A Metabolic Model of *Acetobacterium woodii* for biotechnological purposes

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Aims

Project aims

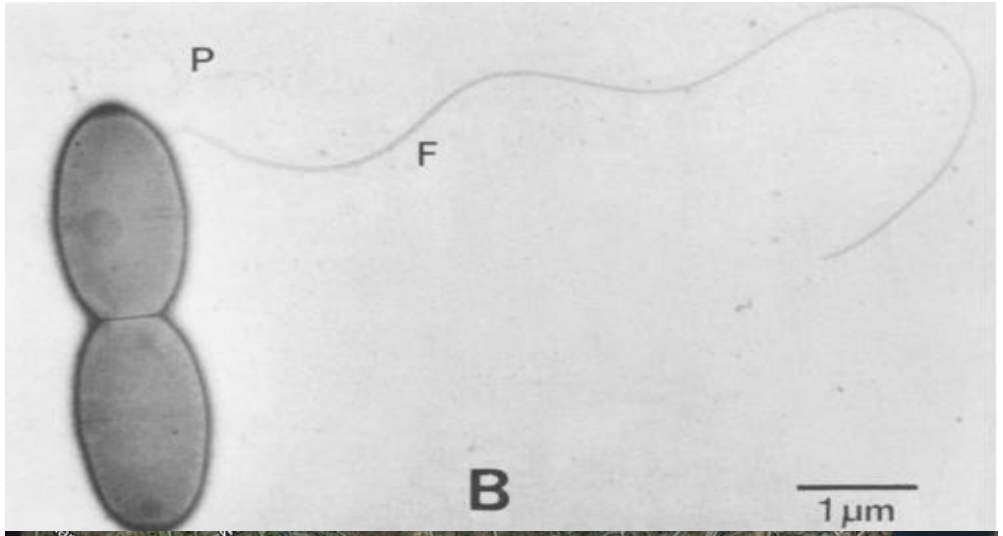
- Gain insights into *A.woodii* metabolism using metabolic modelling.
- *In silico* strain engineering to contribute to the effort in producing an industrially relevant *A.woodii.*

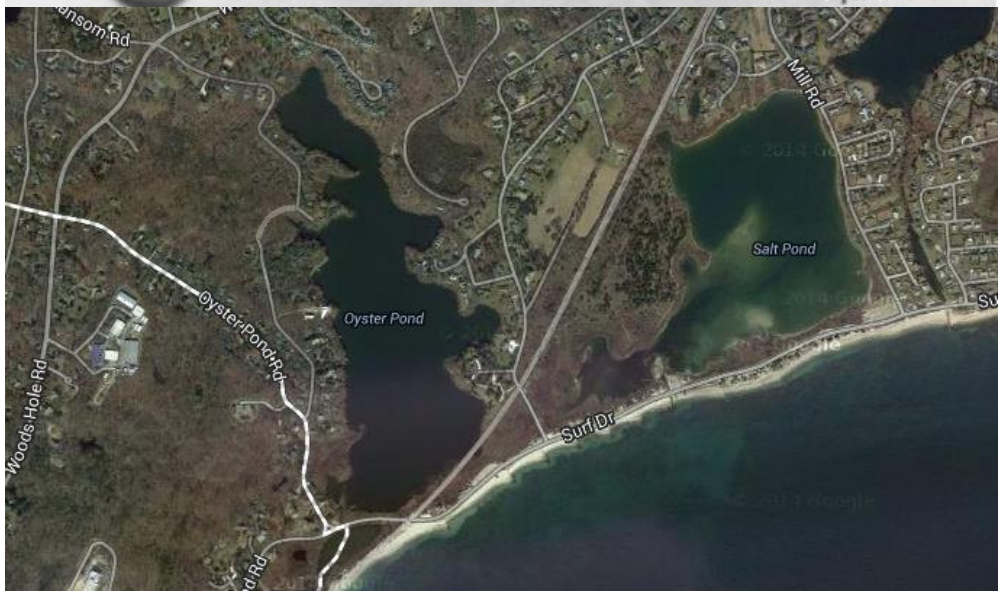
Current study aims

- Survey the feasibility of producing “platform chemicals” with *A.woodii*
- Use model guided results to plan wet lab experiments

A.woodii Background

- Belongs to group of bacteria called acetogens, of which it is a model organism.
- All acetogens are able to fix CO_2 with H_2 for growth and energy purposes with the Woods-Ljungdahl Pathway (WLP).
- Much bioenergetic work done on *A.woodii* ; the only acetogen with all bioenergetically relevant reactions in WLP characterised.
- Categorised as a Rnf type Na^+ dependant acetogen (along with *E.limosum*)



