SOFTWARE

The JBEI Quantitative Metabolic Modeling library (jQMM): a Python library for modeling microbial metabolism. SUPLEMENTARY MATERIAL

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Mathematical description of algorithms

This section describes the inputs, outputs and optimization problems for each of the flux analysis techniques used: FBA, ^{13}C MFA and 2S-^{13}C MFA. Mathematical details for PCAP analysis of proteomic data can be found in Jupyter notebook B5 and in the original publication[1].

Flux Balance Analysis

The mathematical details for FBA are the same as for other implementations[2], and are provided here for comparison with other methods. We only implemented them in jQMM so as to provide an equivalent comparison in situations like Fig. 9 in Garcia Martin *et al*[3].

Input

The inputs for FBA are: a genome-scale model, measurements for exchange fluxes (e.g. glucose input, acetate output, etc), and an objective function (typically growth rate maximization).

Optimization problem

$$\text{Maximize } v_{obj} \tag{1}$$

Subject to:

$$\sum_j S_{ij} v_j = 0 \qquad \forall i \in I^N, j \in J \tag{2}$$

$$lb_j \leq v_j \leq ub_j \qquad \forall j \in J \tag{3}$$

where:

Sets

- $I^N \subset I$: Set of non-exchange metabolites.
- $J = \{j\}$: Set of fluxes.

Parameters

- S_{ij} : Stoichiometry matrix.
- ub_i, lb_i : Upper and lower bounds for reaction i.

Variables

- v_i : Flux value of reaction i, in mmol/gdw/h.

and obj is the objective flux, for maximum growth rate $obj = BiomassEcoli$ and for ATP maximization $obj = ATPM$[4].

Output

The output involves the flux profile $v_j \; \forall j \in J$

^{13}C Metabolic Flux Analysis

^{13}C MFA equations are included here because they are slightly different to the equations used in Garcia Martin *et al*[3]. The results are the same, but the new equations are able to handle reactions with repeated reactants.

Input

The inputs for ^{13}C MFA are a set of transitions (e.g. `PDH: pyr --> co2 + accoa ; abc --> a + bc` as explained above) and measurements for exchange fluxes (as for FBA above).

Optimization problem

$$\text{Minimize } OF = \sqrt{\sum_{e \in E_{\text{meas}}} \left(\sum_{m \in M_e} \left(\frac{f_{em}^{exp} - f_{em}}{\Delta_{em}} \right)^2 \Big/ |M_e| \right) \Big/ |E_{\text{meas}}|} \tag{4}$$

Subject to:

$$\sum_j S_{ij}^* V_j = 0 \qquad \forall i \in I^N, j \in J^B \tag{5}$$

$$LB_j \leq V_j \leq UB_j \qquad \forall j \in J^B \tag{6}$$

$$\sum_{m \in M_e} f_{em} = 1 \qquad \forall e \in E \tag{7}$$

$$\sum_{e' \in E} \left(\left(\sum_{j | EMM_{e'->e}^j > 0} EMM_{e'->e}^j S_{ij}^* V_j \right) f_{e'm} \right) + \left(\sum_{j | S_{ij}^* < 0} S_{ij}^* V_j \right) f_{em} = 0 \qquad \forall m \in M_e, e \in E_i, i \in I^N \tag{8}$$

$$f_{em} = \sum_{w \in W_{em}} \prod_{n=1}^{|E_e|} f_{e_n m_n} \qquad \forall m \in M_e, e \in E^c \tag{9}$$

where:

Sets

$I \equiv \{i\}$	: Set of all metabolites.
$I^N \subset I$	: Set of non-exchange metabolites.
J^B	: Set of fluxes with backward and forward fluxes differentiated, e.g. PGI_f, PGI_b, PGL .... etc.
$E = \{e\}$	: Elementary Metabolite Units (EMUs).
$E^c \subset E$	: Combined EMUs.
$E_i \subset E$	: EMUs from metabolite $i \in I$.
$E_e \subset E$	: EMUs that produce combined EMU e.
$E_{meas} \subset E$	: EMUs corresponding to measured EMUs.
W_{em}	: Set of every possible mass isotopomer multiplet of E_e that produce the mass isotopomer m of e.
M_e	: m values for MDV of emu $e : 0, 1, \ldots,$# of carbons in e .

