E.coli (iJO1366) "Tyrosine, Tryptophan, and Phenylalanine Metabolism" Subsystem

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INTRODUCTION

The purpose of this tutorial is to review the basic structure and capabilities of the Tyrosine, Tryptophan, and Phenylalanine Metabolism Subsystem of the iJO1366 E.coli model.

MATERIALS

This tutorial is based on the Constraint-Based Reconstruction and Analysis (COBRA) Toolbox [1,2]. To use this tutorial requires the 2016a or newer version of Matlab (https://www.mathworks.com/) and the COBRA toolbox software that can be downloaded from https://opencobra.github.io/cobratoolbox/latest/index.html. The installation instructions and troubleshooting tips are also available on this website.

EQUIPMENT SETUP

Initialize the COBRA toolbox.

clear;
initCobraToolbox

> Checking if git is installed ... Done.
> Checking if the repository is tracked using git ... Done.
> Checking if curl is installed ... Done.
> Checking if remote can be reached ... Done.
> Initializing and updating submodules ... Done.
> Adding all the files of the COBRA Toolbox ... Done.
> Define CB map output... set to svg.
> Retrieving models ... Done.
> TranslateSBML is installed and working properly.
> Configuring solver environment variables ...
  * [-++] ILOG_CPLEX_PATH : --> set this path manually after installing the solver ( see instructions )
  * [-++] GUROBI_PATH : C:\gurobi\7.0\win64\matlab
  * [-++] TOMLAB_PATH : --> set this path manually after installing the solver ( see instructions )
  * [-++] MOSEK_PATH : --> set this path manually after installing the solver ( see instructions )
  Done.
> Checking available solvers and solver interfaces ... Done.
> Setting default solvers ... Done.
> Saving the MATLAB path ... Done.
  * The MATLAB path was saved in the default location.

> Summary of available solvers and solver interfaces

<table>
<thead>
<tr>
<th></th>
<th>Support</th>
<th>LP</th>
<th>MILP</th>
<th>QP</th>
<th>MIQP</th>
<th>NLP</th>
</tr>
</thead>
<tbody>
<tr>
<td>cplex_direct</td>
<td>full</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>
% changeCobraSolver('glpk','all');
% changeCobraSolver('gurobi17','all');

% CBT_LP_SOLVER has been set to gurobi.
% CBT_MILP_SOLVER has been set to gurobi.
% CBT_QP_SOLVER has been set to gurobi.
% CBT_NLP_SOLVER has been set to gurobi.

% changeCobraSolver('tomlab_cplex','all');
% changeCobraSolver('gurobi6','all');

Load the **E.coli iJO1366** model.

load('iJO1366.mat');
saved_model = iJO1366;
model = saved_model;

**PROCEDURE**

1. **Tyrosine, Tryptophan, and Phenylalanine Metabolism**

The purpose of this tutorial is to identify and review the structure and capabilities of the "Tyrosine, Tryptophan, and Phenylalanine Metabolism" subsystem of the **E.coli iJO1366** model. This will
begin with an overview of the complete subsystem. This overview will be followed by more detailed descriptions of the individual L-Tyrosine, L-Tryptophan, and L-Phenylalanine biosynthesis pathways. It will conclude with a simulation that shows the maximum flux that each of these amino acids can produce in a range from anaerobic to aerobic conditions.

2. Tyrosine, Tryptophan, and Phenylalanine Metabolism Subsystem

The reactions associated with the "Tyrosine, Tryptophan, and Phenylalanine Metabolism" subsystem can be extracted from the model as shown below.

```plaintext
model = saved_model;
tyrosineTryptophanPhenylalanineSubSystems = {'Tyrosine, Tryptophan, and Phenylalanine Metabolism'};
tyrosineTryptophanPhenylalanineSubSystemReactions = model.rxs(ismember(model.subSystems, tyrosineTryptophanPhenylalanineSubSystems));
tmp,tyrosineTryptophanPhenylalanineSubSystem_rxnID] = ismember(tyrosineTryptophanPhenylalanineSubSystemReactions, model.rxs);
reactionNames = model.rxNames(tyrosineTryptophanPhenylalanineSubSystem_rxnID);
reactionFormulas = printRxnFormula(model, tyrosineTryptophanPhenylalanineSubSystemReactions, 0);
% T = table(reactionNames, reactionFormulas, 'RowNames', tyrosineTryptophanPhenylalanineSubSystemReactions);
fid = 1;
fprintf(fid, '%-12s %-60s %-50s
', 'Reaction', 'Reaction Name', 'Reaction Formula');

[nrows, ncols] = size(tyrosineTryptophanPhenylalanineSubSystemReactions);
for row = 1:nrows
    fprintf(fid, '%-12s %-60s %-50s
', tyrosineTryptophanPhenylalanineSubSystemReactions(row,:), reactionNames(row,:), reactionFormulas(row,:));
end

Table 1. The names and formulas of the reactions associated with the "Tyrosine, Tryptophan, and Phenylalanine Metabolism" subsystem.

<table>
<thead>
<tr>
<th>Reaction Name</th>
<th>Reaction Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetyl-CoA:anthranilate acetyltransferase</td>
<td>accoa_c + anth_c -&gt; acanth_c + coa_c</td>
</tr>
<tr>
<td>AnPRT</td>
<td>Anthranilate phosphoribosyltransferase</td>
</tr>
<tr>
<td>ANS</td>
<td>Anthranilate synthase</td>
</tr>
<tr>
<td>CHROM</td>
<td>Chorismate mutase</td>
</tr>
<tr>
<td>CHORS</td>
<td>Chorismate synthase</td>
</tr>
<tr>
<td>DDPA</td>
<td>3-deoxy-D-arabino-heptulosonate 7-phosphate synthetase</td>
</tr>
<tr>
<td>DHQS</td>
<td>3-dehydroquininate synthase</td>
</tr>
<tr>
<td>DHQI</td>
<td>3-dehydroquininate dehydratase, irreversible</td>
</tr>
<tr>
<td>IGPS</td>
<td>Indole-3-glycerol-phosphate synthase</td>
</tr>
<tr>
<td>PHETA1</td>
<td>Phenylalanine transaminase</td>
</tr>
<tr>
<td>PPND</td>
<td>Prephenate dehydrogenase</td>
</tr>
<tr>
<td>PPNDH</td>
<td>Prephenate dehydratase</td>
</tr>
<tr>
<td>PRAI1</td>
<td>Phosphoribosylanthranilate isomerase (irreversible)</td>
</tr>
<tr>
<td>PSCVT</td>
<td>3-phosphoshikimate 1-carboxyvinyltransferase</td>
</tr>
<tr>
<td>QUINDH</td>
<td>Quinate dehydrogenase</td>
</tr>
<tr>
<td>SHK30r</td>
<td>Shikimate dehydrogenase</td>
</tr>
<tr>
<td>SHKK</td>
<td>Shikimate kinase</td>
</tr>
<tr>
<td>TRPAS2</td>
<td>Tryptophanase (L-tryptophan)</td>
</tr>
<tr>
<td>TRPS1</td>
<td>Tryptophan synthase (indoleglycerol phosphate)</td>
</tr>
<tr>
<td>TRPSS</td>
<td>Tryptophan synthase (indole)</td>
</tr>
<tr>
<td>TYPRp</td>
<td>Phospho-L-tyrosine phosphatase (periplasmic)</td>
</tr>
<tr>
<td>TTYRTA</td>
<td>Tyrosine transaminase</td>
</tr>
</tbody>
</table>
```

The map for the "Tyrosine, Tryptophan, and Phenylalanine Metabolism" subsystem created by the Escher visualization tool [3] is shown below.
As can be seen in this figure, there are two potential pathways that can create the metabolite 'chor_c' which is common to the production of all three amino acids. This pathway as shown in this subsystem begins with either/or D-Erythrose 4-phosphate ('e4p_c') or quinate ('quin_c') and ends with the production of 'chor_c'. The primary pathway, which begins with 'e4p_c', include the reactions 'DDPA', 'DHQS', 'DHQTI', 'SHK3Dr', 'SHKK', 'PSCVT', 'CHORS'. The second pathway begins with 'quin_c' and includes the reactions 'QUINDH', 'DHQTI', 'SHK3Dr', 'SHKK', 'PSCVT', 'CHORS'.

The pathway from this intermediate metabolite ('chor_c') to L-Tryptophan then begins with 'chor_c' and passes through 'ANS' (where it adds its amino group through the conversion of L-Glutamine to L-Glutamate), ANPRT', 'PRAI', and 'IGPS'. The pathway then provides three options; the primary pathway includes 'TRPS3' and 'TRPAS2', alternates include TRPS3 and 'TRPS2'.
or 'TRPS1'. Both of these alternate pathways will require L-Serine to produce L-Tryptophan. This pathway of the subsystem also include the dead-end reaction 'ACANTHAT'. The feeding metabolite for this reaction, 'acanth_c', is not produced in the iJO1366 model.

