Engineering microbial communities for understanding and applications

Orkun S Soyer

European Space Agency, May 2016

OSS LAB

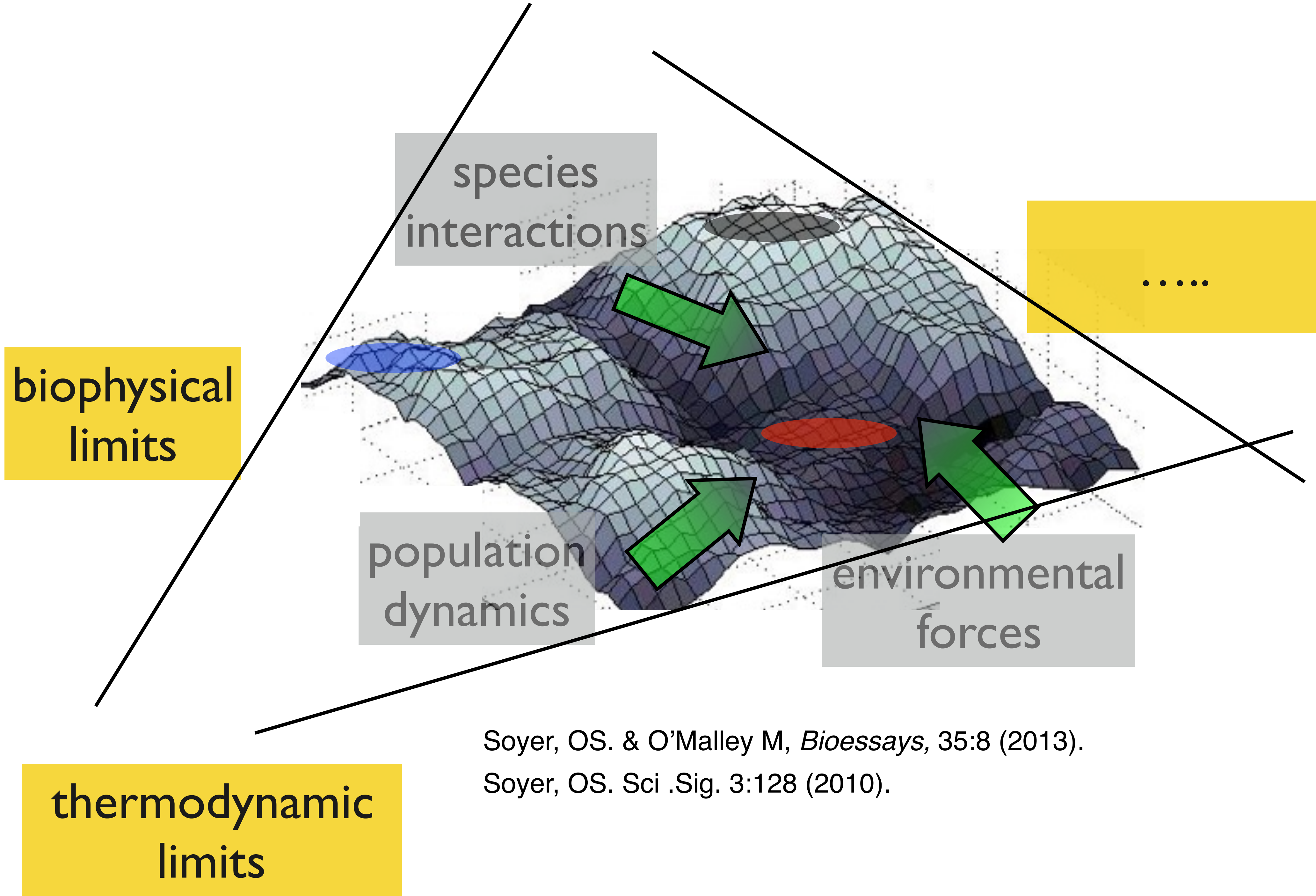
WARWICK CENTRE FOR
INTEGRATIVE SYNTHETIC BIOLOGY

EPSRC & BBSRC Centre for Doctoral
Training in Synthetic Biology

SynBio CDT

WARWICK
THE UNIVERSITY OF WARWICK

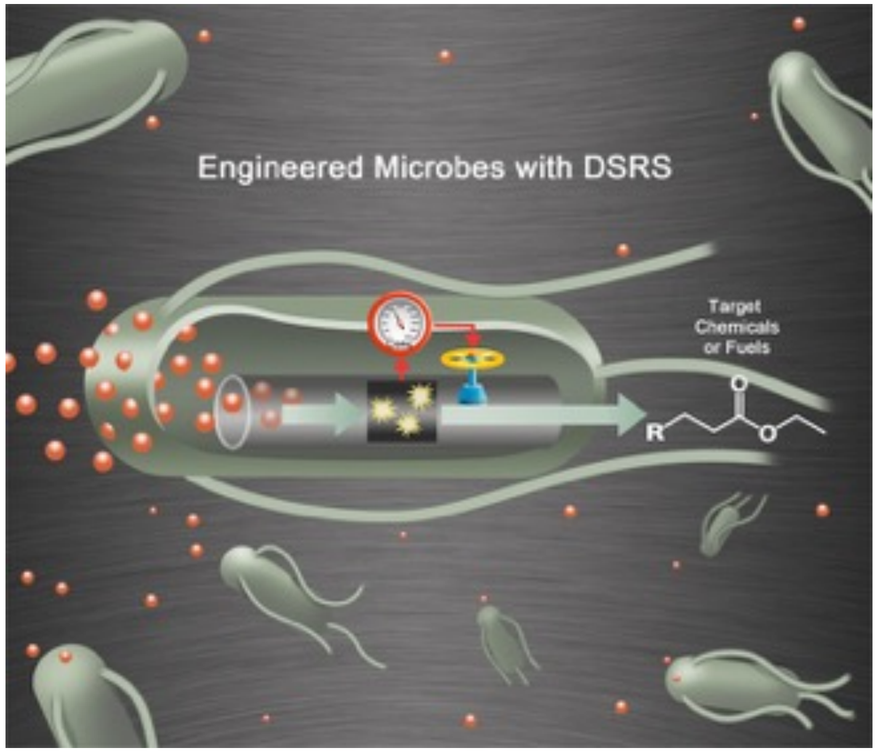
System Design in Biology:
Evolution and the biological design space

