METHODOLOGY

Identifying model error in metabolic flux analysis – A generalized least squares approach

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Additional file 2 – Model definition
The model used in this work was largely taken from [1] with only minimal changes (listed in the Materials and Methods section of the manuscript). A full list of reactions is presented below with a rough outline of material flow in figure 1.

Glycolysis and PPP

| Reaction | Species |
|----------|---------|
| HK       | Glc + ATP $\rightarrow$ ADP + G6P |
| FBA      | G6P + ATP $\rightarrow$ ADP + 2 DHAP |
| PK       | DHAP + 2 ADP + NAD + P $\rightarrow$ 2 ATP + NADH + Pyr + H$_2$O |
| PPP      | G6P + 2 NADP + H$_2$O $\rightarrow$ 2 NADPH + R5P + CO$_2$ |

**HK** hexokinase

**FBA** fructose-biphosphate aldolase

**PK** pyruvate kinase

**PPP** pentose phosphate pathway

Pyr and AcCoA

| Reaction | Species |
|----------|---------|
| LDH      | Pyr + NADH $\rightarrow$ Lac + NAD |
| ACAS     | mAcCoA + $\rightarrow$ mCoA + AcOH |
| ME       | Mal + NADP $\rightarrow$ Pyr + CO$_2$ + NADPH |
| PDH      | mPyr + mCoA + NAD $\rightarrow$ mAcCoA + NADH + CO$_2$ |

**LDH** lactate dehydrogenase

**ACAS** acetyl-coa synthetase

**ME** NADP-malic enzyme

**PDH** pyruvate dehydrogenase

TCA cycle

| Reaction | Species |
|----------|---------|
| CS       | mAcCoA + mOxal + H$_2$O $\rightarrow$ mCit + mCoA |
|          | mCit + NAD $\rightarrow$ mAKG + NADH + CO$_2$ |
| AKGDH    | mAKG + mCoA + NAD $\rightarrow$ mSucCoA + NADH + CO$_2$ |
| SCS      | ADP + mSucCoA + P $\rightarrow$ ATP + mCoA + mSuc |
|          | mFAD + mSuc + H$_2$O $\rightarrow$ mFADH + mMal |
| mMalDH   | mMal + NAD $\rightarrow$ mOxal + NADH |
| cMalDH   | NADH + Oxal $\rightarrow$ Mal + NAD |
CS citrate synthase
AKGDH alpha-ketoglutarate dehydrogenase
SCS succinyl-coa synthetase
mMalDH mitochondrial malate dehydrogenase
cMalDH cytosolic malate dehydrogenase

Glutaminolysis

| ALT | Glu + Pyr $\rightarrow$ AKG + Ala |
| GLDH | mGlu + NAD + H$_2$O $\rightarrow$ Amm + mAKG + NADH |
| GS | Glu + ATP + Amm $\rightarrow$ Gln + ADP + P |
| AST | Glu + Oxal $\rightarrow$ Asp + AKG |
| | Glu $\rightarrow$ Pgl |

ALT alanine transaminase
GLDH glutamate dehydrogenase
GS glutamine synthetase
AST aspartate transaminase

