CytoCopasi: A Chemical Systems Biology Target and Drug Discovery Visual Data Analytics Platform

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1- Step-by-step Model Construction for Fuentes et al. [1] (BIOMD0000000092)

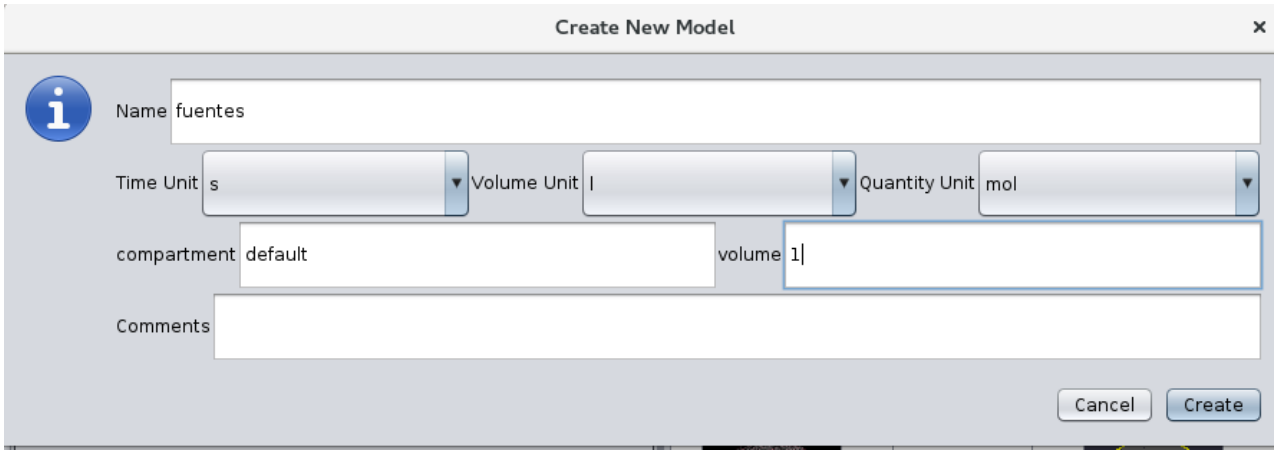


Figure S1: Creating a new model to replicate the model BIOMD0000000092

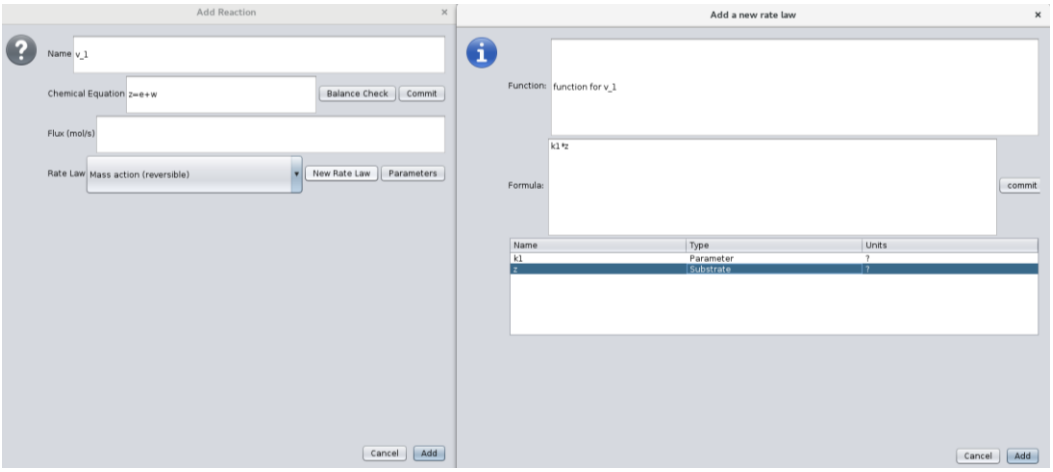
Add Reaction:

v_1

z=e+w

Function for v_1:

k1*z ; k1 = 0.004 l/s



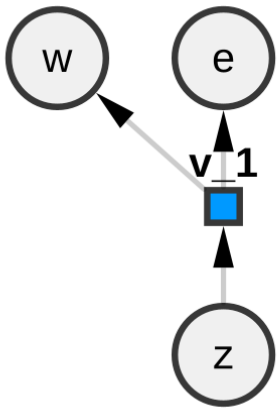


Figure S2: The reaction specifics for v_1 entered into CytoCopasi and the resulting network view

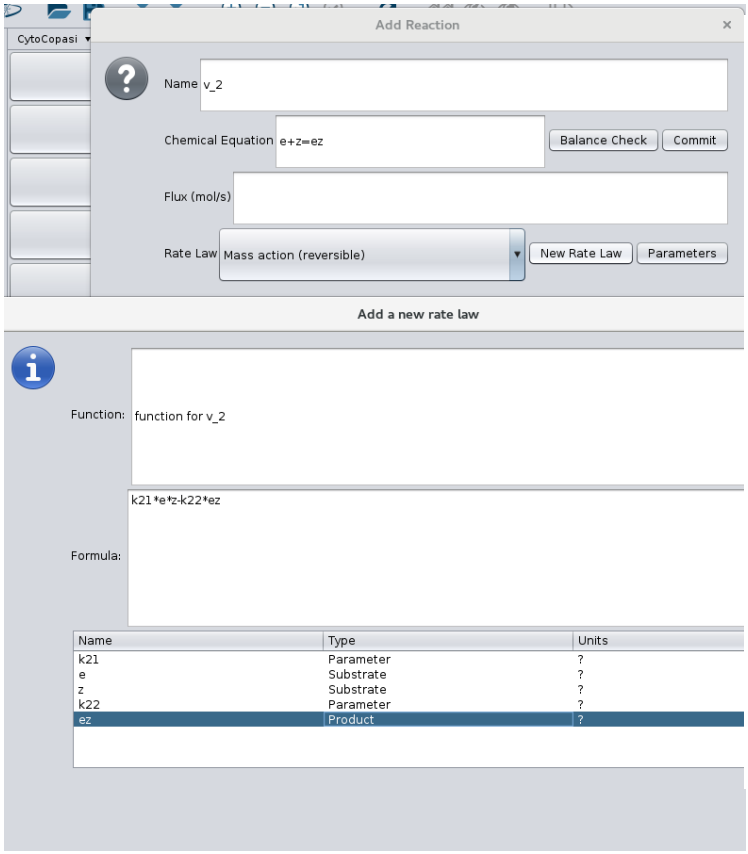
Reaction: v_2

e+z=ez

Function for v_2

k21*e*z-k22*ez

k21 = 100 l^2/(mol*s)
k22 = 0.00021 l/s



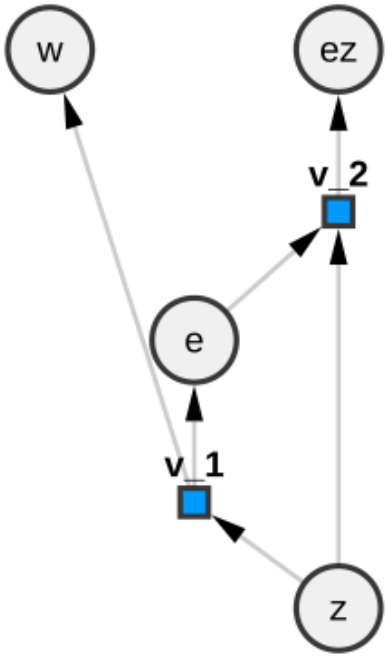


Figure S3: The reaction specifics for v_2 entered into CytoCopasi and the resulting network view including reactions v_1 and v_2