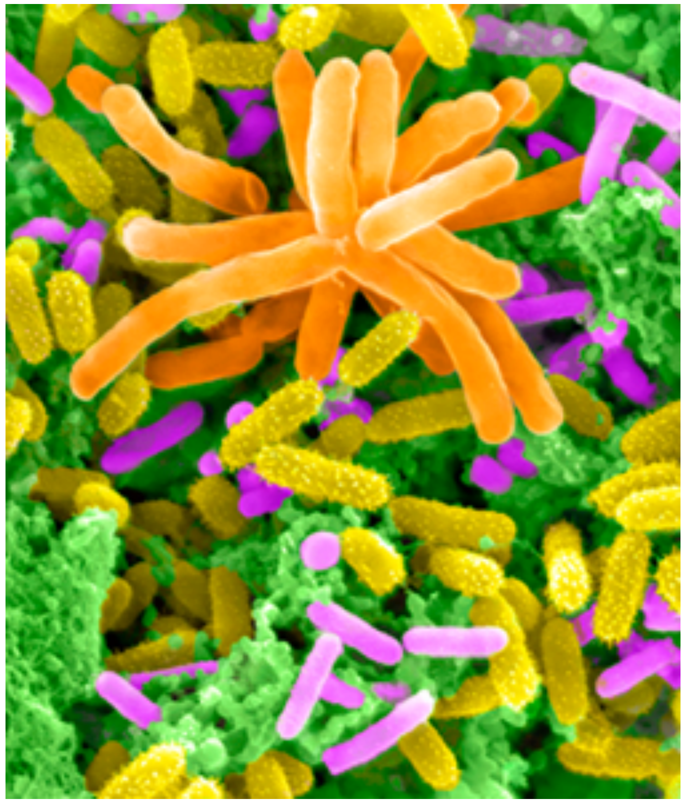


Soyer, OS. & O'Malley M, *Bioessays,* 35:8 (2013).

Soyer, OS. Sci .Sig. 3:128 (2010).

Design principles of metabolism?





Super Bug vs **Microbial Community**

How to (re)engineer metabolism? How to (re)engineer an ecosystem?

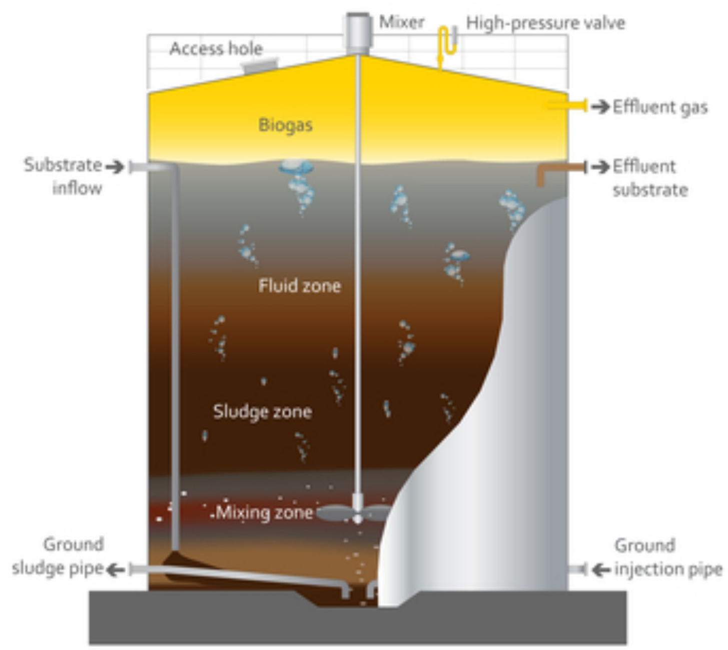
e.g. Bernstein, HC, Paulson SD, Carlson RP. J of Biotech 157:1 (2012)
Santala, S, Karp M, Santala V. PloS One 9:12 (2014)
Zhou, K, Qiao K, Edgar S, Stephanopoulos G. Nature Biotech (2015)

sLoLa

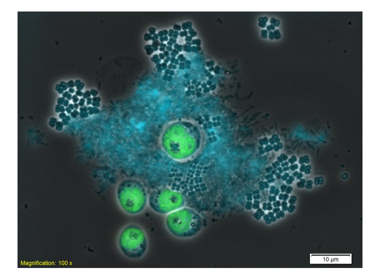
Engineering Synthetic Microbial Communities for Biomethane Production

