

## *Ab Initio* Whole Cell Kinetic Model of *Yarrowia lipolytica* CLIB122 (yliYKY24)

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### Abstract

*Yarrowia lipolytica* is an oleaginous yeast with potential probiotics properties that may be engineered for various industrial applications and the presence of mathematical models (genome-scale models or GSMs, and kinetic models or KMs) will be a valuable tool to guide engineering approaches. To date, there are several GSMs for *Y. lipolytica* but no whole-cell KM. Here, we present a whole cell simulatable KM of *Y. lipolytica*, yliYKY24, based on the genome of *Y. lipolytica* CLIB122. The resulting model consists of 931 metabolites, 703 enzymes with corresponding transcriptions and translations, and 856 enzymatic reactions; which can be a baseline model for incorporating other cellular and growth processes, or as a system to examine cellular resource allocations necessary for engineering.

### Introduction

*Yarrowia lipolytica* is an aerobic oleaginous yeast commonly found in oil-rich habitats [1]. It is capable of storing large amount of lipids [2], and can produce a variety of organic acids [3]. Generally recognized as safe [4], *Y. lipolytica* is currently used in the food industry as flavour enhancers [5], and considered for its potential probiotics properties [6, 7]. It also possess potential industrial applications; such as, bio-oil production [8], wastewater treatment [9], and platform chemical production [10]. These often require metabolic engineering or genetic engineering [11, 12].

Mathematical modelling is an important tool in metabolic engineering [13] as it can predict biological phenotypes under metabolic perturbations, which can be used to guide engineering approaches [14, 15]. Genome-scale models (GSMs, also known as constraint-based models) and kinetic models (KMs) are the two main modelling approaches [16, 17]. GSMs are steady-state stoichiometric models which lacks enzymatic regulation [18] and is difficult to add genes (transgenes) into the system as its original purpose is to evaluate changes in native gene expression on its metabolism [19, 20]. On the other hand, kinetic models (KMs) can have regulation and is much easier to add transgenes. Furthermore, KMs can predict both rates and yield of metabolites [21] while GSMs are primarily for rates. At the same time, large-scale KMs; such as, whole cell KMs; offer more intricate details [22] and have important applications [23, 24] compared to smaller-scale KMs; such as, KMs of pathways. There are a number of GSMs for *Y. lipolytica*; for example, iMK735 [25], and iYli21 [26]. However, there is no whole cell KM of *Y. lipolytica* to date.

Therefore, this study presents the construction a KM of *Y. lipolytica* CLIB122 using *ab initio* approach by identifying enzymes from its genome [27], and linking to its corresponding reaction from KEGG [28]. The result is a whole cell KM of *Y. lipolytica* CLIB122, named as yliYKY24 using the nomenclature proposed by Cho and Ling [29], which consists of 931 metabolites, 703 enzymes with corresponding transcriptions and translations, and 856 enzymatic reactions.

## Methods

### Reactome Identification

Enzymatic genes were identified from the genome of *Yarrowia lipolytica* CLIB122 (NCBI RefSeq GCF\_000002525.2) using the process adapted from previous studies [30, 31]. Briefly, each enzymatic gene was identified as a presence of complete Enzyme Commission (EC) number directly from Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Database for *Y. lipolytica* CLIB122 (Figure 1) or indirectly from UniprotKB and GenBank via the coding sequence's protein ID or locus tag. Each EC number is then mapped into reaction IDs via KEGG Enzyme Database for Enzyme Nomenclature [28].

