Metabolic Modelling of *Acetobacterium woodii* for Biotechnological Purposes

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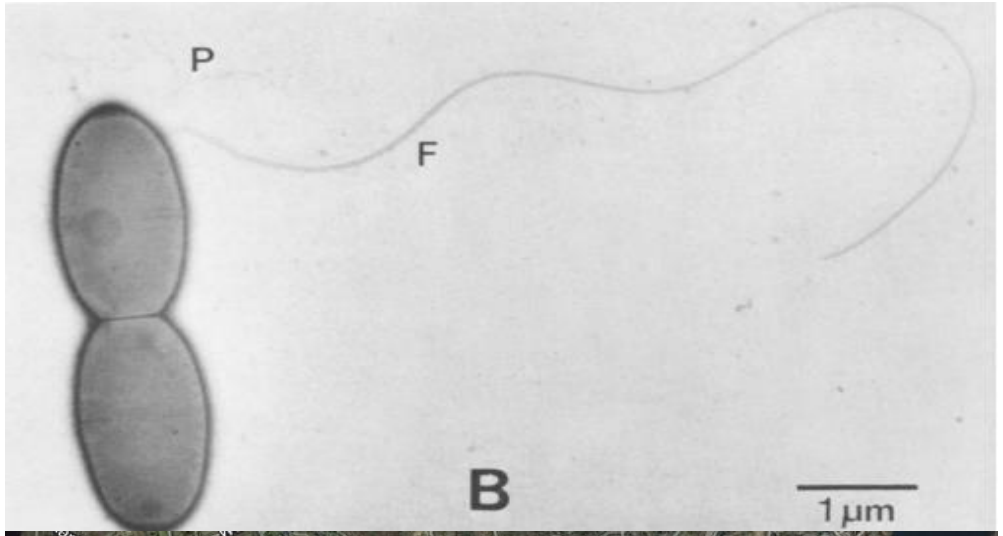
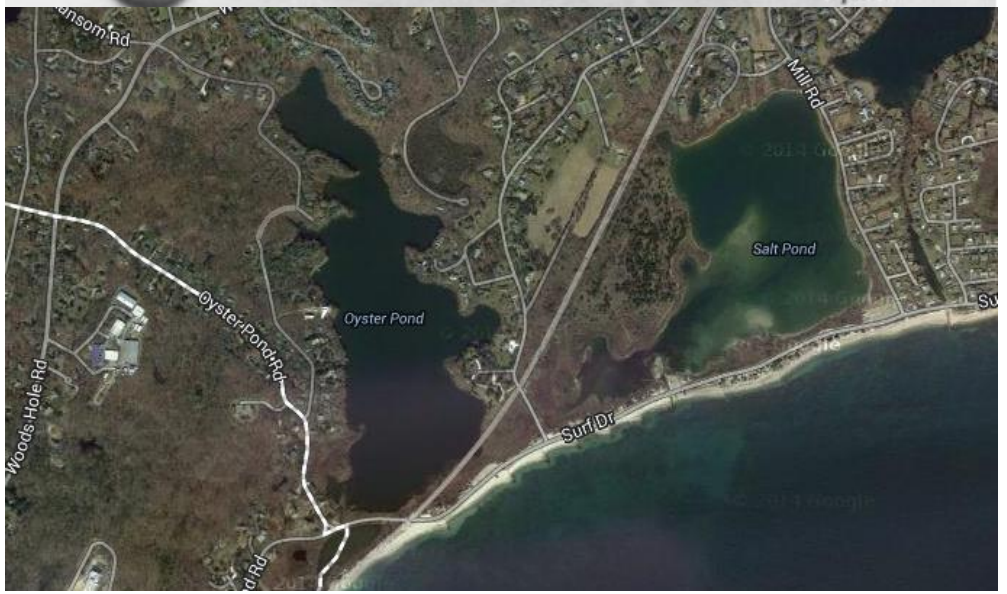
A.woodii Background

- 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*)