Decomplexify natural systems

Anaerobic Digestion



http://www.mannvit.com/Markets/UnitedKingdom/AnaerobicDigestion/

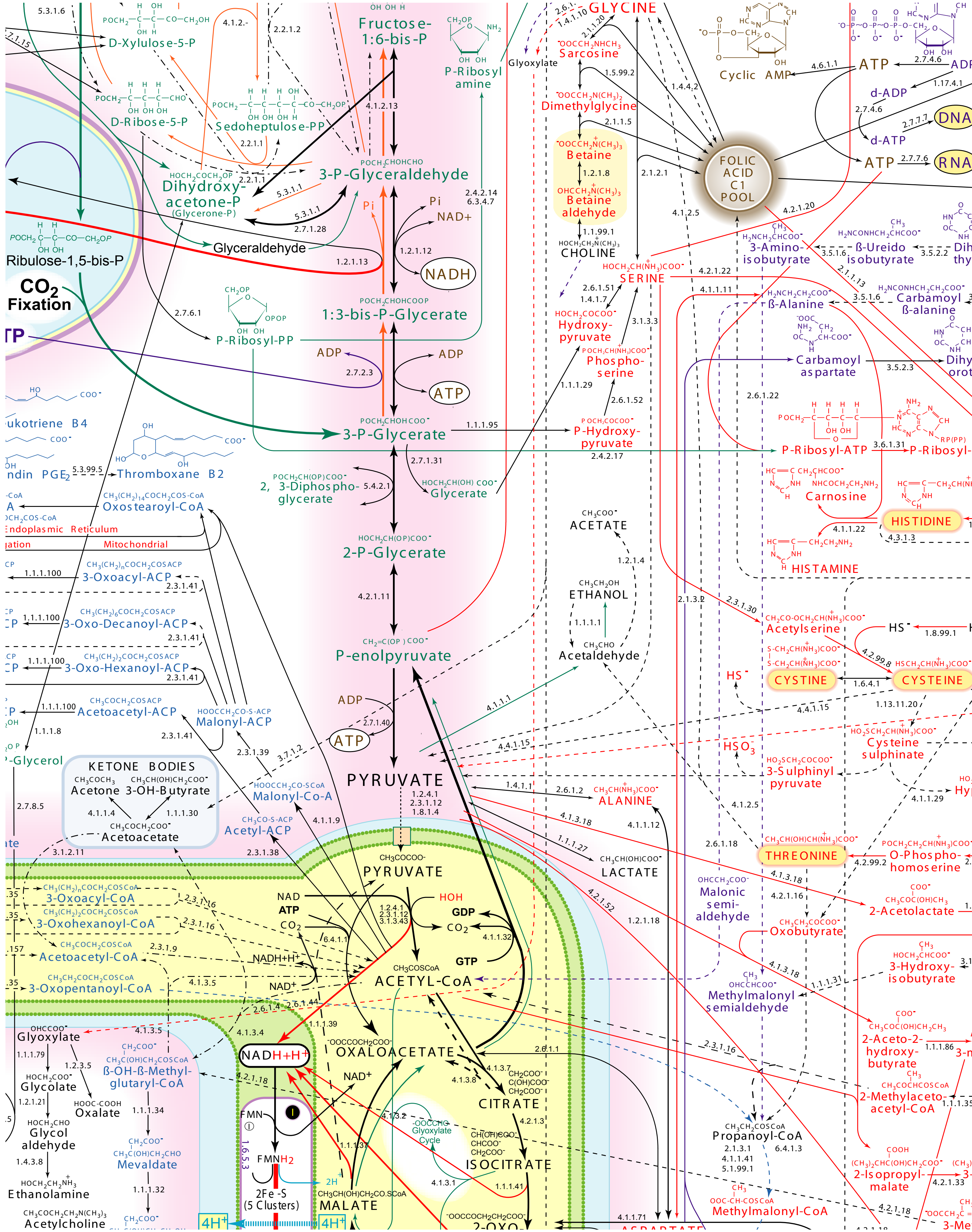


Synthetic Communities

