tutorial - 0 - Get data, model, export, simulate, plot, and manipulate data

September 22, 2020

[1]:
```
# expand cells to the 95% of the display width
from IPython.core.display import display, HTML
display(HTML("<style>.container { width: 95% !important; }</style>"))
```

```
<IPython.core.display.HTML object>
```

1 Tutorial: Automatic rule-based modeling of metabolism, protein-protein interactions, and regulation of gene expression employing *Atlas*

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Notes: This tutorial was created for the manuscript "*Atlas*: Automatic modeling of regulation of bacterial gene expression and metabolism using rule-based languages", first submitted for peer-review to Bioinformatics on May, 2020.

1.1 Prerequisites

0. The tutorial was prepared and executed on Ubuntu 20.04, PathwayTools version 24, and docker engine version 19.03.8.

1. PathwayTools must be installed and running to obtain data from the EcoCyc database. Please, run `pathway-tools -lisp -python-local-only` before to obtain any data. (Optional) The PathwayTools software could be executed in the background, with help of `nohup pathway-tools -lisp -python-local-only > /dev/null 2> /dev/null &`. Please follow instructions at http://pathwaytools.org/ to obtain a licensed copy of the software from https://biocyc.org/download-bundle.shtml. However, data could be manually formatted using a text-based editor or a spreadsheet software.

   Note: If you ran into the `pathway-tools/ptools/24.0/exe/aclssl.so: undefined symbol: CRYPTO_set_locking_callback` error, please follow instructions here:

https://github.com/networkbiolab/atlas/tree/master/PTools-Docker. Instructions will guide you to install a docker image that is able to run pathway tools, but does not include it, so you still need to obtain the software with a valid license.

2. (Highly recommended) Install Docker. Please follow instructions for a supported Operating System https://docs.docker.com/engine/install/: On Ubuntu, install it with `apt-get install docker.io`. On Win10, install Docker Desktop with WSL2 support https://docs.docker.com/docker-for-windows/wsl/. On MacOS, install Docker Desktop https://docs.docker.com/docker-for-mac/install/. The Docker `networkbiolab/pleiades` installs the python packages, the jupyter server, and the stochastic simulators.

3. (Recommended) Jupyter notebook. We recommend the use of Anaconda3 https://www.anaconda.com/products/individual because of the easier installation of the stochastic simulators from https://anaconda.org/alubbock.

4. (Optional) A stochastic simulator, supported by the pySB python package (BNG2, NFsim, KaSim or Stochkit). pySB requires BNG2 to simulate models with NFsim.

5. (Optional) Cytoscape to visualize metabolic networks and others.

6. (Optional) A deterministic simulator: pySB supports ODE integration via scipy.integrate.ode, BioNetGen ODE integration, and CUDA-accelerated ODE integration with Marco S. Nobile's cupSODA software (https://github.com/aresio/cupSODA). If the user feel comfortable with SBML models, pySB could export to SBML and deterministic simulation done with libRoadRunner (http://libroadrunner.org/), Tellurium (http://tellurium.analogmachine.org/), COPASI (http://copasi.org/), etc.

1.2 Installation

0. If you are running the docker image "pleiades", please go directly to the section "Preamble".
1. To install, please follow one of the following steps:
   1. Install the docker image "pleiades" using `docker pull networkbiolab/pleiades`. The container is based on the Anaconda3 software and it installs Atlas, and the stochastic simulators BNG2, NFsim, KaSim, and Stochkit. After building the image, please run the container with `docker run --detach --publish 10000:8888 networkbiolab/pleiades`, and go to `localhost:10000` in your preferred browser. The required password is `pleiades`.
   2. Download or clone the Github repository from https://github.com/networkbiolab/pleiades with `git clone https://github.com/networkbiolab/pleiades foo` (where `foo` is an absolute or relative path). Then, you could build the docker image with `docker build foo --tag pleiades` and run it with `docker run --detach --publish 10000:8888 pleiades`. Finally, go to `localhost:10000` in your preferred browser. The required password is `pleiades`.
   3. Install with pip3: `sudo -H python3 -m pip install pleiades` or `python3 -m pip install pleiades --user`. Pleiades is a meta-package that install Atlas (the rule-based modeller), Pleione (a genetic algorithm for parameter calibration of RBMs, compatible with SLURM), Alcyone (to perform identifiability analysis of parameters), and Sterope (to perform sensitivity analysis of parameters in kappa RBMs, compatible with SLURM). You should install, configure, and run the jupyter notebook on your own: example `sudo -H pip3 install jupyter && nohup python3 -m jupyter notebook`

`--port=8888 --no-browser --port-retries=0 > /dev/null 2> /dev/null &`.

4. Download or clone the Github repository from https://github.com/networkbiolab/atlas with `git clone https://github.com/networkbiolab/atlas foo` (where `foo` is an absolute or relative path). Requisites must be fulfilled manually with pip3: `sudo -H python3 -m pip install pandas pysb pythoncyc jupyter seaborn` or `python3 -m pip install pandas pysb pythoncyc jupyter seaborn --user`.

1.3 Objectives

1. Get metabolic data: enzyme names, substrates, products, and location of enzymes.
2. Basic manipulations of metabolic data: change reversibility, change enzyme location.
3. Get composition of complexes for protein-protein interactions.
4. Basic manipulations of interaction data: add and remove interactions.
5. Perform simulation, plot of variables, and export of models.
6. Get DNA architecture and build a model from a manually written network.
7. Get protein-DNA and TF-metabolite interactions and build a model from manually written networks.
8. Add gene regulatory interactions to transcription rules: Activation of transcription.

1.4 Preamble: load *Atlas*

[2]:
```
# testing source code
# required if atlas was cloned from GitHub and this notebook is executed from␣
↪the tutorial directory.
import sys
sys.path.append("..")

import atlas_rbm.atlas as atlas
import atlas_rbm.utils as utils
import atlas_rbm.export as export
import atlas_rbm.simulation as simulation
```

[3]:
```
utils.checkPathwayTools()
```

```
PathwayTools is not running.
Please, execute execPathwayTools(path) or execPToolsDocker(dockername).
```

[3]:
```
False
```

[4]:
```
utils.execPToolsDocker('ptools-v24')
# execute this inside the docker will fail.
# Please, execute `docker run --rm -d --network host ptools-v24` in a terminal
```

```
Docker ptools-v24 is running (ID
6e8c47d8fa14b1b362c1f7bf7c4727fd721d4132045e127bd7ac6384ea068638)
PathwayTools is running. Available PGDB are: META, ECOLI
```

1.5 Getting data to model metabolism

In this tutorial, we will obtain data from EcoCyc to model the lactose degradation that ocurr in *Escherichia coli*. We choose the lactose metabolism since it was discovered in the decade of 1960s and it is a common model of gene regulation with more than 50 years of biochemical information. In an side note, the characterization of the lactose operon and others rewarded their authors the 1965 Nobel Prize in Physiology or Medicine (https://www.nobelprize.org/prizes/medicine/1965/summary/)

The lactose operon from *E. coli* consists of three genes: the β-galactosidase gene lacZ, the lactose permease gene lacY (also known as lactose-proton symporter), and the galactoside O-acetyltransferase gene lacA.

Note: The location is informed for all gene products, including the location of complexes formed by the encoded protein. Therefore, the location may reflect the location of the enzyme or its components before complex assembly. Still, there are 154 enzymatic and 3 transport reactions without known gene, and the location will set to unknown.

Note: The location could be changed later with help of an utilitary function. The function `utils.metabolicNetwork.FromEnzymeList()` produces data in three formats for the `GENE OR COMPLEX` column: 1. The `product` key produces networks with the name of the enzyme, but the model will not be compatible with the modeling of gene expression. **Note**: The `product` key will try to retrieve the location of the enzyme or transporter. If not, the location refer to the location of the monomers of the complex, e.g. "BETAGALACTOSID-CPLX". 2. The `gene` key produces networks with the name of the gene (is the default), but the model will not be compatible with modeling of protein-protein interactions, specially for the modeling of heteromers. **Note**: For enzyme complexes, the `genes` key will decompose the complex into its genes and locations of their products, e.g. the araFGH transporter ("ABC-2-CPLX") where the araG product locates to the cytoplasm and the inner membrane when interact with araH. 3. The `complex` key produces hypernetworks (brackets denote subnetworks, and we will subsequently call it as *hypergraph notation*), and in that way, Atlas produces a model that will be compatible with the modeling of protein-protein interactions and gene expression. **Note**: For enzyme complexes, the `complex` key will decompose the complex into its genes AND write the stoichiometry of the complex in the *hypergraph notation*. If one or more monomers have two or more locations, the output will show as many rows to show all combinations. Be aware that some could be not physiologically possible.

[8]:
```
%time network = utils.metabolicNetwork.FromEnzymeList('ECOLI',␣
↪['GALACTOACETYLTRAN-CPLX', 'LACY-MONOMER', 'BETAGALACTOSID-CPLX',␣
↪'ABC-2-CPLX'], fmt = 'product')
network
```

```
CPU times: user 50.5 ms, sys: 40.3 ms, total: 90.8 ms
Wall time: 877 ms
```

[8]:
```
         GENE OR COMPLEX ENZYME LOCATION               REACTION  \
0             ABC-2-CPLX  inner membrane             ABC-2-RXN
1    BETAGALACTOSID-CPLX         cytosol      BETAGALACTOSID-RXN
2    BETAGALACTOSID-CPLX         cytosol             RXN0-5363
3    BETAGALACTOSID-CPLX         cytosol             RXN-17726
4    BETAGALACTOSID-CPLX         cytosol             RXN0-7219
5  GALACTOACETYLTRAN-CPLX        cytosol   GALACTOACETYLTRAN-RXN
```

```
6             LACY-MONOMER  inner membrane           TRANS-RXN-24
7             LACY-MONOMER  inner membrane           TRANS-RXN-94
8             LACY-MONOMER  inner membrane              RXN0-7215
9             LACY-MONOMER  inner membrane              RXN0-7217
10            LACY-MONOMER  inner membrane              RXN-17755

                      SUBSTRATES                             PRODUCTS  \
0           WATER,ATP,L-ARABINOSE         Pi,ADP,PROTON,L-ARABINOSE
1                 CPD-15972,WATER          GALACTOSE,Glucopyranose
2                   Alpha-lactose                      ALLOLACTOSE
3                  CPD-3561,WATER         GALACTOSE,Fructofuranose
4                  CPD-3785,WATER             GALACTOSE,D-ARABINOSE
5   Beta-D-Galactosides,ACETYL-COA  6-Acetyl-Beta-D-Galactosides,CO-A
6            PROTON,Alpha-lactose             PROTON,Alpha-lactose
7                PROTON,MELIBIOSE                 PROTON,MELIBIOSE
8                 PROTON,CPD-3561                  PROTON,CPD-3561
9                 PROTON,CPD-3785                  PROTON,CPD-3785
10                PROTON,CPD-3801                  PROTON,CPD-3801

    FWD_RATE  RVS_RATE
0        1.0       0.0
1        1.0       0.0
2        1.0       1.0
3        1.0       0.0
4        1.0       0.0
5        1.0       0.0
6        1.0       1.0
7        1.0       1.0
8        1.0       1.0
9        1.0       1.0
10       1.0       1.0
```

Note: Using the `utils.metabolicNetwork.FromEnzymeList()` function with the `all_enzymes()` and `all_transporters()` functions from the pythoncyc package, we could obtain a **draft** network and a **draf** rule-based model of metabolism for the considered organism:

