# pcYeast Generation and Formulation

# Summary

This manual describes how data were collected, how the model was reconstructed, and finally how the optimization problem was generated as LP file formats. We will describe latter in Part III each step in details. The first part gives an overview of the main Excel and MATLAB files that were used to generate and simulate the model.

## Table S1.xlsx: contains all of the collected information.

* **Annotation:** gene names and information.
* **Intron:** Intron information for the genes in the model
* **Length:** The protein length and scanning length in 5’UTR
* **MW:** The molecular mass for the proteins in the model.
* **ElementalComposition:** number of N, O, H, C, P, and S atoms in each protein
* **Subunit:** stoichiometric composition for the metabolic complexes.
* **Chaperone\_Network:** a chaperone and its substrates from chaperone atlas.
* **Folding:** a protein with its chaperones from the Chaperone\_Networksheet.
* **KcatGEKO:** kcat values from GECKO method.
* **Kcat:** kcat values from (Nilsson & Nielsen 2016) .
* **PlasmaMembrane:** The complexes that are located in the plasma membrane.
* **Mito\_Intermembrane\_proteins:** The complexes that are located in the inner mitochondrial membrane.
* **Cytosolic\_complex:** The complexes that are located in the cytosol.
* **Amino Acids:** free amino acids compositions.
* **Lipid:** Lipid compositions that are used in biomass.
* **Medium:** The composition of YPD, SC, and SD medium.

## tRNA\_modification\_position\_1.xlsx: this contains all of collected information about tRNA.

* **Sequence:** information about cytosolic tRNA sequence, codons, and charged tRNA in the model.
* **Position:** tRNA modification types and their positions.
* **Reaction:** Chemical reaction for each tRNA modification.
* **MitoSequence:** information about mitochondrial tRNA sequence, codons, and charged tRNA in the model.
* **Position\_mito:** tRNA modification types and their positions.

## Two main MATLAB files:

# Laptop Part (model reconstruction) Main\_final\_thermal.m: This script generates the new model. The run time is about six hours on a normal laptop. This file extracts the information in the Excel files and uses other functions to make the new model. The new model is connected and is ready for simulation.

# The generated model is stored as “YeastnextGeneration.mat” and an Excel file “NewYeastnextGeneration.xlsx”

**Server part (model simulation)**

We ran the following on a Linux server with Soplex (Scip et al. 2017)as an optimization solver installed.

**GlucoseLimit\_GEKO.m:** This script calls the model generator function.

**modelGenerationGeko.m:** This function generates the model as LP format and calls Soplex to solve the problem. In Part III, we describe each constraint in the optimization problem.

# Part I: Information Collecting

## **1.1 mRNA**

The RNA polymerase II transcribes a gene from Transcription Start Sites (TSS) to Poly Adenine Sites (PAS), while the ribosome decodes only the nucleotides in the coding region (CDS). The 5’UTR length determines the cost of the scanning process in translation initiation. To determine TSS for each gene,we used SMORE-seq (Parky et al. 2014)**.** For a gene that has no measured TSS, we assume that its 5’UTR length is equal to 50 nts (median of 5’UTR length).For a gene that has TSS inside the CDS, we assume that its 5’UTR length is equal to zero.Finally, we used the yeast genome R64-1-1 from SGD website (Engel et al. 2014) to determine the start and end position in CDS and intron regions.

### Excel Files

We compiled all information in the Excel file TableS1.xlsx, in sheets: *annotation*, *Intron*, and *Length*.

### MATLAB Function

We used the MATLAB function*fiveUTRLength(n,m)* to measure the length of 5’UTR for each gene in the *annotation* sheet in TableS1.xlsx file, where *n* and *m* are indices of the first and last gene in this sheet.

## **1.2 tRNA**

tRNA carries an amino acid corresponding to a current codon in the ribosome. tRNAs are modified with different modification types (El Yacoubi et al. 2012). We downloaded tRNA sequence from GtRNAdb database (Chan & Lowe 2009), and each nucleotide modification type for each tRNA species from MODOMICS database (Machnicka et al. 2013). We curated each modification reaction based on RHEA database and MetaCyC database. Finally, the number of tRNA genes is less than the number of codons. We adopted the codon table proposed by Roth (Roth 2012).

### Excel Files

We compiled all information into the Excel file tRNA\_modification\_position\_1.xlsx.  
  
*Matlab*

We used our MATLAB function*addtRNA2Model\_test (model)* to add the tRNA modifications and charged reactions

## **1.3 Cytoplasmic Ribosome**

### Subunits

We used the annotation for cytoplasmic ribosomal subunits from Cruz et al (de la Cruz et al. 2015).

### Excel Files

We compiled all ribosomal subunits in the Excel file TableS1.xlsx, in the *annotation* sheet.

MatlabWe used our two MATLAB functions *addRibosome(model)* to include the cytoplasmic ribosomes into the model.