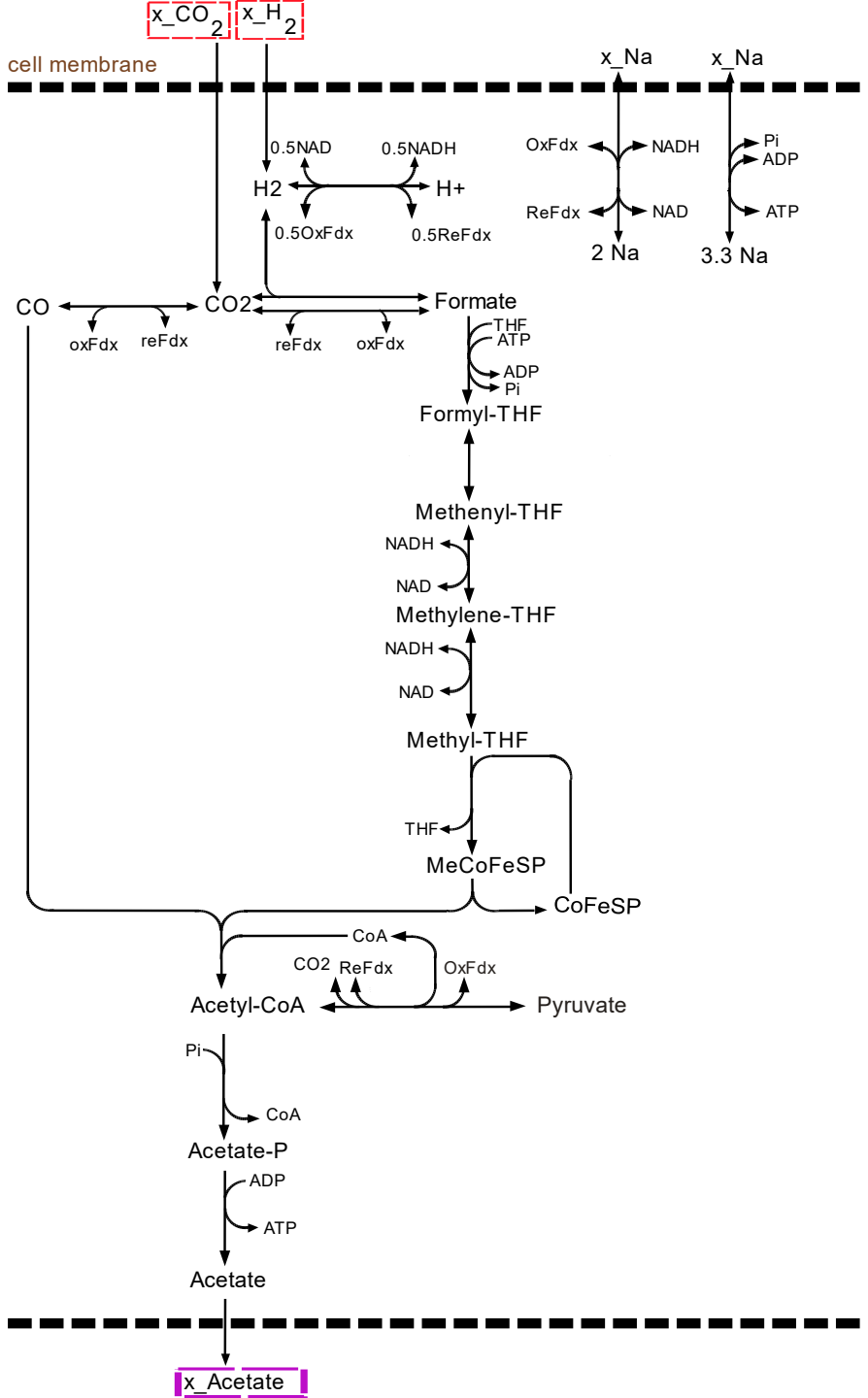




Balch, W. E., et al., 1977

Woods-Ljungdahl Pathway

$2\ CO_2 + 4\ H_2 \rightarrow 1\ Acetate + 0.15\ ATP$



Project aims

- Construct a genome-scale metabolic model of *A.woodii* using a sequenced strain (Poehlin, 2012)
- 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.*