[6]:
```
import pythoncyc
%time network = utils.metabolicNetwork.FromEnzymeList('ECOLI', pythoncyc.
 ↪select_organism('ECOLI').all_transporters())
%time utils.metabolicNetwork.expand_network(network, 'ecocyc-v24-tps-cytoscape.
 ↪txt')
network.to_csv('ecoli-tps-v24.txt', sep = '\t', index = False)
%time atlas.construct_model_from_metabolic_network('ecoli-tps-v24.txt',␣
 ↪noObservables=True, noInitials=True, toFile = 'model-transporters.py')
len(network[network['ENZYME LOCATION'].str.match('unknown')]) # how many␣
 ↪reactions are not mapped to a gene product
```

```
CPU times: user 3.05 s, sys: 2.73 s, total: 5.78 s
Wall time: 55.6 s
CPU times: user 7.97 ms, sys: 213 µs, total: 8.18 ms
Wall time: 35.6 ms
It was found duplicated reaction names in the network.
Please check the conflicting_reactions.txt and correct them if necessary.
CPU times: user 559 ms, sys: 41.3 ms, total: 600 ms
Wall time: 626 ms
```

```
[6]: 3
```

```
[7]: import pythoncyc
     %time network = utils.metabolicNetwork.FromEnzymeList('ECOLI', pythoncyc.
      ↪select_organism('ECOLI').all_enzymes())
     %time utils.metabolicNetwork.expand_network(network, 'ecocyc-v24-enz-cytoscape.
      ↪txt')
     network.to_csv('ecoli-enz-v24.txt', sep = '\t', index = False)
     %time atlas.construct_model_from_metabolic_network('ecoli-enz-v24.txt',␣
      ↪noObservables=True, noInitials=True, toFile = 'model-enzymes.py')
     len(network[network['ENZYME LOCATION'].str.match('unknown')]) # how many␣
      ↪reactions are not mapped to a gene product
```

```
Unable to retrieve data for CPLX0-7889. Please, review the information at
https://biocyc.org/ECOLI/NEW-IMAGE?object=CPLX0-7889 and post an issue at
https://github.com/networkbiolab/atlas if you believe it is a software error.
CPU times: user 9.49 s, sys: 9.5 s, total: 19 s
Wall time: 3min
CPU times: user 38.9 ms, sys: 0 ns, total: 38.9 ms
Wall time: 38.8 ms
It was found duplicated reaction names in the network.
Please check the conflicting_reactions.txt and correct them if necessary.
CPU times: user 1.14 s, sys: 19.8 ms, total: 1.16 s
Wall time: 1.16 s
```

```
[7]: 155
```

However, we must know the exact enzyme ID from the database. As an alternative, we could obtain the metabolic reactions from the `ECOLI` database from PathwayTools with help of the `utils.metabolicNetwork.FromGeneList()` function. The function produce data in three formats for the `GENE OR COMPLEX` column: 1. The `product` key produces networks with the name of the enzyme, but the model will not be compatible with the modeling of gene expression. **Note**: The `product` key will find the product that perform the enzymatic reaction, e.g. lacZ produces monomers that interact to produce the enzyme. Also, it will write the location of the product(s) of the queried gene. It is a good practice to query only one gene of the complex. 2. The `gene` key produces networks with the name of the gene (is the default), but the model will not be compatible with modeling of protein-protein interactions, specially for the modeling of heteromers. **Note**: The `gene` key will write the location of the product(s) of the queried gene., e.g. araF, araG, and araH. 3. The `complex` key produces hyper-networks, and in that way, Atlas will produce a model that is compatible with the modeling of protein-protein interactions and gene expression. **Note**: The `complex` key will write the stoichiometry of the enzymatic complex of the queried gene, and the mapped location of the complex, all in *hypergraph notation*. It is a good practice query only one gene of the complex.

```
[8]: %time utils.metabolicNetwork.FromGeneList('ECOLI', ['lacZ', 'lacA', 'lacY',␣
     ↪'araF', 'araG', 'araH'], fmt = 'product')
```

```
CPU times: user 2.55 s, sys: 2.39 s, total: 4.94 s
Wall time: 51.4 s
```

```
[8]:            GENE OR COMPLEX    ENZYME LOCATION                 REACTION  \
0                ABC-2-CPLX  periplasmic space              ABC-2-RXN
1                ABC-2-CPLX     inner membrane              ABC-2-RXN
2                ABC-2-CPLX            cytosol              ABC-2-RXN
3                ABC-2-CPLX     inner membrane              ABC-2-RXN
4   GALACTOACETYLTRAN-CPLX            cytosol  GALACTOACETYLTRAN-RXN
5             LACY-MONOMER     inner membrane            TRANS-RXN-24
6             LACY-MONOMER     inner membrane            TRANS-RXN-94
7             LACY-MONOMER     inner membrane              RXN0-7215
8             LACY-MONOMER     inner membrane              RXN0-7217
9             LACY-MONOMER     inner membrane              RXN-17755
10     BETAGALACTOSID-CPLX            cytosol     BETAGALACTOSID-RXN
11     BETAGALACTOSID-CPLX            cytosol              RXN0-5363
12     BETAGALACTOSID-CPLX            cytosol              RXN-17726
13     BETAGALACTOSID-CPLX            cytosol              RXN0-7219

                         SUBSTRATES                            PRODUCTS  \
0             WATER,ATP,L-ARABINOSE          Pi,ADP,PROTON,L-ARABINOSE
1             WATER,ATP,L-ARABINOSE          Pi,ADP,PROTON,L-ARABINOSE
2             WATER,ATP,L-ARABINOSE          Pi,ADP,PROTON,L-ARABINOSE
3             WATER,ATP,L-ARABINOSE          Pi,ADP,PROTON,L-ARABINOSE
4   Beta-D-Galactosides,ACETYL-COA  6-Acetyl-Beta-D-Galactosides,CO-A
5              PROTON,Alpha-lactose                PROTON,Alpha-lactose
6                  PROTON,MELIBIOSE                    PROTON,MELIBIOSE
7                   PROTON,CPD-3561                     PROTON,CPD-3561
8                   PROTON,CPD-3785                     PROTON,CPD-3785
9                   PROTON,CPD-3801                     PROTON,CPD-3801
10                  CPD-15972,WATER            GALACTOSE,Glucopyranose
11                    Alpha-lactose                         ALLOLACTOSE
12                   CPD-3561,WATER            GALACTOSE,Fructofuranose
13                   CPD-3785,WATER               GALACTOSE,D-ARABINOSE

    FWD_RATE  RVS_RATE
0        1.0       0.0
1        1.0       0.0
2        1.0       0.0
3        1.0       0.0
```

```
4         1.0       0.0
5         1.0       1.0
6         1.0       1.0
7         1.0       1.0
8         1.0       1.0
9         1.0       1.0
10        1.0       0.0
11        1.0       1.0
12        1.0       0.0
13        1.0       0.0
```

Note: The function is particularly slow since it needs to build a dataframe to map gene names (e.g. *lacY*) to the internal identification name that PathwayTools understand: `EG10526` <-> `lacY`. With that internal identification, the function looks for enzymes, reactions of those enzymes, the substrates and products of those reactions, and the location of the enzymes. We could accelerate the process (useful for repeated gathering of data) if we execute `utils.returnCommonNames()` and utilize that precalculated dataframe and pass to the utilitary functions.

```
[9]: # %time utils.getData('ECOLI', 'EG10526')
     %time utils.getData('ECOLI', 'EG10526')['common_name'] # for simplicity of the␣
      ↪output
```

```
CPU times: user 1.61 ms, sys: 1.24 ms, total: 2.86 ms
Wall time: 13.6 ms
```

```
[9]: 'lacY'
```

```
[10]: %time df_genes = utils.returnCommonNames('ECOLI')
```

```
CPU times: user 2.49 s, sys: 2.18 s, total: 4.67 s
Wall time: 46.9 s
```

```
[11]: %time utils.metabolicNetwork.FromGeneList('ECOLI', ['lacZ', 'lacA', 'lacY',␣
       ↪'araF', 'araG', 'araH'], fmt = 'product', precalculated = df_genes)
      # ~35 times faster
```

```
CPU times: user 61.6 ms, sys: 64.4 ms, total: 126 ms
Wall time: 1.29 s
```

```
[11]:            GENE OR COMPLEX    ENZYME LOCATION                REACTION  \
      0              ABC-2-CPLX  periplasmic space              ABC-2-RXN
      1              ABC-2-CPLX     inner membrane              ABC-2-RXN
      2              ABC-2-CPLX            cytosol              ABC-2-RXN
      3              ABC-2-CPLX     inner membrane              ABC-2-RXN
      4   GALACTOACETYLTRAN-CPLX            cytosol  GALACTOACETYLTRAN-RXN
      5            LACY-MONOMER     inner membrane            TRANS-RXN-24
      6            LACY-MONOMER     inner membrane            TRANS-RXN-94
      7            LACY-MONOMER     inner membrane              RXN0-7215
```

```
8            LACY-MONOMER     inner membrane            RXN0-7217
9            LACY-MONOMER     inner membrane            RXN-17755
10      BETAGALACTOSID-CPLX          cytosol     BETAGALACTOSID-RXN
11      BETAGALACTOSID-CPLX          cytosol            RXN0-5363
12      BETAGALACTOSID-CPLX          cytosol            RXN-17726
13      BETAGALACTOSID-CPLX          cytosol            RXN0-7219

                        SUBSTRATES                            PRODUCTS  \
0            WATER,ATP,L-ARABINOSE          Pi,ADP,PROTON,L-ARABINOSE
1            WATER,ATP,L-ARABINOSE          Pi,ADP,PROTON,L-ARABINOSE
2            WATER,ATP,L-ARABINOSE          Pi,ADP,PROTON,L-ARABINOSE
3            WATER,ATP,L-ARABINOSE          Pi,ADP,PROTON,L-ARABINOSE
4   Beta-D-Galactosides,ACETYL-COA  6-Acetyl-Beta-D-Galactosides,CO-A
5             PROTON,Alpha-lactose               PROTON,Alpha-lactose
6                 PROTON,MELIBIOSE                   PROTON,MELIBIOSE
7                  PROTON,CPD-3561                    PROTON,CPD-3561
8                  PROTON,CPD-3785                    PROTON,CPD-3785
9                  PROTON,CPD-3801                    PROTON,CPD-3801
10                 CPD-15972,WATER            GALACTOSE,Glucopyranose
11                   Alpha-lactose                        ALLOLACTOSE
12                  CPD-3561,WATER           GALACTOSE,Fructofuranose
13                  CPD-3785,WATER              GALACTOSE,D-ARABINOSE

    FWD_RATE  RVS_RATE
0        1.0       0.0
1        1.0       0.0
2        1.0       0.0
3        1.0       0.0
4        1.0       0.0
5        1.0       1.0
6        1.0       1.0
7        1.0       1.0
8        1.0       1.0
9        1.0       1.0
10       1.0       0.0
11       1.0       1.0
12       1.0       0.0
13       1.0       0.0
```

[12]:
```
%time utils.metabolicNetwork.FromGeneList('ECOLI', ['lacZ', 'lacA', 'lacY',␣
↪'araF', 'araG', 'araH'], fmt = 'genes', precalculated = df_genes)
```

```
CPU times: user 74.5 ms, sys: 57.8 ms, total: 132 ms
Wall time: 1.39 s
```

