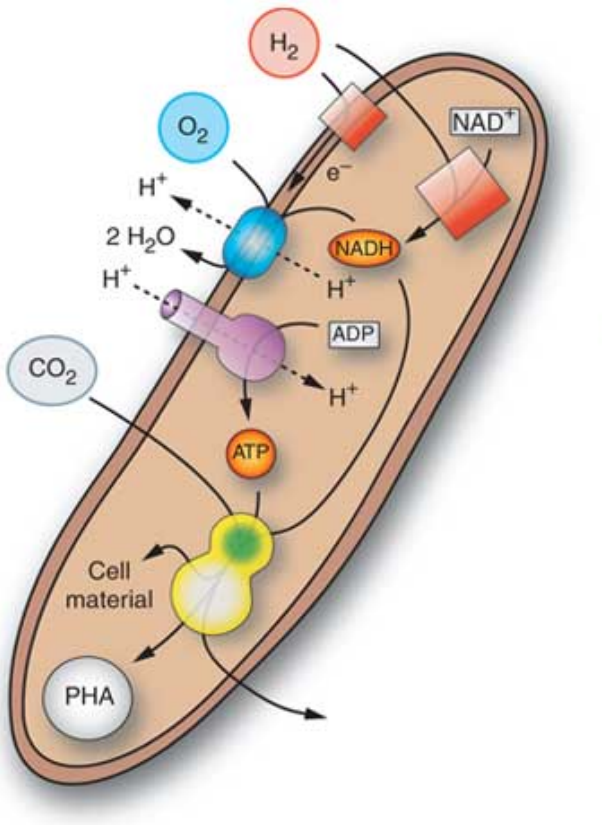
Elementary modes analysis for 3-hydroxypropanoic acid production in *Cupriavidus necator*

Lithoautotrophic metabolism of *Cupriavidus necator*

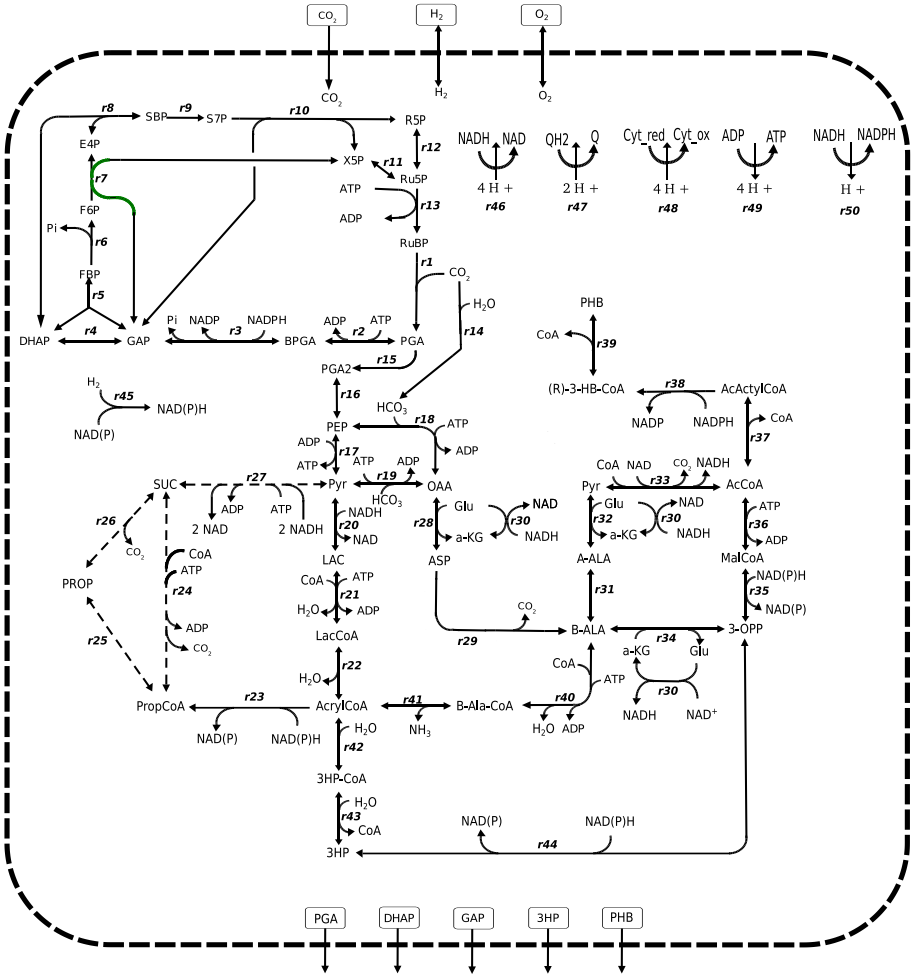
- Carbon dioxide is fixed via the Calvin cycle
- Membrane bound hydrogenase directly connected to the electron transport chain (ETC) for generating ATP
- Soluble hydrogenase that is coupled to NADH synthesis that is required for the Calvin cycle or ETC
- Oxygen final electron acceptor (under anaerobic conditions nitrate is used)



