Computing path vectors

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INTRODUCTION

[klamt_elementary_2017].

During this tutorial, you will learn how to compute elementary (flux) modes / elementary (flux) vectors and a minimal generating set (convex basis) of flux cones or flux polyhedra associated with mass-flow networks [1-2-3].

Let $S \in R^{m \times n}$ be a stoichiometric matrix of a metabolic network with m metabolites and n reactions. The steady-state assumption leads to

$$Sv = 0 \qquad (1)$$

where v is the n-dimensional vector of net reaction rates. Therefore, each flux vector v satisfying equation (1) is in the nullspace of S. Let Δ be the set of all Irreversible reactions of the aforementioned biochemical network, i.e.,

$$v_i \geq 0 \ \forall i \in \Delta \qquad (2)$$

The set Φ of flux vectors v satisfying equations (1) and (2)

$$\Phi = \{v \in R^n | Sv = 0, \ v_i \geq 0 \ \forall \ i \in \Delta\} \qquad (3)$$

is a subset of the nullspace of S, which is called convex polyhedral cone or flux cone. *Elementary modes* are defined as the nonzero vectors of the flux cone Φ with minimal support. Extreme pathways are a set of convex basis vector for stoichiometric matrix S [2]. From a geometrical point of view, extreme pathways are generator vectors (edges) of the cone Φ. As opposed to the computation of elementary modes, one needs to decouple reversible reactions into two irreversible reactions for computing extreme pathways. Thus, extreme pathways are a subset of elementary modes.

We introduce an interface to software that enables the computation of the elementary modes, or extreme pathways, given a network and user-defined reaction bounds.

MATERIALS

- *Please ensure that the COBRA Toolbox has been properly installed and initialised.*
- *Also, you should install CNA (CellNetAnalyzer) software and initialise it. CNA web site (with manual):* https://www2.mpi-magdeburg.mpg.de/projects/cna/cna.html

EQUIPMENT SETUP

Requirements for using CellNetAnalyzer are:

- MATLAB Version 7.5 (Release 18) or higher.

- some functions require an LP or (M)ILP solver; CNA supports the optimization toolbox of MATLAB, GLPKMEX, and CPLEX).
- More information can be found on: https://www2.mpi-magdeburg.mpg.de/projects/cna/cna.html where also a how-to tutorial on CellNetAnalyzer is provided.

PROCEDURE

Before you start with these codes, you should initialise The COBRA Toolbox and CNA software by the following commands

```
initCobraToolbox;
```

```
                                                                       |
     _____   _____   _____   _____      _____                          |
    /  ___| /  _  \ |  _  \ |  _  \    / ___ \                         |   COnstraint-Based Reconstruction and Analysis
    | |     | | | | | |_| | | |_| |   | |___| |                        |   The COBRA Toolbox - 2017
    | |     | | | | |  _  { |  _  /   |  ___  |                        |
    | |___  | |_| | | |_| | | | \ \   | |   | |                        |   Documentation:
    \_____| \_____/ |_____/ |_|  \_\ |_|   |_|                        |   http://opencobra.github.io/cobratoolbox
                                                                       |

> Checking if git is installed ...  Done.
> Checking if the repository is tracked using git ...  Done.
> Checking if curl is installed ...  Done.
> Checking if remote can be reached ...  Done.
> Initializing and updating submodules ... Done.
> Adding all the files of The COBRA Toolbox ...  Done.
> Define CB map output... set to svg.
> Retrieving models ...   Done.
> TranslateSBML is installed and working properly.
> Configuring solver environment variables ...
  - [-*--] ILOG_CPLEX_PATH: /Users/bmp00065/Applications/IBM/ILOG/CPLEX_Studio1271/cplex/matlab
  - [----] GUROBI_PATH :  --> set this path manually after installing the solver ( see instructions )
  - [----] TOMLAB_PATH :  --> set this path manually after installing the solver ( see instructions )
  - [----] MOSEK_PATH :  --> set this path manually after installing the solver ( see instructions )
  Done.
> Checking available solvers and solver interfaces ... Done.
> Setting default solvers ... Done.
> Saving the MATLAB path ... Done.
  - The MATLAB path was saved in the default location.

> Summary of available solvers and solver interfaces

  Support     LP   MILP     QP   MIQP    NLP
------------------------------------------------------------------------
cplex_direct  full              0      0      0      0      -
dqqMinos      full              1      -      -      -      -
glpk          full              1      1      -      -      -
gurobi        full              0      0      0      0      -
ibm_cplex     full              0      0      0      -      -
matlab        full              1      -      -      -      1
mosek         full              0      0      0      -      -
pdco          full              1      -      1      -      -
quadMinos     full              1      -      -      -      1
tomlab_cplex  full              0      0      0      0      -
qpng          experimental      -      -      1      -      -
tomlab_snopt  experimental      -      -      -      -      0
gurobi_mex    legacy            0      0      0      0      -
lindo_old     legacy            0      -      -      -      -
lindo_legacy  legacy            0      -      -      -      -
lp_solve      legacy            1      -      -      -      -
opti          legacy            0      0      0      0      0
------------------------------------------------------------------------
```