[12]:
```
   GENE OR COMPLEX    ENZYME LOCATION              REACTION  \
0             araF  periplasmic space             ABC-2-RXN
```

```
1             araG     inner membrane             ABC-2-RXN
2             araG            cytosol             ABC-2-RXN
3             araH     inner membrane             ABC-2-RXN
4             lacA            cytosol  GALACTOACETYLTRAN-RXN
5             lacY     inner membrane          TRANS-RXN-24
6             lacY     inner membrane          TRANS-RXN-94
7             lacY     inner membrane             RXN0-7215
8             lacY     inner membrane             RXN0-7217
9             lacY     inner membrane             RXN-17755
10            lacZ            cytosol     BETAGALACTOSID-RXN
11            lacZ            cytosol             RXN0-5363
12            lacZ            cytosol             RXN-17726
13            lacZ            cytosol             RXN0-7219

                      SUBSTRATES                            PRODUCTS  \
0           WATER,ATP,L-ARABINOSE         Pi,ADP,PROTON,L-ARABINOSE
1           WATER,ATP,L-ARABINOSE         Pi,ADP,PROTON,L-ARABINOSE
2           WATER,ATP,L-ARABINOSE         Pi,ADP,PROTON,L-ARABINOSE
3           WATER,ATP,L-ARABINOSE         Pi,ADP,PROTON,L-ARABINOSE
4   Beta-D-Galactosides,ACETYL-COA  6-Acetyl-Beta-D-Galactosides,CO-A
5             PROTON,Alpha-lactose              PROTON,Alpha-lactose
6                 PROTON,MELIBIOSE                  PROTON,MELIBIOSE
7                  PROTON,CPD-3561                   PROTON,CPD-3561
8                  PROTON,CPD-3785                   PROTON,CPD-3785
9                  PROTON,CPD-3801                   PROTON,CPD-3801
10                 CPD-15972,WATER           GALACTOSE,Glucopyranose
11                   Alpha-lactose                       ALLOLACTOSE
12                  CPD-3561,WATER          GALACTOSE,Fructofuranose
13                  CPD-3785,WATER             GALACTOSE,D-ARABINOSE

    FWD_RATE  RVS_RATE
0        1.0       0.0
1        1.0       0.0
2        1.0       0.0
3        1.0       0.0
4        1.0       0.0
5        1.0       1.0
6        1.0       1.0
7        1.0       1.0
8        1.0       1.0
9        1.0       1.0
10       1.0       0.0
11       1.0       1.0
12       1.0       0.0
13       1.0       0.0
```

```
[13]: %time utils.metabolicNetwork.FromGeneList('ECOLI', ['lacZ', 'lacA', 'lacY',␣
       ↪'araF', 'araG', 'araH'], fmt = 'complex', precalculated = df_genes)
```

```
CPU times: user 121 ms, sys: 96.9 ms, total: 218 ms
Wall time: 2.14 s
```

```
[13]:              GENE OR COMPLEX  \
      0   [araG,araG,araH,araH,araF]
      1   [araG,araG,araH,araH,araF]
      2   [araG,araG,araH,araH,araF]
      3   [araG,araG,araH,araH,araF]
      4   [araG,araG,araH,araH,araF]
      5   [araG,araG,araH,araH,araF]
      6             [lacA,lacA,lacA]
      7                         lacY
      8                         lacY
      9                         lacY
      10                        lacY
      11                        lacY
      12       [lacZ,lacZ,lacZ,lacZ]
      13       [lacZ,lacZ,lacZ,lacZ]
      14       [lacZ,lacZ,lacZ,lacZ]
      15       [lacZ,lacZ,lacZ,lacZ]

                                      ENZYME LOCATION               REACTION  \
      0   [inner membrane,inner membrane,inner membrane,…            ABC-2-RXN
      1   [cytosol,cytosol,inner membrane,inner membrane…            ABC-2-RXN
      2   [inner membrane,inner membrane,inner membrane,…            ABC-2-RXN
      3   [cytosol,cytosol,inner membrane,inner membrane…            ABC-2-RXN
      4   [inner membrane,inner membrane,inner membrane,…            ABC-2-RXN
      5   [cytosol,cytosol,inner membrane,inner membrane…            ABC-2-RXN
      6                           [cytosol,cytosol,cytosol]  GALACTOACETYLTRAN-RXN
      7                                     inner membrane          TRANS-RXN-24
      8                                     inner membrane          TRANS-RXN-94
      9                                     inner membrane            RXN0-7215
      10                                    inner membrane            RXN0-7217
      11                                    inner membrane            RXN-17755
      12                  [cytosol,cytosol,cytosol,cytosol]     BETAGALACTOSID-RXN
      13                  [cytosol,cytosol,cytosol,cytosol]            RXN0-5363
      14                  [cytosol,cytosol,cytosol,cytosol]            RXN-17726
      15                  [cytosol,cytosol,cytosol,cytosol]            RXN0-7219

                         SUBSTRATES                     PRODUCTS  \
      0          WATER,ATP,L-ARABINOSE       Pi,ADP,PROTON,L-ARABINOSE
      1          WATER,ATP,L-ARABINOSE       Pi,ADP,PROTON,L-ARABINOSE
      2          WATER,ATP,L-ARABINOSE       Pi,ADP,PROTON,L-ARABINOSE
      3          WATER,ATP,L-ARABINOSE       Pi,ADP,PROTON,L-ARABINOSE
```

```
4            WATER,ATP,L-ARABINOSE          Pi,ADP,PROTON,L-ARABINOSE
5            WATER,ATP,L-ARABINOSE          Pi,ADP,PROTON,L-ARABINOSE
6   Beta-D-Galactosides,ACETYL-COA  6-Acetyl-Beta-D-Galactosides,CO-A
7             PROTON,Alpha-lactose               PROTON,Alpha-lactose
8                 PROTON,MELIBIOSE                   PROTON,MELIBIOSE
9                  PROTON,CPD-3561                    PROTON,CPD-3561
10                 PROTON,CPD-3785                    PROTON,CPD-3785
11                 PROTON,CPD-3801                    PROTON,CPD-3801
12                  CPD-15972,WATER           GALACTOSE,Glucopyranose
13                   Alpha-lactose                        ALLOLACTOSE
14                   CPD-3561,WATER          GALACTOSE,Fructofuranose
15                   CPD-3785,WATER               GALACTOSE,D-ARABINOSE

    FWD_RATE  RVS_RATE
0        1.0       0.0
1        1.0       0.0
2        1.0       0.0
3        1.0       0.0
4        1.0       0.0
5        1.0       0.0
6        1.0       0.0
7        1.0       1.0
8        1.0       1.0
9        1.0       1.0
10       1.0       1.0
11       1.0       1.0
12       1.0       0.0
13       1.0       1.0
14       1.0       0.0
15       1.0       0.0
```

The output is a pandas dataframe that could be exported with `network.to_csv(path)`, or in a two-columns format that Cytoscape could interpret as a network. The `utils.metabolicNetwork.expand_network` function reorders and exports the dataframe as a text file (in this case to `./tutorial.txt`).

[14]:
```
%time network = utils.metabolicNetwork.FromGeneList('ECOLI', ['lacZ', 'lacA',␣
↪'lacY'], fmt = 'genes', precalculated = df_genes)
%time utils.metabolicNetwork.expand_network(network, './
↪lactose-metabolism-cytoscape-v1.txt')
```

```
CPU times: user 46.5 ms, sys: 39.9 ms, total: 86.4 ms
Wall time: 884 ms
CPU times: user 0 ns, sys: 1.03 ms, total: 1.03 ms
Wall time: 1.03 ms
```

The following image was prepared from the `lactose-metabolism-cytoscape-v1.txt` file, and you could reproduce it with Cytoscape: 1. Click on the `Import Network from File System` icon or click on `File -> Import -> Network from File...`. 2. Navigate to the file and click on `Open`. 3. SOURCE, TARGET, and EDGE ATTRIBUTE are OK, but the 4th columns must be the SOURCE NODE ATTRIBUTE and the 5th column the TARGET NODE ATTRIBUTE. Click on the header and change it to the correct attribute. The attributes will help later to filter and to add format to nodes and edges. 4. Click on `Filter` (on the right), then on the `+` icon and finally on `Column Filter`: 1. On the selector, click on `Edge: EDGE_ATTRIBUTE` and change `contains` to `is`. 1. Write `NO_REVERSIBLE` that will select edges that correspond to irreversible reactions. Click on `Style`, then `Edge` (in the bottom), and click on the 3rd column to bypass the format of the `Target Arrow Shape` and select your favorite arrow shape. 2. Write `REVERSIBLE` and bypass the format of the `Source Arrow Shape` AND `Target Arrow Shape`, and select your favorite arrow shape. 2. On the selector, click on `Node: SOURCE_NODE_ATTRIBUTE`: 1. Write `RXN` that will select nodes enconding the reactions. Click on `Style`, then on `Node` and bypass the `Fill Color`. In the new window, you could set-up the color, e.g. #00AA50 2. Write `GENE_PROD` that will select nodes encoding the gene name, protein name, or the enzyme name. Click on `Style`, then on `Node` and bypass the `Fill Color`. In the new window, you could set-up the color, e.g. #CC0033 3. Write `MET` that will select nodes encoding substrate metabolites. Click on `Style`, then on `Node` and bypass the `Fill Color`. In the new window, you could set-up the color, e.g. #00ABDD. Also, set a shape for nodes, to differentiate substrates from products. 3. On the selector, click on `Node: TARGET_NODE_ATTRIBUTE`: 1. Write `MET` that will select nodes encoding product metabolites. Click on `Style`, then on `Node` and bypass the `Fill Color`. In the new window, you could set-up the color, e.g. #00ABDD

The result will be similar to

If we inspect the network, we could highlight four things: 1. The lacA reaction is disconnect from the network formed by the lacZ and lacY reactions; 2. The lacY reactions do not inform the metabolite compartment, so substrates and products refer to the same node; 3. The utilization of *internal codes* for certain compounds; and 4. The impossibility of alpha-lactose degradation into glucose (glucopyranose) and galactose.

We could retrieve information from the reaction and metabolite nodes with the `utils.getData()`function:

```
[15]: # utils.get_data('ECOLI', 'CPD-3561')
      %time utils.getData('ECOLI', 'CPD-3561')['names'] # for simplicity of the output
```

```
CPU times: user 2.86 ms, sys: 0 ns, total: 2.86 ms
Wall time: 15.9 ms
```

```
[15]: ['lactulose', 'CEPHULAC (TN)']
```

We observe that `CPD-3561` correspond to `lactulose`. Therefore, the EcoCyc database informs that LacY is able to incorporate alpha-lactose, melibiose, lactulose (`CPD-3561`), 3-O-galactosylarabinose (`CPD-3785`), and melibionate (`CPD-3801`) into the cell cytoplasm. Interestingly, the common synthetic activator IPTG (o-nitrophenyl- -galactoside) is mentioned in the description for the lactose transporter (https://biocyc.org/gene?orgid=ECOLI&id=EG10526#), but there is no inclusion of the IPTG transport in the reactions of LacY.

```
[16]: # utils.get_data('ECOLI', 'CPD-15972')
      %time utils.getData('ECOLI', 'CPD-15972')['names'] # for simplicity of the␣
       ↪output
```

```
CPU times: user 1.53 ms, sys: 0 ns, total: 1.53 ms
Wall time: 14.6 ms
```

```
[16]: ['lactose',
       '&beta;-D-galactopyranosyl-(1&rarr;4)-D-glucopyranose',
       'D-lactose',
       '&beta;-D-Galp-(1&rarr;4)-D-Glcp']
```

Next, EcoCyc informs that LacZ could metabolize lactose (CPD-15972) into galactose and glucopyranose, lactulose into -galactose and fructofuranose, and 3-O-galactosylarabinose into -galactose and arabinose. The difference in metabolite names between the lacY and lacZ reactions is enough to make a Section **??**. Specifically, the name lactose refers to the two anomers α- and β-lactose because of EcoCyc uses generic names when enzymes show no stereoselectivity. In addition, galactose has also an anomeric center, but the database refers to it as β-D-galactose without indication the enzyme could produce α-galactose from α-lactose.

```
[17]: %time print(utils.getData('ECOLI', 'GALACTOSE')['common_name'])
      print()
      %time print(utils.getData('ECOLI', 'GALACTOSE')['synonyms'])
```

```
&beta;-D-galactopyranose
CPU times: user 1.64 ms, sys: 0 ns, total: 1.64 ms
Wall time: 24.8 ms

['&beta;-D-galactopyranose', '&beta;-D-galactose', 'cerebrose',
'6-(hydroxymethyl)tetrahydropyran-2,3,4,5-tetraol']
CPU times: user 2.81 ms, sys: 0 ns, total: 2.81 ms
Wall time: 21.4 ms
```

The advantage of the procedure is the ability to modify the data programatically using python functions (https://pandas.pydata.org/) or export the data and manipulate it using a text processor or a spreadsheet software. For routinary changes, we included utilitary functions to make batch manipulation to the data:

```
[18]: # Transport reactions: Add compartments to substrates and/or products
      %time network = utils.metabolicNetwork.setTransport(network, geneLst =␣
       ↪['lacY'], fromLst = ['PER'], toLst = ['CYT'])
      network
```

```
CPU times: user 7.39 ms, sys: 0 ns, total: 7.39 ms
Wall time: 6.49 ms
```

```
[18]:   GENE OR COMPLEX ENZYME LOCATION               REACTION  \
      0             lacA         cytosol  GALACTOACETYLTRAN-RXN
```

```
1          lacY  inner membrane          TRANS-RXN-24
2          lacY  inner membrane          TRANS-RXN-94
3          lacY  inner membrane            RXN0-7215
4          lacY  inner membrane            RXN0-7217
5          lacY  inner membrane            RXN-17755
6          lacZ         cytosol      BETAGALACTOSID-RXN
7          lacZ         cytosol            RXN0-5363
8          lacZ         cytosol            RXN-17726
9          lacZ         cytosol            RXN0-7219

                              SUBSTRATES                              PRODUCTS  \
0  Beta-D-Galactosides,ACETYL-COA  6-Acetyl-Beta-D-Galactosides,CO-A
1    PER-PROTON,PER-Alpha-lactose                 PROTON,Alpha-lactose
2        PER-PROTON,PER-MELIBIOSE                     PROTON,MELIBIOSE
3         PER-PROTON,PER-CPD-3561                      PROTON,CPD-3561
4         PER-PROTON,PER-CPD-3785                      PROTON,CPD-3785
5         PER-PROTON,PER-CPD-3801                      PROTON,CPD-3801
6                  CPD-15972,WATER          GALACTOSE,Glucopyranose
7                    Alpha-lactose                           ALLOLACTOSE
8                   CPD-3561,WATER          GALACTOSE,Fructofuranose
9                   CPD-3785,WATER             GALACTOSE,D-ARABINOSE

   FWD_RATE  RVS_RATE
0       1.0       0.0
1       1.0       1.0
2       1.0       1.0
3       1.0       1.0
4       1.0       1.0
5       1.0       1.0
6       1.0       0.0
7       1.0       1.0
8       1.0       0.0
9       1.0       0.0
```

Note: By default, Atlas interprets the default location of monomers as cytoplasmatic. When setting the location to `CYT`, the `setTransport()` function will delete a previous compartment or append nothing to the name of the monomer.