For the production of L-Tyrosine begins with 'chor_c' and includes 'CHORM', 'PPND', and 'TYRTA' (provides the amino group from L-Glutamate). The subsystem also include the periplasmic reaction 'TYRPpp' which converts phosphotyrosine ('tyrp_p') to periplasmic L-Tyrosine ('tyr_L_p').

Finally, the production of L-Phenylalanine ('phe_L_c') begins with 'chor_c' and includes the reactions 'CHORM', 'PPNDH', and 'PHETA1' (provides the amino group from L-Glutamate).

The flux flowing through the primary pathways for each of these amino acids can be mapped onto the Escher [3] map of the subsystem using the file "Aerobic_Reaction_Flux.csv" that contains the flux data for aerobic operation with a maximum glucose uptake rate of \(-10 \text{ mmol} \cdot \text{gDW}^{-1} \cdot \text{hr}^{-1}\) and an unlimited oxygen uptake rate.

**Figure 2.** The flux flowing through the primary pathways for each of these amino acids can be mapped onto the Escher map of the subsystem using the file "Aerobic_Reaction_Flux.csv" that contains the flux data for aerobic operation with a maximum glucose uptake rate of \(-10 \text{ mmol} \cdot \text{gDW}^{-1} \cdot \text{hr}^{-1}\) and a maximum oxygen uptake rate of \(-30 \text{ mmol} \cdot \text{gDW}^{-1} \cdot \text{hr}^{-1}\). The thickness of the lines is proportional to the amount of flux flowing through each reaction. The "red" lines refer to flux flowing in the forward direction through a reaction while the "blue" lines refer to flux.
flowing in the reverse direction through a reaction. (Tyrosine_Tryptophan_PhenylalanineSubsystem_Tyrosine_Tryptophan_PhenylalanineSubsystem_Aerobic.svg)

More detail biosynthesis pathways for each of these amino acids will be described in more detail below.

3. L-Tyrosine Biosynthesis

The chemical structure for L-Tyrosine (C9H11NO3) is shown below.

![Figure 3. The chemical structure of L-Tyrosine(C9H11NO3) - Wikipedia](image)

3.1. L-Tyrosine Biosynthesis Pathways

As can be seen in Figure 2, the primary biosynthesis pathway for the production of L-Tyrosine begins with the precursor D-Erythrose 4-phosphate (‘e4p_c’) and includes the following reactions ‘DDPA’, ‘DHQS’, ‘DHQT’, ‘SHK3Dr’, ‘SHKK’, ‘PSCVT’, ‘CHORS’, ‘CHORM’, ‘PPND’, ‘TYRTA’.

To explore the possibility of other L-Tyrosine production pathways we can use the sufNet function we can identify all the reactions that can directly produce L-Tyrosine.
Table 2. List of the producers and consumers of L-Tyrosine.

A Escher figure showing all the reactions that can be used to produce L-Tyrosine is shown below.
Figure 4. A network map of all the reactions and pathways that can be used to produce L-Tyrosine (Tyrosine_Biosynthesis.json, Tyrosine_Biosynthesis.png, Tyrosine_Biosynthesis.svg).
Note that in addition to the primary pathway (‘DDPA’, ‘DHQS’, ‘DHQTi’, ‘SHK3Dr’, ‘SHKK’, ‘PSCVT’, ‘CHORS’, ‘CHORM’, ‘PPND’, ‘TYRTA’) there are several other pathways that can lead to the increased production of L-Tyrosine. On the top of the figure there is an alternate pathway (‘EX_quin_e’, ‘QUIN2tex’, ‘QUIN2pp’, ‘QUINDDH’) that begins by the diffusion of quinate (‘quin_c’) into the periplasm (‘quin_p’) and then transported into the cytoplasm (‘quin_c’) where it adds to the pool of 3-Dehydroquinolate (‘3dhq_c’) which is an intermediate step in the biosynthesis of L-Tyrosine. Another pathway (‘EX_skme’, ‘SKMtex’, ‘SKM2pp’) that can contribute to the front end of primary pathway involves the diffusion (‘skm_p’) and transport of Shikimic acid (‘skm_e’) and transport of L-Tyrosine can also be added to the cell through the proton powered transporter ‘TYRT2pp’ which bring periplasmic L-Tyrosine (tyr__l_p) that is either diffused into the cell via ‘TYRTex’ or converts diffused (‘TYRTPex’) phosphotyrosine (‘tyr_p’) to L-Tyrosine (‘tyr__l_p’) through ‘TYRTpp’.

The formulas for these L-Tyrosine producing and consuming reactions can be found as shown below.

```
model = saved_model;
TyrosineBiosynthesisReactions = transposes({'DDPA', 'DHQS', 'DHQTi', 'SHK3Dr', 'SHKK', 'PSCVT', 'CHORS', 'CHORM', 'PPND', 'TYRTA', ...
    'EX_quin_e', 'QUIN2tex', 'QUIN2pp', 'QUINDDH', 'EX_skme', 'SKMtex', 'SKM2pp', 'EX_tyr__l_e', 'TYRTex', 'TYRT2pp', ...
    'EX_tyrp_e', 'TYRTPex', 'TYRTpp'});
[tmp,TyrosineBiosynthesisReactions_rxnID] = ismember(TyrosineBiosynthesisReactions,model.rxns);
reactionNames = model.rxnNames(TyrosineBiosynthesisReactions_rxnID);
reactionFormulas = printRxnFormula(model,TyrosineBiosynthesisReactions,B);
% T = table(reactionNames, reactionFormulas, 'RowNames', TyrosineBiosynthesisReactions)
fid = 1;
fprintf(fid, '%18s %7s %5s %5s\n', 'Reaction', 'Reaction Name', 'Reaction Formula');
[rnrows,ncols] = size(TyrosineBiosynthesisReactions);
for row = 1:rnnrows
    fprintf(fid, '%18s %7s %5s %5s\n', TyrosineBiosynthesisReactions(row,:), reactionNames(row,:), reactionFormulas(row,:));
end
```

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Reaction Name</th>
<th>Reaction Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDPA</td>
<td>3-deoxy-D-arabino-heptulosonate 7-phosphate synthetase</td>
<td>e4p_c + h2o_c + pep_c -&gt; 2dda7p_c + pl_c</td>
</tr>
<tr>
<td>DHQS</td>
<td>3-dehydroquinolate synthase</td>
<td>2dda7p_c -&gt; 3dhq_c + pl_c</td>
</tr>
<tr>
<td>DHQTi</td>
<td>3-dehydroquinolate dehydratase, irreversible</td>
<td>3dhq_c -&gt; 3dhsk_c + h2o_c</td>
</tr>
<tr>
<td>SHK3Dr</td>
<td>Shikimate dehydrogenase</td>
<td>3dhsk_c + h_c + nadph_c &lt;-&gt; nadp_c + skm_c</td>
</tr>
<tr>
<td>SHKK</td>
<td>Shikimate kinase</td>
<td>adp_c + skm_c -&gt; adp_c + h_c + skm5p_c</td>
</tr>
<tr>
<td>PSCVT</td>
<td>3-phosphoshikimate 1-carboxyvinyltransferase</td>
<td>pep_c + skm5p_c &lt;-&gt; 3pse_c + pl_c</td>
</tr>
<tr>
<td>CHORS</td>
<td>Chorismate synthase</td>
<td>3pse_c -&gt; chor_c + pi_c</td>
</tr>
<tr>
<td>CHORM</td>
<td>Chorismate mutase</td>
<td>chor_c -&gt; pphn_c</td>
</tr>
<tr>
<td>PPND</td>
<td>Prephenate dehydrogenase</td>
<td>nad_c + pphn_c -&gt; 34hcpp_c + co2_c + nadh_c</td>
</tr>
<tr>
<td>TYRTA</td>
<td>Tyrosine transaminase</td>
<td>axg_c + tyr__l_c &lt;-&gt; 34hcpp_c + glu__l_c</td>
</tr>
<tr>
<td>EX_quin_e</td>
<td>Quinic acid exchange</td>
<td>quin_e -&gt;</td>
</tr>
<tr>
<td>QUIN2tex</td>
<td>Quinic acid transport via diffusion (extracellular to periplasm)</td>
<td>quin_e &lt;-&gt; quin_p</td>
</tr>
<tr>
<td>QUIN2pp</td>
<td>Quinic acid transport (periplasm)</td>
<td>quin_p &lt;-&gt; quin_c</td>
</tr>
<tr>
<td>QUINDDH</td>
<td>Quinate dehydrogenase</td>
<td>nad_c + quin_c &lt;-&gt; 3dhq_c + 2 h_c + nadh_c</td>
</tr>
<tr>
<td>EX_skme</td>
<td>Shikimate exchange</td>
<td>skm_e -&gt;</td>
</tr>
<tr>
<td>SKMtex</td>
<td>Shikimate transport via diffusion (extracellular to periplasm)</td>
<td>skm_e &lt;-&gt; skm_p</td>
</tr>
<tr>
<td>SKM2pp</td>
<td>Shikimate transport in via proton symport (periplasm)</td>
<td>skm_p + h2o_p -&gt; h_c + skm_c</td>
</tr>
<tr>
<td>EX_tyr__l_e</td>
<td>L-Tyrosine exchange</td>
<td>tyr__l_e -&gt;</td>
</tr>
<tr>
<td>TYRTex</td>
<td>L-tyrosine transport via diffusion (extracellular to periplasm)</td>
<td>tyr__l_e &lt;-&gt; tyr__l_p</td>
</tr>
<tr>
<td>TYRT2pp</td>
<td>L-tyrosine reversible transport via proton symport (periplasm)</td>
<td>h2o_p + tyr__l_p -&gt; h_c + tyr__l_c</td>
</tr>
<tr>
<td>EX_tyrp_e</td>
<td>Phosphotyrosine exchange</td>
<td>tyrp_e -&gt;</td>
</tr>
<tr>
<td>TYRTPex</td>
<td>Phospho-L-tyrosine transport via diffusion (extracellular to periplasm)</td>
<td>tyrp_e &lt;-&gt; tyrp_p</td>
</tr>
<tr>
<td>TYRTpp</td>
<td>Phospho-L-tyrosine phosphatase (periplasmic)</td>
<td>h2o_p + tyrp_p -&gt; pi_p + tyr__l_p</td>
</tr>
</tbody>
</table>