### **1.4 Translation initiation**

Translation initiation in yeast has been reviewed intensively to reveal translation initiation in Eukaryotes

(Hinnebusch 2014). In conclusion, the translation initiation factors (eIF4A, -4B, -4E, -4G) need one ATP molecule to bind to mRNA and form the mRNA activation complex. A charged Met-tRNAi binds with eIF2b-GTP to form the Ternary Complex (TC). The ribosomal subunit 40S binds with the translation initiation factors (eIF1, -1A, -3, -5) and TC to form PIC assembly complex that binds with mRNA activation complex to form 43S/mRNA complex. This complex scans 5’UTR region until finding the first codon AUG to form the 48S complex. Berthelot et al (Berthelot et al. 2004) proposed a random walk algorithm to estimate the number of steps in the 5’UTR region, where each step hydrolyses one ATP molecule. Finally, the complex 48S binds with the ribosomal subunit 60S and the translation initiation factor eIF5B-GTP to form the elongation complex and releases all translation initiation factors.

Total ATP cost  
Translation initiation process requires one ATP molecule for binding mRNA with initiation factors, one ATP molecule for every step in the scanning process, and two GTP molecules for eIF5b and eIF2b.

### Excel Files

We compiled all translation initiation factors in the Excel file TableS1.xlsx, in the *annotation* sheet.

MATLAB FunctionsWe used our MATLAB function *randomScanning(length,.9,0.002)* to estimate estimated these steps. Where length is the length of 5’UTR (From Table S1) and 0.9 is the probability of moving towards AUG and 0.002 is the probability of leaving mRNA and starting again from the beginning.

The MATLAB function *addTranslationFactors* () adds the translation factors to the model.  
 **1.5 Translation Elongation**Translation elongation is studied intensely in yeast (Dever & Green 2012). In summary, the translation elongation factor 1B (eEF1B) binds eEF1A with one GTP molecule to form eEF1A-GTP that binds with charged-tRNA to form eEF1A-GTP-aatRNA. It binds with accepter site (A) of a ribosome. When eEF1A-GTP-aatRNA recognizes the corresponding code, tRNA hydrolyzes one GTP molecule, releases eEF1A and accommodates aatRNA into A-site of a ribosome. After that, GTPase eEF2 assists the ribosome to release tRNA. Additionally, yeast has another translation elongation factor (eEF3A) that assists eEF2 in releasing tRNA from the ribosome (Andersen et al. 2006). eEF3 is Two ABC-type ATPase (Chakraburtty 2001). Andersen et al (Andersen et al. 2006) proposed that eEF3 fellows the ATP switch model for ABC transporters (Andersen et al. 2006), so eEF3 needs two ATP molecules to release tRNA from the ribosome.

Total costFor each codon (amino acid), two ATP molecules and two GTP molecules are required.

### Excel Files

We compiled all translation elongation factors in the Excel file TableS1.xlsx, in the *annotation* sheet.

MatlabWe used our MATLAB function *elnogation(seq)* to return the number of ATP and GTP molecules required, products and substrates of the protein biosynthesis reaction, and estimated number of steps, where *seq* is a protein sequence without the first and last codon.

### **1.6 Translation termination**

We adopted a termination model proposed by Dever and Green (Dever & Green 2012). In conclusion, the translation termination factor (eRF1) binds with GTP form of eRF3 to form the complex eRF1.eRF3-GTP that recognizes the stop codon and binds with the ribosome. The ATPase Rli1 assist in ribosome recycling.

Total costFor each peptide, one ATP molecule and one GTP molecule are required.

### Excel Files

We compiled all translation termination factors in the Excel file TableS1.xlsx, in the *annotation* sheet.

### **1.7 Reaction for Cytosolic Translation**

MatlabWe used our MATLAB function *addProtein2Model\_test (.)* to include a reaction that describes the cost of translated peptide.

## **1.8 Mitochondrial Ribosome**

### Subunit

We collected mitochondrial ribosomal subunits from Woellhaf et al (Woellhaf et al. 2014), and Greber and Ban (Greber & Ban 2016).

### Excel Files

We compiled all ribosomal subunits in the Excel file TableS1.xlsx, in the *annotation* sheet.

MatlabWe used our MATLAB function *addMitoRibosome(model)* that include the mitochondrial ribosome into the model.

### **1.9 Translation initiation in Mitochondrial**

Mitochondrial translation initiation is simpler than the cytoplasmic translation initiation. The GTP-bound mitochondrial translation initiation factor 2 (IFM1) directs the first Met-tRNA to bind with the mitochondrial ribosome.   
  
Total cost  
One GTP molecule is required

### Excel Files

We compiled all translation initiation factors in the Excel file TableS1.xlsx, in the *annotation* sheet.

**1.10 Translation Elongation in Mitochondrial**Mitochondrial translation elongation is similar with bacterial translation elongation. Yeast mitochondria has two translation elongation factors: MEF1 and TUF1.

Total costTwo GTP molecules are required.

### Excel Files

We compiled all translation initiation factors in the Excel file TableS1.xlsx, in the *annotation* sheet.

MatlabWe used our MATLAB function *elnogationMito(seq)* to return the number of ATP and GTP molecules, products and substrates on the protein biosynthesis reaction, and estimated number of steps, where *seq* is a protein sequence without first and last codon.

### **1.11 Translation termination in Mitochondrial**

Total costFor each peptide, one ATP molecule and one GTP molecule are required.

### Excel Files

We compiled all translation initiation factors in the Excel file TableS1.xlsx, in the *annotation* sheet.