Pohlmann *et al*., Nature biotechnology, 2006.

Structural model of *Cupriavidus necator* for 3HP production

1. Calvin cycle (13)
2. Electron transport chain (6)
3. PHB production (3)
4. 3HP production (34)
5. Transporters (10)



V Kumar, Biotechnology advances, 2013

Elementary modes analysis for increasing 3HP production

Computed elementary modes of the small model for:

- Calculating **3HP yields** and **energy requirements** of the different solutions.
- Identify target **knockouts** for redirecting flux towards the optimal route for 3HP using **minimal cut set analysis**.

Calculating maximum 3HP yield and energy requirements of EMs

Pathway	Number of Modes	Theoretical max. yield (mol/mol CO_2)	O_2 requirements (mol/mol CO_2)	H_2 requirements (mol/mol CO_2)
PHB	614	0.25	[0.86, 9.63]	[6.17, 7.33]
3HP	9708	0.33	[0.58, 7.67]	[4.0, 4.44]
3HP + PHB	151	0.20 + 0.10	[0.93, 1.8]	[4.09, 4.55]

Net stoichiometry example:

$$3.0\ CO_2 + 12.0\ H_2 + 3.0\ O_2 \rightarrow 1.0\ 3HP + 1.0\ H_2O$$

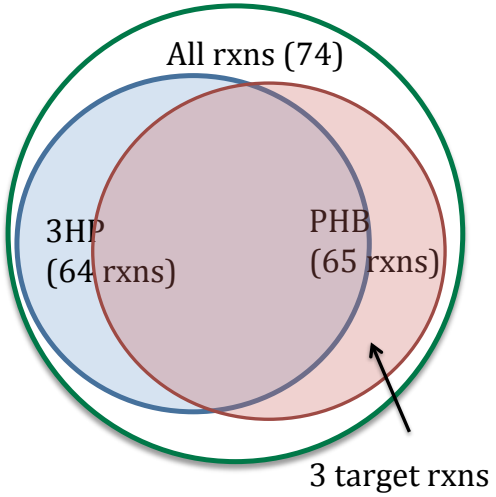
Higher oxygen and hydrogen requirements correspond to higher energy requirements of pathway.

