j=1}^{m} N_j$. We obtain the average intensity $\bar{\lambda} = k\bar{X}_j = N(t)kk_{\lim}/k_j$. In the situations (III) or (IV) the average intensities of the branching reactions are $k\bar{X}_j = N(t)kk_{\lim}/k_j$ and $k_j\bar{X}_j = N(t)k_{\lim}$, respectively.

The result of the cycle averaging can be represented as a simplification of the mechanism (cycle glueing), applied only to non-broken cycles:

- "glue" the cycle into a single node C having the total mass N
- replace the exit reaction of the type i) $A_j \to B$ of rate constant k by a reaction $C \to B$ of effective constant $k' = kk_{\lim}/k_j$.
- replace the reaction of the type ii) $A_j \to A_j + B$ or rate constant k by a reaction $C \to C+B$ of effective constant $k' = kk_{\lim}/k_j$.
- replace the reaction of the type iii) $A_j \to A_{j+1} + B$ of rate constant k_j by a reaction $C \to C + B$ of effective constant $k' = k_{\lim}$.

As a possible design rule, notice that, unless k_j is the limiting step in the cycle, one has $k_{\lim}/k_j << 1$. Then, the average intensity of the exit reaction of the type i) or ii) is weak and could represent a source of intermittence in the system. This situation should be avoided for less noise in the system, or created when noise is wanted.

III. Applications

A. NFκB oscillations

The transcription factor NF-κB is involved in a wide diversity of domains such as the immune and inflammatory responses, cell survival and apoptosis, cellular stress and neuro-degenerative diseases, cancer and development. NF-κB is sequestered in the cytoplasm by inactivating proteins named IκB. Upon signalling, IκB molecules are phosphorylated by a kinase complex, then ubiquitinylated, and finally degraded by the proteasomal complex. NF-κB released from IκB molecules is then transported to the nucleus to activate its target genes, among which its inhibitor IκB. The produced IκB enters the nucleus, binds to, back-translocates and re-sequester NF-κB in the cytosol. This delayed negative feed-back is responsible for oscillations of NF-κB activity.

The biochemical models for NFκB signalling discussed in [2] contain linear sub-networks that were simplified using the algorithm described in sub-section IIA. After simplification, a mapping has been constructed between parameters of the initial model and the parameters of the simpler model. This mapping allowed us to find the critical parameters and to asses their influence on the capacity of the system to undergo sustained oscillations. Thus, many reactions are dominated and not critical. The precise values of their constants are not important for the dynamics, although their relative order matters. The details of this analysis can be found in [2].

B. Stochastic bursting of repressed operon

To illustrate reduction of stochastic models, we present here a new application.

Under strong repression, protein production from a bacterium operon undergoes stochastic bursting. The phenomenon has been modelled by [5], see Fig.1. In this model the bacterium is considered to be in exponential growth phase, increasing size and dividing normally. Cell growth is simulated by a linear increase of the volume in time. During replication the nuclear material doubles (variables D,D.R,DRNAP). At fission the nuclear material

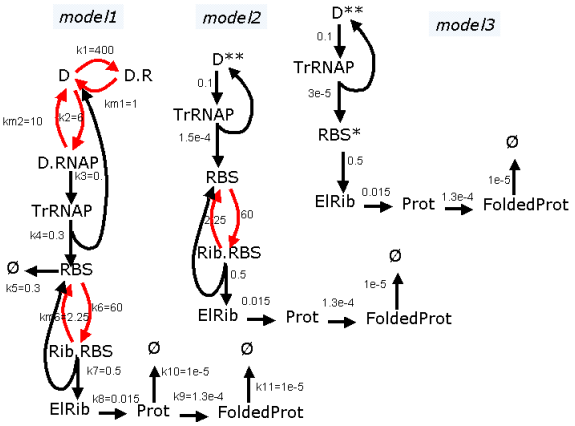

Fig. 1. Repressed operon models. The averaged cycles are in red.

