Fast computation of gradient and sensitivity in 13C metabolic flux analysis instationary experiments using the adjoint method

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Abstract

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1. Motivation

The overall dynamics of a CLE can be described by a cascade of differential equations of the following form (see e.g. [1]):

\[ \dot{X}_k(m) x_k = f_k(v, x_k, \ldots, x_1, x_k^{\text{input}}), \quad k = 1 \ldots n, \quad t \in [0, T] \]  

(1)

where the states \( x_k \) are functions of time \( t \) and take their values in \( \mathbb{R}^{n_k} \) (they represent the cumomer fractions of each metabolite) and the constant vectors \( x_k^{\text{input}} \) are vectors of \( \mathbb{R}^{n_k^{\text{input}}} \) depend on the labeling of the input substrates. The vector \( v \in \mathbb{R}^m \) denotes the unknown fluxes and \( X_k \) are diagonal matrices containing unknown pool sizes corresponding to to cumomer fractions of weight \( k \).

The particular form taken by the functions \( f_k \) depends on the transition pattern of carbon atoms occurring for each reaction in the metabolic network. Writing down by hand the expression of these functions is quite easy for a small sample network but becomes untractable for a realistic network. As far as numerical computations are concerned (direct problem solving or identification) the real concern is to write some specific computer code computing these functions and their exact derivatives with respect of states \( x_1, \ldots, x_n \) and \( v \), in a target language. The formal expression of the overall system could be interesting for testing the identifiability of the flux vector \( v \) and the pool sizes, but previous work shows that the size of realistic networks prevents the use of classical algorithms based on symbolic computations.

Nowadays, the most efficient way of describing a metabolic network is to use the Systems Biology Markup Language (SBML, see [2], [3]), as it has become the de facto standard, used by a growing number of commercial or...
open source applications. The SBML markup language is a dedicated dialect of XML (see e.g. [4]) with a specific structure which allows to describe the different compartments, species, and the kinetics of reactions occurring between these species. Transformations can be applied to the SBML file describing the network, described in another XML dialect, the eXtendted Stylesheet Language (XSL), and the kind of transformations we are interested in, are those which will allow to generate the specific numerical code we need to solve the identification problem (stationary and instationary). The generated computer code is specific for each particular metabolic network and associated CLE, and thus more efficient, readable and resusable, than a general application written to cover all possible cases.

The target language which has been chosen is Scilab (see [5,6]) because it is an open-source Matlab compatible language, allowing high-level programming together with compiled libraries with performant differential equation solvers, optimization routines and efficient sparse matrix algebra. The GUI of the final application is also described in XML, using another specific dialect called XMLlab (see [7]), which is available under the form of an official Scilab Toolbox (see Scilab www site). The description of the GUI is obtained with another pass of XSL transformations on the original SBML file describing the network. This GUI allows the biologist to enter the input data (label input of the substrate, known fluxes or a priori relationships between them, label observation), and launch the optimization process solving the identification problem.

Throughout this paper, we will use a very small example to illustrate our approach. This is the branching network used by Wiechert and Isermann in [8] (see Figure 1). The SBML file corresponding to this network can be found in Figures A.1 and A.2 in the appendix section. As it can be seen, the description is very verbose. The added material describing some informations on the CLE (label input, label observation, carbon atom mapping) are entered in the species and reaction notes, directly from the CellDesigner interface. In the future, we plan to develop a plugin to directly enter these information from within CellDesigner and create XML annotation in the SBML file.

2. Mathematical modelling in the stationary case

It has been shown in [1] that the actual state equation is in fact a succession of linear ordinary differential equation, where for each $k$ the non homogeneous part $b_k$ of the right handside depends on $x_1, \ldots, x_{k-1}$, giving the following cascade

$$X_k(m)x_k(t) = M_k(v)x_k(t) + b_k(v, x_{k-1}(t), \ldots, x_1(t), x_k^{\text{input}}), \quad k = 1 \ldots n, \quad t > 0.$$  

Each component of vectors $x_k(t)$ represents a cumomer fraction of weight $k$ of a given species (for a proper definition of cumomer and cumomer weight see [1]). The constant vectors $x_k^{\text{input}}$ contain the cumomer fractions of weight $k$ of species which are input metabolites. The diagonal matrices $X_k(m)$ depend on the stationary concentrations.
of metabolites. The matrix $M_k(v)$ and the vector $b_k$ are constructed by considering the balance equation for each cumomer of the vector $x_k$. Constructing these matrix by hand is a very tedious task but can be automated if adequate data structures are used, in order to represent the metabolic network and the carbon transition map for each reaction (we will explain later how we deal with this particular information).

In the stationary case, the CLE is considering the asymptotic behaviour of the system (2),

$$0 = M_k(v)x_k + b_k(v, x_{k-1}, \ldots, x_1(t), x_k^{input}), \quad k = 1 \ldots n,$$

in this case the states $x_k$ do not depend on time anymore and the identification problem is restricted to the determination of the flux vector $v$ such that some cost function is minimized. For a given flux vector $v$ this cost function can be classically defined as the squared norm of the difference between an observation vector $y^{meas} \in \mathbb{R}^{n_{meas}}$ and the corresponding synthetic observation $y(v)$ computed by solving the state equation (3) for the given value of the fluxes. In the following, we will consider that this observation is composed of isotopomer and cumomer fractions of given species, which can always be computed as linear combination of cumomers, i.e. there exists $n$ non zero matrices $C_1, C_2, \ldots, C_n$ such that

$$y(v) = \sum_{k=1}^n C_kx_k(v),$$

where we have used $x_k(v)$ to denote the solutions of the state equation (3) for a given flux vector $v$.

