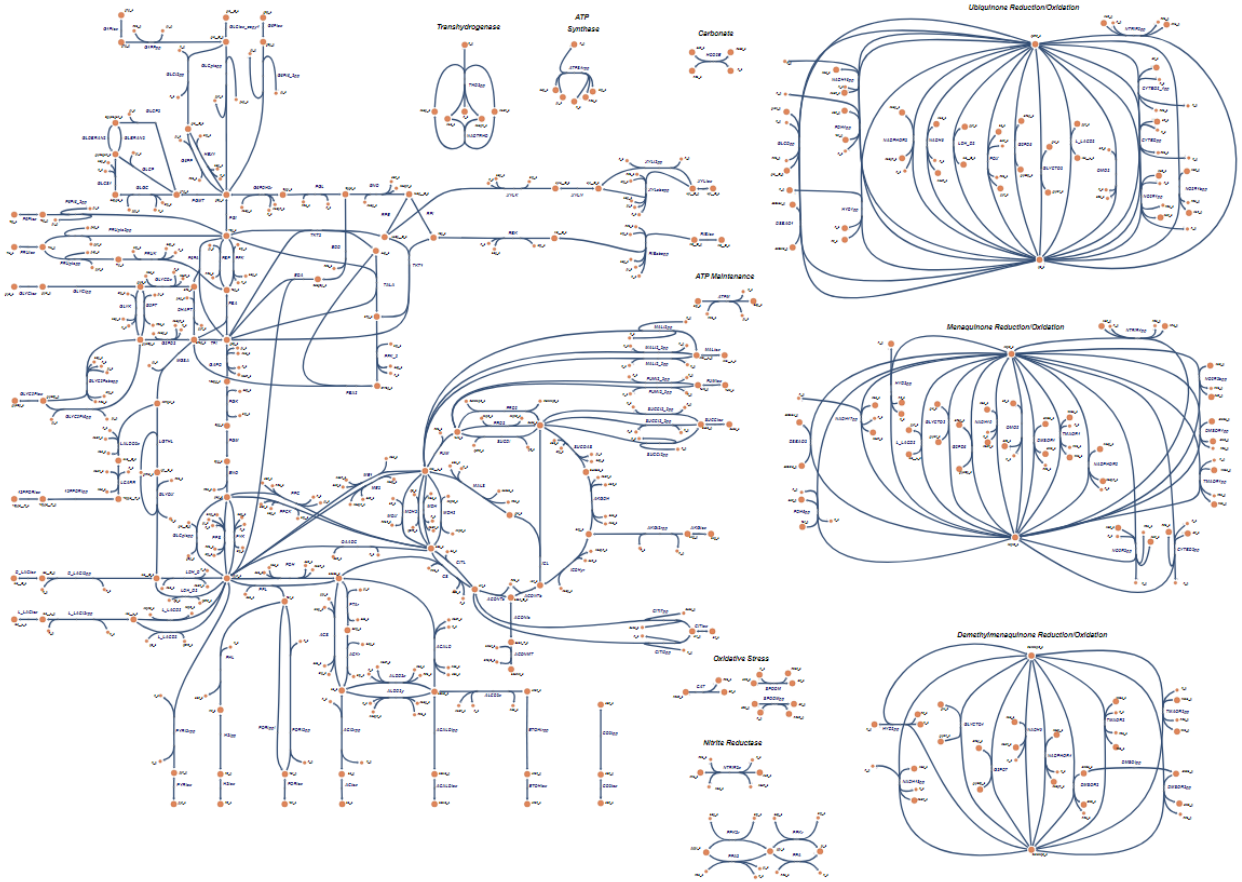
Understanding COBRAme & ECOLIme

Lesson Outline

- Metabolism (M) vs. Metabolism & Expression (ME) Models
- COBRAme
  - Working Environment (Docker)
  - ME Model Architecture
  - Macromolecular Coupling
  - Reaction Lumping
- ECOLIme
  - Macromolecular Reactions
  - Transcription
  - Translation
  - COBRAme Execution
  - ECOLIme Operation

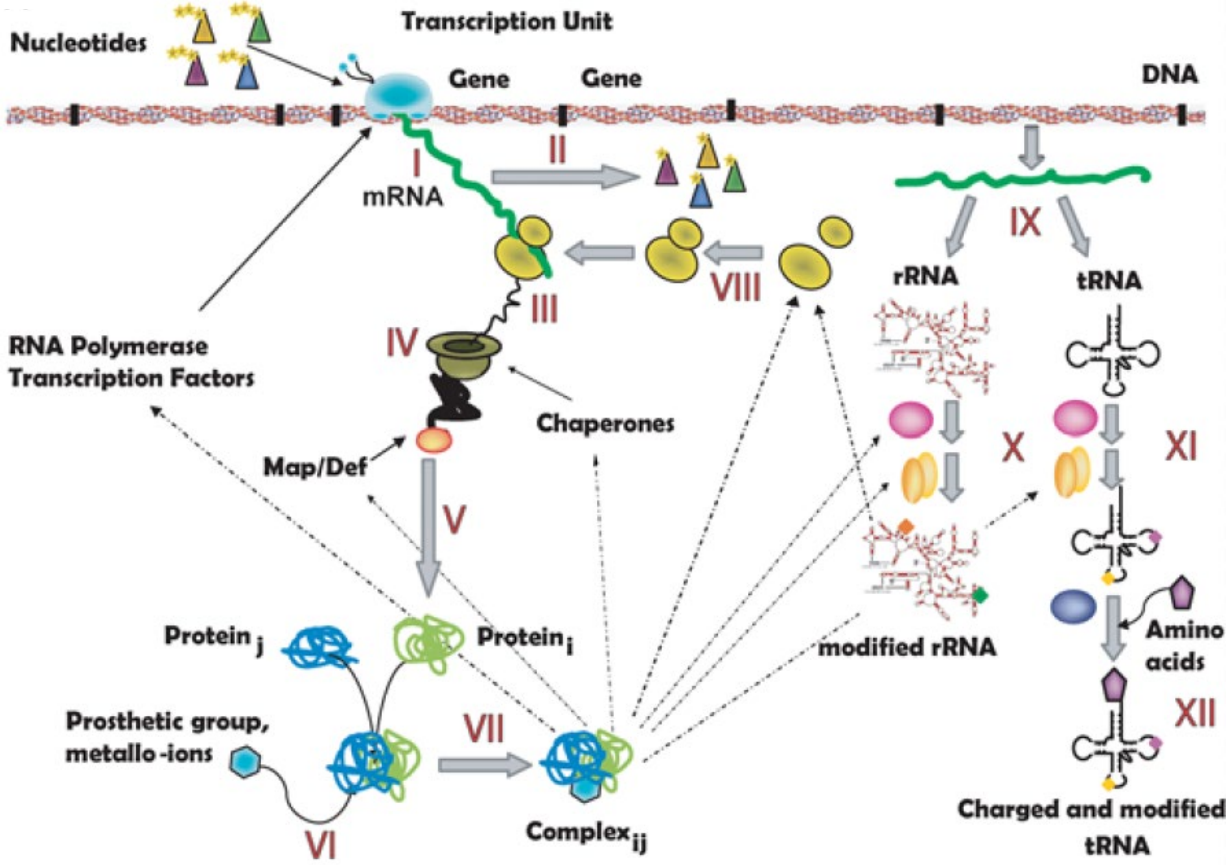
M vs ME Constraint-based Models

Metabolism (M) Model



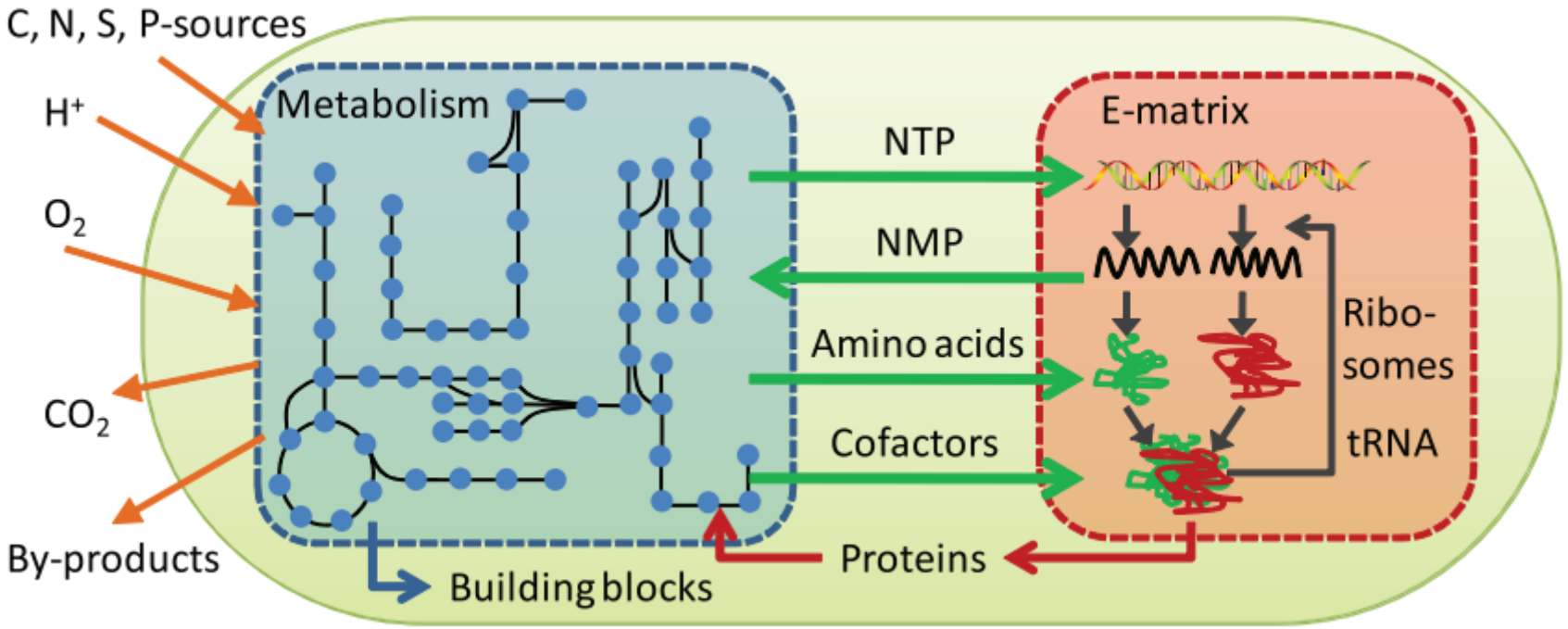
Orth JD, Conrad TM, Na J, Lerman JA, Nam H, Feist AM, Palsson BØ. A comprehensive genome-scale reconstruction of Escherichia coli metabolism--2011. Mol Syst Biol. 2011 Oct 11;7:535. doi: 10.1038/msb.2011.65.

Metabolism and Expression (ME) Model



Thiele I, Jamshidi N, Fleming RMT, Palsson BØ (2009) Genome-Scale Reconstruction of Escherichia coli's Transcriptional and Translational Machinery: A Knowledge Base, Its Mathematical Formulation, and Its Functional Characterization. PLoS Comput Biol 5(3): e1000312. doi:10.1371/journal.pcbi.1000312"

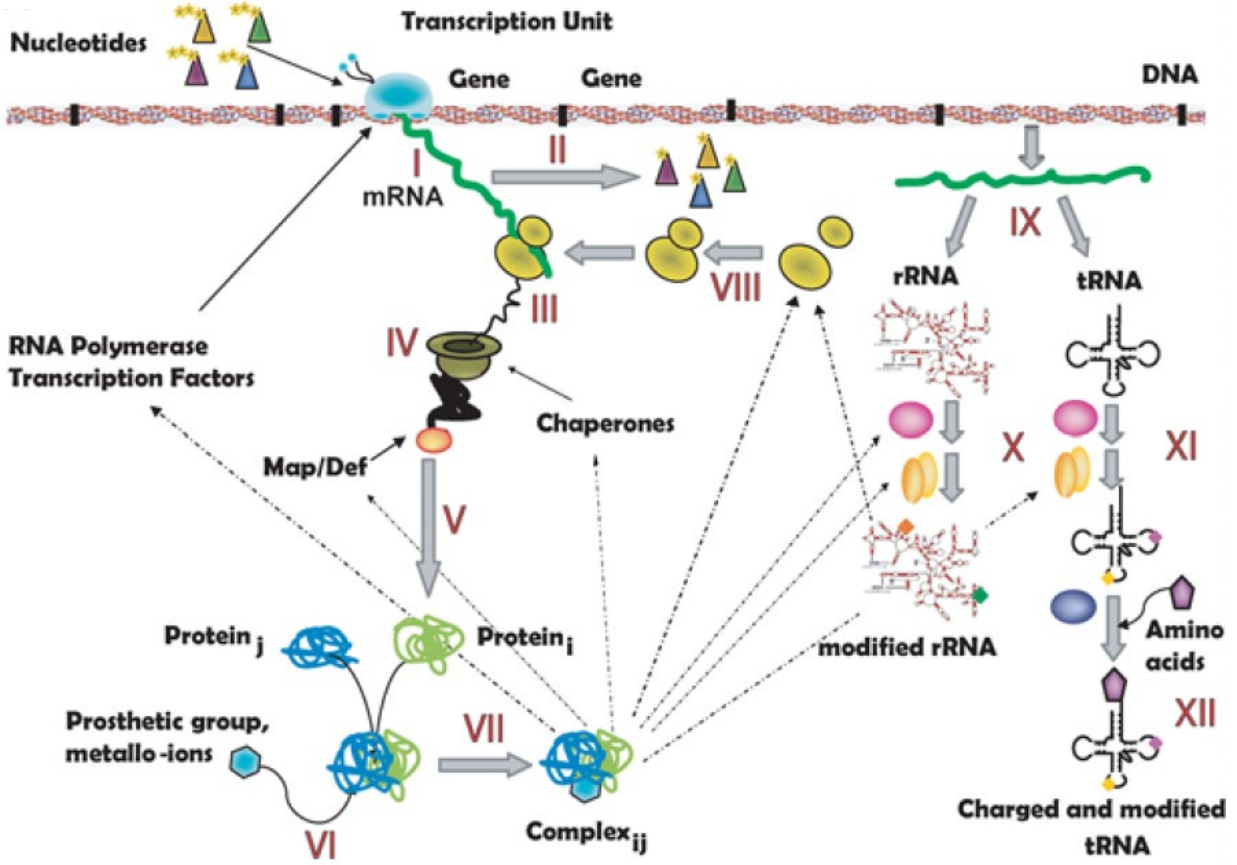
Functional Synergy Between Metabolism and Macromolecular Synthesis



Thiele I, Fleming RMT, Que R, Bordbar A, Diep D, Palsson BO (2012) Multiscale Modeling of Metabolism and Macromolecular Synthesis in E. coli and Its Application to the Evolution of Codon Usage. PLoS ONE 7(9)

M vs ME Constraint-based Models

- **M**etabolism models (M-models) can be extended to include the synthesis of the gene expression machinery which enables models to compute the entire metabolic and gene expression proteome in a growing cell.
- ME-models integrate **M**etabolism and **E**xpression on the genome scale, and are capable of computing a large percentage of the proteome by mass.
- ME-models not only compute optimal metabolic flux states, as with M-models, but they additionally compute the optimal proteome composition required to sustain the metabolic phenotype.
- COBRAme, a software tool for creating ME models.
- ECOLIme is a tool to create the COBRAme-based ME-model of *E. coli*.



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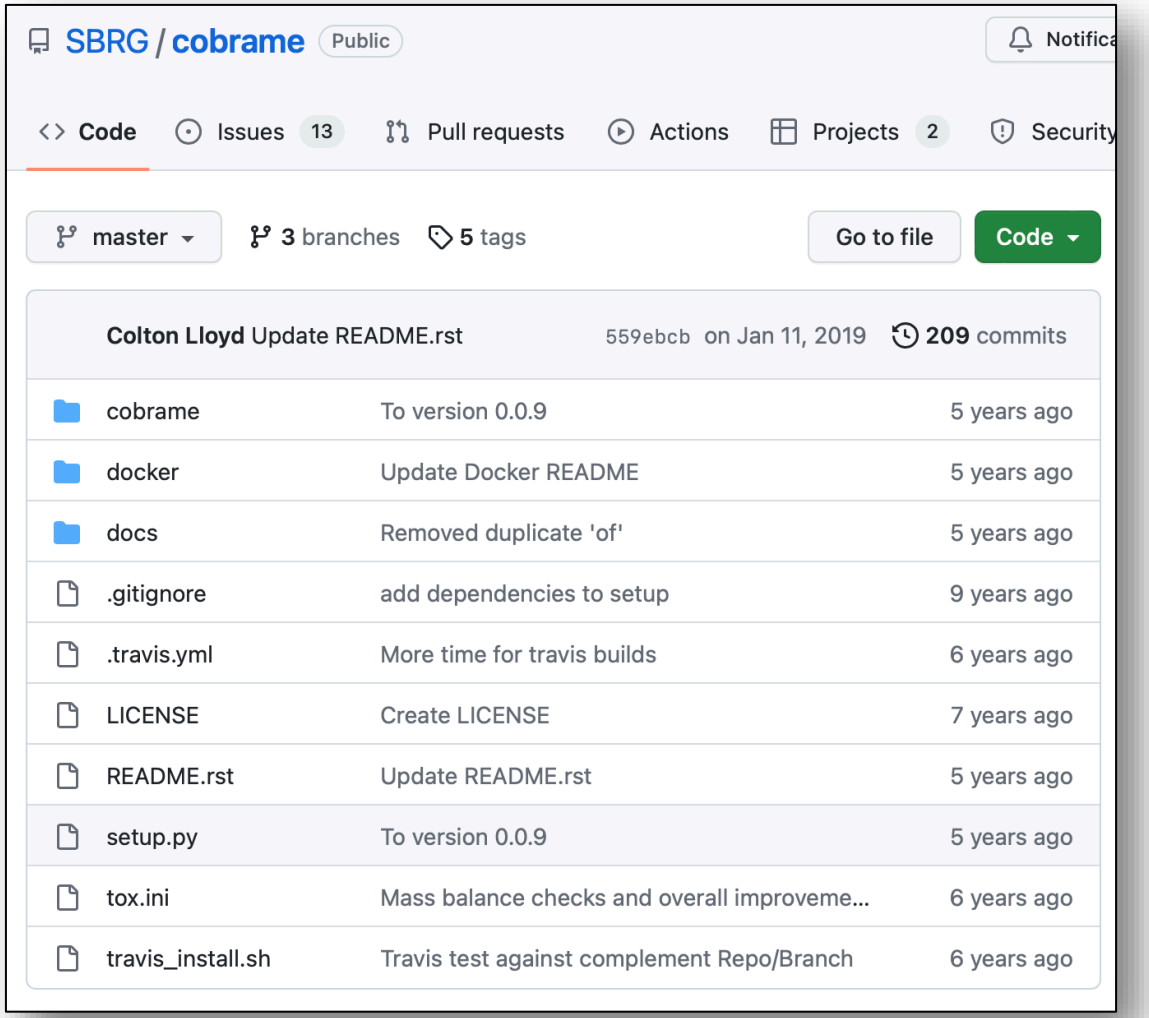
COBRAme

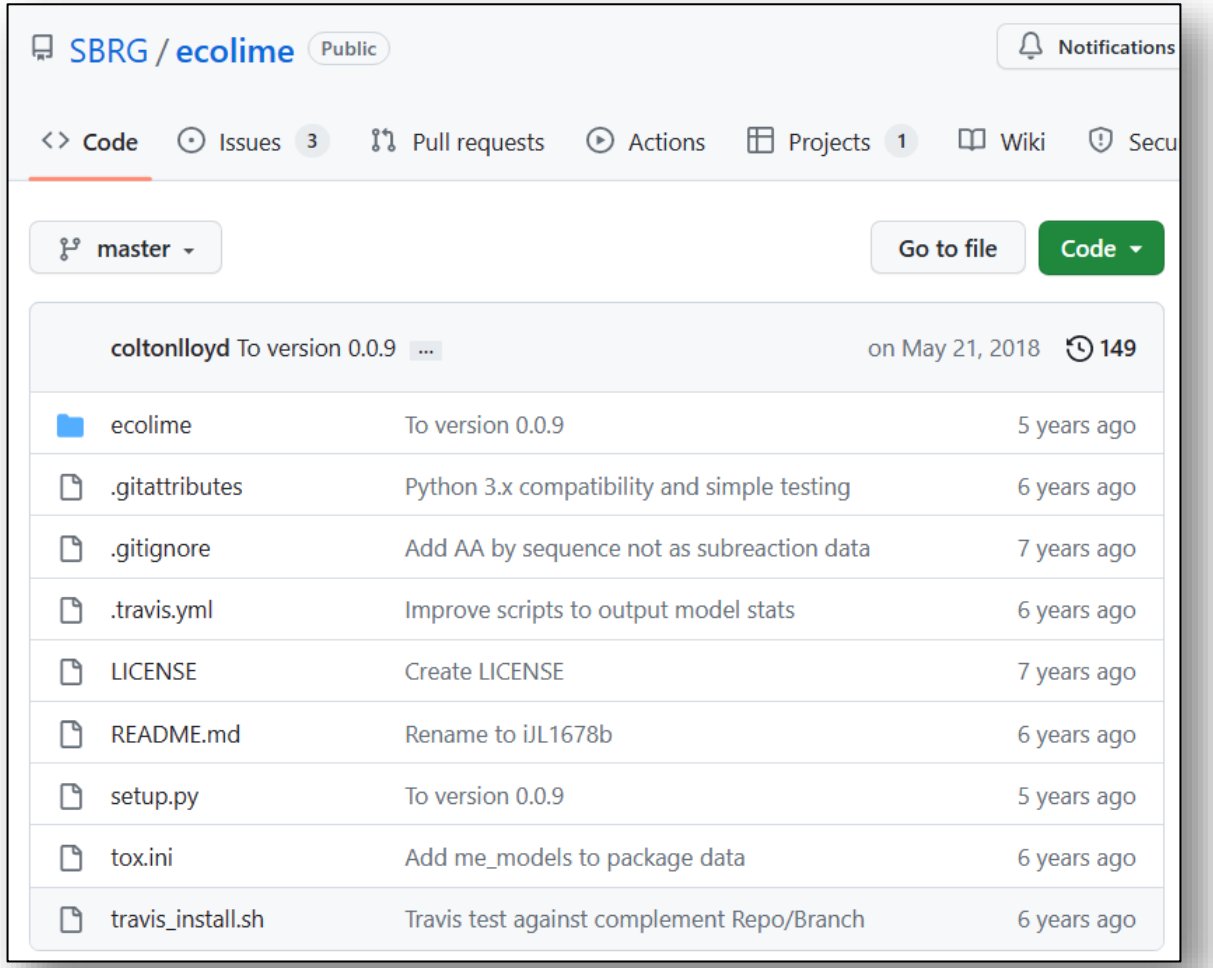
- COBRAme, a software tool for creating ME models, is designed to:
  1. support any organism with an existing M-model;
  2. use protocols and commands familiar to current users of COBRApy;
  3. represent ME-models with an intuitive collection of Python classes;
  4. solve FBA simulations orders of magnitude faster than previous ME-models
- COBRAme is written in Python and extends the widely used COBRApy software that only supports M-models.
- COBRAme was developed by C. J. Lloyd *et al* from the Bernard Palsson's group at UCSD.
- COBRAme publication: "Lloyd CJ, Ebrahim A, Yang L, King ZA, Catoiu E, O'Brien EJ, et al. (2018) COBRAme: A computational framework for genome-scale models of metabolism and gene expression. PLoS Comput Biol 14(7)"



https://github.com/SBRG/cobrame

COBRAme vs ECOLIme

- The ME-model of *E. coli* is reconstructed using the two Python packages: COBRAme and ECOLIme.
- COBRAme contains the class definitions and necessary methods to facilitate building and editing a working ME-model. It supplies the computational framework underlying the ME-model
- COBRAme is written to be organism-agnostic so that it can be applied to ME-models for any organism.
- ECOLIme contains the *E. coli* specific information (e.g., the *E. coli* ribosome composition) as well as functions required to process files containing *E. coli* reaction information (e.g., the text file containing transcription unit definitions) and associate them with the ME-model being constructed.
- ECOLIme is required to assemble the reaction and gene expression information that comprises iJL1678b-ME.
- The package composition along with further demonstrations of the utility of each of these packages is outlined in the COBRAme Documentation.



https://github.com/SBRG/ecolime

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Docker/GitHub Working Environment

1. Start Docker Desktop
2. In a Windows terminal window load the COBRAme Docker image (converts Windows terminal window to Linux window)

   docker run -p 8888:8888 --rm -i -t sbrg/cobrame:master bash

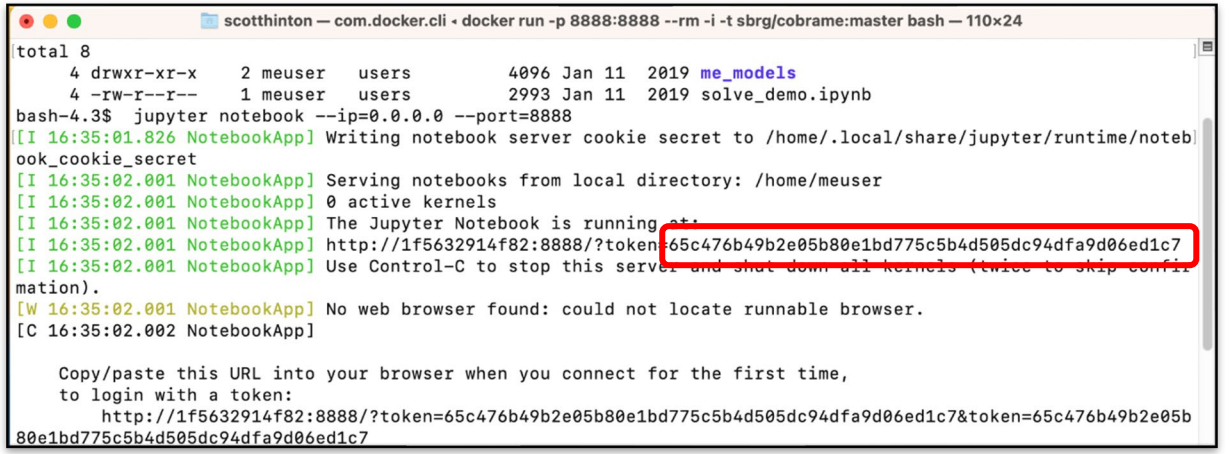
3. In a Docker terminal window clone the GitHub repository (associated with the "Container" page of Docker Desktop)

