BioModTool – Install and Usage

1. BioModTool installation

Use pip to install BioModTool from PyPI (https://pypi.org/project/BioModTool/).

pip install BioModTool

BioModTool source code can also be downloaded or cloned from GitHub (https://github.com/Total-RD/BioModTool/tree/main/BioModTool). You can run the following to install BioModTool.

pip install <path-to-BioModTool-repo>

2. BioModTool usage

Usage of BioModTool is illustrated using the example of *E. Coli* iML1515 GEM (Monk et al. 2017). Data used to determine *E. Coli* biomass composition come from S4_Biomass_Composition supplementary file from Beck, Hunt et Carlson 2018.

2.1 BOF structure and Biomass composition

(Beck, Hunt et Carlson 2018) provide monomer and macromolecular composition of the biomass for: DNA, RNA, proteins, lipids and polysaccharides. Regarding lipids, three classes are defined: PE (Phosphatidylethanolamine), PG (Phosphatidylglycerol) and CLPN (Cardiolipin). A single fatty acid profile is available. Same fatty acid profile for the three lipid classes was therefore considered. Given available data, BOF is structured as follows (with s_i representing stoichiometric coefficients):

$$s_1\,\text{DNA} + s_2\,\text{RNA} + s_3\,\text{PROTEINS} + s_4\,\text{LIPIDS} + s_5\,\text{POLYSACCHARIDES} \rightarrow 1\,\text{BIOMASS (Level 1)}$$

Level 2 is composed of five pseudo-reactions, one for each aforementioned macromolecules, with lipid pseudo-reaction defined as:

$$s_6\,\text{PE} + s_7\,\text{PG} + s_8\,\text{CLPN} \rightarrow 1\,\text{LIPIDS (Level 2 - lipids)}$$

Finally, level 3 is defined by three pseudo-reactions: one for each lipid class.

(Beck, Hunt et Carlson 2018) data were used to complete BioModTool Excel file. For example, "BIOMASS" sheet was filled based on macromolecular/polymer composition of biomass (Table 1).

Table 1. Macromolecular composition of E. Coli (Beck, Hunt et Carlson 2018)

Macromolecule	$g_{polymer} \cdot g_{DW}^{-1}$
DNA	1.0
RNA	17.2
proteins	35.2
lipids	6.7
polysaccharides	4.2

Raw data are given in $g_{polymer} \cdot g_{DW}^{-1}$, so unit "g per …" was selected in "D12 cell" drop-down list. No energy or other cofactors are required in BOF level 1, so Table 2 was left empty. BIOMASS sheet fully completed is illustrated in (Figure 1). A level_2 sheet was created and named "DNA" (Figure 2). *E. Coli* genome GC-content was used to detail DNA composition in Table 1 (24.6% A, 24.6% T, 25.4% C and 25.4% G) using metabolites identifiers (datp_c, dttp_c, dctp_c and dgtp_c) from in iML1515 GEM (Monk et al. 2017). DNA polymerization produces one diphosphate per dNTP addition, therefore production of one mole of ppi_c metabolite per mole of DNA was added in DNA sheet Table 2. Same methodology was applied to create and fill the nine sheets required to generate the BOF matching the structure described above (BIOMASS, DNA, RNA, PROTEINS, LIPIDS, POLYSACCHARIDES, PE, PG and CLPN). In Beck, Hunt et Carlson 2018 polymer lengths for the macromolecular synthesis reactions was set to 10 NTPs for RNA, 10 glucose-1-phosphates for polysaccharides and 100 amino acids for proteins. Polymerization energy requirements and byproducts in Beck, Hunt et Carlson 2018 data are given on the basis of these polymers lengths. On the other hand in BioModTool all polymers are considered to be one monomer long, stoichiometric coefficients of energy and byproducts requirement reported by Beck, Hunt et Carlson 2018 were therefore adjusted.
Fully completed Excel file is available as (Supplemental File S4).