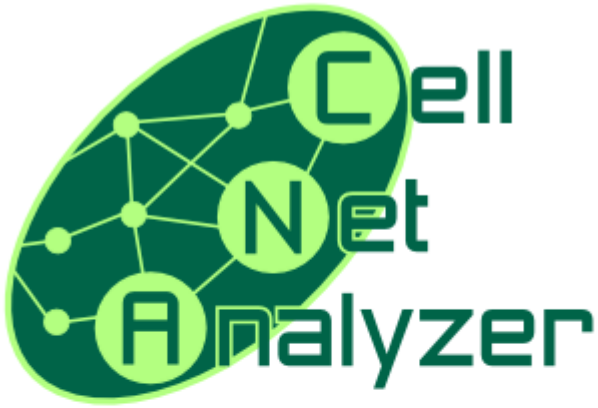
```
Total         -                  6      1      2      0      2

+ Legend: - = not applicable, 0 = solver not compatible or not installed, 1 = solver installed.


> You can solve LP problems using: 'dqqMinos' - 'glpk' - 'matlab' - 'pdco' - 'quadMinos' - 'lp_solve'
> You can solve MILP problems using: 'glpk'
> You can solve QP problems using: 'pdco' - 'qpng'
> You can solve MIQP problems using:
> You can solve NLP problems using: 'matlab' - 'quadMinos'

> Checking for available updates ...
--> You cannot update your fork using updateCobraToolbox(). [6cf35a @ develop].
    Please use the MATLAB.devTools (https://github.com/opencobra/MATLAB.devTools).
```

```
% Add path to Cell Net Analyzer
CNAPath = '~/CellNetAnalyzer';
addpath(genpath(CNAPath));
startcna
```



Version 2017.3

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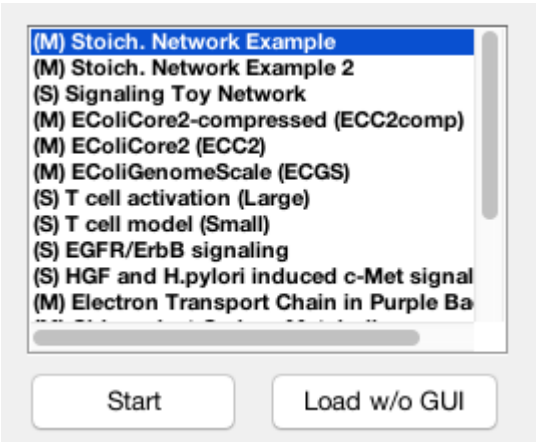
OK