We have also natural constraints on the flux vector which result of the particular structure of the metabolic network (the stoichiometric balances) and some other kinds of linear constraints on the fluxes, which can express that some of the fluxes are fixed, for example the flux of input substrate, or some more specific information, e.g. some linear combination of fluxes which should be zero. Thus, the constraints on $v$ take an affine form

$$Av = w.$$

There is usually some measured extracellular fluxes $v^{meas}$, which has to be compared with the actual value of these fluxes, which can be always be expressed as linear function of $v$. The mostly used cost function is the Chi-Square function

$$J(v) = \frac{1}{2} \sigma^{-1} (y(v) - y^{meas})^2 + \frac{1}{2} \alpha^{-1} (Ev - v^{meas})^2$$

where $\sigma$ and $\alpha$ are diagonal positive definite matrices containing the standard deviation for each observation. Minimizing the Chi-Square function, under the hypothesis of gaussian distribution, is equivalent to maximizing the likelihood of measurements.

$$\begin{cases}
\hat{v} = \arg \min_{v \in \mathbb{R}^m} J(v), \\
Av = w, \\
v \geq 0,
\end{cases}$$

(\text{P}_\varepsilon)

where $y(v) = \sum_{k=1}^n C_kx_k(v)$ and $x_k(v), \quad k = 1 \ldots n$ are the solutions of the state equation (3).

2.1. \textit{Parametrisation of the admissible fluxes subspace}

The subspace of admissible fluxes is determined by the system of equations and inequations

$$\begin{cases}
Av = w, \\
v \geq 0,
\end{cases}$$

(4)

In order to detect any redundancy or incompatibilities due to the eventual complimentary constraints added by the user, an admissibility test is done on the system. We do it by solving a trivial linear program, which allows to test if $w$ is in the range of $A$ and then if the subspace (4) is non-void.
Then there are two possibilities to obtain a parametrization: by computing an orthonormal basis \( \{V^1, \ldots, V^r\} \) of the kernel of \( A \) (where \( p = \text{rank } A \)) and the minimum norm solution \( v_0 \) of \( Av = w \), any \( v \) satisfying (4) can be expressed as
\[
v = Vq + v_0,
\]
where \( q \) is a vector of size \( m - p \).

The classical parametrization, using the free fluxes, can be found by computing the QR factorization of \( A \). There exists an \( m \times m \) permutation matrix \( P = [P_1, P_2] \) and an orthogonal square matrix \( Q = [Q_1, Q_2] \), where \( P_1 \) and \( Q_1 \) represent the first \( p \) columns of \( P \) and \( Q \), and a full rank \( p \times p \) upper-triangular matrix \( R_1 \) such that
\[
AP = Q \begin{bmatrix} R_1 & R_2 \\ 0 & 0 \end{bmatrix},
\]
where the lower right zero block is absent if \( A \) has full rank. The free fluxes in the vector are given by \( q = P_2^T v \) and the complimentary dependent fluxes are given by \( P_1^T v \). Straightforward computations give the parametrization \( v = Vq + v_0 \), where \( q \) has \( m - p \) components and
\[
V = P_2 - P_1R_1^{-1}R_2, \quad v_0 = P_1R_1^{-1}Q_1^Tw.
\]

Such parametrizations remove any redundancy in the constraints, and allow to identify which constraints in \( Vq + v_0 \geq 0 \) are equality constraints blocking the value of some fluxes. Some of them are not specified in the initial system (4) but are added de facto in the parametrization because of the implicit fluxes balancing constraints. This situation can be detected when a row of \( V \) is equal to zero. Hence, if we define the sets \( I = \{i, \ V_i \neq 0\} \) and the set \( D \) containing the indices of dependent fluxes, the parametrized optimization problem takes the form
\[
\begin{cases}
\tilde{q} = \arg \min_{q \in \mathbb{R}^{m-p}} J_\varepsilon(Vq + v_0), \\
q_i \geq 0, \ i = 1 \ldots m - p, \\
V_iq + (v_0)_i \geq 0, \ i \in I \cap D,
\end{cases}
\]
and we have \( \tilde{v} = V\tilde{q} + v_0 \). The gradient of the parametrized cost function can be expressed via the chain rule as
\[
\left( \frac{d}{dq} J_\varepsilon(Vq + v_0) \right)^T = V^T \nabla J_\varepsilon(Vq + v_0).
\]
The type of parametrization (orthogonal or free fluxes) used in the optimization does not seem to influence the conditioning of the algorithm, so the free fluxes parametrization is used, because of its biological interpretation.

2.2. Identifiability and regularization

When \( \varepsilon = 0 \) the constraints on \( q \) are not enough to ensure existence of a solution because the problem may be unbounded. In fact, existence and unicity of a solution will occur if the fluxes are identifiable. A general discussion about this subject can be found in [8], where the authors propose an algorithm based on integer arithmetics to test the structural identifiability of metabolic networks. The most encountered problematic situation corresponds to bidirectional reactions, such as
\[
v_1 : A \rightarrow B, \quad v_2 : B \rightarrow A
\]
where \( v_1 - v_2 \) (the net flux) is identifiable but \( v_1 \) and \( v_2 \) are not individually identifiable. The counterpart of such a situation is that the optimal \( v_1 \) and \( v_2 \) tend to infinity when \( \varepsilon \rightarrow 0 \), and the cost function \( J_\varepsilon \) is ill-conditioned when \( \varepsilon \) is too small, leading to convergence problems in the optimization phase. The change of variables proposed in [9] considers the net flux \( v_{\text{net}} = v_1 - v_2 \) and the exchange fluxes \( v_{\text{xch}} = \min(v_1, v_2) \) and a "compacification" of \( v_{\text{xch}} \) defined by
\[
v_{[0,1]} = \frac{v_{\text{xch}}}{\beta + v_{\text{xch}}},
\]
where \( \beta > 0 \). The above change of variables maps \([0, +\infty]\) to \([0, 1]\) and thus is interesting from a numerical point of view. Although these new variables make sense from a metabolic point of view, it remains that the overall mapping from \((v_1, v_2)\) to \((v_{net}, v_{[0,1]})\) is not differentiable and thus needs to be approximated. A more systematic approach is proposed in [10] where all free fluxes \( q_i \geq 0 \) are mapped to \( r_i \in [0, 1] \) with the change of variables \( q = q(r) \), where