Reaction: v_3

ez = 2*e+w

Function for v_3:

k3*ez; k3 = 0.00054 l/s

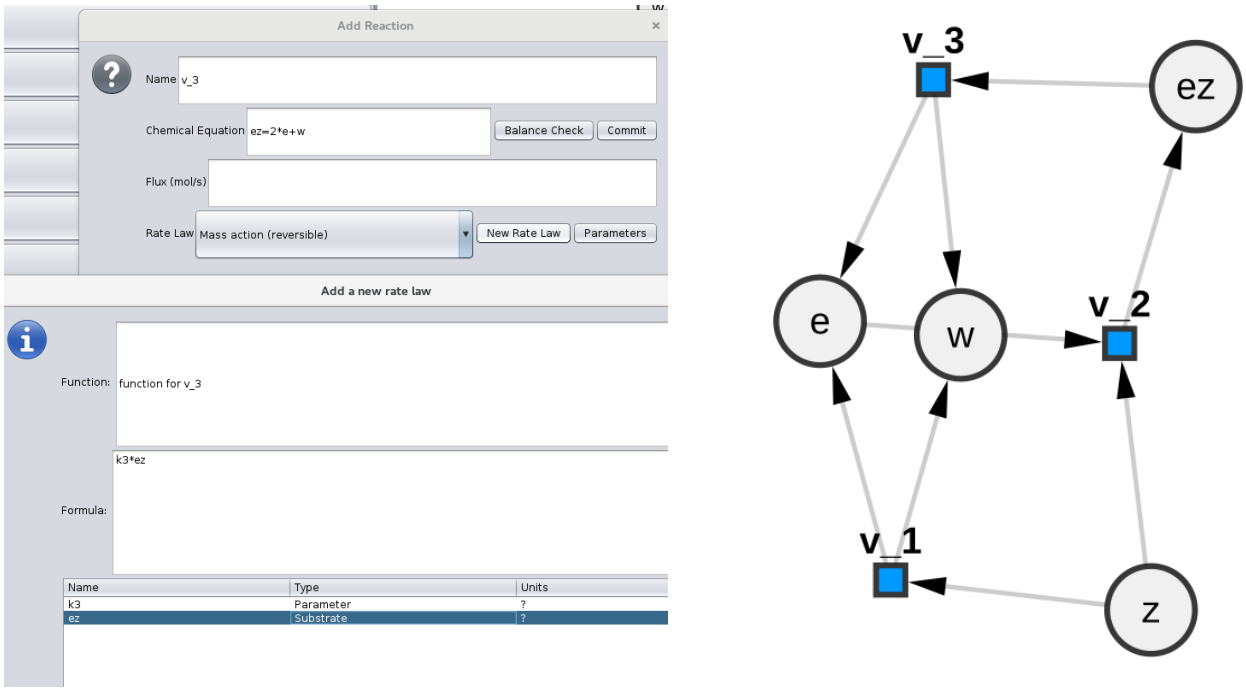


Figure S4: The reaction specifics for v_3 entered into CytoCopasi and the resulting network view including all three reactions

When reactions are created, initial metabolite concentrations are set to 1.0 and status is set to "Reactions". Double-click on the metabolite you want to edit.

$[e]_0$, $[ez]_0$, and $[w]_0$ = 0.0 mol/l

$[z]_0 = 2.4 \times 10^{-5}$ mol/l

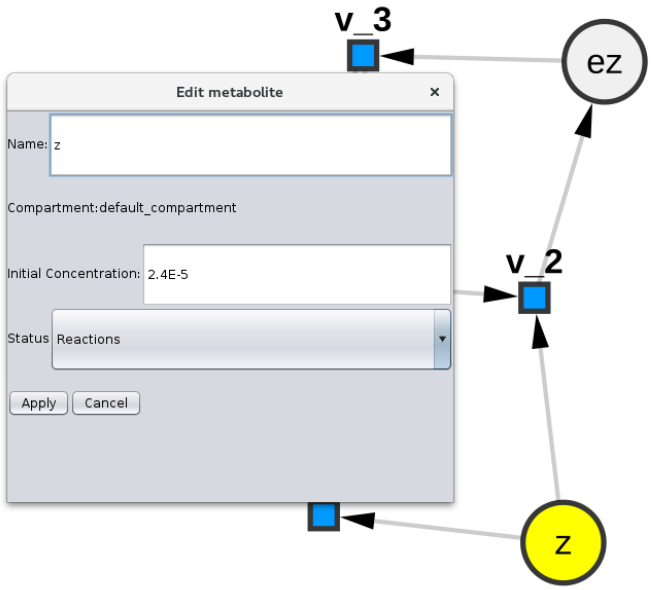


Figure S5: Editing the initial concentrations of metabolites

Now, run the time course to confirm that you constructed the model correctly.

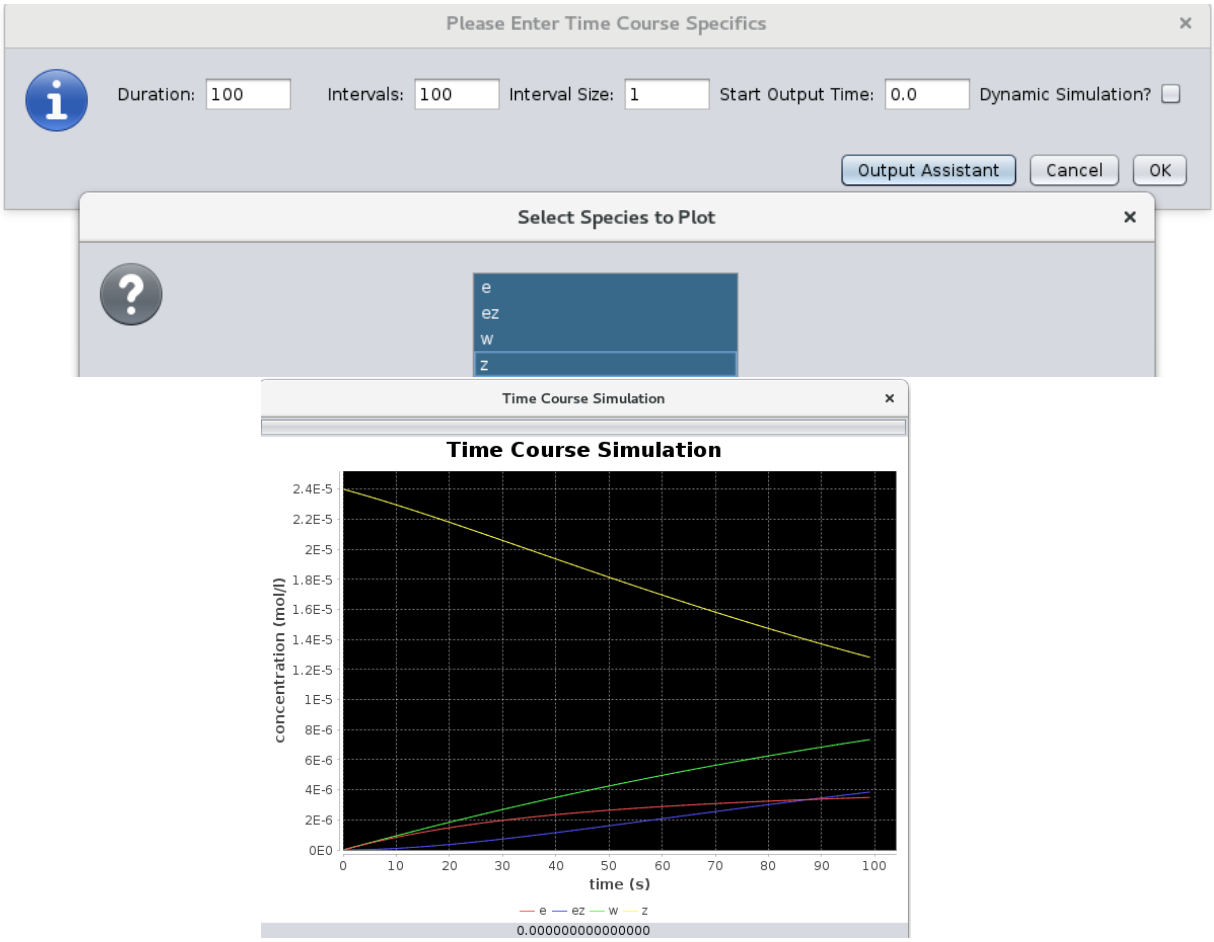


Figure S6: Running a time course simulation for 100 seconds to display the concentration profiles of the metabolites

2- Replicating Lambeth et al. [2] from the KEGG Glycolysis Model

Steps

- Renaming the metabolites: Although this is optional, shortening the metabolite names can make rate law definitions involving those metabolites easier to compute.