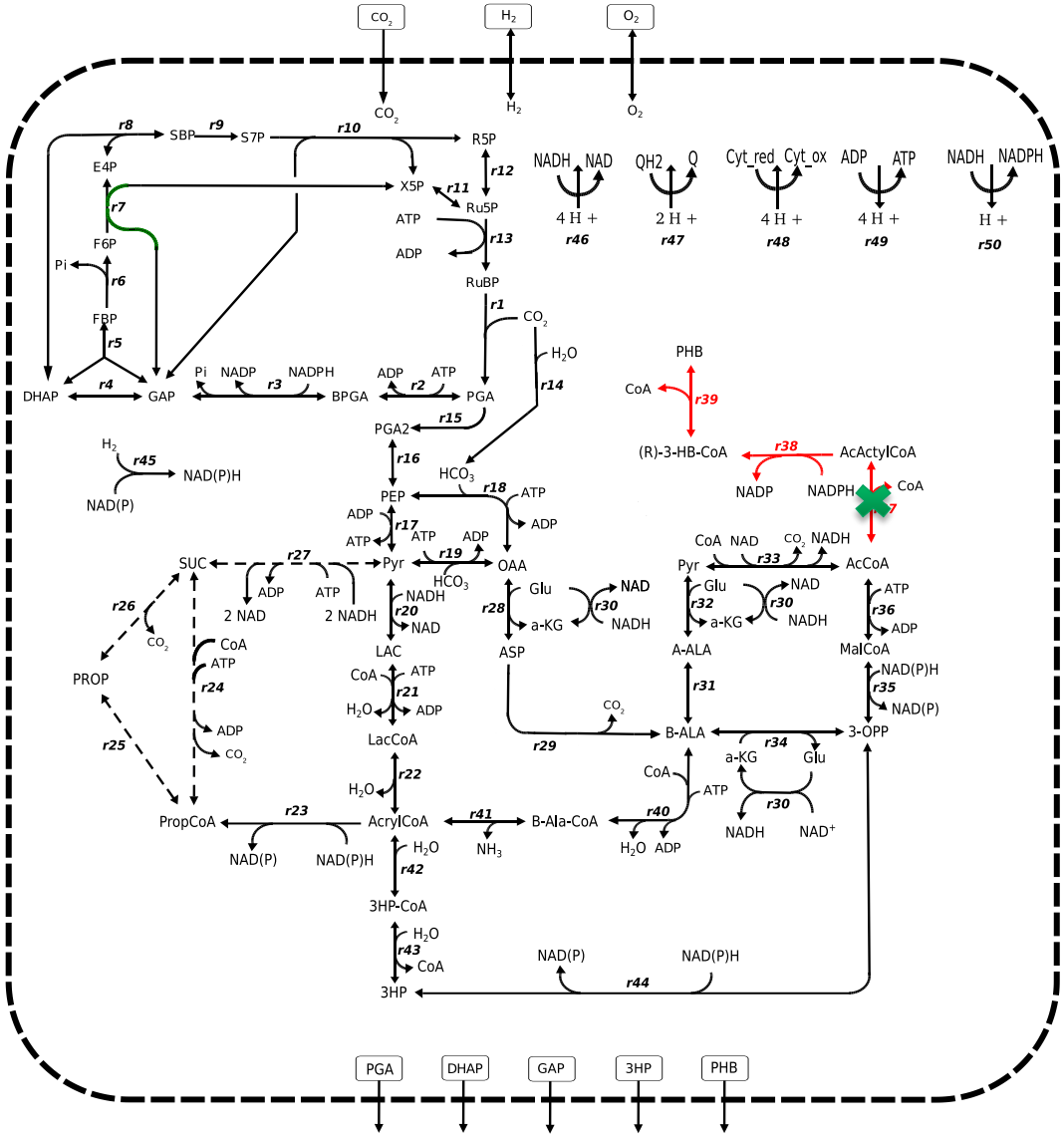
Identifying target knockouts for increasing 3HP production

Minimal Cut Sets

A **minimal** set of reactions whose **deletion** results in **failure** of certain **network functions**.

- Searched for reactions whose deletion **blocks** PHB production.
- Found only 3 possible target knock outs.
- The reaction **r37** would be the **primary target** to avoid the accumulation of unwanted intermediates and locking up CoA in a dead-end.