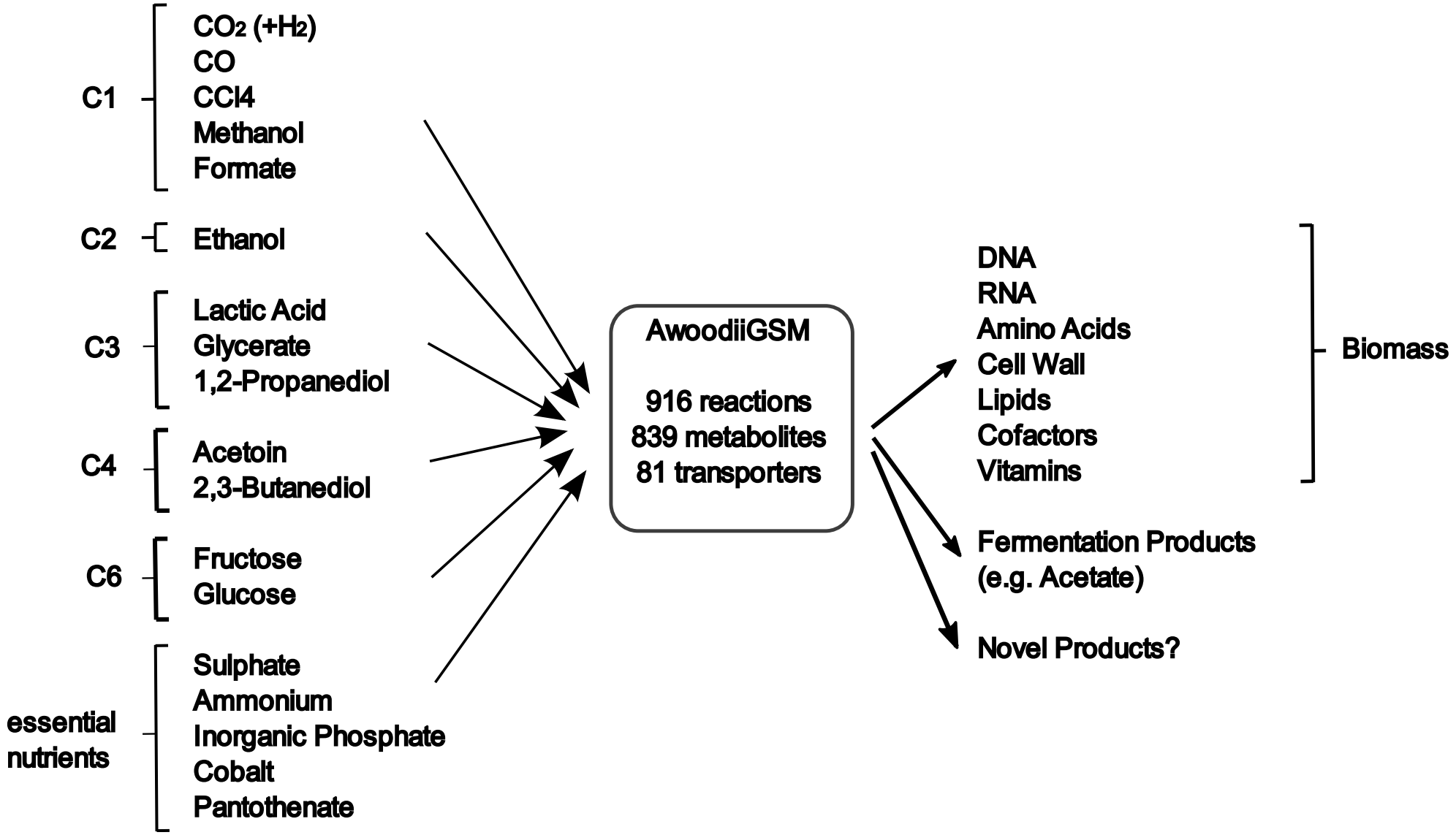


Balch, W. E., et al., 1977

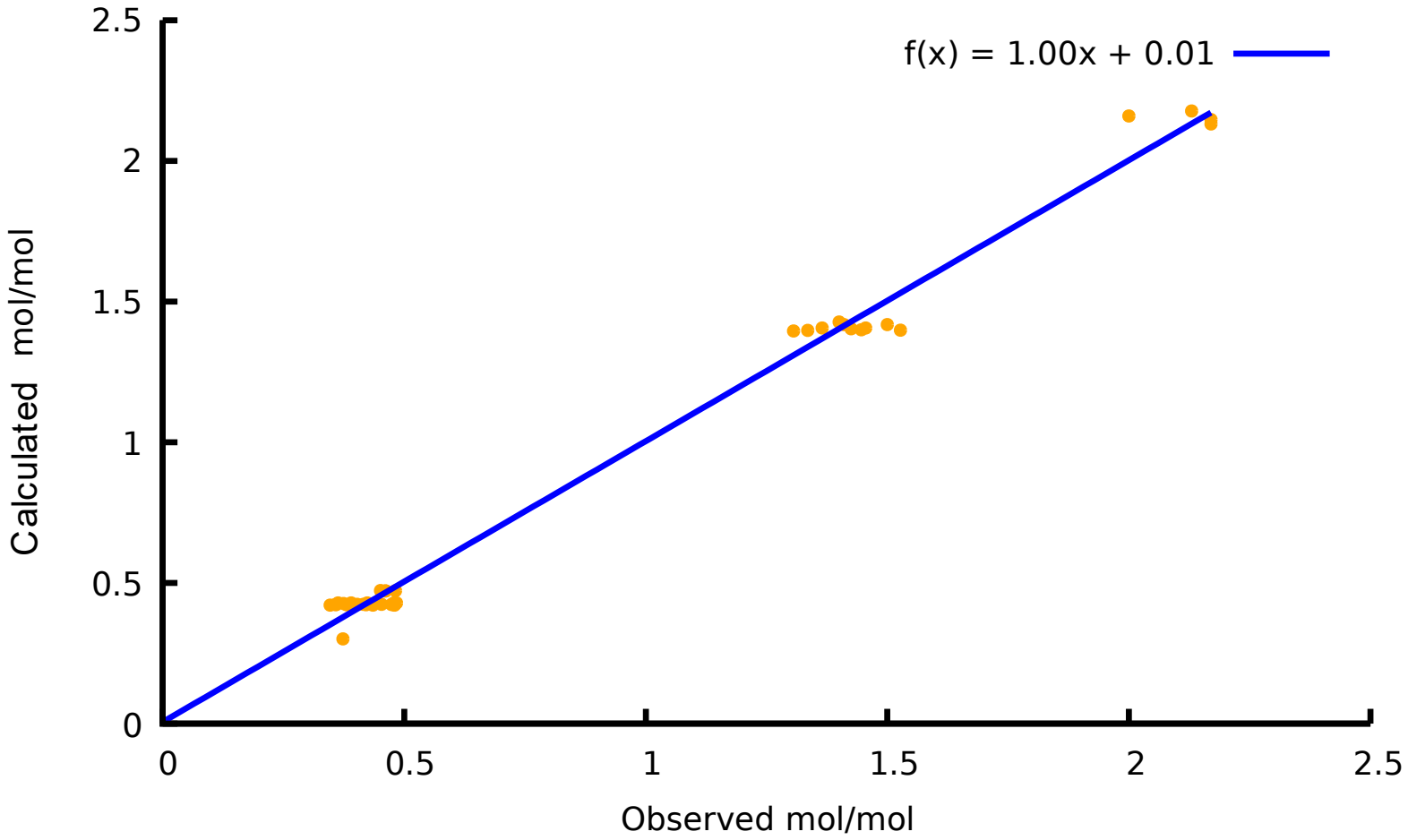
Modelling Approaches

- Structural modelling. Metabolism at **steady state**; where the **change in concentration** of internal metabolites and the **change in reaction** fluxes is zero.
- **Construction** of a genome-scale metabolic model of *A.woodii* using a recently sequenced genome of strain DSMZ1030.
- Model **validation**
- **Extraction of a sub-model** from the genome-scale model for elementary mode analysis

Genome-scale Model of *A.woodii*



Model Predicted Acetate Yields vs Observed Acetate Yields



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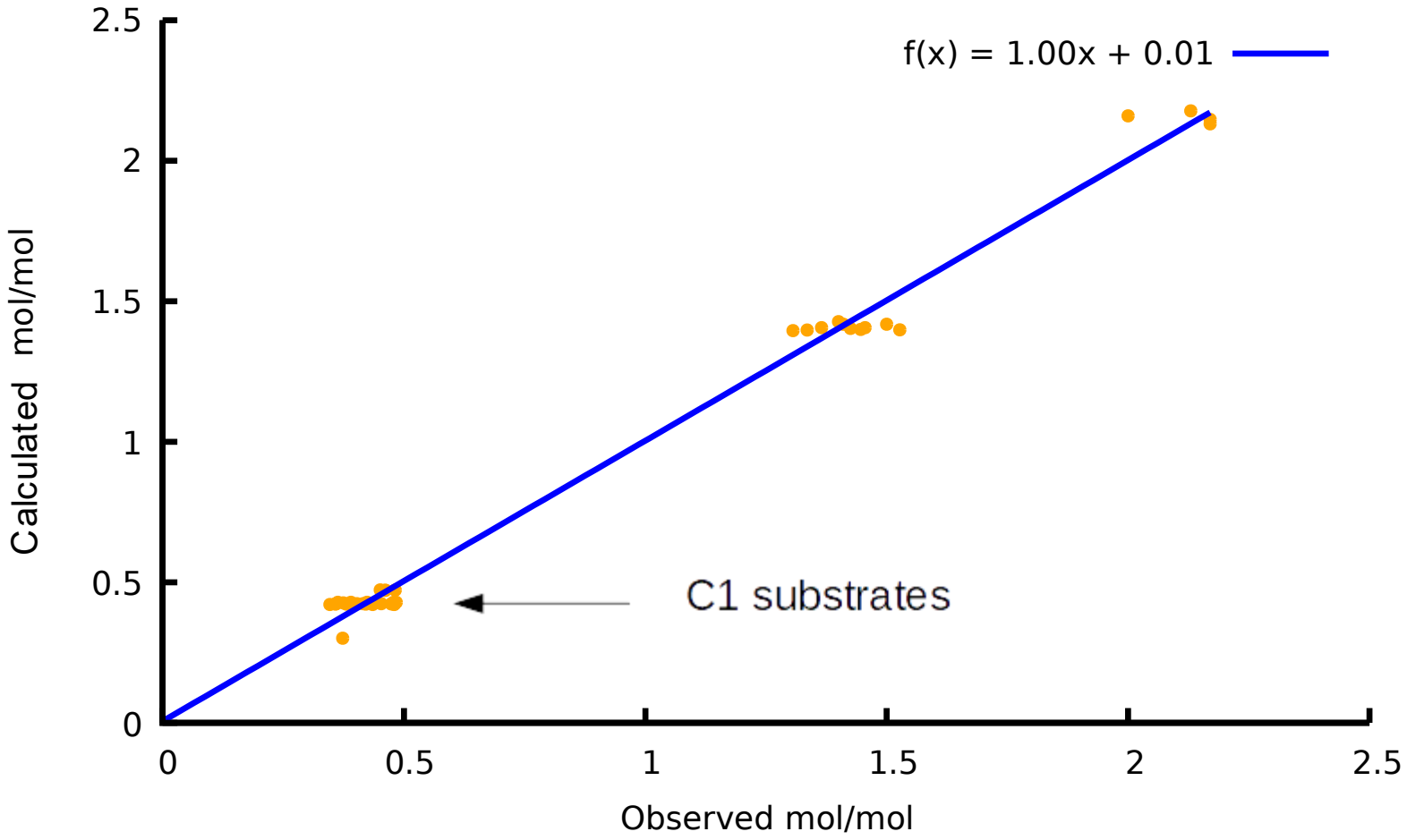