- Adding or removing reactions: The metabolites outside the KEGG subnetwork must be added manually with their corresponding reactions. By the same logic, KEGG network reactions not included in the original SBML model must be deleted. Examples for Lambeth et al. include the reactions for glycogen (Ph + GLY = G1P) and lactate (PYR + NADH = LAC + NAD)

- Changing the chemical equation of an existing reaction: One might want to integrate ADP to ATP or NAD to NADH conversions into existing reactions. Because KEGG networks do not include these side products, we changed the chemical equation for those reactions and selected the appropriate rate law to introduce the new metabolites and reflect the changes in the reaction scheme.

- Changing the rate law for existing reactions: KEGG networks lack the kinetic rate law information, in which case CytoCopasi sets mass action as the default rate law. That's why we modified the rate law for some of the reactions without changing the chemical equation.

The original SBML file

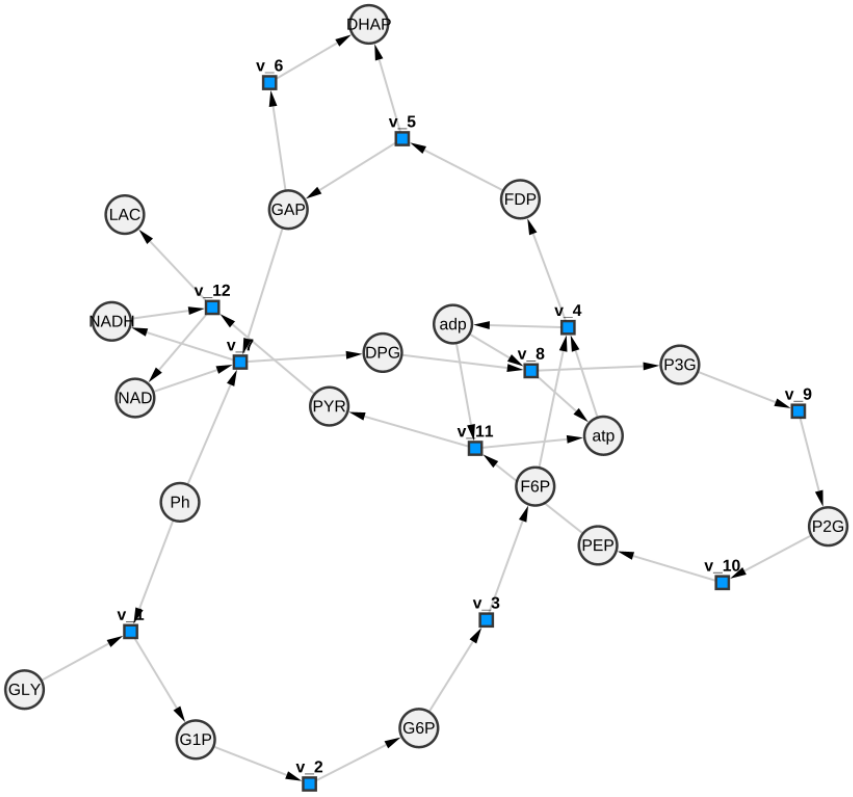


Figure S7: The Cytoscape network of the original model

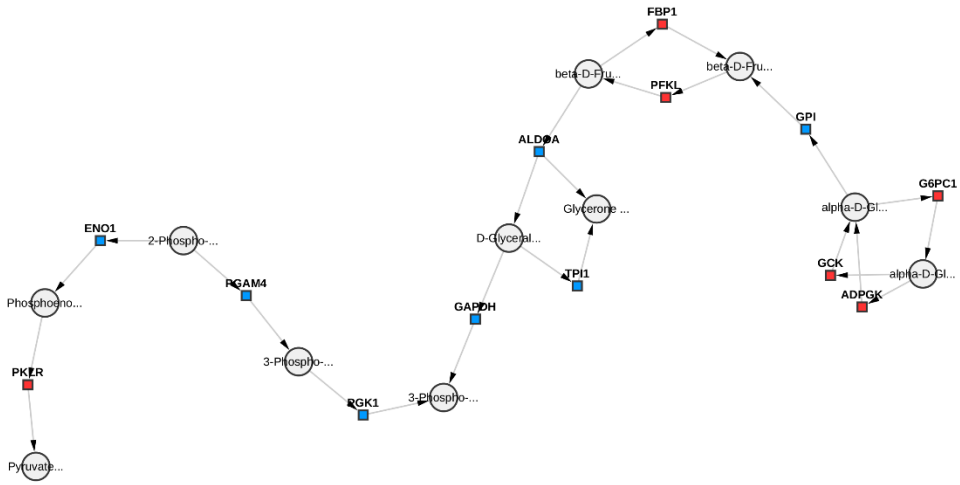


Figure S8: The KEGG Glycolysis module M00002 (core module involving three-carbon compounds)

2.1 Metabolite Names

Step 1: Rename the Metabolites

Nomenclature:

GLY: glycogen
G1P: glucose 1-phosphate
G6P: glucose 6-phosphate
F6P: fructose 6-phosphate
FBP: fructose 1,6-bisphosphate
DHAP: glycerone phosphate (1,3-dihydroxyacetone phosphate)
GAP: D-glyceraldehyde 3-phosphate
DPG: 3-phospho-D-glyceryl phosphate
3PG: 3-phospho-D-glycerate
2PG: 2-phospho-D-glycerate
PEP: Phosphoenolpyruvate
PYR: Pyruvate
LAC: Lactate
P_h: inorganic phosphate