**Table 3.** Reactions names and formulas for L-Tyrosine biosynthesis reactions.
We can also find the subsystems that are associated with each of these reactions.

```plaintext
reactionSubsystems = model.subSystems(TyrosineBiosynthesis_rxnID);
% T = table(reactionNames, reactionSubsystems, 'RowNames', MethionineBiosynthesisReactions)
fid = 1;
fprintf(fid, '%-18s %-75s %-50s\r\n', 'Reaction', 'Reaction Name', 'Reaction Subsystem');

[rrows,ncols] = size(TyrosineBiosynthesisReactions);
for row = 1:rrows
    fprintf(fid, '%-18s %-75s %-50s\r\n', TyrosineBiosynthesisReactions(row,:), reactionNames(row,:), reactionSubsystems(row,:));
end
```

Table 4. Reactions names and subsystems for L-Tyrosine biosynthesis reactions.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Reaction Name</th>
<th>Reaction Subsystem</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDPA</td>
<td>3-deoxy-D-arabino-heptulosonate 7-phosphate synthetase</td>
<td>Tyrosine, Tryptophan, and Phenylalanine Metabolism</td>
</tr>
<tr>
<td>DHQS</td>
<td>3-dehydroquinate synthase</td>
<td>Tyrosine, Tryptophan, and Phenylalanine Metabolism</td>
</tr>
<tr>
<td>DHQI</td>
<td>3-dehydroquinate dehydratase, irreversible</td>
<td>Tyrosine, Tryptophan, and Phenylalanine Metabolism</td>
</tr>
<tr>
<td>SHK3Dr</td>
<td>Shikimate dehydrogenase</td>
<td>Tyrosine, Tryptophan, and Phenylalanine Metabolism</td>
</tr>
<tr>
<td>SHKK</td>
<td>Shikimate kinase</td>
<td>Tyrosine, Tryptophan, and Phenylalanine Metabolism</td>
</tr>
<tr>
<td>PSCVT</td>
<td>3-phosphoshikimate 1-carboxyvinyltransferase</td>
<td>Tyrosine, Tryptophan, and Phenylalanine Metabolism</td>
</tr>
<tr>
<td>CHORS</td>
<td>Chorismate synthase</td>
<td>Tyrosine, Tryptophan, and Phenylalanine Metabolism</td>
</tr>
<tr>
<td>CHORM</td>
<td>Chorismate mutase</td>
<td>Tyrosine, Tryptophan, and Phenylalanine Metabolism</td>
</tr>
<tr>
<td>PPND</td>
<td>Prephenate dehydrogenase</td>
<td>Tyrosine, Tryptophan, and Phenylalanine Metabolism</td>
</tr>
<tr>
<td>TYRTA</td>
<td>Tyrosine transaminase</td>
<td>Tyrosine, Tryptophan, and Phenylalanine Metabolism</td>
</tr>
<tr>
<td>EX_gln_e</td>
<td>Quinolate exchange</td>
<td>Extracellular exchange</td>
</tr>
<tr>
<td>QUIN2tex</td>
<td>Quinate transport via diffusion (extracellular to periplasm)</td>
<td>Transport, Outer Membrane Porin</td>
</tr>
<tr>
<td>QUIN2tp</td>
<td>Quinate transport (periplasm)</td>
<td>Transport, Inner Membrane</td>
</tr>
<tr>
<td>QUINDH</td>
<td>Quinate dehydrogenase</td>
<td>Tyrosine, Tryptophan, and Phenylalanine Metabolism</td>
</tr>
<tr>
<td>EX_skm_e</td>
<td>Shikimate exchange</td>
<td>Extracellular exchange</td>
</tr>
<tr>
<td>SKMtex</td>
<td>Shikimate transport via diffusion (extracellular to periplasm)</td>
<td>Transport, Outer Membrane Porin</td>
</tr>
<tr>
<td>SMK2pp</td>
<td>Shikimate transport in via proton symport (periplasm)</td>
<td>Transport, Inner Membrane</td>
</tr>
<tr>
<td>EX_tyr__L_e</td>
<td>L-Tyrosine exchange</td>
<td>Extracellular exchange</td>
</tr>
<tr>
<td>TYRTex</td>
<td>L-tyrosine transport via diffusion (extracellular to periplasm)</td>
<td>Transport, Outer Membrane Porin</td>
</tr>
<tr>
<td>TYRT2pp</td>
<td>L-tyrosine reversible transport via proton symport (periplasm)</td>
<td>Transport, Inner Membrane</td>
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<tr>
<td>EX_tyrp_e</td>
<td>Phosphotyrosine exchange</td>
<td>Extracellular exchange</td>
</tr>
<tr>
<td>TYPtx</td>
<td>Phospho-L-tyrosine transport via diffusion (extracellular to periplasm)</td>
<td>Transport, Outer Membrane Porin</td>
</tr>
<tr>
<td>TYPtp</td>
<td>Phospho-L-tyrosine phosphatase (periplasm)</td>
<td>Tyrosine, Tryptophan, and Phenylalanine Metabolism</td>
</tr>
</tbody>
</table>

3.2 L-Tyrosine Aerobic Operation

Now let's explore the flux through this pathway under aerobic conditions.

```plaintext
model = saved_model;
model = changeRxnBounds(model,'EX_g1c_0_e','-10','1'); % Set maximum glucose uptake
model = changeRxnBounds(model,'EX_o2_e','-30','1'); % Set oxygen uptake
model = changeRxnBounds(model,'Biomass_Ec_iJO1366_WT_53p95M','-0','b'); % Disable WT biomass reaction
model = changeObjective(model,'Biomass_Ec_iJO1366_core_53p95M'); % Set the objective function
FBASolution = optimizeBModel(model, 'max', 0, 0); % Perform FBA

tyrosineReactions = transpose({'DDPA', 'DHQS', 'DHQI', 'SHK3Dr', 'SHKK', 'PSCVT', 'CHORS', 'CHORM', 'PPND', 'TYRTA', ...
    'EX_gln_e', 'QUIN2tex', 'QUIN2tp', 'QUINDH', 'EX_skm_e', 'SKMtex', 'SMK2pp', 'EX_tyr__L_e', 'TYRTex', 'TYRT2pp', ...
    'EX_tyrp_e', 'TYPtx', 'TYPtp'});
[tmp,tyrosine_rxnID] = ismember(tyrosineReactions, model.rxns);
printlabeledData(tyrosineReactions, round(FBASolution.x(tyrosine_rxnID)),3)
```
<table>
<thead>
<tr>
<th></th>
<th>0.374</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDA</td>
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<tr>
<td>DHQ5</td>
<td>0.374</td>
</tr>
<tr>
<td>DHQ71</td>
<td>0.374</td>
</tr>
<tr>
<td>SHK3D</td>
<td>0.374</td>
</tr>
<tr>
<td>SHK</td>
<td>0.374</td>
</tr>
<tr>
<td>PSCVT</td>
<td>0.374</td>
</tr>
<tr>
<td>CHORS</td>
<td>0.374</td>
</tr>
<tr>
<td>CHORM</td>
<td>0.318</td>
</tr>
<tr>
<td>PPNQ</td>
<td>0.136</td>
</tr>
<tr>
<td>TYRTA</td>
<td>-0.136</td>
</tr>
<tr>
<td>EX_quin_e</td>
<td>0</td>
</tr>
<tr>
<td>QUIN2tex</td>
<td>0</td>
</tr>
<tr>
<td>QUIN2tpp</td>
<td>0</td>
</tr>
<tr>
<td>QUINDH</td>
<td>0</td>
</tr>
<tr>
<td>EX_skn_e</td>
<td>0</td>
</tr>
<tr>
<td>SKMtex</td>
<td>0</td>
</tr>
<tr>
<td>SKM2pp</td>
<td>0</td>
</tr>
<tr>
<td>EX_tyr__L_e</td>
<td>0</td>
</tr>
<tr>
<td>TYRtex</td>
<td>0</td>
</tr>
<tr>
<td>TYRt2pp</td>
<td>0</td>
</tr>
<tr>
<td>EX_tyrp_e</td>
<td>0</td>
</tr>
<tr>
<td>TYRptex</td>
<td>0</td>
</tr>
<tr>
<td>TYRpp</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 5.** Flux values for L-Tyrosine pathways under normal aerobic conditions.