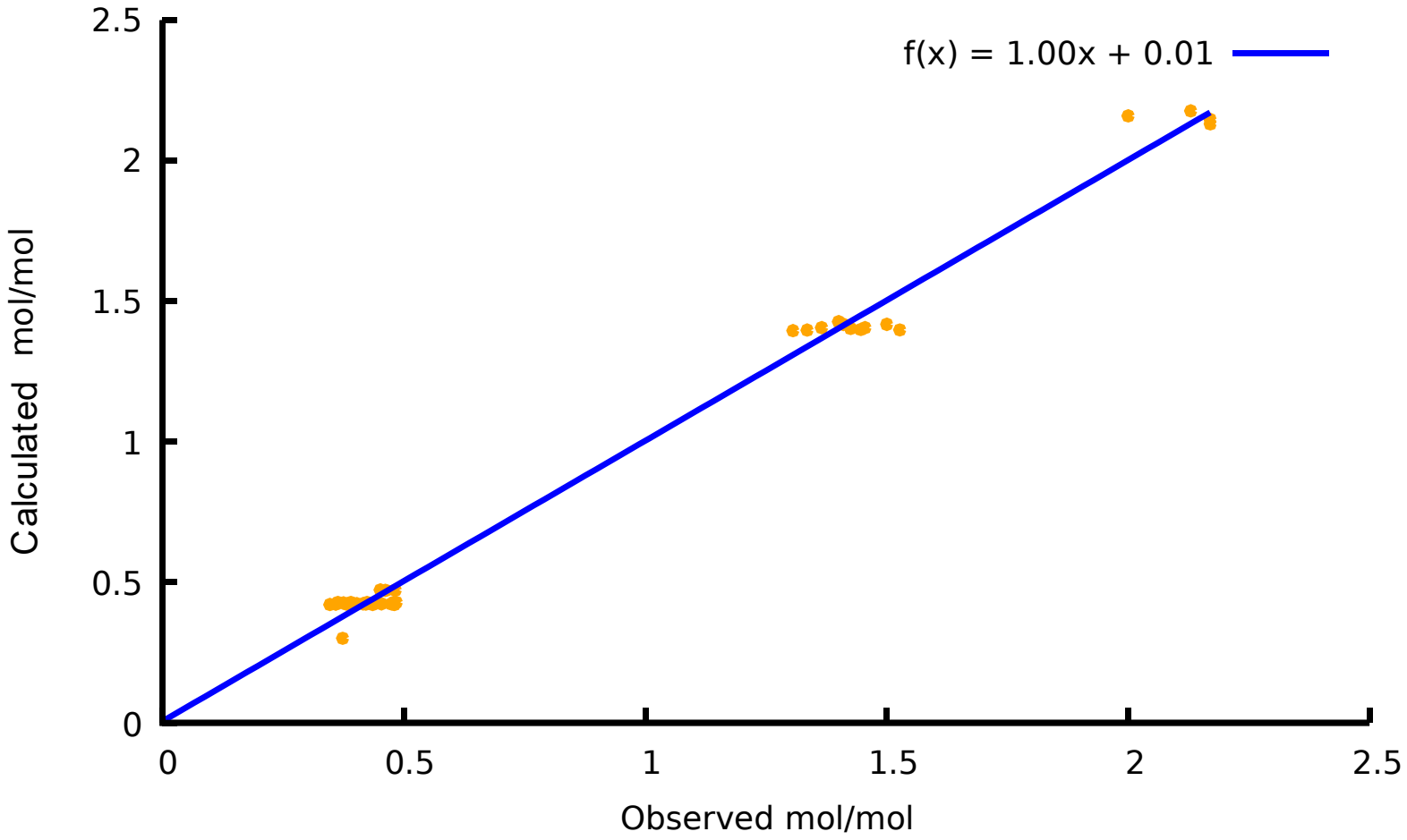
Modelling Approaches

- 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

Model Validations

- Conservation of mass. Carbon, nitrogen, phosphorus, sulphur atoms cannot be produced unless appropriate substrate is supplied
- Conservation of energy. ATP cannot be produced without substrate, and redox reactions cannot occur unless appropriate substrates are supplied
- Substrates reported to support growth in the literature where tested against what is supported in the model
- Compared published acetate yields in several conditions to model produced acetate yields in same conditions.

Model Predicted Acetate Yields vs Observed Acetate Yields



Substrates Used

Fructose,
$CO_2 + H_2$,
Lactate + H2 + CO_2,
Lactate + CO_2,
Methanol + CO_2,
Methanol + Formate

(changing growth rates)