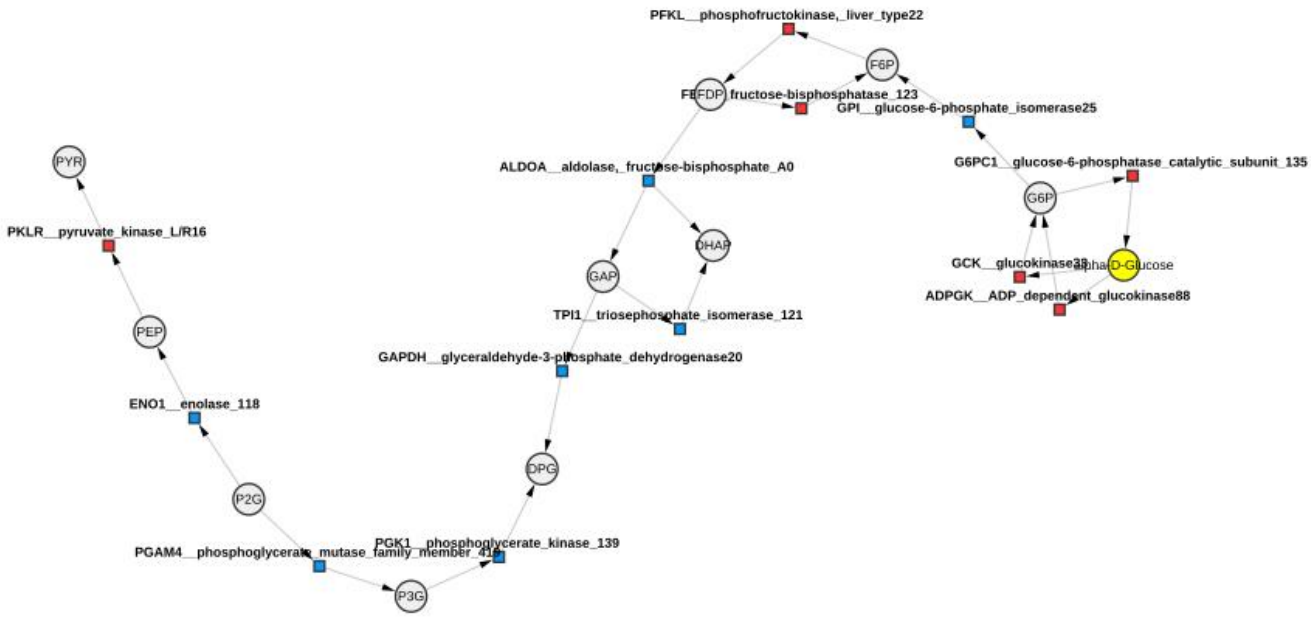


Figure S9: Metabolites have been renamed for ease of visualizing and customization

2.2 ADDING/REMOVING REACTIONS, CHANGING REACTION CHEMICAL EQUATIONS TO ADD NEW METABOLITES

Note: All the kinetics parameters are given in mM unless stated otherwise

Step 2: Remove the reactions between G6P and alpha-D-Glucose

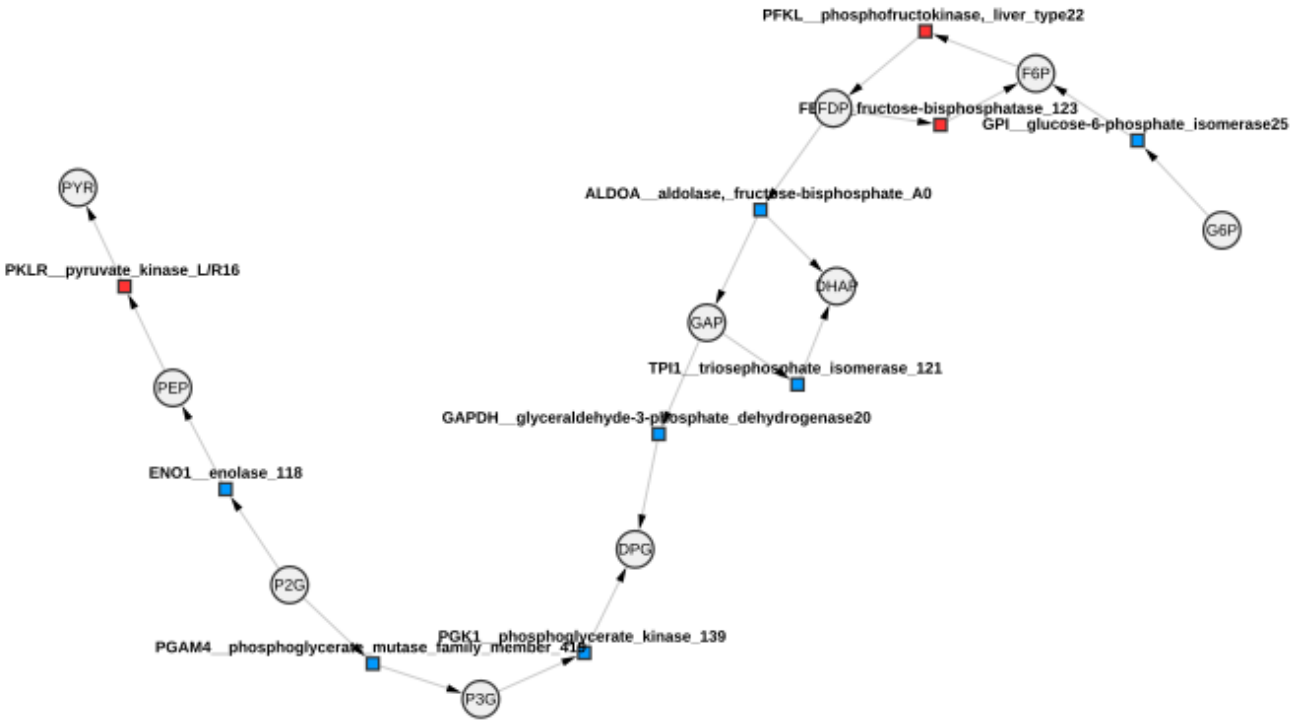


Figure S10: Remove the reactions between G6P and alpha-D-Glucose

Step 3: Add v_2

G1P = G6P

((Vfpglm*G1P/Kpglmg1p)-(Vfpglm*Kpglmg6p/(Kpglmg1p*16.62))*G6P/Kpglmg6p)/(1 + G1P/Kpglmg1p + G6P/Kpglmg6p)

Brenda Keyword: Phosphoglucomutase

EC to select: 5.4.2.2

It did not return parameters for Oryctolagus cuniculus (rabbit) nor Homo sapiens (human), therefore we pulled both parameters from the paper.

Kpglmg1p= 0.063 [3]
Kpglmg6p= 0.03 [3]