BIOMASS (level 1) - Instructions

Please fill the sheet according to the following instructions:

1 - Fill metabolite IDS	***Table1 :*** **Pseudo-metabolite ID (also used for pseudo-reaction ID).** **/!\ Add a sheet for each pseudo metabolite. (Sheet name = pseudo-metabolite ID)**			
	Additional informations can be added. *Names will be added to pseudo-metabolites and pseudo-reactions (optional).* *Comments/Source cells are not taken into account by BioModTool.*			
2 - Indicate data unit	***Table 1:*** Choose appropriate unit **(in D12 cell).**	Possible units:	**g per …**	for example g/gDCW, g/cells, mass% …
			mol per …	for example mol/gDCW, mol/cells, mol% …
3 - Fill coefficients	***Table 1:*** coefficient value for each pseudo-metabolite (macromolecule/polymer). **/!\ Coefficient >= 0**			

BIOMASS (level 1) - Data read by BioModTool

Table 1: Basic level metabolites

coefficient >= 0

Coefficients will be converted and normalized by BioModTool.

Metabolite name	Metabolite ID in model	Coefficient	Unit	Comments/Source
DNA pseudo-metabolite	**DNA**	1	**g per …**	data in gpolymer/gDW Beck et al., 2018
RNA pseudo-metabolite	**RNA**	17.2	g per …	Beck et al., 2018
PROTEINS pseudo-metabolite	**PROTEINS**	35.2	g per …	Beck et al., 2018
LIPIDS pseudo-metabolite	**LIPIDS**	6.7	g per …	Beck et al., 2018
POLYSACCHARIDES pseudo-metabolite	**POLYSACCHARIDES**	4.2	g per …	Beck et al., 2018
			g per …	
			g per …	

Figure 1. Example of a "BIOMASS" sheet of BioModTool Excel filled with *E. coli* data from Beck et al., 2018.

DNA (level 2) - Instructions

Please fill the sheet according to the following instructions:

1 - Fill metabolite IDS	***Tables 1 & 2: Metabolite IDs.*** **/!\ Must correspond to a metabolite of the chosen model.**			
	Additional information can be added. Names will be added to pseudo-metabolites and pseudo-reactions (optional). Comments/Source cells are not taken into account by BioModTool.			
2 - Indicate data unit	***Table 1:*** Choose appropriate unit **(in D12 cell).**	Possible units:	**g per …**	for example g/gDCW, g/cells, mass% …
			mol per …	for example mol/gDCW, mol/cells, mol% …
3 - Fill coefficients	***Table 1:*** coefficient value for each pseudo-metabolite (macromolecule/polymer). **/!\ Coefficient >= 0**			

DNA (level 2) - Data read by BioModTool

Table 1: Basic level metabolites

coefficient >= 0

Coefficients will be converted and normalized by BioModTool.

Metabolite name	Metabolite ID in model	Coefficient	Unit	Comments/Source
dATP	**datp_c**	24.6000	**mol per …**	data in gpolymer/gDW Beck et al., 2018
dCTP	**dctp_c**	25.4000	mol per …	Beck et al., 2018
dGTP	**dgtp_c**	25.4000	mol per …	Beck et al., 2018
dTTP	**dttp_c**	24.6000	mol per …	Beck et al., 2018
			mol per …	

Figure 2. Example of a "DNA" sheet of BioModTool Excel filled with *E. coli* data from Beck et al., 2018. *(Continue next page)*

DNA (level 2) - Instructions

***Tables 1 & 2:* Metabolite IDs.** **/!\ Must correspond to a metabolite of the chosen model.**
Additional information can be added. Names will be added to pseudo-metabolites and pseudo-reactions (optional). Comments/Source cells are not taken into account by BioModTool.
Table 2: Unit can not be changed (mmol/gDW)
Table 2: coefficient value for each metabolite. **/!\ Coefficient < 0 if the metabolite is consumed and >0 if metabolite is produced by the reaction.**

DNA (level 2) - Data read by BioModTool

Table 2: Intermediate and advanced levels metabolites (optional)

if consumed: coeff < 0,
if produced coeff >0
/!\ Coefficients will be directly used in pseudo-reaction (no conversion).

Metabolite name	Constant metabolite ID in model	Constant metabolite Coefficient	Constant metabolite Unit	Comments/Source
Diphosphate	ppi_c	1.0000	mol/mol macromolecule	Beck et al., 2018
			mol/mol macromolecule	

End of Figure 2.

2.2 Run BioModTool

BioModTool can be run either in Python command-lines or using the user interface (implemented with Tkinter).

2.2.1 Line-command version

The following section presents an example of a Jupyter Notebook to run BioModTool. This Jupyter Notebook is available: Ecoli_iML1515_script_BioModTool_add_biomass_reaction.ipynb at https://github.com/Total-RD/BioModTool/tree/main/Application_examples/1_iML1515_ecoli/test_with_jupyternotebook.