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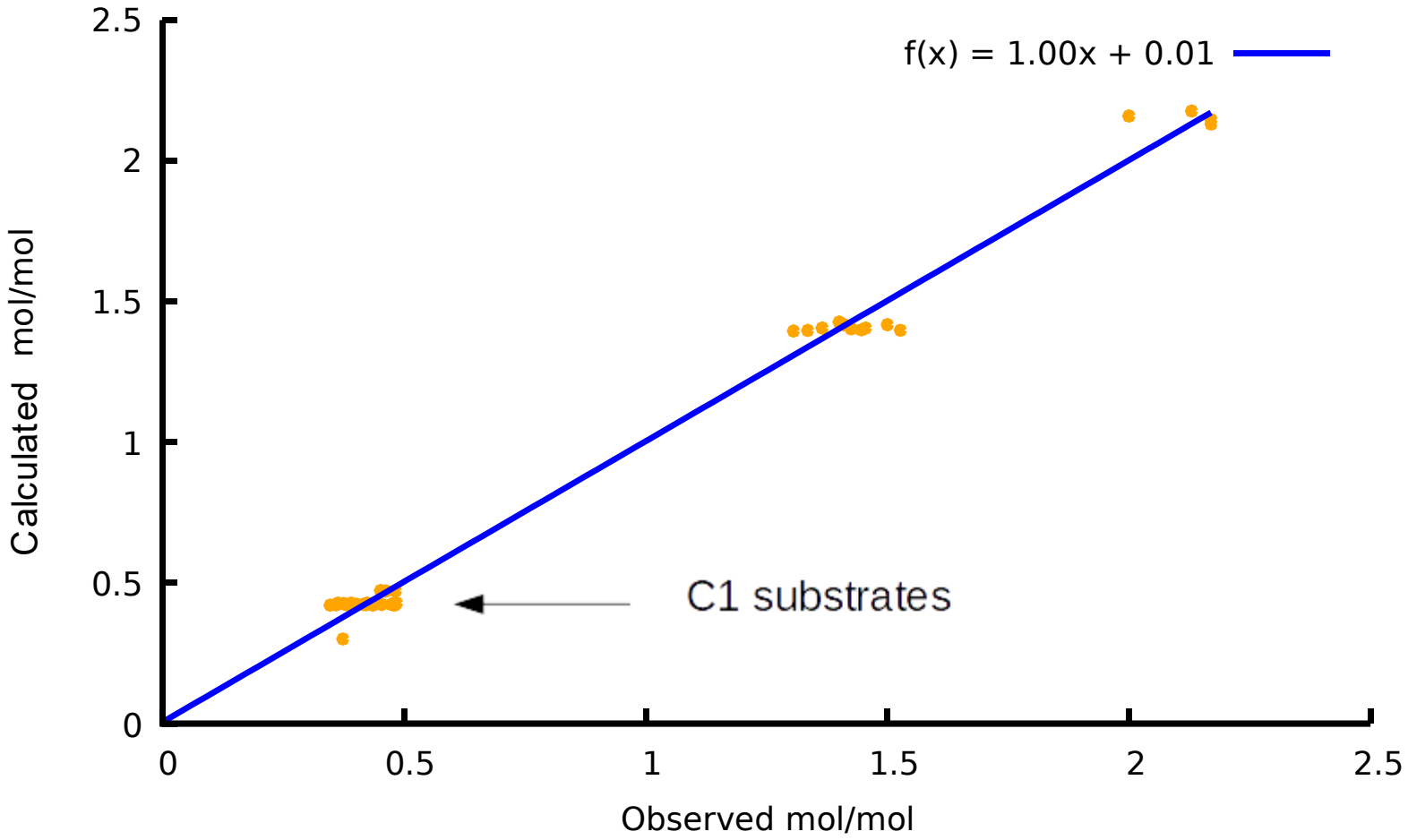

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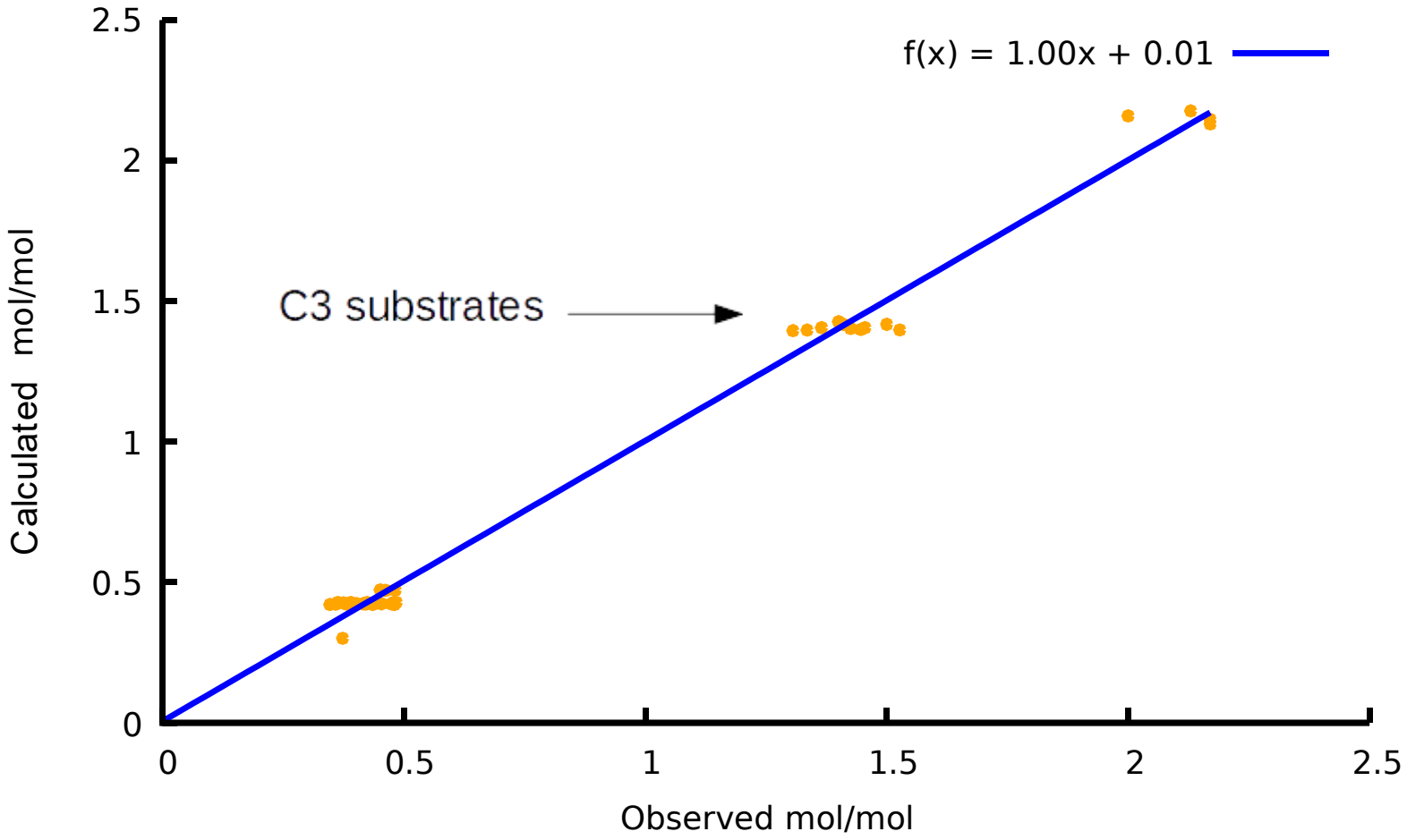