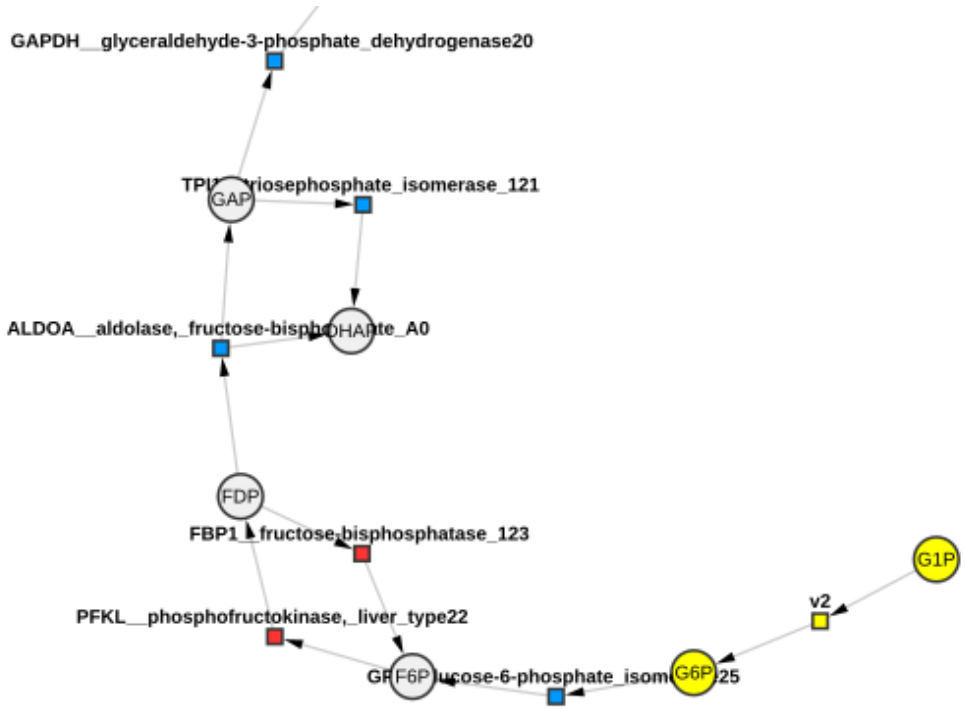


Figure S11: Adding the reaction G1P = G6P

Step 4: Add v_1

Ph + GLY = G1P

fracA*((Vfgly*Ph*GLY/(KgpAigly*KgpApi))/(1 + GLY/KgpAglyf + Ph/KgpApi + GLY*Ph/(KgpAglyf*KgpAipi) + GLY/KgpAglyb + G1P/KgpAg1p + GLY*G1P/(KgpAig1p*KgpAglyb)) - ((Vfgly*KgpAglyb*KgpAig1p/(KgpAigly*KgpApi*0.31))*G1P*GLY/(KgpAglyb*KgpAig1p))/ (1 + GLY/KgpAglyf + Ph/KgpApi + GLY*Ph/ (KgpAglyf*KgpAipi) + GLY/KgpAglyb + G1P/KgpAg1p + GLY*G1P/(KgpAig1p*KgpAglyb))) + fracB*((((amp^nH)/((Kgpamp^nH)*0.02))/ (1 + (amp^nH)/((Kgpamp^nH)*0.02))*(Vfgly*Ph*GLY/(KgpBiglyf*KgpBpi))/((1 + GLY/KgpBipi + Ph/KgpBiglyf + GLY/KgpBiglyb + G1P/KgpBig1p + GLY*Ph/(KgpBiglyf*KgpBpi) + GLY*G1P/(KgpBg1p*KgpBiglyb)))) - (((amp^nH)/((Kgpamp^nH)*0.02))/(1 + (amp^nH)/((Kgpamp^nH)*0.02))*((Vfgly*KgpBg1p*KgpBiglyb/(KgpBiglyf*KgpBpi*0.31))*G1P*GLY/(KgpBg1p*KgpBiglyb))/((1 + GLY/KgpBipi + Ph/KgpBiglyf + GLY/KgpBiglyb + G1P/KgpBig1p + GLY*Ph/(KgpBiglyf*KgpBpi) + GLY*G1P/(KgpBg1p*KgpBiglyb)))))

Keyword: Glycogen Phosphorylase

EC to select: 2.4.1.1

The K_m values are pulled entirely from BRENDA, while the K_i values for Glycogen Phosphorylase B are from BRENDA, and those for Glycogen Phosphorylase A are from the paper.

K_m:

KgpApi: 28 [4]
KgpAglyf: 0.62 [5]
KgpAglyb: 0.15 [5]
KgpAg1p: 1.7 [6]
Kgpamp: 0.074 [7]
KgpBpi: 11 [7]
KgpBg1p: 5.5 [7]

K_i: look at the commentary column to see what the inhibitor is being used against

KgpBipi= 0.71 [7]
KgpBig1p = 0.44 [7]
KgpBiglyf = 0.0028 [8]
KgpBiglyb = 5.9 [9]

We did not find inhibition constants for Glycogen Phosphorylase A, but the papers that contain Km constants can be checked by the user to see if they contain inhibition constants as well. For now, we are going to use the values from the original paper.

KgpAigly = 2 [10]
KgpAiPi = 4 [10]
KgpAig1p = 10.1 [10]

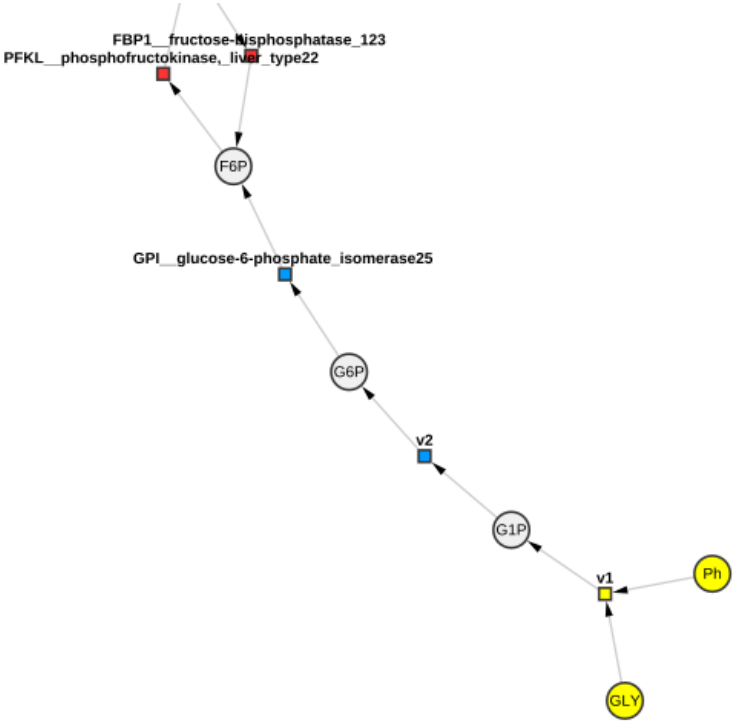


Figure S12: Adding the reaction Ph + GLY = G1P

Step 5: The reaction F6P=FDP is defined as one reversible reaction in the SBML model, whereas Kegg has a separate reaction node for the reverse reaction (because of different enzymes being

involved). So: Delete FBP1 – fructose biphosphatase and Change the Chemical equation for PFKL_phosphofructokinase

F6P + atp = FDP + adp

(Vfpfk*atp*F6P*(1 + (Kpfkatp*Kpfkf6p*Lo*(1 + (en*amp)/Kpfkamp)^4*(1 + atp/Kpfkiatp)^4*((1 + atp/KpfkatpT)*(1 + F6P/Kpfkf6pT) + FDP/KpfkfdpT + (adp*(1 + FDP/KpfkfdpT))/KpfkadpT)^3)/(KpfkatpT*Kpfkf6pT*(1 + amp/Kpfkamp)^4*(1 + (dn*atp)/Kpfkiatp)^4*((1 + atp/Kpfkatp)*(1 + F6P/Kpfkf6p) + FDP/Kpfkfdp + (adp*(1 + FDP/Kpfkfdp))/Kpfkadp)^3)))/(Kpfkatp*Kpfkf6p*((1 + atp/Kpfkatp)*(1 + F6P/Kpfkf6p) + FDP/Kpfkfdp + (adp*(1 + FDP/Kpfkfdp))/Kpfkadp)*(1 + (Lo*(1 + (en*amp)/Kpfkamp)^4*(1 + atp/Kpfkiatp)^4*((1 + atp/KpfkatpT)*(1 + F6P/Kpfkf6pT) + FDP/KpfkfdpT + (adp*(1 + FDP/KpfkfdpT))/KpfkadpT)^4)/((1 + amp/Kpfkamp)^4*(1 + (dn*atp)/Kpfkiatp)^4*((1 + atp/Kpfkatp)*(1 + F6P/Kpfkf6p) + FDP/Kpfkfdp + (adp*(1 + FDP/Kpfkfdp))/Kpfkadp)^4))) - (0.004117429077284144*Vfpfk*adp*FDP*(1 + (Kpfkatp*Kpfkf6p*Lo*(1 + (en*amp)/Kpfkamp)^4*(1 + atp/Kpfkiatp)^4*((1 + atp/KpfkatpT)*(1 + F6P/Kpfkf6pT) + FDP/KpfkfdpT + (adp*(1 + FDP/KpfkfdpT))/KpfkadpT)^3)/(KpfkatpT*Kpfkf6pT*(1 + amp/Kpfkamp)^4*(1 + (dn*atp)/Kpfkiatp)^4*((1 + atp/Kpfkatp)*(1 + F6P/Kpfkf6p) + FDP/Kpfkfdp + (adp*(1 + FDP/Kpfkfdp))/Kpfkadp)^3)))/(Kpfkatp*Kpfkf6p*((1 + atp/Kpfkatp)*(1 + F6P/Kpfkf6p) + FDP/Kpfkfdp + (adp*(1 + FDP/Kpfkfdp))/Kpfkadp)*(1 + (Lo*(1 + (en*amp)/Kpfkamp)^4*(1 + atp/Kpfkiatp)^4* ((1 + atp/KpfkatpT)*(1 + F6P/Kpfkf6pT) + FDP/KpfkfdpT + (adp*(1 + FDP/KpfkfdpT))/KpfkadpT)^4)/((1 + amp/Kpfkamp)^4*(1 + (dn*atp)/Kpfkiatp)^4*((1 + atp/Kpfkatp)*(1 + F6P/Kpfkf6p) + FDP/Kpfkfdp + (adp*(1 + FDP/Kpfkfdp))/Kpfkadp)^4)))