This can be seen using an Escher [3] plot with the "Aerobic_Reaction_Flux.csv" data.
Figure 5. L-Tyrosine production under aerobic conditions. The thickness of a line is proportional to the amount of flux flowing through a reaction, the "red" lines correspond to reactions operating in the forward direction while the "blue" lines correspond to reactions operating in the reverse direction (Tyrosine_Biosynthesis_Aerobic.png and Tyrosine_Biosynthesis_Aerobic.svg).

In this simple case only the primary pathway supports active flux.
3.3 Excess L-Tyrosine Production

When a cell is producing a recombinant protein, it might be required to produce additional L-tyrosine for the desired bioproduct. What is the maximum amount of L-tyrosine that can be produced for a given growth-rate?

```matlab
model = saved_model;
model = changeRxnsBounds(model,'EX_glc__D_e',-10,'l'); % Set maximum glucose uptake
model = changeRxnsBounds(model,'EX_o2_e',-30,'l'); % Set oxygen uptake
model = changeRxnsBounds(model,'BIOMASS_Ec_iJO1366_WT_53p95M',0,'b'); % Disable WT biomass reaction
model = changeRxnsBounds(model,'BIOMASS_Ec_iJO1366_core_53p95M'); % Set the objective function
FBA_solution = optimizeModel(model,'max'); % Perform FBA to find optimal growth-rate
model = changeRxnsBounds(model,'BIOMASS_Ec_iJO1366_WT_53p95M',FBA_solution.f,'b'); % Set fixed growth-rate
model = addDemandReaction(model,'tyr_L_c');

DM_tyr___L_c   tyr___L_c   ->
[tmp,tyrosine_MAX_rxnID] = ismember({'DM_tyr___L_c'},model.rxns);
model = changeObjective(model,'DM_tyr___L_c'); % Set the objective function
FBA_solution = optimizeModel(model,'max'); % Perform FBA to find optimal growth-rate
%xMax = xMax; %printLabeledData({'DM_tyr___L_c'},round(FBA_solution_tyrrosine.x(tyrosine_MAX_rxnID),3))
%xMin = 0.5;
xMax = FBA_solution.f;
xInc = (xMax - xMin)/20;
x = xMin;
excessTyrosine = [];
for i = 1:21
    model = changeRxnsBounds(model,'BIOMASS_Ec_iJO1366_WT_53p95M',x,'b'); % Set fixed growth-rate
    FBA_solution_tyrrosine = optimizeModel(model,'max'); % Perform FBA
    excessTyrosine(i) = FBA_solution_tyrrosine.f;
    growthRate(i) = x;
    x = x + xInc;
end
% growthRate = 0:20;
figure(1);
plot(growthRate,excessTyrosine)
title('Excess L-Tyrosine');
xlabel('Growth-rate (h-1)'); ylabel('Excess Tyrosine (mmol.gDW-1.h-1)');
```

Figure 6. The maximum amount of excess L-Tyrosine that can be produced for a given growth-rate.

This figure illustrates that as the need for excess L-Tyrosine increases the growth-rate will need to decrease. In this figure we can see that the excess L-Tyrosine flux can increase from 0.02426 mmol·gDW\(^{-1}\)·hr\(^{-1}\) when the cell is at maximum growth-rate to 2.817 mmol·gDW\(^{-1}\)·hr\(^{-1}\) when it is at 50% of that optimal growth-rate. Finally, to increase the L-Tyrosine flux beyond these levels will require using the pathways that allow for the transport of L-Tyrosine from the extracellular media.

4. L-Tryptophan Biosynthesis

The chemical structure for L-Tryptophan ($C_9H_9N_4O_2$) is shown below.

![L-Tryptophan structure](image)

Figure 7. The chemical structure of L-Tryptophan($C_9H_9N_4O_2$) - Wikipedia

3.1. L-Tryptophan Biosynthesis Pathways

As can be seen in the Figure 1 the primary biosynthesis pathway for the production of L-Tryptophan begins with the precursor ‘e4p_c’ and includes the following reactions ‘DDPA’, ‘DHQS’, ‘DHQTi’, ‘SHK3Dr’, ‘SHKK’, ‘PSCTV’, ‘CHORS’, ‘ANS’, ‘ANPRT’, ‘PRAII’, ‘IGPS’, ‘TRPS1’, ‘TRPS2’, ‘TRPS3’, ‘TRPAS2’, and ‘QUINDH’. To explore the possibility of other L-Tyrosine production pathways we can use the surfNet function we can identify all the reactions that can directly produce L-Tyrosine.

```
surfNet(model, 'trp_l_c')
```

Met #977 trp_l_c, L-Tryptophan, C13H12N2O2
Consuming reactions:
Biomass_Ec_iJO1366_53p95M_E. coli biomass objective function (iJO1366) - WT - with 53.95 GAM estimate

0.000223 10thr_c + 0.000223 2dwdhq18_c + 2.5e-05 2fe2s_c + 0.00024 4fe4s_c + 0.000223 5nhf_c + 0.000279 accoa_c + 0.000223 adocb1_c + 0.49915 ala_l_c + 0.000223 amet_c + 0.28742 arg_l_c + 0.23423 asn_l_c + 0.23423 asp_l_c + 54.12 atp_c + 0.000116 bncodgp_c + 2e-06 btrn_c + 0.00049 c2a1_c + 0.000223 chor_c + 0.00049 cl_c + 0.000168 coa_c + 2.6e-05 cobalt2_c + 0.1298 ctp_c + 0.000674 cu2_c + 0.88988 cys_l_c + 0.004886 datp_c + 0.025612 dctp_c + 0.025612 dgtp_c + 0.024806 dtpp_c + 0.000223 enter_c + 0.000223 fad_c + 0.000638 fe3c_c + 0.007428 fe3_c + 0.0002335 gln_l_c + 0.25571 glu_c + 0.5953 gln_c + 0.15419 glycogen_c + 0.000223 gndh_c + 0.28912 gp_c + 48.7529 h2o_c + 0.000223 hemo_c + 0.000223 kcs_c + 0.28231 lie_c + 0.18569 k_c + 0.43778 leu_l_c + 3e-05 ligo_p_c + 0.33345 lys_l_c + 3.1e-05 malcoa_c + 0.14934 met_l_c + 0.0008253 mg2_c + 0.000223 mlthf_c + 0.000658 mn2_c + 7e-06 mobd_c + 7e-06 noocdcp_c + 7e-06 noocogp_c + 0.000223 nq18_c + 0.001787 nad_c + 4.5e-05 nadh_c + 0.000112 nadp_c + 0.000335 nadph_c + 0.012379 nh4_c + 0.000307 n12_c + 0.023366 pe161_c + 0.000618 pe1611_c + 0.000497 pe181_c + 0.005767 pg160_c + 0.000439 pg161_c + 0.002288 pg1611_c + 0.18002 phe_l_c + 0.000223 phene_c + 0.2148 pro__c + 0.0337 ptrc_c + 0.000223 pydxp5p_c + 0.000234 q2h2_c + 0.000223 ribrflv_c + 0.20968 ser_l_c + 0.000223 shene_c + 0.000417 so4_c + 0.000674 spnf_c + 9.8e-05 sucacoa_c + 0.000223 thr_c + 0.000223 thmp_c + 0.24651 thr_l_c + 0.055234 trpl_c + 0.13999 tyr_l_c + 5.6e-05 udcdph_c + 0.1401 utpc_c + 0.04118 val_l_c + 0.000324 zn2_c + 0.0008151 collapa_e + 0.002944 clp160_p + 0.00229 clp161_p + 0.00111 clp181_p + 0.001345 murein3xp4p_p + 0.000005 murein3xp4p_p + 0.000673 murein3xp4p4p_p + 0.031798 pe160_p + 0.024732 pe161_p + 0.012747 pe181_p + 0.004892 pg160_p + 0.003085 pg161_p + 0.001661 pg181_p -> 53.95 adp_c + 53.95 h_c + 53.9459 pi_c + 0.7493 ppl_c