Addition of a new BOF to iML1515 using BioModTool:

- Cobra model: iML1515 (*E. coli*) (Monk et al. 2017)
- Biomass composition data from (Beck, Hunt et Carlson 2018)

Imports

In [1]:

```
import BioModTool.load
import BioModTool.main_add_biomass_objective_function
import BioModTool.save
```

1 - Load Genome Scale Metabolic Model

1.a) Model directory

In [2]:

```
path_to_model = "path_to_model_repository\\iML1515.xml"
```

In [3]:

```
original_model = BioModTool.load.load_cobra_model(path_to_model)
```

1.b) Calculate formula and/or charge ?

/!\ Note that if you select formula = False, the molecular weight cannot be calculated from the metabolite formula preventing unit conversion.

- Level 1 data must be given in mmol.gDW-1

- Levels 2 and 3 data must be given in mol per ...

In [4]:

```
calculate_formula = True
calculate_charge = True
```

1.c) Choose a compartment to add biomass reactions

- Reactions and pseudo metabolites will be added in compartment given by the user.
- Compartment must be chosen among model's compartments (key of cobra_model.compartments dictionary)
- Biomass reactions are commonly added in cytosol ("_c").

In [5]:

```
# Compartments in model:
original_model.compartments
```

Out[5]:

```
{'c': 'cytosol', 'e': 'extracellular space', 'p': 'periplasm'}
```

In [6]:

```
BOF_compartment = "c"
```

2 – Biomass composition data

2.a) Define data file

Data must be given in an Excel file with a specific format. See Supplemental Files S1-S3.

In [7]:

```
path_to_data = "Biomass_composition_ecoli_Beck2018.xlsx"
```

3 – Structure of biomass objective function

3.a) Biomass reaction structure

Dictionary defining BOF structure:
Must be defined in accordance with Excel data file (sheet names).

- Mandatory:
  - one and only one key with value = "level_1"
- Optional:
  - if desired structure contains two or more levels:
    - one or several key(s) with value = "level_2"
  - if desired structure contains three levels:
    - one and only one key with value = "level_2_lipid"
    - one or several key(s) with value = "level_3"

In [8]:

```
dict_pool_id_Ecoli = {'BIOMASS': 'level_1',
  'POLYSACCHARIDES': 'level_2',
  'DNA': 'level_2',
  'RNA': 'level_2',
  'PROTEINS': 'level_2',
  'LIPIDS': 'level_2_lipid',
  'PG': "level_3",
  'PE': "level_3",
  'CLPN': "level_3"}
```

3.b) Choose a suffix

- Expected suffix: string containing only alphanumeric characters or _
- Test performed in BioModTool: re.match("^[a-zA-Z0-9_]*$",suffix)
- All added pseudo-reactions and pseudo-metabolites will contain the given suffix.

In [9]:

```
suffix = "BioModTool_beck2018"
```

4 – Create and add the new biomass objective function

In [10]:

```
updated_model =
BioModTool.main_add_biomass_objective_function.add_biomass_objective_function(
      cobra_model = original_model,
      path_to_data = path_to_data,
      suffix = suffix,
      dict_structure = dict_structure_BOF,
      user_compartment = BOF_compartment,
      calculate_charge = calculate_charge,
      calculate_formula = calculate_charge,
      saving_final_data = True)
```

Out[10]:

```
Metabolite POLYSACCHARIDES_BioModTool_beck2018_c (formula: C6.0H10.0O5.0, charge:
0) added to model.
Reaction POLYSACCHARIDES_BioModTool_beck2018_c added to model.
atp_c + g1p_c --> POLYSACCHARIDES_BioModTool_beck2018_c + adp_c + ppi_c
--------------------------------------------------------------------------------
Metabolite   DNA_BioModTool_beck2018_c   (formula:   C9.746H11.246O6.0N3.754P1.0,
charge: -1) added to model.
Reaction DNA_BioModTool_beck2018_c added to model.
0.246   datp_c   +   0.254   dctp_c   +   0.254   dgtp_c   +   0.246   dttp_c   -->
DNA_BioModTool_beck2018_c + ppi_c
--------------------------------------------------------------------------------
[…]
--------------------------------------------------------------------------------
Metabolite                BIOMASS_BioModTool_beck2018_c                (formula:
C40.59259727004123H62.339155478773726O16.850976170654622N10.09717316334253P1.0379
95606777102S0.2007301352, charge: -1) added to model.
Reaction BIOMASS_BioModTool_beck2018_c added to model.
0.0505 DNA_BioModTool_beck2018_c + 0.13999 LIPIDS_BioModTool_beck2018_c + 0.40285
POLYSACCHARIDES_BioModTool_beck2018_c + 5.02831 PROTEINS_BioModTool_beck2018_c +
0.84039 RNA_BioModTool_beck2018_c --> BIOMASS_BioModTool_beck2018_c
--------------------------------------------------------------------------------
```