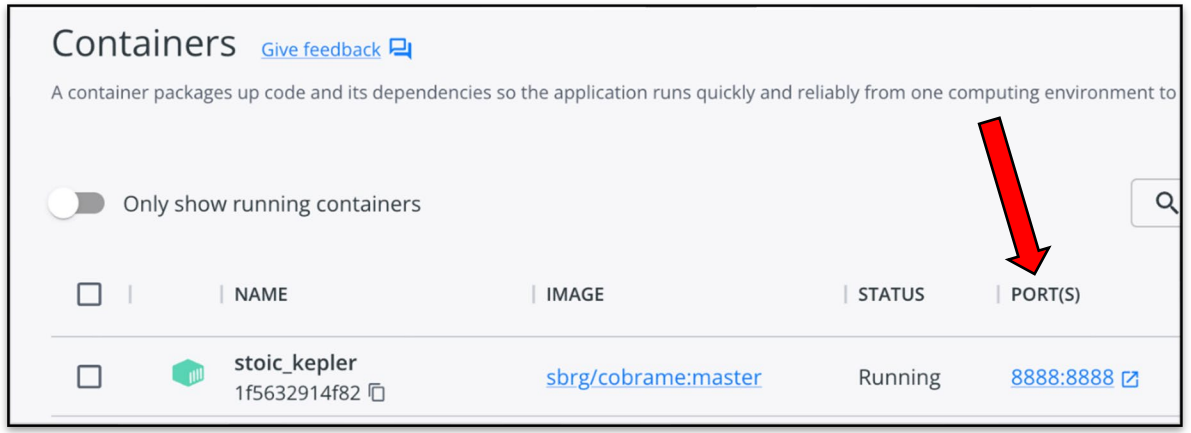
   git clone https://github.com/hscotthinton/me_files

4. Identify the token needed for the Jupyter notebook from the Docker's new created "bash" window

   jupyter notebook --ip=0.0.0.0 --port=8888

5. Click the port link on the Docker "Container" page to start the Jupyter notebook and enter the token





Docker Development Environment

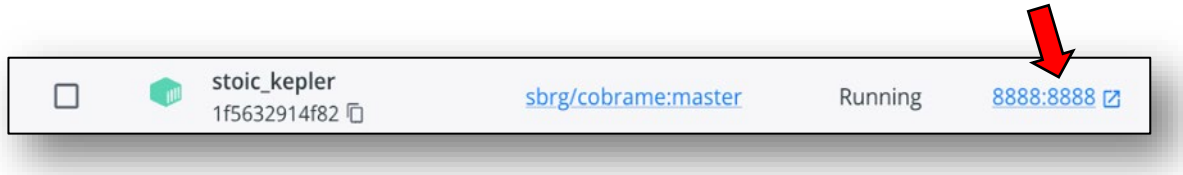
1. Start Docker Desktop
2. Open a terminal window and move to the shared drobox folder C:\Users\hinton\Dropbox\me-share
3. Load the COBRAme Docker image (converts Windows "terminal" window to Linux "bash" window)

   ```
   docker run -p 8888:8888 --rm -it -v ${PWD}:/home/meuser/me-share/ -t sbrg/cobrame:master bash
   ```

4. Using the Linux Bash window, identify the Jupyter security token with

   ```
   jupyter notebook --ip=0.0.0.0 --port=8888
   ```

5. Click the port link on the Docker "Container" page to start the Jupyter notebook



6. After the Jupyter window opens and asks for the token, enter the token generated from the previous command

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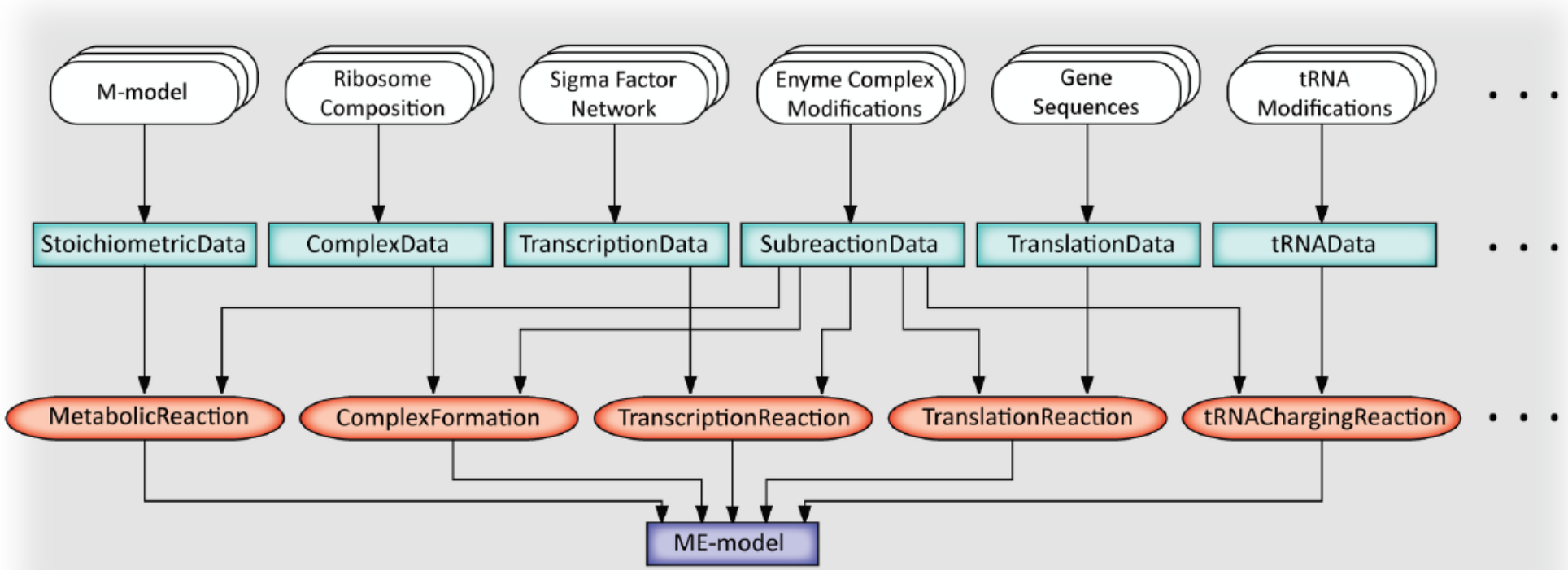
Flow of Information from Input Data to the ME-Model

- The 'build_me_model' workflow uses the ECOLIme package to load and process the *E. coli* M-model along with all supplied files containing information defining gene expression processes/reactions (white ovals).
- This information is then used to populate the different ProcessData classes (turquoise boxes) and link them to the appropriate ME Reaction classes (red ovals), all of which are defined in the COBRAme package.
- The entirety of the ME Reactions comprise a working ME-model.



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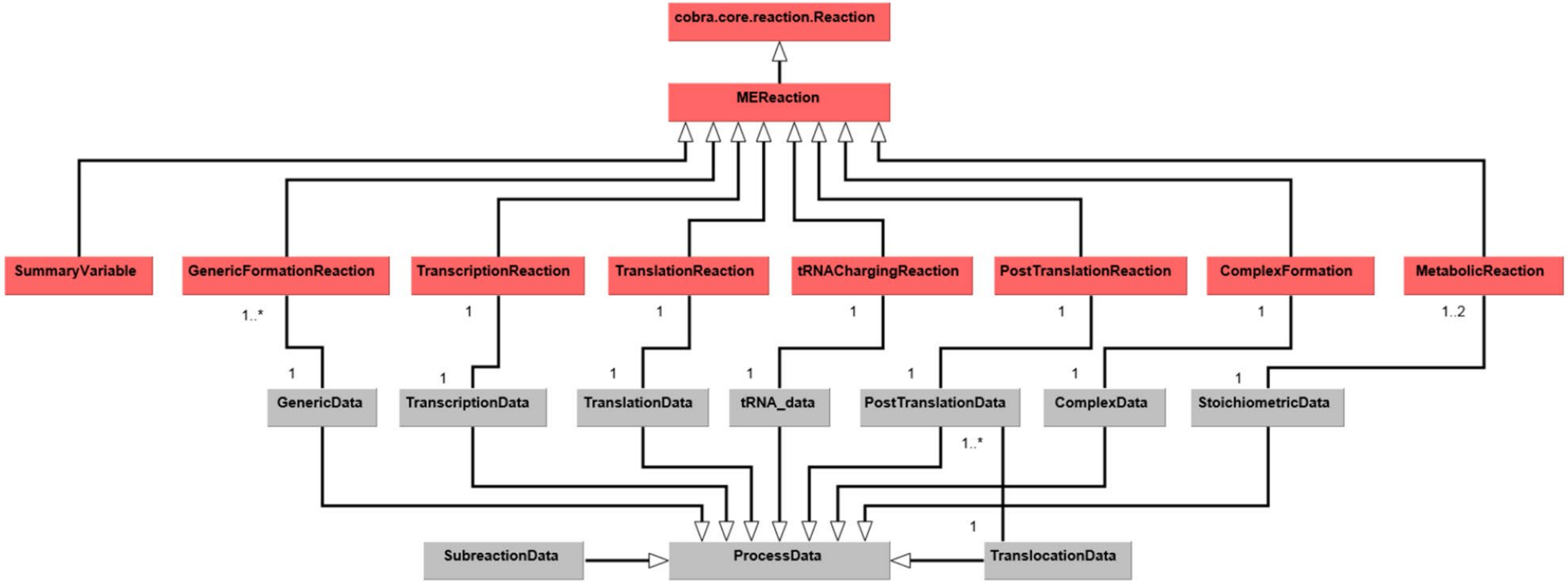
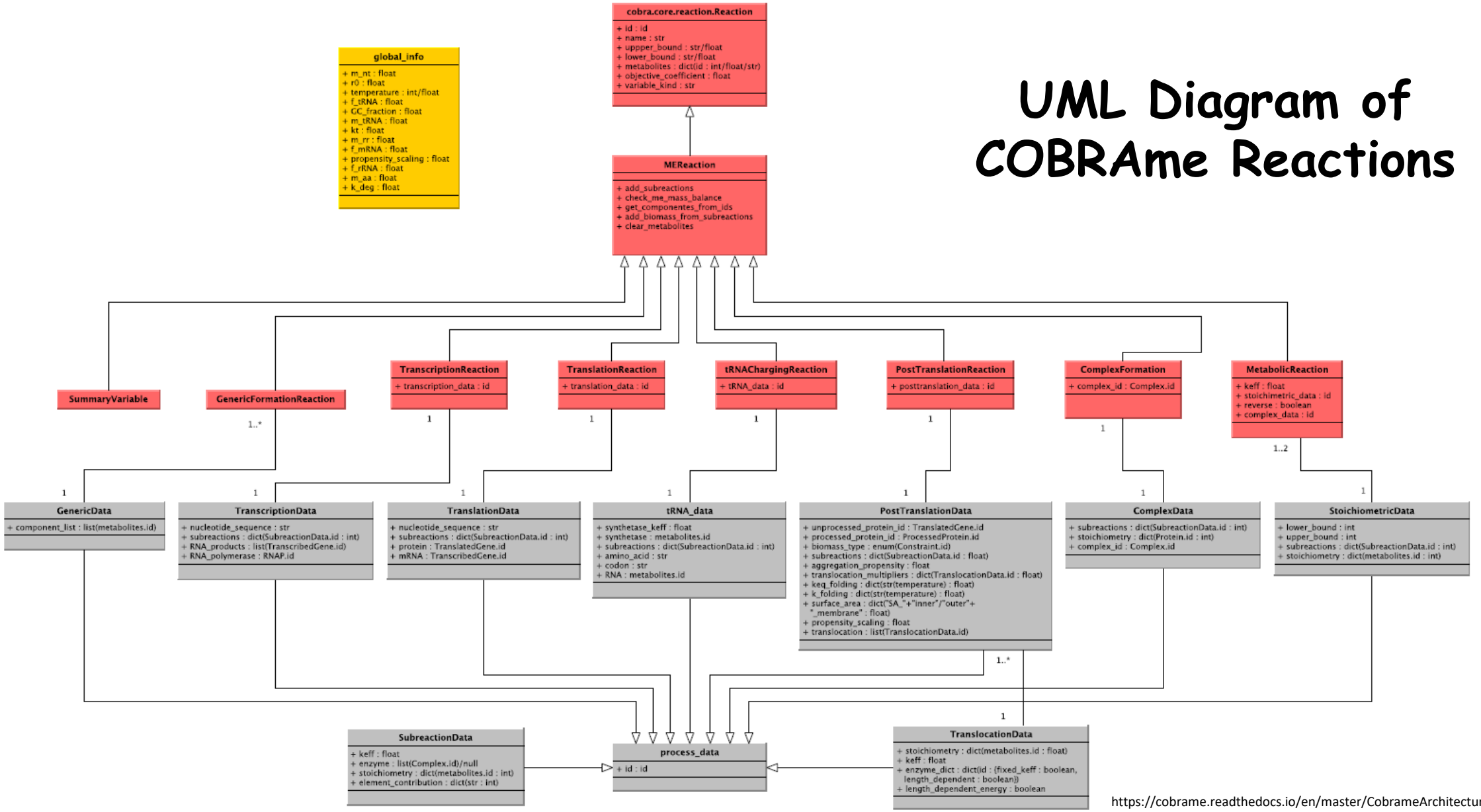
UML Diagram of COBRAme Architecture (Reactions)



https://cobrame.readthedocs.io/en/master/CobrameArchitectureOverview.html

UML Diagram of COBRAme Reactions



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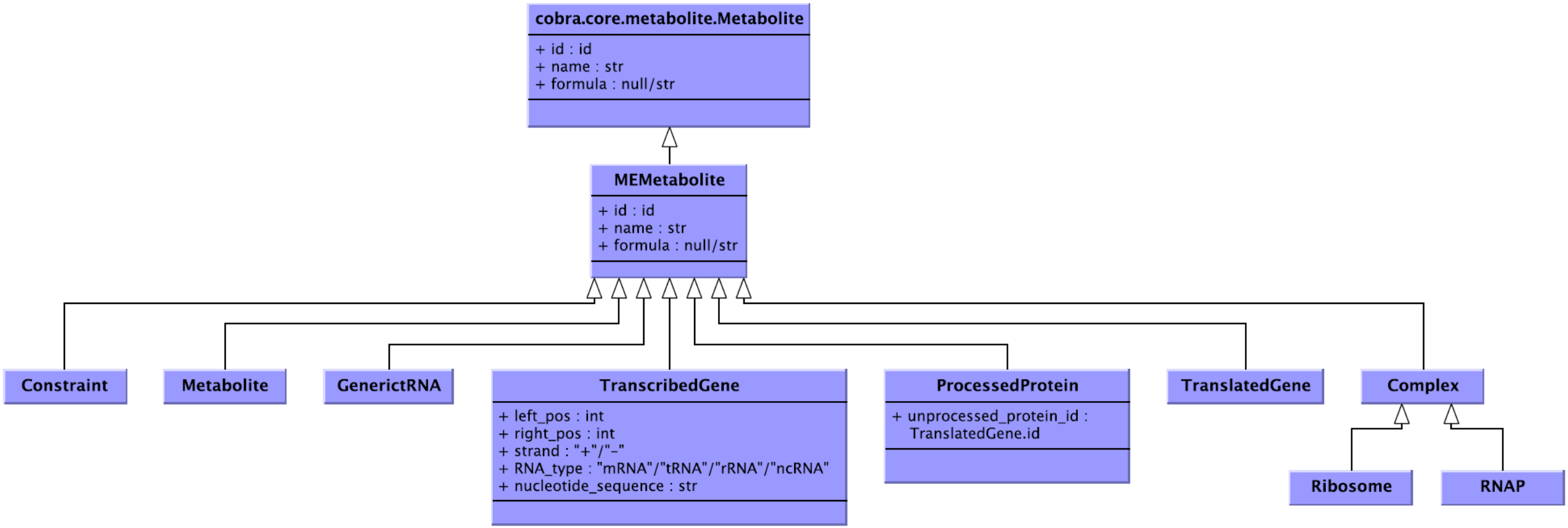
UML Diagram of COBRAme Metabolites



cobrame_full_uml.pdf

cobrame_full_uml.pdf

Classes of ME-Model Metabolites

- COBRAme supports 10 metabolite classes, they include:
  1. GenericComponent - Broadly Used Metabolites
  2. ProcessedProtein - Lipoprotein
  3. GenerictRNA - Charged tRNA
  4. Constraint - Biomass Metabolites
  5. Complex - Complex Metabolites
  6. Metabolite - M-Model Metabolites
  7. RNAP - RNA Polymerases
  8. Ribosome - Ribosomes
  9. TranscribedGene - mRNA, rRNA, ncRNA, tRNA
  10. TranslatedGene - Protein peptide
- Most metabolites effectively behave the same as a typical COBRA metabolite. The main exception is the "TranscribedGene" metabolite type which contains additional sequence information about the RNA it represents. In addition, each metabolite includes the types of ME Reactions that they participate in.
- The COBRAme compartments include: "p"="Periplasm", "e"= "Extra-organism" , "c"="Cytosol", "im"= 'Inner Membrane ", "om"= "Outer Membrane", "mc"="ME-model Constraint", "m"="Membrane"!
- See the online documentation (https://cobrame.readthedocs.io) and look at the ME metabolites table (S7) included in the supplementary material (https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1006302)

ME-Model Metabolite Classes

Metabolite Class	Purpose	Primary Producing Reaction Types	Primary Consuming Reaction Types
GenericComponent	Broadly Used Metabolites	GenericFormationReaction	ComplexFormation, …
ProcessedProtein	Lipoprotein	ComplexFormation	PostTranslationReaction
GenerictRNA	Charged tRNA	tRNAChargingReaction	TranslationReaction
Constraint	Biomass Metabolites	TranscriptionReaction, …	SummaryVariable
Complex	Complex Metabolites	MetabolicReactions, …	ComplexFormation, …
Metabolite	M-Model Metabolites	MetabolicReactions, …	MetabolicReactions, …
RNAP	RNA Polymerases	ComplexFormation	TranscriptionReaction
Ribosome	Ribosomes	ComplexFormation	TranslationReaction
TranscribedGene	mRNA, rRNA, ncRNA, tRNA	TranscriptionReaction	TranslationReaction, …
TranslatedGene	Protein peptide	TranslationReaction	ComplexFormation, …

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Classes of ME-Model Reactions

- COBRAme supports 9 classes of reactions, they include:
  1. MetabolicReaction - Create M-Model Metabolic Reactions
  2. GenericFormationReaction - Synthesize GenericComponents
  3. TranscriptionReaction - Synthesize Transcribed Genes
  4. tRNAChargingReaction - Synthesize Charged tRNA
  5. TranslationReaction - Synthesize Protein Peptides
  6. PostTranslationReaction - Synthesize Membrane Proteins
  7. ComplexFormation - Synthesize Protein Complexes
  8. SummaryVariable - Biomass Formation
  9. MEReaction - Create Demand/Exchange Reactions
- See the online documentation (https://cobrame.readthedocs.io) and look at the ME metabolites table (S7) included in the supplementary material (https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1006302)

ME-Model Reaction Classes

Reaction Class	Purpose	Primary Reactant Metabolite Classes	Primary Product Metabolite Classes
MetabolicReaction	Create M-Model Metabolic Reactions	Metabolite	Metabolite
GenericFormationReaction	Synthesize GenericComponents	Complex, …	GenericComponent
TranscriptionReaction	Synthesize Transcribed Genes	RNAP, …	TranscribedGene, …
tRNAChargingReaction	Synthesize Charged tRNA	TranscribedGene, …	GenerictRNA, …
TranslationReaction	Synthesize Protein Peptides	Ribosome, TranscribedGene, Metabolite, GenerictRNA, …	TranslatedGene, …
PostTranslationReaction	Synthesize Membrane Proteins	TranslatedGene, Complex, …	ProcessedProtein, …
ComplexFormation	Synthesize Protein Complexes	TranslatedGene, ProcessedProtein	Complex
SummaryVariable	Biomass Formation	Constraint, …	Biomass
MEReaction	Create Demand/Exchange Reactions	Metabolite	-

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Coupling Constraints for Metabolic Reactions

- Coupling constraints are required in an ME-model in order to **couple the synthesis of a reaction's enzyme (macromolecule) flux to the value of the metabolic reaction flux.**
- The coupling of enzyme synthesis cost to metabolic reaction flux scales with the growth-rate (μ) to represent the dilution of enzymes as they are passed on the daughter cells.
- For a metabolic reaction, The flux of the catalyzing enzyme, ν_{enzyme}, is calculated from

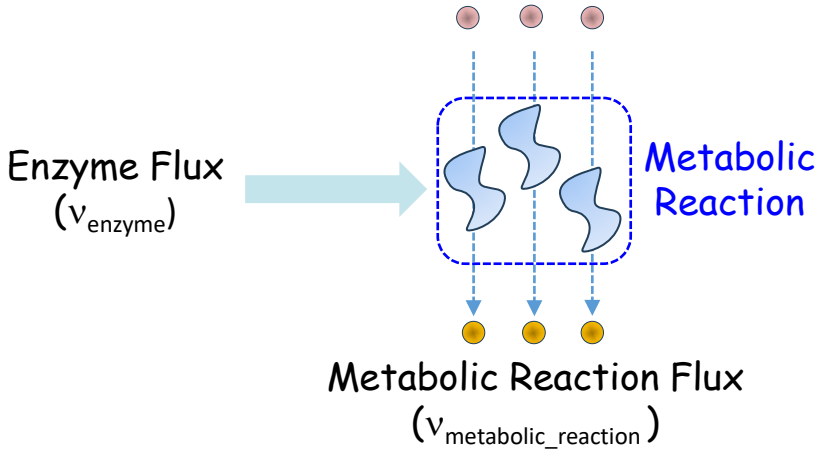
$$\nu_{enzyme} = (\mu/k_{eff})*\nu_{metabolic\_reaction}$$

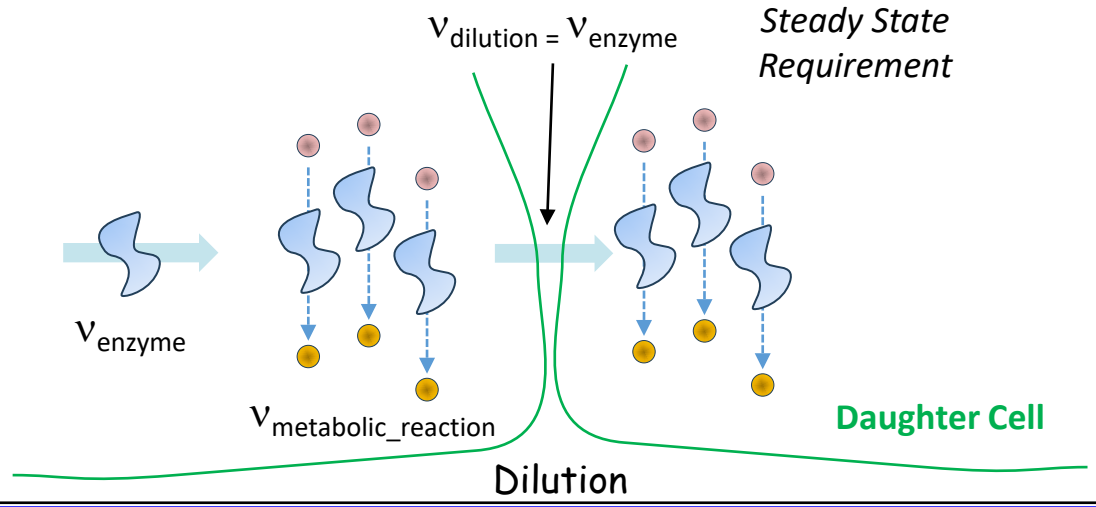
where $\nu_{metabolic\_reaction}$ is the flux passing through the metabolic reaction and k_{eff} is the effective turnover rate, which is the number of metabolic products the enzyme can catalyze per cell division.