Elementary modes and extreme pathways

The mandatory inputs for computing elementary modes and extreme pathways are a model and a directory where the CNA model is going to be saved. A model should be a COBRA model, a simple MATLAB `struct` with fields defined in the Documentation.

```
% define the model
global CBTDIR
addpath([CBTDIR filesep 'tutorials' filesep 'pathVectors'])
load('smallmodel.mat')
% define the directory (the place that CNA model will be saved there)
directory = 'Pathwaysvector';
[E, id, ir, rev, modelOut] = pathVectors(smallmodel, directory)
```

```
Field 'type' not defined. Initialized with '1' (for mass-flow).
Field 'nums' not defined. Initialized with 15.
Field 'numr' not defined. Initialized with 6.
Field 'specNotes' not defined. Initialized empty 'specNotes'.
Field 'specExternal' not defined. Initialized with zero vector (i.e. all species are configured as inter
Field 'reacNotes' not defined. Initialized empty 'reacNotes'.
Field 'reacVariance' not defined. Initialized for all reactions a variance level of 0.01.
Field 'reacDefault' not defined. Initialized with NaN vector (empty default values).
Field 'mue' not defined. Initialized according to existence of string 'mue' in 'reacID'.
Field 'reacBoxes' not defined. Initialized with default values.
Field 'macroComposition' not defined. Initialized as empty matrix (no macromolecules defined; other fiel
Field 'epsilon' not defined. Initialized with '1e-10'.
Field 'has_gui' not defined. Initialized with 'false'.
Saving ....
Could not open file: reactions



Final number of elementary modes: 2

Removing 0 external metabolites
E =
    -1     0
     1     0
     0     1
     0    -1
    -1    -1
id =
     2
     3
     4
     5
     6
•
ir =
     1
     1
•
rev =
     1     1
•
modelOut =
    description: 'COBRA model converted from a CNA project'
           rxns: {6×1 cell}
           mets: {15×1 cell}
```

```
             S: [15×6 double]
            lb: [6×1 double]
            ub: [6×1 double]
           rev: [6×1 double]
             c: [6×1 double]
      rxnNames: {6×1 cell}
      metNames: {15×1 cell}
             b: [15×1 double]
```

Optional inputs

The function can have some optional inputs which depend on computing elementary modes or extreme pathways. We explain the most important optional inputs in the following.

- `constraints`: is a matrix specifying homogeneous `0` and inhomogeneous `1` constraints on the reaction rates;
- `mexVersion`: there are four options for mexversion, that the default value is `4`.

- 1: use CNA mex files,

- 2: use Metatool mex files,

- 3: use both CNA and Metatool mex files

- 4: use Marco Terzer's EFM tool (see http://www.csb.ethz.ch/tools/index)

- `irrevFlag` : if set to `0`, considers the reversibility of reactions and set to `1` if it does not consider reversibility.
- `convbasisFlag`: if set to `0`, all elementary modes/vectors are calculated and if set to `1`, only a minimal generating set (convex basis) is calculated (default: `0`).
- `isoFlag` : considers isoenzymes (parallel reactions) only once or not; the default value is (default: `0`).
- `cMacro`: vector containing the concentrations (g/gDW) of the macromolecules if a variable biomass composition has been defined (cnap.mue not empty). It can be empty when cnap.mue or cnap.macroComposition is empty. If it is empty and cnap.mue is not empty then cnap.macroDefault is used. In CNA models default is cnap.macroDefault, but in COBRA models if it is not contain mu (biomass composition) then the default value is empty.
- `display`: controls the detail of console output; the default value is '`None`'. Other options are `'Iteration'`, `'All'` or `'Details'`.
- `positivity`: whether a non-negative convex basis `1` or not `0`. If yes it converts every reversible reaction to two irreversible reactions.