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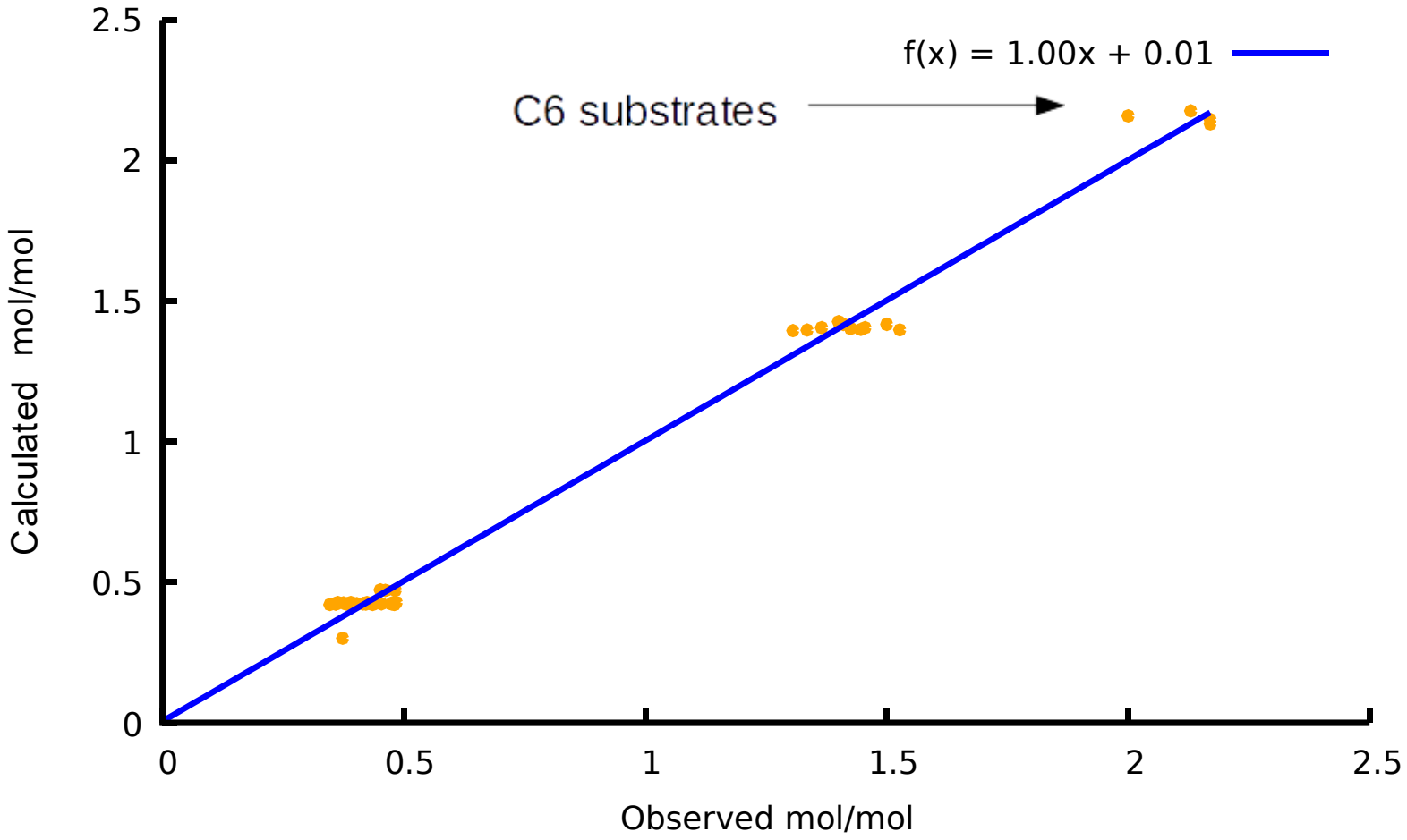