\[
q_i = \beta \frac{r_i}{1 - r_i}, \quad i = 1 \ldots m - p,
\]

where \( \beta > 0 \) is a scaling parameter. In this case, the inequality constraints in (5) become non-linear and the new optimization problem is

\[
\begin{align*}
\hat{r} &= \arg \min_{r \in \mathbb{R}^{m-p}} J_e(Vq(r) + v_0), \\
1 - \delta \geq r_i &\geq 0, \quad i = 1 \ldots m - p, \\
Vq(r) + (v_0), &\geq 0, \quad i \in I \cap D,
\end{align*}
\]

(6)

where \( \delta > 0 \) can be arbitrary small. The gradient of the cost function is given by

\[
\left( \frac{d}{d\tilde{r}} J_e(Vq(r) + v_0) \right)^\top = q'(r)^\top V^\top J_e(Vq(r) + v_0).
\]

2.3. Multiple experiences

In the following we will also consider the case where multiple CLE are done with the same metabolic network but with different labeling of the input metabolites, given by \( x_{input,i} \) for \( i = 1 \ldots n_{exp} \). Thus, we will consider the cost function

\[
J_e(v) = \frac{1}{2} \sum_{i=1}^{n_{exp}} \left( \| \sigma^{-1} \left( y(v, x_{input,i}) - y_{meas,i} \right) \|^2 + \| \alpha^{-1} \left( Ev - v_{obs,i} \right) \|^2 \right) + \frac{c}{2} \| v \|^2
\]

where \( y_{meas,i} \) is the observation of labeled material for experience \( i \), \( v_{obs,i} \) is the vector of measured extracellular fluxes and

\[
y(v, x_{input,i}) = \sum_{k=1}^{n} C_k x_k(v, x_{input,i})
\]

and \( x_k(v, x_{input,i}), k = 1 \ldots n \) is the solution of

\[
0 = M_k(v)x_k + b_k(v, x_{k-1}, \ldots, x_1(t), x_{k}^{input,i}), \quad k = 1 \ldots n,
\]

(7)

2.4. Computation of the gradient of the cost function

The computation of the gradient of \( J(v) \) needs the derivative of \( x(v) \) with respect to \( v \). In the stationary case, it makes sense to compute this derivative by implicit differentiation of the state equation (3). To this purpose, we adopt the notation

\[
f_k(v, x_k, \ldots, x_1, x_{k}^{input,i}) = M_k(v)x_k + b_k(v, x_{k-1}, \ldots, x_1, x_{k}^{input,i})
\]

and

\[
x_k(v, x_k, \ldots, x_1, x_{k}^{input,i}) = 0, \quad k = 1 \ldots n.
\]

By differentiating these equations with respect to \( v \), when \( x = x^i(v) \), we obtain for \( k = 1 \ldots n \)

\[
0 = \frac{df_k}{dv}(v, x_k, \ldots, x_1, x_{k}^{input,i}) = \frac{\partial f_k}{\partial v}(v, x_k, \ldots, x_1, x_{k}^{input,i}) + \sum_{l=1}^{k} \frac{\partial f_l}{\partial x_l}(v, x_k, \ldots, x_1, x_{k}^{input,i}) \frac{\partial x_l^i}{\partial v}.
\]

(9)
Hence, the key ingredients in the computation of the derivatives of \( \mathbf{x}_k \) whose right hand side is a function of \( \mathbf{x}_l \) for \( l = 1 \ldots k \):

\[
M_k(\mathbf{v}) \frac{\partial \mathbf{x}_k}{\partial \mathbf{v}} = \frac{\partial f_k}{\partial \mathbf{v}}(\mathbf{v}, \mathbf{x}_k, \ldots, \mathbf{x}_1, \mathbf{x}_k^{\text{input},i}) - \sum_{l=1}^{k-1} \frac{\partial b_k}{\partial \mathbf{x}_l}(\mathbf{v}, \mathbf{x}_k, \ldots, \mathbf{x}_1, \mathbf{x}_k^{\text{input}}) \frac{\partial \mathbf{x}_l}{\partial \mathbf{v}}, \quad k = 1 \ldots n. \tag{10}
\]

Hence, the key ingredients in the computation of the derivatives of \( \mathbf{x}_k \) are the derivatives \( \frac{\partial b_k}{\partial \mathbf{x}_l} \) for \( l < k \) and the derivatives \( \frac{\partial f_k}{\partial \mathbf{v}} \). This will be one of the main tasks of the automatically generated computer code, together with the assembly of matrices \( M_k(\mathbf{v}) \). Since the gradient of the cost function \( J(\mathbf{v}) \) will be required at each iteration of the optimization algorithm, these matrix will be assembled as sparse matrices in order to speed up the computations, particularly the resolution of the linear systems (10).