Parameters

$EMM^j_{e'->e}$	: $= \frac{1}{k}$ if e' produces e through reaction $j \in J^B$, 0 otherwise. See below.
S^*_{il}	: Stoichiometry matrix with backward and forward fluxes differentiated.
UB_j, LB_j	: Upper and lower bounds for reaction j.
$f^{exp}_{em} \in [0, 1]$	: Experimentally measured MDV(m)[5] for emu e.
Δ_{em}	: Measurement error for f^{exp}_{em}.

Variables

V_l	: Flux value of reaction $i \in J^B$, normalized to glucose input rate.
$f_{em} \in [0, 1]$	: MDV for emu e from metabolite $m \in M_e$.

Output

The output involves a flux profile $v_j \; \forall j \in J$ with confidence intervals. For example: `PDH: [ 0.3 :0.4 : 0.6 ]` Where 0.5 mmol/gdw/hr is the best fit for the data, and 0.3 mmol/gdw/hr and 0.6 mmol/gdw/hr are the minimum and maximum flux values compatible with the labeling data as determined through ^{13}C FVA[3].

2S-^{13}C Metabolic Flux Analysis

2S-^{13}C MFA is a hybrid of FBA and ^{13}C MFA where the stoichiometry constraints are applied to the full genome-scale network, as in the case of FBA, and the labeling constraints are applied only to the core set of reactions and metabolites, as is the case for ^{13}C MFA[3].

Input

The inputs for 2S-^{13}C MFA are: a genome-scale model, a set of transitions and measurements for exchange fluxes.

Optimization problem

In the notation of [6]:

$$\text{Minimize } OF = \sqrt{\left(\sum_{\substack{e\in E_{meas}\\ m\in M_e}} \left(\frac{f_{em}^{exp} - f_{em}}{\Delta_{em}}\right)^2 / |M_e|\right) / |E_{meas}|} \tag{10}$$

Subject to:

$$\sum_j S_{ij} v_j = 0 \qquad \forall i \in I^N, j \in J \tag{11}$$

$$lb_j \le v_j \le ub_j \qquad \forall j \in J \tag{12}$$

$$\sum_{m\in M_e} f_{em} = 1 \qquad \forall e \in E_{co} \tag{13}$$

$$\sum_{e'\in E_{co}} \left(\left(\sum_{l|EMM^l_{e'->e}>0} EMM^l_{e'->e} S^*_{il} V_l\right) f_{e'm}\right) + \left(\sum_{l|S^*_{il}<0} S^*_{il} V_l\right) f_{em} = 0 \qquad \forall m \in M_e, e \in E_i, i \in I^N_{co} \tag{14}$$

$$f_{em} = \sum_{w\in W_{em}} \prod_{n=1}^{|E_e|} f_{e_n m_n} \qquad \forall m \in M_e, e \in E^c_{co} \tag{15}$$

$$v_j = \sum_{l\in J^B_{co}} map_{jl} V_l \qquad \forall j \in J \tag{16}$$

where:

Sets

$I \equiv \{i\}$	: Set of all metabolites.
$I_{co} \subset I$	: Set of core metabolites.
$I_{co}^{N} \subset I_{co}$	: Set of non-exchange core metabolites.
J	: Set of fluxes.
$J_{co} \subset J$	: Set of core fluxes.
J^{B}	: Set of fluxes with backward and forward fluxes differentiated, e.g. PGI_f, PGI_b, PGL .... etc.
$J_{co}^{B} \subset J^{B}$	: Set of core fluxes for J^{B} .
$E = \{e\}$	: Elementary Metabolite Units (EMUs).
$E^{c} \subset E$	: Combined EMUs.
$E_{i} \subset E$	: EMUs from metabolite $i \in I$.
$E_{co}^{c} \subset E^{c}$	: Core combined EMUs.
$E_{e} \subset E$	: EMUs that produce combined EMU e.
$E_{co} \subset E$	: EMUs corresponding to core metabolites.
$E_{meas} \subset E$	: EMUs corresponding to measured EMUs.
W_{em}	: Set of every possible mass isotopomer multiplet of E_e that produce the mass isotopomer m of e.
M_{e}	: m values for MDV of emu $e : 0, 1, \ldots,$# of carbons in e .