- With COBRAme the dilution of coupled macromolecules to the daughter cell is accounted for by applying a coupling coefficient directly in the reaction in which the macromolecule is used. Example,

$$(\mu/k_{eff})\ *\ CPLX0\text{-}3929 + ara5p\_c \dashrightarrow ru5p\_\_D\_c$$

$$3.315e\text{-}6*mu\ CPLX0\text{-}3929 + ara5p\_c \dashrightarrow ru5p\_\_D\_c$$





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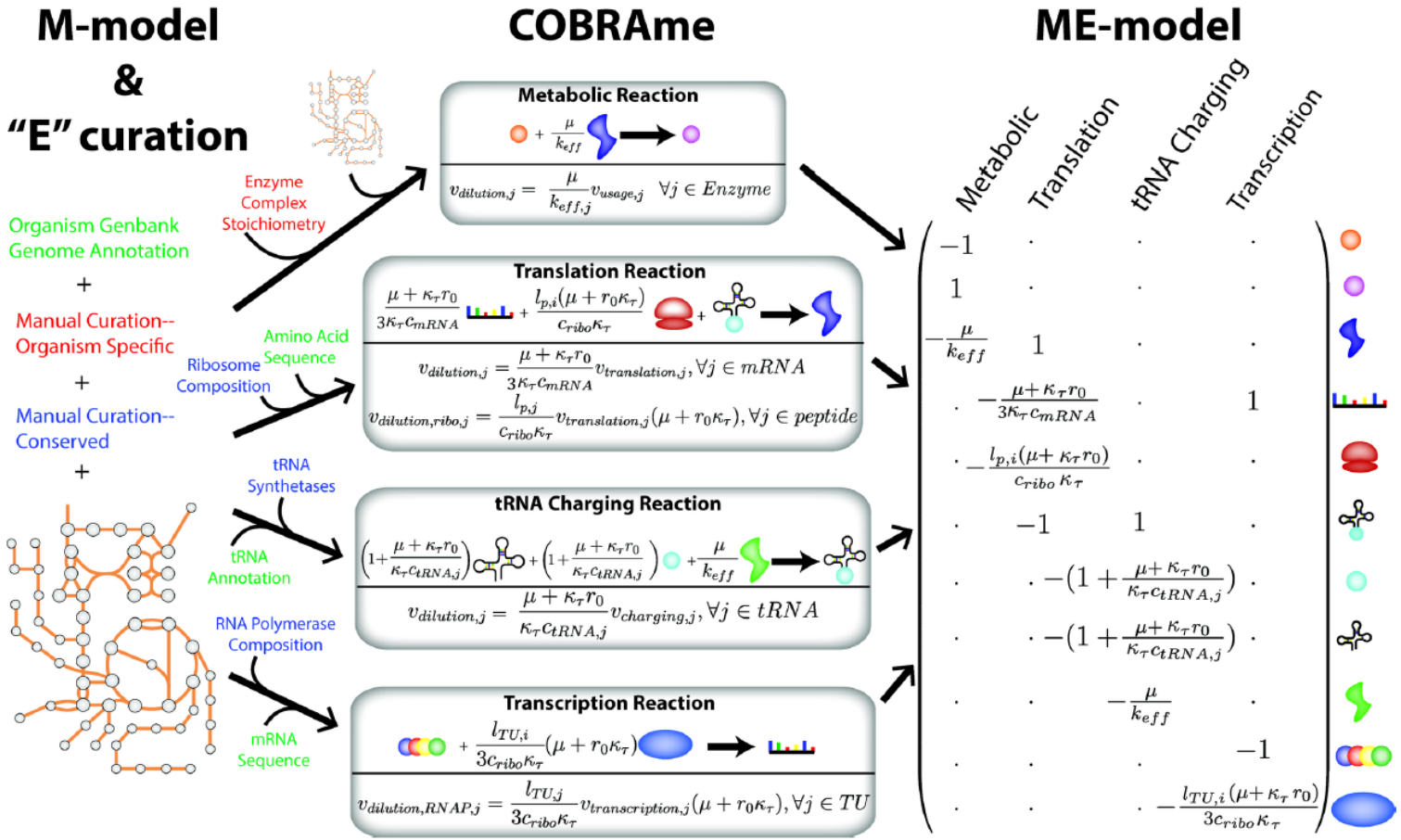
COBRAme Dilution and Optimization



$$\max_{v,\mu} \mu$$

s.t. $Sv = 0$

$$v_{\text{formation,Ribosome}} - \sum_{i \in Peptide} \left(\frac{l_{p,i}}{c_{\text{ribo}} \kappa_\tau} (\mu + r_0 \kappa_\tau) \cdot v_{\text{translation},i} \right) = 0$$

$$v_{\text{formation,RNAP}} - \sum_{i \in TU} \left(\frac{l_{\text{TU},i}}{3 c_{\text{ribo}} \kappa_\tau} (\mu + r_0 \kappa_\tau) \cdot v_{\text{transcription},i} \right) = 0$$

$$v_{\text{formation},j} - \sum_{i \in \text{generic\_tRNA}_{AA}} \left(\left(1 + \frac{\mu}{k_{eff,tRNA}} \right) \frac{\mu}{k_{eff,charging}} v_{\text{charging},i} \right) = 0, \quad \forall j \in Synthetase$$

$$v_{\text{formation},j} - \sum_{i \in \text{enzymatic reaction}} \left(\frac{\mu}{k_{ij}^{\text{eff}}} v_{\text{usage},i} \right) = 0, \quad \forall j \in Enzyme$$

$$v_{\text{formation},j} - \sum_{i \in \text{tRNA anticodons}} \frac{(\mu + \kappa_\tau r_0)}{\kappa_\tau c_{tRNA,j}} v_{charging,i} = 0, \quad \forall j \in tRNA$$

$$v_{\text{degredation},j} - \frac{k_{deg,j}}{3 \kappa_\tau c_{mRNA}} \cdot \frac{\mu + \kappa_\tau r_0}{\mu} v_{translation,j} = 0, \quad \forall j \in mRNA$$

$$v_{\text{formation},j} - \frac{(\mu + \kappa_\tau r_0)}{3 \kappa_\tau c_{mRNA}} v_{translation,j} = 0, \quad \forall j \in mRNA$$

$$v^L \leq v \leq v^U$$

$$\mu \leq v_{biomass\_dilution} \leq \mu$$

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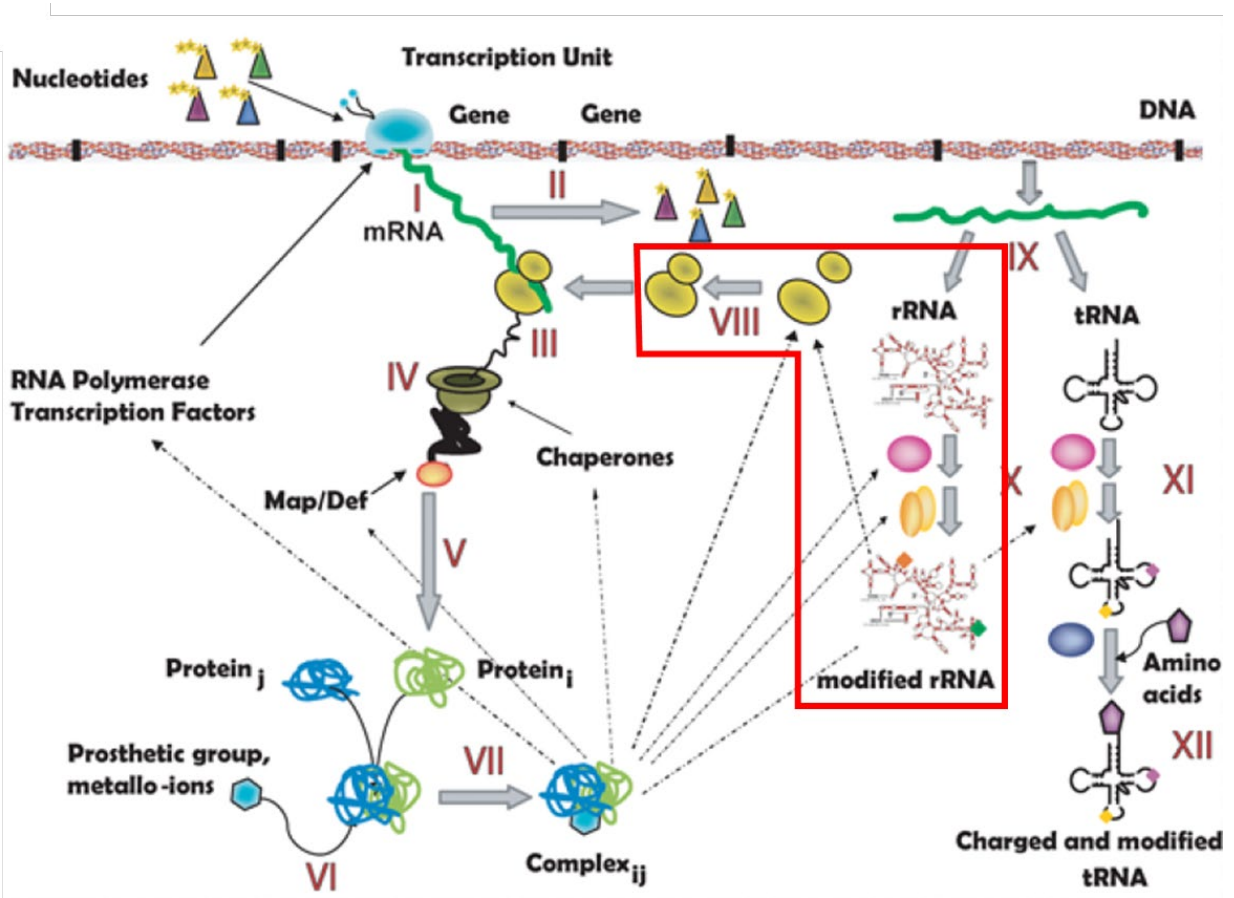
Reaction Lumping

- Splitting the model into ProcessData and ME Reactions allows for a variety of model simplifications. For example, reactions that occur in a number of individual steps or sub-reactions (i.e., ribosome formation, translation, etc.) can be lumped into a single reaction.
- The single lumped ME Reaction can be constructed by associating it with the multiple ProcessData instances that detail the individual sub-reactions involved in the overall reaction.
- All sub-reaction information is further accessible through the ME Reaction instance itself which allows the information to be queried, edited, and updated throughout the reaction.
- If the sub-reaction participates in many different reactions, the sub-reaction changes can be applied throughout the entire model.
- This lumping has the obvious benefit of reducing the number of model reactions, thus shortening the solve time.
- Lumping intricate reactions has the added benefit of making the ME-model much more modular in nature.
- This simplifies the process of adding or removing new processes associated with the ME-model reaction.



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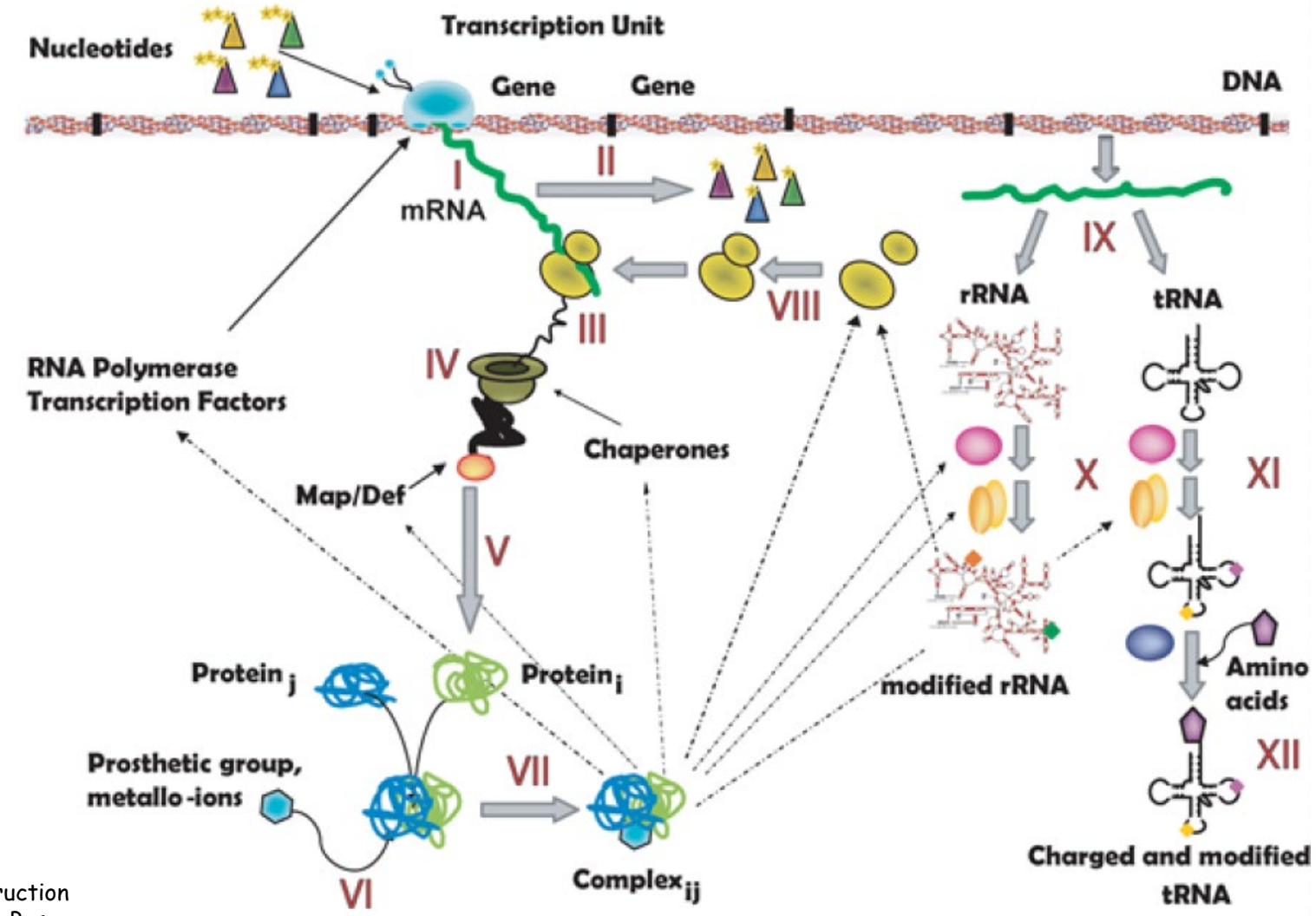
Expression Content

Schematic representation of the network components and reactions is shown. In addition to the macromolecular synthesis of RNA and proteins, rRNA and tRNA processing reactions were included in the reconstruction.

I: transcription;
II: mRNA degradation;
III: translation;
IV: protein maturation;
V: protein folding;
VI: metallo-ion binding;
VII: protein complex formation;
VIII: ribosome assembly;
IX: RNA processing;
X: rRNA modification;
XI: tRNA modification;
XII: tRNA charging.



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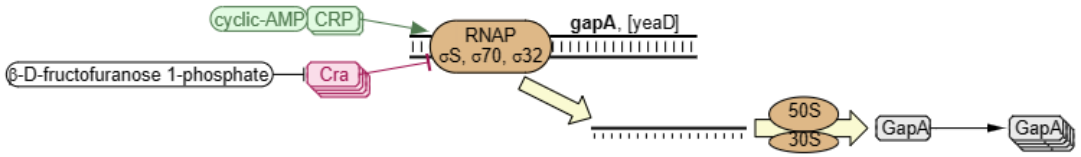
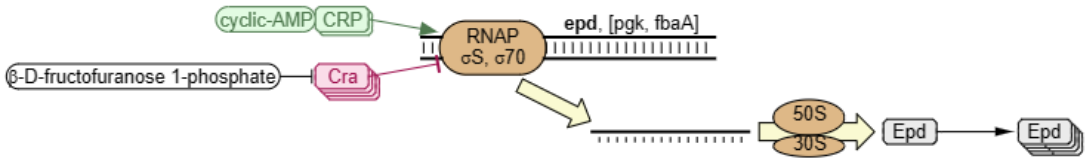
EcoCyc Transcription Units for gapA (b1779)

https://www.biocyc.org/gene?orgid=ECOLI&id=EG10367#tab=TU

B1779-based Reactions



https://www.biocyc.org/gene?orgid=ECOLI&id=GAPDH-A-MONOMER



https://www.biocyc.org/gene?orgid=ECOLI&id=EG10368

Nucleotide Sequence — b1779

TranscriptionReaction — transcription_TU0_4904_from_RPOH_MONOMER, transcription_TU0_4905_from_RPOS_MONOMER, transcription_TU0_4905_from_RpoD_mono, transcription_TU0_4906_from_RpoD_mono, or transcription_TU0_4907_from_RpoD_mono.

TranscribedGene — RNA_b1779 → *MEReaction* DM_RNA_b1779 →

TranslationReaction — translation_b1779

TranslatedGene — protein_b1779

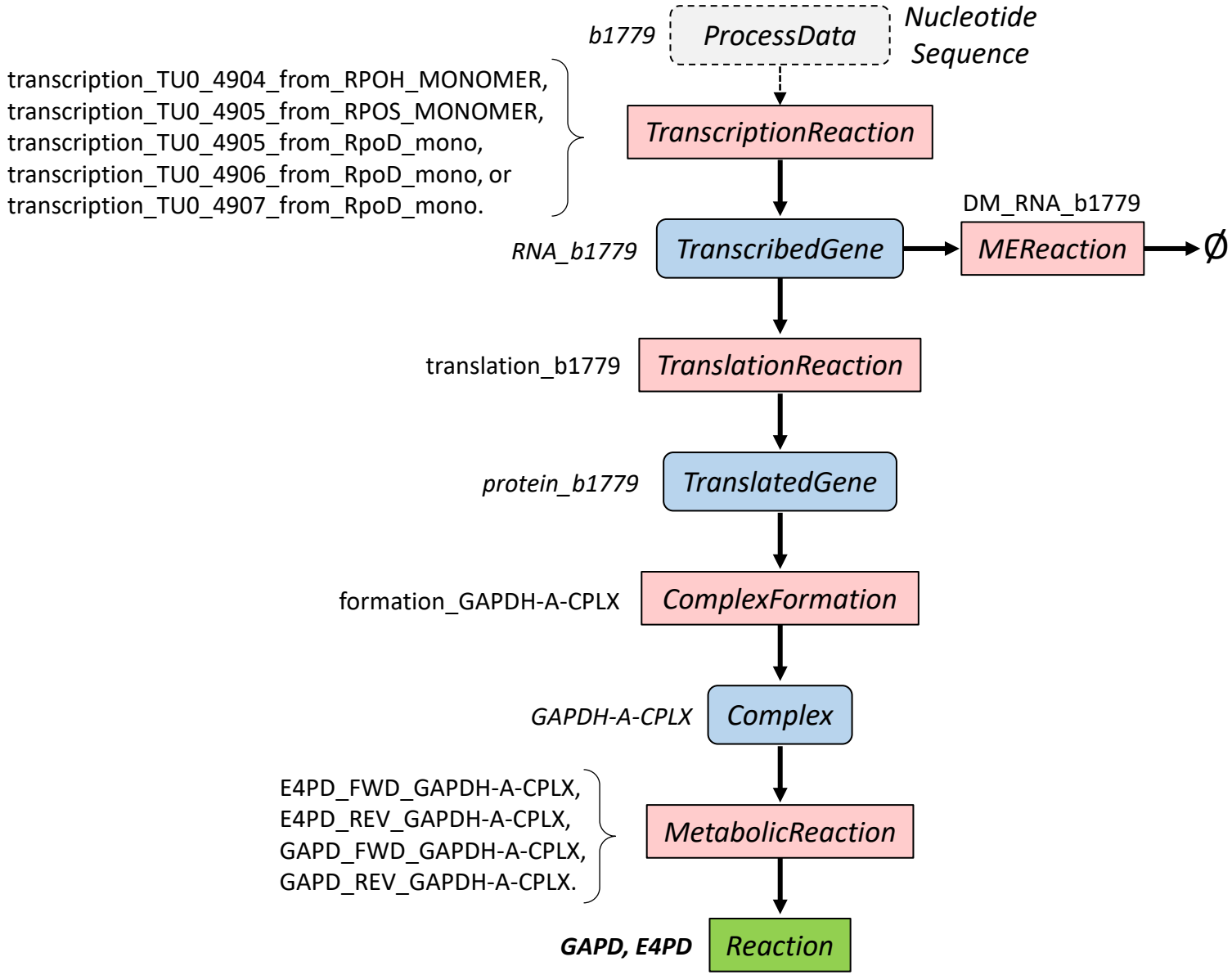
ComplexFormation — formation_GAPDH-A-CPLX

Complex — GAPDH-A-CPLX

MetabolicReaction — E4PD_FWD_GAPDH-A-CPLX, or E4PD_REV_GAPDH-A-CPLX. | GAPD_FWD_GAPDH-A-CPLX, or GAPD_REV_GAPDH-A-CPLX.

Reaction — **E4PD** | **GAPD**

"MetabolicReaction" (Enzyme) Production Process



Reaction

Metabolite

Metabolic Network Reaction

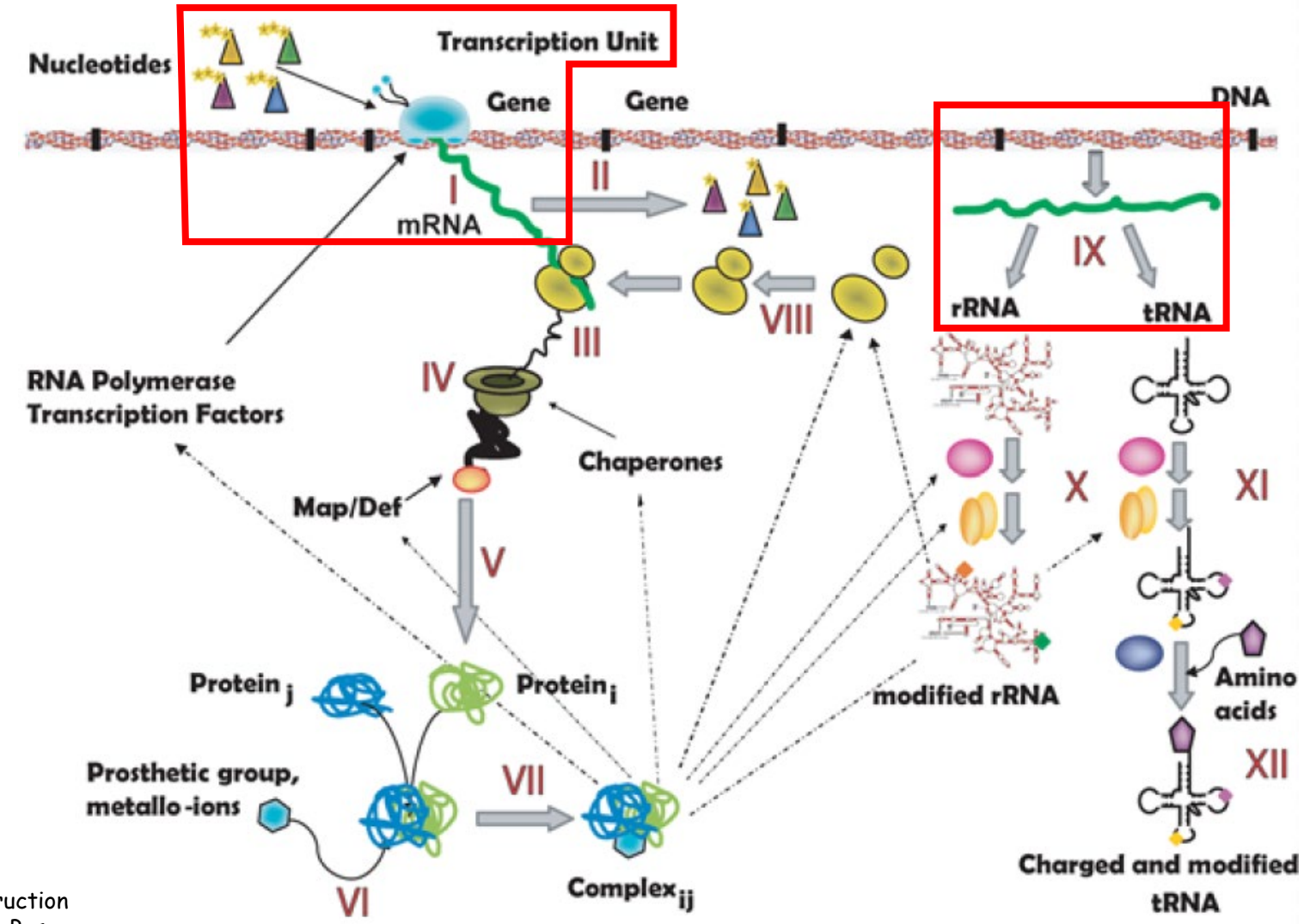
Lesson Outline

- Metabolism (M) vs. Metabolism & Expression (ME) Models
- COBRAme
  - Working Environment (Docker)
  - ME Model Architecture
  - Macromolecular Coupling
  - Reaction Lumping
- ECOLIme
  - Macromolecular Reactions
  - Transcription
  - Translation
  - COBRAme Execution
  - ECOLIme Operation

Transcription Reaction Lumping

Schematic representation of the network components and reactions is shown. In addition to the macromolecular synthesis of RNA and proteins, rRNA and tRNA processing reactions were included in the reconstruction.

I: transcription;
II: mRNA degradation;
III: translation;
IV: protein maturation;
V: protein folding;
VI: metallo-ion binding;
VII: protein complex formation;
VIII: ribosome assembly;
IX: RNA processing;
X: rRNA modification;
XI: tRNA modification;
XII: tRNA charging.

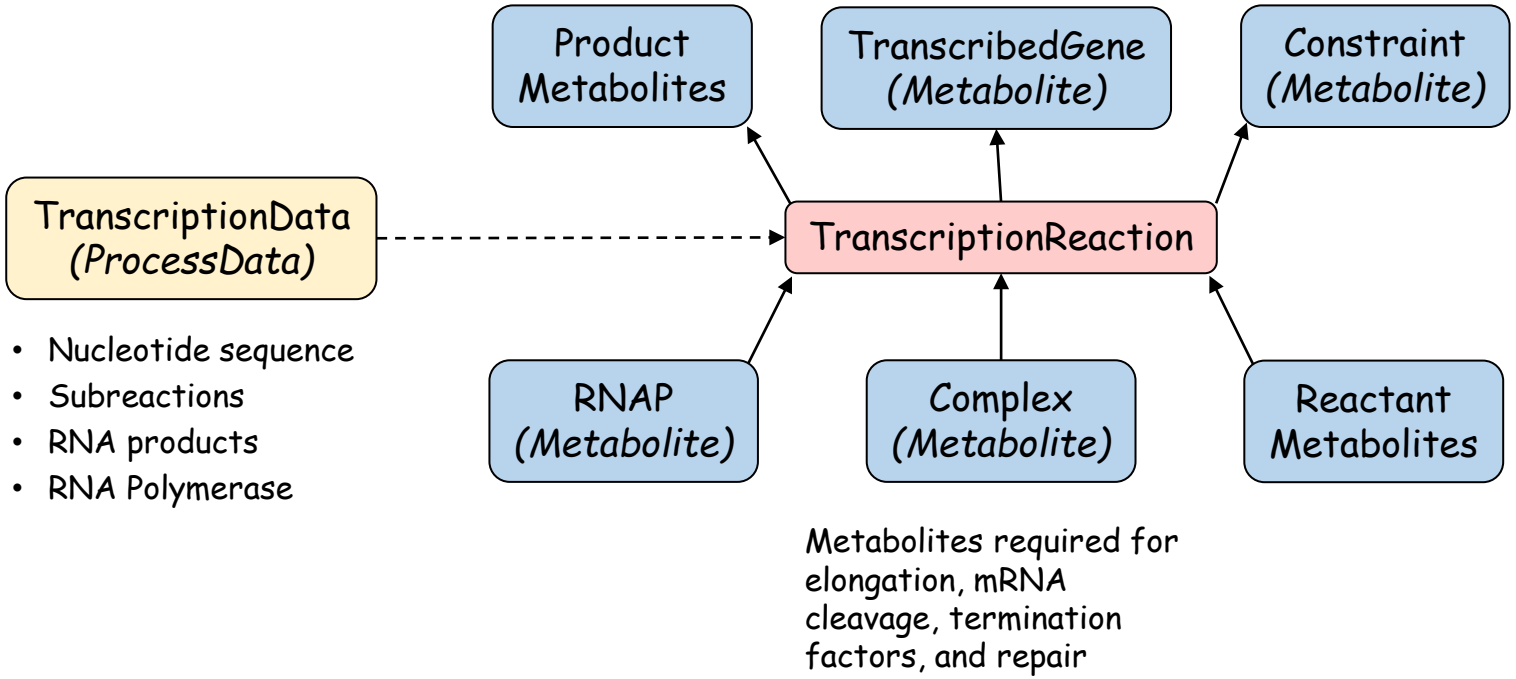


Thiele I, Jamshidi N, Fleming RMT, Palsson BØ (2009) Genome-Scale Reconstruction of Escherichia coli's Transcriptional and Translational Machinery: A Knowledge Base, Its Mathematical Formulation, and Its Functional Characterization. PLoS Comput Biol 5(3): e1000312. doi:10.1371/journal.pcbi.1000312"

TranscriptionReaction

A "TranscriptionReaction" includes all the metabolites required to transcribe the DNA associated with a specific EcoCyc defined transcription unit to either RNA or mRNA. These metabolites include:

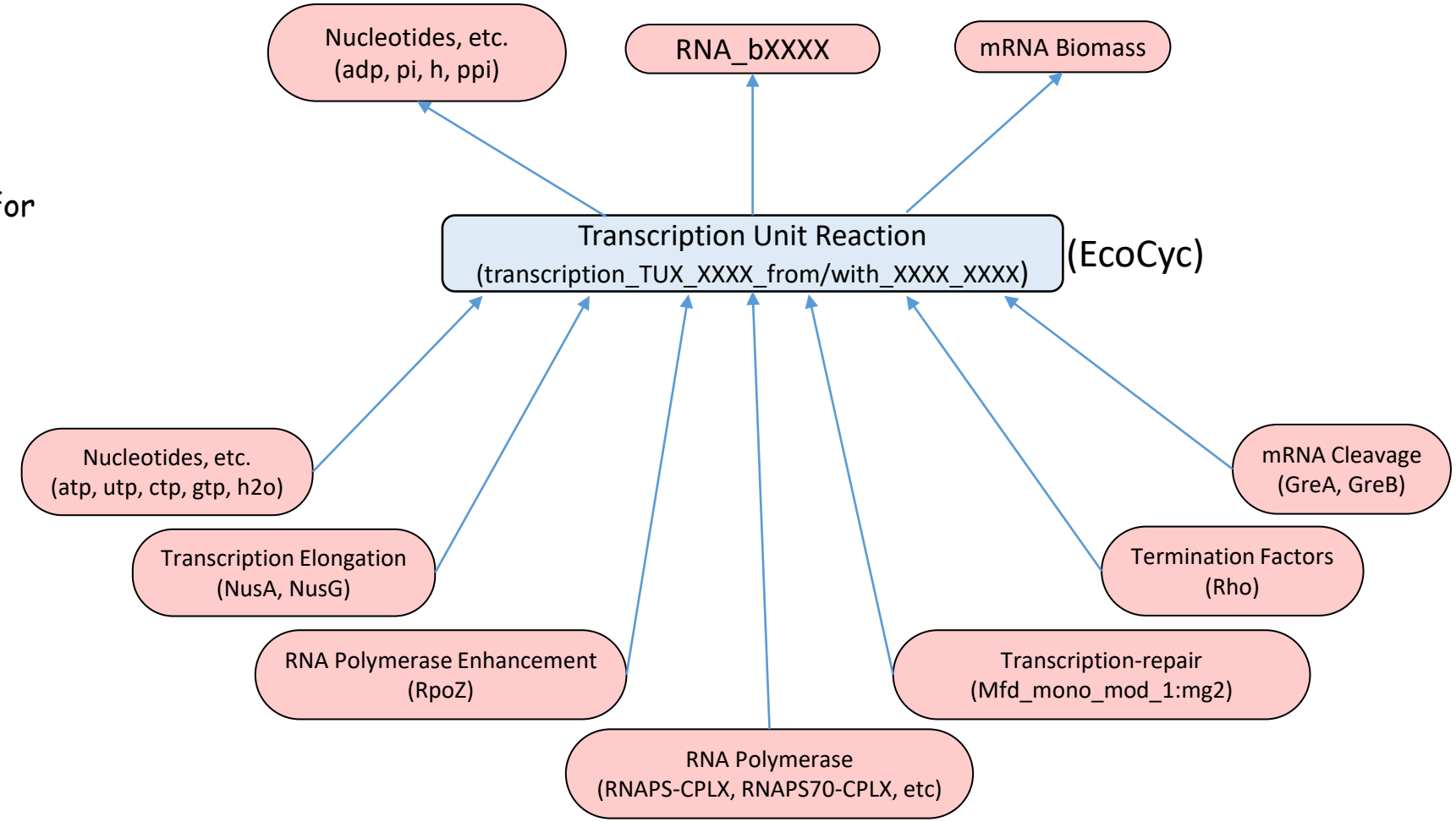
1. The nucleotide metabolites required for the reaction (derived from the nucleotide sequence located in the "processdata" class)
2. The metabolites required for transcription elongation
3. The metabolites required for transcription repair
4. The RNA polymerase and associated metabolites
5. The termination factor metabolites
6. The metabolites required for mRNA cleavage



TranscriptionReactions

The reaction associated with each transcription unit (*TranscriptionReaction*) includes the following:

1. The nucleotide metabolites required for the reaction (derived from the nucleotide sequence located in the "processdata" class)
2. The metabolites required for transcription elongation
3. The metabolites required for transcription repair
4. The RNA polymerase and associated metabolites
5. The termination factor metabolites
6. The metabolites required for mRNA cleavage



List of "TranscriptionReaction" Supporting Metabolites

- **Mfd_mono_mod_1:mg2:** transcription-repair coupling factor
- **NusA_mono:** transcription termination/antitermination protein NusA
- **NusB_mono:** transcription antitermination protein NusB
- **NusG_mono:** transcription termination/antitermination factor NusG
- **GreA_mono:** transcription elongation factor GreA
- **GreB_mono:** transcription elongation factor GreB
- **monocistronic_excision_machinery:** Processing of monocistronic mRNA
- **polycistronic_wout_rRNA_excision_machinery:** Processing of polyocistronic mRNA.
- **Rho_hexa_mod_3:mg2:** transcription termination factor Rho
- **RplC_mono:** 50S ribosomal subunit protein L3
- **RNA_degradosome:** multiprotein complex involved in RNA degradation
- **RplD_mono:** 50S ribosomal subunit protein L4
- **RplM_mono:** 50S ribosomal subunit protein L13
- **RpoZ_mono_mod_1:mg2:** RNA polymerase subunit ω
- **RpsD_mono:** 30S ribosomal subunit protein S4
- **RpsJ_mono:** 30S ribosomal subunit protein S10
- **rRNA_containing_excision_machinery:** processing of rRNA

Transcription.ipynb

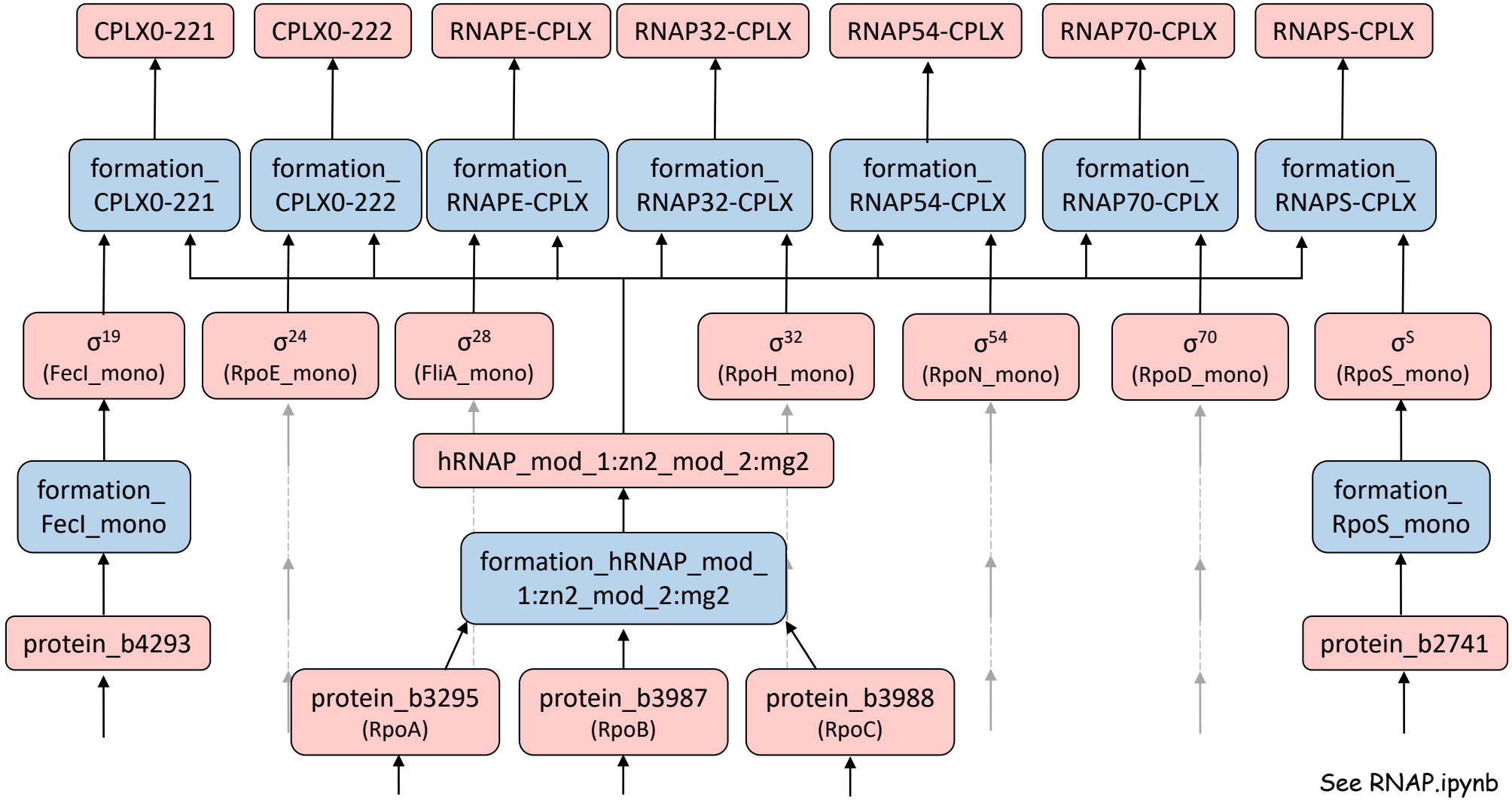
Transcription "Complex" Metabolites

- **Mfd_mono_mod_1:mg2: transcription-repair coupling factor**
  The Mfd protein, also known as transcription-repair coupling factor (TRCF), is responsible for ATP-dependent displacement of stalled RNA polymerase (RNAP) from DNA lesions. Mfd facilitates the repair of template strand lesions. (https://ecocyc.org/gene?orgid=ECOLI&id=EG11619)
- **NusA_mono: transcription termination/antitermination protein NusA**
  Transcription termination/antitermination L factor (NusA) is a key component in both prevention and enhancement of transcriptional termination. It is important in both Rho-dependent and intrinsic termination, as well as in lambda and other phage antitermination systems. (https://ecocyc.org/gene?orgid=ECOLI&id=EG10665)
- **NusG_mono: transcription termination/antitermination factor NusG**
  Transcription termination factor NusG is required for some kinds of Rho-dependent termination as well as for transcription antitermination.NusG is also involved in antitermination during lambda phage transcription (https://ecocyc.org/gene?orgid=ECOLI&id=EG10667)
- **GreA_mono: transcription elongation factor GreA**
  GreA stimulates the mRNA cleavage activity of RNA polymerase, which acts to release a polymerase complex that has stalled or has incorporated an incorrect nucleotide. GreA (and GreB) is also required for wild-type transcription of some regulatory regions within lambda phage. (https://ecocyc.org/gene?orgid=ECOLI&id=EG10415)
- **GreB_mono: transcription elongation factor GreB**
  GreB stimulates the mRNA cleavage activity of RNA polymerase, which acts to release a polymerase complex that has stalled. GreB (and GreA) is also required for wild-type transcription of some regulatory regions within lambda phage.
- **RpoZ_mono_mod_1:mg2: RNA polymerase subunit ω**
  RpoZ copurifies with RNA polymerase and may play a structural role in that complex.
- **Rho_hexa_mod_3:mg2: transcription termination factor Rho**
  Rho is required for one of the two major types of termination of RNA transcription.

RNA Polymerase

The iJL1678b (ECOLIme) model RNA polymerase includes:

- A unique sigma factor is included in each RNAP metabolite
- There are seven different RNAP metabolites, each corresponding to a different sigma factor.



See RNAP.ipynb

Modeling the RNA Polymerases in COBRAme

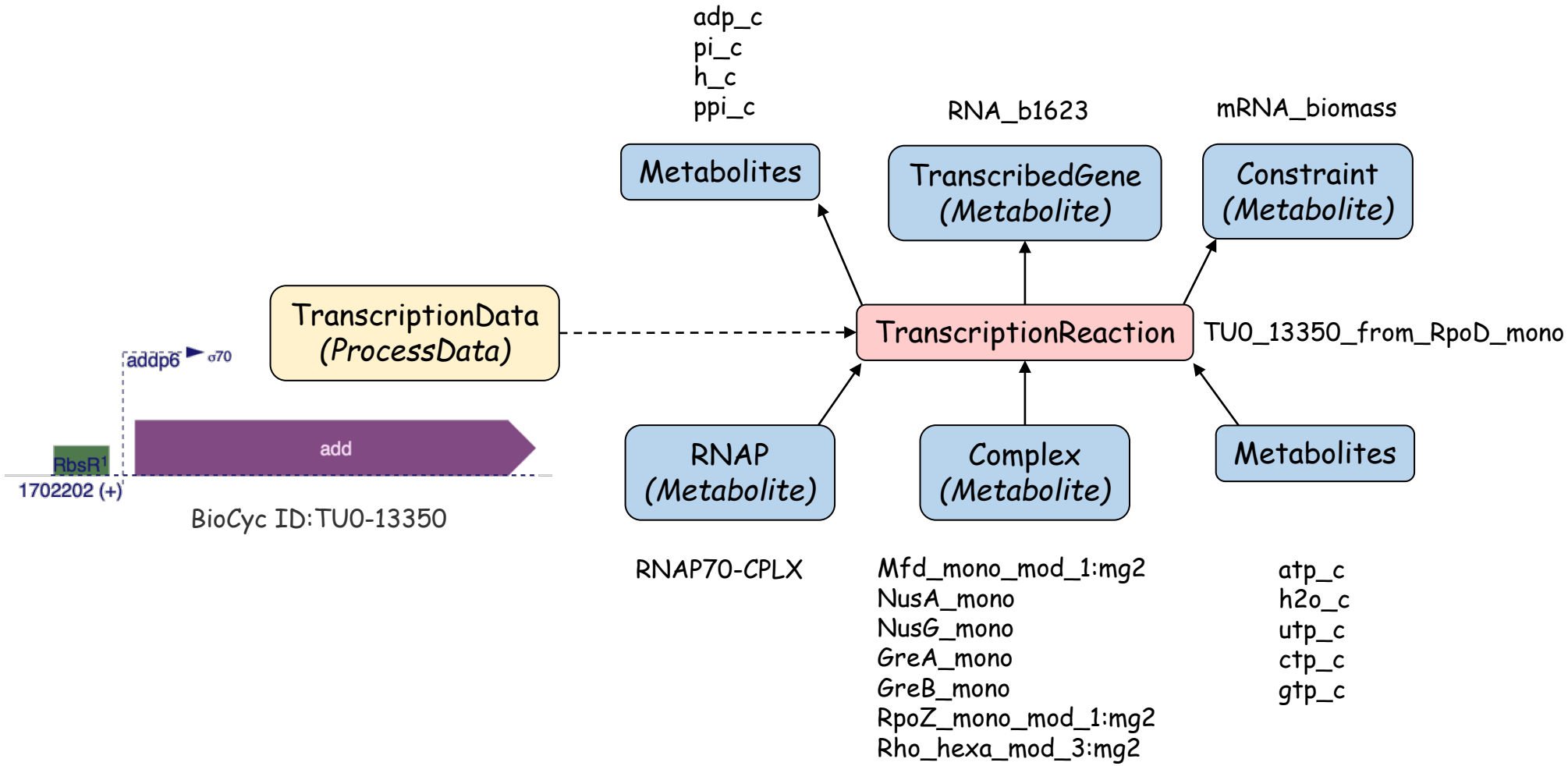
1. Load the python packages

In [1]:
```
from __future__ import print_function, division, absolute_import

# python imports
import re
from os.path import join
from collections import defaultdict
import pickle
import pandas as pd

# third party imports
import pandas
import tabulate
import cobra
pd.set_option('display.max_columns', 100)
pd.set_option('display.width',100)
pd.set_option('display.max_colwidth',100)

# ECOLIme
import ecolime
from ecolime import (transcription, translation, flat_files, generics, formulas, compartments)

# COBRAme
import cobrame
from cobrame.util import building, mu, me_model_interface
#from cobrame.io.json import save_json_me_model, save_reduced_json_me_model
```

RNAP.ipynb

TranscriptionReaction

Example: "transcription_TU0_13350_from_RpoD_mono"



Modeling Transcription in the iJL1678b (ECOLIme) Model

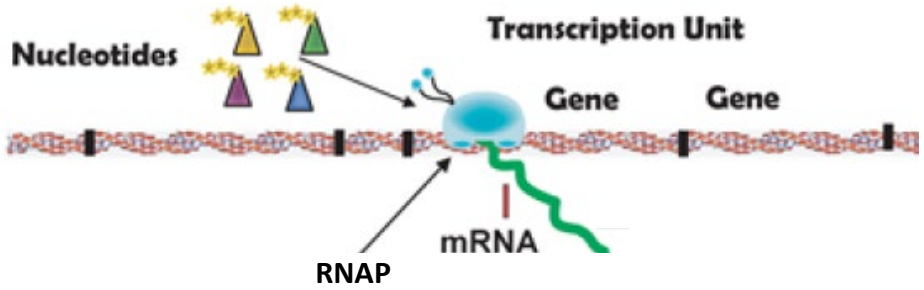
1. Load the python packages

In [1]:
```
from __future__ import print_function, division, absolute_import

# python imports
import re
from os.path import join
from collections import defaultdict
import pickle
import pandas as pd

# third party imports
import pandas
import tabulate
import cobra
pd.set_option('display.max_columns', 100)
pd.set_option('display.width',100)
pd.set_option('display.max_colwidth',100)

# ECOLIme
import ecolime
from ecolime import (transcription, translation, flat_files, generics, formulas, compartments)

# COBRAme
import cobrame
from cobrame.util import building, mu, me_model_interface
#from cobrame.io.json import save_json_me_model, save_reduced_json_me_model
```

Transcription.ipynb

Creating a Transcription Reaction

1. Define the RNA id, rna type, nucleotide sequence
   - gene = cobrame.TranscribedGene('RNA_a', 'mRNA', sequence)
   - me.add_metabolites([gene])
2. Add the transcription data to the model
   - transcription_data = cobrame.TranscriptionData('TU_a',me,rna_products={'RNA_a'})
   - transcription_data.nucleotide_sequence = sequence
3. Update the transcription reaction
   - transcription_rxn.update()
4. Incorporate the RNA Polymerase in the model
   - RNAP = cobrame.RNAP('RNA_polymerase')
   - me.add_metabolites(RNAP)
5. Associate the RNA polymerase with the transcription data
   - for data in me.transcription_data:
   - data.RNA_polymerase = RNAP.id
   - me.reactions.transcription_TU_a.update()
6. Examine reaction
   - 0.00088887053605567*mu + 0.000347992814865795 RNA_polymerase + 86 atp_c + 38 ctp_c + 12 gtp_c + 50 utp_c --> RNA_a + 59.172286 mRNA_biomass + 186 ppi_c



https://cobrame.readthedocs.io/en/master/building_a_model.html

Lesson Outline

- Metabolism (M) vs. Metabolism & Expression (ME) Models
- COBRAme
  - Working Environment (Docker)
  - ME Model Architecture
  - Macromolecular Coupling
  - Reaction Lumping
- ECOLIme
  - Macromolecular Reactions
  - Transcription
  - Translation
  - COBRAme Execution
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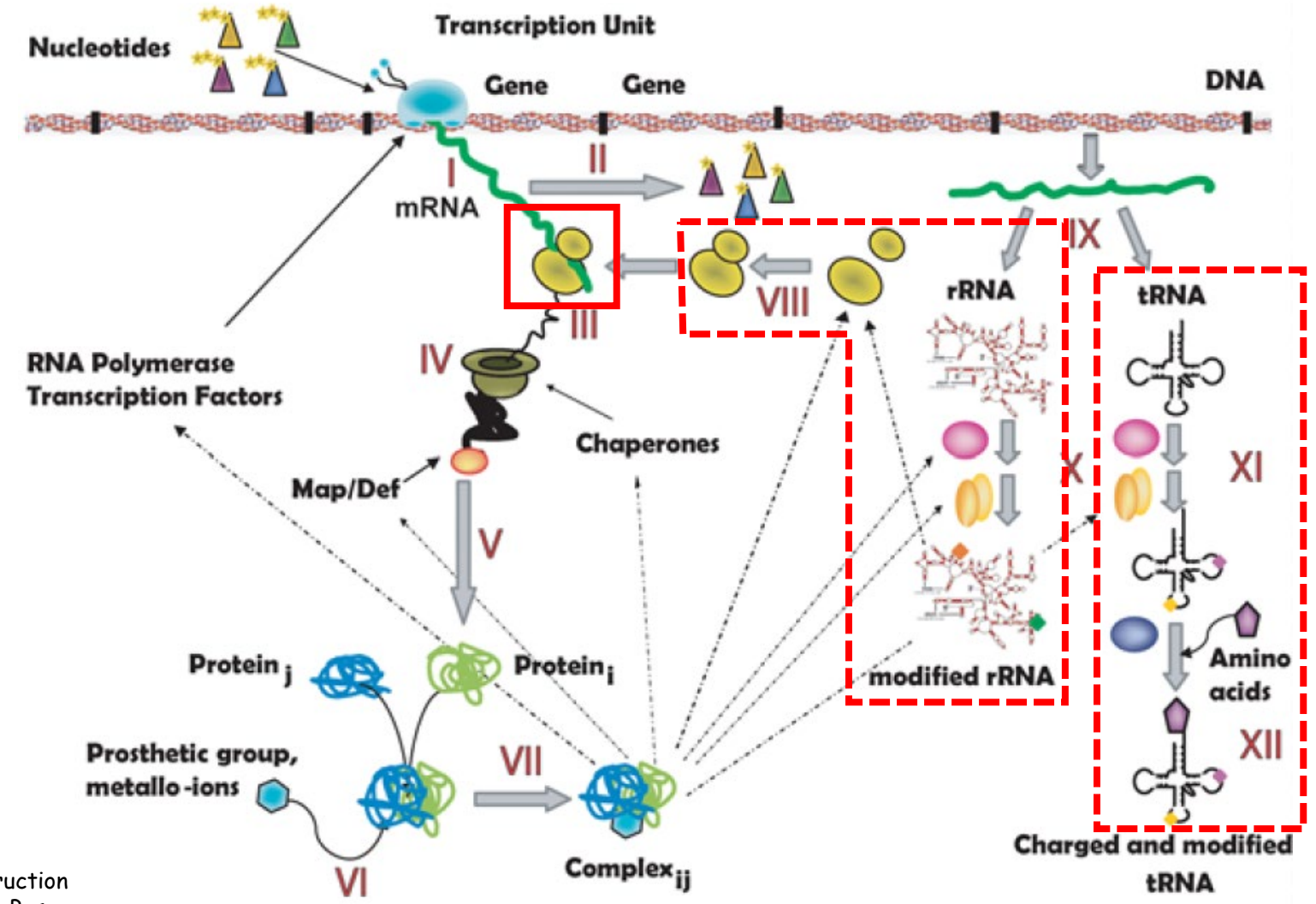
Translation Reaction Lumping

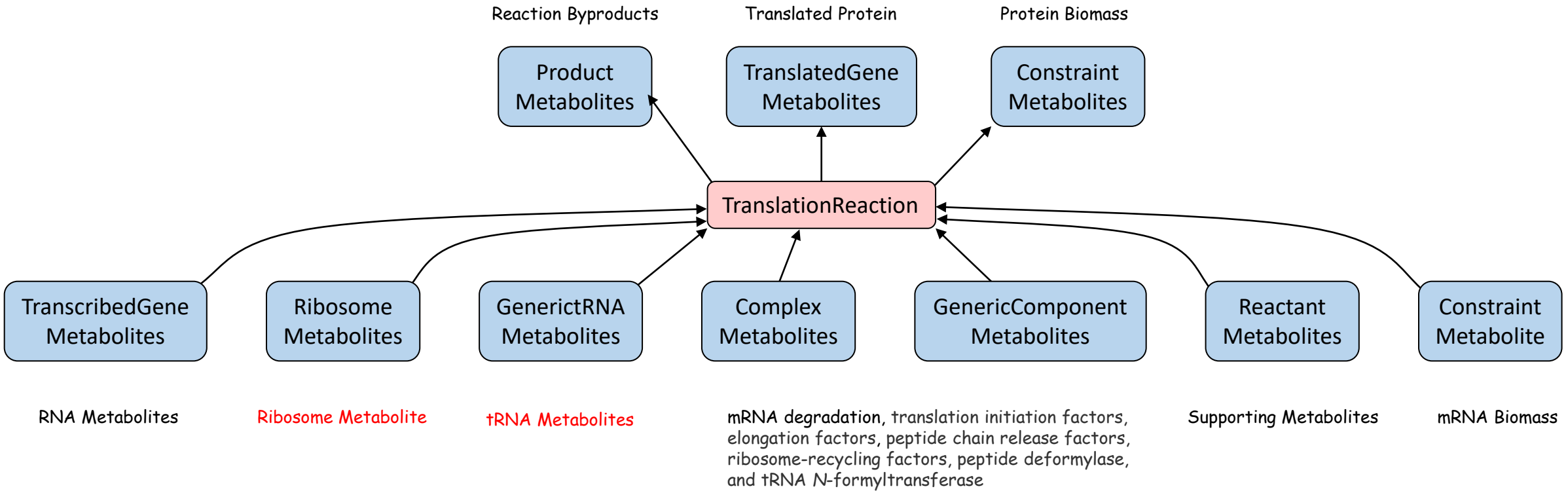
Schematic representation of the network components and reactions is shown. In addition to the macromolecular synthesis of RNA and proteins, rRNA and tRNA processing reactions were included in the reconstruction.

I: transcription;
II: mRNA degradation;
III: translation;
IV: protein maturation;
V: protein folding;
VI: metallo-ion binding;
VII: protein complex formation;
VIII: ribosome assembly;
IX: RNA processing;
X: rRNA modification;
XI: tRNA modification;
XII: tRNA charging.



Thiele I, Jamshidi N, Fleming RMT, Palsson BØ (2009) Genome-Scale Reconstruction of Escherichia coli's Transcriptional and Translational Machinery: A Knowledge Base, Its Mathematical Formulation, and Its Functional Characterization. PLoS Comput Biol 5(3): e1000312. doi:10.1371/journal.pcbi.1000312"

iJL1678b (ECOLIme) Translation Overview



tRNA Metabolites

tRNA Biosynthesis

Schematic representation of the network components and reactions is shown. In addition to the macromolecular synthesis of RNA and proteins, rRNA and tRNA processing reactions were included in the reconstruction.