Keyword: Phosphofructokinase

EC to select: 2.7.1.11

It is best to pull the values from the original paper since all the values were obtained from rabbit.
Kpfkf6p: 0.18 [11]

Kpfkf6pT= 20 [11]

Kpfkatp= 0.08 [11]

KpfkatpT= 0.25 [11]

Kpfkiatp= 0.87 [11]

Kpfkfdp= 4.02 [12]

KpfkfdpT= 4.02 [12]

Kpfkadp= 2.7 [12]

KpfkadpT= 2.7 [12]

Kpfkamp = 0.06 [12]

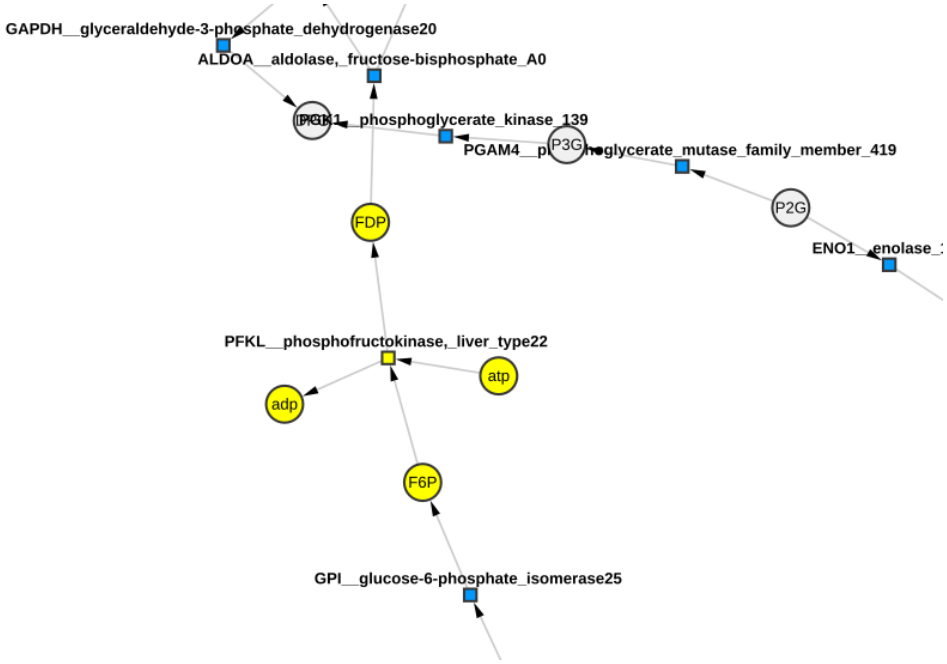


Figure S13: Delete FBP1 – fructose biphosphatase and change the chemical equation for PFKL_phosphofructokinase to account for adp-to-atp conversion

Step 6: Change Reaction GAP=DPG

GAP + NAD + Ph = DPG + NADH

((-11.235955056179776*Vfgad*DPG*NADH)/(Kgapdhgap*Kgapdhnad*Kgapdhpi) + (Vfgad*GAP*NAD*Ph)/(Kgapdhgap*Kgapdhnad*Kgapdhpi))/(1 + DPG/Kgapdh13dpg + GAP/Kgapdhgap + NAD/Kgapdhnad + (GAP*NAD)/(Kgapdhgap*Kgapdhnad) + NADH/Kgapdhnadh + (DPG*NADH)/(Kgapdh13dpg*Kgapdhnadh) + Ph/Kgapdhpi + (GAP*NAD*Ph)/(Kgapdhgap*Kgapdhnad*Kgapdhpi))

Keyword: Glyceraldehyde-3-Phosphate Dehydrogenase
EC to select: 1.2.1.12
Rabbit: **80% complete with BRENDA, one value from the original paper**

Kgapdhnadh=0.012 [13]
Kgapdhnad = 0.06 [13]
Kgapdhgap = 0.82 [13]
Kgapdh13dpg = 0.13 [13]

From paper:
Kgapdhpi = 0.29 [14]

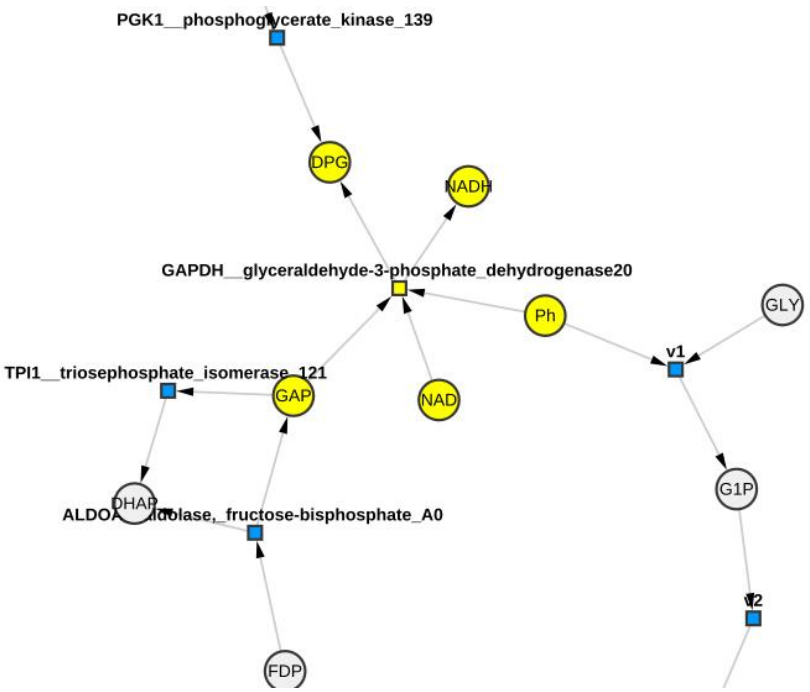


Figure S14: Changing the reaction GAP=DPG to account for the NAD-to-NADH conversion

Step 7: Change Reaction DPG=P3G

DPG + adp = P3G + atp

((57109*Vbpgk*adp*DPG)/(Kpgk3pg*Kpgkatp) - (Vbpgk*atp*P3G)/(Kpgk3pg*Kpgkatp))/(1 + adp/Kpgkadp + atp/Kpgkatp + DPG/Kpgk13dpg + (adp*DPG)/(Kpgk13dpg*Kpgkadp) + P3G/Kpgk3pg + (atp*P3G)/(Kpgk3pg*Kpgkatp))

Keyword: Phosphoglycerate Kinase
EC to select: 2.7.2.3

All the values are pulled from BRENDA (for Homo sapiens)
Kpgk3pg = 0.1 [15]
Kpgkatp = 0.11 [15]
Kpgkadp = 0.1 [15]
Kpgk13dpg = 0.0077 [15]