### **1.12 Reaction for Mitochondrial Translation**

MatlabWe used our MATLAB function *addMitoProtein2Model*()to include a reaction that describes the cost of a translated peptide in the mitochondria.

## **1.13 Proteasome**

### Subunits

We used the annotation for proteasome subunits from Finley et al (Finley et al. 2012).

### Excel Files

We compiled all proteasome subunits in the Excel file TableS1.xlsx, in the *annotation* sheet.

MatlabWe used our two MATLAB function *addProteasome* () that include the proteasome into the model.

### Degradation cost

**1.14 Reaction for protein degradation**MatlabWe used our MATLAB function *addProteinDegradation2Model* () that includes all degradation reactions into the model.

### **1.15 Cytosolic ribosome assembly** Factors **???** Excel Files

We compiled assembly factors in the Excel file TableS1.xlsx, in the *annotation* sheet.

MatlabWe used our MATLAB function *addAssemblyFactors* () that includes the assembly factors into the model.

## **1.16 Chaperones**

### Subunits

We used the annotation for cytosolic chaperones and their substrates from atlas of chaperones (Gong et al. 2009).

### Excel Files

We compiled all proteasome subunits in the Excel file TableS1.xlsx, in the *annotation* sheet and *Folding* sheets.

MatlabWe used our MATLAB function *addChaperone ()* to that include the chaperones into the model.

## **1.17 TOM/TIM complexes**

### Subunits

We used the annotation for TOM/TIM complexes subunits from Fox (Fox 2012).

### Excel Files

We compiled all proteasome subunits in the Excel file TableS1.xlsx, in the *annotation* sheet.

MatlabWe used our three MATLAB functions: *addTOM* (), *addTIM22()*, and *addTIM23()* to that include the TOM/TIM complexes into the model.

## **1.18 Metabolic complexes**

### Subunits

We collected the metabolic subunits from UniProt, MetaCyc, and Protein Data bank databases.

### Excel Files

We compiled all metabolic subunits in the Excel file TableS1.xlsx, in the *subunit* sheet.

MatlabWe used our two MATLAB function *addMetabolicComplex* () that include the metabolic complexes into the model.

## **1.19 Kcats**

We collected Kcat from the yeast GECKO model (Sánchez et al. 2017) in the sheet kcat in the Excel file Table S1.xlsx.

**Part II: Model Reconstruction**

## **2.1 New reactions**

Our MATLAB function *Main\_final\_thermal* () will generate the new model. First, the function converts the reversible reactions to two irreversible reactions. After that, the function includes the next reaction types to the model (See the next figures):

1. Translation reaction ( => peptide)

The reaction name has the keyword “\_translation” for cytosolic translation and the keyword “\_translation\_mitochondrion” for translation in mitochondrial

1. Degradation reaction ( peptide => )

The reaction name has the keyword “\_subunit\_degradation” for a subunit degradation

1. Transporting peptide to a compartment

The reaction name has the keyword “\_importing\_cytoplasm\_compartment” for transporting peptide from a cytoplasm to a compartment.

1. Folding reaction ( peptide => folding peptide)

The reaction name has the keyword “\_folding\_compartment” for a peptide folding. The compartment is replaced by a compartment name, such mitochondrion.

1. Reaction for a complex assembly ( folding peptide 1 + … + folding peptide n => complex

The reaction name has the keyword “\_complex\_formation” for assembling a complex from its subunits.

1. Reaction for complex dilution

The reaction name has the keyword “\_complex\_dilution” for a subunit dilution.

1. Reaction for complex degradation

The reaction name has the keyword “\_complex\_degradation” for a subunit degradation

1. Reaction for transporting a degraded peptide from a compartment to cytosol.

The reaction name has the keyword “\_subunit\_degradation” for a subunit degradation

In addition to these reactions, we added reactions that describe the charging and un-charging of tRNA.

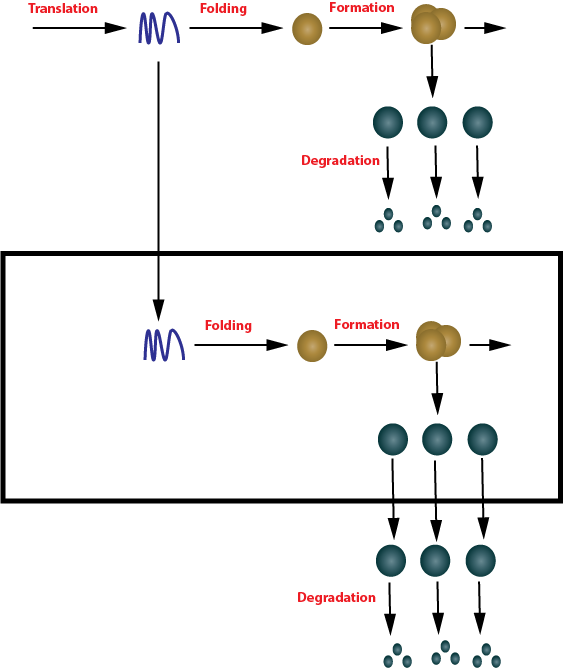


Figure S1. New reactions in the model that describes peptide metabolism.