Parameters

$EMM_{e'->e}^{l}$	$= \frac{1}{k}$ if e' produces e through reaction $l \in J_{co}^{B}$, 0 otherwise. See [6] .
S_{ij}	: Stoichiometry matrix.
S_{il}^{*}	: Stoichiometry matrix with backward and forward fluxes differentiated.
ub_{j}, lb_{j}	: Upper and lower bounds for reaction j.
$f_{em}^{exp} \in [0, 1]$	: Experimentally measured MDV for emu e from metabolite m.
Δ_{em}	: Measurement error for f_{em}^{exp}.
map_{jl}	$= 1 * glucupt$ if l corresponds to forward flux of j.
	$= -1 * glucupt$ if l corresponds to backward flux of j.

Variables

v_{j}	: Flux value of reaction $j \in J$, in mmol/gdw/h.
V_{l}	: Flux value of reaction $l \in J_{co}^{B}$, normalized to glucose input rate.
$f_{em} \in [0, 1]$	: Mass isotopomer fraction (MDV) for emu e from metabolite m.

Notice that S_{ij}^{*} is not the same as S_{ij}, since J and J^{B} are slightly different sets of fluxes. In fact:

$$\begin{array}{ll} S_{il}^{*} = S_{ij} & \text{if } l \text{ is the forward version of } j. \\ S_{il}^{*} = -S_{ij} & \text{if } l \text{ is the backward version of } j. \end{array} \quad (17)$$

Output

The output involves a flux profile $v_{j}\ \forall j \in J$ for all reactions in the genome-scale model with confidence intervals.

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Supplementary Figures

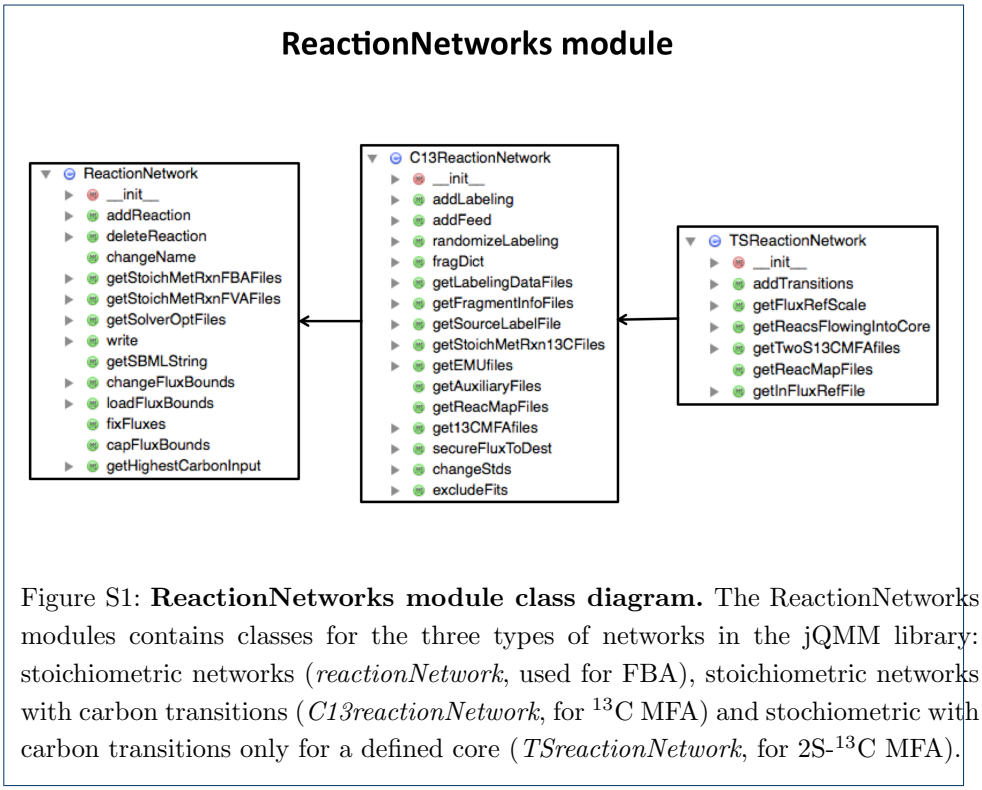

Figure S1: **ReactionNetworks module class diagram.** The ReactionNetworks modules contains classes for the three types of networks in the jQMM library: stoichiometric networks (*reactionNetwork*, used for FBA), stoichiometric networks with carbon transitions (*C13reactionNetwork*, for ^{13}C MFA) and stochiometric with carbon transitions only for a defined core (*TSreactionNetwork*, for 2S-^{13}C MFA).