Biomass_Ec_iJO1366_core_53p95M_E. coli biomass objective function (iJO1366) - core - with 53.95 GAM estimate

0.000223 10thr_c + 2.56e-05 2fe2s_c + 0.000223 2ooph_c + 0.00026 4fe4s_c + 0.51369 ala_l_c + 0.000223 amet_c + 0.29579 arg_l_c + 0.24105 asn_l_c + 0.24105 asp_l_c + 54.1284 atp_c + 0.000122 bncodgp_c + 2e-06 btrn_c + 0.005265 c2a1_c + 0.000265 cl_c + 0.00057 coa_c + 2.5e-05 cobalt2_c + 0.13531 ctp_c + 0.000709 cu2_c + 0.09158 cys_l_c + 0.026166 datp_c + 0.000223 dgtp_c + 0.026166 dtpp_c + 0.000223 fad_c + 0.009672 fe3_c + 0.000708 fe3_c + 0.009672 fe3_c + 0.000708 fe3_c + 0.26316 gln_l_c + 0.26316 glu_l_c + 0.61264 gly_c + 0.2151 gtp_c + 48.6815 h2o_c + 0.094738 his_l_c + 0.29953 lie_c + 0.19519 k_c + 0.45853 leu_l_c + 0.34316 lys_l_c + 0.15369 met_l_c + 0.008675 mg2_c + 0.000223 mlthf_c + 0.000691 mn2_c + 7e-06 mobd_c + 0.0001381 nad_c + 0.000447 nadp_c + 0.00023 nh4_c + 0.00032 n12_c + 0.01786 pe160_c + 0.054154 pe161_c + 0.18527 phe_l_c + 0.000223 phene_c + 0.22106 pro__c + 0.000223 pydxp5p_c + 0.000223 ribrflv_c + 0.21579 ser_l_c + 0.000223 shene_c + 0.004338 spn_c + 0.000223 thr_c + 0.000223 thmp_c + 0.25360 thr_l_c + 0.005843 trpl_c + 0.14139 tyr_l_c + 5.6e-05 udcdph_c + 0.1441 utpc_c + 0.42316 val_l_c + 0.000341 zn2_c + 0.019456 kdo121pd4a_e + 0.013894 murein5xp4p_p + 0.04596 pe160_p + 0.02106 pe161_p -> 53.95 adp_c + 53.95 h_c + 53.9457 pi_c + 0.7739 ppl_c

TRPAS2 Tryptophanase (L-tryptophan)

h2o_c + trp_l_c -> indole_c + nh4_c + pyr_c

TRPTRS Tryptophanyl-tRNA synthetase

atp_c + trnatrp_c + trp_l_c -> ampc_c + ppl_c + trtrna_c

Producing reactions:

MTRPOX N-nethyltrytophan oxidase

Ntrp_c + h2o_c + o2_c -> fald_c + h2o2_c + trp_l_c

TRP51 Tryptophan synthase (Indoleglycerol phosphate)
\[ 3 \text{g3p_c} + \text{ser}_\text{L_c} \rightarrow g3p_c + \text{h2o_c} + \text{trp}_\text{L_c} \]

**#2668** TRPS2  Tryptophan synthase (Indole)
\[ \text{indole_c} + \text{ser}_\text{L_c} \rightarrow \text{h2o_c} + \text{trp}_\text{L_c} \]

**#2471** TRP12pp  L-Tryptophan reversible transport via proton symport (periplasm)
\[ \text{h}_\text{p} + \text{trp}_\text{L_p} \leftrightarrow \text{h}_\text{c} + \text{trp}_\text{L_c} \]

Show previous steps...

**Table 6. List of the producers and consumers of L-Tryptophan**

An Escher figure showing all the reactions that can be used to produce L-Tryptophan is shown below.
In addition to the primary pathway (‘DDPA’, ‘DHQS’, ‘DHQTI’, ‘SHK3Dr’, ‘SHKk’, ‘PSCVT’, ‘CHORS’, ‘ANS’, ‘ANPRT’, ‘PRAII’, ‘IGPS’, ‘TRPS1’, ‘TRPS2’, ‘TRPS3’, ‘TRPAS2’, and ‘QUINH’), it can be seen that there are two other potential pathways for the production of L-tryptophan. On the left of the figure there is an alternate pathway (‘EX_quin_e’, ‘QUIN2tex’, ‘QUIN2hpp’, and ‘QUINH’) that begins by the diffusion of quinate (‘quin_e’) into the periplasm (‘quin_p’) and then transported into the cytoplasm (‘quin_c’) where it adds to the pool of 3-Dehydroquininate (‘3dhq_c’) which is an intermediate step in the biosynthesis of L-tryptophan. Another pathway (‘EX_skm_e’, ‘SKM1ex’, ‘SKM12pp’) based on shikimate (‘skm_c’) can be used to contribute to the front end of primary pathway involves the diffusion (‘skm_e’) and transport of periplasmic shikimate (‘skm_p’). The iJO1366 model includes the dead-end reaction ‘MTRPOX’ since there is no source of N-methyltryptophan oxidase (‘NMtrp_c’) within the model. L-tryptophan can also be added to the cell through the proton powered transporter ‘TRP2trpp’ which bring periplasmic L-
tryptophan (trp L-p) that is diffused into the cell via 'EX_trp L-c' and 'TRPtx'.

The formulas for these L-Tryptophan producing and consuming reactions can be found as shown below.

```r
model = saved_model;
TryptophanBiosynthesisReactions = transpose(('[DDPA','DHQS','DHQTI','SHK3Dn','SHK','PSCTV','CHORS','CHORM','PPND','TYRTA', ...
    'EX_quin_e','QUIN2tex','QUIN2tpp','QUIN2NH','EX_skml_e','SMKtex','SKM2tpp','EX_lyn__L_e','TYRtex','TYR2tpp',...
    'EX_lyn__e','TYRptex','TYRppp'));
[tmp,TryptophanBiosynthesis_rxnID] = ismember(TryptophanBiosynthesisReactions,model.rxn);
reactionNames = model.rxnNames(TryptophanBiosynthesis_rxnID);
reactionFormulas = printRxnFormula(model,TryptophanBiosynthesisReactions,0);
% T = table(reactionNames,reactionFormulas,'RowNames',TryptophanBiosynthesisReactions)
fid = 1;
fprintf(fid,'%18s %7s %5s\n','Reaction','Reaction Name','Reaction Formula');

[nrows,nocols] = size(TryptophanBiosynthesisReactions);
for row = 1:nrows
    fprintf(fid,'%18s %7s %5s\n',TryptophanBiosynthesisReactions(row,:), reactionNames(row,:), reactionFormulas(row,:));
end

DDPA  3-deoxy-D-arabino-heptulosonate 7-phosphate synthetase  e4p_c + h2o_c + pep_c  -> 2dda7p_c + pi_c
DHQS  3-dehydroquininate synthase  2dda7p_c  -> 3dqo_c + pi_c
DHQTI 3-dehydroquininate dehydratase, irreversible  3dqo_c  -> 3dshk_c + h2o_c
SHK3D SHikinate dehydrogenase  3dshk_c + h_c + nadp_c  => nadp_c + skm_c
SHK   Shikinate kinase  3hsk_c + skm_c  -> adp_c + h_c + skm5p_c
PSCTV 3-phosphoshikimate 1-carboxyvinyltransferase  pep_c + skm5p_c  => 3psme_c + pi_c
CHORS Chorismate synthase  3psme_c  -> chom_c + pi_c
CHORM Chorismate mutase  chom_c  -> ppph_c
PPND Prephenate dehydrogenase  nadp_c + ppph_c  -> 34hpp_c + co2_c + nadh_c
TYRTA Tyrosine transaminase  3h2p_c + tyr__l_c  => 34hpp_c + glu_l_c
EX_quin_e Quinate exchange  quin_e  ->
QUIN2tex Quinate transport via diffusion (extracellular to periplasm)  quin_e  <= quin_p
QUIN2tpp Quinate transport (periplasm)  quin_p  <= quin_c
QUIN2NH Quinate dehydrogenase  nad_c + quin_c  <= 3dqo_c + 2 h_c + nadh_c
EX_skml_e Shikinate exchange  skm_e  ->
SKMtex Shikinate transport via diffusion (extracellular to periplasm)  skm_e  <= skm_p
SKM2tpp Shikinate transport in via proton symport (periplasm)  skm_p  -> h_c + skm_c
EX_lyn__L_e L-Tyrosine exchange  h_c + skm_p  -> h_c + skm_c
TYRtex L-Tyrosine transport via diffusion (extracellular to periplasm)  tyr__l_c  -> tyr__l_p
TYR2tpp L-Tyrosine reversible transport via proton symport (periplasm)  h_c + tyr__l_p  <= h_c + tyr__l_c
EX_lyn__e Phosphorylosine exchange  tyr__l_p  ->
TYRptex Phospho-L-Tyrosine transport via diffusion (extracellular to periplasm)  tyr_p  <= tyr_p
TYRppp Phospho-L-Tyrosine phosphatase (periplasmic)  h2o_p + tyr_p  -> pi_p + tyr__l_p
```

Table 7. Reactions names and formulas for L-Tryptophan biosynthesis reactions.