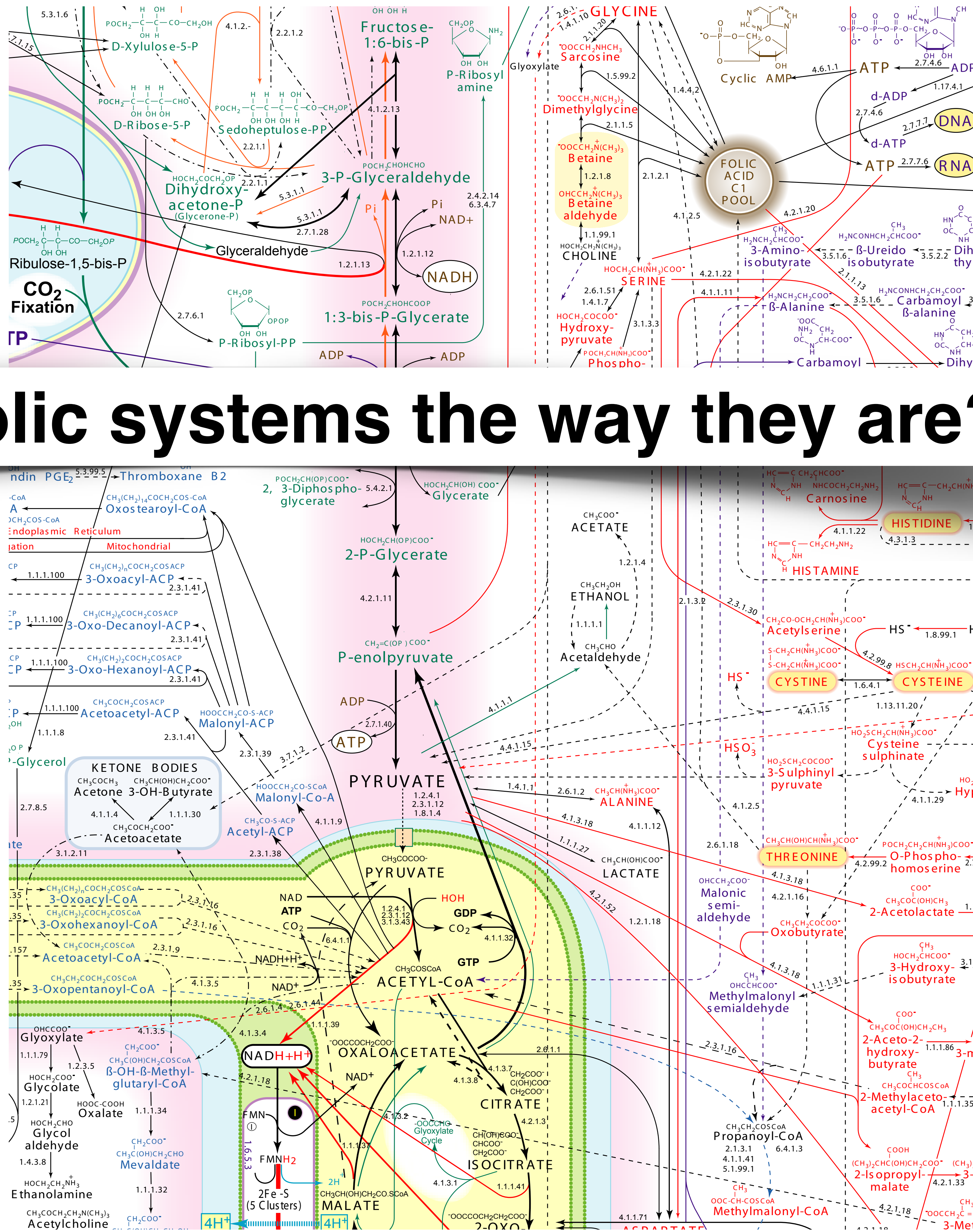
Engineer minimal systems

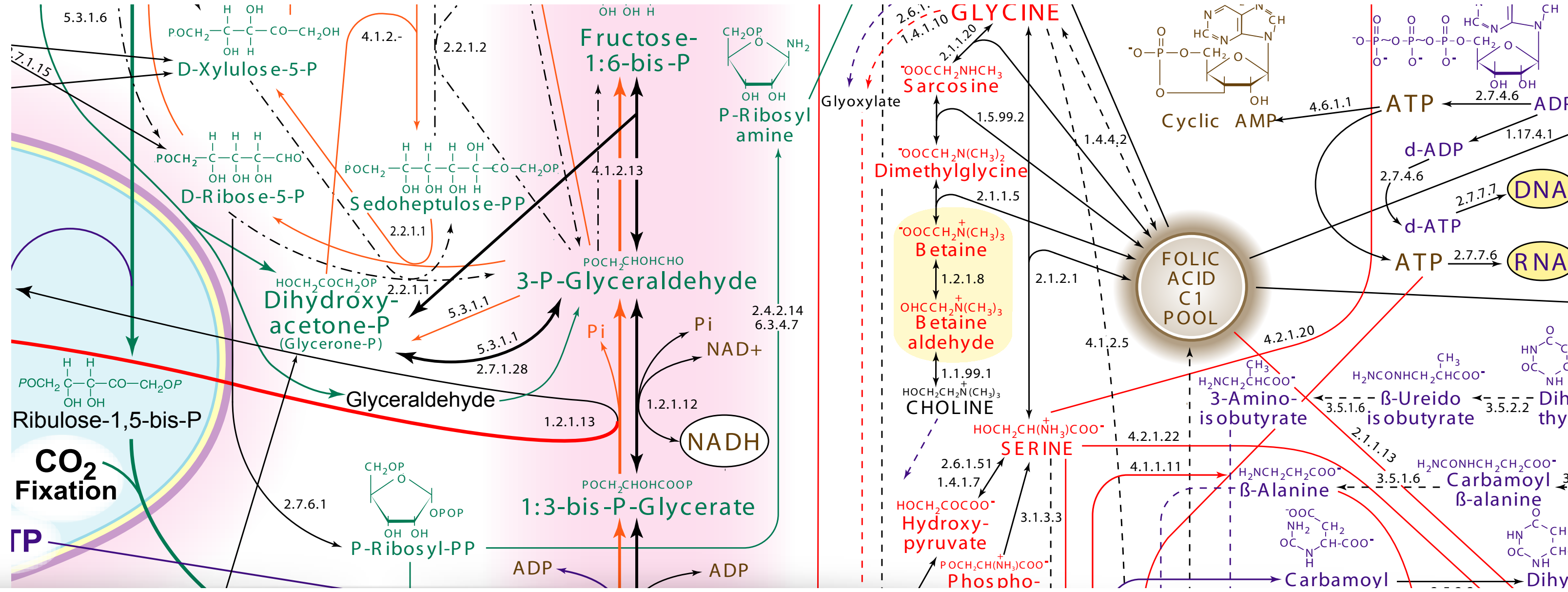
Grosskopf T & Soyer OS, *Curr. Opin. in Microbiol.* 18 (2014)

Widder S *et al.*, *ISME Journal* (2016)