## **2.2 non-modelled protein**

The model does not contain all proteins in yeast. To include non-modelled proteins, we propose the protein YDRUMD1 as a proxy. This protein has 447 amino acids, where the amino acids composition of this protein is similar to amino acids in the biomass reaction. We assumed that YDRUMD1 has 50 nucleotides in its 5’UTR.

**2.3 Biomass reaction**

The biomass reaction is a lumped reaction that describes the concentration of biomass precursors as mmol/gDW (protein precursors are represented by their corresponding amino acids) (Thiele & Palsson 2010). Förster et al (Forster et al. 2003) described how these precursor concentrations were computed from published data. The amino acid concentrations were the major components in the biomass reaction. They determined them from monomer compositions (Oura 1972) and amino acids composition was proposed by Bruinenberg et al (Bruinenberg et al. 1983). These concentrations are conserved to the last yeast GEM (yeast 7.6). Additionally, they computed also the carbohydrates, DNA, and RNA concentrations. Heavner et al. (Heavner et al. 2012) used a lipidomics study that analyzes the global yeast lipidome (Ejsing et al. 2009) to curate more lipid species. They developed an “isa” reaction that describes how a lipid class (i.e. Complex Sphingolipid) is formed from its lipid species (i.e. MIPC). Finally, Nookaew et al (Nookaew et al. 2008) computed the biomass compositions when yeast grows in nitrogen-limited condition.

In GEMs, the composition of the biomass reaction does not change with the growth rate. However, when yeast grows faster, it needs more proteins. The increase in protein composition leads to increase in RNA and decrease in carbohydrates and lipid compositions. Additionally, the biomass compositions are also changed from a strain to a strain. Canelas et al. (Canelas et al. 2011) measured the biomass composition for the strain CEN.PK 113-7D, which is considered a laboratory strain. We collected the biomass composition from their supplementary data and applied basic curve fitting to predict the biomass composition at different growth rates. The proportion of DNA, phosphate, sulfate, and metals is constant at 0.036 g/gDW. For the metals, we divided its ratio to riboflavin, chitin, and heme A, which are biomass components in the model yeast 6.5. The estimated total biomass value is between 0.975 to 1.02 g/gDW.

To make the biomass composition depend on growth rate, we constrained our model to produce the protein ratio (g/gDW) and removed the protein component from the biomass reaction. Additionally, we constrained the new biomass reaction flux (without protein) to equal the corresponding growth rate. For carbohydrates and RNA, we followed the same computation as in Förster et al (Forster et al. 2003), but with different carbohydrates and RNA ratios (g/gDW). To make lipid composition depend on growth rate, we made a lumped reaction consisting of 64 lipid species (Ejsing et al. 2009), where the data were provided as mol/mol lipid \*100. First, we used  the Yeast Metabolome Database (YMDB) (Ramirez-Gaona et al. 2016) and LIPID MAPS (http://www.lipidmaps.org/) to find the molecular mass for each metabolite. We then computed the molecular mass of lipid as g/mol. Finally, we got the concentration of each metabolite as mmol/gDW **(Table S1),** where the lipid ratio in Table S1 is 0.07g/gDW. We updated these compositions with the lipid ratio at different growth rates.

**MATLAB functions**

The MATLAB function *biomass\_CEN\_PK(μ)* returns the biomass reaction for the strain CEN.PK 113-7D, when growth rate equals *μ*.

The MATLAB function *proteinRatio(μ)* returns the protein ratio for the strain CEN.PK 113-7D, when growth rate equals *μ*.

**2.4 Growth Media**

We compiled the media compositions (SC, YPD, and SD) from Snitkin et al (Snitkin et al. 2008) in sheet media in the Excel file.

**2.5 Converting protein concentration as Molecules/cell**

The protein concentration in the model is expressed as mmol/gDW. We use the next calculations to convert 1 protein molecule to mmol/gDW, where a cell weighs 13 pg.

At steady state, protein concentration where and is a flux value in a reaction of a translated protein We convert as Molecules/cell by multiplying [e] by 13\*6.023e8.

**2.6 Protein volume and occupied area**

Erickson (Erickson 2009) assumed that the protein molecule is a sphere. He estimated the radius ) of this sphere as a function of molecular weights where:

We then use to estimate the volume and area occupied by this protein.

**Part III: Model Generation**

We converted our model to LP format (CPLEX format) and used the Soplex solver to solve the problem with high accuracy. The advantage of writing our model as LP format directly is to add constraints directly without any restrictions. Additionally, most solvers read LP format, so we are not restricted to the solvers in RAVEN or COBRA packages. In the following, we describe in detail, how we added each constraint in the model.