For a complete list of optional inputs and their definition, you can run the following command.

```
help pathVectors
```

```
  Computes elementary mode and extreme pathway
  (convex basis) of a COBRA model using the CellNetAnalyzer software
  package [1].

  CellNetAnalyzer can be downloaded at https://www2.mpi-magdeburg.mpg.de/projects/cna/download.html

  INPUTS:
     model:             COBRA model

                          * S  - `m x 1` Stoichiometric matrix
                          * c  - `n x 1` Linear objective coefficients
                          * lb - `n x 1` Lower bounds
                          * ub - `n x 1` Upper bounds
```

```
   directory:         folder path to where the CNA model is going to be
                      saved (required for intermediate computations)

OPTIONAL INPUTS:
   constraints:       empty
                      the number of reactions
                      many rows and up to 4 columns:
                      - COLUMN1 specifies excluded/enforced reactions: if
                        (constraints(i,1)==0) then onlythose modes / rays
                        / points will be computed that do not include
                        reaction i; constraints(i,1)~=0 and
                        constraints(i)~=NaN enforces reaction i, i.e. only
                        those modes / rays / points will be computed that
                        involve reaction i; for all other reactions choose
                        constraint(i,1)=NaN; several reactions may be
                        suppressed/enforced simultaneously
                      - COLUMN2: specifies lower boundaries for the
                        reaction rates (choose NaN if none is active).
                        Note that zero boundaries (irreversibilities) are
                        better described by the vector containg all lower
                        bound of reactions(Lb). In any case, the
                        lower boundary eventually considered will be zero
                        if Lb(i)==0 and constraints(i,2)<0.
                      - COLUMN3: specifies upper boundaries for the
                        reaction rates (choose NaN if none is active)
                      - COLUMN4: specifies equalities for the reaction
                        rates (choose NaN if none is active).
   mexVersion:        (default:4)
                        1, CNA mex files,
                        2, Metatool mex files,
                        3, CNA and Metatool mex files
                        4, Marco Terzer's EFM tool

   irrevFlag:         (default: 1)
                        0, reversible
                        1, irreversible

   convBasisFlag:     (Default: 0)
                        0, elementary modes
                        1, extreme pathways

   isoFlag:           (default: 0)
                        0, not consider isoenzymes
                        1, consider isoenzymes

   cMacro:            (default: the vector containing the default
                        value of the macromolecules)
                        empty
                        MC : A (m*w) matrix defining the stoichiometry
                        of the macromolucules with respect to the
                        metabolietes
```

The following function computes a convex basis for the model by the name smallmodel (the CNA version) and will save it in the `directory` folder.

```
[E, id, ir, rev, modelOut] = pathVectors(smallmodel, directory,'convBasisFlag',1)
```

```
Field 'type' not defined. Initialized with '1' (for mass-flow).
Field 'nums' not defined. Initialized with 15.
Field 'numr' not defined. Initialized with 6.
Field 'specNotes' not defined. Initialized empty 'specNotes'.
Field 'specExternal' not defined. Initialized with zero vector (i.e. all species are configured as inter
Field 'reacNotes' not defined. Initialized empty 'reacNotes'.
```

```
Field 'reacVariance' not defined. Initialized for all reactions a variance level of 0.01.
Field 'reacDefault' not defined. Initialized with NaN vector (empty default values).
Field 'mue' not defined. Initialized according to existence of string 'mue' in 'reacID'.
Field 'reacBoxes' not defined. Initialized with default values.
Field 'macroComposition' not defined. Initialized as empty matrix (no macromolecules defined; other fiel
Field 'epsilon' not defined. Initialized with '1e-10'.
Field 'has_gui' not defined. Initialized with 'false'.
Saving ....
Could not open file: reactions



Final number of convex basis vectors: 2

Removing 0 external metabolites
E =
    -1     0
     1     0
     0     1
     0    -1
    -1    -1
•
id =
     2
     3
     4
     5
     6
•
ir =
     1
     1
•
rev =
     1     1
modelOut =
    description: 'COBRA model converted from a CNA project'
           rxns: {6×1 cell}
           mets: {15×1 cell}
              S: [15×6 double]
             lb: [6×1 double]
             ub: [6×1 double]
            rev: [6×1 double]
              c: [6×1 double]
       rxnNames: {6×1 cell}
       metNames: {15×1 cell}
              b: [15×1 double]
```

Output

The output of `pathVectors.m is`

- `E` : the matrix that contains (row-wise) the elementary modes (or elementary vectors) or a minimal set of generators (lineality space + extreme rays/points), depending on the chosen scenario. The columns correspond to the reactions; the column indices of efms (with respect to the columns in cnap.stoichMat) are stored in the returned variable idx (see below; note that columns are removed in efms if the corresponding reactions are not contained in any mode)
- `rev` : vector indicating for each mode whether it is reversible(0)/irreversible (1)

- `id` : maps the columns in efm onto the column indices in cnap.stoichmat, i.e. idx(i) refers to the column number in cnap.stoichmat (and to the row number in cnap.reacID)
- `ir` : indicates whether the i-th row (vector) in efm is an unbounded (1) or bounded (0) direction of the flux cone / flux polyhedron. Bounded directions (such as extreme points) can only arise if an inhomogeneous problem was defined.