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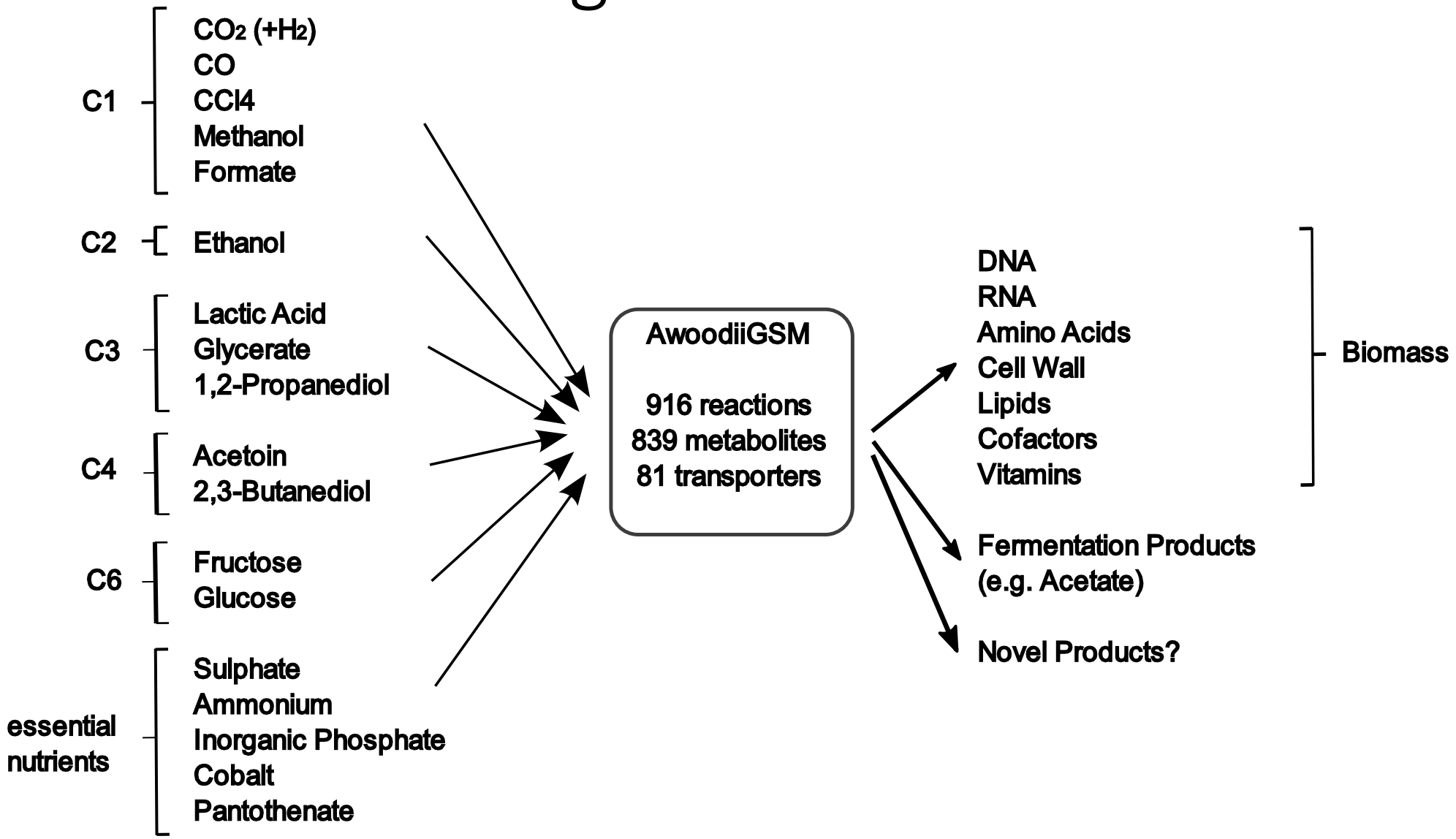
A.woodii Genome-scale Metabolic Model – high level view