I: transcription;
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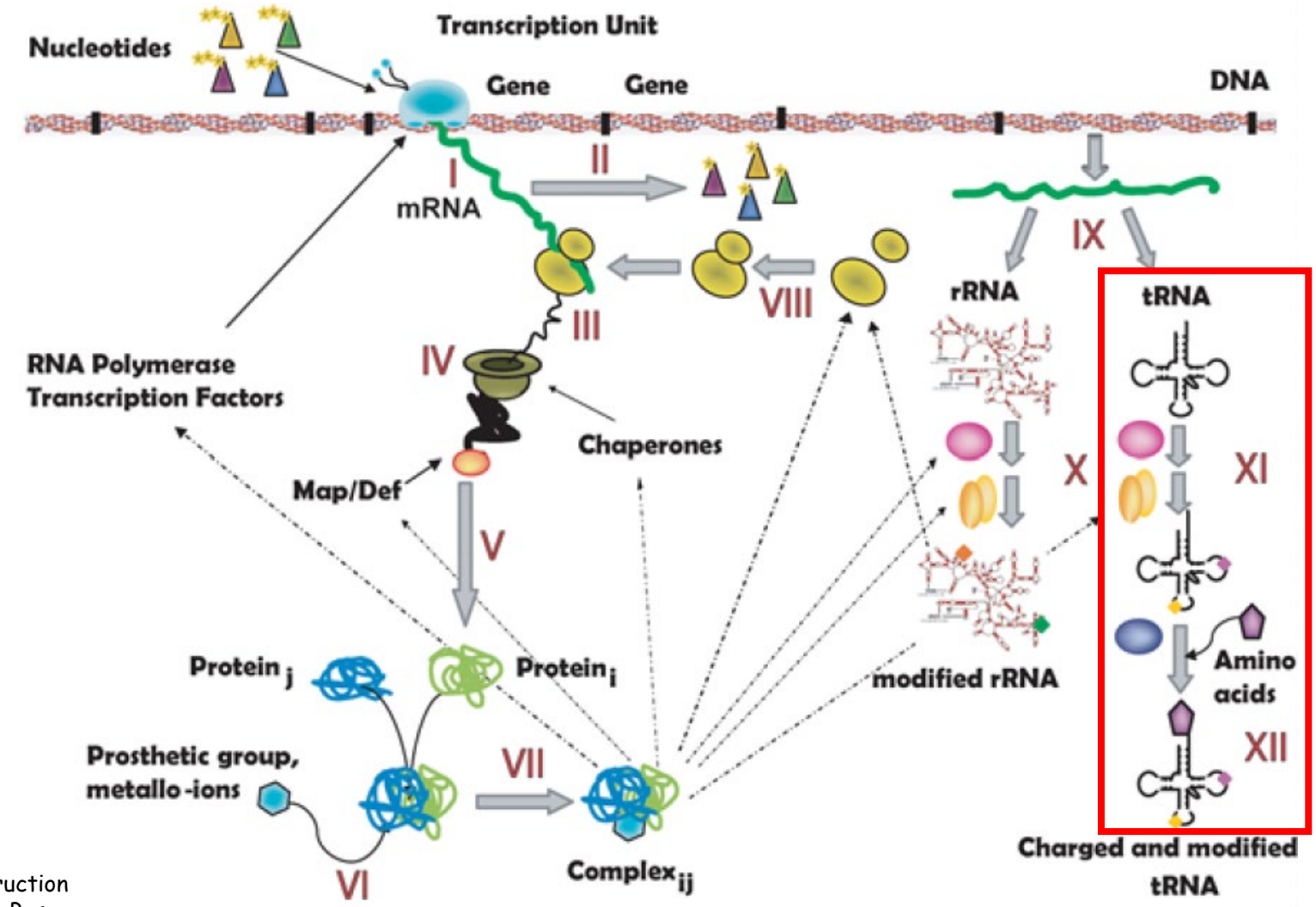
V: protein folding;
VI: metallo-ion binding;
VII: protein complex formation;
VIII: ribosome assembly;
IX: RNA processing;
X: rRNA modification;
XI: tRNA modification;
XII: tRNA charging.



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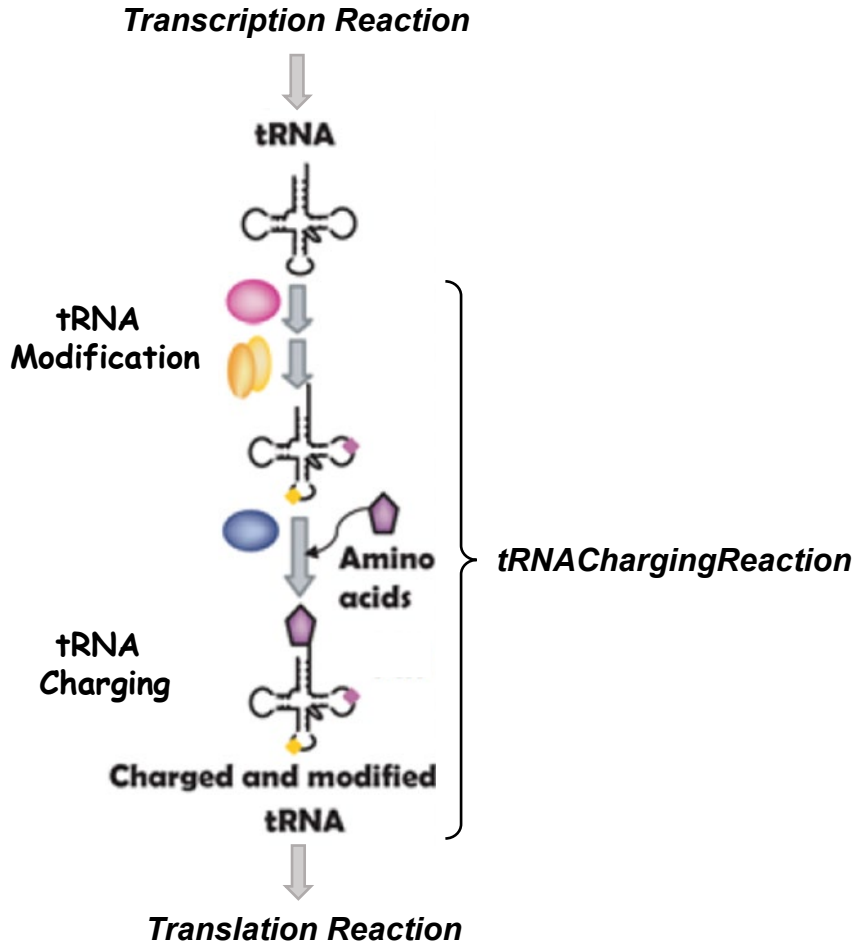
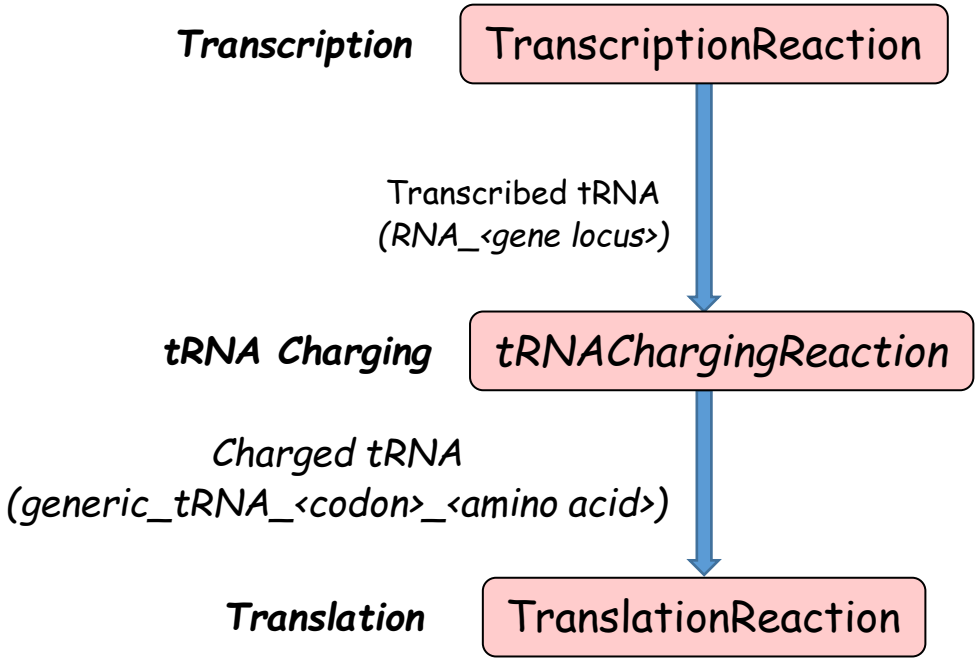
tRNA Bioynthesis in COBRAme Models

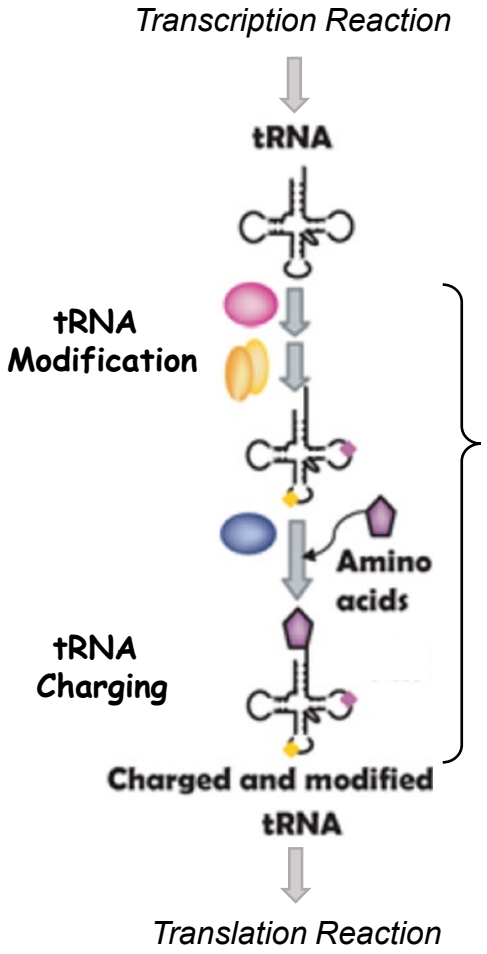


Original ME Model



COBRAme Models

iJL1678b (ECOLIme) tRNA Overview



tRNAChargingReaction

Side Products

Charged tRNA

Metabolites

GenerictRNA

tRNAChargingReaction

TranscribedGene Metabolites

GenericComponent

Complex Metabolites

tRNA Synthetase

Metabolites

Transcribed tRNA

tRNAs are processed to their active, mature forms by RNA cleavage and by modification of their bases. tRNAs are also subject to a wide variety of base modifications catalyzed by proteins

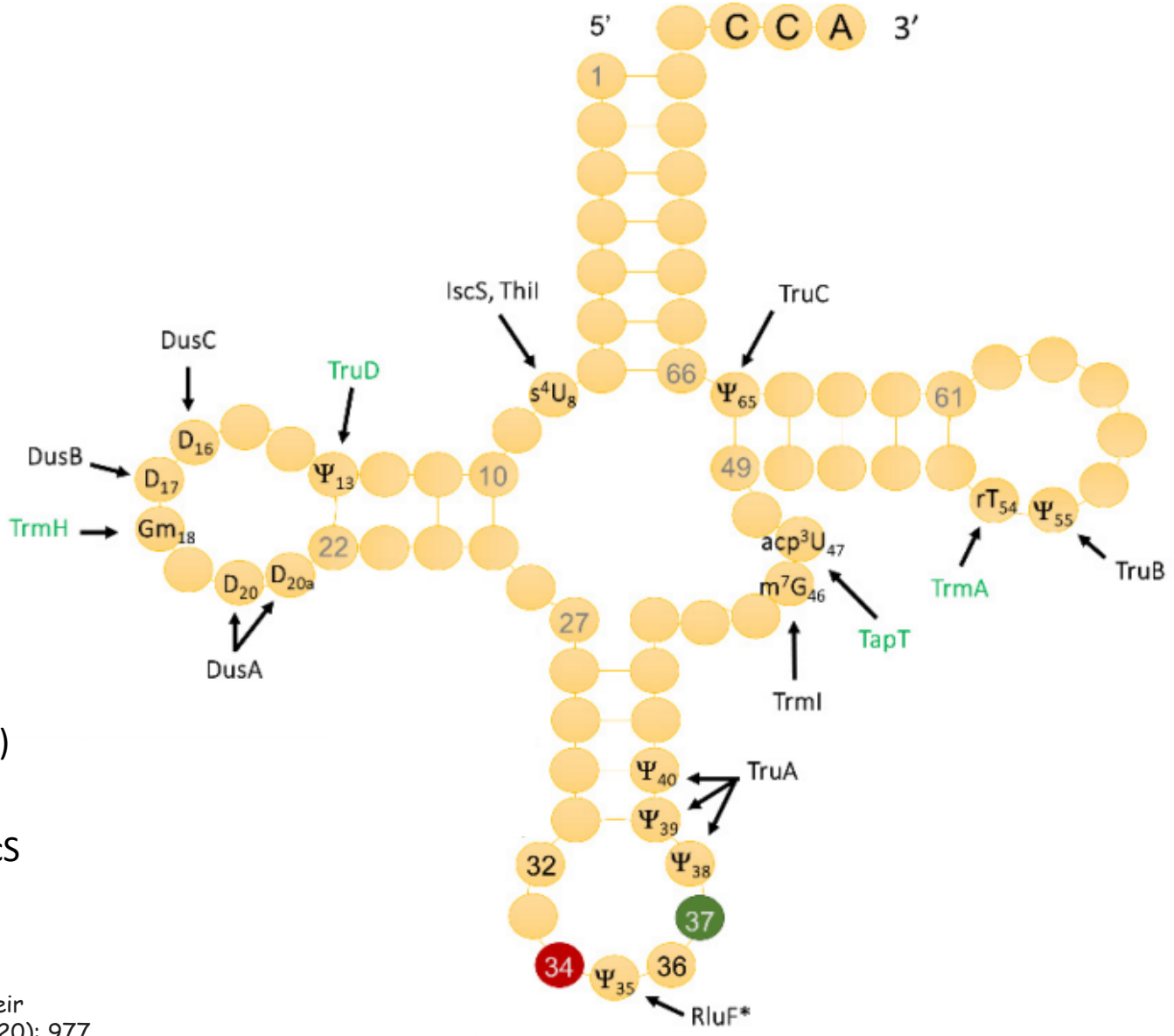
Transcribed tRNA Ligase

Supporting Metabolites

Escherichia coli tRNA Modifications and Corresponding Enzymes

Examples of glycine tRNA modifications:

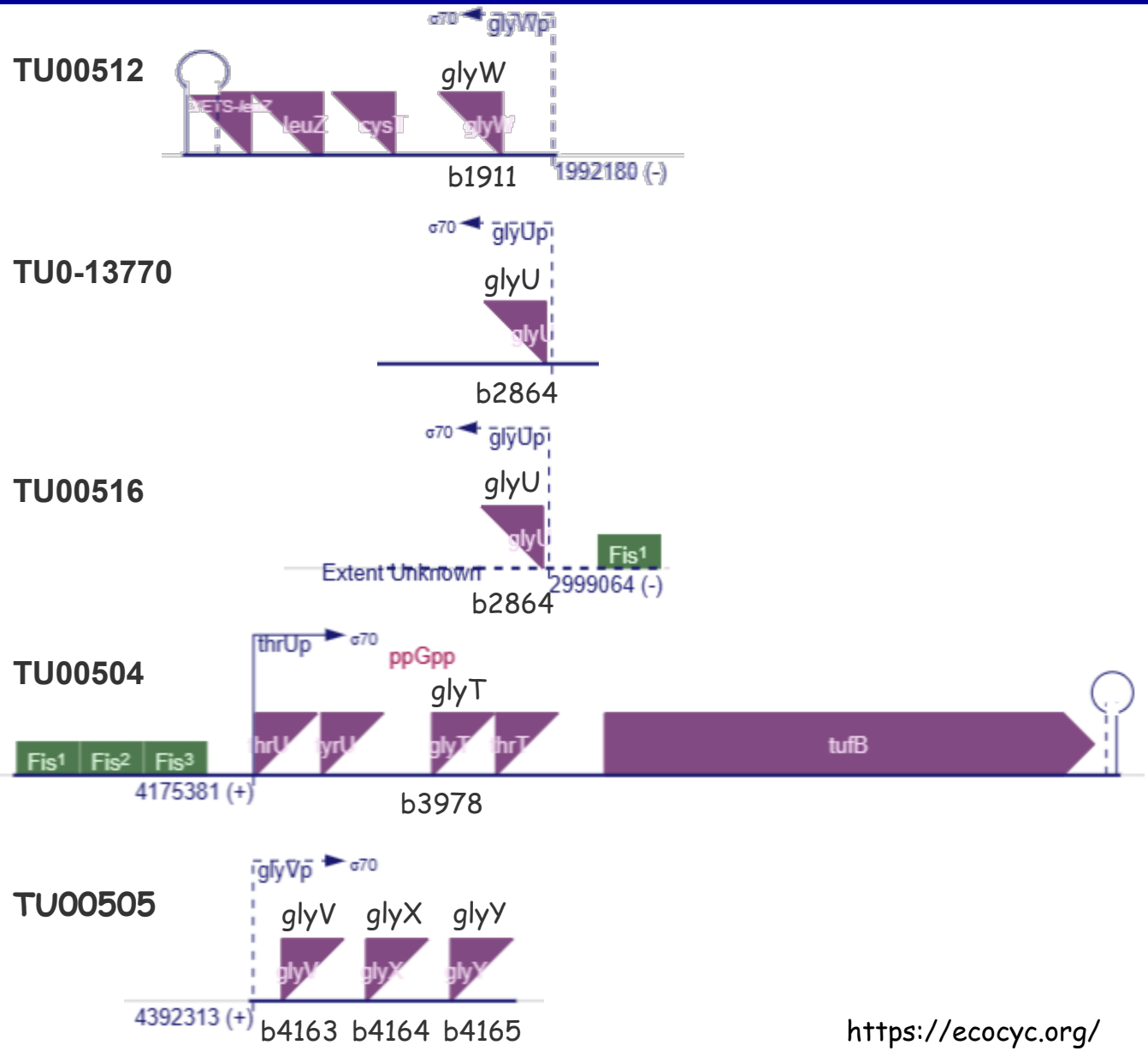
- 'ThiI_mono'- tRNA uridine 4-sulfurtransferase
- 'Gly_RS_tetra'- Glycine—tRNA ligase
- 'generic_Dus'- tRNA-dihydrouridine synthase A
- 'generic_Dus'- tRNA-dihydrouridine synthase B
- 'generic_Dus'- tRNA-dihydrouridine synthase C
- 'YggH_mono'- tRNA m7G46 methyltransferase (also TrmB)
- 'TruB_mono'- tRNA pseudouridine55 synthase
- 'IscS_mod_2:pydx5p_mod_1:SH'- cysteine desulfurase IscS
- 'TrmA_mono'- tRNA m5U54 methyltransferase



de Crecy-Lagard, Valerie, et al. "Survey and validation of tRNA modifications and their corresponding genes in Bacillus subtilis sp subtilis strain 168." *Biomolecules* 10.7 (2020): 977.

Example: Glycine tRNA Transcription Units

tRNA Gene	Glycine tRNA Transcription Reactions	Glycine RNA Metabolite
b1911	[transcription_TU00512_from_RpoD_mono]	RNA_b1911
b2864	[transcription_TU0_13770_from_RpoD_mono, transcription_TU00516_from_RpoD_mono]	RNA_b2864
b3978	[transcription_TU00504_from_RpoD_mono]	RNA_b3978
b4163	[transcription_TU00505_from_RpoD_mono]	RNA_b4163
b4164	[transcription_TU00505_from_RpoD_mono]	RNA_b4164
b4165	[transcription_TU00505_from_RpoD_mono]	RNA_b4165

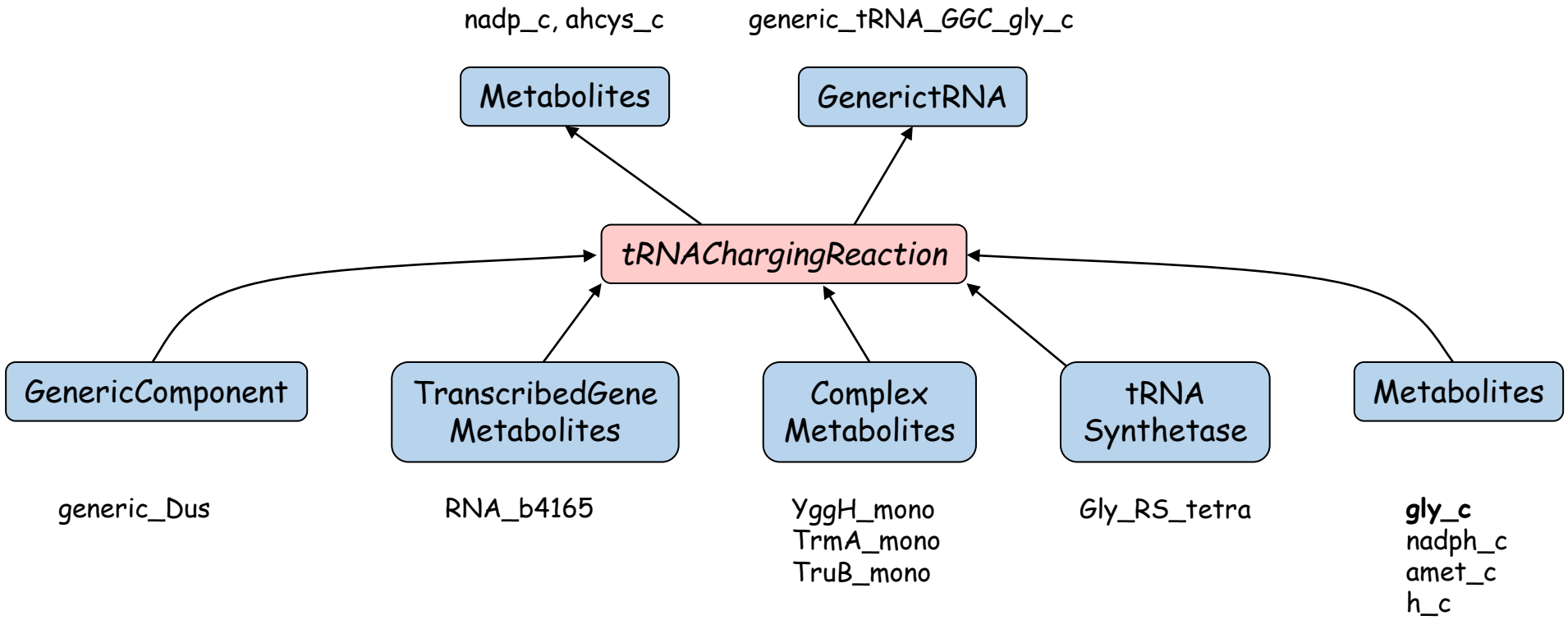


tRNA_glycine_pathways.ipynb

https://ecocyc.org/

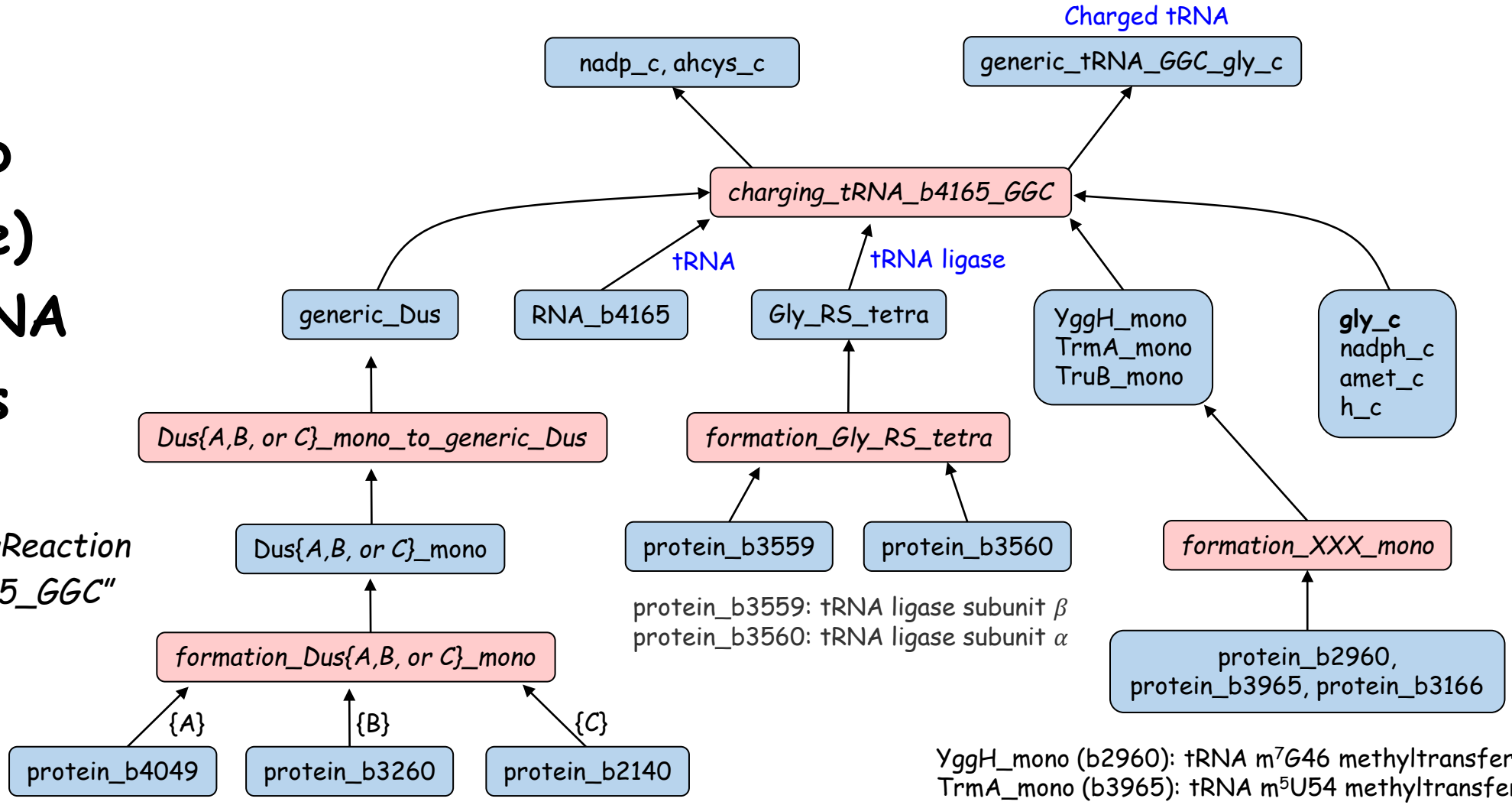
Example: iJL1678-ME ECOLIme Glycine tRNA

Example: tRNAChargingReaction – "charging_tRNA_b4165_GGC"



iJL1678b (ECOLIme) Glycine tRNA Pathways

Example: tRNAChargingReaction "charging_tRNA_b4165_GGC"



protein_b3559: tRNA ligase subunit β
protein_b3560: tRNA ligase subunit α

dusA (b4049): tRNA-dihydrouridine synthase A
dusB (b3260): tRNA-dihydrouridine synthase B
dusC (b2140): tRNA-dihydrouridine^{16} synthase