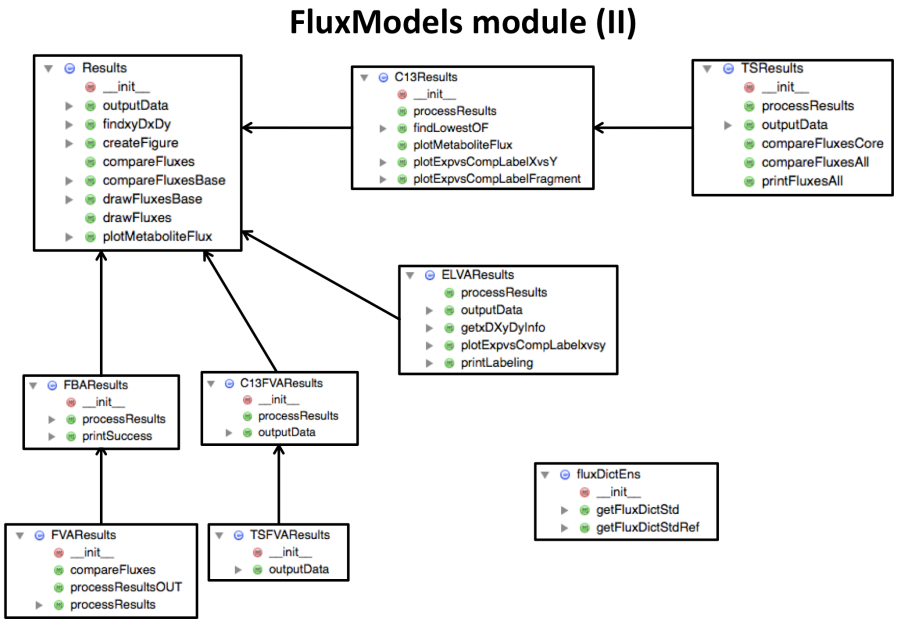

Figure S2: **FluxModels module class diagram (part II).** The FluxModels module also contains classes for storing results for each of the flux analysis types. Arrows indicate derived classes.

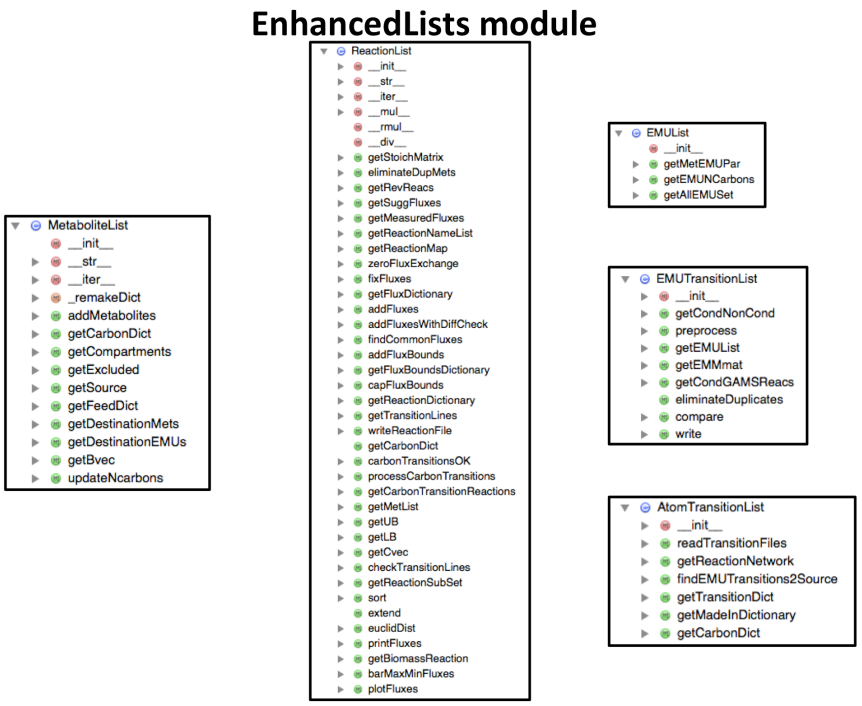

Figure S3: **EnhancedLists module class diagram.** The EnhancedLists module involves lists of special objects from core. For example, a reactionList class is provided that is a list of reactions enhanced with a variety of useful functions. Other enhanced lists include list of metabolites, emus, atom transitions and emu transitions.