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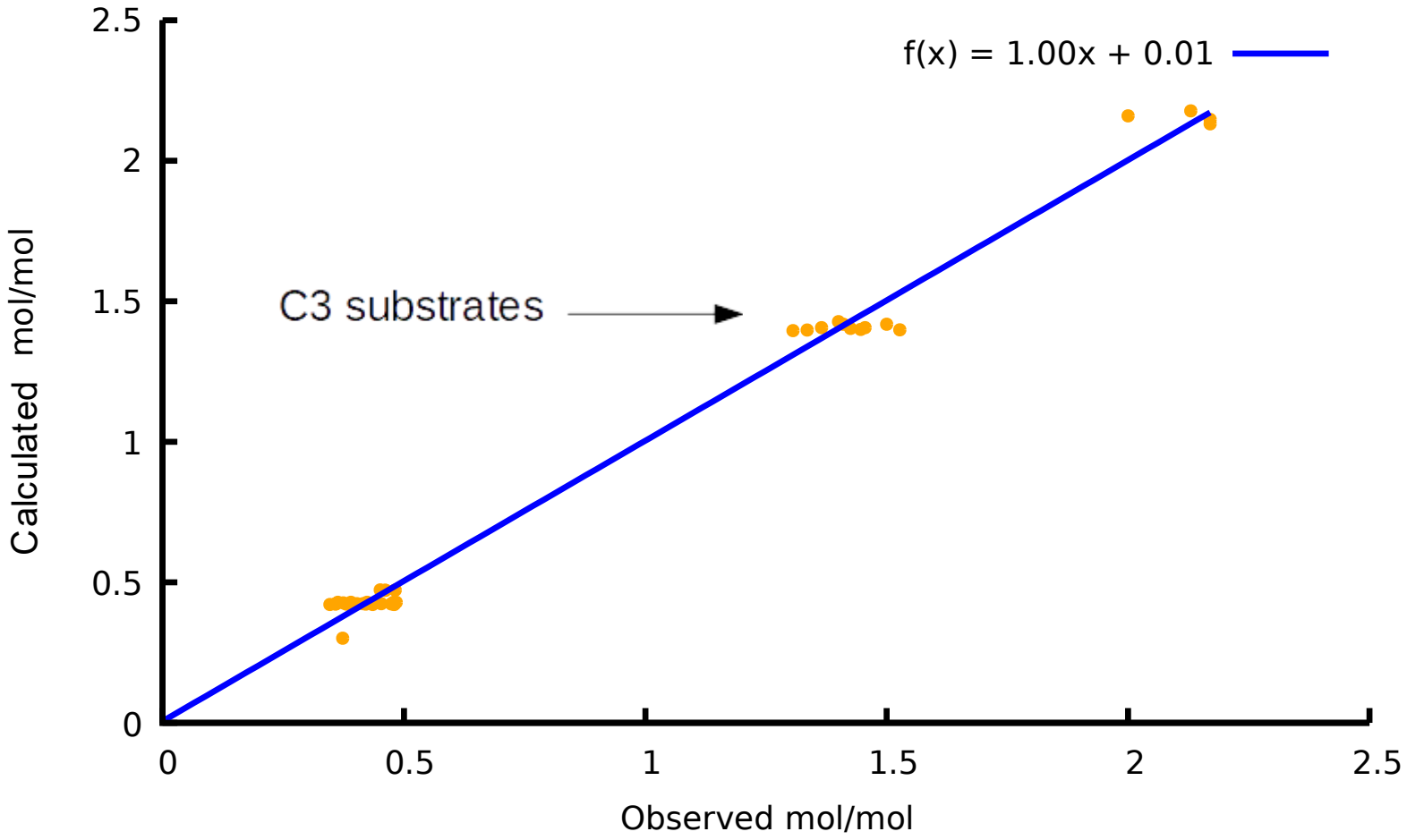

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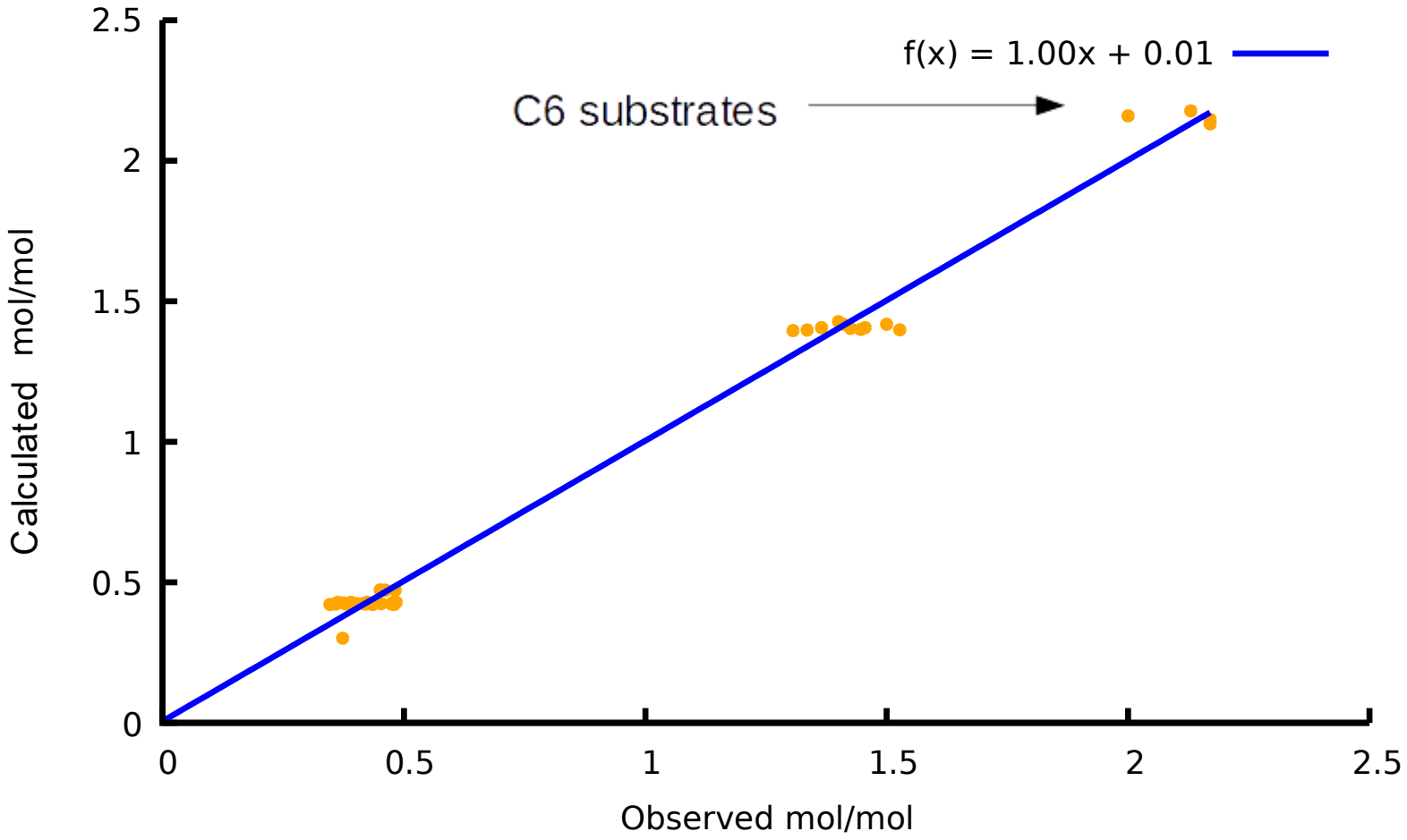

Maximum Y_{ATPs}
(mol/mol carbon)

Substrate	Product	Calculated Yield	Estimated Yield*	Reactions
Fructose	Acetate	0.72	0.72	26
Glycerol	Acetate + Ethanol	0.55	-	27
2,3-Butanediol + CO_2	Acetate	0.24	0.24	22
Ethanol + CO_2	Acetate	0.18	0.18	19
Lactate	Acetate	0.18	0.13	18
1,2-Propanediol	Propanol + Propionate	0.17	0.17	9
$H_2 + CO_2$	Acetate	0.15	0.15	17
2,3-Butanediol	Acetate + Ethanol	0.13	-	10