5 – Save updated model

BioModTool comes with a function to save model both in JSON and SBML formats.

In [11]:

```
BioModTool.save.save_model(updated_model,"iML1515_updated" )
```

2.2.2 Using graphical interface

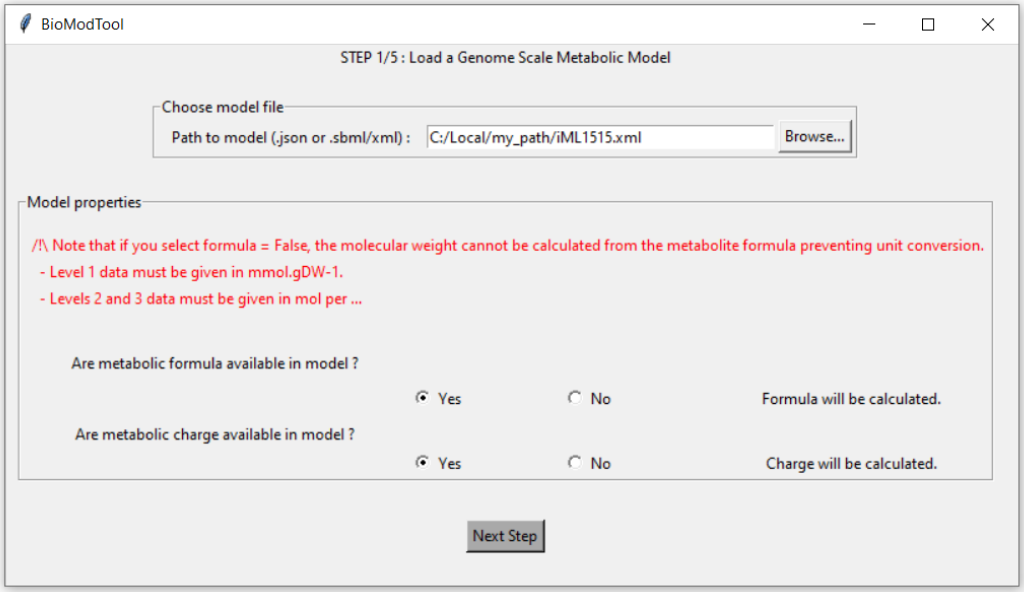
BioModTool comes with a graphical user interface: interface_BioModTool.py. Interface source code is available on GitHub (https://github.com/Total-RD/BioModTool/blob/main/interface_BioModTool.py). The interface can be executed using Python and is compatible with Windows, Linux and MacOS operating systems.

python <path-to-interface_BioModTool.py>

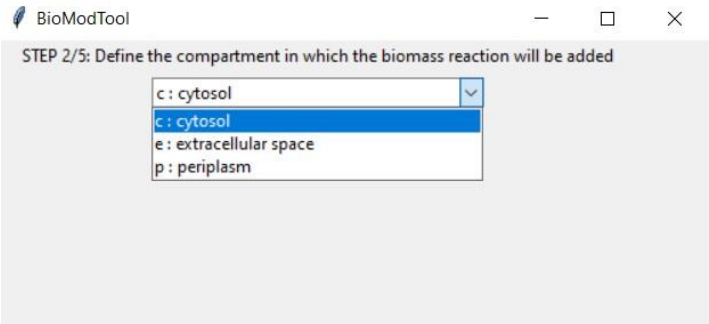
On Windows, the interface can also be launched directly from an executable: interface_BioModTool.exe

