# Metabolic modelling to support synthetic biology in C1Net organisms

C1net POC award

**Andrez Kierzek, Mehdi Khoury, Mark Poolman & David Fell**



July 22, 2016

# Project Plan

## Aim:

Create a genome-scale metabolic model of a C1 gas fermenting microorganism and demonstrate the applicability of predictive modelling to the design of new strains of interest to C1Net.

## Objectives:

1. Selection of target organism
2. Construction of Genome–Scale Metabolic Network Model
3. Model validation and refinement by Flux Balance Analysis
4. Demonstration of model application
5. Model import to web–based simulation environment (Simulocyte, Surrey FBA)
6. Dissemination

# 1. Target Organism



- *Methylococcus capsulatus* (Bath) was selected as the primary target.

# 1. Target Organism

- *Methylococcus capsulatus* (Bath) was selected as the primary target.
- As a secondary objective, advice was given to Rupert Norman (Nottingham) on parallel construction of a model of *Clostridium autothenogenum* (see Poster 15).

# 2. Model Construction



- The *M. capsulatus* model was created and curated from the BioCyc PGDB gene–enzyme–reaction mapping according to the OBU group's standard operating procedures, using the modelling packages ScrumPy and PyoCyc.

# 2. Model Construction



- The *M. capsulatus* model was created and curated from the BioCyc PGDB gene–enzyme–reaction mapping according to the OBU group's standard operating procedures, using the modelling packages ScrumPy and PyoCyc.
- The resulting model has:
  - 965 reactions
  - 896 internal metabolites
  - 63 external metabolites, of which
  - 38 are biomass precursors, and
  - the remainder media components and fermentation products.

# 3. Model Validation

1. Mass balance:
   (a) Individual reactions balanced for C, N, P, S from empirical formulae where available.
   (b) No system–level stoichiometric inconsistencies for C, N, S, P using Gevorgyan's method.

# 3. Model Validation

1. Mass balance:
   (a) Individual reactions balanced for C, N, P, S from empirical formulae where available.
   (b) No system–level stoichiometric inconsistencies for C, N, S, P using Gevorgyan's method.
2. Energetic consistency - No ATP or NAD(P)H production without mass flow.

# 3. Model Validation

1. Mass balance:
   (a) Individual reactions balanced for C, N, P, S from empirical formulae where available.
   (b) No system–level stoichiometric inconsistencies for C, N, S, P using Gevorgyan's method.
2. Energetic consistency - No ATP or NAD(P)H production without mass flow.
3. 15 inconsistent subsets (total 40 reactions) - that is, sets of reactions having conflicting irreversibility. (To be resolved.)

# 3. Model Validation

1. Mass balance:
   (a) Individual reactions balanced for C, N, P, S from empirical formulae where available.
   (b) No system–level stoichiometric inconsistencies for C, N, S, P using Gevorgyan's method.
2. Energetic consistency - No ATP or NAD(P)H production without mass flow.
3. 15 inconsistent subsets (total 40 reactions) - that is, sets of reactions having conflicting irreversibility. (To be resolved.)
4. Essential biomass components can be produced from both glucose and $CH_4$: 20 amino acids; 8 nucleotide bases; coenzymes NAD(P), CoA, MeTHF and flavins; lipid as palmitate. Not implemented: cell wall LPS — enzymes missing from annotation.

**To do:** Adjust biomass composition and nutrient uptake rates to experimentally–determined values; compare model outputs with observed product spectrum.

# 4.1 Model Application — Biomass



- The model predicts optimal growth on glucose in aerobic conditions with formation of $CO_2$ and acetate as by–products, and in anaerobic conditions with formation of $CO_2$, acetate and succinate.
- Optimal growth on methane occurs in aerobic conditions.
- Except for aerobic growth on glucose, some redox balancing occurs involving N and S metabolism

# 4.2 Model Application — Reachable Products



- There are potential routes to lactate, pyruvate, ethanol and succinate as sole products from glucose in aerobic and anaerobic conditions.

# 4.2 Model Application — Reachable Products



- There are potential routes to lactate, pyruvate, ethanol and succinate as sole products from glucose in aerobic and anaerobic conditions.
- There are potential routes to lactate, pyruvate, ethanol and succinate as sole products from methane in aerobic conditions, though with the exception of lactate, these are accompanied by redox balancing in N and S metabolism.

# 4.2 Model Application — Reachable Products



- There are potential routes to lactate, pyruvate, ethanol and succinate as sole products from glucose in aerobic and anaerobic conditions.
- There are potential routes to lactate, pyruvate, ethanol and succinate as sole products from methane in aerobic conditions, though with the exception of lactate, these are accompanied by redox balancing in N and S metabolism.
- **To do:** the routes of redox balancing by N and S metabolism need more investigation, and checking for thermodynamic feasibility.

# 5. Web–based Simulation



The ScrumPy model was transferred to Surrey FBA and run successfully. However, with the departure of Andrezj Kierzek from Surrey, the *M. capsulatum* model has not been released on the server at http://sysbio3.fhms.surrey.ac.uk/.

# 6. Dissemination



- The model is available to C1net members in ScrumPy and SBML formats on request to Mark Poolman or David Fell. See http://mudshark.brookes.ac.uk/Projects/C1netModel for details.

- Training in the use of ScrumPy for FBA has been given in a C1net workshop. It is likely that there will be a further C1net modelling workshop available for those who want to use the model.

# Conclusion

- A first version of a working genome–scale metabolic model of *M. capsulatus* has been completed and is available to C1net members for investigating metabolic engineering designs.

# Conclusion

- A first version of a working genome–scale metabolic model of *M. capsulatus* has been completed and is available to C1net members for investigating metabolic engineering designs.
- Parallel work in Oxford and Nottingham has also generated models of *Acetobacterium woodii, Clostridium autoethanogenum* and *Cupriavidus necator*. All have been created in ScrumPy with BioCyc reaction and metabolite identifiers, so are easily comparable.

# Conclusion

- A first version of a working genome–scale metabolic model of *M. capsulatus* has been completed and is available to C1net members for investigating metabolic engineering designs.
- Parallel work in Oxford and Nottingham has also generated models of *Acetobacterium woodii, Clostridium autoethanogenum* and *Cupriavidus necator*. All have been created in ScrumPy with BioCyc reaction and metabolite identifiers, so are easily comparable.
- These models are beginning to be used for metabolic engineering and synthetic biology designs. Examples of typical model applications can be seen in posters 13, 15 and 17.

# Acknowledgements



- Other members of the OBU Cell Systems Modelling Group, especially Hassan Hartman and Noah Mesfin, also contributed to this work.

- The work was funded by a Proof of Concept grant from the BBSRC–funded NIBB C1net to the University of Surrey.