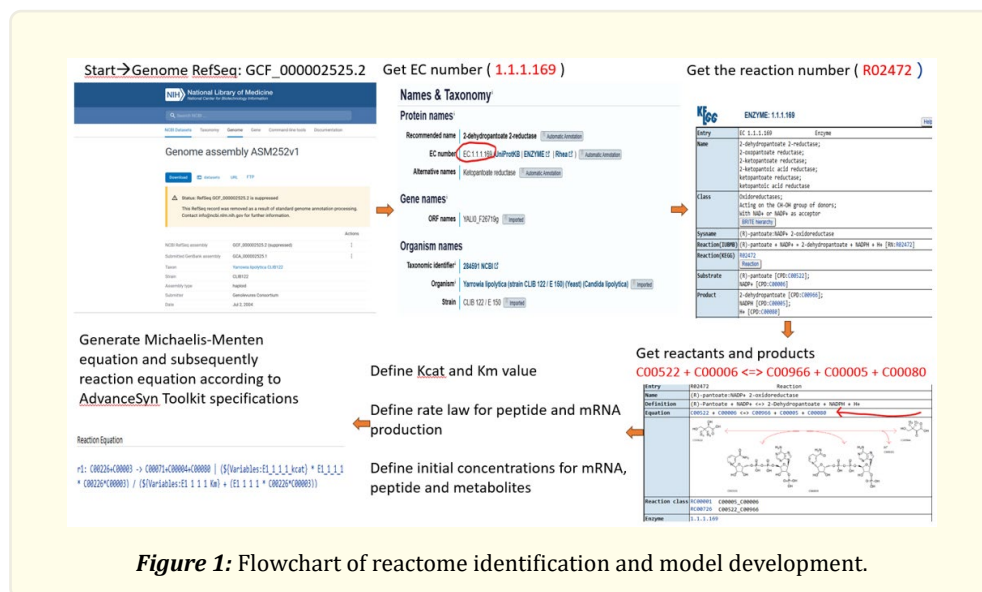


Figure 1: Flowchart of reactome identification and model development.

### Model Development

The production of each enzyme was modelled as a pair of ODEs - an ODE for transcription, and an ODE for translation. Given that each *E. coli* cell has about 750 active units of RNA polymerase (BioNumbers 106199 [32] and 111676 [33]) polymerizing 22 ribonucleotides per second (BioNumbers 104109) [34]. With 339.5 Daltons per ribonucleotide, implying the total mRNA synthesis rate at 5600 kDa per second. One Dalton is 1.66054e-24 gram; hence 5600 kDa per second is 9.3e-18 grams per second. An *E. coli* cell is about 0.7 cubic micrometres [35] or 7e-16 litres with 4225 protein-coding genes (BioNumbers 105443) [36], the total mRNA synthesis rate can be estimated at 2.92 uM per protein-coding genes per second. The average lifespan of mRNA transcripts is 1.79 minutes (BioNumbers 107666) [37] or 107.56 seconds; therefore, 0.93% degraded per second. Therefore, the rate law for mRNA concentration can be written as  $d[mRNA]/dt = (0.00292 - 0.0093[mRNA])$  mM per second. Similarly, the median protein synthesis in mammalian cell culture is 1000 peptides per mRNA transcript per hour (BioNumbers 106382) [38], which equates to 0.278 peptides per mRNA transcripts per second; and the average protein degradation rate for *E. coli* is about 1 percent per hour (BioNumbers 109924) [39], which equates to 0.00000278 per second; the rate law for peptide concentration can be written as  $d[peptide]/dt = (0.278[mRNA] - 0.00000278[peptide])$  uM per second. The reactome was modelled as a set of ordinary differential equations (ODEs) where each ODE

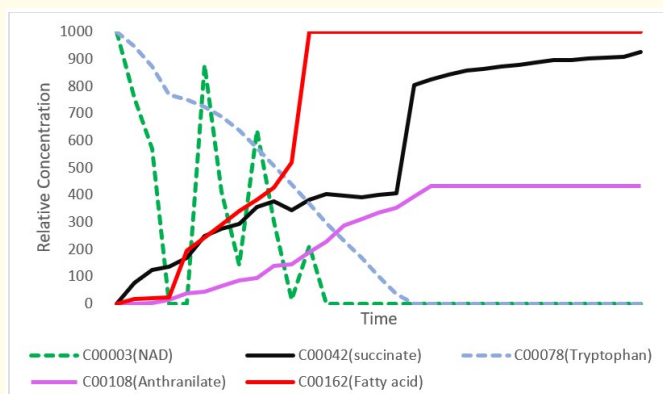
represented one metabolite concentration [30, 40]. The turnover number of enzyme (Kcat) and Michaelis-Menten constant (Km) were set at 13.7 per second and 1 millimolar respectively; which were the median values estimated by Bar-Even et al. [41]. The model was written in AdvanceSyn Model Specification [16].