**3.1 General Parameters setting [Code Lines 45 - 150 in the file modelGenerationGeko.m]:**

* **Growth rate:** the model is generated for each growth rate independently, so growth must be given to generate a model at this growth rate.
* **Metabolic enzyme catalytic rate (Kcat):** We used an average Kcat (110 per second).
* **Sigma factor: We fitted this parameter for each growth rate.**
* **Degradation rate (kdeg):** the average protein degradation rate is 0.045 per hour, at growth rate = 0.1 (1/h) ((Lahtvee et al. 2017)).
* **Cytosolic Ribosomal catalytic rate (kcat\_ribo):** We describe in details this parameter in the part IV.
* **Mitochondrial Ribosomal elongation rate (kcat\_ribo\_mit):** 15 aa/s, as the average value of the mitochondrial and cytosolic ribosome.
* **Protesome catalytic rate:** protesome can degrade 2.7 substrate molecules per min (Peth et al. 2013).
* **Assembly Factor rate:** yeast makes about 2000 per minute (Warner 1999).
* **aa-tRNA synthetases (AARSs) catalytic rate (kcat\_AARS):** 3.5 (1/S). Their kcat vary between 1-6 (1/S)
* **ATP Growth Associated Maintenance (GAM):** 30 mmol/gDW/h. GAM in yeast model equal 59 mmol/gDW/h. Our model uses ATP molecules in protein translation and degradation. We reduced the GAM value to 30 mmol/gDW/h.
* **ATP non-Growth Associated Maintenance (NGAM): 0.5** mmol/gDW/h. NGAM in yeast model equal 1 mmol/gDW/h. Our model uses ATP molecules in protein translation and degradation. We reduced the GAM value to 0.5 mmol/gDW/h.

**3.2 Model preprocessing**

**Biomass reaction [Code Lines 159 - 173 in the file modelGenerationGeko.m]**

In our model, the biomass reaction has no protein ratio and depends on the growth rate. The function biomass\_CEN\_PK(mu) return the biomass reaction. We added this reaction to the model and constrained the upper and lower bound for this reaction to the growth rate value.

**Growth media [Code Lines 183 - 200 in the file modelGenerationGeko.m]**

We fixed upper and lower bounds for exchange reactions based on the growth media.

**GAM and NGAM reactions [Code Lines 202 - 212 in the file modelGenerationGeko.m]**

We added the next two reactions in the model for losing energy that represents energy in un-modeled processes such as transcription and maintenance processes. We constrained the upper and lower bound for these reactions to growth rate value.

**3.3 Objective function [Code Lines 213 - 228 in the file modelGenerationGeko.m]**

**The objective function in our model is to minimize glucose uptake rate.**

**3.4 Constrains**

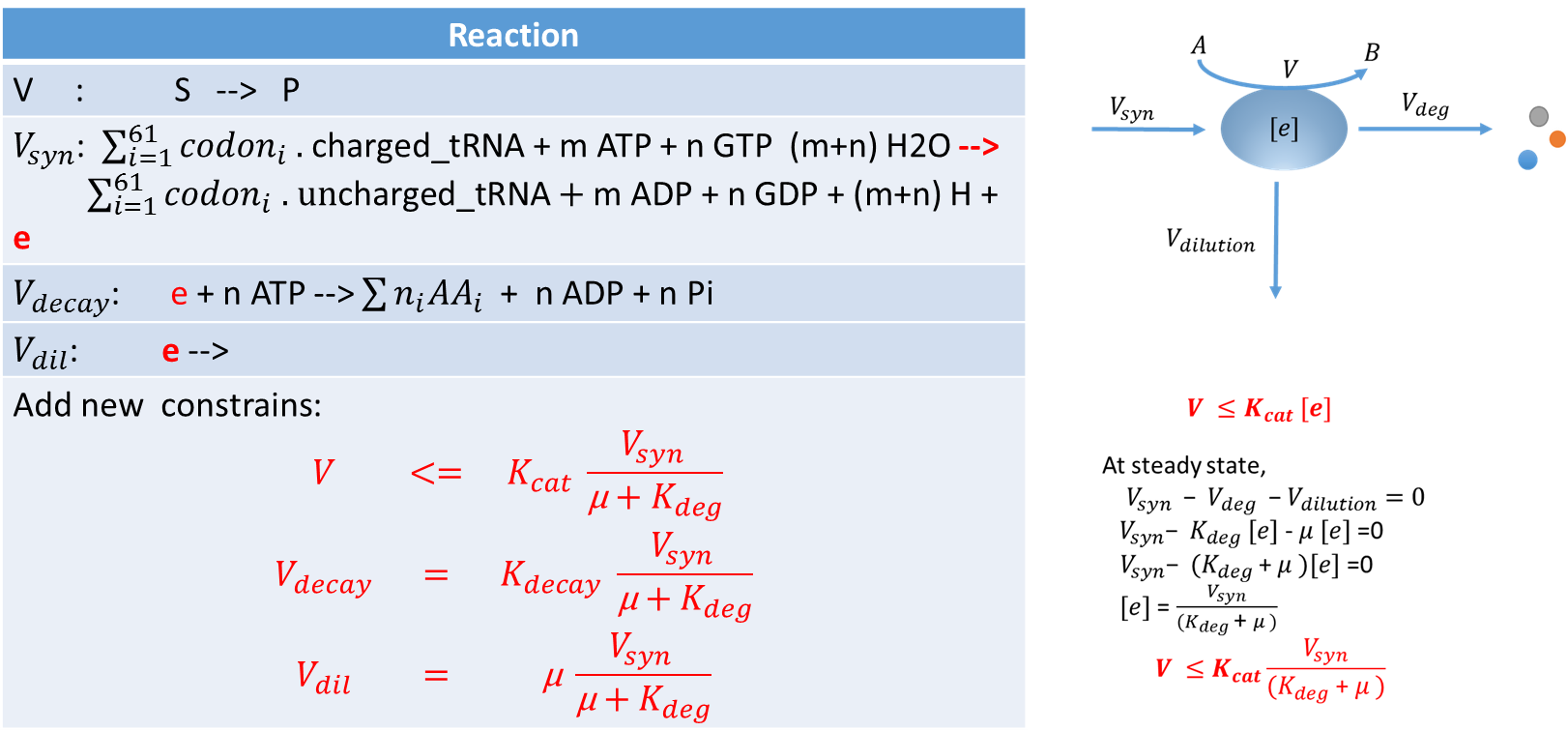
**Constraint 1: Sv = 0 [Code Lines 229 - 255 in the file modelGenerationGeko.m]**  
The first constraint in our model is the steady-state condition (SV=0). This constraint is the main constraint in metabolic models. Each reaction in the model has the variable name *Xi*, where *i* is the ith reaction (*ith* column in *S*). For example, the reaction number 100 is represented as X100. For each metabolite *j* (where *j* is the *jth* row in S), we print Sji Xi = 0 in the LP file, only and only if *Sji* is not equal zero.