YggH_mono (b2960): tRNA m^{7}G46 methyltransferase
TrmA_mono (b3965): tRNA m^{5}U54 methyltransferase
TruB_mono (b3166): tRNA pseudouridine^{55} synthase

tRNA_glycine_pathways.ipynb

Key Glycine tRNA Reactions and Metabolites

tRNA Gene	Glycine tRNA Transcription Reactions	Glycine RNA Metabolite	Glycine Charging Reactions	Glycine Charged tRNA
b1911	[transcription_TU00512_from_RpoD_mono]	RNA_b1911	[charging_tRNA_b1911_GGC, charging_tRNA_b1911_GGU]	[generic_tRNA_GGC_gly_c, generic_tRNA_GGU_gly_c]
b2864	[transcription_TU0_13770_from_RpoD_mono transcription_TU00516_from_RpoD_mono]	RNA_b2864	[charging_tRNA_b2864_GGG]	[generic_tRNA_GGG_gly_c]
b3978	[transcription_TU00504_from_RpoD_mono]	RNA_b3978	[charging_tRNA_b3978_GGA]	[generic_tRNA_GGA_gly_c]
b4163	[transcription_TU00505_from_RpoD_mono]	RNA_b4163	[charging_tRNA_b4163_GGC, charging_tRNA_b4163_GGU]	[generic_tRNA_GGC_gly_c, generic_tRNA_GGU_gly_c]
b4164	[transcription_TU00505_from_RpoD_mono]	RNA_b4164	[charging_tRNA_b4164_GGC, charging_tRNA_b4164_GGU]	[generic_tRNA_GGC_gly_c, generic_tRNA_GGU_gly_c]
b4165	[transcription_TU00505_from_RpoD_mono]	RNA_b4165	[charging_tRNA_b4165_GGC, charging_tRNA_b4165_GGU]	[generic_tRNA_GGC_gly_c, generic_tRNA_GGU_gly_c]

tRNA_glycine_pathways.ipynb

Flux for Key Glycine tRNA Reactions

Transcription Reactions	Transcription Reactions Flux	Charging Reactions	Charging Reactions Flux
transcription_TU00512_from_RpoD_mono	0.000006	charging_tRNA_b1911_GGC	0.044674
transcription_TU0_13770_from_RpoD_mono	0.000005	**charging_tRNA_b1911_GGU**	**0.000000**
transcription_TU00516_from_RpoD_mono	**0.000000**	charging_tRNA_b2864_GGG	0.033864
transcription_TU00504_from_RpoD_mono	0.000003	charging_tRNA_b3978_GGA	0.022934
transcription_TU00505_from_RpoD_mono	0.000012	charging_tRNA_b4163_GGC	0.019518
transcription_TU00505_from_RpoD_mono	0.000012	charging_tRNA_b4163_GGU	0.066318
transcription_TU00505_from_RpoD_mono	0.000012	**charging_tRNA_b4164_GGC**	**0.000000**
		charging_tRNA_b4164_GGU	0.085836
		charging_tRNA_b4165_GGC	0.085836
		charging_tRNA_b4165_GGU	**0.000000**

tRNA_glycine_pathways.ipynb

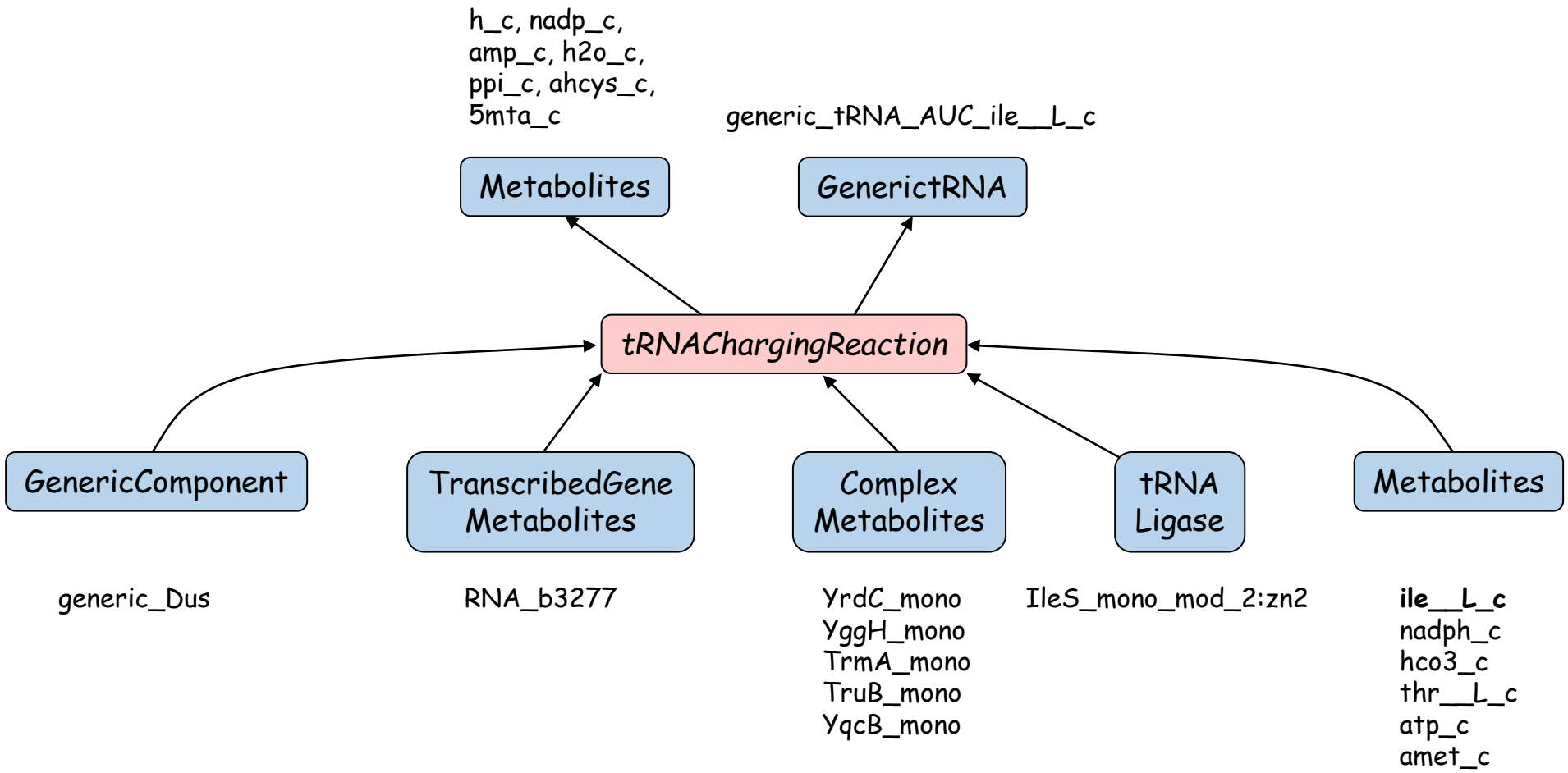
Flux of Support Glycine tRNA Reactions

Glycine Support Metabolites	Glycine Support Reactions	tRNA Modifications	Glycine Support Reactions Flux
Gly_RS_tetra	formation_Gly_RS_tetra	tRNA uridine 4-sulfurtransferase	**1.270606e-06**
TrmA_mono	formation_TrmA_mono	tRNA-dihydrouridine synthase A	**2.284456e-09**
generic_Dus	DusA_mono_to_generic_Dus	Glycine—tRNA ligase	0.000000e+00
generic_Dus	DusB_mono_to_generic_Dus	tRNA-dihydrouridine synthase B	0.000000e+00
generic_Dus	DusC_mono_to_generic_Dus	tRNA-dihydrouridine synthase C	**4.679154e-09**
YggH_mono	formation_YggH_mono	tRNA m7G46 methyltransferase	**1.635159e-09**
ThiI_mono	formation_ThiI_mono	tRNA pseudouridine55 synthase	**1.130280e-09**
TruB_mono	formation_TruB_mono	cysteine desulfurase IscS	**2.339411e-09**
IscS_mod_2:pydx5p_mod_1:SH	ICYSDS1_FWD_CPLX_dummy	tRNA m5U54 methyltransferase	**1.060364e-03**

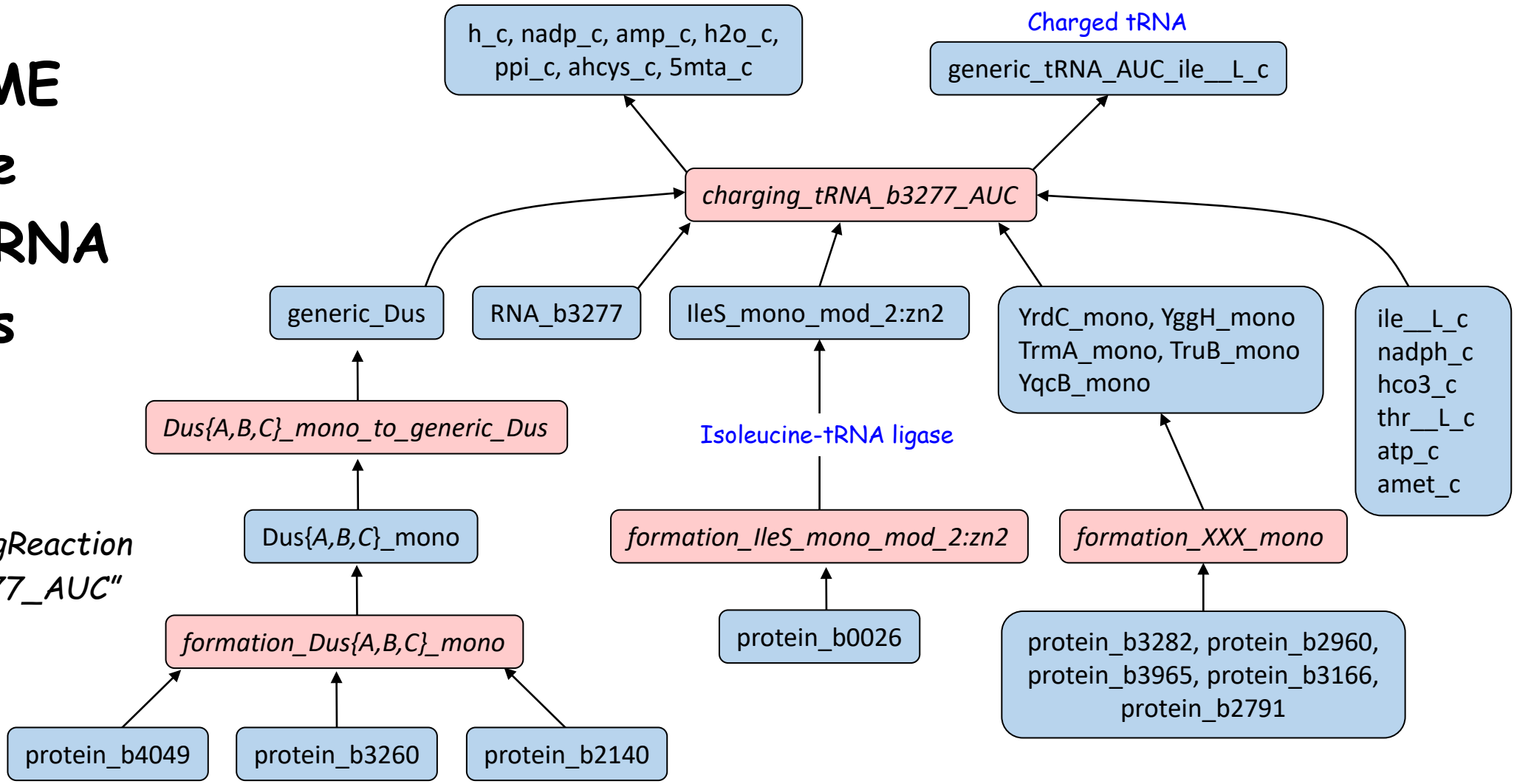
tRNA_glycine_pathways.ipynb

Example: iJL1678-ME ECOLIme Isoleucine tRNA

Example: tRNAChargingReaction – "charging_tRNA_b3277_AUC"



iJL1678-ME ECOLIme Isoleucine tRNA Pathways



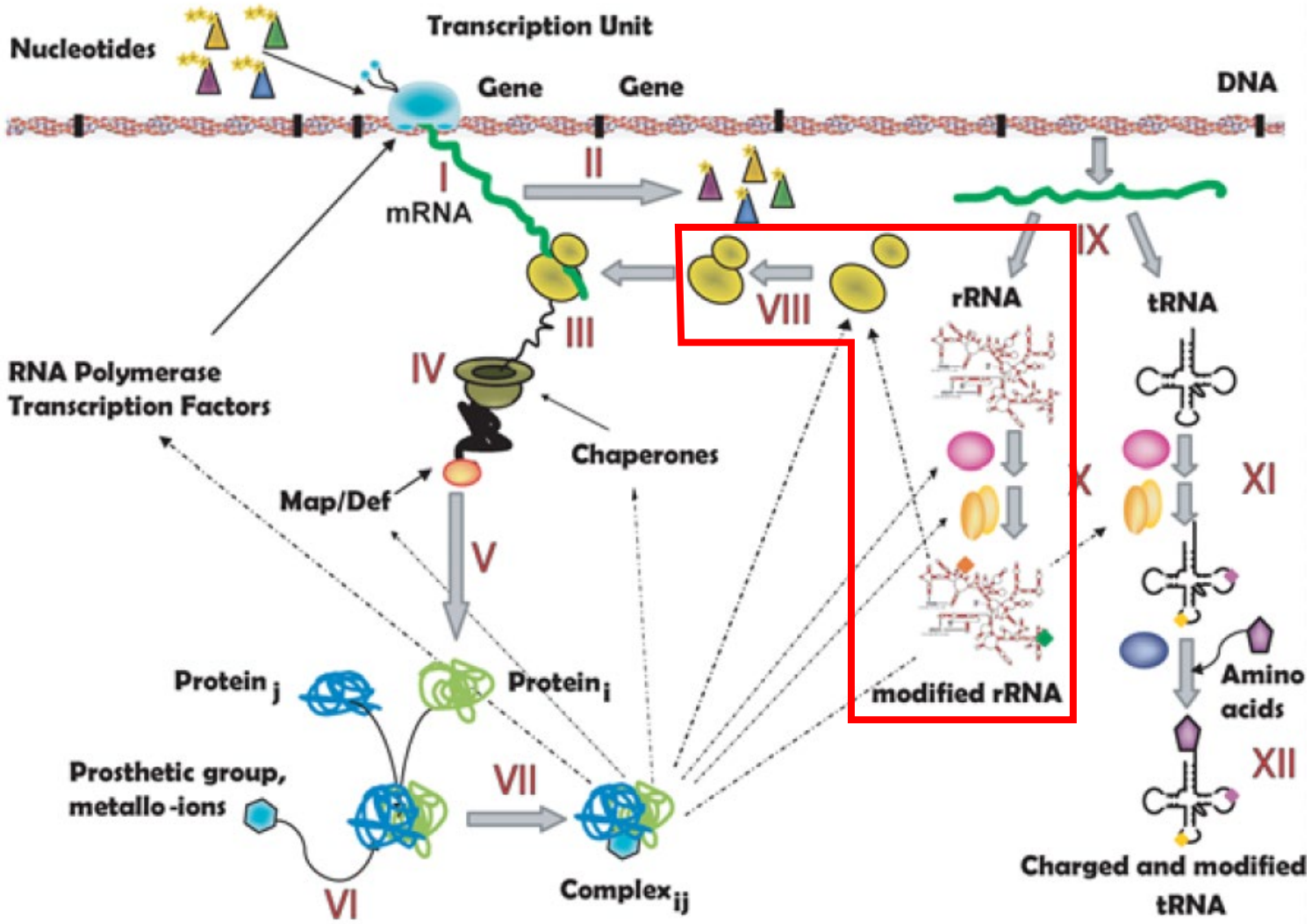
Example: tRNAChargingReaction "charging_tRNA_b3277_AUC"

tRNA.ipynb

Ribosome Assembly

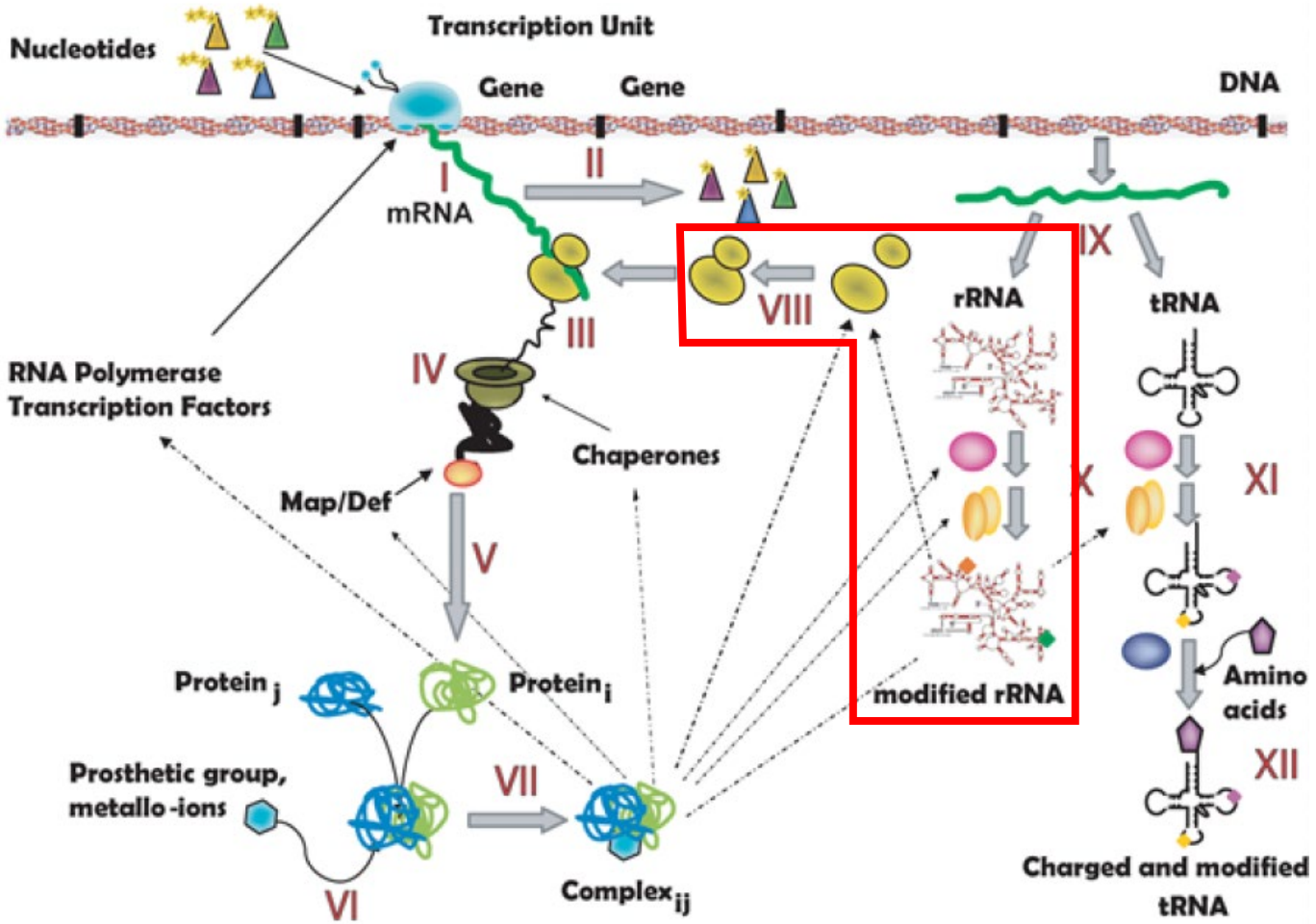
Schematic representation of the network components and reactions is shown. In addition to the macromolecular synthesis of RNA and proteins, rRNA and tRNA processing reactions were included in the reconstruction.

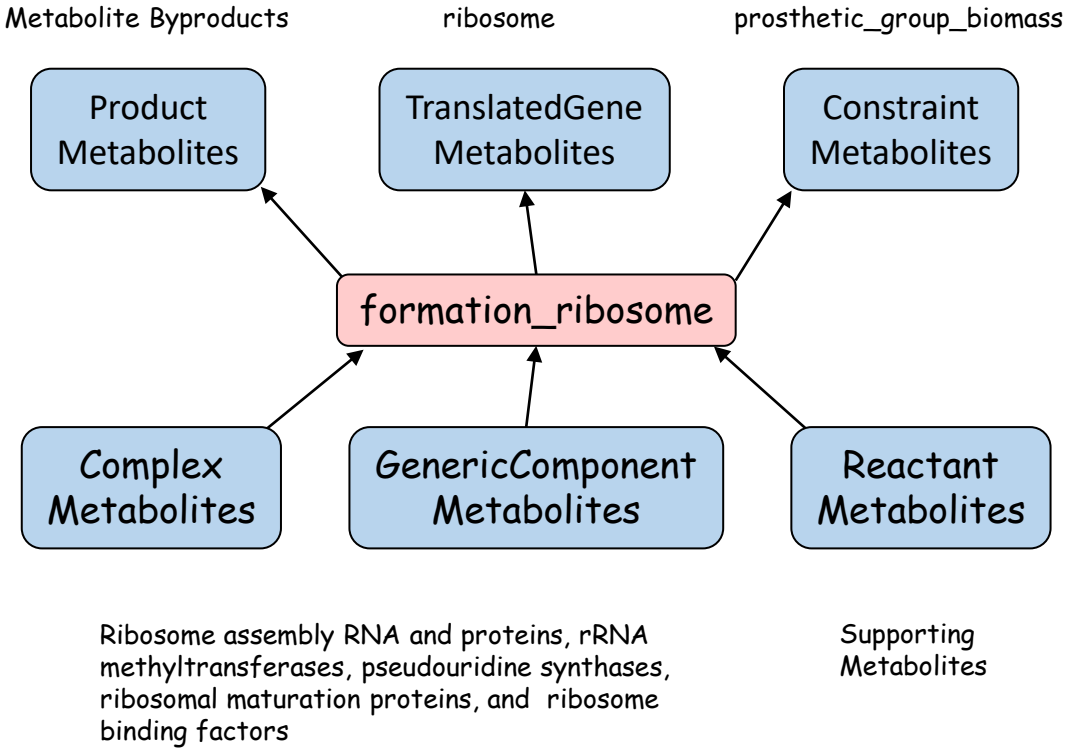
I: transcription;
II: mRNA degradation;
III: translation;
IV: protein maturation;
V: protein folding;
VI: metallo-ion binding;
VII: protein complex formation;
VIII: ribosome assembly;
IX: RNA processing;
X: rRNA modification;
XI: tRNA modification;
XII: tRNA charging.



Thiele I, Jamshidi N, Fleming RMT, Palsson BØ (2009) Genome-Scale Reconstru
of Escherichia coli's Transcriptional and Translational Machinery: A Knowledge Base,
Its Mathematical Formulation, and Its Functional Characterization. PLoS Comput
Biol 5(3): e1000312. doi:10.1371/journal.pcbi.1000312"

iJL1678b (ECOLIme) Ribosome Formation





Ribosome Assembly Genes: "formation_ribosome"

30S ribosomal subunit RNA
B3851

30S ribosomal subunit proteins
b0911, b0169, b3314, b3296, b3303, b4200, b3341, b3306, b3230, b3321, b3297, b3329, b3298, b3307, b3165, b2609, b3311, b4202, b3316, b0023, b3065

50S ribosomal subunit RNA
b3854, b3855

50S ribosomal subunit Protein
(b3984, b3317, b3320, b3319, b3308, b3305, b3986, b3985, b3986, b4203, b3985, b3983, b3986, b3231, b3310, b3301, b3313, b3294, b3304, b2606, b1716, (b3186, b3315, b3318, b3309, b2185, b3185, b3637, b3312, b3302, b3936, b1089, b3636, b3703, b1717, b3299

16S rRNA methyltransferases
b0051, b3289, b4371, b3465, b2946, b1835, b3740

23S rRNA methyltransferases
b1822, b4180, b0859, b2785, b3179, b0807, b3084, b0636, b0967, b0948, b2806, b2517

23S rRNA pseudouridine synthase
b0058, b1269, b1086, b2594, b1135, b4022

16S rRNA pseudouridine synthase
B2183

30S ribosomal maturation
b2566, b2608, b0436, b1480

30S ribosome binding factor
b3167

See Ribosome.ipynb

Ribosome Assembly Reactions: "formation_ribosome"

30S ribosomal subunit RNA

generic_16s_rRNAs,

30S ribosomal subunit proteins

RpsA_mono, RpsB_mono, RpsC_mono, RpsD_mono, RpsE_mono, RpsF_mono, RpsG_mono, RpsH_mono, RpsI_mono, RpsJ_mono, RpsK_mono, RpsL_mono, RpsM_mono, RpsN_mono, RpsO_mono, RpsP_mono, RpsQ_mono, RpsR_mono, RpsS_mono, RpsT_mono, RpsU_mono

50S ribosomal subunit RNA

generic_23s_rRNAs, generic_5s_rRNAs,

50S ribosomal subunit proteins

RplA_mono, RplB_mono, RplC_mono, RplD_mono, RplE_mono, RplF_mono, rpL7/12_mod_1:acetyl, RplJ_mono, RplI_mono, RplJ_mono, RplK_mono, RplL_mono, RplM_mono, RplN_mono, RplO_mono, RplP_mono, RplQ_mono, RplR_mono, RplS_mono, RplT_mono, RplU_mono, RplV_mono, RplW_mono, RplX_mono, RplY_mono, RpmA_mono, RpmB_mono, RpmC_mono, RpmD_mono, RpmE_mono, RpmF_mono, RpmG_mono, RpmH_mono, RpmI_mono, RpmJ_mono

16S rRNA methyltransferases

KsgA_mono, RsmB_mono, RsmC_mono, RsmD_mono, YggJ_dim (rsmE), RsmF_mono, RsmG_mono,

23S rRNA methyltransferases

RrmA_dim_mod_2:zn2, RlmB_dim, RumB_mono_mod_1:4fe4s, RumA_mono_mod_1:4fe4s, RrmJ_mono, RlmF_mono, RlmG_mono, RlmH_dim, RlmI_dim, RlmL_dim, RlmM_mono, RlmN_mono_mod_1:4fe4s,

23S rRNA pseudouridine synthase

RluA_mono, RluB_mono, RluC_mono, RluD_mono_mod_1:mg2, YmfC_mono, YjbC_mono (rluF),