The final computation of the derivative gives

\[
\frac{dJ_c(\mathbf{v})}{\partial \mathbf{v}} = (E\mathbf{v} - \mathbf{v}_{\text{obs},i})^\top \alpha^{-2}\mathbf{E} + \varepsilon \mathbf{v}^\top + \sum_{i=1}^{n_{\text{exp}}} (y(\mathbf{v}, \mathbf{x}_i^{\text{input},i}) - y_{\text{meas}})^\top \sigma^{-2} \sum_{k=1}^{n} C_k \frac{\partial \mathbf{x}_k}{\partial \mathbf{v}}.
\]

3. Architecture of the computer code generation algorithms

The most innovative aspect of this work is the choice of the techniques to generate the code: from the original SBML file edited under Cell Designer (or any other SBML compliant software), only XSL (eXtended Stylesheet Language) transformations are used to generate the Scilab code computing the specific objects for a given Carbon Labeling Experiment. The way transformations are done is described in XSL stylesheets, written in anoth XML dialect. XSL is very different from the typical programming languages in use today. One question that’s being asked frequently is: what kind of programming language is actually XSLT? Until now, the authoritative answer from some of the best specialists is that XSLT is a declarative (as opposed to imperative) language. The XSL stylesheets are thus very explicit, human readable, and easy to debug and maintain.

In the whole process which maps the original SBML file to the computer code and the graphical user interface, successive XSL transformations occur. The first set of transformations aims to translate all the specific information about the CLE into XML markup which can be later used, for example, the carbon atom mapping of each reaction (this step is described in Appendix A). Then two different main paths are followed:

(i) The first series of transformations is dedicated to the computer code generation:

  (a) A main assembly loop is processed, which for each weight \( k \), generates, for \( j = 1 \ldots n_j \), some intermediate XML markup declaring the contribution of each cumomer fraction \( \{ \mathbf{x}_k \}_j \) to the matrices \( M_k(\mathbf{v}), b_k(\mathbf{v}), \frac{\partial f_k}{\partial \mathbf{v}}, \left( \frac{\partial b_k}{\partial \mathbf{x}_l} \right)_{l=1 \ldots k-1} \).

  The contributions to \( M_k(\mathbf{v}) \) are functions of the flux vector \( \mathbf{v} \) only, but the contributions to other matrices are functions of lower weight cumomer components and eventually of \( \mathbf{x}_k^{\text{input}} \), respecting the weight preservation property (see [1]). This intermediate step is event-driven, i.e. contributions to the matrices are dumped in the order they occur. The contributions are gathered for each matrix in the subsequent transformation which produces the Scilab code.

  (b) The Scilab code solving the cascaded linear systems is generated. For each weight \( k \), the matrix \( M_k(\mathbf{v}) \) is stored as a sparse matrix and its sparse \( LU \) factorization is computed (the sparse triangular factors are also retained because they are also needed to compute the derivatives). The linear system giving \( \mathbf{x}_k \) is then solved by using the precomputed sparse \( LU \) factors. Then we compute the matrices \( \frac{\partial f_k}{\partial \mathbf{v}} \) and \( \left( \frac{\partial b_k}{\partial \mathbf{x}_l} \right)_{l=1 \ldots k-1} \), which need the previously computed cumomer vectors \( \mathbf{x}_1, \ldots, \mathbf{x}_{k-1} \) and finally solve the linear system giving \( \frac{\partial \mathbf{x}_k}{\partial \mathbf{v}} \).
(ii) The second series of transformation aims to build the specific graphical user interface for the given metabolic network. To this purpose, an XML file conforming to the XMLlab DTD is generated. The structure of the interface is described in a high level way: there are given sections of the interface, each of these being dedicated to different purposes. The first section is hosting all the fluxes, the second section is hosting the fluxes observations with associated standard deviation, the third section hosts together the label measurements and the label output corresponding to the current fluxes. This is the place where the user can compare the original measurement and the reconstructed measurement after the identification process. The fourth section displays all components of the cumomer vector, sorted by weight and by species name. The last section is reserved to the parameters of the identification method (maximum iteration, regularization parameter, and so on). The structure of the original SBML file, enriched with the specific annotations in the sbml namespace (see Appendix A), allows to perform this step in a very straightforward way.

The generated Scilab code computing the cumomers vector $x$, the derivative matrices, the cost function and its gradient are given on Figures A.6 and A.7 in Appendix A.

4. Numerical results

5. Mathematical modelling in the unstationnary case

In the unstationary case, the CLE is done when the system (2) has not reached its asymptotic behaviour. The measurements

$$y_{\text{meas}, j}, j = 1 \ldots n_t,$$

are done at different values $t_j$ of time, and we can make the hypothesis that the final time $T$ in (2) is equal to the final observation time i.e $T = t_{n_t}$. The fundamental difference with the stationary case is that the stationary concentration of metabolites is also an unknown, i.e. the vector $m$ is also to be determined.

The cost function takes the form

$$J_\varepsilon(v, m) = \frac{1}{2} \sum_{j=1}^{n_t} \left(\| \sigma^{-1} (y(t_j, v, m) - y_{\text{meas}, j}) \|^2 + \frac{1}{2} \| \alpha^{-1} (E v - v_{\text{meas}}) \|^2 \right),$$

where

$$y(t_j, v, m) = \sum_{k=1}^{n} C_k x_k(t_i, v, m),$$

and $x_k(t, v, m), k = 1 \ldots n$ are the solutions of the state equation for a given pair $(v, m)$ of fluxes and pool sizes:

$$X_k(m) \dot{x}_k(t) = M_k(v) x_k(t) + b_k(v, x_{k-1}(t), \ldots, x_1(t), x_{k\text{input}}), k = 1 \ldots n, t > 0. \quad (11)$$

The minimization problem is the following:

$$\begin{align*}
(\hat{v}, \hat{m}) &= \arg \min_{v, m} J_\varepsilon(v, m), \\
A v &= w, \\
v &\geq 0, \\
m &\geq 0.
\end{align*} \quad (P_{\varepsilon}^u)$$

The main difficulty is the computation of the gradient of $J$, which can be done by using the sensitivity matrices $\frac{\partial x_k(t)}{\partial v}$, computed as the solution of a system of differential equations obtained by implicit differentiation of (11) as in the stationary case. The problem is that this approach is computationally intensive (see e.g. [9,11,12]) because it involves a cascade of differential equations where the state is a matrix (instead of a vector).