**Constraint 2: Metabolic coupling (V <= kcat [e]) [Code Lines 256 - 474 in the file modelGenerationGeko.m]**

*v* represents a flux value in a metabolic reaction. We iterated through all metabolic reactions that are catalyzed by enzymes (with concentrations [e]), where

.

Each reaction is associated with a complex, where the complex assembly reaction has a keyword “\_complex\_formation”. We search for this reaction describing the enzyme complex formation (Vsyn). In this case, the enzyme can carry out more than one reaction Xi, Xi1, … Xin, we then print in the LP file



* **Constraint 3: Vdil = mu [e]**

The model needs to dilute with a rate equal to the growth rate. Therefore, we constrain the flux value in the dilution reaction of complex biosynthesis to :

=0

* **Constraint 4: Vdeg = kdeg [e]**

The model needs to degrade with a rate equal to the protein degradation rate. Therefore we constrain the flux value in degradation reaction of complex biosynthesis to

=0.

**Constraint 5: Cytosolic Ribosome and translation factors capacity [Code Lines 477 - 646 in the file modelGenerationGeko.m]**

To couple the ribosome biosynthesis reaction with translated proteins, we constrained each translated protein (including ribosomal proteins) to less equal the ratio of total number of ribosomes as:

Where is a flux value in a translated protein reaction . is the elongation translation rate, and and is the length and number of steps in the scanning at 5’UTR region of a protein . We added also a fixed number of amino acids that represent the cost of translation termination and recycling. These equations were adopted from Pavlov and Ehrenberg (Pavlov & Ehrenberg 2013)  
  
We then searched for each reaction ID ending with “\_translation” to find . We added the coupling constraint in the model LP file:

he ribosome biosynthesis, dilution, and degradation reactions are known, so we added the total ribosome constraint to three constraints in the model LP file.

**Constraint 6: Translation initiation factors:**

We collected all initiation translation factors in one complex (). We simulated the scanning process, where a ribosome can scan about 10 nt per second. During the model reconstruction, we estimated the total number of steps that are required to reach the first codon. Therefore, we constrained that each translated protein takes a ratio of total initiation complexes similar to the previous ribosomal constraint.

Where is a flux value in a translated protein reaction . is the scanning rate, and is the number of steps in the scanning process (see section 1.4 translation initialization).  
  
We then searched for each reaction ID ending with “\_translation” to find . We added the coupling constraint in the model LP file:

he ribosome biosynthesis, dilution, and degradation reactions are known, so we added the total ribosome constraint three constraints in the model LP file.

**Constraint 7: Translation elongation factors (Binding EF1A with GTP and aa-tRNA: 6 (1/S)** Gromadski et al. (Gromadski et al. 2007) estimated that the rate of exchange of eEF1A with GTP is 6 molecules per second. Additionally, they estimated also the association rate of Phe-tRNA with eEF1A as 5.7 ± 0.3. Therefore, we assumed the rate of overall process is equal to 6 (1/S).  
  
To couple the elongation translation factor (eEF1A) biosynthesis reaction with translated proteins, we constrained that each translated protein (including ribosomal proteins) must take a ratio of eEF1A as:

Where is a flux value in a translated protein reaction . is the catalytic rate of and is a length of protein .   
  
We then searched for each reaction ID ending with “\_translation” to find . We added the coupling constraint in the model LP file:

he eEF1A biosynthesis, dilution, and degradation reactions are known, so we added the total eEF1A constraint three constraints in the model LP file.

**Constraint 8: eEF2**

To couple the elongation translation factor (eEF2) biosynthesis reaction with translated proteins, we constrained that each translated protein (including ribosomal proteins) must take a ratio of total amount of eEF2as:

Where is a flux value in a translated protein reaction . is the catalytic rate of and is a length of protein .   
  
We then searched for each reaction ID ending with “\_translation” to find . We added the coupling constraint in the model LP file:

he eEF2 biosynthesis, dilution, and degradation reactions are known, so we added the total eEF2 constraint three constraints in the model LP file.

**Constraint 9: eEF3**

**eEF2 has the rate of elongation rate 10 aa/. We added the next constraint.**To couple the elongation translation factor (eEF3) biosynthesis reaction with translated proteins, we constrained that each translated protein (including ribosomal proteins) must take a ratio of total amount of eEF3as:

Where is a flux value in a translated protein reaction . is the catalytic rate of and is a length of a protein .   
  