*from literature

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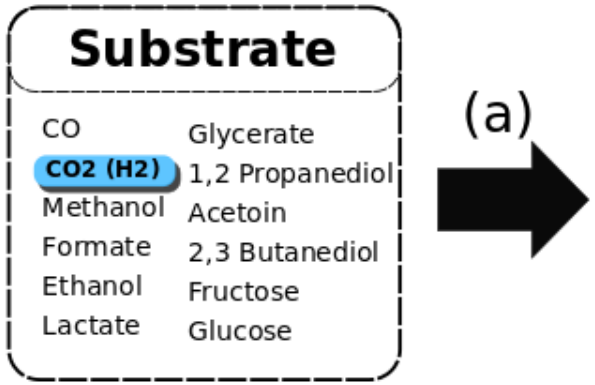
Impact of producing novel compounds on *A.woodii* metabolism

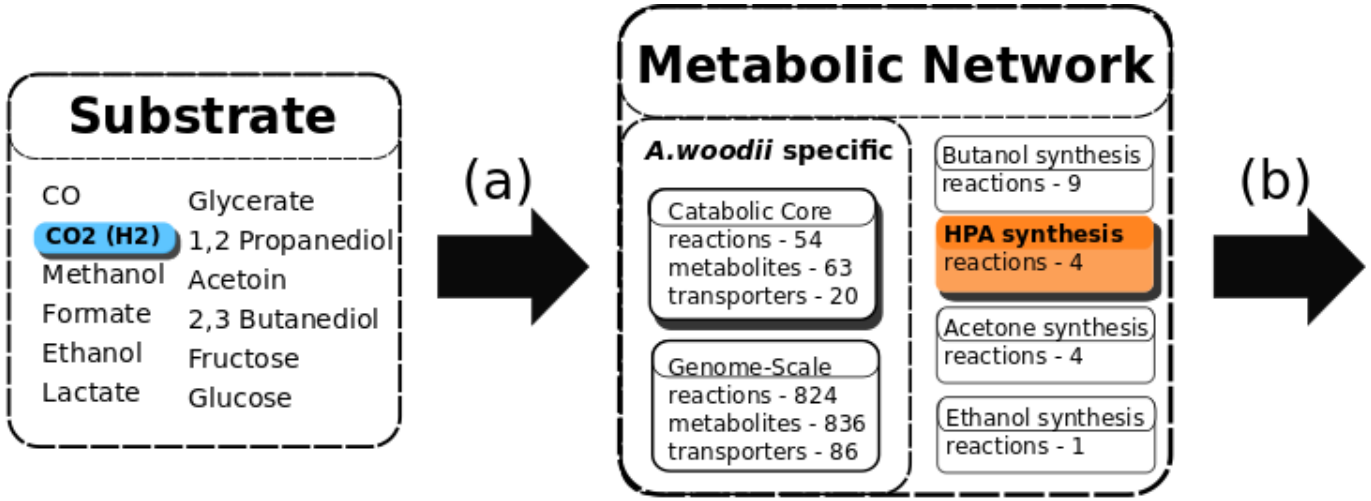
General Workflow



(a)

a) Select substrate(s)

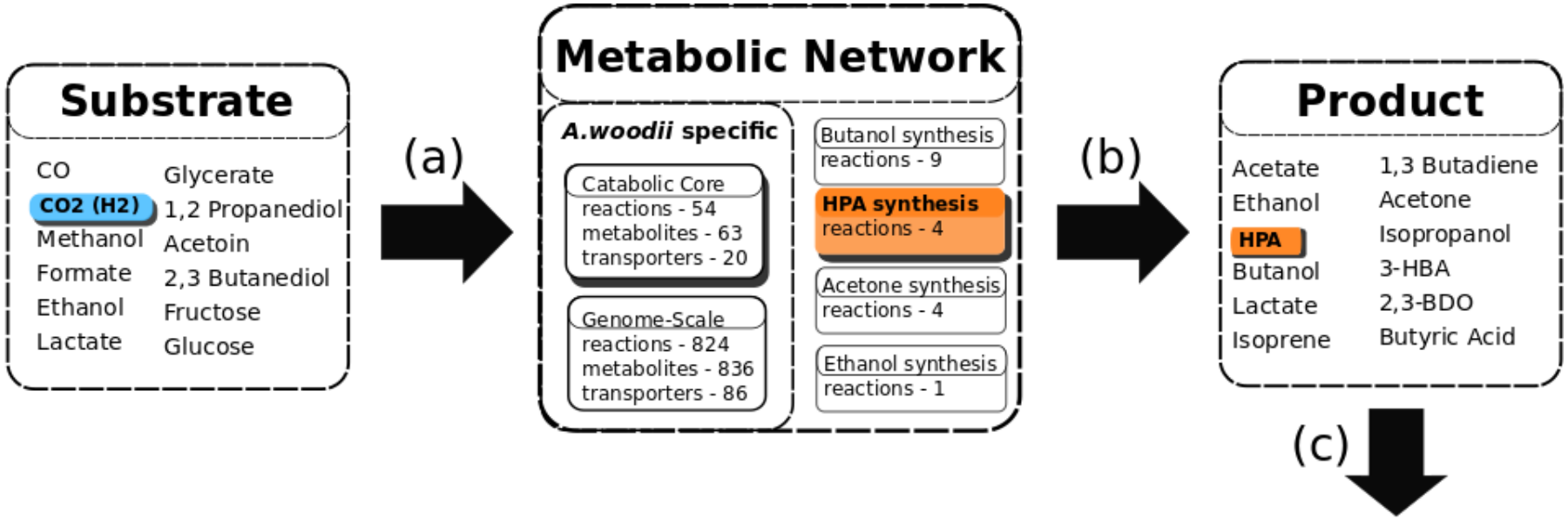
General Workflow



a) Select substrate(s)

b) Select a metabolic configuration

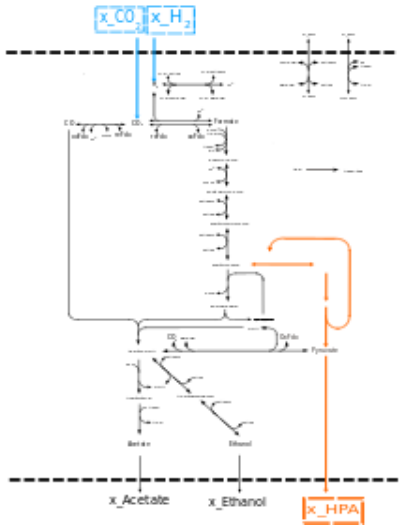
General Workflow



a) Select substrate(s)

b) Select a metabolic configuration

c) Select product(s)



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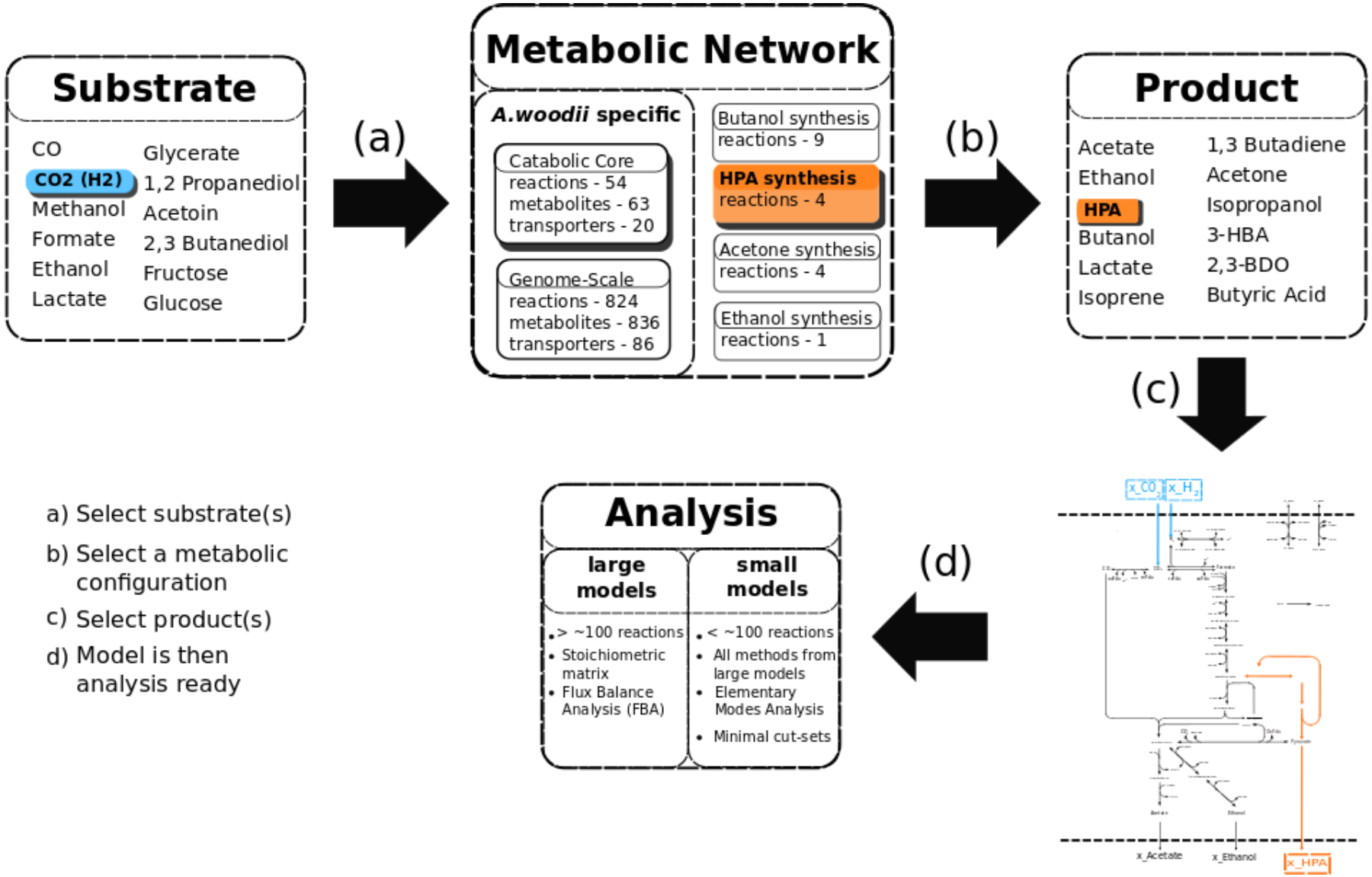