A more suitable approach for non-stationary problems is to use the adjoint state method (see [13,14,15]). If the number of parameters of interest (the fluxes) exceeds the number of model outputs for which the sensitivity is desired, the adjoint method is more efficient than traditional direct methods of calculating sensitivities. The gradient of $J$
can be computed at the same cost as the state equation (11). A far as the statistical evaluation of identified fluxes is concerned, the sensitivity of the model output \( y(t, v, m) \) with respect to \( v \) can be obtained with a cost of \( n_{\text{meas}} \) state equations. Hence, the adjoint method will always outperform the sensitivity method for the computation of the gradient. For the output sensitivity, the dimension of \( y(t, v, m) \) observations is generally lower than the number of fluxes, so the same method should be used. The adjoint state method is best understood in continuous time and the next section is devoted to this presentation. Section 5.2 will detail its practical implementation in discrete time.

5.1. The adjoint equation in continuous time

In order to simplify the presentation of the results, we will adopt the block notation \( x = (x_1; x_2; \ldots x_n) \) for the overall cumomer vector, and for the state equation we will write

\[
X(m)\dot{x}(t) - f(x(t), v) = 0, \quad t \in [0, T],
\]

(12)

where \( f(x, v) = (f_1(x, v); \ldots f_n(x, v)) \) where \( f_k \) is defined by (8). We also define \( C = [C_1, C_2, \ldots, C_n] \) so that \( y(t) = Cx(t) \). Without loss of generality, we consider only one measurement at final time \( T \).

The adjoint state method allows to compute the total derivative with respect to \( v \) and \( m \) of a given function \( I(x(v, m)) \in \mathbb{R} \) where \( x(v, m) \) is the solution of the state equation (12). If the gradient of \( J_e \) is to be computed then we will take

\[
I(x) = \frac{1}{2} \left\| \sigma^{-1} (Cx(T) - y_{\text{meas}}) \right\|^2.
\]

(13)

In the following, we do not consider the quantities in \( J_e \) depending explicitly on \( v \) hence, we define \( J(v, m) = I(x(v, m)) \). Let us define the Lagrangian

\[
L(x, p, v, m) = I(x) + \int_0^T p(t)^\top (X(m)\dot{x}(t) - f(x(t), v)) \, dt,
\]

(14)

where the adjoint state \( p = (p_1; p_2; \ldots p_n) \) has the same block structure as \( x \). The first remark that can be done is that when \( x \) is the solution of the state equation (12), we have

\[
L(x(v, m), p, v, m) = J(v, m),
\]

and when we express the total derivative of \( J(v, m) \) e.g. with respect to \( v \) we have

\[
\frac{dJ(v, m)}{dv} = \frac{\partial L}{\partial x} (x(v, m), p, v, m) \frac{\partial x(v, m)}{\partial v} + \frac{\partial L}{\partial v} (x(v, m), p, v, m).
\]

(15)

The idea of the adjoint state technique is to compute \( p \) such that \( \frac{\partial L}{\partial x} = 0 \). Then the remaining part of the derivative can be computed in a straightforward way. This adjoint equation is a (backward in time) differential equation given by

\[
X(m)\dot{p}(t) = -\left( \frac{\partial f}{\partial x}(x(t), v) \right)^\top p(t), \quad t \in [0, T],
\]

(16)

with the final condition

\[
p(T) = -C^\top \sigma^{-2} (Cx(T) - y_{\text{meas}}).
\]

(17)

Because of the block triangular structure of \( \frac{\partial f}{\partial x} \), the adjoint equation has also a cascade structure, but in reverse order, i.e. \( p_n \) is obtained at first and \( p_1 \) at last:

\[
p_k(T) = -C_k^\top \sigma^{-2} (Cx(T) - y_{\text{meas}}),
\]

(18)

\[
X_k(m)\dot{p}_k(t) = -M_k^\top p_k(t) + \sum_{l=k+1}^n \left( \frac{\partial b_l}{\partial x_k} \right)^\top p_l(t), \quad t \in [0, T],
\]

(19)

for \( k = 1 \ldots n \).
When for a given pair \((v, m)\) the state equations and the adjoint state equations are solved, then the gradient of \(J\) can be readily computed by using (15)

\[
\frac{dJ(v, m)}{dv} = \frac{\partial L(x, p, v, m)}{\partial v} = \sum_{k=1}^{n} \int_{0}^{T} p_k(t)^{\top} \frac{\partial f_k}{\partial v}(v, x(t)) \, dt.
\]

We will give the derivative with respect to \(m\) in the next section.

**Remark 1** The output sensitivity \(\frac{\partial y(T)}{\partial v}\) can be computed in the same way by taking \(I(x) = Cx(T)\). In this case, we have

\[
\frac{\partial y(T)}{\partial v} = \sum_{k=1}^{n} \int_{0}^{T} p_k(t)^{\top} \frac{\partial f_k}{\partial v}(v, x(t)) \, dt,
\]

where the final condition (18) is replaced by \(p_k(T) = -C_k^{\top}\) and the adjoint equation (19) is unchanged, but \(p_k(t)\) is a matrix of size \(n_k \times n_{meas}\).

### 5.2. The adjoint equation in discrete time

The previous sections shows that once the state equation is solved, the gradient of \(J\) can be computed at the cost of one more differential equation (16), which has to be compared to the cost of computing the sensitivity function. But the practical implementation needs to reconsider this approach in discrete time, since we cannot just approximate independently the continuous state and the continuous adjoint state equation, i.e. the discretized adjoint must be the adjoint of the discrete state. This is a reason why high order integration schemes are seldom used in adjoint codes written by hand (otherwise automatic differentiation can be used, see [16]) since the discrete adjoint is obtained by formal derivation of the state integration scheme. Since we have to consider that the state equation could be stiff because of eventual large values of fluxes, a good compromise is the implicit trapezoidal rule, which is of order 2.