### Model Simulation

The constructed model was tested for simulatability using AdvanceSyn Toolkit [16]. Initial concentrations of all mRNA and enzymes were set to 0 mM. Initial concentrations of all metabolites were set to 1 mM except the following which were set to 1000 mM: (i) C00001 (water), (ii) C00002 (ATP), (iii) C00003 (NAD+), (iv) C00004 (NADH), (v) C00005 (NADPH), (vi) C00006 (NADP+), (vii) C00007 (Oxygen), (viii) C00008 (ADP), (ix) C00009 (Phosphate), (x) C00011 (Carbon dioxide), (xi) C00014 (Ammonia), (xii) C00025 (Glutamate), (xiii) C00031 (Glucose), (xiv) C00041 (L-Alanine), (xv) C00047 (L-Lysine), (xvi) C00049 (L-Aspartate), (xvii) C00062 (L-Arginine), (xviii) C00064 (L-Glutamine), (xix) C00065 (L-Serine), (xx) C00078 (L-Tryptophan), (xxi) C00079 (L-Phenylalanine), (xxii) C00082 (L-Tyrosine), (xxiii) C00097 (L-Cysteine), (xxiv) C00123 (L-Leucine), (xxv) C00135 (L-Histidine), (xxvi) C00148 (L-Proline), (xxvii) C00152 (L-Asparagine), (xxviii) C00183 (L-Valine), (xxix) C00188 (L-Threonine) and (xxx) C00407 (L-Isoleucine). The model was simulated using fourth-order Runge-Kutta method [42, 43] from time zero to 3600 seconds with timestep of 0.1 second, and the concentrations of metabolites were bounded between 0 millimolar and 1000 millimolar. The simulation results were sampled every 2 seconds.

### Results and Discussion

The genome of *Yarrowia lipolytica* CLIB122 has 7144 coding sequences, with 1838 EC numbers. Out of 1838 EC numbers, 703 are unique and used to identify 856 reactions consisting of 931 metabolites. The resulting model, denoted as yliYKY24, was simulated using AdvanceSyn Toolkit [16]. The presence of simulation results (Figure 2) suggests that the model is free from syntax error.



**Figure 2:** Selection of simulation results.

Our simulation result shows a gradual increase in anthranilate's concentration with a corresponding drop in tryptophan's concentration, indicating some form of feedback inhibition by end-product tryptophan on anthranilate precursor. This type of feedback inhibition is seen commonly in many biochemical processes, including tryptophan inhibition on anthranilate synthase [44]. NAD and fatty acid concentration also shows an inverse relationship, which might be explained by NAD involvement in beta-oxidation of fatty acid; hence, the increase in fatty acid concentration along with NAD depletion. However, the simulation results cannot be taken at face value since all enzyme kinetics (turnover number and Michaelis-Menten constant) are kept the median levels [41], which implies that

all enzymes are producing at same rate and with the sample activity. Hence, the current model represents a whole cell structural model that contains all the necessary kinetic parameters and can be simulated without errors.

Therefore, we present a simulatable whole cell KM of *Y. lipolytica* CLIB122, which can be a base template for incorporating other cellular and growth processes [45-47] or as a system to examine cellular resource allocations [24, 48-50] as it contains comparable number of metabolites, and reactions (931 metabolites and 856 reactions) compared to iMK735 (1111 metabolites and 1136 reactions) [25]. Future work can extend the number of metabolites and reactions to that of iYli21 [26] - 1868 metabolites and 2285 reactions.

## Conclusion

In this study, we present an *ab initio* whole cell kinetic model of *Y. lipolytica* CLIB122, yliYKY24; comprising of 931 metabolites, 703 enzymes with corresponding transcriptions and translations, and 856 enzymatic reactions.

## Supplementary Materials

Reaction descriptions and model can be download from <https://bit.ly/yliYKY24>.

## Conflict of Interest

The authors declare no conflict of interest.

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