a) Select substrate(s)

b) Select a metabolic configuration

c) Select product(s)

d) Model is then analysis ready

Elementary Modes of 3-HPA production

Elementary Mode	Y_{HPA} (%)	Y_{ATP} (mol/mol carbon)	Number of reactions
1	100	0.07	21
2	100	0	23
3	84.8	0	23
4	72.7	0	25
5	63.6	0	22
6	39.4	0	23

Work done in collaboration with Sheila Ingemann Jensen and Alex Toftgaard Nielsen at Novo Nordisk Foundation Centre for Biosustainability.

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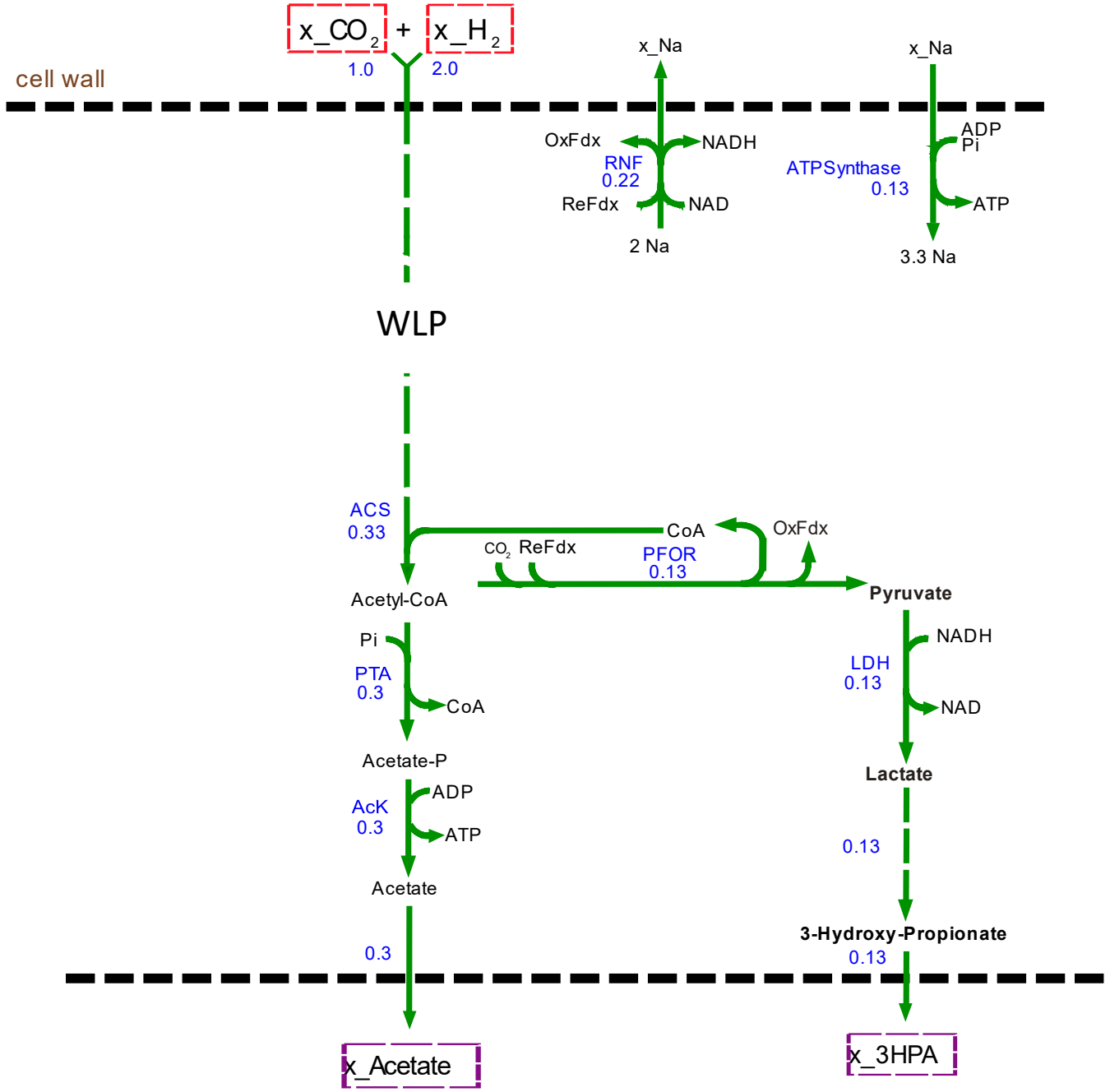

Y_{HPA} = 0.13 (39%)

Y_{ATP} = 0 (-0.3 deficit)

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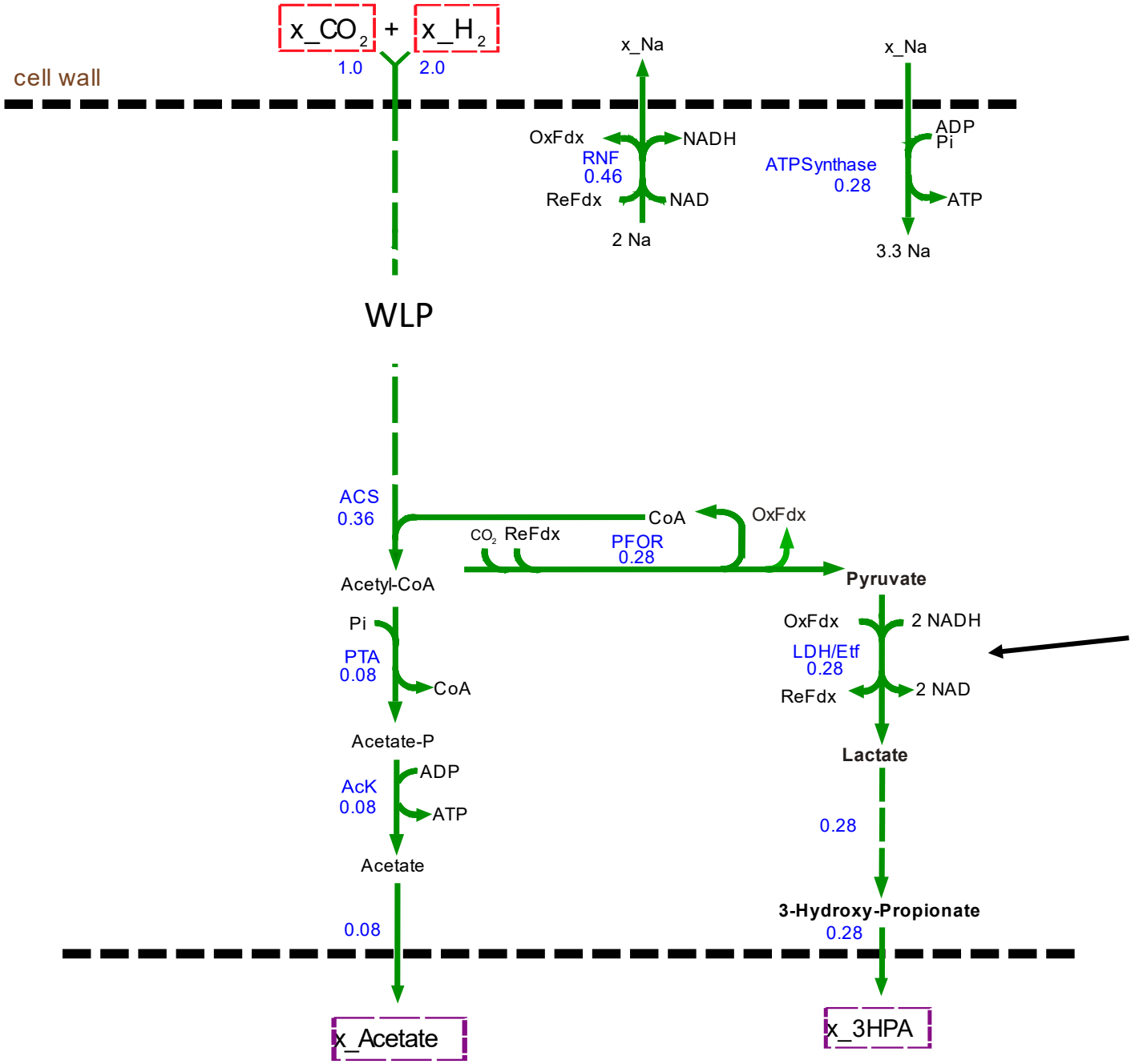