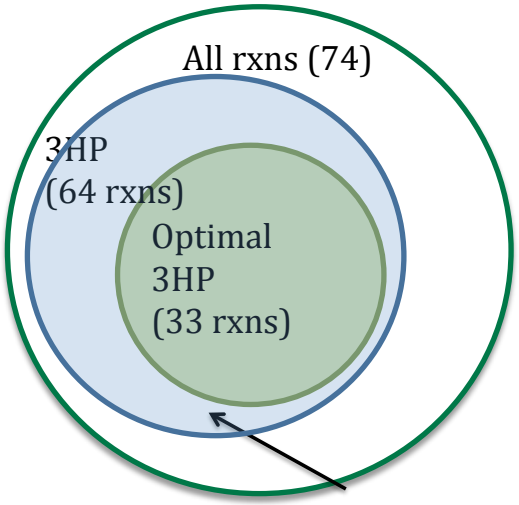

CO2
H2
O2
r8
SBP
r9
S7P
r10
R5P
E4P
X5P
r11
r12
Ru5P
r7
ATP
ADP
r13
F6P
Pi
r6
RuBP
FBP
r1
CO2
r5
H2O
DHAP
r4
GAP
Pi
NADP
r3
NADPH
BPGA
ADP
r2
ATP
PGA
r14
r15
PGA2
r16
HCO3
r45
H2
NAD(P)
NAD(P)H
PEP
r18
ATP
ADP
r17
ATP
ADP
r27
SUC
Pyr
r19
OAA
HCO3
ADP
ATP
2 NAD
2 NADH
NADH
r20
NAD
LAC
r26
CO2
PROP
CoA
ATP
r24
ADP
CO2
r25
CoA
ATP
r21
H2O
ADP
LacCoA
r22
H2O
PropCoA
r23
NAD(P)
NAD(P)H
AcrylCoA
r41
NH3
B-Ala-CoA
H2O
r42
3HP-CoA
H2O
r43
CoA
3HP
r44
NAD(P)
NAD(P)H
Glu
r28
NAD
a-KG
r30
NADH
ASP
r29
CO2
B-ALA
r31
A-ALA
Pyr
Glu
r32
NAD
a-KG
r30
NADH
r33
CoA
NAD
CO2
NADH
AcCoA
ATP
r36
ADP
MalCoA
NAD(P)H
r35
NAD(P)
3-OPP
r34
a-KG
Glu
r30
NADH
NAD+
CoA
ATP
r40
H2O
ADP
NADH
NAD
4 H +
r46
QH2
Q
2 H +
r47
Cyt_red
Cyt_ox
4 H +
r48
ADP
ATP
4 H +
r49
NADH
NADPH
H +
r50
PHB
CoA
r39
(R)-3-HB-CoA
r38
AcActylCoA
NADP
NADPH
CoA
7
PGA
DHAP
GAP
3HP
PHB

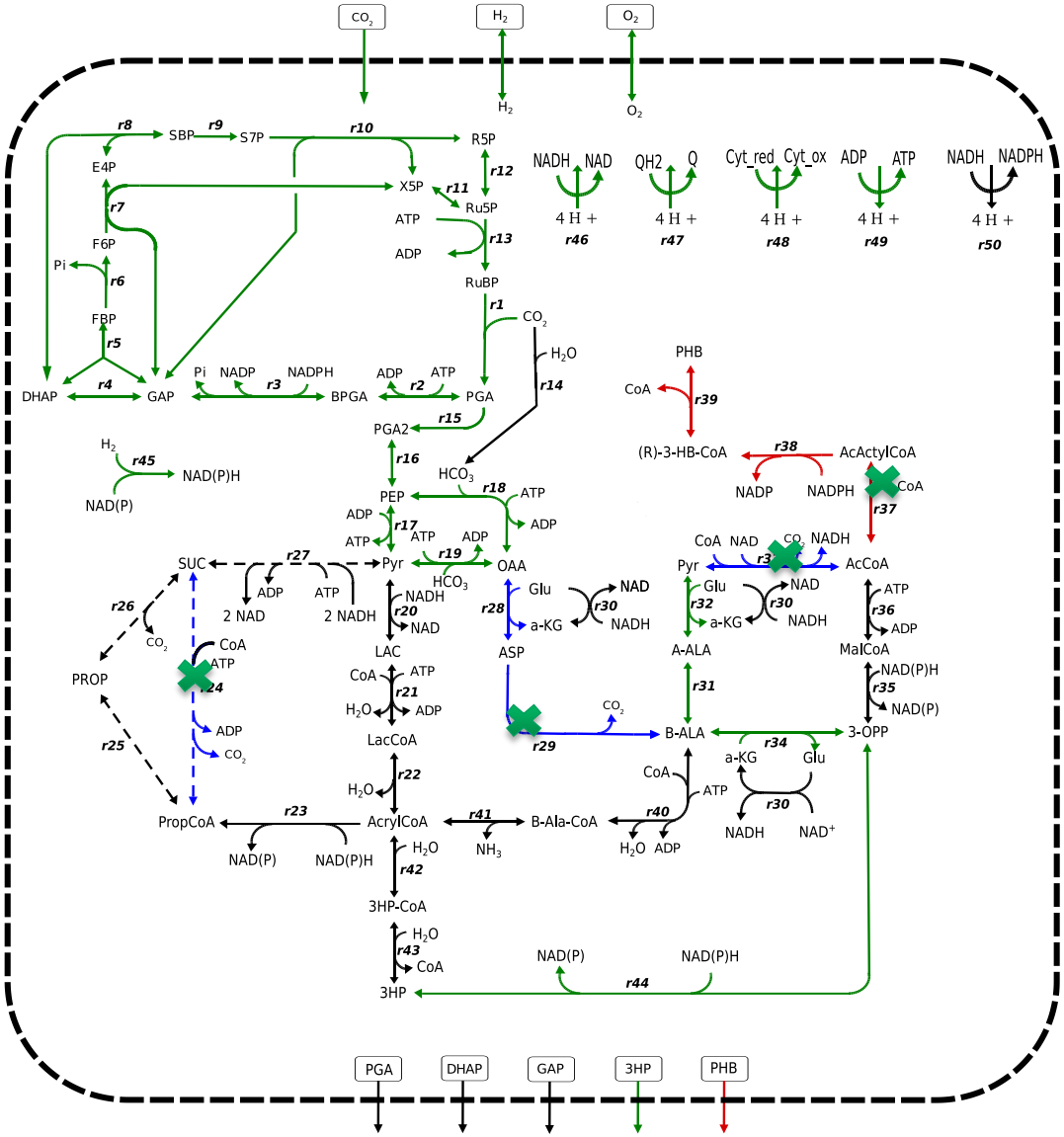
Identifying target knockouts for increasing 3HP production

