Lecture 4: Model checking for ODE models

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• Stoichiometric matrix
• Calculating the mass conservation relations
• Calculating the steady state
• Elementary fluxes
• Sensitivity coefficients
• Define all these concepts in a general framework, on the level of reaction-based models

• The discussion about the mathematical details of these concepts is for ODE-based models
  o Similar definitions can also be given for other math frameworks

• Recall
  o To each reaction we associate a reaction rate – it tells how fast reactants are consumed, products are produced
  o The form of the reaction rate depends on the kinetic law chosen for the reaction
    – Mass-action
    – Michaelis-Menten
    – Inhibition of various types
    – Hill
    – ...

5.11.2014 Advanced computational modeling
Stoichiometry

• The **stoichiometric coefficients** denote the quantitative proportion in which substrate and product molecules are involved in a reaction.

**Examples:**

- For an irreversible reaction $S_1 + S_2 \rightarrow 2P$, the stoichiometric coefficients of $S_1$, $S_2$, and $P$ are -1, -1, and 2, respectively.

  In general, the stoichiometric coefficients are positive for products and negative for reactants.

- For a reversible reaction $S_1 + S_2 \rightleftharpoons 2P$, the stoichiometric coefficients of $S_1$, $S_2$, and $P$ are -1, -1, and 2, respectively.

- For a reaction $S_1 + S_2 \rightleftharpoons 2P + S_2$ the stoichiometric coefficients are -1, 0, and 2, respectively.
The stoichiometric coefficients of a reaction network consisting of $s$ species and $r$ reactions are organized in a so-called stoichiometric matrix, denoted $\mathbf{N}=(n_{ij})_{s \times r}$, where $n_{ij}$ denotes the stoichiometric coefficient of species $S_i$ in reaction $R_j$.

**Example:**

**Reaction network**

- $r_1$: $A \rightleftharpoons 2B$
- $r_2$: $A + C \rightleftharpoons D$
- $r_3$: $D \rightarrow B + E$
- $r_4$: $A + B \rightleftharpoons D + B$

**Stoichiometric matrix**

\[
\begin{bmatrix}
-1 & -1 & 0 & -1 \\
2 & 0 & 1 & 0 \\
0 & -1 & 0 & 0 \\
0 & 1 & -1 & 1 \\
0 & 0 & 1 & 0
\end{bmatrix}
\]
Stoichiometric matrix

- For a reaction network consisting of $s$ substances and $r$ reactions, the dynamics, in particular the change of concentrations in time, is described by the following system of equations:

$$\frac{d}{dt} [S_i] = \sum_{j=1}^{r} n_{ij} v_j,$$

for all $1 \leq i \leq s$, where $v_j$ is the rate of reaction $j$

- This can be rewritten in the matrix notation:

$$\frac{ds}{dt} = Nv,$$

where $S$ is a vector of the concentrations of all the substances in the reaction network, i.e. $S = ([S_1], \ldots, [S_s])^T$, and $v = (v_1, \ldots, v_r)^T$ is the vector of the reaction rates.
Stoichiometric matrix

- Example

\[2A \rightarrow B \ (k_1)\]
\[A + B \rightleftharpoons C \ (k_2^+, k_2^-)\]

**Stoichiometric matrix:**

\[
\mathbf{N} = \begin{bmatrix}
-2 & -1 \\
1 & -1 \\
0 & 1 \\
\end{bmatrix}
\]

**Reaction rates (mass action kinetics):**

\[\nu_1 = k_1[A]^2,\]
\[\nu_2 = k_2^+[A][B] - k_2^-[C]\]

\[
\begin{bmatrix}
\frac{d[A]}{dt} \\
\frac{d[B]}{dt} \\
\frac{d[C]}{dt}
\end{bmatrix} = \begin{bmatrix}
-2 & -1 \\
1 & -1 \\
0 & 1 \\
\end{bmatrix} \cdot \begin{bmatrix}
k_1[A]^2 \\
k_2^+[A][B] - k_2^-[C]
\end{bmatrix}
\]
Stoichiometric matrix

• The stoichiometric matrix contains valuable information about the structure of the network
  o Mass conservation relations
  o Steady states
  o Elementary fluxes
  o Sensitivity coefficients

• Discuss each of them in the rest of this lecture
Mass-conservation relations
Mass conservation relations

• Frequently, the concentrations of several species involved in biochemical reaction networks are included in so-called conservation sums.