To clarify above description for example, if you look at the output `E` is

```
E
```

```
E =
    -1     0
     1     0
     0     1
     0    -1
    -1    -1
```

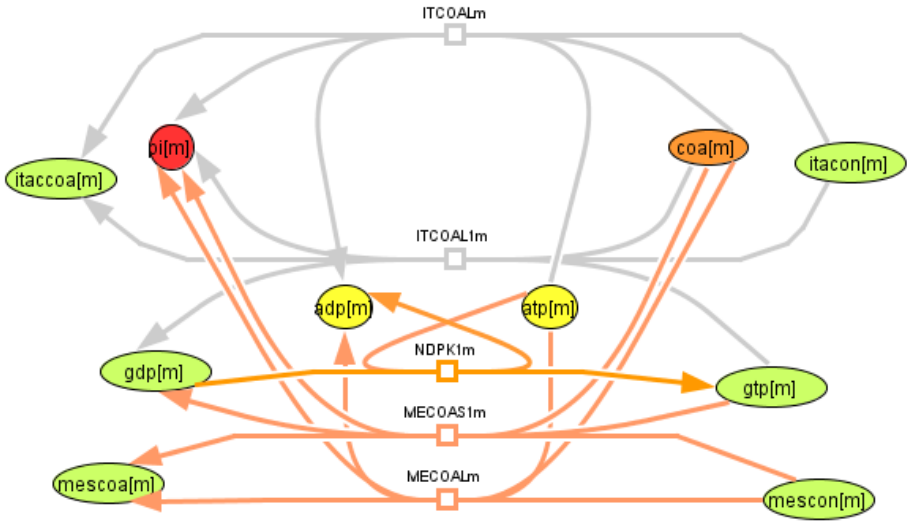
and

```
id
```

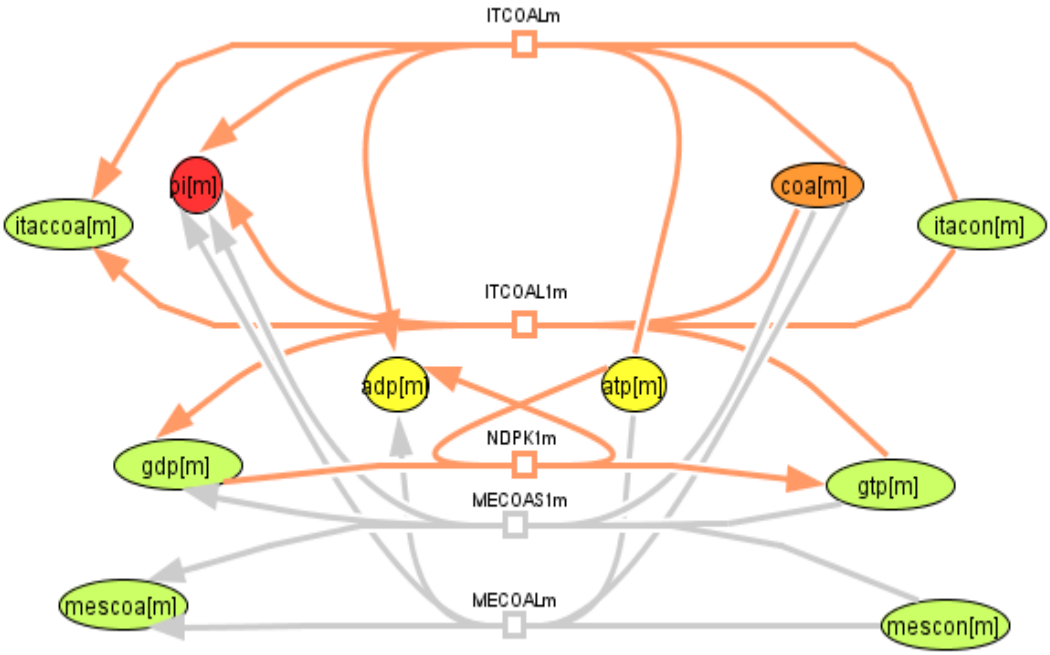
```
id =
     2
     3
     4
     5
     6
```