Complete Growth Stoichiometry

Growth on CO_2 + H_2 ($\mu = 0.2\ h^{-1}$):

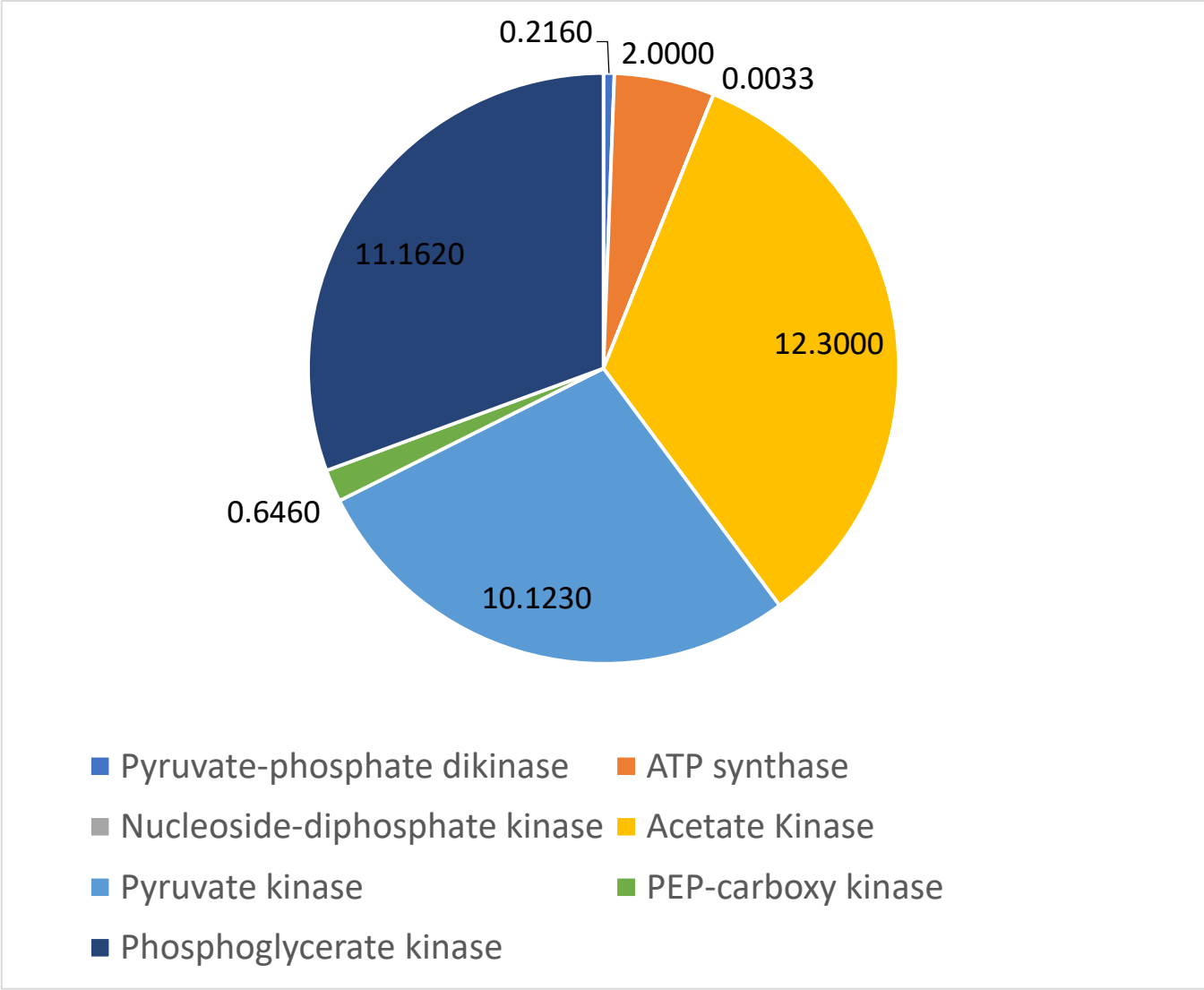
171mM CO_2 + 343mM H_2 + 0.04mM SO_4 + 2.4mM NH_3 + 0.4mM Pi + trace components