[19]:
```
# Irreversibility of reactions per gene: Change the Reverse Rate to zero
%time network = utils.metabolicNetwork.setIrreversibility(network, geneLst =␣
 ↪['lacY', 'lacA'])
network
```

```
CPU times: user 3.98 ms, sys: 0 ns, total: 3.98 ms
Wall time: 3.37 ms
```

```
[19]:   GENE OR COMPLEX ENZYME LOCATION               REACTION  \
     0             lacA         cytosol  GALACTOACETYLTRAN-RXN
     1             lacY  inner membrane           TRANS-RXN-24
     2             lacY  inner membrane           TRANS-RXN-94
     3             lacY  inner membrane             RXN0-7215
     4             lacY  inner membrane             RXN0-7217
     5             lacY  inner membrane             RXN-17755
     6             lacZ         cytosol      BETAGALACTOSID-RXN
     7             lacZ         cytosol             RXN0-5363
     8             lacZ         cytosol             RXN-17726
     9             lacZ         cytosol             RXN0-7219

                             SUBSTRATES                            PRODUCTS  \
     0  Beta-D-Galactosides,ACETYL-COA  6-Acetyl-Beta-D-Galactosides,CO-A
     1    PER-PROTON,PER-Alpha-lactose                 PROTON,Alpha-lactose
     2        PER-PROTON,PER-MELIBIOSE                     PROTON,MELIBIOSE
     3         PER-PROTON,PER-CPD-3561                      PROTON,CPD-3561
     4         PER-PROTON,PER-CPD-3785                      PROTON,CPD-3785
     5         PER-PROTON,PER-CPD-3801                      PROTON,CPD-3801
     6                  CPD-15972,WATER              GALACTOSE,Glucopyranose
     7                     Alpha-lactose                          ALLOLACTOSE
     8                   CPD-3561,WATER           GALACTOSE,Fructofuranose
     9                   CPD-3785,WATER              GALACTOSE,D-ARABINOSE

        FWD_RATE  RVS_RATE
     0       1.0       0.0
     1       1.0       0.0
     2       1.0       0.0
     3       1.0       0.0
     4       1.0       0.0
     5       1.0       0.0
     6       1.0       0.0
     7       1.0       1.0
     8       1.0       0.0
     9       1.0       0.0
```

```
[20]: # Irreversibility of reactions per reaction. The beta-galactosidase has also␣
       ↪isomerase activity (reversible reaction)
      %time network = utils.metabolicNetwork.setIrreversibility(network, rxnLst =␣
       ↪['BETAGALACTOSID-RXN', 'RXN-17726', 'RXN0-7219'])
      network
```

```
CPU times: user 4.89 ms, sys: 504 µs, total: 5.39 ms
Wall time: 5.47 ms
```

```
[20]:   GENE OR COMPLEX ENZYME LOCATION               REACTION  \
     0             lacA         cytosol  GALACTOACETYLTRAN-RXN
```

```
1             lacY  inner membrane           TRANS-RXN-24
2             lacY  inner membrane           TRANS-RXN-94
3             lacY  inner membrane              RXN0-7215
4             lacY  inner membrane              RXN0-7217
5             lacY  inner membrane              RXN-17755
6             lacZ         cytosol     BETAGALACTOSID-RXN
7             lacZ         cytosol              RXN0-5363
8             lacZ         cytosol              RXN-17726
9             lacZ         cytosol              RXN0-7219

                          SUBSTRATES                            PRODUCTS  \
0  Beta-D-Galactosides,ACETYL-COA  6-Acetyl-Beta-D-Galactosides,CO-A
1    PER-PROTON,PER-Alpha-lactose                PROTON,Alpha-lactose
2        PER-PROTON,PER-MELIBIOSE                    PROTON,MELIBIOSE
3         PER-PROTON,PER-CPD-3561                     PROTON,CPD-3561
4         PER-PROTON,PER-CPD-3785                     PROTON,CPD-3785
5         PER-PROTON,PER-CPD-3801                     PROTON,CPD-3801
6                 CPD-15972,WATER             GALACTOSE,Glucopyranose
7                   Alpha-lactose                         ALLOLACTOSE
8                  CPD-3561,WATER            GALACTOSE,Fructofuranose
9                  CPD-3785,WATER               GALACTOSE,D-ARABINOSE

   FWD_RATE  RVS_RATE
0       1.0       0.0
1       1.0       0.0
2       1.0       0.0
3       1.0       0.0
4       1.0       0.0
5       1.0       0.0
6       1.0       0.0
7       1.0       1.0
8       1.0       0.0
9       1.0       0.0
```

[21]:
```
# Compartment of reactions. The lacY gene is a protein located to the inner␣
↪membrane of E.coli
%time network = utils.metabolicNetwork.setEnzymeLocation(network, geneLst =␣
↪['lacY'], compartmentLst = ['iMEM'])
network
```

```
CPU times: user 786 µs, sys: 611 µs, total: 1.4 ms
Wall time: 1.27 ms
```

[21]:
```
  GENE OR COMPLEX ENZYME LOCATION                REACTION  \
0            lacA         cytosol  GALACTOACETYLTRAN-RXN
1            lacY  inner membrane           TRANS-RXN-24
2            lacY  inner membrane           TRANS-RXN-94
```

```
3            lacY  inner membrane            RXN0-7215
4            lacY  inner membrane            RXN0-7217
5            lacY  inner membrane            RXN-17755
6            lacZ         cytosol     BETAGALACTOSID-RXN
7            lacZ         cytosol            RXN0-5363
8            lacZ         cytosol            RXN-17726
9            lacZ         cytosol            RXN0-7219

                          SUBSTRATES                            PRODUCTS  \
0  Beta-D-Galactosides,ACETYL-COA  6-Acetyl-Beta-D-Galactosides,CO-A
1    PER-PROTON,PER-Alpha-lactose                 PROTON,Alpha-lactose
2        PER-PROTON,PER-MELIBIOSE                     PROTON,MELIBIOSE
3         PER-PROTON,PER-CPD-3561                      PROTON,CPD-3561
4         PER-PROTON,PER-CPD-3785                      PROTON,CPD-3785
5         PER-PROTON,PER-CPD-3801                      PROTON,CPD-3801
6                 CPD-15972,WATER           GALACTOSE,Glucopyranose
7                    Alpha-lactose                      ALLOLACTOSE
8                  CPD-3561,WATER         GALACTOSE,Fructofuranose
9                  CPD-3785,WATER            GALACTOSE,D-ARABINOSE

   FWD_RATE  RVS_RATE
0       1.0       0.0
1       1.0       0.0
2       1.0       0.0
3       1.0       0.0
4       1.0       0.0
5       1.0       0.0
6       1.0       0.0
7       1.0       1.0
8       1.0       0.0
9       1.0       0.0
```

[22]:
```
%time utils.metabolicNetwork.expand_network(network, './
 ↪lactose-metabolism-cytoscape-v2.txt')
```

```
CPU times: user 243 µs, sys: 1.14 ms, total: 1.38 ms
Wall time: 1.09 ms
```

[23]:
```
%time model = atlas.construct_model_from_metabolic_network(network, verbose =␣
 ↪False) # verbose = True will print the pySB functions needed to reproduce␣
 ↪the model
model
```

```
CPU times: user 408 ms, sys: 0 ns, total: 408 ms
Wall time: 407 ms
```

```
[23]: <Model 'atlas_rbm.construct_model_from_metabolic_network' (monomers: 2, rules:
10, parameters: 40, expressions: 0, compartments: 0) at 0x7f61cfc5e790>
```

```
[24]: utils.analyseConnectivity(model, '/opt/git-repositories/KaSim.Kappa-Dev/KaSa')
# Do not worry, later we will set the initial condition.
# Due to the multiple instances, we create only metabolites at cytoplasm and␣
↪the transport reactions will fail.
```

```
There are some non applicable rules:
rule TRANS_RXN_24 (File "_HXNUD8P608.kappa", line 47, characters 15-448:) will
never be applied.
rule TRANS_RXN_24_rev (File "_HXNUD8P608.kappa", line 48, characters 19-452:)
will never be applied.
rule TRANS_RXN_94 (File "_HXNUD8P608.kappa", line 49, characters 15-440:) will
never be applied.
rule TRANS_RXN_94_rev (File "_HXNUD8P608.kappa", line 50, characters 19-444:)
will never be applied.
rule RXN0_7215 (File "_HXNUD8P608.kappa", line 51, characters 12-432:) will
never be applied.
rule RXN0_7215_rev (File "_HXNUD8P608.kappa", line 52, characters 16-436:) will
never be applied.
rule RXN0_7217 (File "_HXNUD8P608.kappa", line 53, characters 12-432:) will
never be applied.
rule RXN0_7217_rev (File "_HXNUD8P608.kappa", line 54, characters 16-436:) will
never be applied.
rule RXN_17755 (File "_HXNUD8P608.kappa", line 55, characters 12-432:) will
never be applied.
rule RXN_17755_rev (File "_HXNUD8P608.kappa", line 56, characters 16-436:) will
never be applied.

Every monomer and complex of monomers may occur in the model.
```

Note: Even with an initial condition of zero, KaSA will report reactions as reacheables because it analyzes the connectivity of the network. Unreacheable rules or agents will those that: first, monomers are not instantiated (missing `%init:` statements in `kappa`), and two, *Rules* not producing the monomers in the required state (e.g. phosphorylation). For sake of completeness, we produced kappa files that fail the connectivity analysis:

```
[25]: utils.analyseConnectivity('fail_rules.kappa', path = '/opt/git-repositories/
↪KaSim.Kappa-Dev/KaSa')
# Missing instantiation of the lacA trimer, so impossibility to catalyze its␣
↪reactions
```

```
There are some non applicable rules:
rule GALACTOACETYLTRAN_RXN_galactose (File "fail_rules.kappa", line 187,
characters 34-534:) will never be applied.
rule GALACTOACETYLTRAN_RXN_galactose_rev (File "fail_rules.kappa", line 188,
characters 38-538:) will never be applied.
```

```
Every monomer and complex of monomers may occur in the model.
```

[26]:
```
utils.analyseConnectivity('fail_agents.kappa', path = '/opt/git-repositories/
 ↪KaSim.Kappa-Dev/KaSa')
# Missing instantiation of Acetyl-CoA and CoA, substrate and product of the␣
 ↪lacA trimer.
# It does not matter if the rate of the reaction is zero.
# (by design, Atlas writes reversible reactions even if the rate for the␣
 ↪reverse reaction is zero)
```

```
There are some non applicable rules:
rule GALACTOACETYLTRAN_RXN_galactose (File "fail_agents.kappa", line 187,
characters 34-534:) will never be applied.
rule GALACTOACETYLTRAN_RXN_galactose_rev (File "fail_agents.kappa", line 188,
characters 38-538:) will never be applied.

Every monomer and complex of monomers may occur in the model.
```