Amino acid degradation

| SDH | Ser $\rightarrow$ Pyr + Amm |
| SHMT | Ser + FH$_4$ $\rightarrow$ Gly + N$_5$N$_{10}$methyleneFH$_4$ + H$_2$O |
| GCS | CO$_2$ + Amm + N$_5$N$_{10}$methyleneFH$_4$ + NAD $\rightarrow$ Gly + FH$_4$ + NAD |
| | Thr + mCoA + NAD $\rightarrow$ Gly + mAcCoA + NADH |
| | Cys + AKG $\rightarrow$ Glu + Pyr |
| | His + FH$_4$ + NADPH + 2 H$_2$O $\rightarrow$ |
| | Glu + N$_5$N$_{10}$methyleneFH$_4$ + NADP + 2 Amm |
| | Arg + AKG + NAD + 2 H$_2$O $\rightarrow$ 2 Glu + NADH |
| | Pro + 2 NAD + 2 H$_2$O $\rightarrow$ Glu + 2 NADH |
| ASNS | Asn + H$_2$O $\rightarrow$ Amm + Asp |
| | Ile + AKG + ATP + 2 mCoA + mFAD + 2 NAD + H$_2$O $\rightarrow$ |
| | mSucCoA + Glu + mAcCoA + ADP + P + mFADH + 2 NADH |
| | Leu + ATP + AKG + NAD + mCoA + mFAD + H$_2$O + mSucCoA $\rightarrow$ |
| | 2 mAcCoA + mSuc + Glu + NADH + mFADH + ADP + P |
| | Lys + AKG + 2 mCoA + 0.5 O$_2$ + 2 H$_2$O + 2 mFAD + 2 NAD $\rightarrow$ |
| | Glu + 2 mAcCoA + 2 CO$_2$ + Amm + 2 mFADH + 2 NADH |
| | Met + Ser + 3 ATP + FH$_4$ + 2 NAD + ATP + mCoA + 4 H$_2$O $\rightarrow$ |
| | mSucCoA + Cys + Amm + 3 ADP + 3 P + N$_5$N$_{10}$methyleneFH$_4$ + 2 NADH + ADP |
| PAH | Phe + NADPH + O$_2$ $\rightarrow$ Tyr + NADP + H$_2$O |
| | Trp + 2 mCoA + 3 O$_2$ + 4 H$_2$O + NADPH + 3 NAD + mFAD $\rightarrow$ |
| | 2 mAcCoA + 4 CO$_2$ + Ala + NADP + 3 NADH + mFADH + Amm |
| | Tyr + AKG + mCoA + mSucCoA + 2 O$_2$ + 2 H$_2$O $\rightarrow$ |
| | Glu + Mal + 2 mAcCoA + mSuc + CO$_2$ |
| | Val + AKG + 2 ATP + mCoA + mFAD + 3 NAD + 4 H$_2$O $\rightarrow$ |
| | mSucCoA + Glu + 2 ADP + 2 P + mFADH + 3 NADH + CO$_2$ |
SDH  serine dehydratase
SHMT  serine hydroxymethyltransferase
GCS  glycine cleavage system
ASNS  asparagine synthetase
PAH  phenylalanine hydroxylase

Macromolecules

| Carb | G6P + 3.5 ATP \(\rightarrow\) Carb + ADP
| OA | 9 mCit + 9 Mal + 17 ATP + 17 NADPH + 9 NADH + O\(_2\) \(\rightarrow\)
| DNA | 1.9 Gln + 1.3 Asp + 7.5 ATP + 0.5 Gly + 1.3 N\(_5\)N\(_{10}\)methyleneFH\(_4\) +
| RNA | 2.091 Gln + 1.194 Asp + 7.487 ATP + 0.489 Gly + 0.978 N\(_5\)N\(_{10}\)methyleneFH\(_4\) +
| Prot | 0.095 Ala + 0.048 Asp + 0.039 Asn + 0.063 Arg + 0.028 Cys +

Misc.

| mMal + AKG \(\rightarrow\) mAKG + Mal
| mMal + Cit \(\rightarrow\) mCit + Mal
| GLAST  Glu \(\rightarrow\) mGlu
| MPC  Pyr \(\rightarrow\) mPyr
| FH\(_4\) + FoOH + ATP + NADH \(\rightarrow\) ADP + P + NAD + N\(_5\)N\(_{10}\)methyleneFH\(_4\)

GLAST  glutamate aspartate transporter
MPC  mitochondrial pyruvate carrier

Phosphorylation

| 3 ADP + NADH + 0.5 O\(_2\) + 3 P \(\rightarrow\) 3 ATP + NAD + 4 H\(_2\)O
| 2 ADP + mFADH + 0.5 O\(_2\) + 2 P \(\rightarrow\) 2 ATP + mFAD + 2 H\(_2\)O
Figure 1 Outline of metabolic network used in this work. A number of intermediate compounds have been omitted to clarify overall material flow.
References
1. Altamirano, C., Illanes, A., Casablancas, A., Gámez, X., Cairó, J.J., Gódia, C.: Analysis of CHO cells metabolic redistribution in a glutamate-based defined medium in continuous culture. Biotechnology Progress 17(6), 1032–41 (2001). doi:10.1021/bp0100981