We then searched for each reaction ID ending with “\_translation” to find . We added the coupling constraint in the model LP file:

he eEF3 biosynthesis, dilution, and degradation reactions are known, so we added the total eEF3 constraint three constraints in the model LP file.

* **Constraint 10: Translation Termination factors:**

To couple the ribosome biosynthesis reaction with translated proteins, we constrained that each translated protein (including ribosomal proteins) must take a ratio of total amount of eRFas:

Where is a flux value in a translated protein reaction . is the catalytic rate of

We then searched for each reaction ID ending with “\_translation” to find . We added the coupling constraint in the model LP file:

he ribosome biosynthesis, dilution, and degradation reactions are known, so we added the total constraint three constraints in the model LP file.

**Constraint 11: Mitochondrial ribosome capacity: [Code Lines 647 - 703 in the file modelGenerationGeko.m]**

To couple mitochondrial ribosome biosynthesis with mitochondrial protein translation involving 7 proteins for oxidative phosphorylation systems and one subunit in the mitochondrial ribosome, we constrained that these proteins should be less than all ribosomes in the model (. We used the same constraints as in cytosolic ribosomes (constraint 5). The mitochondrial proteins were translated by reactions with “\_translation\_ mitochondrial” in their IDs to find .

**Constraint 12: Total protein weight: [Code Lines 704 - 708 in the file modelGenerationGeko.m]**

The sum of all protein concentration should be equal to the protein ratio at the given growth rate. In the previous two steps (cytosolic and mitochondrial ribosome capacity constrained), we searched for andand collected all protein concentrations as in the next equation:

We added this constraint to the LP file.

**Constraint 13: Total number of proteins molecules per cell:** The sum of all the protein molecules should be equal the total protein number at the given growth rate. In the previous two steps (cytosolic and mitochondrial ribosome capacity constrained), we searched for andand collected all protein concentrations as in the next equation:

We added this constraint to the LP file.

**Constraint 14: Chaperone capacity [Code Lines 7028 - 804 in the file modelGenerationGeko.m]:** We implemented encoded the information included in the chaperon atlas (Gong et al. 2009) as a binary matrix, where a row is a substrate and a column is a cytosolic chaperones (RAC, SSB, SSA, SSE, HSP40, HSP90, PFD, and CCT) (see the file Table S1.xlsx sheet Folding). We added three constraints for each chaperone.

**Constraint 15: Cytoplasmic volume constraint : [Code Lines 825 - 836 in the file modelGenerationGeko.m]**

We collected all cytosolic proteins in the metabolic model and translation machinery proteins. We added this constraint to the generated LP file:

We assumed that all proteins in the model are cytosolic proteins except the proteins that are translated by the mitochondrial ribosome.

Yeast cell volume is constant at different growth rates (Canelas et al. 2011) and is about . The cytoplasmic volume is about 50% of the whole cell volume. From a molecular crowding view, the occupied volume in the cytoplasm is about 30% of the cytoplasmic volume. Additionally, we assume that 50% of occupied volume is filled by proteins. Therefore, we assumed that cytoplasmic proteins occupy about .

**Constraint 16: Mitochondria volume constraint**

We collected all mitochondrial proteins in the metabolic model (including mitochondrial ribosomes and import machinery). We added this constraint to the generated LP file:

**Constraint 17: Plasma Membrane**

Using a minimal medium, the model consumes these five nutrients: glucose, ammonia, phosphate, sulfate and oxygen. Oxygen can only be transported by diffusion but the other need transporting proteins. The cell volume is constant at different growth rates [REF], so we assume the plasma membrane surface area is also constant over different growth rates.

|  |  |  |
| --- | --- | --- |
| **Metabolite** | **Comment** | **kcat (1/s)** |
| Glucose | Ye et al (Ye et al. 2001) and Kruckeberg et al (Kruckeberg et al. 1999) estimated that the catalytic rate () for HXT7 and HXT2 are equal to 200 and 53 Molecules/Sec, respectively. HXT7 has a paralog HXT4. | 200 |
| Phosphate | Shokrollahzadeh et al (Shokrollahzadeh et al. 2007) reported that Pho84 has the specific activity as 6 µ mol/gDW/min.  We assume that the concentration of Pho84 is about 300,000 molecules/cell, so we can estimate as: | 3 |
| Ammonia | Wacker et al (Wacker et al. 2014) measured the rate of transported ammonia as 30–300 **ions** per s per trimer. | 300 |
| Sulfate | Holt et al (Holt et al. 2017) reported that the is equal to 1.01 nmol/mg cells/min.  Thetotal concentration of mRNA for sulphate transporters is equal to 4.5 molecules/pg ((Lahtvee et al. 2017)). One cell weights 13 pg, so the mRNA concentration is equal to 58.5 molecules/cell. We assume that 1 mRNA molecule is translated to 10,000 protein copies. We therefore assume that there are about 60,000 sulfate transporters in each cell. Therefore, we can estimate as: | 2.5 |
| Proton (H+) | Sampedro et al (Sampedro et al. 2007) measured that the kcat for Plasma Membrane H+-ATPase in *KluyVeromyces lactis* is equal to 36 per second. | 36 |
| Fructose | We added a new reaction that HXT2 transports fructose to cytoplasm through HXT2 without a proton. | 200 |

**Constraint 18: Ribosome Assembly constraints [Code Lines 839 - 857 in the file modelGenerationGeko.m]**

Ribosome assembly factors make a new ribosome. We couple a ribosome biosynthesis reaction with ribosome assembly factor reaction as

**Constraint 19: Proteasome capacity [Code Lines 858 - 900 in the file modelGenerationGeko.m]**

To couple the proteasome biosynthesis reaction with degradation proteins, we constrained that the sum of all fluxes in the degradation protein reactions (is less than [Proteasome] as:

Where is the ’s catalytic rate.