To simulate, we need to set the initial condition:

[27]:
```
# initial condition
# for metabolites
simulation.set_initial.met(model, 'Alpha_lactose', 'per', 100)
# Message of "Initial not found" explained: By default, Atlas creates initials␣
 ↪for cytosolic metabolites, proteins, and complexes (DNA and RNAs are always␣
 ↪cytosolic)
simulation.set_initial.met(model, 'PROTON', 'per', 100) # required for lactose␣
 ↪transport
simulation.set_initial.met(model, 'WATER', 'cyt', 100) # required for lactose␣
 ↪hydrolysis

# for proteins
simulation.set_initial.prot(model, 'lacY', 'imem', 1) # required for lactose␣
 ↪transport.
simulation.set_initial.prot(model, 'lacZ', 'cyt', 1) # required for lactose␣
 ↪isomerization
```

```
Initial t0_met_Alpha_lactose not found. Creating Initial t0_met_Alpha_lactose…
Initial t0_met_PROTON not found. Creating Initial t0_met_PROTON…
Initial t0_prot_lacY not found. Creating Initial t0_prot_lacY…
```

[27]:
```
<Model 'atlas_rbm.construct_model_from_metabolic_network' (monomers: 2, rules:
10, parameters: 43, expressions: 0, compartments: 0) at 0x7f61cfc5e790>
```

[28]:
```
utils.analyseConnectivity(model, '/opt/git-repositories/KaSim.Kappa-Dev/KaSa')
```

```
Every rule may be applied.
Every monomer and complex of monomers may occur in the model.
```

Once the model has a suitable initial condition, the user could export it to a variety of formats or simulate directly inside the notebook

[29]:
```python
# export to
%time export.to_sbml(model, 'export-to-sbml.sbml')
%time export.to_matlab(model, 'export-to-matlab.m')
%time export.to_mathematica(model, 'export-to-mathematica.wl')
%time export.to_potterswheel(model, 'export-to-potterswheel.m')
%time export.to_bngl(model, 'export-to-bngl.bngl')
%time export.to_bngnet(model, 'export-to-bngnet.net')
%time export.to_kappa(model, 'export-to-kappa.kappa')
%time export.to_python(model, 'export-to-python.py')
%time export.to_pysb(model, 'export-to-pysb.py')
# %time export.to_stochkit(model, 'export-to-stochkit.xml') # pySB error
%time export.to_json(model, 'export-to-json.json')
```

```
CPU times: user 454 ms, sys: 55.7 ms, total: 510 ms
Wall time: 1.28 s
CPU times: user 72.8 ms, sys: 0 ns, total: 72.8 ms
Wall time: 74.9 ms
CPU times: user 178 ms, sys: 0 ns, total: 178 ms
Wall time: 179 ms
CPU times: user 165 ms, sys: 0 ns, total: 165 ms
Wall time: 166 ms
CPU times: user 3.46 ms, sys: 0 ns, total: 3.46 ms
Wall time: 5.12 ms
CPU times: user 12.5 ms, sys: 0 ns, total: 12.5 ms
Wall time: 510 ms
CPU times: user 3.6 ms, sys: 0 ns, total: 3.6 ms
Wall time: 3.42 ms
CPU times: user 166 ms, sys: 0 ns, total: 166 ms
Wall time: 168 ms
CPU times: user 1.36 ms, sys: 1.65 ms, total: 3.01 ms
Wall time: 4.7 ms
CPU times: user 3.34 ms, sys: 0 ns, total: 3.34 ms
Wall time: 3.23 ms
```

[30]:
```python
# simulation
bng = '/opt/git-repositories/bionetgen.RuleWorld/bng2/'
kasim = '/opt/git-repositories/KaSim4.Kappa-Dev/'
cupsoda = '/opt/git-repositories/cupSODA.aresio/'
stochkit = '/opt/git-repositories/StochKit.StochSS' # not the bin folder

%time data0 = simulation.scipy(model, start = 0, finish = 10, points = 2000)
%time data1 = simulation.cupsoda(model, start = 0, finish = 10, points = 2000,␣
 ↪path = cupsoda) # only if you have a GPU NVIDIA; comment if not.
```

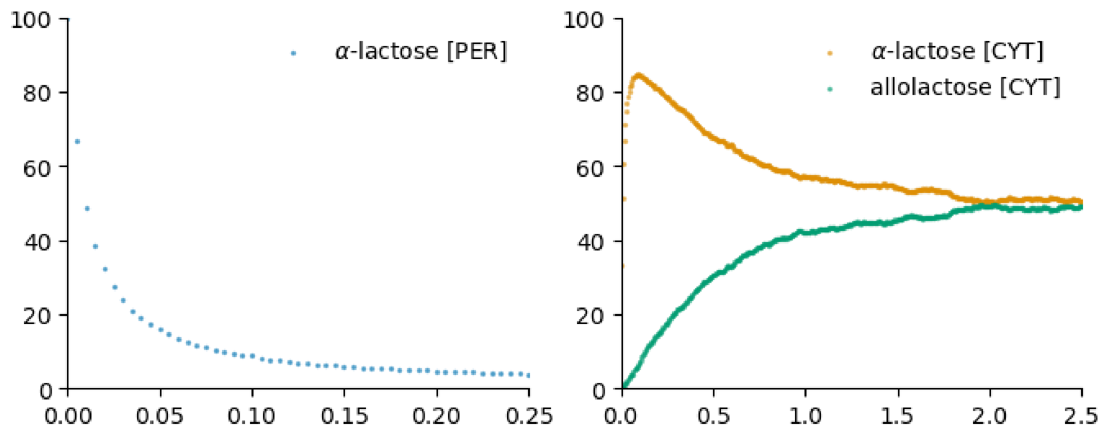
```
%time data2 = simulation.bngODE(model, start = 0, finish = 10, points = 2000,␣
 ↪path = bng)
%time data3 = simulation.bngSSA(model, start = 0, finish = 10, points = 2000,␣
 ↪n_runs = 20, path = bng)
# %time data4 = simulation.bngPLA(model, start = 0, finish = 10, points = 2000,␣
 ↪n_runs = 20, path = bng) # requires refinement of the model
%time data5 = simulation.bngNF(model, start = 0, finish = 10, points = 2000,␣
 ↪n_runs = 20, path = bng)
%time data6 = simulation.kasim(model, start = 0, finish = 10, points = 2000,␣
 ↪n_runs = 20, path = kasim)
# %time data7 = simulation.stochkit(model, start = 0, finish = 10, points =␣
 ↪2000, n_runs = 20, path = stochkit) # pySB error
```

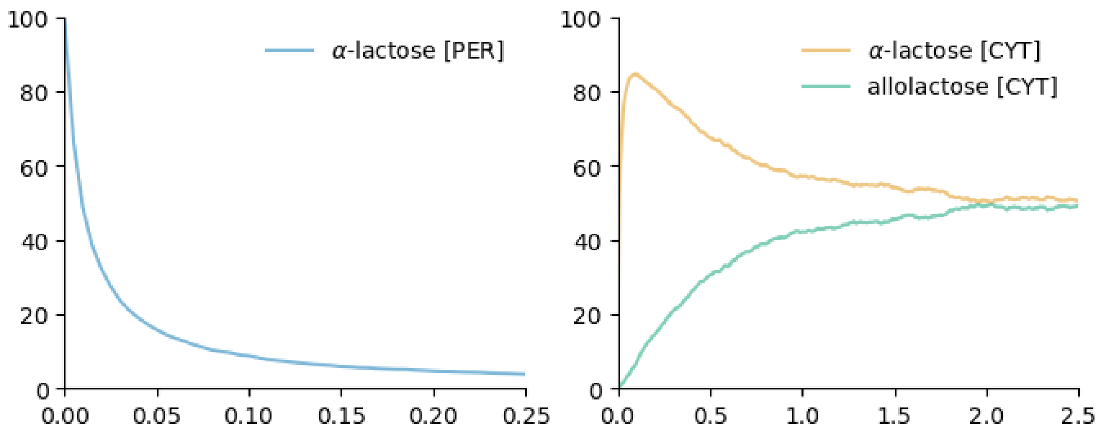
```
CPU times: user 3.64 s, sys: 1.32 s, total: 4.96 s
Wall time: 3.39 s
CPU times: user 1.43 s, sys: 2.52 ms, total: 1.43 s
Wall time: 2.28 s
CPU times: user 1.5 s, sys: 0 ns, total: 1.5 s
Wall time: 2.28 s
CPU times: user 3.74 s, sys: 23.7 ms, total: 3.76 s
Wall time: 6.04 s
CPU times: user 2.91 s, sys: 108 ms, total: 3.02 s
Wall time: 5.21 s
CPU times: user 2.4 s, sys: 181 ms, total: 2.58 s
Wall time: 3.94 s
```

Note: The ODE and SSA simulations require the execution of the network generation, while KaSim and NFsim are network-free simulators.

Finally, we plot the simulation results. The result of the `simulation.scipy()`, `simulation.scipy()` function is a pandas dataframe. In the case of stochastic simulations (SSA, KaSim, NFsim, Stochkit), the function returns a dictionary with a list of dataframes for each simulations (`sims` key), a dataframe with the average (`avrg` key) and a dataframe with the standard deviation (`stdv` key) of those simulations. Currently, we included three kind of plots, although the user could access the dataframes and plot diretly with methods in the seaborn package (https://seaborn.pydata.org/), in the pandas package (https://pandas.pydata.org/pandas-docs/stable/reference/api/pandas.DataFrame.plot.html), or with matplotlib (https://matplotlib.org/).