• A characteristic feature of such species is that
  o their gain and loss rates are equal;
  o they can form complexes with other species or be part of other species.

• A mass conservation relation is a **constant linear combination** of some of the species of the model.
Mass conservation relations

- Example

  reactions:
  
  \[2A \rightleftharpoons A_2\]
  \[A_2 + B \rightleftharpoons A_2:B\]
  \[A_2:B \rightarrow C + A_2:B\]
  \[C \rightarrow\]

  species:
  
  \[A, A_2, B, A_2:B, C\]

  The total amounts of A and B are conserved in time. Neither of them is produced nor degraded.

  \[1 \times \#A + 2 \times \#A_2 + 2 \times \#A_2:B = \text{const.}\]
  \[1 \times \#B + 1 \times \#A_2:B = \text{const.}\]
Mass conservation relations

• To identify the conservation relations we solve the following equation in matrix $G$:

$$GN = 0$$

where $N$ is the stoichiometric matrix

  - Indeed, for such $G$: 

  $$G \frac{dS}{dt} = GNv = 0.$$

• Example (continued):

$$S = \begin{bmatrix} A \\ A_2 \\ B \\ A_2:B \\ C \end{bmatrix}, \quad N = \begin{bmatrix} -2 & 0 & 0 & 0 \\ 1 & -1 & 0 & 0 \\ 0 & -1 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & -1 \end{bmatrix}, \quad G = \begin{bmatrix} 1 & 2 & 0 & 2 & 0 \\ 0 & 0 & 1 & 1 & 0 \end{bmatrix}, \quad GN = 0$$
Recall from linear algebra

- The number of independent rows of $G$, i.e. the number of conservation relations, is equal to $s$-Rank($N$).
  - In the example $s=5$ and Rank($N$)$=3$. It follows that $G$ contains 2 independent rows, i.e., there are two mass conservation relations.
  - Observation: if the stoichiometric matrix is full rank, it follows that the system has no conservation relations.
Mass conservation relations

• Conservation relations can be used to reduce the system of differential equations \( \frac{dS}{dt} = Nv \) describing the dynamics of a reaction network.

• Each conservation relation leads to one more dependent variable, that can be expressed in terms of the independent variables and eliminated from the system of ODEs.
Mass conservation relations

- Example
  reactions:
  
  \[2A \rightleftharpoons A_2\]
  
  \[A_2 + B \rightleftharpoons A_2:B\]
  
  \[A_2:B \rightarrow C + A_2:B\]
  
  \[C \rightarrow \_\]

  \[S = \begin{bmatrix}
  A \\
  A_2 \\
  B \\
  A_2:B \\
  C 
  \end{bmatrix}, \quad
  N = \begin{bmatrix}
  -2 & 0 & 0 & 0 \\
  1 & -1 & 0 & 0 \\
  0 & -1 & 0 & 0 \\
  0 & 1 & 0 & 0 \\
  0 & 0 & 1 & -1 
  \end{bmatrix}, \quad
  G = \begin{bmatrix}
  1 & 2 & 0 & 2 & 0 \\
  0 & 0 & 1 & 1 & 0 
  \end{bmatrix}\]

  \[\text{GN} = 0\]

- The mass conservation relations (based on G):
  
  \[[A] + 2[A_2] + 2[A_2:B] = K\]
  
  \[[B] + [A_2:B] = K'\]

- In other words:
  
  
  \[[B] = K' - [A_2:B]\]

- The initial system of 5 ODEs in [A], [A_2], [B], [A_2:B], [C] is reduced to a system of 3 independent ODEs in [A_2], [A_2:B], [C]
Steady states
Steady state

- **Steady state** – one of the basic concepts of dynamical systems theory, extensively utilized in modelling.