That means reactions 2, 3, 4 ,5 and 6 participate in the elementary modes. For example, reactions 2, 3 and 6 are made elementary mode one in `E(1),` which in every elementary mode two of reaction use reversible reactions.

These two elementary modes are shown in figuers 1 and 2 respectively as following



Figuer 1: elementary 1 which is involves reactions 1, 2 and 5.



Figuer 2: elementary 2 which is involves reactions 3, 4 and 5.

TIMING

Running, the codes are dependent on the size of models may take long from 30 seconds to few hours. But in addition to the time of running you should allow 60 seconds to start-up the CNA software.

ANTICIPATED RESULTS

If the user just wants to compute extreme pathways with non-negative elements, every reversible reaction has to be converted into two irreversible reactions. This will increase the dimensions of the problem.

```
[E, id, ir, rev, modelOut] = pathVectors(smallmodel, directory, 'convBasisFlag', 1, 'irrevFlag
```

```
Field 'type' not defined. Initialized with '1' (for mass-flow).
Field 'nums' not defined. Initialized with 15.
Field 'numr' not defined. Initialized with 10.
Field 'specNotes' not defined. Initialized empty 'specNotes'.
Field 'specExternal' not defined. Initialized with zero vector (i.e. all species are configured as inter
Field 'reacNotes' not defined. Initialized empty 'reacNotes'.
Field 'reacVariance' not defined. Initialized for all reactions a variance level of 0.01.
Field 'reacDefault' not defined. Initialized with NaN vector (empty default values).
Field 'mue' not defined. Initialized according to existence of string 'mue' in 'reacID'.
Field 'reacBoxes' not defined. Initialized with default values.
Field 'macroComposition' not defined. Initialized as empty matrix (no macromolecules defined; other fiel
Field 'epsilon' not defined. Initialized with '1e-10'.
Field 'has_gui' not defined. Initialized with 'false'.
Saving ....
Could not open file: reactions



Final number of convex basis vectors: 6

Removing 0 external metabolites
```

```
E =
     1     0     0     0     0     0
     0     1     0     0     0     0
     0     0     1     0     0     0
     0     0     0     1     0     0
     0     0     0     0     1     0
     0     0     0     0     0     1
     1     0     0     0     0     0
     0     1     1     0     0     0
     0     0     0     1     1     0
     0     0     1     1     0     1
```

•

```
id =
     1
     2
     3
     4
     5
     6
     7
     8
     9
    10
```