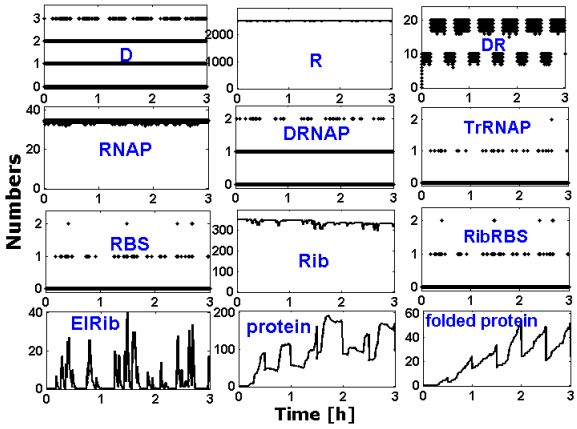

Fig. 2. Trajectories obtained by SSA

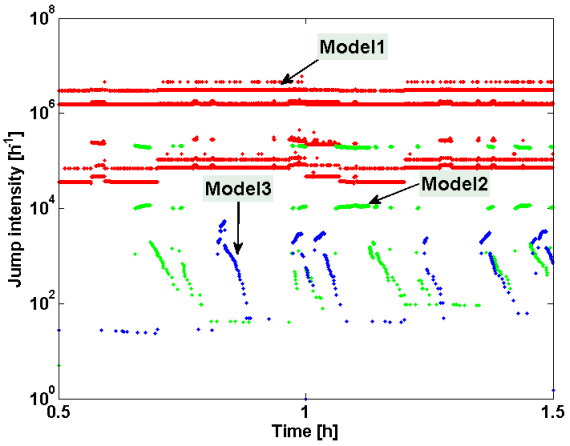

Fig. 3. Jump intensities for the 3 models.

is halved and all other components are divided among daughter cells according to a binomial distribution.

The cycle averaging procedure can be applied three times:

1.1 The cycle $D, D.R$ is not-broken. It is glued to the node D^* whose total mass is equal to the mass of D and $D.R$.

1.2 The limiting step of the cycle is $k_{lim} = k_{m1} << k_1$.

1.3 The branching reaction $D \rightarrow D.RNAP$ is replaced by $D^* \rightarrow D.RNAP$ of effective constant $k_2' = \frac{k_{m1}}{k_1} k_2$.

2.1 The cycle $D^*, D.RNAP$ is not-broken. It is glued to the node D^{**} whose total mass is equal to the mass of D and $D.R$ and $D.RNAP$.

2.2 We have $k_2' << k_{m2}$ hence the limiting step of the cycle is k_2'.

2.3 The branching reaction $D.RNAP \rightarrow TrRNAP$ is replaced by $D^{**} \rightarrow TrRNAP$ of effective constant $k_3' = \frac{km1}{km2} \frac{k2}{k1} k3$.

3.1 The cycle $RBS, Rib.RBS$ is not-broken. It is glued to the node RBS^* whose total mass is the one of RBS and of $Rib.RBS$.

3.2 The limiting step is $k_{m6} << k_6$.

3.3 The branching reaction $Rib.RBS \rightarrow ElRib + RBS$ is replaced by the reaction $RBS^* \rightarrow ElRib + RBS^*$ of effective constant $k7^* \approx k7$.

Notice that a loss of accuracy should be expected from the application of the third averaging step. The separation of the branching and cycling reactions is not that good. Indeed, $k_7/k_{m6} \approx 0.22$ while in theory we need $k_7/k_{m6} << 1$. The trajectories obtained by SSA (Fig.2) show the bursting phenomenon that can be now understood by the resulting low intensity of the reaction $TrRNAP \rightarrow RBS$. The reduced models reproduce the same behavior (with good accuracy for model 2, only qualitatively for model 3).

In order to compare the performance of the models (in terms of time complexity) we have represented the total jump intensities for three models (exact SSA, second and third averaged steps models) as functions of time on a trajectory. The model that demands the least computer time is the one with the smallest jump intensity. In Fig.3, we notice a decrease of several orders of magnitudes of the total intensity from exact SSA to the second and third averaging steps.

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