- Steady states (*stationary states, fixed points, equilibrium points*) are determined by the fact that the values of all state variables remain constant in time: $S(t) = S_0$

- If $S(0) = S_0$, then $\frac{dS}{dt} = Nv_0 = 0$

- Solve the equation in the unknown $S_0$ (a vector with $s$ components, one for each variable)
  - $N$ is the stoichiometric matrix
  - $v_0$ is a function of the components of $S_0$
  - An algebraic (system of) equation(s) for the typical kinetics, e.g. mass-action or MM
Steady state

- Example (mass action kinetics)

\[ 2A \rightarrow B \ (k_1) \]
\[ A + B \rightleftharpoons C \ (k_{+2}, k_{-2}) \]

Steady state algebraic equations ([A]_0, [B]_0, and [C]_0 are unknowns)

\[
\begin{bmatrix}
0 \\
0 \\
0
\end{bmatrix} = 
\begin{bmatrix}
-2 & -1 \\
1 & -1 \\
0 & 1
\end{bmatrix} \cdot 
\begin{bmatrix}
k_1[A]_0^2 \\
k_2^+[A]_0[B]_0 - k_2^-[C]_0
\end{bmatrix}
\]

or

\[
\begin{align*}
0 &= -2k_1[A]_0^2 - k_2^+[A]_0[B]_0 + k_2^-[C]_0 \\
0 &= k_1[A]_0^2 - k_2^+[A]_0[B]_0 + k_2^-[C]_0 \\
0 &= k_2^+[A]_0[B]_0 - k_2^-[C]_0
\end{align*}
\]
Elementary fluxes
Elementary flux modes

• Concept of elementary flux mode
  o a minimal set of enzymes (or, in other words, reactions) that can operate at steady state
  o the smallest sub-networks that allow a bionetwork to function at steady state
  o a *minimal* combination of reactions whose combined effect maintains the network in steady state
    - any subset of it does not maintain the steady state
  o they offer a key insight into the objectives of the network
  o each elementary flux mode should have a clear biological interpretation in terms of the objectives of the network
  o determines whether a given set of enzymes/reactions are feasible at steady states

• Larger flux modes can be obtained by composing several flux modes: steady-state flux distributions
Calculating the elementary flux modes

• We are interested in linear combinations of reactions whose combined effect is to preserve the steady state
  
  - denote \( w_i \) the weight of reaction \( i \) in the flux mode
  
  - Recall: \( \frac{ds}{dt} = Nv \), where \( N \) is the stoichiometric matrix and \( v \) is the vector of fluxes
  
  - We are interested in combinations of fluxes \( (w_1, ..., w_r) \) that ensure \( \frac{ds}{dt} = 0 \)
  
  - In other words, solve the equation \( Nw = 0 \) in the unknown \( w \)
    - Recall from linear algebra: the solution is called the kernel (or the null space) of matrix \( N \)
    - In general, the solution is a vectorial space – we are interested in its base; all other solutions are linear combinations of the elements in the base
• Stoichiometric matrix: \[
\begin{pmatrix}
1 & -1 & 0 & -1 \\
0 & 2 & -1 & 0 \\
0 & 0 & 0 & 1
\end{pmatrix}
\]

• NK=0 yields solution \( K = (1 \ 1 \ 2 \ 0) \)

  ◦ \( v_4 = 0 \) means that in any steady state, the rates of production and degradation of \( S_3 \) must be equal
Sensitivity coefficients
Local sensitivity analysis

• **Local sensitivity analysis** is a method to estimate the changes brought into the system through small perturbations in the parameters of the model.

• It provides means to:
  - estimate the robustness of the model against small changes in the model,
  - identify possibilities for bringing a certain desired change in the system.

• We write the system of ODEs describing the dynamics of a reaction network in the following form:

\[
\frac{d}{dt}[S_i] = f_i([S_1], \ldots, [S_N], \kappa), \quad \text{for all } 1 \leq i \leq N,
\]

where \(\kappa=(k_1,\ldots,k_M)^T\) is the parameter vector.
Local sensitivity analysis

- **First order local concentration sensitivity coefficients**: how the solution depends on small variations in the parameter values

- We denote \( \mathbf{S}(t, \kappa) = ([S_1](t, \kappa), [S_2](t, \kappa), \ldots, [S_N](t, \kappa))^T \) the solution of the system with respect to the parameter vector \( \kappa \). The concentration sensitivity coefficients are the time functions

\[
\frac{\partial[S_i]}{\partial k_j}(t),
\]

for all \( 1 \leq i \leq N, \ 1 \leq j \leq M \).
Local sensitivity analysis

- Very often however, the focus is on sensitivity analysis around steady states.