N.B.: interface_BioModTool.exe is in the repository **BioModTool\dist**

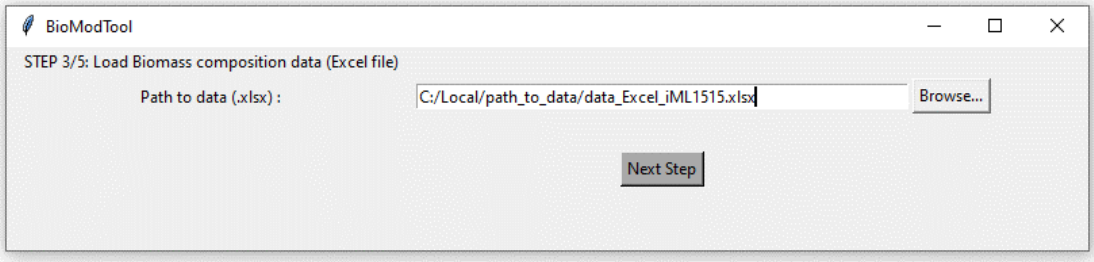
Step 1: Browse GEM model file (JSON or SBML format) and select via radio buttons if formula and/or charge are available for all metabolites consumed in the BOF.



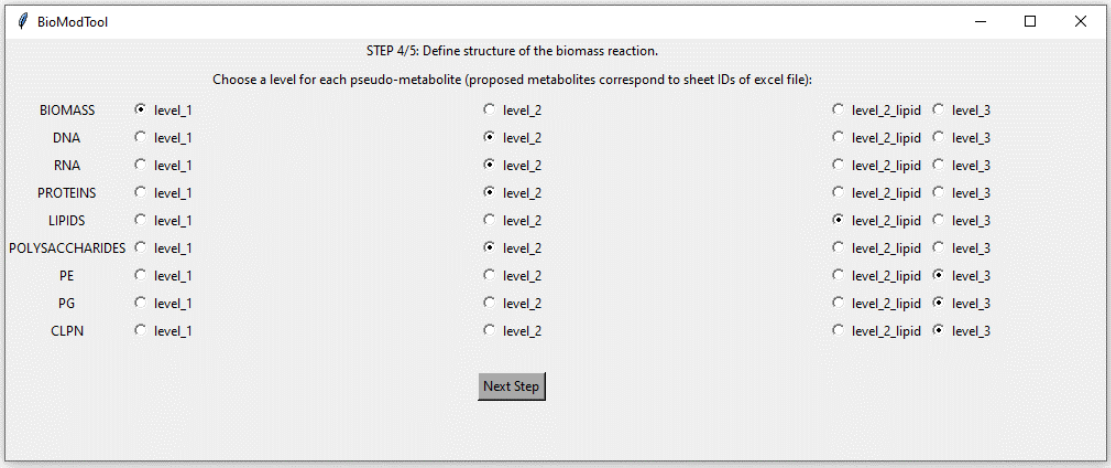
Step 2: Select a compartment in the drop-down list. Biomass objective function will be added to the selected compartment.



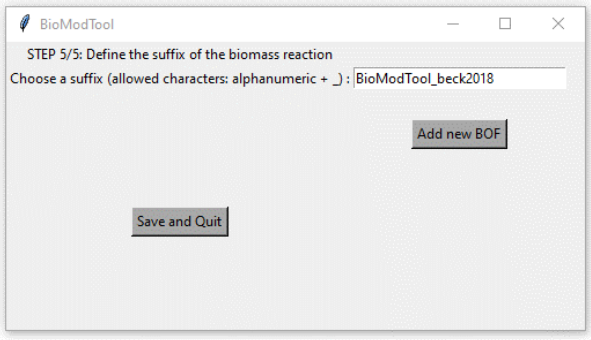
Step 3: Browse biomass composition data (Excel file, *.xlsx).



Step 4: Based on Excel data file structure (available sheets), define BOF structure. To do so, indicate desired level for each pseudo-metabolite by selecting desired level: level_1, level_2, level_2_lipide or level_3.



Step 5: Define a suffix, only alphanumeric characters (and "_") are accepted. Add the BOF, save and quit BioModTool.



Updated model is saved in SBML and JSON formats in a directory chosen by the user. Excel file with calculations details is saved in the working directory.

2.3 Remove a BOF with BioModTool

A function removing a BioModTool formatted BOF from GEM is available (remove_biomass_objective_function). The function takes a model and a suffix as parameters, remove all reactions harboring the given suffix ("_suffix_") in their ID and returns updated model.