**Constraint 20: Un-modelled protein: [Code Lines 901 - 915 in the file modelGenerationGeko.m]**

The model should translate and decay proteins that are not in the model. These protein represents a ratio (

**Constraint 21: TOM complex capacity [Code Lines 916 - 928 in the file modelGenerationGeko.m]:**

**Constraint 22: Over-expressed protein (such as GFP) [Code Lines 946 - 955 in the file modelGenerationGeko.m]:**

The model contains three fluorescent proteins: GFP, YFP, and mCherry proteins, and hemoglobin complex. We converted the number of molecules to mmol/gDW. For GFP example, we added these three constraints.

**Part IV (Translation rate)**

Translation time of a ribosome

The total protein biosynthesis rate () was used to estimate the protein translation rate (elongation rate in the previous studies) at different growth rates, where (gram protein /gDW/h). Canelas et al (Canelas et al. 2011) measured protein ratio at different growth rates, so we can estimate the rate pf protein biosynthesis at different growth rates. We generated a MTALAB function proteinRatio() that returns the protein ration at growth rate We can convert the protein ratio P to a string of amino acids by dividing by the average molecular weight of amino acid This average was reported as 107 g/mol. We can revise this value by the amino acids compositions in the biomass reaction of PaxDB, because the amino acids has different ratio in total protein mass. We find the MW(aa) =126 and 128 (gram/mol) in biomass reaction and PaxDB. So we used MW(aa) =127 (gram/mol). By applying the ribosome capacity constraint, we get where is the moles of active ribosome. From this we can formulate the next equaltion (Dai et al. 2016):

(1)

By using alternative expressions, the previous equation the fraction of active ribosome is linear dependent on growth rate.

We assume that the total ribosome fraction consists of two parts: the active ribosomal fraction andthe inactive ribosomal fraction . In the next equations the total ribosomal fraction is linearly dependent on the growth rate, where the 1/slope is time of translation of one ribosome by only one ribosome. Additionally, the equation shows that representing of fraction of in-active ribosomal fraction. However, our equation shows that the cost of protein degradation is included also in fraction of inactive ribosomal fraction.

The results show that changes and depends on growth condition. We expected that protein degradation rate can increase or decrease the value

K at different growth rate

The equation (1) can be used to find the protein translation rate by dividing by we get:

(4)

If we assume that all rRNA are used in active ribosomes, we can use the RNA ratio to estimate protein content at different growth rates. We used estimate the number of ribosome from [RNA] . We assumed that the [rRNA] = 0.85 [RNA] (von der Haar 2008). To estimate the number of ribosomes, we divided by the molecular weight of rRNA into one ribosome , where , so . By substituting in equation (4)

(5)

The equation (5) shows that the protein translation rate depends on the ratio between protein to RNA content. We used the protein and RNA measurements at different growth rates from Canelas et al (Canelas et al. 2011). We plotted RNA/P protein ratio with growth rate. From the regression line we get , where and . From this line, we get . Finally, by substituting in equation (5), we get

(6)

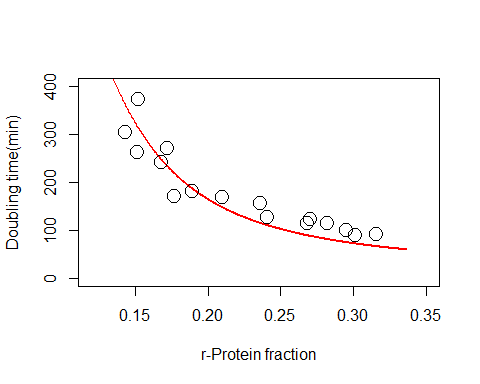
Using Equation (6) we can estimate the protein translation rate at given growth rate .

Prediction of ribosomal proteome fraction

We have revised, he time of translation of one ribosome by another ribosome, where. We can estimate at given growth rate, equation (6), and we estimate L=19093 amino acids. Therefore, we can estimate at any given growth rate. Using, we can follow equations to predict the fraction of ribosomal fraction. They proposed ratio of time that represents the ratio of to doubling time T (min), where

(7)

The ratio of time ratio of time represents also the fraction of ribosomal proteome. To validate , we used ribosomal protein fraction from published data (Metzl-Raz et al. 2017). The red line represents the prediction of the fraction of ribosomal proteome by equation 7. The prediction is consistent with the ribosomal proteome fraction using general parameters without any parameter fitting. We can conclude that the ribosomal proteome fraction only depends on growth rate and is not dependent on other environmental conditions.



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