- In case of asymptotically stable steady states, consider $\lim_{t \to \infty} (\partial S/\partial k_j)(t)$ – stationary sensitivity coefficients.
  
  - reflects the dependency of the steady state on the parameters of the model.
Scaled sensitivity coefficients

• When used for comparing the relative effect of a parameter change in two or more variables, the sensitivity coefficients must have the same physical dimension or be dimensionless.

• One simply considers the matrix $C$ of (dimensionless) normalized (also called scaled) sensitivity coefficients:

$$C_{ij} = \frac{k_j}{S_i(t, \kappa)} \cdot \frac{\partial [S_i](t, \kappa)}{\partial k_j} = \frac{\partial \ln ([S_i](t, \kappa))}{\partial \ln (k_j)}$$

• Numerical estimations of the normalized sensitivity coefficients for a steady state may be computed in software applications such as
  - COPASI (http://www.copasi.org) or
  - SBML-SAT (http://sysbio.molgen.mpg.de/SBML-SAT), a tool for MATLAB (http://www.mathworks.com/).
• Note: a similar analysis can also be done for the dependency with respect to the initial conditions
  o Skip it here
Local sensitivity analysis – example

- Model: $A \rightarrow B$, $B \rightarrow A$

- ODEs:
  \[
  \frac{d[A]}{dt} = -k_1[A] + k_2[B] \\
  \frac{d[B]}{dt} = k_1[A] - k_2[B]
  \]

- Numerical setup:
  $[A](0) = 10 \, \text{mmol/ml},$  
  $[B](0) = 10 \, \text{mmol/ml},$  
  $k_1=0.5 \, \text{s}^{-1},$  
  $k_2=0.3 \, \text{s}^{-1}$

- Steady state concentrations:
  $[A]_{ss} = 7.5 \, \text{mmol/ml},$  
  $[B]_{ss} = 12.5 \, \text{mmol/ml}$
Local sensitivity analysis

• Scaled stationary sensitivity coefficients:

<table>
<thead>
<tr>
<th>C_{ij}</th>
<th>k_1</th>
<th>k_2</th>
</tr>
</thead>
<tbody>
<tr>
<td>[A]</td>
<td>-0.62461</td>
<td>0.624766</td>
</tr>
<tr>
<td>[B]</td>
<td>0.374766</td>
<td>-0.374859</td>
</tr>
</tbody>
</table>

• Interpretation:
  - increasing $k_1$ from 0.5 by 1% yields a decrease in the level of $[A]$ at the steady state by $\sim 0.6\%$.
  - Note: these results are accurate only for infinitesimally small changes!
Local sensitivity analysis

• Verification (1% change):
  o \(k_1\) set to 0.505 (increase by 1% with respect to the original value)
  o new steady state:
    \([A]_{\text{new ss}}=7.45342 \text{ mmol/ml}, [B]_{\text{new ss}}=12.5466 \text{ mmol/ml}\)
  o \((A)_{\text{new ss}}-[A]_{\text{ss}}/[A]_{\text{ss}}=7.45342/7.5-1 \approx 0.993789-1 \approx -0.006 = -0.6\%\)
  o \((B)_{\text{new ss}}-[B]_{\text{ss}}/[B]_{\text{ss}}=12.5466/12.5-1=1,003728-1 \approx 0.37\%\)

  o To be compared with \(C_{11}= -0.62461(\%)\) and \(C_{21}= 0.374766(\%)\)

• Verification (10% change):
  o \(k_2\) set to 0.33 (increase by 10% with respect to the original value)
  o new steady state:
    \([A]_{\text{new ss}}=7.95181 \text{ mmol/ml}, [B]_{\text{new ss}}=12.0482 \text{ mmol/ml}\)
  o \((A)_{\text{new ss}}-[A]_{\text{ss}}/[A]_{\text{ss}}=7.95181/7.5-1 = 1,06024-1 = 6,024\%\)
  o \((B)_{\text{new ss}}-[B]_{\text{ss}}/[B]_{\text{ss}}=12.0482/12.5-1=0,963856-1 = -3,61\%\)

To be compared with \(10 \times C_{21} = 6.24766(\%)\) and \(10 \times C_{22} = -3.74859(\%)\)