Y_{HPA} = 0.28 (84%)

Y_{ATP} = 0 (-0.08 deficit)

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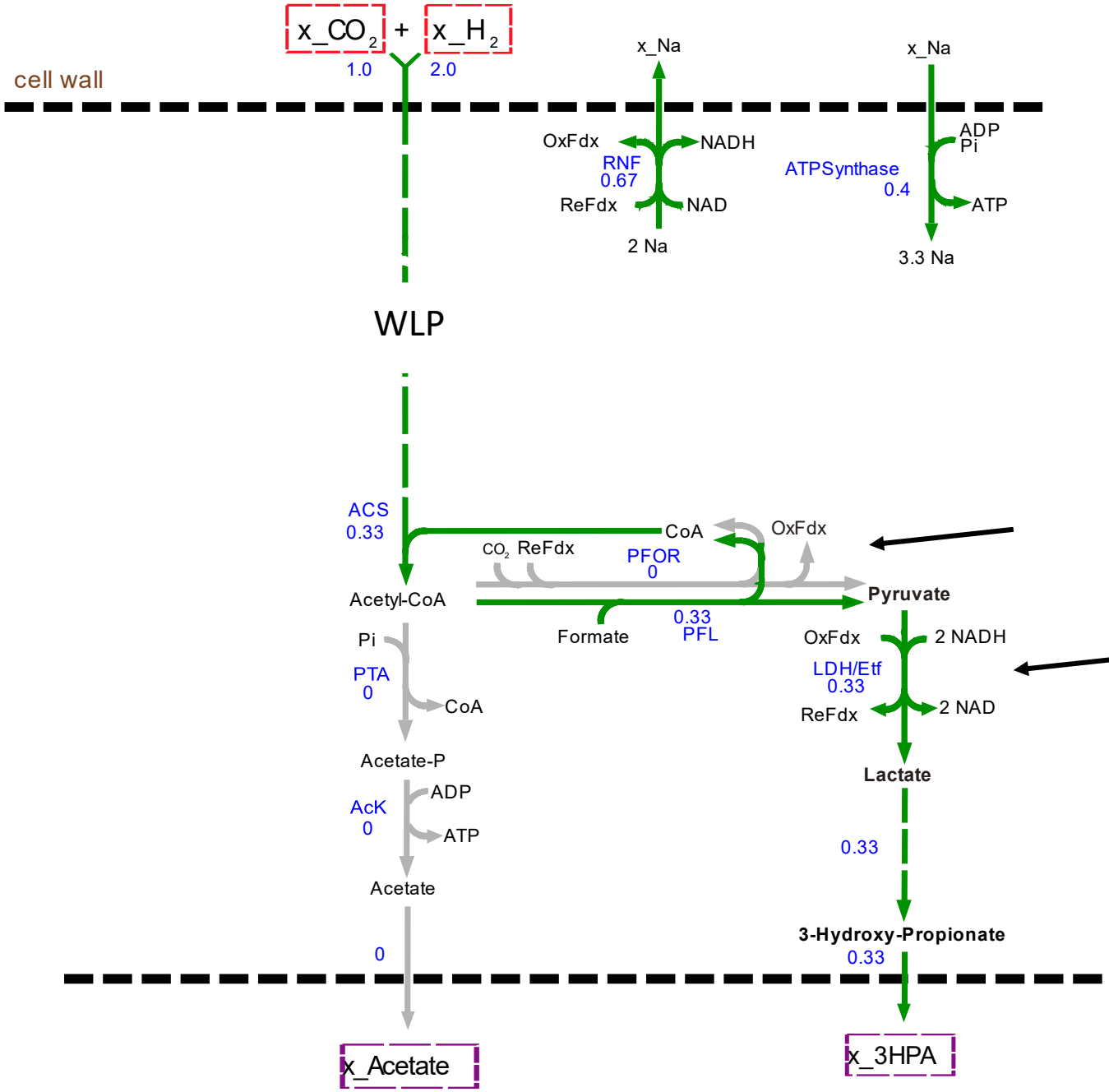

Y_{HPA} = 0.33 (100%)

Y_{ATP} = 0.07 (surplus)

Redox Agent Turnover

Y_{HPA} (%)	NADH (mol/mol carbon)	ReFdx (mol/mol carbon)
100	1.33	1
84.8	1.27	1.1
39.4	1	0.78

Redox Agent Turnover

Y_{HPA} (%)	NADH (mol/mol carbon)	ReFdx (mol/mol carbon)	RNF (flux/mol carbon)
100	1.33	1	0.67
84.8	1.27	1.1	0.46
39.4	1	0.78	0.22