16S rRNA pseudouridine synthase

RsuA_mono,

30S ribosomal maturation

Era_dim, RimM_mono, Tig_mono, Sra_mono,

30S ribosome binding factor

RbfA_mono,

Metabolites

gtp_c, amet_c, nadh_c, h2o_c, mg2_c

See Ribosome.ipynb

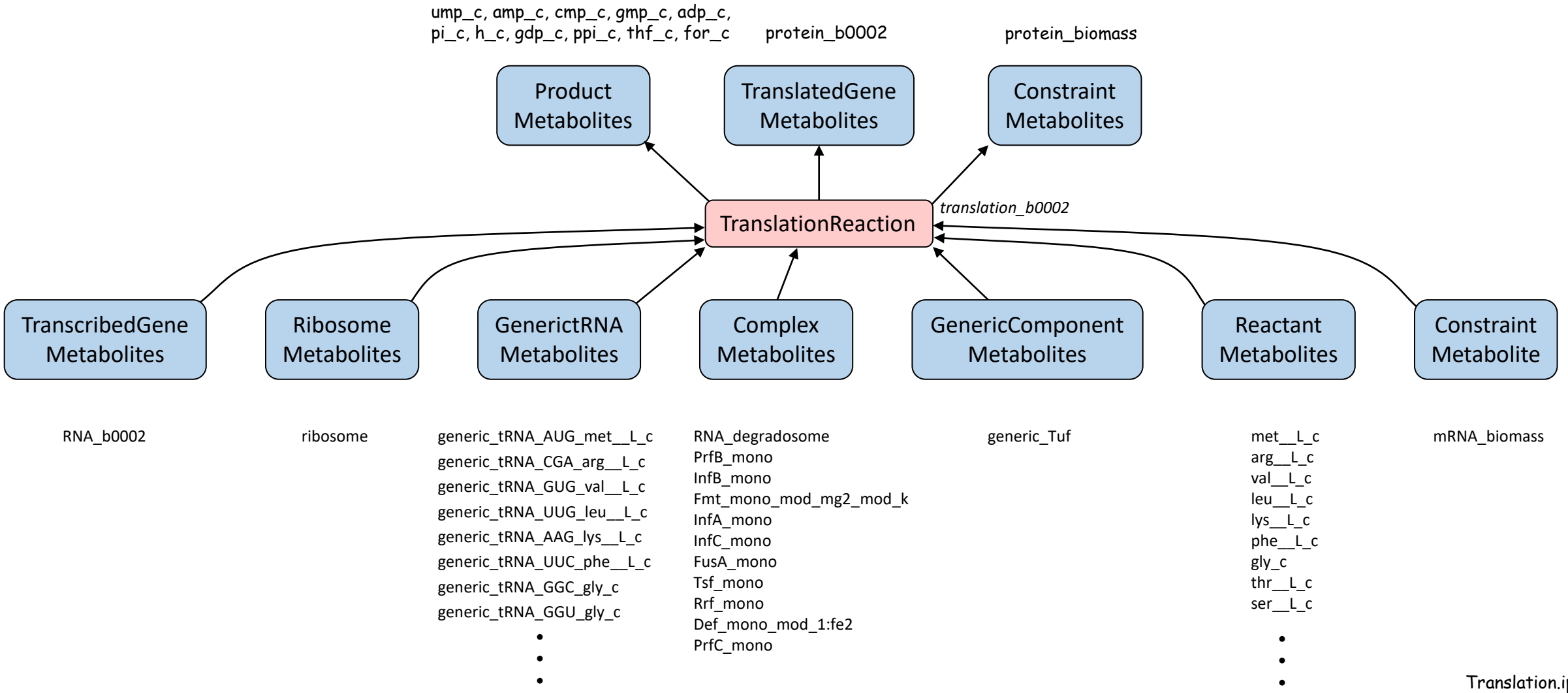
Ribosome Reaction (formation_reaction)

4.27350427350427e-6*mu Era_dim + 8.54700854700855e-6*mu KsgA_mono + 4.27350427350427e-6*mu RbfA_mono + 4.27350427350427e-6*mu RimM_mono + 4.27350427350427e-6*mu RlmB_dim + 4.27350427350427e-6*mu RlmF_mono + 4.27350427350427e-6*mu RlmG_mono + 4.27350427350427e-6*mu RlmH_dim + 4.27350427350427e-6*mu RlmI_dim + 8.54700854700855e-6*mu RlmL_dim + 4.27350427350427e-6*mu RlmM_mono + 4.27350427350427e-6*mu RlmN_mono_mod_1:4fe4s + 4.27350427350427e-6*mu RluA_mono + 4.27350427350427e-6*mu RluB_mono + 1.28205128205128e-5*mu RluC_mono + 1.28205128205128e-5*mu RluD_mono_mod_1:mg2 + RplA_mono + RplB_mono + RplC_mono + RplD_mono + RplE_mono + RplF_mono + RplI_mono + RplJ_mono + RplK_mono + RplM_mono + RplN_mono + RplO_mono + RplP_mono + RplQ_mono + RplR_mono + RplS_mono + RplT_mono + RplU_mono + RplV_mono + RplW_mono + RplX_mono + RplY_mono + RpmA_mono + RpmB_mono + RpmC_mono + RpmD_mono + RpmE_mono + RpmF_mono + RpmG_mono + RpmH_mono + RpmI_mono + RpmJ_mono + RpsA_mono + RpsB_mono + RpsC_mono + RpsD_mono + RpsE_mono + RpsF_mono + RpsG_mono + RpsH_mono + RpsI_mono + RpsJ_mono + RpsK_mono + RpsL_mono + RpsM_mono + RpsN_mono + RpsO_mono + RpsP_mono + RpsQ_mono + RpsR_mono + RpsS_mono + RpsT_mono + RpsU_mono + 4.27350427350427e-6*mu RrmA_dim_mod_2:zn2 + 4.27350427350427e-6*mu RrmJ_mono + 4.27350427350427e-6*mu RsmB_mono + 4.27350427350427e-6*mu RsmC_mono + 4.27350427350427e-6*mu RsmD_mono + 4.27350427350427e-6*mu RsmF_mono + 4.27350427350427e-6*mu RsmG_mono + 4.27350427350427e-6*mu RsuA_mono + 4.27350427350427e-6*mu RumA_mono_mod_1:4fe4s + 4.27350427350427e-6*mu RumB_mono_mod_1:4fe4s + Sra_mono + Tig_mono + 4.27350427350427e-6*mu YggJ_dim + 4.27350427350427e-6*mu YjbC_mono + 4.27350427350427e-6*mu YmfC_mono + 27.0 amet_c + 4.27350427350427e-6*mu generic_16Sm4Cm1402 + generic_16s_rRNAs + generic_23s_rRNAs + generic_5s_rRNAs + 3.0 gtp_c + 2.0 h2o_c + 171.0 mg2_c + nadh_c + 2.0 rpL7/12_mod_1:acetyl

--> 27.0 ahcys_c + 2.0 gdp_c + 28.0 h_c + nad_c + 2.0 pi_c + 4.5368885400000005 prosthetic_group_biomass + ribosome

See Ribosome.ipynb

Example: iJL1678b (ECOLIme) RNA_b0002 Translation



Translation.ipynb

Translation Supporting Metabolites

- RNA_degradosome: Degradation of mRNA
- InfA_mono: **translation initiation factor IF-1**
- InfB_mono: **translation initiation factor IF-2β'**
- InfC_mono: **translation initiation factor IF-3**
- PrfB_mono: **peptide chain release factor RF2**
- PrfC_mono: **peptide chain release factor RF3**
- Fmt_mono_mod_mg2_mod_k: **10-formyltetrahydrofolate:L-methionyl-tRNA^{fMet} *N*-formyltransferase**
- FusA_mono: **elongation factor *G***
- Tsf_mono: **protein chain elongation factor EF-Ts**
- Rrf_mono: **ribosome-recycling factor**
- Def_mono_mod_1:fe2: **peptide deformylase**

ecocyc.org

Lesson Outline

- Metabolism (M) vs. Metabolism & Expression (ME) Models
- COBRAme
  - Working Environment (Docker)
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  - Macromolecular Coupling
  - Reaction Lumping
- ECOLIme
  - Macromolecular Reactions
  - Transcription
  - Translation
  - COBRAme Execution
  - ECOLIme Operation

Executing the iJL1678b model

```
from __future__ import print_function, division, absolute_import
# python imports
import re
from os.path import join
from collections import defaultdict
import pickle
import pandas as pd
# third party imports
import tabulate
import cobra
# ECOLIme
import ecolime
from ecolime import (transcription, translation, flat_files, generics, formulas, compartments)
# COBRAme
import cobrame
from cobrame.util import building, mu, me_model_interface
```

```
# Load the iJL1678b model
with open('iJL1678b.pickle', 'rb') as f:
    me = pickle.load(f)
# Methods
def solve_me_model(me, max_mu, precision=1e-6, min_mu=0, using_soplex=False,
                   compiled_expressions=None):
    if using_soplex:
        from cobrame.solve.algorithms import binary_search
        binary_search(me, min_mu=min_mu, max_mu=max_mu, debug=True, mu_accuracy=precision,
                      compiled_expressions=compiled_expressions)
    else:
        from qminospy.me1 import ME_NLP1
        # The object containing solveME methods--composite that uses a ME model object
        me_nlp = ME_NLP1(me, growth_key='mu')
        # Use bisection for now (until the NLP formulation is worked out)
        muopt, hs, xopt, cache = me_nlp.bisectmu(precision=precision, mumax=max_mu)
        me.solution.f = me.solution.x_dict['biomass_dilution']

solve_me_model(me, 1., min_mu = .1, precision=1e-2, using_soplex=False)
```

solve_demo.ipynb

solve_demo.ipynb

```
import pickle
import cobrame
from cobrame.io.json import load_reduced_json_me_model, load_json_me_model
```

```
with open('./me_models/iJL1678b.pickle', 'rb') as f:
    me = pickle.load(f)
```

```
def solve_me_model(me, max_mu, precision=1e-6, min_mu=0, using_soplex=True,
                   compiled_expressions=None):
    if using_soplex:
        from cobrame.solve.algorithms import binary_search
        binary_search(me, min_mu=min_mu, max_mu=max_mu, debug=True, mu_accuracy=precision,
                      compiled_expressions=compiled_expressions)
    else:
        from qminospy.me1 import ME_NLP1
        # The object containing solveME methods--composite that uses a ME model object
        me_nlp = ME_NLP1(me, growth_key='mu')
        # Use bisection for now (until the NLP formulation is worked out)
        muopt, hs, xopt, cache = me_nlp.bisectmu(precision=precision, mumax=max_mu)
        me.solution.f = me.solution.x_dict['biomass_dilution']

def show_escher_map(me, solution=None):
    import escher
    view = escher.Builder("iJO1366.Central metabolism")
    view.reaction_data = me.get_metabolic_flux(solution=solution)
    return view
```

```
solve_me_model(me, 1., min_mu = .1, precision=1e-2, using_soplex=False)
```

```
# Display metabolic flux on escher map
show_escher_map(me).display_in_notebook()
```

Solving a COBRAme Model

```
import pickle
import cobrame
from cobrame.io.json import load_reduced_json_me_model, load_json_me_model
```

```
with open('./me_models/iJL1678b.pickle', 'rb') as f:
    me = pickle.load(f)
```

```
def solve_me_model(me, max_mu, precision=1e-6, min_mu=0, using_soplex=True,
                   compiled_expressions=None):
    if using_soplex:
        from cobrame.solve.algorithms import binary_search
        binary_search(me, min_mu=min_mu, max_mu=max_mu, debug=True, mu_accuracy=precision,
                      compiled_expressions=compiled_expressions)
    else:
        from qminospy.me1 import ME_NLP1
        # The object containing solveME methods--composite that uses a ME model object
        me_nlp = ME_NLP1(me, growth_key='mu')
        # Use bisection for now (until the NLP formulation is worked out)
        muopt, hs, xopt, cache = me_nlp.bisectmu(precision=precision, mumax=max_mu)
        me.solution.f = me.solution.x_dict['biomass_dilution']

def show_escher_map(me, solution=None):
    import escher
    view = escher.Builder("iJO1366.Central metabolism")
    view.reaction_data = me.get_metabolic_flux(solution=solution)
    return view
```

solve_demo.ipynb

```
solve_me_model(me, 1., min_mu = .1, precision=1e-2, using_soplex=False)
```

```
iter    muopt           a       b       mu1             stat1
Finished compiling expressions in 67.532865 seconds
Finished substituting S,lb,ub in 4.933790 seconds
Finished makeME_LP in 0.852235 seconds
Getting MINOS parameters from ME_NLP...
1 0.5 0.5 1.0 0.5 optimal
Finished substituting S,lb,ub in 5.459582 seconds
Finished makeME_LP in 0.926348 seconds
Getting MINOS parameters from ME_NLP...
2 0.75 0.75 1.0 0.75 optimal
Finished substituting S,lb,ub in 5.111826 seconds
Finished makeME_LP in 0.894545 seconds
Getting MINOS parameters from ME_NLP...
3 0.75 0.75 0.875 0.875 1
Finished substituting S,lb,ub in 4.831290 seconds
Finished makeME_LP in 0.847434 seconds
Getting MINOS parameters from ME_NLP...
4 0.8125 0.8125 0.875 0.8125 optimal
Finished substituting S,lb,ub in 4.792310 seconds
Finished makeME_LP in 0.844745 seconds
Getting MINOS parameters from ME_NLP...
5 0.8125 0.8125 0.84375 0.84375 1
Finished substituting S,lb,ub in 4.794153 seconds
Finished makeME_LP in 0.848020 seconds
Getting MINOS parameters from ME_NLP...
6 0.828125 0.828125 0.84375 0.828125 optimal
Finished substituting S,lb,ub in 5.176051 seconds
Finished makeME_LP in 0.852741 seconds
Getting MINOS parameters from ME_NLP...
7 0.828125 0.828125 0.8359375 0.8359375 1
Bisection done in 242.881 seconds
```

Escher Plot of COBRAme Simulation

```
import pickle
import cobrame
from cobrame.io.json import load_reduced_json_me_model, load_json_me_model
```

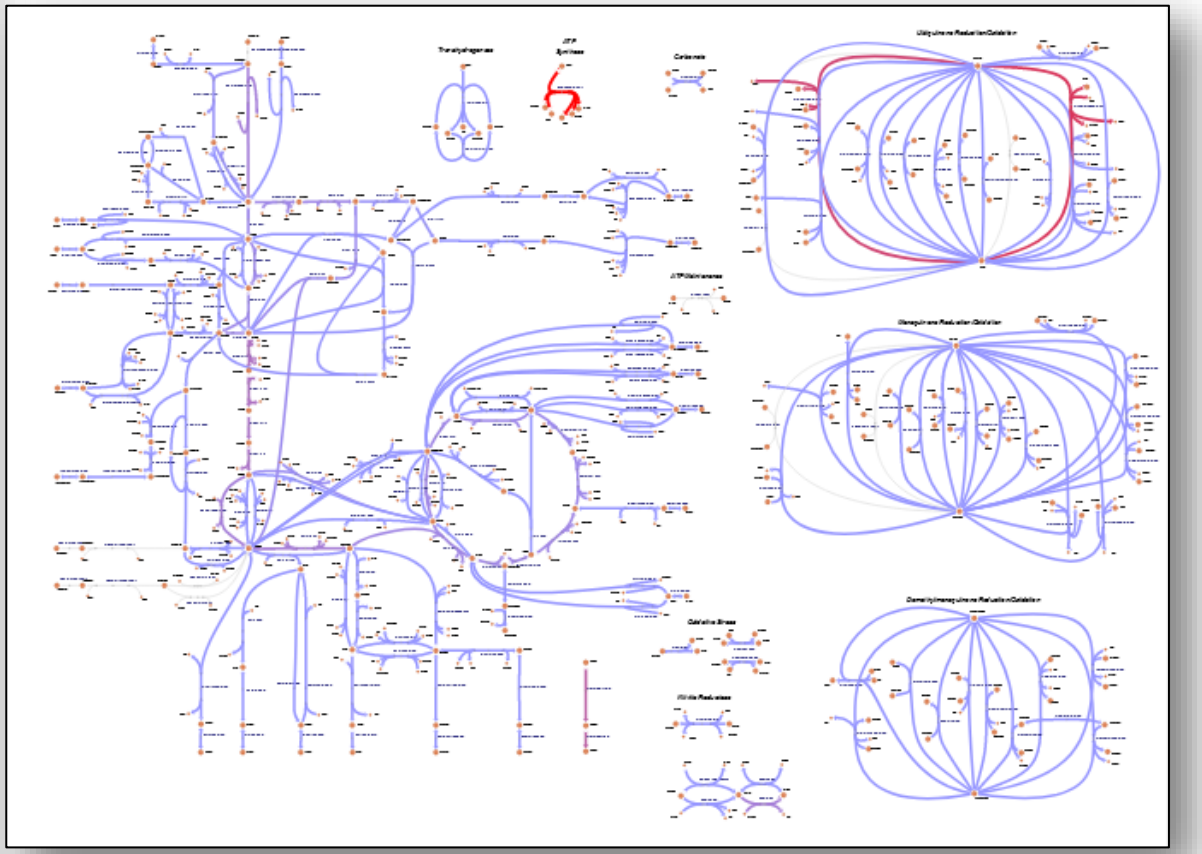
```
with open('./me_models/iJL1678b.pickle', 'rb') as f:
    me = pickle.load(f)
```

```
def solve_me_model(me, max_mu, precision=1e-6, min_mu=0, using_soplex=True,
                   compiled_expressions=None):
    if using_soplex:
        from cobrame.solve.algorithms import binary_search
        binary_search(me, min_mu=min_mu, max_mu=max_mu, debug=True, mu_accuracy=precision,
                      compiled_expressions=compiled_expressions)
    else:
        from qminospy.me1 import ME_NLP1
        # The object containing solveME methods--composite that uses a ME model object
        me_nlp = ME_NLP1(me, growth_key='mu')
        # Use bisection for now (until the NLP formulation is worked out)
        muopt, hs, xopt, cache = me_nlp.bisectmu(precision=precision, mumax=max_mu)
        me.solution.f = me.solution.x_dict['biomass_dilution']

def show_escher_map(me, solution=None):
    import escher
    view = escher.Builder("iJO1366.Central metabolism")
    view.reaction_data = me.get_metabolic_flux(solution=solution)
    return view
```

```
solve_me_model(me, 1., min_mu = .1, precision=1e-2, using_soplex=False)
```

```
# Display metabolic flux on escher map
show_escher_map(me).display_in_notebook()
```



solve_demo.ipynb

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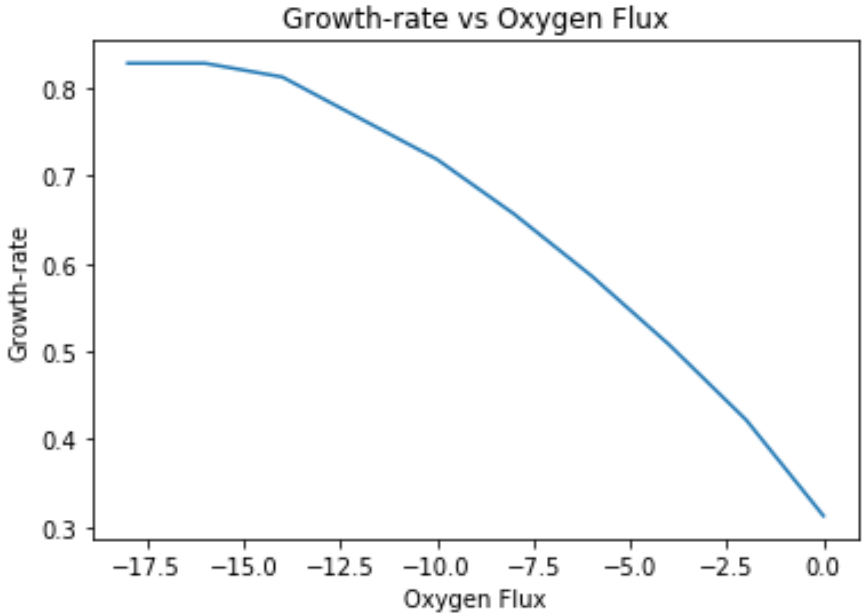
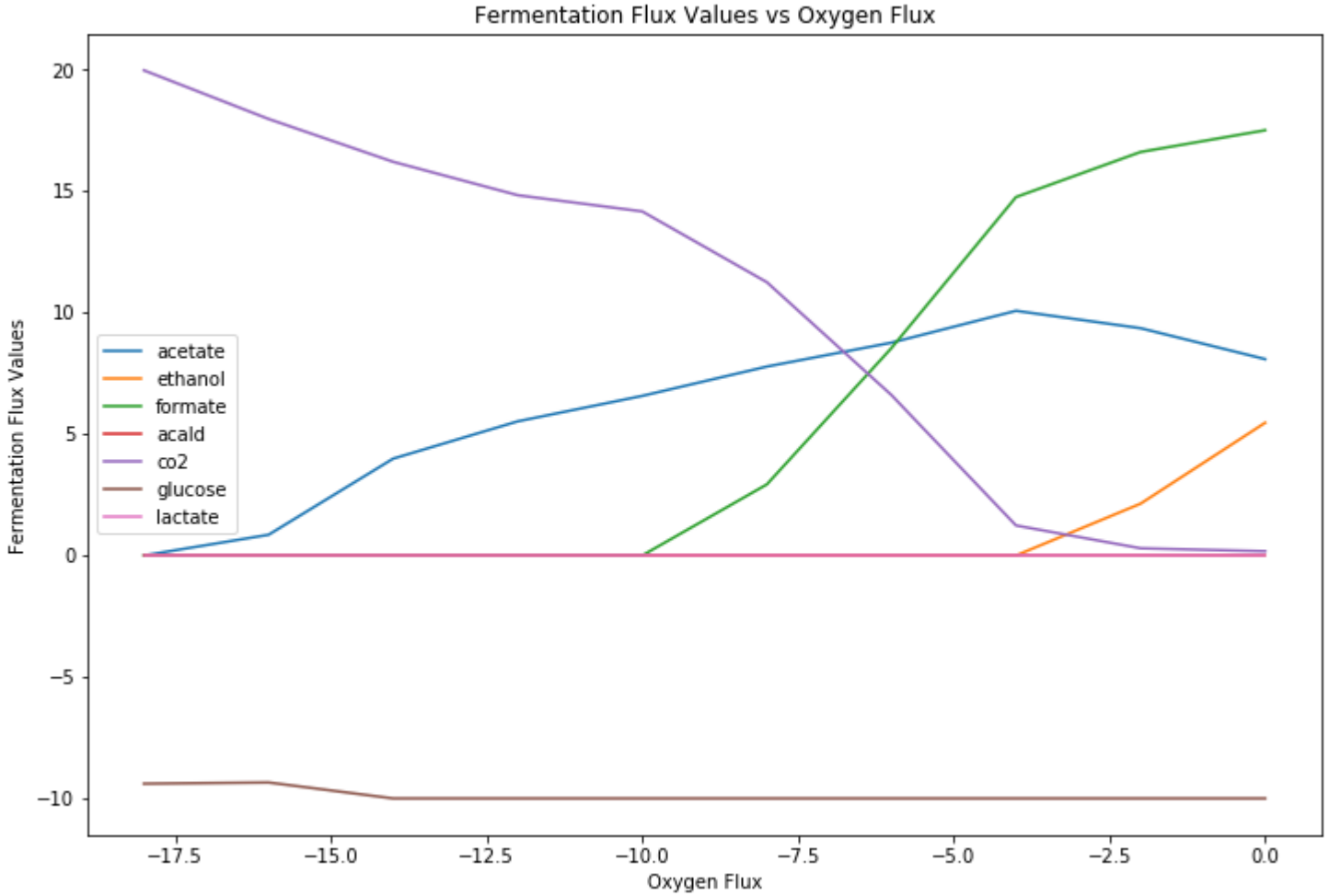
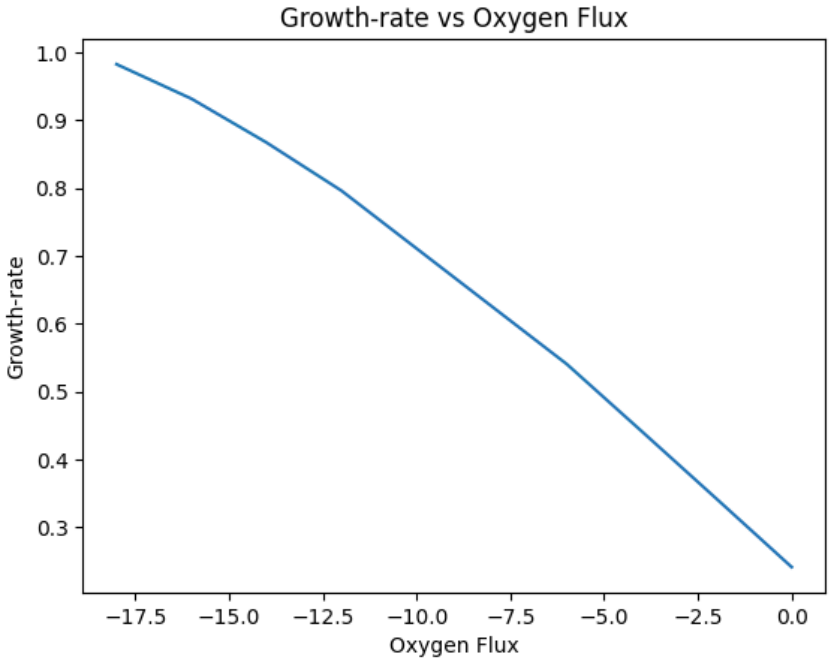
iJL1678b Fermentation