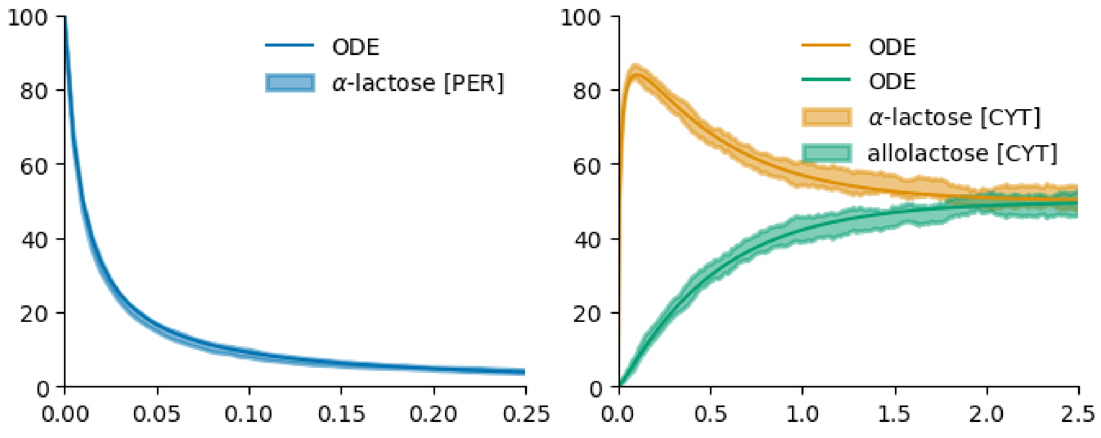
[31]:
```
import seaborn
import matplotlib.pyplot as plt

palette = seaborn.color_palette('colorblind')

for kind in ['scatter', 'plot']:
    # first plot, periplasmic concentration
    fig, ax = plt.subplots(1, 2, figsize = (4*2, 3*1), dpi = 100)
    simulation.plot.metabolite(data3['avrg'], 'Alpha_lactose', 'per', ax =␣
↪ax[0], **{'kind' : kind},
        plt_kws = {'s' : 2, 'color' : palette[0], 'label' : r'$\alpha$-lactose␣
↪[PER]', 'alpha' : .5})

    # second plot, cytoplasmic concentration
    simulation.plot.metabolite(data3['avrg'], 'Alpha_lactose', 'cyt', ax =␣
↪ax[1], **{'kind' : kind},
        plt_kws = {'s' : 2, 'color' : palette[1], 'label' : r'$\alpha$-lactose␣
↪[CYT]', 'alpha' : .5})

    # second plot, cytoplasmic concentration
    simulation.plot.metabolite(data3['avrg'], 'ALLOLACTOSE', 'cyt', ax = ax[1],␣
↪**{'kind' : kind},
        plt_kws = {'s' : 2, 'color' : palette[2], 'label' : r'allolactose␣
↪[CYT]', 'alpha' : .5})

    ax[0].set_xlim((0,.25))
    ax[1].set_xlim((0,2.5))
    ax[0].set_ylim((0,100))
    ax[1].set_ylim((0,100))

    seaborn.despine()
```





```
[32]: import seaborn
      import matplotlib.pyplot as plt

      palette = seaborn.color_palette('colorblind')

      # first plot, periplasmic concentration
      fig, ax = plt.subplots(1, 2, figsize = (4*2, 3*1), dpi = 100)
      simulation.plot.metabolite(data3, 'Alpha_lactose', 'per', ax = ax[0], **{'kind'␣
       ↪: 'fill_between', 'weight' : .5},
         plt_kws = {'s' : 2, 'color' : palette[0], 'label' : r'$\alpha$-lactose␣
       ↪[PER]', 'alpha' : .5})

      # second plot, cytoplasmic concentration
      simulation.plot.metabolite(data3, 'Alpha_lactose', 'cyt', ax = ax[1], **{'kind'␣
       ↪: 'fill_between', 'weight' : .5},
         plt_kws = {'s' : 2, 'color' : palette[1], 'label' : r'$\alpha$-lactose␣
       ↪[CYT]', 'alpha' : .5})

      # second plot, cytoplasmic concentration
      simulation.plot.metabolite(data3, 'ALLOLACTOSE', 'cyt', ax = ax[1], **{'kind' :␣
       ↪'fill_between', 'weight' : .5},
         plt_kws = {'s' : 2, 'color' : palette[2], 'label' : r'allolactose [CYT]',␣
       ↪'alpha' : .5})

      # first plot, periplasmic concentration
      simulation.plot.metabolite(data0, 'Alpha_lactose', 'per', ax = ax[0], **{'kind'␣
       ↪: kind},
         plt_kws = {'s' : 2, 'color' : palette[0], 'label' : r'ODE'})

      # second plot, cytoplasmic concentration
```

```
simulation.plot.metabolite(data0, 'Alpha_lactose', 'cyt', ax = ax[1], **{'kind'␣
↪: kind},
    plt_kws = {'s' : 2, 'color' : palette[1], 'label' : r'ODE'})

# second plot, cytoplasmic concentration
simulation.plot.metabolite(data0, 'ALLOLACTOSE', 'cyt', ax = ax[1], **{'kind' :␣
↪kind},
    plt_kws = {'s' : 2, 'color' : palette[2], 'label' : r'ODE'})

ax[0].set_xlim((0,.25))
ax[1].set_xlim((0,2.5))
ax[0].set_ylim((0,100))
ax[1].set_ylim((0,100))

seaborn.despine()
```



As expected, the degradation of lactose into glucose and galactose is impossible due to a Section **??**. The disconnected pathway will be manually corrected and explained in a second tutorial. Also, we must note that we considered the enzymatic reactions are performed by the monomers (e.g. one monomer of LacZ catalyze one reaction), although biochemical information informs the monomers are catalytically active only when the complex is assembled (e.g. one LacZ tetramer catalyzes four reactions). This will be considered next, when we will model protein-protein interactions.

1.6 Modeling protein-protein interactions

We could consult the stoichiometry of complexes with PythonCyc, and vice versa, quering which complexes are formed by a certain gene product (although indirectly):

[33]:
```
import pythoncyc
print(pythoncyc.select_organism('ECOLI').
↪monomers_of_protein('BETAGALACTOSID-CPLX'))
```

```
# we obtained the code "BETAGALACTOSID-CPLX" with the gathering of the␣
 ↪metabolic data in "product" format.
print(pythoncyc.select_organism('ECOLI').
 ↪monomers_of_protein('GALACTOACETYLTRAN-CPLX'))
# Similarly, the code "GALACTOACETYLTRAN-CPLX".

print(pythoncyc.select_organism('ECOLI').
 ↪genes_of_protein('BETAGALACTOSID-CPLX'))
print(pythoncyc.select_organism('ECOLI').
 ↪genes_of_protein('GALACTOACETYLTRAN-CPLX'))

print(pythoncyc.select_organism('ECOLI').all_products_of_gene('EG10527'))
print(pythoncyc.select_organism('ECOLI').all_products_of_gene('EG10524'))
```

```
[['|BETAGALACTOSID-MONOMER|'], [4]]
[['|GALACTOACETYLTRAN-MONOMER|'], [3]]
['|EG10527|']
['|EG10524|']
['|BETAGALACTOSID-MONOMER|', '|BETAGALACTOSID-CPLX|']
['|GALACTOACETYLTRAN-MONOMER|', '|GALACTOACETYLTRAN-CPLX|']
```

We included the function `utils.interactionNetwork.FromGeneList()` to write possible mechanism of assembly from the stoichiometry data of complexes.

[34]:
```
%time network = utils.interactionNetwork.FromGeneList('ECOLI', genes = ['lacZ',␣
 ↪'lacA', 'lacY'], precalculated = df_genes)
network
```

```
CPU times: user 10.3 ms, sys: 2.89 ms, total: 13.1 ms
Wall time: 56 ms
```

[34]:
```
  SOURCE            TARGET                          LOCATION  FWD_RATE  \
0   lacA              lacA                 [cytosol,cytosol]       1.0
1   lacA       [lacA,lacA]         [cytosol,cytosol,cytosol]       1.0
2   lacZ              lacZ                 [cytosol,cytosol]       1.0
3   lacZ       [lacZ,lacZ]         [cytosol,cytosol,cytosol]       1.0
4   lacZ  [lacZ,lacZ,lacZ]  [cytosol,cytosol,cytosol,cytosol]       1.0

   RVS_RATE
0       1.0
1       1.0
2       1.0
3       1.0
4       1.0
```

[35]:
```
atlas.read_network('network-lac-ProtProt.tsv')
```

[35]:
```
          SOURCE        TARGET  FWD_RATE  RVS_RATE LOCATION
0           lacZ          lacZ       1.0       0.0  cytosol
1    [lacZ,lacZ]   [lacZ,lacZ]       1.0       0.0  cytosol
2           lacA          lacA       1.0       0.0  cytosol
3           lacA   [lacA,lacA]       1.0       0.0  cytosol
4           lacI          lacI       1.0       0.0  cytosol
```

Despite the effort, the function could retrieve interactions that would be hard to observe in nature (parsimony). Also, because we obtain from BioCyc the protein composition of complexes and not intermediates, we could miss interactions or add interactions that never occur because we programmed the complete enumeration of possible ordered mechanisms. From the network, we could remove interaction by its indexes, and add interactions defining its participants:

[36]:
```
network = utils.interactionNetwork.removeInteraction(network, index = [3,4])
network = utils.interactionNetwork.addInteraction(network, source = ['lacZ',␣
 ↪'lacZ'], target = ['lacZ', 'lacZ'])
# we supposed a dimer-dimer interaction is more plausible than a trimer-monomer␣
 ↪interaction to form a tetramer
network
```

[36]:
```
          SOURCE        TARGET                   LOCATION  FWD_RATE  RVS_RATE
0           lacA          lacA          [cytosol,cytosol]       1.0       1.0
1           lacA   [lacA,lacA]  [cytosol,cytosol,cytosol]       1.0       1.0
2           lacZ          lacZ          [cytosol,cytosol]       1.0       1.0
3    [lacZ,lacZ]   [lacZ,lacZ]                    cytosol       1.0       1.0
```

Note: If the location of complexes do not match the number of components, Atlas will suppose the location (or the first defined location) is valid for all components of the final complex.

[37]:
```
%time model = atlas.construct_model_from_interaction_network(network, verbose =␣
 ↪False)
model
```

```
CPU times: user 177 ms, sys: 0 ns, total: 177 ms
Wall time: 176 ms
```

[37]:
```
<Model 'atlas_rbm.construct_model_from_interaction_network' (monomers: 1, rules:
4, parameters: 14, expressions: 0, compartments: 0) at 0x7f620e07bd00>
```

[38]:
```
utils.analyzeConnectivity(model)
```

```
Every rule may be applied.
Every monomer and complex of monomers may occur in the model.
```

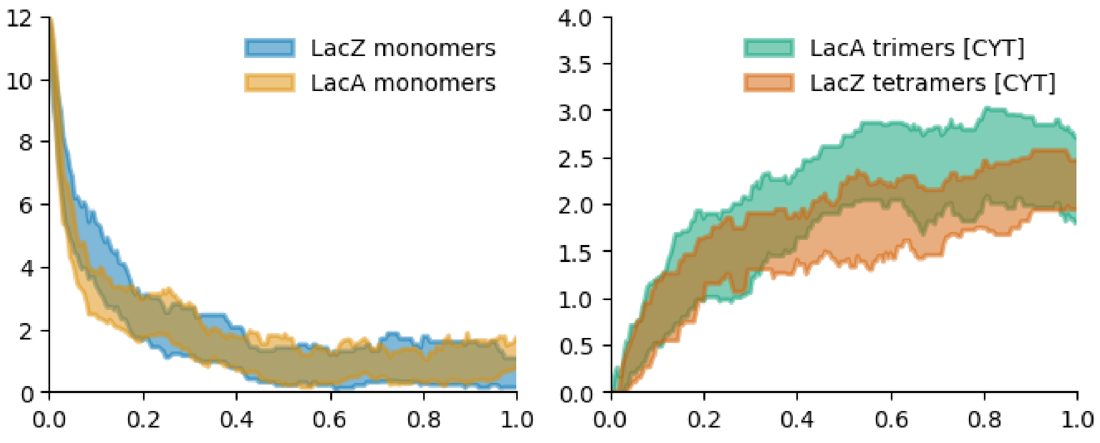
[39]:
```
# initial condition
# for proteins
simulation.set_initial.prot(model, 'lacZ', 'cyt', 12)
simulation.set_initial.prot(model, 'lacA', 'cyt', 12)
```

```
# simulation
bng = '/opt/git-repositories/bionetgen.RuleWorld/bng2/'
kasim = '/opt/git-repositories/KaSim4.Kappa-Dev/'
cupsoda = '/opt/git-repositories/cupSODA.aresio/'
stochkit = '/opt/git-repositories/StochKit.StochSS' # not the bin folder

%time data0 = simulation.scipy(model, start = 0, finish = 10, points = 2000)
%time data1 = simulation.cupsoda(model, start = 0, finish = 10, points = 2000,␣
 ↪path = cupsoda)
%time data2 = simulation.bngODE(model, start = 0, finish = 10, points = 2000,␣
 ↪path = bng)
%time data3 = simulation.bngSSA(model, start = 0, finish = 10, points = 2000,␣
 ↪n_runs = 20, path = bng)
# %time data4 = simulation.bngPLA(model, start = 0, finish = 10, points = 2000,␣
 ↪n_runs = 20, path = bng)
%time data5 = simulation.bngNF(model, start = 0, finish = 10, points = 2000,␣
 ↪n_runs = 20, path = bng)
%time data6 = simulation.kasim(model, start = 0, finish = 10, points = 2000,␣
 ↪n_runs = 20, path = kasim)
# %time data7 = simulation.stochkit(model, start = 0, finish = 10, points =␣
 ↪2000, n_runs = 20, path = stochkit) # pySB error
```

```
CPU times: user 335 ms, sys: 11.4 ms, total: 346 ms
Wall time: 750 ms
CPU times: user 282 ms, sys: 12.2 ms, total: 294 ms
Wall time: 819 ms
CPU times: user 311 ms, sys: 9.77 ms, total: 321 ms
Wall time: 763 ms
CPU times: user 1.39 s, sys: 38.1 ms, total: 1.43 s
Wall time: 2.72 s
CPU times: user 957 ms, sys: 72.6 ms, total: 1.03 s
Wall time: 2.32 s
CPU times: user 859 ms, sys: 181 ms, total: 1.04 s
Wall time: 1.67 s
```