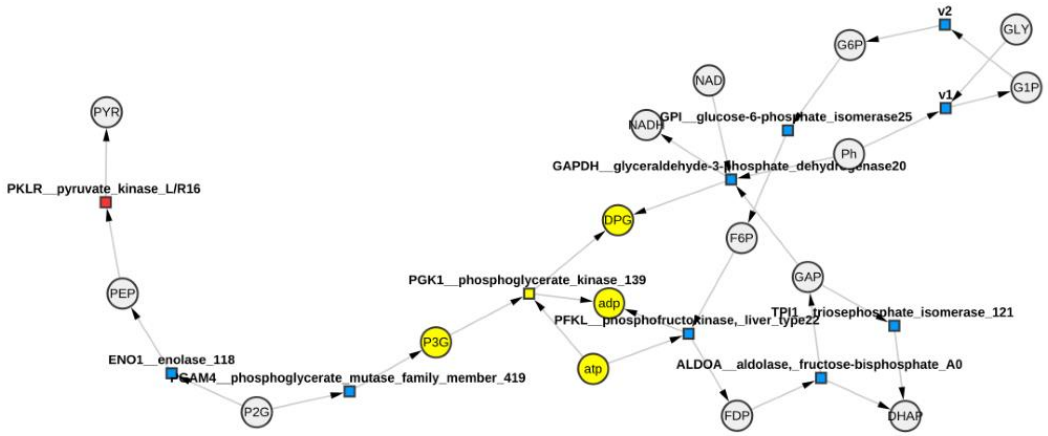


Figure S15: Changing the reaction DPG=P3G to account for the ATP-to-ADP conversion

Step 8: Change PEP->PYR

PEP + adp = PYR + atp

(Vfpk*PEP*adp/(Kpkpep*Kpkadp)-
(Vfpk*Kpkpyr*Kpkatp/(Kpkpep*Kpkadp*10304))*PYR*atp/(Kpkpyr*Kpkatp))/(1+PEP/Kpkpep+adp/Kpkadp + PEP*adp/(Kpkpep*Kpkadp) + atp/Kpkatp + PYR/Kpkpyr + PYR*atp/(Kpkpyr*Kpkatp))

Keyword: Pyruvate Kinase, organism: Oryctolagus cuniculus
EC no to select: 2.7.1.40

The K_m values for PEP and ADP are pulled from BRENDA, and those for PYR and ATP are from the original paper.

Kpkpep = 0.076 [16]
Kpkadp = 0.357 [16]

Kpkpyr = 7.05 [17]
Kpkatp = 1.13 [17]

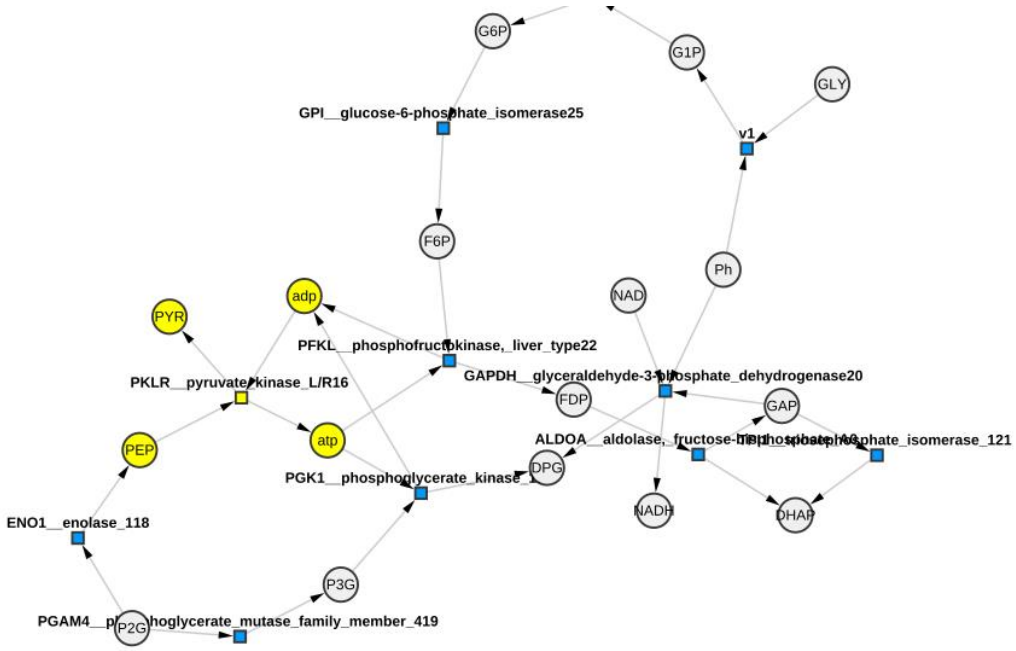


Figure S16: Changing the reaction PEP->PYR to account for the ADP-to-ATP conversion

Step 9: Add the Reaction for Lactate

PYR + NADH = LAC + NAD

((Vfldh*PYR*NADH/(Kldhpyr*Kldhnadh))-
((Vfldh*Kldhlac*Kldhnad/(Kldhpyr*Kldhnadh*16198))*LAC*NAD/(Kldhlac*Kldhnad)))/(1 +
PYR/Kldhpyr + NADH/Kldhnadh + PYR*NADH/(Kldhpyr*Kldhnadh) + LAC/Kldhlac + NAD/Kldhnad +
LAC*NAD/(Kldhlac*Kldhnad))

Keyword: Lactate dehydrogenase; Organism: Homo sapiens
EC no: 1.1.1.28
3 out of 4 parameters were available on BRENDA:
Kldhpyr= 0.398 [18]
Kldhnad = 0.5 [19]
Kldhlac = 10.73 [19]

From the original paper:

Kldhnadh = 0.002 [20]

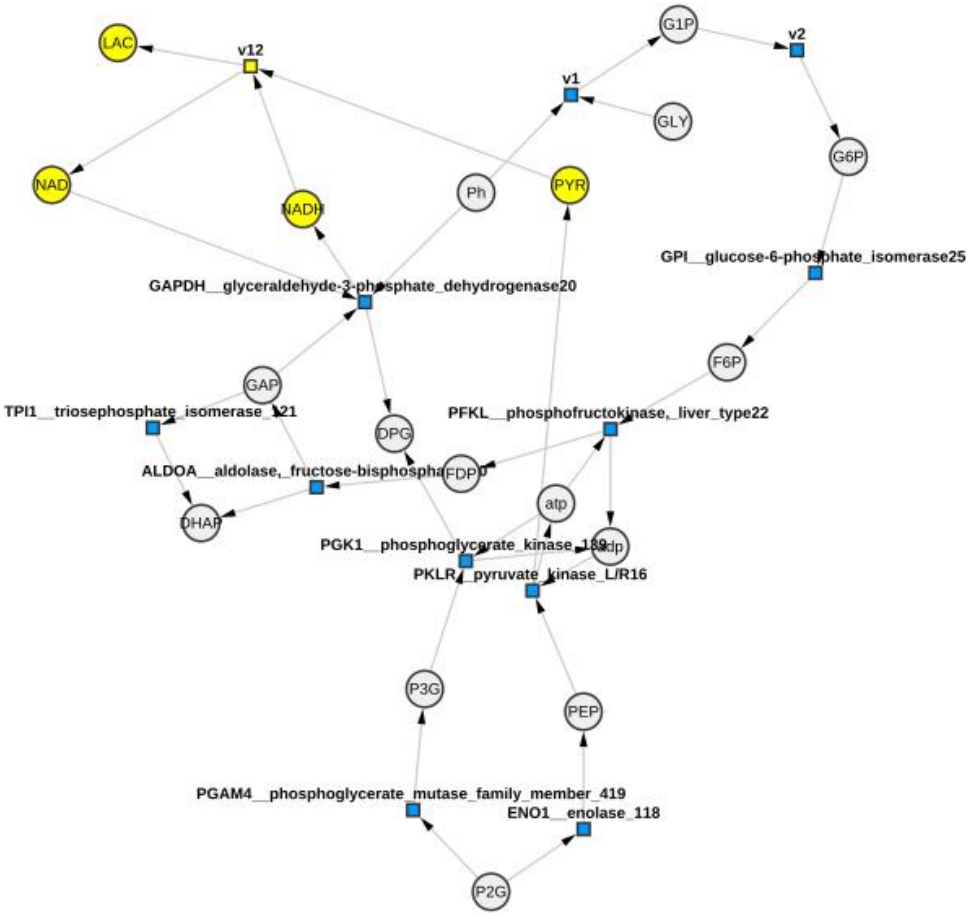


Figure S17: Adding the reaction for lactate synthesis from pyruvate

Final view (with Hierarchical Layout instead of force-directed)