Minimal Cut Sets

A **minimal** set of reactions whose **deletion** results in **failure** of certain **network functions**.

- Searched for reactions that removed **non-optimal** 3HP-producing EMs.
- Identified 10 **optimal** modes in terms of **energy requirements**, and number of **non-native** reactions.
- 3-combinations **r24, r28, r33** and **r24, r29, r33** removed a **9593** of the **9849** non-optimal modes.




CO2
H2
O2
r8
SBP
r9
S7P
r10
R5P
E4P
X5P
r11
r12
Ru5P
ATP
ADP
r13
RuBP
F6P
Pi
r6
r7
FBP
r5
DHAP
r4
GAP
Pi
NADP
r3
NADPH
BPGA
ADP
ATP
r2
PGA
r1
CO2
H2O
r14
PGA2
r15
r16
HCO3
PEP
r18
ATP
ADP
r17
ATP
ADP
r19
OAA
HCO3
SUC
r27
Pyr
ADP
ATP
2 NAD
2 NADH
r20
NADH
NAD
LAC
r26
CO2
CoA
ATP
r24
PROP
r25
ADP
CO2
CoA
ATP
r21
H2O
ADP
LacCoA
r22
H2O
PropCoA
r23
AcrylCoA
NAD(P)
NAD(P)H
r41
NH3
B-Ala-CoA
r42
H2O
3HP-CoA
r43
H2O
CoA
3HP
r44
NAD(P)
NAD(P)H
Glu
NAD
r28
a-KG
r30
NADH
ASP
r29
CO2
B-ALA
r31
Pyr
Glu
NAD
r32
a-KG
r30
NADH
A-ALA
r34
a-KG
Glu
r30
NADH
NAD+
CoA
ATP
r40
H2O
ADP
3-OPP
NAD(P)H
r35
NAD(P)
CoA
NAD
CO2
NADH
r33
AcCoA
ATP
r36
ADP
MalCoA
PHB
CoA
r39
(R)-3-HB-CoA
r38
NADP
NADPH
AcActylCoA
CoA
r37
H2
r45
NAD(P)
NAD(P)H
NADH
NAD
QH2
Q
Cyt_red
Cyt_ox
ADP
ATP
NADH
NADPH
4 H +
r46
r47
r48
r49
r50
PGA
DHAP
GAP
3HP
PHB

Summary

- Using the **net stoichiometry** of each elementary mode we can calculate the **theoretical yield** and **energy requirements**, which are useful for assessing different pathways for producing a target chemical.

- **Minimal cut-set analysis** identifies a minimal set of reactions that **prevents** the network from carrying out a function (e.g. PHB production) to ensure only the desirable solutions (e.g. 3HP producing solutions) are feasible.

- Since we are only using a small model of metabolism, any results should be tested in a **genome scale model** for validation.