[40]:
```
import seaborn
import matplotlib.pyplot as plt

palette = seaborn.color_palette('colorblind')

# first plot, periplasmic concentration
fig, ax = plt.subplots(1, 2, figsize = (4*2, 3*1), dpi = 100)
simulation.plot.protein(data3, 'lacZ', 'cyt', ax = ax[0], **{'kind' :␣
 ↪'fill_between', 'weight' : .5},
    plt_kws = {'s' : 2, 'color' : palette[0], 'label' : 'LacZ monomers',␣
 ↪'alpha' : .5})
```

```
simulation.plot.protein(data3, 'lacA', 'cyt', ax = ax[0], **{'kind' :␣
↪'fill_between', 'weight' : .5},
    plt_kws = {'s' : 2, 'color' : palette[1], 'label' : 'LacA monomers',␣
↪'alpha' : .5})

simulation.plot.cplx(data3, 'lacAx3', 'cyt', ax = ax[1], **{'kind' :␣
↪'fill_between', 'weight' : .5},
    plt_kws = {'s' : 2, 'color' : palette[2], 'label' : r'LacA trimers [CYT]',␣
↪'alpha' : .5})

simulation.plot.cplx(data3, 'lacZx4', 'cyt', ax = ax[1], **{'kind' :␣
↪'fill_between', 'weight' : .5},
   plt_kws = {'s' : 2, 'color' : palette[3], 'label' : r'LacZ tetramers [CYT]',␣
↪'alpha' : .5})

ax[0].set_xlim((0,1))
ax[1].set_xlim((0,1))
ax[0].set_ylim((0,12))
ax[1].set_ylim((0,4))

seaborn.despine()
```



1.7 Modeling gene expression

To model gene expression (transcription and translation) we must first retrieve data for the genomic architecture of the considered genes. For this purpose, we could use the `operon_of_gene` method from the pythoncyc package to obtain the operons of a gene:

[41]:

```
import pythoncyc
TUs = pythoncyc.select_organism('ECOLI').operon_of_gene(df_genes['gene␣
 ↪name']['lacZ'])
TUs
```

```
[41]: ['|TU0-4703|', '|TU00036|', '|TU0-4701|', '|TU0-4702|', '|TU0-14521|']
```

```
[42]: components = []
      for TU in TUs:
          x = utils.getData('ECOLI', TU)['components']
          print(TU, '=', x)
          components.append(x)
```

```
|TU0-4703| = ['|TERM0223|', '|TERM0222|', '|EG10524|', '|EG10526|', '|EG10527|',
'|PM0-4943|']
|TU00036| = ['|BS0-5469|', '|BS0-5468|', '|BS0-5467|', '|BS0-5463|',
'|BS0-5462|', '|BS0-5461|', '|TERM0223|', '|TERM0222|', '|PM00045|',
'|BS0-3622|', '|BS00104|', '|BS00105|', '|BS00106|', '|EG10524|', '|EG10526|',
'|EG10527|']
|TU0-4701| = ['|TERM0223|', '|TERM0222|', '|BS0-3622|', '|EG10524|',
'|EG10526|', '|EG10527|', '|PM0-4941|']
|TU0-4702| = ['|TERM0223|', '|TERM0222|', '|BS0-3622|', '|EG10524|',
'|EG10526|', '|EG10527|', '|PM0-4942|']
|TU0-14521| = ['|TERM0223|', '|TERM0222|', '|EG10524|', '|EG10526|',
'|PM0-9804|']
```

Note: An operon is a functional unit of DNA under the control of a promoter. In bacteria, operons could be policistronic (two or more genes under the control of a unique promoter) or monocistronic (only one gene under the control of a promoter). However, the information from the pythoncyc could led to misinterpretation of the genomic architecture, and we do not provide a function to cover all possibilities. Please, look at https://ecocyc.org/gene?orgid=ECOLI&id=EG10527#tab=TU and similar webpages to construct by yourself the network using a spreadsheet or text editor software.

Here is an example depicting the DNA architecture of the lactose operons (see above note an output of `operon_of_gene`). Using brackets, Atlas understand that DNA promoters, ribosome binding sites (RBS), coding DNA sequences (CDS), and terminators are part of the same architectural unit, and therefore, Atlas defines the RNA products and protein products. From the example, Atlas will write rules for the synthesis of 10 molecules of RNA (five promoters times two terminators), which produce three proteins:

```
[43]: utils.read_network('network-lac-operon-arq.tsv')
```

```
[43]:         UPSTREAM      DOWNSTREAM  RNAP_FWD_DOCK_RATE  RNAP_RVS_DOCK_RATE  \
      0      [lacZ-pro4      lacZ-pro3                 0.0                 1.0
      1       lacZ-pro3      lacZ-pro2                 0.0                 1.0
      2       lacZ-pro2   BS-lacI-72-92                0.0                 1.0
      3   BS-lacI-72-92    BS-lacI-21-1                NaN                 NaN
      4    BS-lacI-21-1      lacZ-pro1                 NaN                 NaN
5         lacZ-pro1         lacZ-rbs                 1.0                 1.0
6          lacZ-rbs         lacZ-cds                 NaN                 NaN
7          lacZ-cds  BS-lacI-422-402                 NaN                 NaN
8   BS-lacI-422-402        lacY-pro1                 NaN                 NaN
9         lacY-pro1         lacY-rbs                 0.0                 1.0
10         lacY-rbs         lacY-cds                 NaN                 NaN
11         lacY-cds         lacA-rbs                 NaN                 NaN
12         lacA-rbs         lacA-cds                 NaN                 NaN
13         lacA-cds        lacA-ter1                 NaN                 NaN
14        lacA-ter1       lacA-ter2]                 NaN                 NaN

    RNAP_FWD_SLIDE_RATE  RNAP_FWD_FALL_RATE  RIB_FWD_DOCK_RATE  \
0                     1                 NaN                NaN
1                     1                 NaN                NaN
2                     1                 NaN                NaN
3                     1                 NaN                NaN
4                     1                 NaN                NaN
5                     1                 NaN                NaN
6                     1                 NaN                1.0
7                     1                 NaN                NaN
8                     1                 NaN                NaN
9                     1                 NaN                NaN
10                    1                 NaN                1.0
11                    1                 NaN                NaN
12                    1                 NaN                1.0
13                    1                 1.0                NaN
14                    1                 1.0                NaN

    RIB_RVS_DOCK_RATE  RIB_FWD_SLIDE_RATE  RIB_FWD_FALL_RATE
0                 NaN                 NaN                NaN
1                 NaN                 NaN                NaN
2                 NaN                 NaN                NaN
3                 NaN                 NaN                NaN
4                 NaN                 NaN                NaN
5                 NaN                 NaN                NaN
6                 1.0                 1.0                1.0
7                 NaN                 NaN                NaN
8                 NaN                 NaN                NaN
9                 NaN                 NaN                NaN
10                1.0                 1.0                1.0
11                NaN                 NaN                NaN
12                1.0                 1.0                1.0
13                NaN                 NaN                NaN
14                NaN                 NaN                NaN
```

Notes:

- `RNAP_FWD_DOCK_RATE`s and `RNAP_RVS_DOCK_RATE`s are valid values only for the description of the reversible interaction of the RNA Polymerase to the promoters.

- `RNAP_FWD_SLIDE_RATE`s are valid values for the transition of the RNA Polymerase from the `UPSTREAM` to the `DOWNSTREAM` DNA parts. We describe rules where the RNA Polymerase could not move back.
- `RNAP_FWD_FALL_RATE`s are valid values only for the description of the unbinding of the RNA Polymerase and the `UPSTREAM` identifiying a DNA terminator.
- `RIB_FWD_DOCK_RATE`s and `RIB_RVS_DOCK_RATE`s are valid values only for the description of the reversible interaction of the bacterial Ribosome to the RBS.
- `RIB_FWD_SLIDE_RATE`s are valid values for the transition of the Ribosome from the `UPSTREAM` to the `DOWNSTREAM` RNA parts. We describe rules where the Ribosome could not move back.
- `RIB_FWD_FALL_RATE`s are valid values only for the description of the unbinding of the Ribosome and the `UPSTREAM` identifiying a CDS.
- Encode DNA parts as: "name of the gene"-"type of DNA part" (`pro#`: Promoter, `rbs`: Ribosome Binding Site, `cds`: Coding DNA Sequence, `ter#`: (transcriptional) Terminator). Multiple promoters and terminators must have a numeric identifier (to replace `#`). For instance, `lacZ-pro1` identifies the most proximal promoter to the `lacZ` gene.
- In the case of Binding Sites (`BS`) for the interaction of Transcriptional Factors with DNA, please use the notation: BS-"name of the gene or regulator"-"upstream genomic coordinate"-"downstream genomic coordinate". The coordinates could be relatives or absolutes. For instance `BS-araC-56-72` describe the binding site located upstream the transcription start site of the araC gene, from the -56 nucleotide to the -72 nucleotide.

We provide two functions to reconstruction transcription and translation from the network of DNA parts (known as a "genome graph"). Here, we will use the first of these two functions. The `construct_model_from_genome_graph()` function is an automatization of the Kappa BioBrick Framework (https://www.sciencedirect.com/science/article/pii/S1571066111001289). The function writes rules for transcription of DNA mediated by a `RNAP-CPLX` agent (to keep notation with BioCyc) and translation of RNA mediated by a `RIBOSOME-CPLX` agent:

[44]:
```
%time model = atlas.construct_model_from_genome_graph('network-lac-operon-arq.
 ↪tsv')
model
```

```
CPU times: user 804 ms, sys: 19.2 ms, total: 824 ms
Wall time: 823 ms
```

[44]:
```
<Model 'atlas_rbm.construct_model_from_genome_graph' (monomers: 4, rules: 31,
parameters: 53, expressions: 0, compartments: 0) at 0x7f61cc307250>
```

[45]:
```
%time model = atlas.construct_model_from_genome_graph('network-ara-operons-arq.
 ↪tsv')
model
```

```
CPU times: user 1.07 s, sys: 0 ns, total: 1.07 s
Wall time: 1.16 s
```

```
[45]: <Model 'atlas_rbm.construct_model_from_genome_graph' (monomers: 4, rules: 50,
      parameters: 78, expressions: 0, compartments: 0) at 0x7f61cc28efd0>
```

1.8 Modeling regulation of gene expression

The second function that describe gene transcription and translation also consider the specificity of sigma factors for bacterial promoters. In bacteria, there is only one RNA polymerase that synthetize mRNA, rRNA, and tRNA. To control which genes are in active transcription, promoters show differential specificity for one or several sigma factors. In the case of *E. coli*, the bacteria has seven of those sigma factors: rpoD (exponential growth phase), rpoE (heat and protein misfolding stress), rpoH (heat stress), rpoN (nitrogen homeostasis), rpoS (stationary phase), fecI (iron starvation), and fliA (flagela synthesis) that interact with the core RNA polymerase (rpoAABC) and form an interaction interface for the holoenzyme with promoters.

```
[46]: utils.read_network('network-lac-sigma-specificity.tsv')
```

```
[46]:                       SOURCE     TARGET  FWD_DOCK_RATE  RVS_DOCK_RATE  \
      0  [rpoA,rpoA,rpoB,rpoC,rpoD]  lacZ-pro4            1.0            1.0
      1  [rpoA,rpoA,rpoB,rpoC,rpoD]  lacZ-pro3            1.0            1.0
      2  [rpoA,rpoA,rpoB,rpoC,rpoD]  lacZ-pro2            1.0            1.0
      3  [rpoA,rpoA,rpoB,rpoC,rpoD]  lacZ-pro1            1.0            1.0
      4  [rpoA,rpoA,rpoB,rpoC,rpoD]  lacY-pro1            1.0            1.0

         FWD_SLIDE_RATE
      0             1.0
      1             1.0
      2             1.0
      3             1.0
      4             1.0
```