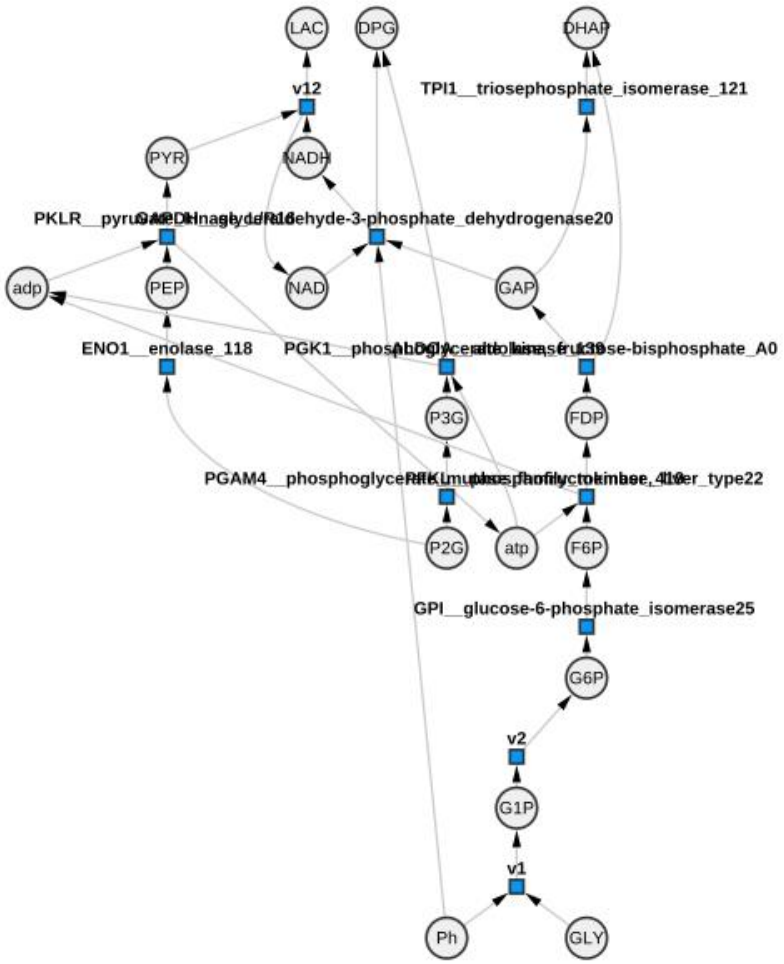


Figure S18: Final view (with Hierarchical Layout instead of force-directed)

2.3 MODIFYING RATE LAWS FOR THE OTHER REACTIONS (WHOSE EQUATIONS DID NOT NEED TO BE CHANGED, BUT HAD CUSTOM RATE LAWS)

Step 10: G6P = F6P

(((Vbpgi*Kpgig6p/Kpgif6p*0.45)*G6P/Kpgig6p)-(Vbpgi*F6P/Kpgif6p))/(1+F6P/Kpgif6p + G6P/Kpgig6p)

Keyword: Phosphoglucoisomerase; Organism: Oryctolagus cuniculus

EC no: 5.3.1.9

Both K_m values are available on BRENDA
Kpgif6p = 0.01 [21]

Kpgig6p = 0.03 [21]

Step 11: FDP = DHAP + GAP

((Vfald*FDP/Kaldfdp)-((Vfald*Kaldgap*Kalddhap/(Kaldfdp*0.000095))*DHAP*GAP/(Kaldgap*Kalddhap)))/(1+FDP/Kaldfdp+GAP/Kaldgap + DHAP/Kalddhap)

Keyword: Aldolase; Organism: Oryctolagus cuniculus

EC=4.1.2.13

All three K_m values are available on BRENDA.

Kaldfdp = 0.06 [22]
Kaldgap = 1 [22]
Kalddhap = 2 [22]

Step 12: GAP = DHAP

((Vftpi*GAP/Ktpigap)-((Vftpi*Ktpidhap/(Ktpigap*19.2))*DHAP/Ktpidhap))/(1 + GAP/Ktpigap + DHAP/Ktpidhap)

Keyword: triose-phosphate isomerase; Organism: Oryctolagus cuniculus

E.C. 5.3.1.1

Both K_m values are available on BRENDA

Ktpigap = 0.42 [23]
Ktpidhap = 0.75 [23]

Step 13: P3G = P2G

((Vfpgm*P3G/Kpgm3pg)-((Vfpgm*Kpgm2pg/(Kpgm3pg*0.18))*P2G/Kpgm2pg))/(1+P3G/Kpgm3pg + P2G/Kpgm2pg)

Keyword: Phosphoglycerate Mutase; Organism: Homo sapiens

EC: 5.4.2.11

From BRENDA

Kpgm3pg = 0.4 [24]

No value of Kpm2pg for homo sapiens on BRENDA. That said, there are very few results available with P2G as the substrate.

From paper: 0.014 (chicken) [25]

Step 14: P2G = PEP

((Vfen*P2G/Ken2pg)-((Vfen*Kenpep/(Ken2pg*0.49))*PEP/Kenpep))/(1+P2G/Ken2pg + PEP/Kenpep)

Keyword: Enolase; Organism: Oryctolagus cuniculus
EC no: 4.2.1.11

Ken2pg = 0.061 [26]
Kenpep = 0.25 [26]

3- Reactions for Drug Treatment with Vemurafenib or Dabrafenib

3.1 Treatment with Vemurafenib

The following reaction was added to ERK_Akt_Wnt_SBML-BRaf.cps to construct the Vemurafenib treated version ERK_Akt_Wnt_SBML-BRaf-Vem.cps

$$pBRaf \rightarrow BRaf; Vem \tag{1}$$

Rate Law: Modified Michaelis-Menten

$$\frac{Kcat * modifier * substrate}{km + substrate} \tag{2}$$

Kcat = 1/s
Km = 100 nM
[Vem]_0 = 1000 nM

3.2 Treatment with Dabrafenib

The following reactions were added to ERK_Akt_Wnt_SBML-BRaf.cps to construct the Dabrafenib treated version ERK_Akt_Wnt_SBML-BRaf-DFB.cps
Note: Mass action (reversible) is selected as the rate law for all the reactions below.

$$BRaf + DBF \underset{d_4}{\overset{a_4}{\rightleftharpoons}} BRaf.DBF$$

$$BRaf.MEK + DBF \underset{d_4}{\overset{a_4}{\rightleftharpoons}} BRaf.MEK.DBF$$

$$BRaf.pMEK + DBF \underset{d_4}{\overset{a_4}{\rightleftharpoons}} BRaf.pMEK.DBF$$

$$BRaf.DFB \underset{d_2}{\overset{a_2}{\rightleftharpoons}} BRaf.MEK.DBF$$

$$BRaf.DBF + pMEK \underset{d_2}{\overset{a_2}{\rightleftharpoons}} BRaf.pMEK.DFB$$

Constant	Value	Reference
a_2	0.106 $\mu M^{-1}s^{-1}$	[27]
d_2	0.02385 s^{-1}	[28]
a_4	0.106 $\mu M^{-1}s^{-1}$	[27]
d_4	0.0000593 s^{-1}	[28]

Table S1: Kinetic Constants used for Dabrafenib-related reactions

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