We can also find the subsystems that are associated with each of these reactions.

```r
reactionSubsystems = model.subSystems(TryptophanBiosynthesis_rxnID);
% T = table(reactionNames,reactionSubsystems,'RowNames',TryptophanBiosynthesisReactions)
fid = 1;
fprintf(fid,'%18s %7s %5s\n','Reaction','Reaction Name','Reaction Subsystem');
```
### Table 8. Reactions names and subsystems for L-Tryptophan biosynthesis reactions.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Reaction Name</th>
<th>Reaction Subsystem</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDPA</td>
<td>3-deoxy-D-arabino-heptulosonate 7-phosphate synthetase</td>
<td>Tyrosine, Tryptophan, and Phenylalanine Metabolism</td>
</tr>
<tr>
<td>DHQS</td>
<td>3-dehydroquinase synthase</td>
<td>Tyrosine, Tryptophan, and Phenylalanine Metabolism</td>
</tr>
<tr>
<td>DHQTl</td>
<td>3-dehydroquinase dehydratase, irreversible</td>
<td>Tyrosine, Tryptophan, and Phenylalanine Metabolism</td>
</tr>
<tr>
<td>SHKDr</td>
<td>Shikimate dehydrogenase</td>
<td>Tyrosine, Tryptophan, and Phenylalanine Metabolism</td>
</tr>
<tr>
<td>SHK</td>
<td>Shikimate kinase</td>
<td>Tyrosine, Tryptophan, and Phenylalanine Metabolism</td>
</tr>
<tr>
<td>PSCVT</td>
<td>3-phosphoshikimate 1-carboxyvinyltransferase</td>
<td>Tyrosine, Tryptophan, and Phenylalanine Metabolism</td>
</tr>
<tr>
<td>CHORS</td>
<td>Chorismate synthase</td>
<td>Tyrosine, Tryptophan, and Phenylalanine Metabolism</td>
</tr>
<tr>
<td>CHORM</td>
<td>Chorismate mutase</td>
<td>Tyrosine, Tryptophan, and Phenylalanine Metabolism</td>
</tr>
<tr>
<td>PPND</td>
<td>Prephenate dehydrogenase</td>
<td>Tyrosine, Tryptophan, and Phenylalanine Metabolism</td>
</tr>
<tr>
<td>TYRITA</td>
<td>Tyrosine transaminase</td>
<td>Tyrosine, Tryptophan, and Phenylalanine Metabolism</td>
</tr>
<tr>
<td>EX_quin_e</td>
<td>Quinate exchange</td>
<td>Extracellular exchange</td>
</tr>
<tr>
<td>QUIN2tex</td>
<td>Quinate transport via diffusion (extracellular to periplasm)</td>
<td>Transport, Outer Membrane Porin</td>
</tr>
<tr>
<td>QUIN2tpp</td>
<td>Quinate transport (periplasm)</td>
<td>Transport, Inner Membrane</td>
</tr>
<tr>
<td>QUINNDH</td>
<td>Quinate dehydrogenase</td>
<td>Tyrosine, Tryptophan, and Phenylalanine Metabolism</td>
</tr>
<tr>
<td>EX_skm_e</td>
<td>Shikimate exchange</td>
<td>Extracellular exchange</td>
</tr>
<tr>
<td>SKMtex</td>
<td>Shikimate transport via diffusion (extracellular to periplasm)</td>
<td>Transport, Outer Membrane Porin</td>
</tr>
<tr>
<td>SKMtpp</td>
<td>Shikimate transport in via proton symport (periplasm)</td>
<td>Transport, Inner Membrane</td>
</tr>
<tr>
<td>EX_tyr__l_e</td>
<td>L-Tyrosine exchange</td>
<td>Extracellular exchange</td>
</tr>
<tr>
<td>TYRtex</td>
<td>L-tyrosine transport via diffusion (extracellular to periplasm)</td>
<td>Transport, Outer Membrane Porin</td>
</tr>
<tr>
<td>TYRtpp</td>
<td>L-tyrosine reversible transport via proton symport (periplasm)</td>
<td>Transport, Inner Membrane</td>
</tr>
<tr>
<td>EX_tyrp_e</td>
<td>Phosphotyrosine exchange</td>
<td>Extracellular exchange</td>
</tr>
<tr>
<td>TRPtex</td>
<td>Phospho-L-tyrosine transport via diffusion (extracellular to periplasm)</td>
<td>Transport, Outer Membrane Porin</td>
</tr>
<tr>
<td>TRPp</td>
<td>Phospho-L-tyrosine phosphatase (periplasmic)</td>
<td>Tyrosine, Tryptophan, and Phenylalanine Metabolism</td>
</tr>
</tbody>
</table>

### 4.2 L-Tryptophan Aerobic Operation

Now let's explore the flux through this pathway under aerobic conditions.

```plaintext
model = saved_model;
model = changeRxnBounds(model,'EX_glc_D_e-',10,'1'); % Set maximum glucose uptake
model = changeRxnBounds(model,'EX_o2_e','-30','1'); % Set oxygen uptake
model = changeRxnBounds(model,'Biomass_Ec_1301366_WT_S3p95M',-0,'b'); % Disable WT biomass reaction
model = changeObjective(model,'Biomass_Ec_1301366_core_S3p95M'); % Set the objective function
FBAsolution = optimizeCoModel(model,'max',0,0); % Perform FBA
tryptophanReactions = transpose(['DDPA','DHQS','DHQTl','SHK3Dr','SHK','PSCVT','CHORS','ANS','ANPRt','PRAIi',
                                 'TIGS','TRPS1','TRPS2','TRPS5','TRPAS2','EX_quin_e','QUIN2tex','QUIN2tpp','QUINNDH','MTPOX',
                                 'EX_trp__l_e','TRPtex']);
[tmp,tryptophan_rxnID] = ismember(tryptophanReactions,model.rxns);
printLabeledData(tryptophanReactions, round(FBAsolution.x(tryptophan_rxnID),3))
```

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDPA</td>
<td>0.374</td>
</tr>
<tr>
<td>DHQS</td>
<td>0.374</td>
</tr>
<tr>
<td>DHQTl</td>
<td>0.374</td>
</tr>
<tr>
<td>SHK3Dr</td>
<td>0.374</td>
</tr>
<tr>
<td>SHK</td>
<td>0.374</td>
</tr>
<tr>
<td>PSCVT</td>
<td>0.374</td>
</tr>
</tbody>
</table>
Table 9. Flux values for L-Tryptophan pathways under normal aerobic conditions.

This can be seen using an Escher [3] plot with the "Aerobic_Reaction_Flux.csv" data.
Figure 9. L-Tryptophan production under aerobic conditions. The thickness of a line is proportional to the amount of flux flowing through a reaction, the "red" lines correspond to reactions operating in the forward direction while the "blue" lines correspond to reactions operating in the reverse direction (Tryptophan_Biosynthesis_Aerobic.png and Tryptophan_Biosynthesis_Aerobic.svg).

In this simple case only the primary pathway ('DDPA', 'DHQS', 'DHQTI', 'SHK3Dr', 'SHK2', 'PSCVT', 'CHORS', 'ANS', 'ANPRT', 'PRAII', 'IGPS', 'TRPS3', 'TRPS2') supports active flux.