Note: The `TARGET` name must match the `UPSTREAM` name in the network that describe the genomic architecture:

```
[47]: network = utils.read_network('network-lac+rpoABCD-operons-arq.tsv')
      network[['UPSTREAM', 'DOWNSTREAM']][network['UPSTREAM'].str.contains('pro')] #␣
       ↪shows the UPSTREAM and DOWNSTREAM columns where the UPSTREAM column contains␣
       ↪the 'pro' string
```

```
[47]:       UPSTREAM DOWNSTREAM
      0   [lacZ-pro4  lacZ-pro3
      1    lacZ-pro3  lacZ-pro2
      2    lacZ-pro2  lacZ-pro1
      3    lacZ-pro1   lacZ-rbs
      6    lacY-pro1   lacY-rbs
      12  [rpoA-pro1   rpoA-rbs
      15  [rpoB-pro1   rpoB-rbs
      20  [rpoD-pro1   rpoD-rbs
```

[48]:
```
%time model = atlas.
↪construct_model_from_sigma_specificity_network('network-lac-sigma-specificity.
↪tsv', 'network-lac+rpoABCD-operons-arq.tsv')
model
```

```
CPU times: user 1.42 s, sys: 0 ns, total: 1.42 s
Wall time: 1.41 s
```

[48]:
```
<Model 'atlas_rbm.construct_model_from_sigma_specificity_network' (monomers: 4,
rules: 55, parameters: 91, expressions: 0, compartments: 0) at 0x7f620e2fe280>
```

[49]:
```
%time model = atlas.
↪construct_model_from_sigma_specificity_network('network-ara-sigma-specificity.
↪tsv', 'network-ara+rpoABCD-operons-arq.tsv')
```

```
CPU times: user 1.82 s, sys: 0 ns, total: 1.82 s
Wall time: 1.88 s
```

However, sigma factors is only one of the many mechanism a bacteria has to control gene expression. Canonically speacking, Transcription Factors (TFs) drive the control of gene expression. TFs are proteins that bind to DNA or unbind from DNA at speciliazed sequences. Most TFs are sensors of metabolites: lacI senses allolactose, araC senses arabinose, crp senses cyclic AMP, etc. We employ an interaction network to model such information:

[50]:
```
net1 = utils.read_network('network-lac+ara-TFs+DNA.tsv')
# net1 # for better visualization
net1[0:1]
```

[50]:
```
                              SOURCE        TARGET  FWD_RATE  RVS_RATE LOCATION
0  [crp,SMALL-CAMP,crp,SMALL-CAMP]  BS-crp-51-72       1.0       1.0  cytosol
```

Note: We appended the prefix `SMALL-` to tell Atlas that the interaction partner is a metabolite and not a protein (an interaction network is interpreted by default as a protein-protein interaction network). We employs the *hypergraph notation* to denote a complex of agents.

[51]:
```
net2 = utils.read_network('network-lac+ara-ProtMet.tsv')
net3 = utils.read_network('network-lac-ProtProt.tsv')
```

Networks of the same kind (except metabolic networks) must be concatenated to produce valid models. Atlas numbers the rules and there is a probability to produce non unique rule names. In the case of metabolic networks, we use the reaction name to name rules (and check for unique names prior to write rules).

[52]:
```
import pandas
network = pandas.concat([net1, net2, net3]).reset_index(drop = True)
# rules are numbered and networks of the same kind cannot be processed␣
↪separately to then combine them into one bigger model (uniqueness of rule␣
↪names)
```

```
# reset index is optional, but we used it to show the model produce all 30␣
↪interactions (index start at zero)
network[-5:]
```

[52]:
```
          SOURCE       TARGET  FWD_RATE  RVS_RATE LOCATION
31          lacZ         lacZ       1.0       0.0  cytosol
32   [lacZ,lacZ]  [lacZ,lacZ]       1.0       0.0  cytosol
33          lacA         lacA       1.0       0.0  cytosol
34          lacA  [lacA,lacA]       1.0       0.0  cytosol
35          lacI         lacI       1.0       0.0  cytosol
```

[53]:
```
%time model = atlas.construct_model_from_interaction_network(network)
model
```

```
CPU times: user 1.13 s, sys: 0 ns, total: 1.13 s
Wall time: 1.13 s
```

[53]:
```
<Model 'atlas_rbm.construct_model_from_interaction_network' (monomers: 3, rules:
36, parameters: 92, expressions: 0, compartments: 0) at 0x7f620e13c430>
```

Data from the BioCyc database concerning regulation of gene expression could be obtained with help of the `utils.getData()` function. After obtaining the components of an operon, we could obtain the genomic coordinates of *most* of the DNA parts, and the center position of DNA binding sites:

[54]:
```
for DNA_part in components[1]:
    data = utils.getData('ECOLI', DNA_part)
    print(DNA_part, data['left_end_position'], data['abs_center_pos'],␣
↪data['right_end_position'])
```

```
|BS0-5469| None 366410 None
|BS0-5468| None 366397 None
|BS0-5467| None 366374 None
|BS0-5463| None 366348 None
|BS0-5462| None 366338 None
|BS0-5461| None 366320 None
|TERM0223| 361140 None 361179
|TERM0222| 361212 None 361236
|PM00045| None None None
|BS0-3622| None 366404.5 None
|BS00104| None 365932 None
|BS00105| None 366425 None
|BS00106| None 366333 None
|EG10524| 361249 None 361860
|EG10526| 361926 None 363179
|EG10527| 363231 None 366305
```

```
[55]: print(TUs[1])
for DNA_part in components[1]:
    data = utils.getData('ECOLI', DNA_part)
    if data['involved_in_regulation'] != None:
#         print(data['involved_in_regulation'][0])
        regulation = utils.getData('ECOLI', data['involved_in_regulation'][0])
        print(DNA_part, regulation['regulator'], regulation['mode'])
print()

print(TUs[2])
for DNA_part in components[2]:
    data = utils.getData('ECOLI', DNA_part)
    if data['involved_in_regulation'] != None:
#         print(data['involved_in_regulation'][0])
        regulation = utils.getData('ECOLI', data['involved_in_regulation'][0])
        print(DNA_part, regulation['regulator'], regulation['mode'])
print()

print(TUs[3])
for DNA_part in components[3]:
    data = utils.getData('ECOLI', DNA_part)
    if data['involved_in_regulation'] != None:
#         print(data['involved_in_regulation'][0])
        regulation = utils.getData('ECOLI', data['involved_in_regulation'][0])
        print(DNA_part, regulation['regulator'], regulation['mode'])
```

```
|TU00036|
|BS0-5469| |PD00288| ['-']
|BS0-5468| |PD00288| ['-']
|BS0-5467| |PD00288| ['-']
|BS0-5463| |PD00288| ['-']
|BS0-5462| |PD00288| ['-']
|BS0-5461| |PD00288| ['-']
|BS0-3622| |CPLX0-226| ['-']
|BS00104| |PD00763| ['-']
|BS00105| |PD00763| ['-']
|BS00106| |PD00763| ['-']

|TU0-4701|
|BS0-3622| |CPLX0-226| ['-']

|TU0-4702|
|BS0-3622| |CPLX0-226| ['-']
```

We hope you could format data by hand, as many interactions with a Transcription factor and a small metabolite are described as metabolic reactions, meanwhile we formatted that data as interaction networks:

[56]:
```
print(utils.getData('ECOLI', 'CPLX0-226')['components']) # CPLX0-226 is the␣
↪CRP-cAMP complex
print(utils.getData('ECOLI', 'CPLX0-226')['consensus_sequence']) # we could␣
↪obtain the consensus DNA sequence if known
print(utils.getData('ECOLI', 'PC00004')['appears_in_left_side_of']) # in␣
↪contrast to the "catalyzes" slot of gene products
print(utils.getData('ECOLI', 'BETAGALACTOSID-CPLX')['catalyzes'])
print(utils.getData('ECOLI', 'CPLX0-226')['appears_in_right_side_of']) #␣
↪equivalent output
print(' + '.join(utils.getData('ECOLI', 'RXN0-269')['left']) + '->' + ' + '.
↪join(utils.getData('ECOLI', 'RXN0-269')['right']))
```

```
['|PC00004|', '|CAMP|']
['AAATGTGAtctagaTCACATTT']
['|RXN0-269|']
['|BETAGALACTOSID-ENZRXN|', '|ENZRXN0-6526|', '|ENZRXN0-8150|',
'|ENZRXN0-8152|']
['|RXN0-269|']
|PC00004| + |CAMP|->|CPLX0-226|
```

Finally, we will incorporate regulatory relationships through the copy of rules discribing the docking or sliding of the RNAP through the DNA. For the inactivation of transcription by competition of a TF and a RNAP for the same or overlapping binding site, we simple add interactions of the TF and copy the docking rule of the RNAP with a condition:

[57]:
```
%time network = utils.read_network('network-lac+ara-TFs+DNA.tsv')
network[0:5]
```

```
CPU times: user 2.48 ms, sys: 947 µs, total: 3.43 ms
Wall time: 2.81 ms
```

[57]:
```
                              SOURCE          TARGET  FWD_RATE  RVS_RATE  \
0  [crp,SMALL-CAMP,crp,SMALL-CAMP]     BS-crp-51-72       1.0       1.0
1  [crp,SMALL-CAMP,crp,SMALL-CAMP]      BS-crp-22-1       1.0       1.0
2                             lacI  BS-lacI-422-402       1.0       1.0
3                             lacI     BS-lacI-21-1       1.0       1.0
4                             lacI    BS-lacI-72-92       1.0       1.0

  LOCATION
0  cytosol
1  cytosol
2  cytosol
3  cytosol
4  cytosol
```

[58]:
```
%time model1 = atlas.
↪construct_model_from_interaction_network('network-lac+ara-TFs+DNA.tsv')
model1
```

```
CPU times: user 536 ms, sys: 0 ns, total: 536 ms
Wall time: 533 ms
```

```
[58]: <Model 'atlas_rbm.construct_model_from_interaction_network' (monomers: 3, rules:
      24, parameters: 53, expressions: 0, compartments: 0) at 0x7f61cc2e0970>
```

```
[59]: %time model2 = atlas.
       ↪construct_model_from_sigma_specificity_network('network-lac-sigma-specificity.
       ↪tsv', 'network-lac+rpoABCD-operons-arq.tsv')
      model2
```

```
CPU times: user 1.38 s, sys: 0 ns, total: 1.38 s
Wall time: 1.38 s
```

```
[59]: <Model 'atlas_rbm.construct_model_from_sigma_specificity_network' (monomers: 4,
      rules: 55, parameters: 91, expressions: 0, compartments: 0) at 0x7f61cc26ff10>
```

```
[60]: %time model = atlas.combine_models([model1, model2], verbose = False)
      model
```

```
CPU times: user 1.9 s, sys: 0 ns, total: 1.9 s
Wall time: 1.9 s
```

```
[60]: <Model 'atlas_rbm.atlas' (monomers: 5, rules: 79, parameters: 144, expressions:
      0, compartments: 0) at 0x7f61cc2e05b0>
```

```
[61]: # repression: the DNA binding site for CRP must be free
      atlas.add_regulation(model, name = 'docking_2_lacZ_pro3',
                     conditions = ['BS-crp-51-72', 'BS-crp-22-1'], replace = True)
```

```
[61]: <Model 'atlas_rbm.atlas' (monomers: 5, rules: 79, parameters: 146, expressions:
      0, compartments: 0) at 0x7f61cc2e05b0>
```

```
[62]: # repression: the DNA binding site for CRP must be free
      atlas.add_regulation(model, name = 'docking_3_lacZ_pro2',
                     conditions = ['BS-crp-51-72', 'BS-crp-22-1'], replace = True)
```

```
[62]: <Model 'atlas_rbm.atlas' (monomers: 5, rules: 79, parameters: 148, expressions:
      0, compartments: 0) at 0x7f61cc2e05b0>
```

```
[63]: # activation: the CRP-CAMP complex is required for cooperative docking of RNAP␣
       ↪to a promoter.
      atlas.add_regulation(model, name = 'docking_4_lacZ_pro1',
                     conditions = ['[crp,SMALL-CAMP,crp,SMALL-CAMP,BS-crp-51-72]',
                                   '[crp,SMALL-CAMP,crp,SMALL-CAMP,BS-crp-22-1]'],␣
       ↪replace = True)
```

```
[63]: <Model 'atlas_rbm.atlas' (monomers: 5, rules: 79, parameters: 150, expressions:
      0, compartments: 0) at 0x7f61cc2e05b0>
```