Net Stoichiometry of *A.woodii* Electron Transport Chain

ReFdx + 0.6 ADP + 0.6 Pi + NAD ↔ OxFdx + NADH + 0.6 ATP

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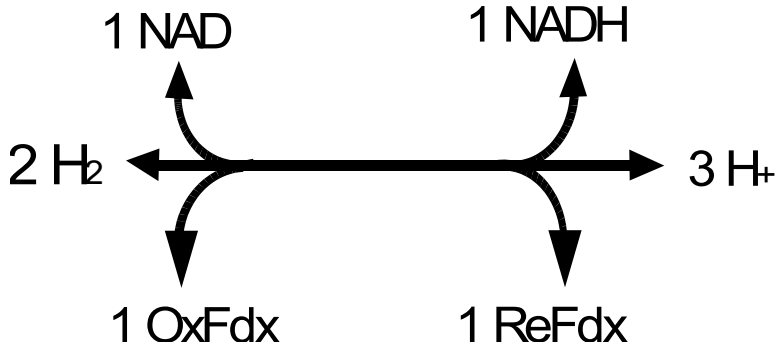
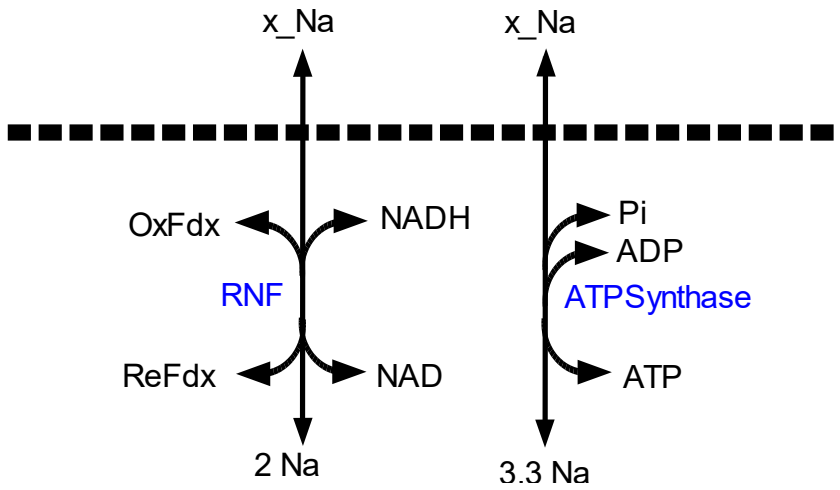
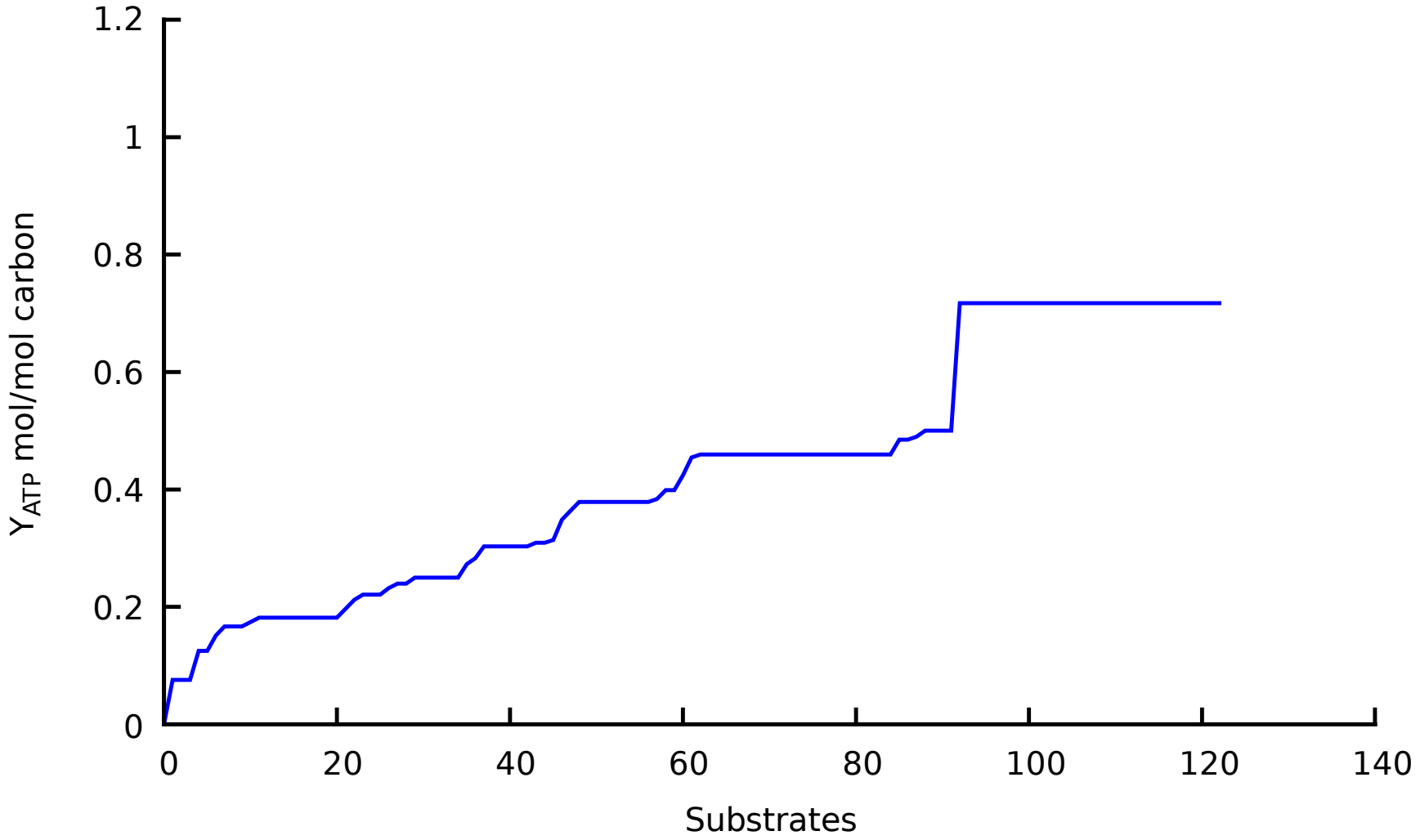
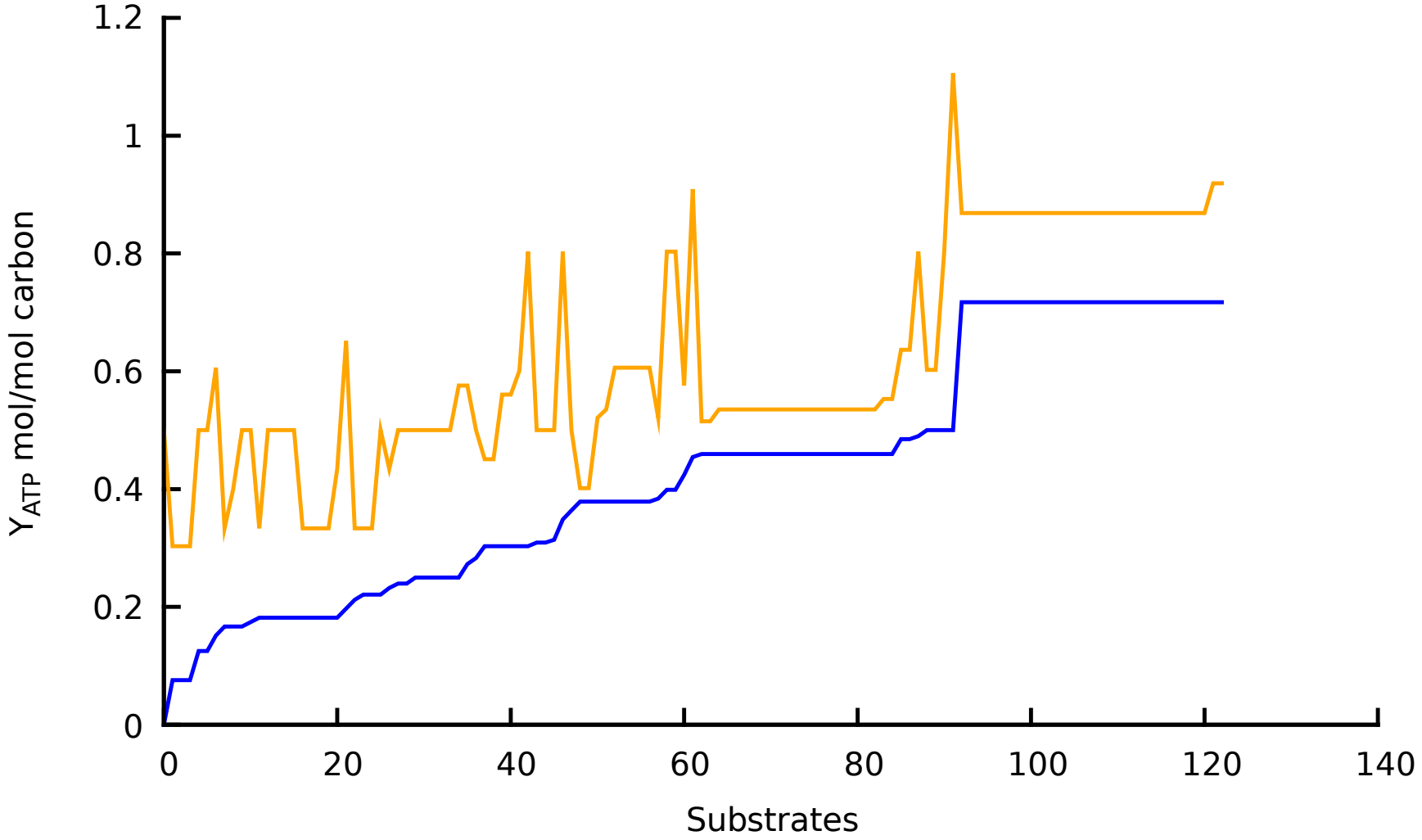