Hence, we consider a discretization of the interval \([0, T]\) by considering \(t_i = (i-1)h\), for \(i = 1 \ldots N\) and \(h = T/(N-1)\), and we denote by \(x^i\) the approximation of \(x(t_i)\). The implicit trapezoidal rule applied to equation (12) gives

\[
D(m)(x^{i+1} - x^i) - \frac{h}{2}(f(x^{i+1}, v) + f(x^i, v)) = 0, \quad i = 1 \ldots N - 1,
\]

and we still denote by \(x(v, m)\) the solution of (20). We consider that for each measurement time \(\{\tau_j\}_{1 \leq j \leq n_s}\) there exists \(\theta(j)\) such that \(\tau_j = t_{\theta(j)}\), with \(\theta(n_s) = N\), and we define the cost function \(J(v, m) = I(x(v, m))\) where

\[
I(x) = \frac{1}{2} \sum_{j=1}^{n_s} \sigma^{-1} \left( Cx^{\theta(j)} - y^{meas, j} \right)^{\top} \sigma^{-1} \left( Cx^{\theta(j)} - y^{meas, j} \right).
\]

The discrete Lagrangian is defined by

\[
L(x, p, v, m) = I(x) + \sum_{i=1}^{N-1} (p^i)^{\top} (D(m)(x^{i+1} - x^i) - \frac{h}{2}(f(x^{i+1}, v) + f(x^i, v))),
\]

where \(p^i\) is the adjoint state for time \(i\). Straightforward computations show that the adjoint equation is given by

\[
\left( X(m) - \frac{h}{2} \frac{\partial f}{\partial x}(x^i, v)^{\top} \right) p^{i-1} = \left( X(m) + \frac{h}{2} \frac{\partial f}{\partial x}(x^i, v)^{\top} \right) p^i - \left( \frac{\partial I}{\partial x^i} \right)^{\top}, \quad 1 < i < N,
\]

with the final condition

\[
\left( X(m) - \frac{h}{2} \frac{\partial f}{\partial x}(x^N, v)^{\top} \right) p^{N-1} = -\left( \frac{\partial I}{\partial x^N} \right)^{\top},
\]

where \(\frac{\partial I}{\partial x^i} = 0\) if \(\theta(j) \neq i\) for all \(j = 1 \ldots n_s\) and

\[
\frac{\partial I}{\partial x^i} = - \left( Cx^i - y^{meas, \theta^{-1}(i)} \right)^{\top} \sigma^{-2} C
\]
otherwise. Once the state and the adjoint state equations are solved, the gradient is given by

\[
\left( \frac{dJ(v, m)}{dv} \right)^\top = -\frac{\hbar}{2} \sum_{i=1}^{N-1} \left( \frac{\partial f}{\partial v}(x^{i+1}, v) + \frac{\partial f}{\partial v}(x^i, v) \right)^\top p^i,
\]

and

\[
\left( \frac{dJ(v, m)}{dm} \right)^\top = \sum_{i=1}^{N-1} \left( \frac{\partial}{\partial m} (X(m)(x^{i+1} - x^i)) \right)^\top p^i,
\]

where for a given vector \( z \) the matrix \( \frac{\partial}{\partial m} (X(m)z) \) is defined by

\[
\left( \frac{\partial}{\partial m} (X(m)z) \right)_{ij} = \begin{cases} 
z_i, & \text{if the cumomer fraction } z_i \text{ belongs to metabolite } j, \\
0, & \text{otherwise.} \end{cases}
\]

(24)

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Appendix A. Workflow of SBML markup to Scilab code

The specific annotation in the private namespace `xmlns:smtb="http://www.utc.fr/sysmetab"` namespace concerning the carbon atom mapping of each reaction is done as follows: for example, for the reaction corresponding to flux \( v_2 \),
\( v_2: \ A \rightarrow D + D \)

\(#ij \rightarrow #i + #j\)

some specific markup is generated from the string \( \text{IJ} \rightarrow I+J \) found in the reaction \(<\text{notes}>\) element, as depicted in Figure A.3.

For each intermediate species, we also add some markup specifying the exhaustive list of its cumomers. For example, for species \( A \) in the branching network, we have the cumomers \( A_{x1}, A_{1x}, A_{11} \) (we don’t take into account \( A_{xx} \) which is equal to 1), and the \(<\text{species}>\) element corresponding to \( A \) is enriched as depicted in Figure A.4. Each \(<\text{smtb:cumomer}>\) element has an id of the form \( A_n \) where \( n \) is equal to the number whose base 2 representation is equal to the cumomer pattern when replacing the \( x \)'s by zeros. Each \(<\text{smtb:carbon}>\) element denotes a 13 neutrons isotope at position given by the \text{position} attribute in the molecule.