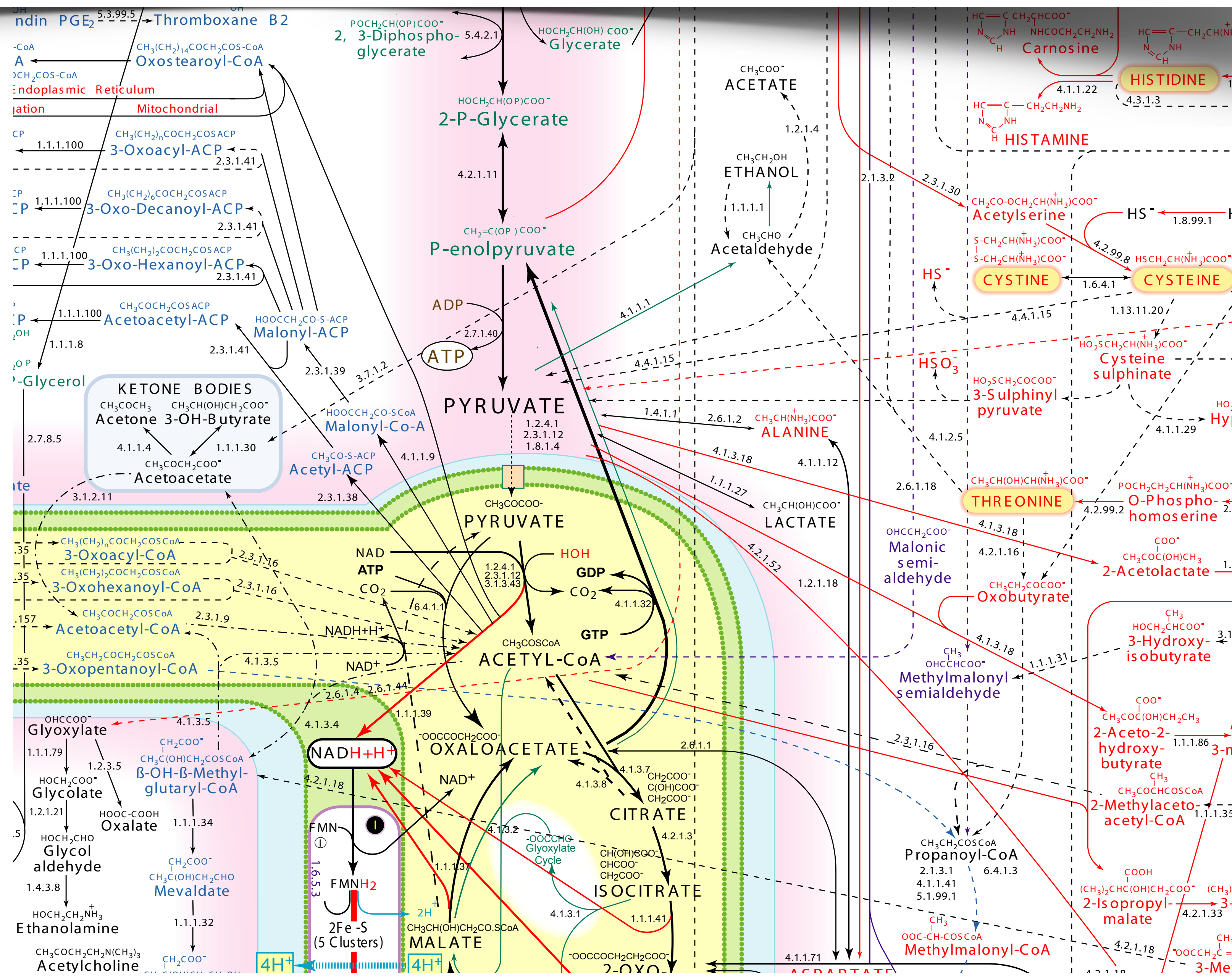

D-Xylulose-5-P
D-Ribose-5-P
Sedoheptulose-PP
Fructose-1:6-bis-P
P-Ribosyl amine
GLYCINE
Sarcosine
Glyoxylate
Dimethylglycine
Betaine
Betaine aldehyde
CHOLINE
Cyclic AMP
ATP
ADP
d-ADP
d-ATP
DNA
RNA
FOLIC ACID C1 POOL
Dihydroxy-acetone-P (Glycerone-P)
3-P-Glyceraldehyde
Glyceraldehyde
Ribulose-1,5-bis-P
CO2 Fixation
Pi
NAD+
NADH
1:3-bis-P-Glycerate
P-Ribosyl-PP
ATP
SERINE
Hydroxy-pyruvate
Phospho-serine
3-Amino-isobutyrate
ß-Ureido isobutyrate
ß-Alanine
Carbamoyl ß-alanine
Carbamoyl aspartate
Dihydro orotate
3-P-Glycerate
P-Hydroxy-pyruvate
P-Ribosyl-ATP
P-Ribosyl-AMP
Leukotriene B4
Prostaglandin PGE2
Thromboxane B2
2, 3-Diphospho-glycerate
Glycerate
Carnosine
HISTIDINE
HISTAMINE
Oxostearoyl-CoA
Endoplasmic Reticulum
Mitochondrial
ACETATE
2-P-Glycerate
3-Oxoacyl-ACP
3-Oxo-Decanoyl-ACP
ETHANOL
3-Oxo-Hexanoyl-ACP
P-enolpyruvate
Acetaldehyde
Acetylserine
HS-
CYSTINE
CYSTEINE
Acetoacetyl-ACP
Malonyl-ACP
ADP
ATP
Cysteine sulphinate
HSO3-
3-Sulphinyl pyruvate
Hypotaurine
P-Glycerol
KETONE BODIES
Acetone
3-OH-Butyrate
Acetoacetate
Malonyl-Co-A
Acetyl-ACP
PYRUVATE
ALANINE
THREONINE
O-Phospho-homoserine
LACTATE
2-Acetolactate
Malonic semi-aldehyde
Oxobutyrate
3-Hydroxy-isobutyrate
Methylmalonyl semialdehyde
3-Oxoacyl-CoA
3-Oxohexanoyl-CoA
Acetoacetyl-CoA
3-Oxopentanoyl-CoA
NAD
ATP
CO2
HOH
GDP
CO2
GTP
NADH+H+
NAD+
ACETYL-CoA
Glyoxylate
Glycolate
Oxalate
Glycol aldehyde
Ethanolamine
Acetylcholine
ß-OH-ß-Methyl-glutaryl-CoA
Mevaldate
NADH+H+
OXALOACETATE
CITRATE
ISOCITRATE
Glyoxylate Cycle
MALATE
FMN
FMNH2
2Fe-S (5 Clusters)
4H+
2H
2-Aceto-2-hydroxy-butyrate
2-Methylaceto-acetyl-CoA
Propanoyl-CoA
2-Isopropyl-malate
Methylmalonyl-CoA
ASPARTATE
2-OXO-

Why are metabolic systems the way they are?





Why are metabolic systems the way they are?