Y_{ATP}^{MAX} of 124 Substrate Combinations



Hypothetical NADH Sink



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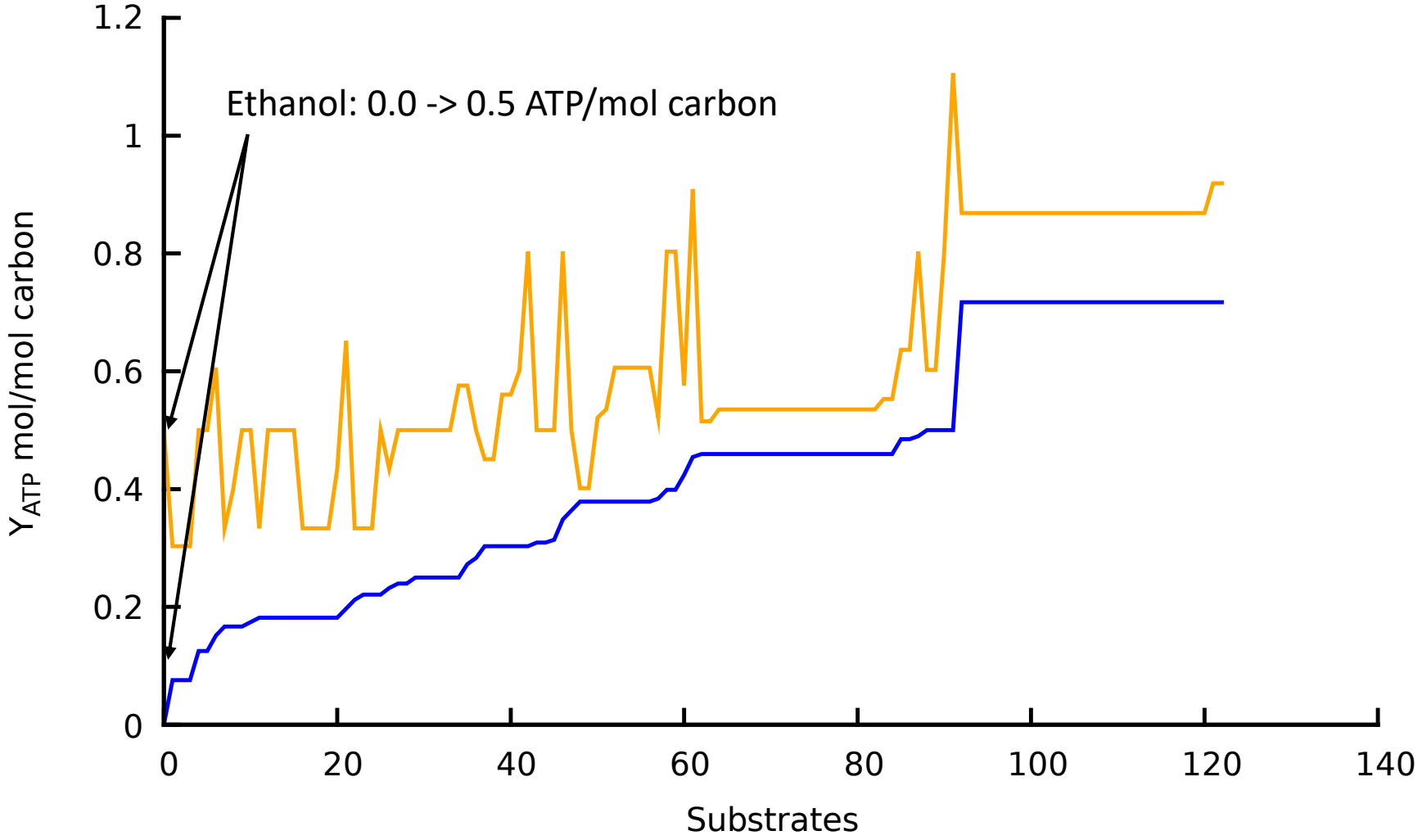

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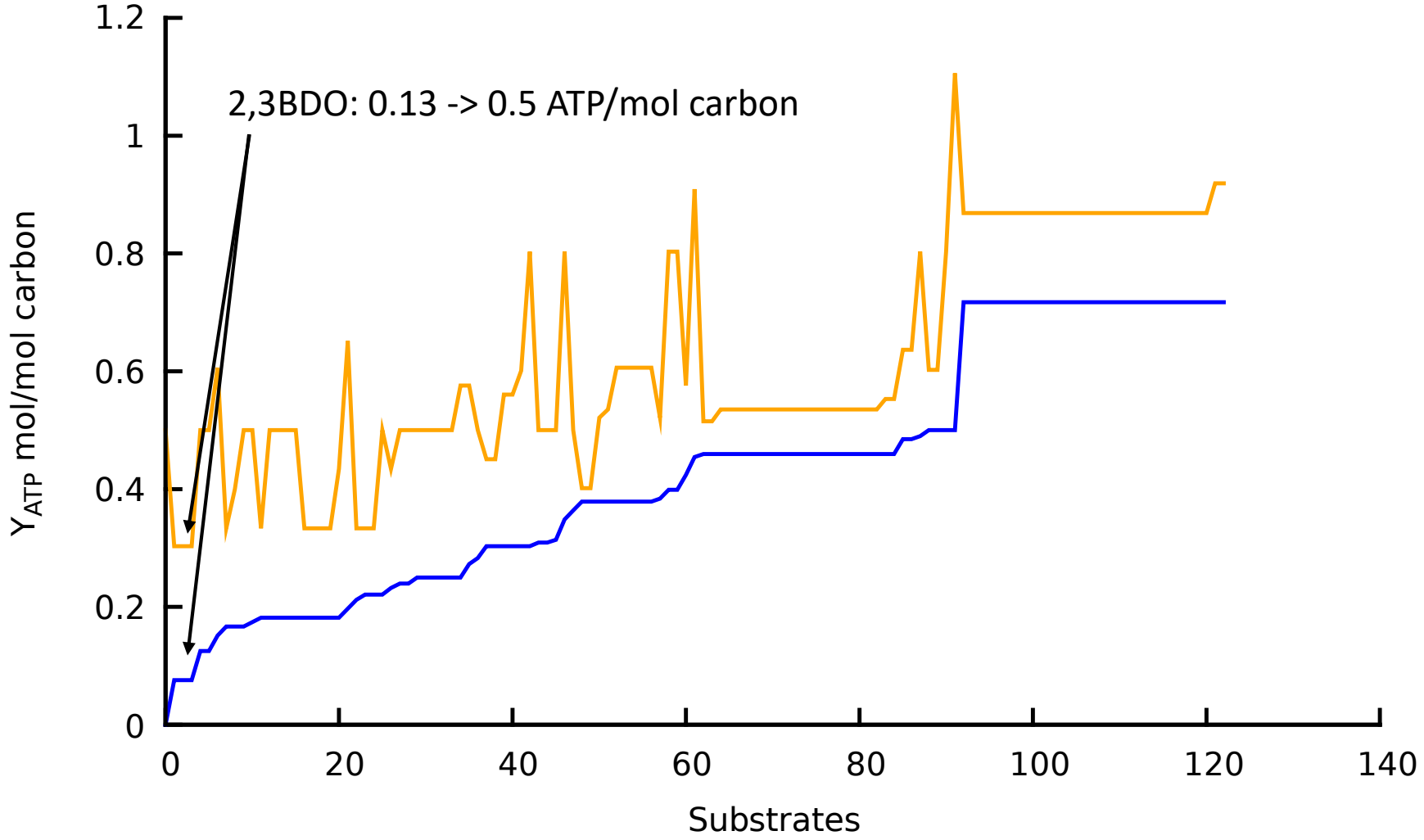

Alternative Electron Acceptor Y_{ATP}^{MAX}

Acetogens are known to reduce a variety of electron acceptors, including several nitrogen sources (e.g. nitrite) and sulphur sources as well as lignin degradation products (e.g. caffeic acid).

Substrate	- (no growth)	CO2	NADH oxidase	Nitrite
Ethanol	0	0.18	0.5	0.5
2,3 Butanediol	0.13	0.24	0.5	0.5

Model analysis shows ethanol and 2,3 BDO are not dependent on CO_2 for growth when using alternative electron acceptors. Also, better growth yields are achieved with electron acceptors such as nitrite and caffeic acid (results not shown here).

Summary

- Metabolic models are a flexible platform for surveying potential target compounds.
- Adding even a few heterologous reactions for HPA production gives rise to several new metabolic regimes, which are identified with elementary modes analysis.
- Insights into the impact of redox balances on bioenergetics is helping guide program of experiments.
- Producing more reduced compounds would be favoured bioenergetically in *A.woodii.*

Acknowledgments

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Novo Nordisk Foundation: Sheila Ingemann Jensen, Alex Toftgaard Nielsen

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