When we consider the vectors of intermediate species cumomer fractions \( x_k \) for weights up to 2 for the branching network, we have

\[
x_1 = \left( A_{x1}, A_{1x}, D_1, F_{x1}, F_{1x} \right)^T, \quad x_2 = (A_{11}, F_{11})^T
\]

A redundant enumeration is also generated (see the \(<\text{smtb:listOfIntermediateCumomers}>\) element on Figure A.5) giving the ordering of all cumomers sorted by weight, allowing to keep the correspondence between components of vectors \( x_1, x_2 \) and corresponding species cumomers (this information is needed in the subsequent transformations). A similar enumeration is also generated for input species cumomers in the \(<\text{smtb:listOfInputCumomers}>\), giving the correspondence between the components of vectors \( x_k^{\text{input}} \) and corresponding cumomers:

\[
x_1^{\text{input}} = (A_{\text{out}x1}, A_{\text{out}1x})^T, \quad x_2^{\text{input}} = (A_{\text{out}11})^T
\]
<xml version="1.0" encoding="UTF-8"?>
<sbml level="2" version="1" xmlns="http://www.sbml.org/sbml/level2"
  xmlns:ss="http://www.sbml.org/sbml/level2"
  xmlns:ns="http://www.sbml.org/2001/ns/celldesigner">
  <model id="branching">
    <listOfCompartments>
      <compartment id="default"/>
    </listOfCompartments>
    <listOfSpecies>
      <species compartment="default" id="A"/>
      <species compartment="default" id="D"/>
      <species compartment="default" id="F">
        <notes>
          <html xmlns="http://www.w3.org/1999/xhtml">
            <body>LABEL_MEASUREMENT 1x,x1,11</body>
          </html>
        </notes>
      </species>
      <species compartment="default" id="G"/>
      <species compartment="default" id="A_out">
        <notes>
          <html xmlns="http://www.w3.org/1999/xhtml">
            <body>LABEL_INPUT 01,10,11</body>
          </html>
        </notes>
      </species>
    </listOfSpecies>
    <listOfReactions>
      <reaction id="v1" reversible="false">
        <notes>
          <html xmlns="http://www.w3.org/1999/xhtml">
            <body>I J &gt; I J</body>
          </html>
        </notes>
        <listOfReactants>
          <speciesReference species="A"/>
        </listOfReactants>
        <listOfProducts>
          <speciesReference species="F"/>
        </listOfProducts>
      </reaction>
      <reaction id="v2" reversible="false">
        <notes>
          <html xmlns="http://www.w3.org/1999/xhtml">
            <body>I J &gt; 1+J</body>
          </html>
        </notes>
        <listOfReactants>
          <speciesReference species="A"/>
        </listOfReactants>
        <listOfProducts>
          <speciesReference species="D" stoichiometry="2.0"/>
        </listOfProducts>
      </reaction>
    </listOfReactions>
  </model>
</sbml>

Figure A.1. The XML file describing the branching network, with added information concerning the carbon mapping for each reaction, and the detail of input and measured label (lines 1 to 61)
Figure A.2. The XML file describing the branching network, with added information concerning the carbon mapping for each reaction, and the detail of input and measured label (lines 62 to 121).
Figure A.3. Fragment of the XML file transformed after the first set of XSL transformation: the atom mapping is translated into XML markup in the namespace `smtb`.

```xml
<reaction position="2" id="v2" name="v2" reversible="false">
  <listOfReactants>
    <speciesReference species="A">
      <smtb:carbon position="2" destination="1" occurrence="1" species="D"/>
      <smtb:carbon position="1" destination="1" occurrence="2" species="D"/>
    </speciesReference>
    <listOfProducts>
      <speciesReference species="D">
        <smtb:carbon position="1" destination="2" occurrence="1" species="A"/>
      </speciesReference>
      <speciesReference species="D">
        <smtb:carbon position="1" destination="1" occurrence="1" species="A"/>
      </speciesReference>
    </listOfProducts>
  </listOfReactants>
</reaction>
```

Figure A.4. Fragment of the XML file transformed after the first set of XSL transformation: the cumomers of a given intermediate species are named and their labeling is described using `smtb:carbon` elements. in the namespace `smtb`.

```xml
<species compartment="default" id="A" name="A" type="intermediate" carbons="2">
  <smtb:cumomer id="A_1" species="A" weight="1" pattern="x1">
    <smtb:carbon position="1"/>
  </smtb:cumomer>
  <smtb:cumomer id="A_2" species="A" weight="1" pattern="1x">
    <smtb:carbon position="2"/>
  </smtb:cumomer>
  <smtb:cumomer id="A_3" species="A" weight="2" pattern="11">
    <smtb:carbon position="2"/>
    <smtb:carbon position="1"/>
  </smtb:cumomer>
</species>
```
Figure A.5. Fragment of the XML file transformed after the first set of XSL transformation: enumeration of all cumomers sorted by weight and type (intermediate or input species).
function [x1, x2, dx1_dv, dx2_dv] = solveCumomers(v, x1_input, x2_input)

n1 = 5; // Weight 1 cumomers
M1_ijv = [1, 1, -(v(1)+v(2)+v(3))
2, 2, -(v(1)+v(2)+v(3))
3, 2, v(2)
3, 1, v(2)
3, 3, -(v(4)+v(4))
4, 1, v(1)
4, 2, v(3)
4, 3, v(4)
4, 4, -v(5)
5, 2, v(1)
5, 1, v(3)
5, 3, v(4)
5, 5, -v(5)];
M1 = sparse(M1_ijv(:, 1:2), M1_ijv(:, 3), [n1, n1]);
b1_ijv = [1, 1, v(6)*x1_input(1, :)
2, 1, v(6)*x1_input(2, :)];
b1_l = s_full(b1_ijv(:, 1:2), b1_ijv(:, 3), [n1, 1]);