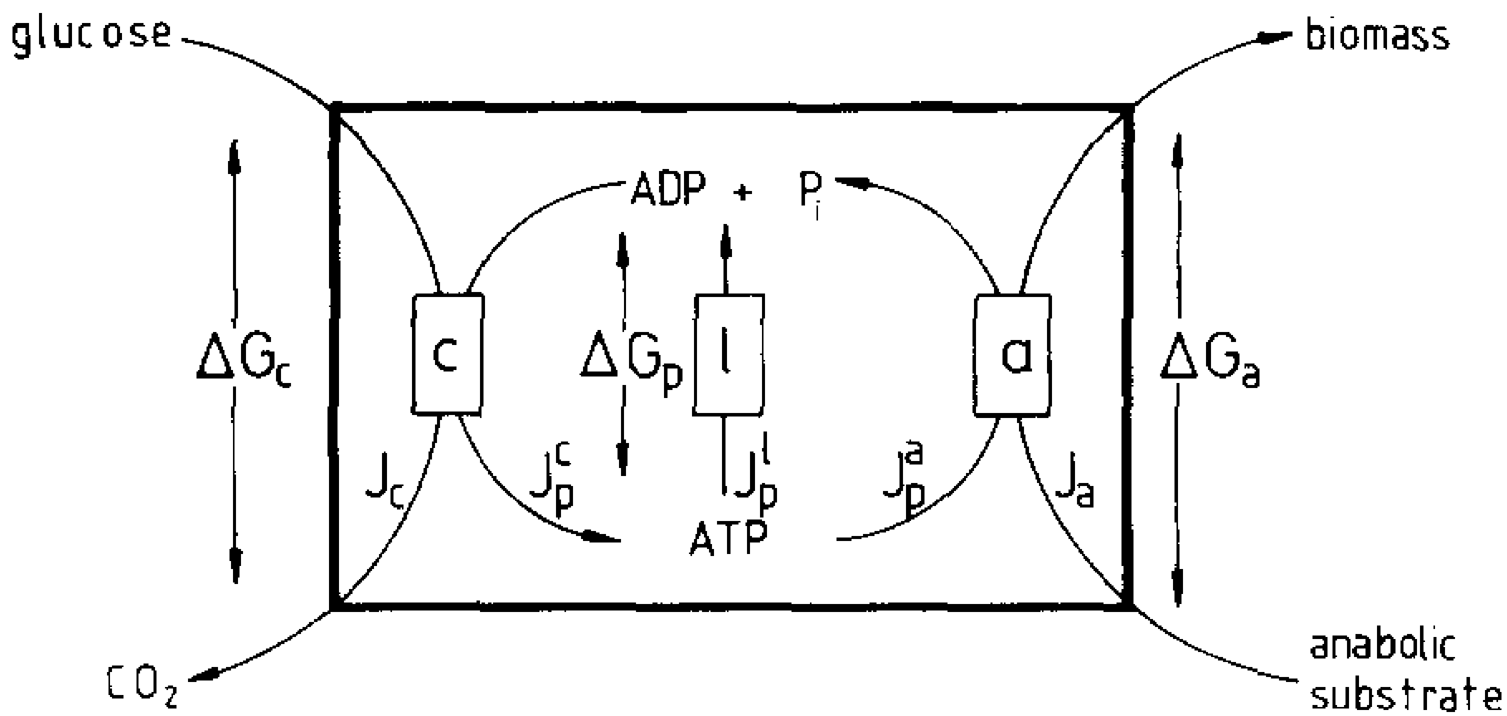
The role of constraints?

Klumpp, S, Z Zhang, Hwa T. Cell 139:7 (2009)

Molenaar, D, Berlo Rv, Ridder Dd, Teusink B. MSB 5 (2009)

Basan, M, et al. Nature 528 (2015)

The most fundamental constrain on metabolism is thermodynamics



Hellingwerf, K J, et al FEMS Microbiol. Lett 15 (1982)

Substrate

Products

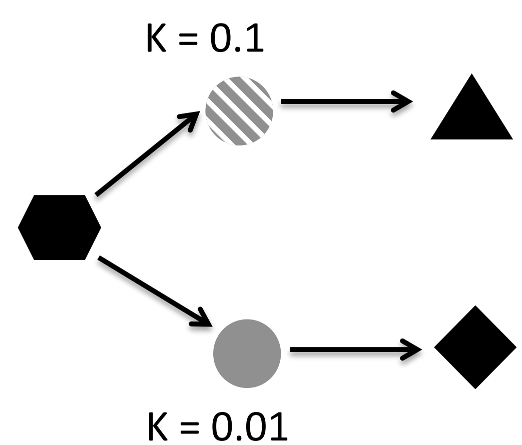
$$\Delta G_{rxn} = \Delta G_{rxn}^{0'} + R \cdot T \cdot \ln\left(\frac{\prod_i a_i^{m,i}}{\prod_j a_j^{m,j}} \right)$$