4.3 Excess L-Tryptophan Production

When a cell is producing a recombinant protein, it might be required to produce additional L-Tryptophan for the desired bioproduct. What is the maximum amount of L-Tryptophan that can be produced for a given growth-rate?

```matlab
model = saved_model;
model = changeRxnBounds(model,'EX_glc_D_e','-10','1'); % Set maximum glucose uptake
```
```matlab
model = changeRxnBounds(model,'EX_o2_e',-30,'1'); % Set oxygen uptake
model = changeRxnBounds(model,'BIOMASS_Ec_iJO1366_WT_S3p95M',-0,'b'); % Disable WT biomass reaction
model = changeObjective(model,'BIOMASS_Ec_iJO13166_core_S3p95M'); % Set the objective function
FBA_solution = optimizeCbModel(model,'max'); % Perform FBA to find optimal growth-rate
model = changeRxnBounds(model,'BIOMASS_Ec_iJO1366_WT_S3p95M',FBA_solution.f,'b'); % Set fixed growth-rate
model = addDemandReaction(model, 'trp__L_c');

[tmp,tryptophan_MAX_rxnID] = ismember({{'DM_trp__L_c'},model.rxns});
model = changeObjective(model,'DM_trp__L_c'); % Set the objective function
FBA_solution_tryptophan = optimizeCbModel(model,'max'); % Perform FBA to find optimal growth-rate
xMin = 0.5;
xMax = FBA_solution.f;
xInc = (xMax - xMin)/20;
x = xMin;
excessTryptophan = [];
growthRate = [];
for i = 1:21
    model = changeRxnBounds(model,'BIOMASS_Ec_iJO1366_WT_S3p95M',x,'b'); % Set fixed growth-rate
    FBA_solution_tryptophan = optimizeCbModel(model,'max'); % Perform FBA
    excessTryptophan(i) = FBA_solution_tryptophan.f;
growthRate(i) = x;
x = x + xInc;
end
plot(growthRate,excessTryptophan)
title('Excess L-Tryptophan');
xlabel('Growth-rate (h^-1)'); ylabel('Excess Tryptophan (mmol.gDW^-1.h^-1)');
```

![Graph showing the relationship between growth rate and excess L-Tryptophan](image)
Figure 10. The maximum amount of excess L-Tryptophan that can be produced for a given growth-rate.

This figure illustrates that as the need for excess L-Tryptophan increases the growth-rate will need to decrease. In this figure we can see that the excess L-Tryptophan flux can increase from 0.0195 mmol · gDW⁻¹ · hr⁻¹ when the cell is at maximum growth-rate to 2.27 mmol · gDW⁻¹ · hr⁻¹ when it is at 50% of that optimal growth-rate. Finally, to increase the L-Tryptophan flux beyond these levels will require using the pathways that allow for the transport of L-Tryptophan from the extracellular media.

5. L-Phenylalanine Biosynthesis

The chemical structure for L-Phenylalanine (C₆H₇NO₂) is shown below.

![Chemical Structure of L-Phenylalanine](image)

5.1. L-Phenylalanine Biosynthesis Pathways

As can be seen in Figure 1 the primary biosynthesis pathway for the production of L-Phenylalanine begins with the precursor D-Erythrose 4-phosphate ('e4p_c') and includes the following reactions 'DDPA', 'DHQS', 'DHQTI', 'SHK3D', 'SHKK', 'PSCVT', 'CHORS', 'CHORM', 'PPNDH', 'PHETA1'.

To explore the possibility of other L-Phenylalanine production pathways we can use the sufNet function we can identify all the reactions that can directly produce L-Phenylalanine.
0.005381 murein4npd_p + 0.00548 murein4npd_p + 0.00673 murein4npd_p
+ 0.000187 pe161_p + 0.004732 pe161_p + 0.017477 pe181_p + 0.004892 pe180_p
+ 0.00380 pg161_p + 0.001961 pg181_p -> 53.95 adp_c + 53.95 h_c + 53.95 psi_c + 0.74983 psi_c
#Biomass_Ec_iJ01366_core_53p95M E. coli biomass objective function (1J01366) - core - with 53.95 GAM estimate
0.000223 10thf_c + 2.6e-05 2fe2s_c + 0.000223 2ohph_c + 0.00026 4fe4s_c
+ 0.51369 1al__l_c + 0.000223 amet_c + 0.29579 arg__l_c + 0.24105 asn__l_c
+ 0.24105 asp__l_c + 54.1248 atp_c + 0.000122 bmecogdp_c + 2e-06 btm_c
+ 0.00520 ca2_c + 0.00520 cli_c + 0.000576 cao_c + 2.5e-05 cobalt2_c
+ 0.13351 ctp_c + 0.000789 cu2_c + 0.09158 cyu__l_c + 0.026166 datp_c
+ 0.027017 dctp_c + 0.027017 dgtc_p + 0.026166 dttbp_c + 0.000223 rad_c
+ 0.006715 fe2_c + 0.007808 fe3_c + 0.26316 gln__l_c + 0.26316 glu__l_c
+ 0.61264 gly_c + 0.2151 gtp_c + 48.6015 h2o_c + 0.094738 his__l_c
+ 0.29953 1le__l_c + 0.19519 k_c + 0.45053 leu__l_c + 0.34316 lys__l_c
+ 0.15369 met__l_c + 0.008675 mg2_c + 0.000223 mnthf_c + 0.000691 mn2_c
+ 7e-06 nobd_c + 0.001831 nad_c + 0.000447 naph_c + 0.013013 nh3_c
+ 0.00032 n12_c + 0.017868 pe161_c + 0.054154 pe161_c + 0.18527 phe__l_c
+ 0.000223 phene_c + 0.21106 pro__l_c + 0.000223 pyoxyp_c + 0.000223 ribflv_c + 0.21579 ser__l_c + 0.000223 sheme_c + 0.04338 so4_c + 0.000223 thr__l_c + 0.000223 thmpc_c + 0.25369 thr__l_c + 0.056843 trp__l_c + 0.1379 tyr__l_c + 5.5e-05 ucdpdp_c + 0.1441 utp_c + 0.42316 val__l_c + 0.000341 zn2_c + 0.019456 kdo21lipd4_e + 0.013894 murein5px4p_p + 0.045946 pe160_p
+ 0.02106 pe161_p -> 53.95 adp_c + 53.95 h_c + 53.95 psi_c + 0.7739 psi_c
#2466 TRPAS2 Tryptophanase (L-tryptophan)
    h2o_c + trp__l_c <-> indole_c + nh4_c + pyr_c
#2470 TRPTRS5 Tryptophanyl-tRNA synthetase
    atp_c + trnLnc_c + trp__l_c -> ampc_c + psi_c + tprtrna_c
#2584 DM_trp__l_c DM_trp__l_c
    trp__l_c ->
Producing reactions:
#1851 MTRPOX N-methyltryptophan oxidase
    Ntrp_c + h2o2_c + o2_c -> fald_c + h2o2_c + trp__l_c
#2467 TRP51 Tryptophan synthase (indoleglycerol phosphate)
    3ig3p_c + ser__l_c -> g3p_c + h2o2_c + trp__l_c
#2658 TRP12 Tryptophan synthase (indole)
    indole_c + ser__l_c -> h2o2_c + trp__l_c
#2471 TRPt2rpp L-tryptophan reversible transport via proton symport (periplasm)
    h_c + trp__l_c <-> h_c + trp__l_c
Show previous steps...

Table 10. List of the producers and consumers of L-Phenylalanine

An Escher figure showing all the reactions that can be used to produce L-Phenylalanine is shown below.
Figure 12. A network map showing all the pathways and reactions that can produce L-Phenylalanine (Phenylalanine_Biosynthesis.json, Phenylalanine_Biosynthesis.png, Phenylalanine_Biosynthesis.svg).

In addition to the primary pathway ('DDPA', 'DHQS', 'DHQTI', 'SHK3Dr', 'SHK', 'PSCVT', 'CHORS', 'CHORM', 'PPNDH', 'PHETA1'), it can be seen that there are other potential pathways for the production of L-Phenylalanine. On the top of the figure there is an alternate pathway ('EX_quin_e', 'QUIN2tex', 'QUIN2pp', and 'QUINDH') that begins by the diffusion of quinate ('quin_e') into the periplasm ('quin_p') and then transported into the cytoplasm ('quin_c') where it adds to the pool of 3-Dehydroquinate ('3dqha_c') which is an intermediate step in the biosynthesis of L-Phenylalanine. Another pathway ('EX_skm_e', 'SKMtex', 'SKM2pp') based on shikimate (skm_c) can be used to contribute to the front end of primary pathway involves the diffusion ('skm_e') and transport of periplasmic shikimate ('skm_p'). L-phenylalanine can also be added to the cell through the proton powered transporter 'PHEI2rpp' which bring periplasmic L-Phenylalanine ('phe__l_p') that is diffused into the cell via 'EX_phe__l_e' and 'PHEItex'.