•

```
ir =
     1
     1
     1
     1
     1
     1
```

•

```
rev =
     1     1     1     1     1     1
```

```
modelOut =
    description: 'COBRA model converted from a CNA project'
           rxns: {10×1 cell}
           mets: {15×1 cell}
              S: [15×10 double]
             lb: [10×1 double]
             ub: [10×1 double]
            rev: [10×1 double]
              c: [10×1 double]
       rxnNames: {10×1 cell}
       metNames: {15×1 cell}
              b: [15×1 double]
```

```
[E, id, ir, rev, modelOut] = pathVectors(smallmodel, directory, 'convBasisFlag', 1, 'irrevFlag
```

```
Field 'type' not defined. Initialized with '1' (for mass-flow).
Field 'nums' not defined. Initialized with 15.
Field 'numr' not defined. Initialized with 6.
Field 'specNotes' not defined. Initialized empty 'specNotes'.
Field 'specExternal' not defined. Initialized with zero vector (i.e. all species are configured as inter
Field 'reacNotes' not defined. Initialized empty 'reacNotes'.
Field 'reacVariance' not defined. Initialized for all reactions a variance level of 0.01.
Field 'reacDefault' not defined. Initialized with NaN vector (empty default values).
Field 'mue' not defined. Initialized according to existence of string 'mue' in 'reacID'.
Field 'reacBoxes' not defined. Initialized with default values.
Field 'macroComposition' not defined. Initialized as empty matrix (no macromolecules defined; other fiel
Field 'epsilon' not defined. Initialized with '1e-10'.
Field 'has_gui' not defined. Initialized with 'false'.
Saving ....
Could not open file: reactions



Final number of convex basis vectors: 2

Removing 0 external metabolites
```

```
E =
    -1     0
     1     0
     0     1
     0    -1
    -1    -1
```

```
id =
     2
     3
     4
     5
     6
```

```
ir =
     1
     1
```

```
rev =
     1     1
```

```
modelOut =
    description: 'COBRA model converted from a CNA project'
           rxns: {6×1 cell}
           mets: {15×1 cell}
              S: [15×6 double]
             lb: [6×1 double]
             ub: [6×1 double]
            rev: [6×1 double]
              c: [6×1 double]
       rxnNames: {6×1 cell}
       metNames: {15×1 cell}
              b: [15×1 double]
```

If the user wants to compute elementary modes, then the conBasisFlag has to be 0 as in `convBasisFlag = 0,` while other variables are optional.

```
[E, id, ir, rev, modelOut] = pathVectors(smallmodel, directory, 'convBasisFlag', 0)
```

```
Field 'type' not defined. Initialized with '1' (for mass-flow).
Field 'nums' not defined. Initialized with 15.
Field 'numr' not defined. Initialized with 6.
Field 'specNotes' not defined. Initialized empty 'specNotes'.
Field 'specExternal' not defined. Initialized with zero vector (i.e. all species are configured as inter
Field 'reacNotes' not defined. Initialized empty 'reacNotes'.
Field 'reacVariance' not defined. Initialized for all reactions a variance level of 0.01.
Field 'reacDefault' not defined. Initialized with NaN vector (empty default values).
Field 'mue' not defined. Initialized according to existence of string 'mue' in 'reacID'.
Field 'reacBoxes' not defined. Initialized with default values.
Field 'macroComposition' not defined. Initialized as empty matrix (no macromolecules defined; other fiel
Field 'epsilon' not defined. Initialized with '1e-10'.
Field 'has_gui' not defined. Initialized with 'false'.
Saving ....
Could not open file: reactions



Final number of elementary modes: 2

Removing 0 external metabolites
E =
    -1     0
     1     0
     0     1
     0    -1
    -1    -1
•
id =
     2
     3
     4
     5
     6
•
ir =
     1
     1
rev =
     1     1
•
modelOut =
```

```
      description: 'COBRA model converted from a CNA project'
             rxns: {6×1 cell}
             mets: {15×1 cell}
                S: [15×6 double]
               lb: [6×1 double]
               ub: [6×1 double]
              rev: [6×1 double]
                c: [6×1 double]
         rxnNames: {6×1 cell}
         metNames: {15×1 cell}
                b: [15×1 double]
```

TROUBLESHOOTING

*To compute elementary modes, you should **not** use the `mexVersion 2`, because of this option is valid for computing convex basis and you might get this message.*

`Undefined function or variable 'to_bits_c'.`

`Error in elmodes_calc`

`Error in compute_elmodes`

REFERENCES

[1] Klamt, S. et al. Algorithmic approaches for computing elementary modes in large biochemical reaction networks. IEE Proc. Syst. Biol., 152, 249–255 (2005).

[2] Klamt, S. et al. From elementary flux modes to elementary flux vectors: Metabolite pathway analysis with arbitary linear flux constraints. PLOS Computational Biology, 13(4):e1005409, April 2017. 00002.

[3] Kamp. A. and Klamt S. Enumeration of smallest intervention strategies in genome-scale metabolic networks. PLoS Computational Biology, 10:e1003378. (2014).