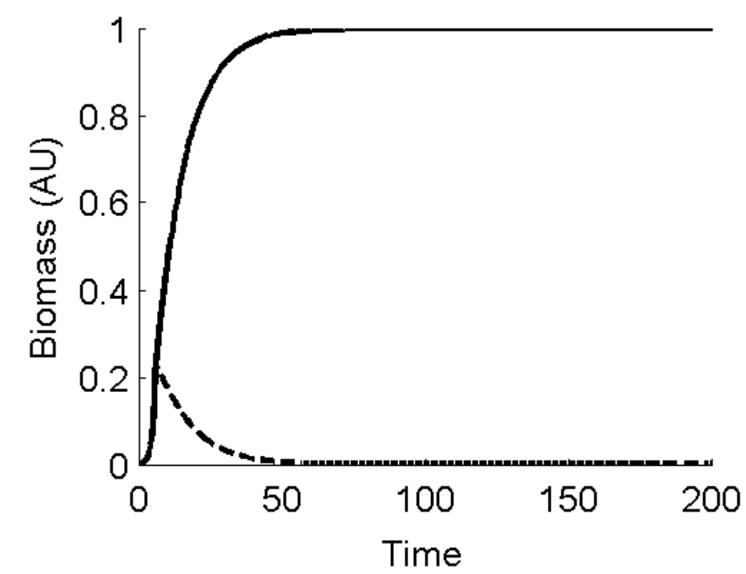
Thermodynamic inhibition on metabolism

Exclusion principle revisited

Kinetics dominated world

$$v = \frac{v_{max} \cdot [S]}{K + [S]}$$



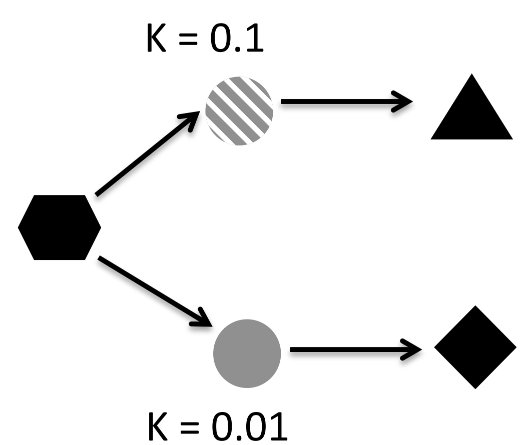


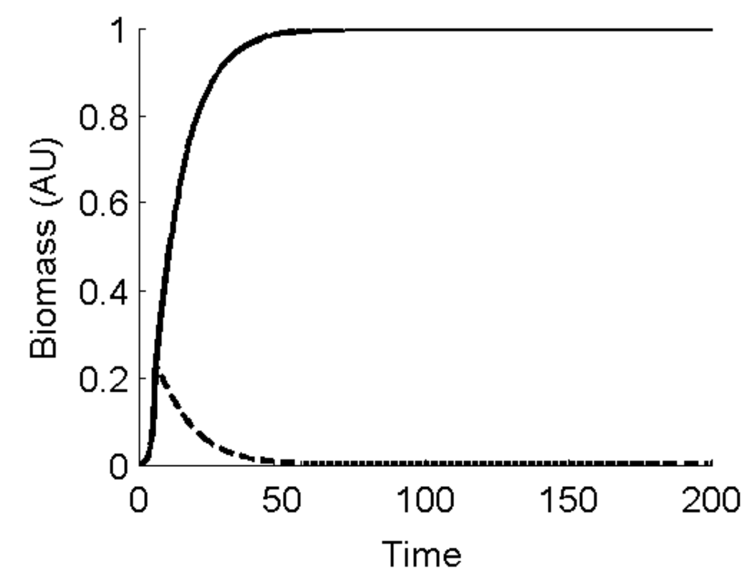
Thermodynamic inhibition on metabolism

Exclusion principle revisited

Kinetics dominated world

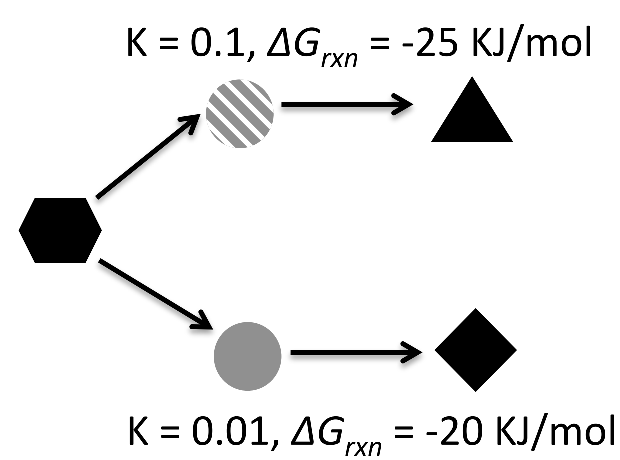
$$v = \frac{v_{\max} \cdot [S]}{K + [S]}$$

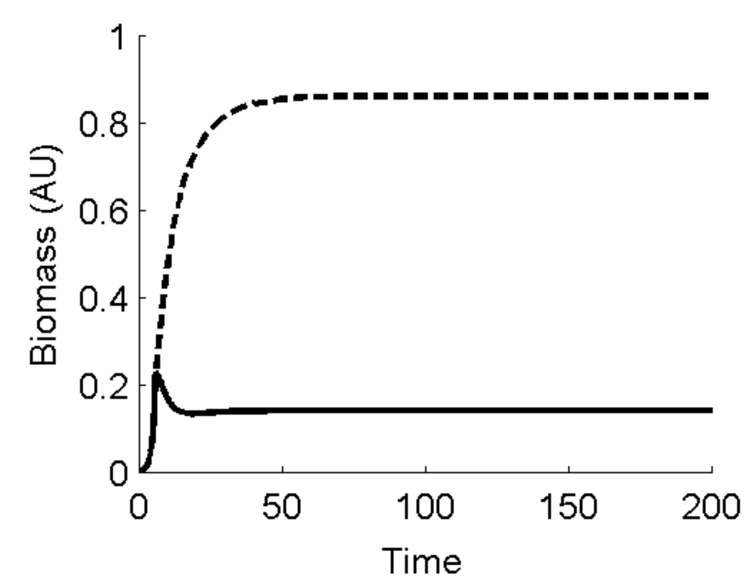




Thermodynamics dominated world

$$v = \frac{v_{\max} \cdot [S] \cdot \left(1 - \exp(\Delta G_{rxn})\right)}{K + [S] \cdot \left(1 + k_r \cdot \exp(\Delta G_{rxn})\right)}$$





Hoh, C Y, R Cord-Ruwisch. Biotech & Bioeng 51:5 (1996)

Thermodynamic inhibition as a general principle for explaining microbial communities / diversity