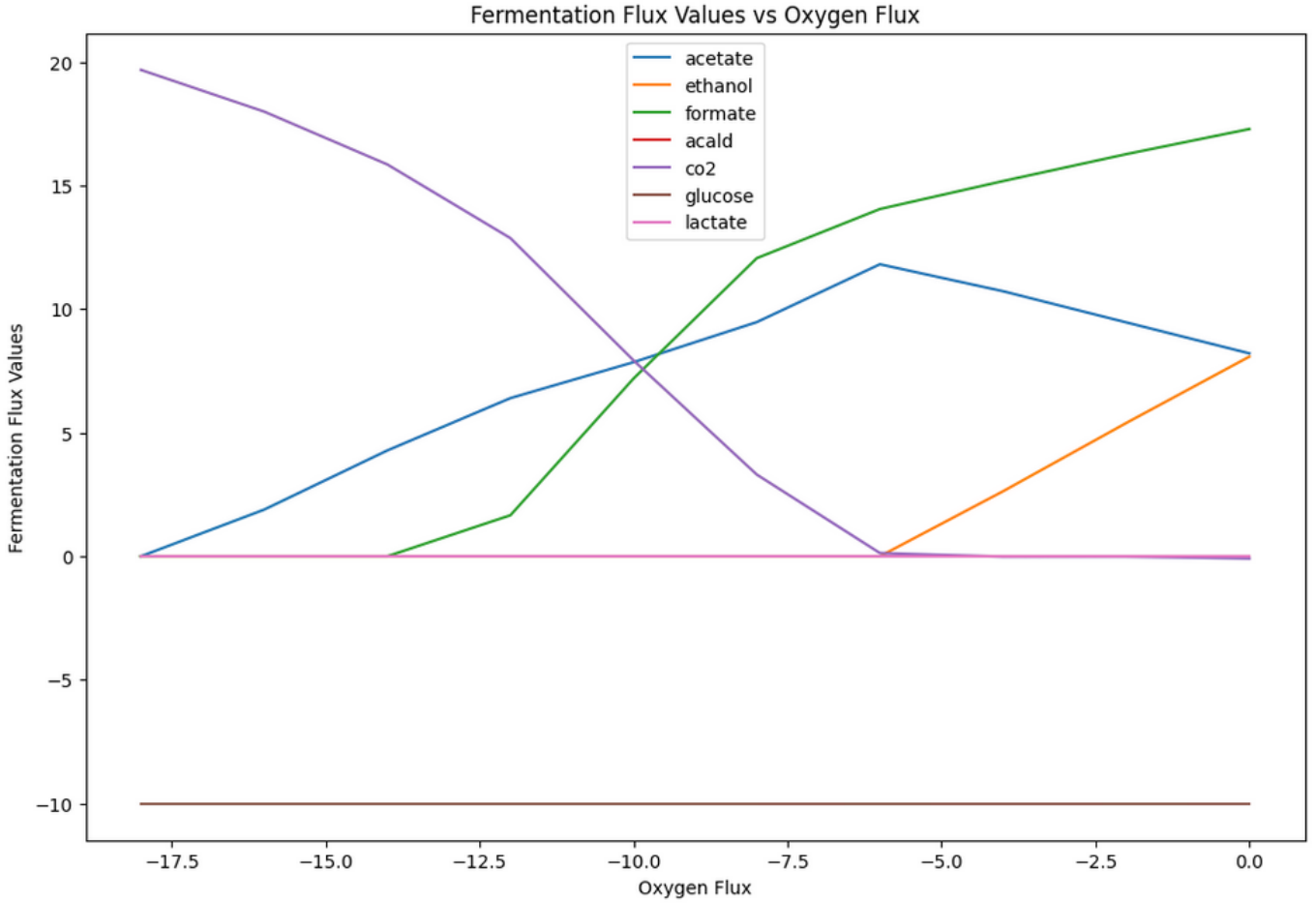




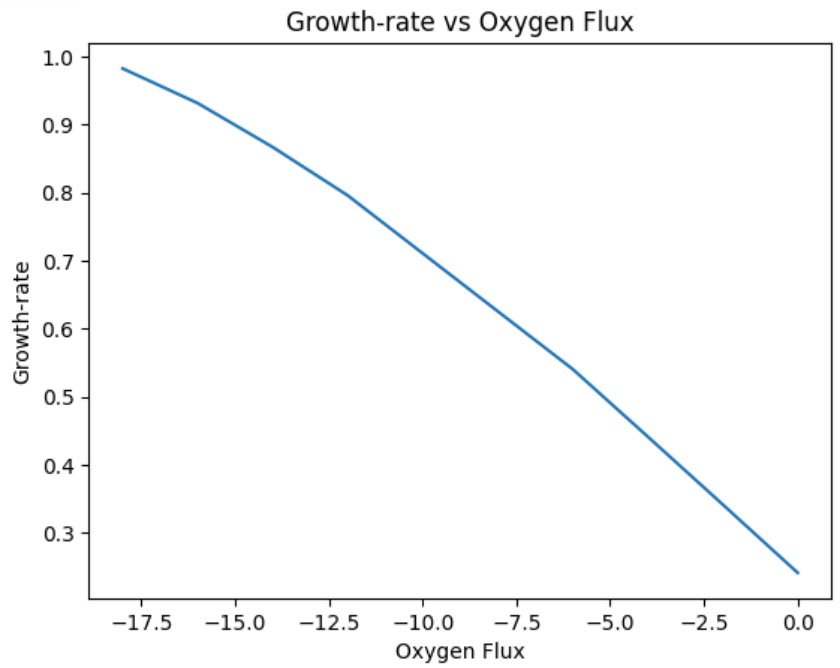
me.reactions.get_by_id('EX_glc__D_e').lower_bound = -10

iJO1366 Fermentation

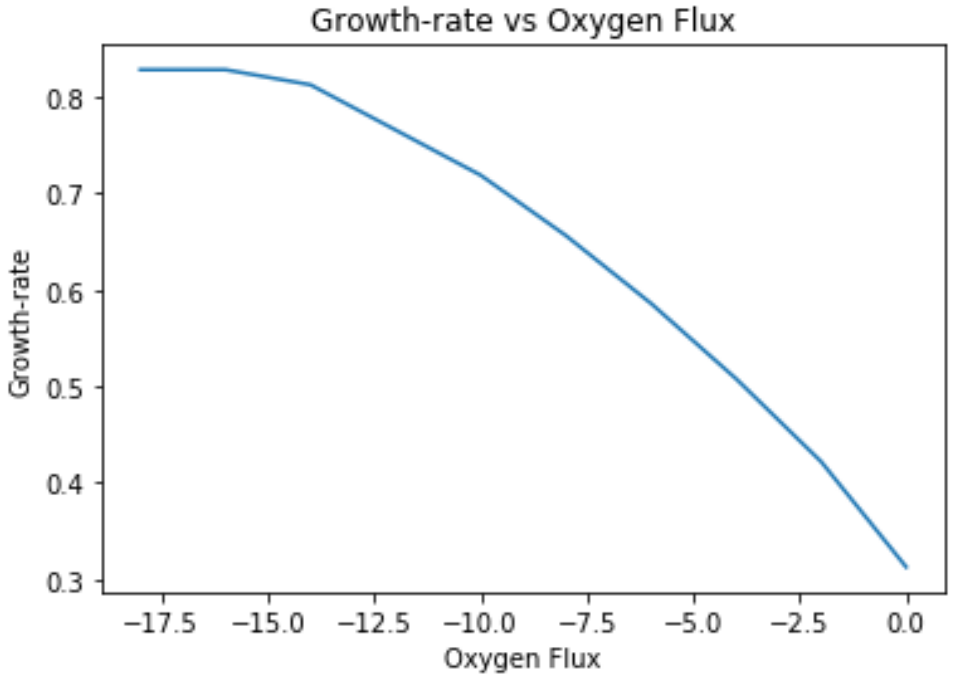




iJO1366 vs iJL1678b Growth-rate



iJO1366

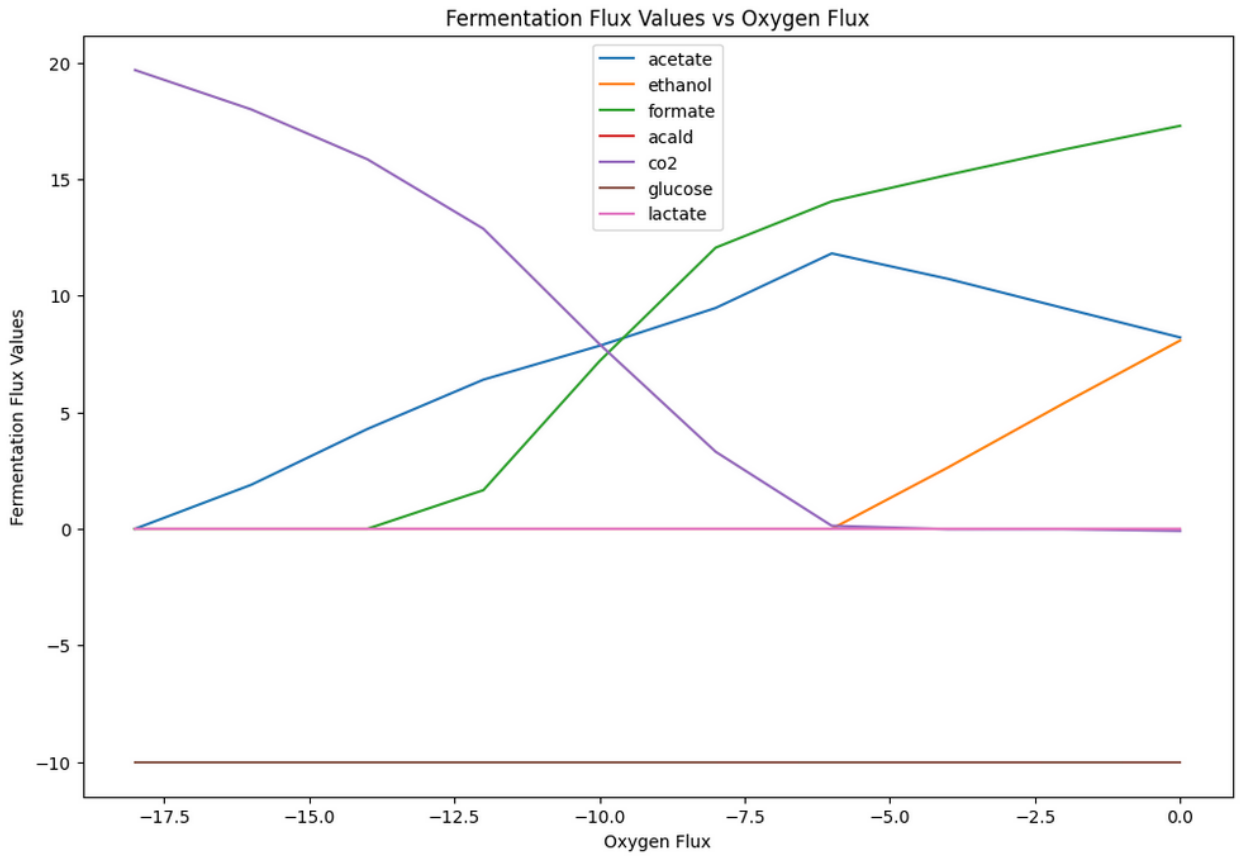


iJL1678b

Oxygen_Impact_iJO13166.ipynb

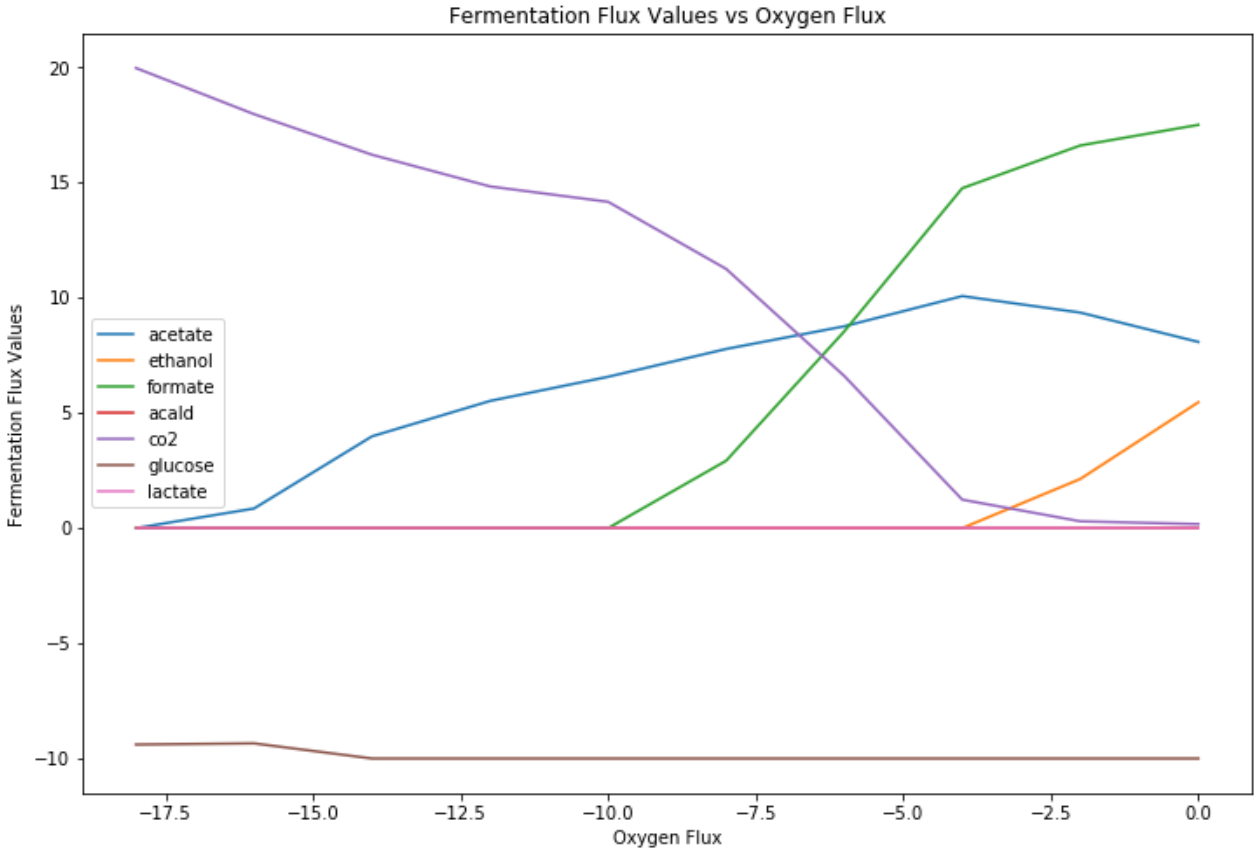
Oxygen_Impact_iJL1678b.ipynb

iJO1366 vs iJL1678b Oxygen Impact



iJO1366

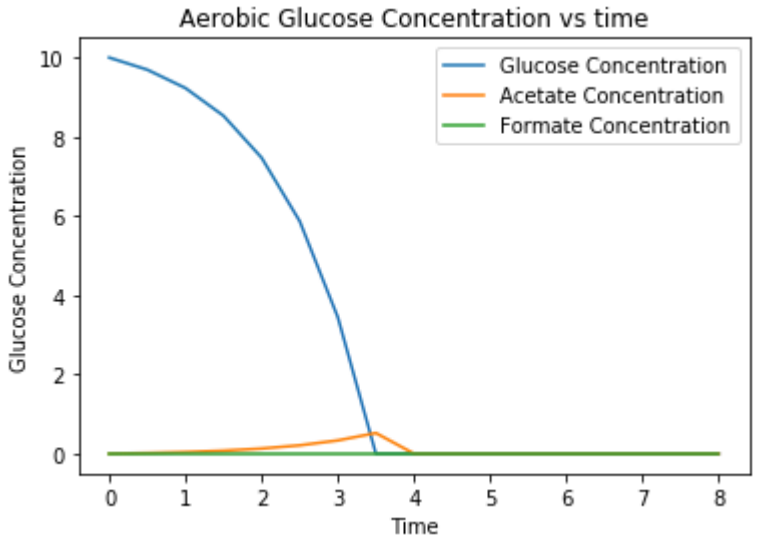
Oxygen_Impact_iJO13166.ipynb

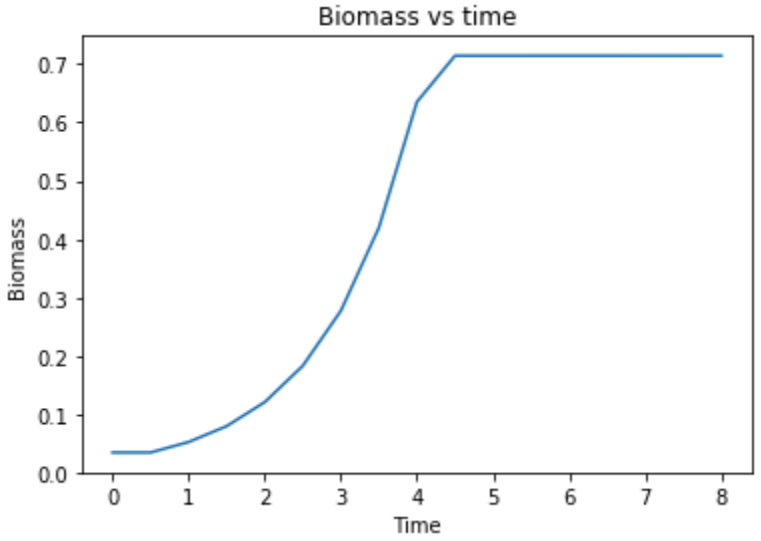


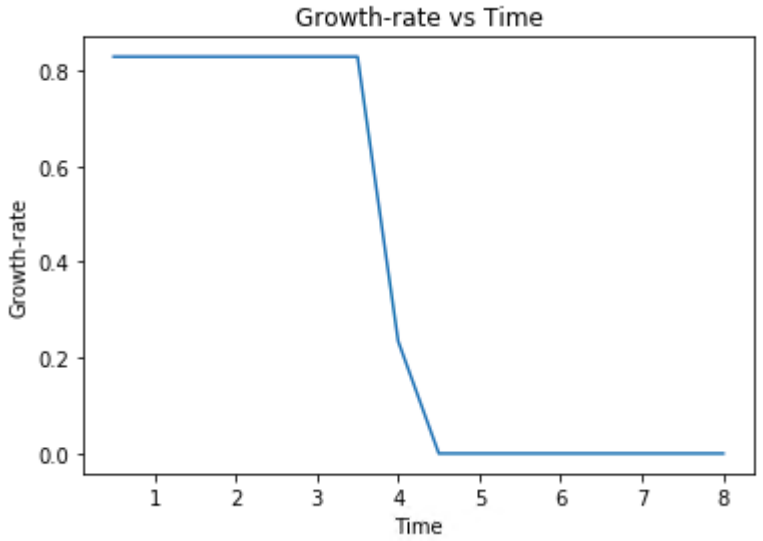
iJL1678b

Oxygen_Impact_iJL1678b.ipynb

iJL1678b Dynamic Flux Balance Analysis (dFBA) (Aerobic Growth)





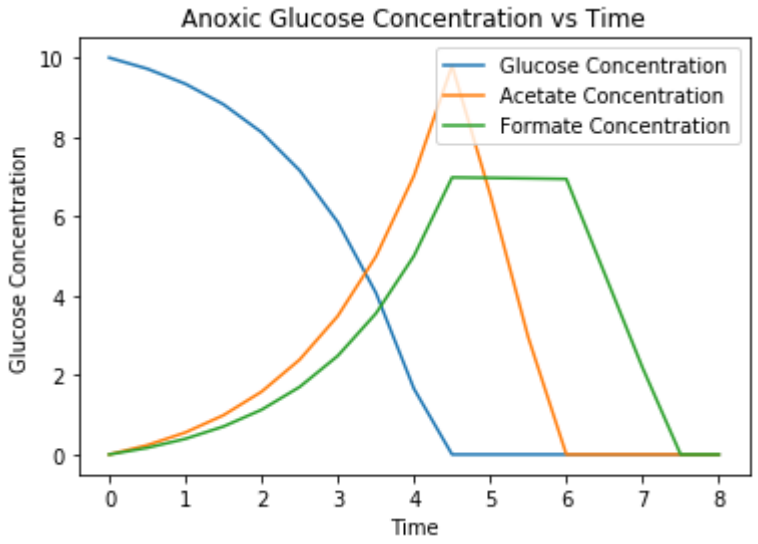


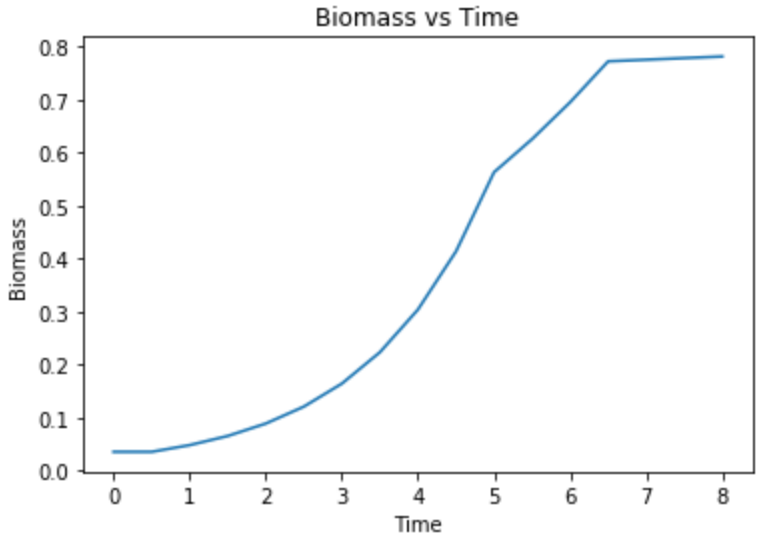

```
model.reactions.EX_o2_e.lower_bound = -20
model.reactions.EX_ac_e.lower_bound = -10
model.reactions.EX_for_e.lower_bound = -10
```

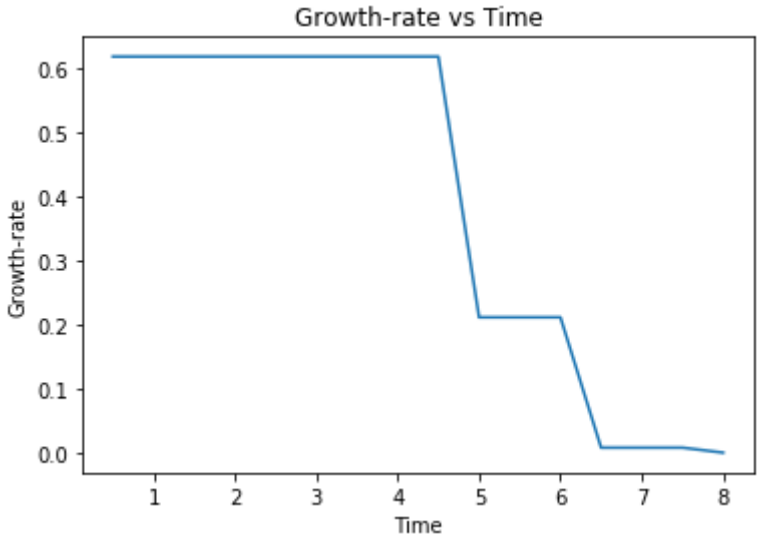
```
# Initial Constants
biomass_0 = 0.035 # Initial biomass
biomass_t = biomass_0
time_total = 8 # Hours
samples = 16 # Total number of samples
```

dFBA_COBRAme_Aerobic.ipynb

iJL1678b Dynamic Flux Balance Analysis (dFBA) (Anoxic Growth)





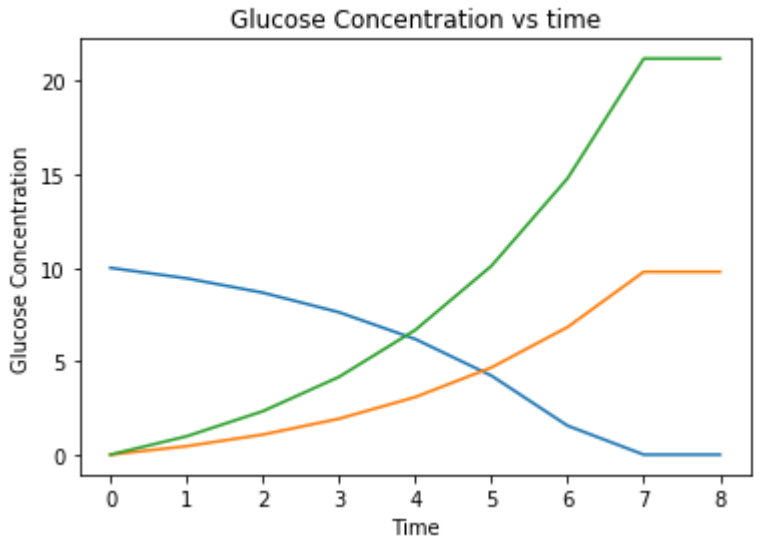


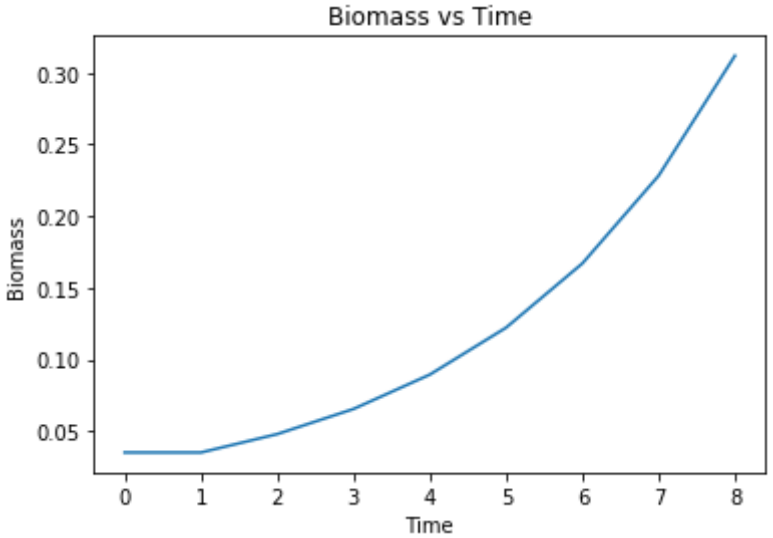

```
model.reactions.EX_glc__D_e.lower_bound = -10
model.reactions.EX_o2_e.lower_bound = -7
model.reactions.EX_ac_e.lower_bound = -10
model.reactions.EX_for_e.lower_bound = -10
```

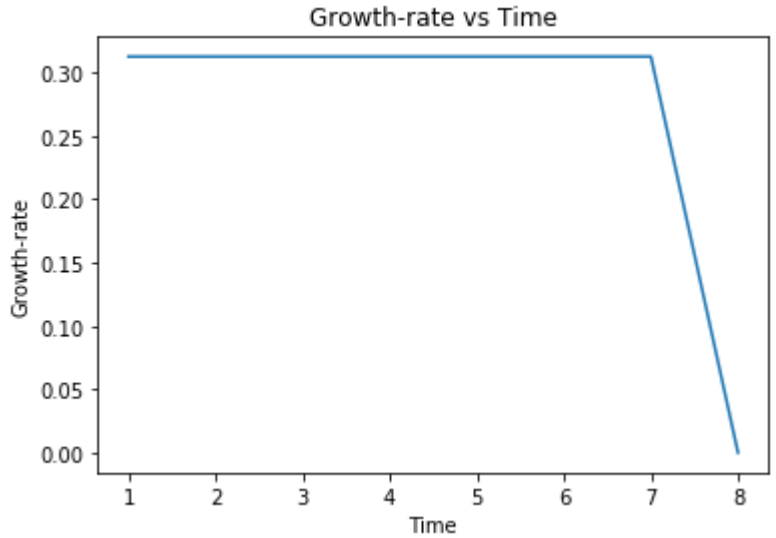
```
# Initial Constants
biomass_0 = 0.035 # Initial biomass
biomass_t = biomass_0
time_total = 8 # Hours
samples = 16 # Total number of samples
```

dFBA_COBRAme_Anoxic.ipynb

iJL1678b Dynamic Flux Balance Analysis (dFBA) (Anaerobic Growth)








```
model.reactions.EX_glc__D_e.lower_bound = -10
model.reactions.EX_o2_e.lower_bound = 0
model.reactions.EX_ac_e.lower_bound = -10
model.reactions.EX_for_e.lower_bound = -10
```

```
# Initial Constants
biomass_0 = 0.035 # Initial biomass
biomass_t = biomass_0
time_total = 8 # Hours
samples = 8 # Total number of samples
```

dFBA_COBRAme_Anaerobic.ipynb

Lesson Outline

- Metabolism (M) vs. Metabolism & Expression (ME) Models
- COBRAme
  - Working Environment (Docker)
  - ME Model Architecture
  - Macromolecular Coupling
  - Reaction Lumping
- ECOLIme
  - Macromolecular Reactions
  - Transcription
  - Translation
  - COBRAme Execution
  - ECOLIme Operation