1g Biomass + 80.3mM Acetate + trace components

93.8% Carbon in acetate

6.1% Carbon in biomass

ATP producing reactions during growth – Fructose



Extracting a Bioenergetic Core

Rationale:

- Useful for analysis not possible on large model
- Would represent all catabolic reactions involved with energy metabolism

Method:

- Available substrates are combined in all possible pairs

precondition - substrate pair must support growth (e.g. a pair like H2 + caffeate would be excluded)

- ATP extraction is computed with 4 LP formulations per substrate (1,084 total)
- All reactions which show up at least once are saved
- Yield of ATP is calculated as ATP/C-mol

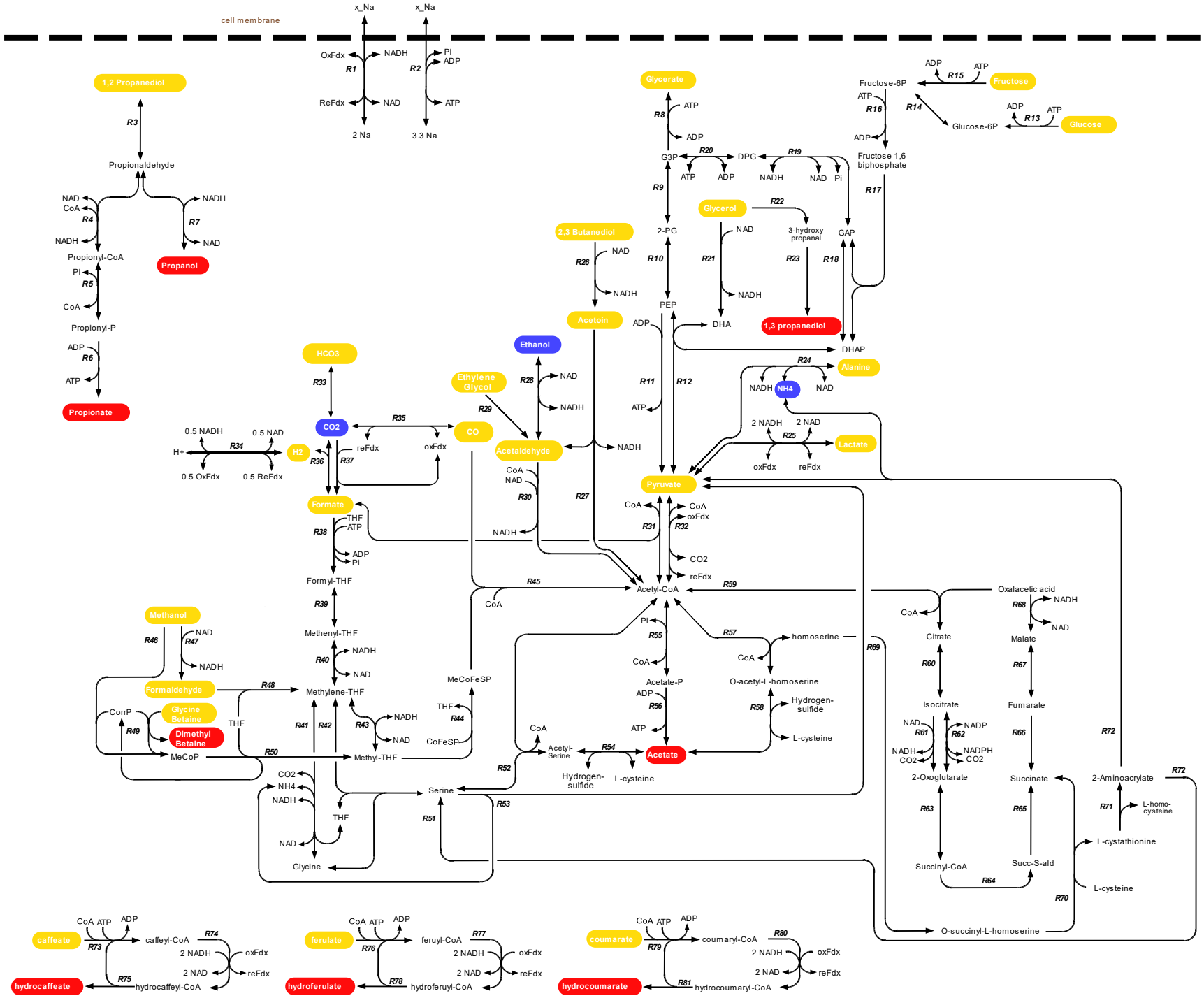
Bioenergetic Core -draft

Reactions: 837 -> 83

Metabolites: 848 -> 95

Comprises all of the reactions needed to extract ATP from 271 growth substrates.

Amenable to elementary modes analysis



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