[M1_handle, M1_rank] = lu_fact(M1);
x1 = lusolve(M1_handle, -[b1_l]);

df1_dv_ijv = [1, 6, x1_input(1, :)
1, 1, -x1(1, :)
1, 2, -x1(1, :)
1, 3, -x1(1, :)
2, 6, x1_input(2, :)
2, 1, -x1(2, :)
2, 2, -x1(2, :)
2, 3, -x1(2, :)
3, 2, x1(2, :)
3, 2, x1(1, :)
3, 4, -x1(3, :)
3, 4, -x1(3, :)
4, 1, x1(1, :)
4, 3, x1(2, :)
4, 4, x1(3, :)
4, 5, -x1(4, :)
5, 1, x1(2, :)
5, 3, x1(1, :)
5, 4, x1(3, :)
5, 5, -x1(5, :)];
df1_dv_l = s_full(df1_dv_ijv(:, 1:2), df1_dv_ijv(:, 3), [n1, 6]);
dx1_dv(:, 1) = lusolve(M1_handle, -df1_dv_l);
ludel(M1_handle);

n2 = 2; // Weight 2 cumomers
M2_ijv = [1, 1, -(v(1)+v(2)+v(3))
2, 1, v(1)
2, 1, v(3)
2, 2, -v(5)];
M2 = sparse(M2_ijv(:, 1:2), M2_ijv(:, 3), [n2, n2]);
b2_ijv = [1, 1, v(6)*x2_input(1, :)
2, 1, v(4)*x1(3, :)*x1(3, :)];
b2_l = s_full(b2_ijv(:, 1:2), b2_ijv(:, 3), [n2, 1]);

[M2_handle, M2_rank] = lu_fact(M2);
x2 = lusolve(M2_handle, -[b2_l]);

Figure A.6. The generated Scilab code for the Branching example (continued on Figure A.7)
df2_dv_ijv=[1,6,x2_input(1,:)]
1.1.-x2(1,:)
1.2.-x2(1,:)
1.3.-x2(1,:)
2.1.x2(1,:)
2.3.x2(1,:)
2.4.x1(3,:).*x1(3,:)
2.5.-x2(2,:));
df2_dv_l=full(df2_dv_ijv(:,1:2),df2_dv_ijv(:,3),[n2,6]);
db2_dx1_ijv=[2,3,x1(3,:).*v(4)
2,3,x1(3,:).*v(4)];
db2_dx1_l=sparse(db2_dx1_ijv(:,1:2),db2_dx1_ijv(:,3),[n2,n1]);
dx2_dv=zeros(n2,6,1);
dx2_dv(:,:,:)=lusolve(M2_handle,-(df2_dv_l+db2_dx1_l*dx1_dv(:,:,1)));
endfunction

function [cost,grad]=costAndGrad(v)
[x1,x2,dx1_dv,dx2_dv]=solveCumomers(v,x1_input,x2_input);
e_label=(C1*x1+C2*x2)-yobs;
e_flux=E*v-vobs;
cost=0.5*(sum(delta.*e_flux.^2)+sum(alpha(:,:,1).*e_label(:,:,1).^2));
grad=(delta.*e_flux)'*E+(alpha(:,:,1).*e_label(:,:,1))'*(C1*dx1_dv(:,:,1)+C2*dx2_dv(:,:,1));
endfunction

Figure A.7. The generated Scilab code for the Branching example (begining of code is on Figure A.6)
Appendix B. Computation of the gradient in the non stationnary case

The discrete state equation in its cascade form is easily obtained from (20) and the definition of \( f \) as

\[
\left( X_k - \frac{h}{2} M_k \right) x_i^{k+1} = \left( X_k + \frac{h}{2} M_k \right) x_i^k + \frac{h}{2} \left( b_k(x^i) + b_k(x^{i+1}) \right), \quad 1 \leq i < N, \quad (B.1)
\]

for \( k = 1 \ldots n \). We recall that \( b_k(x) \) only depends on \( x_l \) for \( l < k \), so that the right-hand side of (B.1) is already known at stage \( k \). To obtain \( x_i^{k+1} \) at each time step \( i \) we just have to solve a sparse linear system with a matrix whose \( LU \) factors need to be determined only once before the iterations. The cascade structure of discretized state and adjoint state equations is easily recovered. The discretized state equations (20) for weights \( k = 1 \ldots n \), by

\[
\left( X_k - \frac{h}{2} M_k^T \right) p_N^{k-1} = \frac{h}{2} \sum_{l=k+1}^n \left( \frac{\partial b_l}{\partial x_k} (x^N) \right)^T p_N^{l-1} - \frac{\partial I}{\partial x_k^N}, \quad (B.2)
\]

\[
\left( X_k - \frac{h}{2} M_k^T \right) p_i^{k-1} = \left( X_k + \frac{h}{2} M_k^T \right) p_i^k + \frac{h}{2} \sum_{l=k+1}^n \left( \frac{\partial b_l}{\partial x_k} (x^i) \right)^T (p_i^l + p_i^{l-1}), \quad 1 < i < N. \quad (B.3)
\]

As in the continous case, the adjoint states \( p_k \) are obtained in decreasing weight order. The two components of the gradient are finally obtained by

\[
\left( \frac{dJ(v, m)}{dv} \right)^T = \left( \frac{h}{2} \sum_{i=1}^{N-1} \sum_{k=1}^n \left( \frac{\partial f_k}{\partial x_i} (x^{i+1}) + \frac{\partial f_k}{\partial x_i} (x^i) \right)^T p_k^i \right),
\]

and

\[
\left( \frac{dJ(v, m)}{dm} \right)^T = \sum_{i=1}^{N-1} \sum_{k=1}^n \left( \frac{\partial}{\partial m} (D_k(m) (x_{i+1}^k - x_i^k)) \right)^T p_k^i,
\]

where for a given vector \( z \in \mathbb{R}^{n_k} \) the matrix \( \frac{\partial}{\partial m} (X_k(m)z) \) is defined by

\[
\left( \frac{\partial}{\partial m} (X_k(m)z) \right)_{ij} = \begin{cases} 
  z_i, & \text{if the cumomer fraction } z_i \text{ belongs to metabolite } j, \\
  0, & \text{otherwise.} 
\end{cases} \quad (B.4)
\]