This functionality is available in line command version.

In [12]:

```
model_wo_BOF = remove_biomass_objective_function(updated_model,suffix)
```

3. Implementation

First, BioModTool loads and tests user inputs. Then, stoichiometric coefficients are calculated from data. For levels 2 and 3 pseudo-reactions, final stoichiometric coefficients are in molar fraction ($mol.mol^{-1}$). For each pseudo-reaction if user data are in "g per …", data are converted in "mol per …" as follows:

$$coeff_{mol} = \frac{coeff_g}{MW}$$

With $coeff_{mol}$ molar coefficient in "mol per …", $coeff_g$ initial mass coefficient in "g per …" and MW molecular weight of the metabolite calculated from metabolite formula in the GEM.

In BioModTool, MW are calculated using COBRApy function *formula_weight* from metabolite instances (Ebrahim et al. 2013). Molar coefficients are then normalized so that the sum of all molar fractions is one, and rounded to the fifth decimal place.

By definition, produced biomass must represent one gram of dry weight. Therefore, for level 1 pseudo-reaction (typically biomass), final stoichiometric coefficients are in mmol.gDW^{-1}. Before being converted in mmol.gDW^{-1}, coefficients given by the user are first converted in g.gDW^{-1} to allow their normalization. Data in "mol per …" are converted in "g per …" as follows:

$$coeff_g = coeff_{mol} * MW$$

With $coeff_g$ mass coefficient in "g per …", $coeff_{mol}$ initial molar coefficient in "mol per …" and MW molecular weight of the metabolite obtained from metabolite formula. Mass coefficients are then normalized such that the sum of metabolite mass fraction is one, before being converted in final unit mmol.gDW^{-1} as follows:

$$coeff_{mmol/gDW} = 1000 * \frac{coeff_{g/gDW}}{MW}$$

With $coeff_{mmol/gDW}$ final stoichiometric coefficient in mmol.gDW^{-1}, $coeff_{g/gDW}$ mass fraction in g.gDW^{-1} and MW molecular weight of the metabolite. Final coefficients are rounded to fifth decimal place. BioModTool calculates pseudo-metabolites charge and formula (when possible and desired by the user), and instantiates pseudo-metabolites and pseudo-reactions. Annotation being an important feature in GEM models, SBO terms "SBO:0000247" and "SBO:0000629" are respectively added to metabolites and reactions annotations (Lieven et al. 2020).

In accordance with the FAIR (Findable, Accessible, Interoperable, Reusable) principles and to offer users a comprehensible overview of the calculations executed by BioModTool, the results of the various calculations steps are recorded in an Excel file. The pseudo-metabolites created, along with their charge and chemical formula, are also summarized in this Excel file (worksheet: added_metabolites). In addition, BioModTool performs a charge and mass balance test of all the reactions making up the biomass function. Results of this test are saved in the Excel file (worksheet: mass_charge_balance).

Steps that require loading, updating, saving GEM and manipulating metabolites and reactions rely on COBRApy packages (Ebrahim et al. 2013). Full codes are available at (https://github.com/Total-RD/BioModTool).

References

Beck, Ashley; Hunt, Kristopher; Carlson, Ross (2018): Measuring Cellular Biomass Composition for Computational Biology Applications. In Processes 6 (5), p. 38. DOI: 10.3390/pr6050038.

Ebrahim, Ali; Lerman, Joshua A.; Palsson, Bernhard O.; Hyduke, Daniel R. (2013): COBRApy: COnstraints-Based Reconstruction and Analysis for Python. In BMC Syst Biol 7 (1), p. 74. DOI: 10.1186/1752-0509-7-74.

Lieven, Christian; Beber, Moritz E.; Olivier, Brett G.; Bergmann, Frank T.; Ataman, Meric; Babaei, Parizad et al. (2020): MEMOTE for standardized genome-scale metabolic model testing. In Nature biotechnology 38 (3), pp. 272–276. DOI: 10.1038/s41587-020-0446-y.

Monk, Jonathan M.; Lloyd, Colton J.; Brunk, Elizabeth; Mih, Nathan; Sastry, Anand; King, Zachary et al. (2017): iML1515, a knowledgebase that computes Escherichia coli traits. In Nature biotechnology 35 (10), pp. 904–908. DOI: 10.1038/nbt.3956.