The formulas for these L-Phenylalanine producing and consuming reactions can be found as shown below.

```python
model = saved_model;
PhenylalanineBiosynthesisReactions = transpose([[HSST', 'SHSL1', 'CYSITL', 'METS', 'HCYSMT', 'HCYSMT2', ...
    'TYRL', 'LIPOS', 'CPPP6G02', 'BTSS', 'EX_metsox_S_L_e', 'METSOX1tex', 'METSOX1abcpp', 'METSOX1R1', ...
    'EX_metsox_R_L_e', 'METSOX2tex', 'METSOX2abcpp', 'METSOX2R2', 'EX_met__l_e', 'METtex', 'METabcpp']);
```
Table 11. Reactions names and formulas for L-Phenylalanine biosynthesis reactions.

We can also find the subsystems that are associated with each of these reactions.
Table 12. Reactions names and subsystems for L-Phenylalanine biosynthesis reactions.

5.2 L-Phenylalanine Aerobic Operation

Now let’s explore the flux through this pathway under aerobic conditions.

```matlab
model = saved_model;
model = changeRxnBounds(model,'EX_glc_D_e','-10','1'); % Set maximum glucose uptake
model = changeRxnBounds(model,'EX_o2_e','-30','1'); % Set oxygen uptake
model = changeRxnBounds(model,'Biomass_Ec_iJO1366_WT_3p95M',-1,'b'); % Disable WT biomass reaction
model = changeRxnBounds(model,'Biomass_Ec_iJO1366_core_3p95M'); % Set the objective function
FBAsolution = optimizeChModel(model,'max',0,0); % Perform FBA
phenylalanineReactions = transpose(['DDPA','DHQS','DHQT1','SHK3Dr','SHKX','PSCVT','CHORS','CHORM','PPNDH',... 'PHETA1','EX_quin_e','QUIN2tex','QUIN2tp','QUINHD','EX_phe__e','PHEtedx','PHEt2rpp']);
[tmp,phenylalanine_rxnID] = ismember(phenylalanineReactions,model.rxns);
printLabeledData(phenylalanineReactions,round(FBAsolution.x(phenylalanine_rxnID),3))
```

Table 13. Aerobic flux values for L-Phenylalanine biosynthesis reactions.
These fluxes can be seen in the following Escher-based visualization [3].

Figure 13. Aerobic fluxes flowing through the L-Phenylalanine biosynthesis pathways. The thickness of a line is proportional to the amount of flux flowing through a reaction, the "red" lines correspond to reactions operating in the forward direction while the "blue" lines correspond to reactions operating in the reverse direction (Phenylalanine_Biosynthesis_Aerobic.svg or Phenylalanine_Biosynthesis_Aerobic.png).

Again we can see, that under aerobic conditions, it is the primary pathway that supports all the flux to production of L-Phenylalanine.

5.3 Excess L-Phenylalanine Production
When a cell is producing a recombinant protein, it might be required to produce additional L-Phenylalanine for the desired bioproduct. What is the maximum amount of L-Pphenylalanine that can be produced for a given growth-rate?

```matlab
model = saved_model;
model = changeRxnBounds(model,'EX_glc_D_e',-10,'l'); % Set maximum glucose uptake
model = changeRxnBounds(model,'EX_o2_e',-30,'l'); % Set oxygen uptake
model = changeRxnBounds(model,'BIOMASS_Ec_iJO1366_WT_53p95M',-0,'b'); % Disable WT biomass reaction
model = changeObjective(model,'BIOMASS_Ec_iJO1366_core_53p95M'); % Set the objective function
FBSolution = optimizeCvModel(model,'max'); % Perform FBA to find optimal growth-rate
model = changeRxnBounds(model,'BIOMASS_Ec_iJO1366_WT_53p95M',FBSolution.f,'b'); % Set fixed growth-rate
```
model = addDemandReaction(model, 'phe_L-c');

DM_phe_L-c  phe_L-c  ->

[tmp,phenylalanine_MAX_rxnID] = ismember({'DM_phe_L-c'},model.rxns);
model = changeObjective(model,'DM_phe_L-c');  % Set the objective function
FBA_solution_phenylalanine = optimizeCbModel(model,'max');  % Perform FBA to find optimal growth-rate
% printlabeledData({'DM_phe_L-c'}, round(FBA_solution_tyrosine.x(tyrosine_MAX_rxnID),3))
xMin = 0.5;
xMax = FBA_solution.f;
xInc = (xMax - xMin)/20;
x = xMin;
excessPhenylalanine = [];
growthRate = [];
for i = 1:21
    model = changeRxnBounds(model,'BIOMASS_Ec_iJO1366 WT_53p95M',x,'b');  % Set fixed growth-rate
    FBA_solution_phenylalanine = optimizeCbModel(model,'max');  % Perform FBA
    excessPhenylalanine(i) = FBA_solution_phenylalanine.f;
    growthRate(i) = x;
    x = x + xInc;
end
plot(growthRate,excessPhenylalanine)
title('Excess L-Phenylalanine');
xlabel('Growth-rate (h-1)'); ylabel('Excess Phenylalanine (mmol.gDW-1.hr-1)');

Figure 14. A plot showing the maximum amount of L-Phenylalanine that can be produced for a given growth-rate. This figure illustrates that as the need for excess L-Phenylalanine increases the growth-rate will need to decrease. In this figure we can see that the excess L-Phenylalanine flux can increase from $0.0233 \text{ mmol \cdot gDW}^{-1} \cdot \text{hr}^{-1}$ when the cell is at maximum growth-rate to $2.705 \text{ mmol \cdot gDW}^{-1} \cdot \text{hr}^{-1}$ when it is at 50% of that optimal growth-rate. Finally, to increase the L-Phenylalanine flux beyond these levels will require using the pathways that allow for the transport of L-Phenylalanine from the extracellular media.
6. Aerobic vs Anaerobic Amino Acid Production

Now let's look at the total amount of flux that is created for each of these amino acids as the oxygen content varies from anaerobic to aerobic.

```matlab
model = saved_model;
model = changeRxnBounds(model,'EX_glc__D_e',-10,'l'); % Set maximum glucose uptake
model = changeRxnBounds(model,'BIOMASS_Ec_iJO1366_WT_53p95M',-0,'b'); % Disable WT biomass reaction
model = changeObjective(model,'BIOMASS_Ec_iJO1366_core_53p95M'); % Set the objective function

for k = 1:131
    model = changeRxnBounds(model,'EX_o2_e',-(k-1),'b'); % Set oxygen uptake
    FBA solution = optimizeModel(model,'max'); % Perform FBA
    [P, C, vP, vC] = computeFluxSpills(model, {'tyr__L_c'}, FBA solution.x);
    tyr_flux(k) = sum(vP);
    [P, C, vP, vC] = computeFluxSpills(model, {'trp__L_c'}, FBA solution.x);
    trp_flux(k) = sum(vP);
    [P, C, vP, vC] = computeFluxSpills(model, {'phe__L_c'}, FBA solution.x);
    phe_flux(k) = sum(vP);
    growthRate(k) = FBA solution.f;
end

figure(1)
oFlux = 0:30;
ax1 = subplot(2,1,1); % top subplot
ax2 = subplot(2,1,2); % middle subplot
ax3 = subplot(2,1,3); % bottom subplot
ax4 = subplot(2,1,4); % bottom subplot

plot(ax1,oFlux,tyr_flux)
title(ax1,'Total L-Tyrosine Flux');
xlabel(ax1,'Oxygen uptake flux (mmol.gDW-1.h-1)'); ylabel(ax1,'tyr-L Flux');

plot(ax2,oFlux,trp_flux);
title(ax2,'Total L-Tryptophan Flux');
xlabel(ax2,'Oxygen uptake flux (mmol.gDW-1.h-1)'); ylabel(ax2,'trp-L Flux');

plot(ax3,oFlux,phe_flux);
title(ax3,'Total L-Phenylalanine Flux');
xlabel(ax3,'Oxygen uptake flux (mmol.gDW-1.h-1)'); ylabel(ax3,'phe-L Flux');

plot(ax4,oFlux,growthRate);
title(ax4,'Growth-rate');
xlabel(ax4,'Oxygen uptake flux (mmol.gDW-1.h-1)'); ylabel(ax4,'Growth-rate');
```
Figure 15. A plot showing the total amount of flux that is can be created for each of these amino acids as the oxygen content varies from anaerobic to aerobic.

6. Conclusion

The purpose of this tutorial was to identify and review the structure and capabilities of the “Tyrosine, Tryptophan, and Phenylalanine Metabolism” subsystem of the E.coli iJO1366 model. It began with an overview of the complete subsystem. This was followed by more detailed descriptions of the individual L-Tyrosine, L-Tryptophan, and L-Phenylalanine biosynthesis pathways. It concluded with a simulation showing the maximum flux that each of these amino acids can produce in a range from anaerobic to aerobic conditions.

References