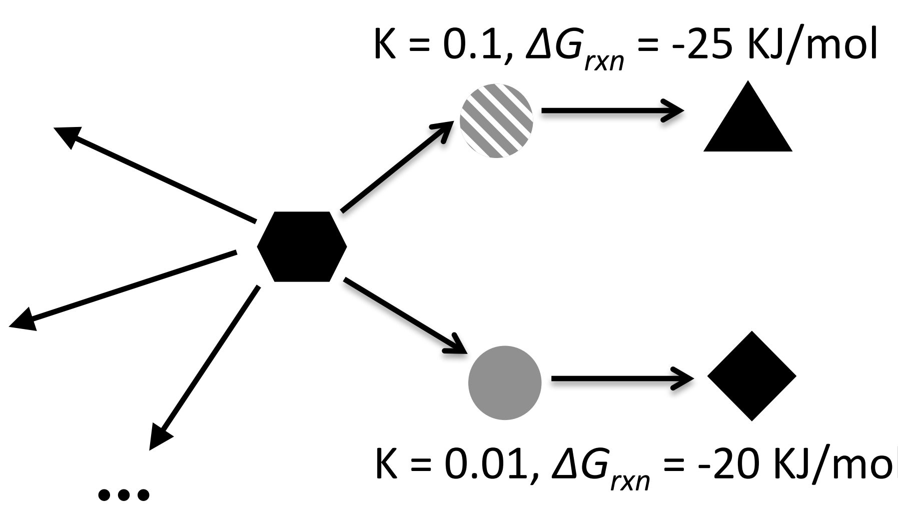
$$[X_1]' = Y_1 \cdot [X_1] \cdot \frac{v_1 \cdot [S] \cdot \left(1 - \exp\left(\Delta G_{rxn,1}\right)\right)}{K_1 + [S] \cdot \left(1 - k \cdot \exp\left(\Delta G_{rxn,1}\right)\right)} - \lambda \cdot [X_1]$$

$$[X_2]' = Y_2 \cdot [X_2] \cdot \frac{v_2 \cdot [S] \cdot \left(1 - \exp\left(\Delta G_{rxn,2}\right)\right)}{K_2 + [S] \cdot \left(1 - k \cdot \exp\left(\Delta G_{rxn,2}\right)\right)} - \lambda \cdot [X_2]$$

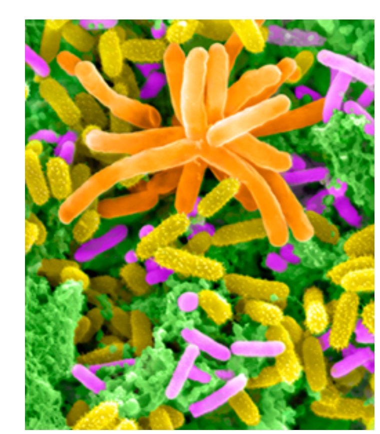
…

…

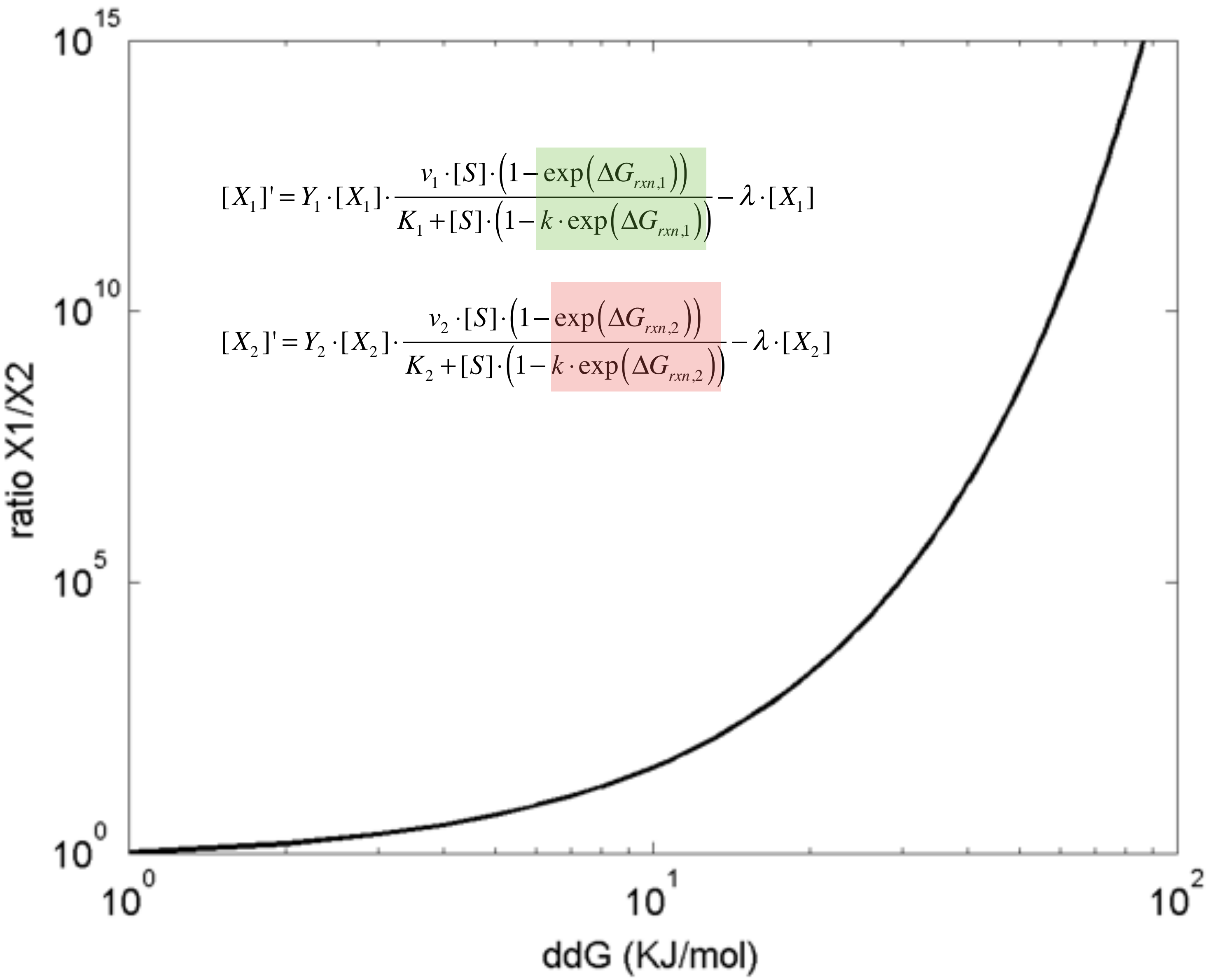
…



Many microbes can co-exist utilizing the same substrate, as long as they convert it into different end-products.

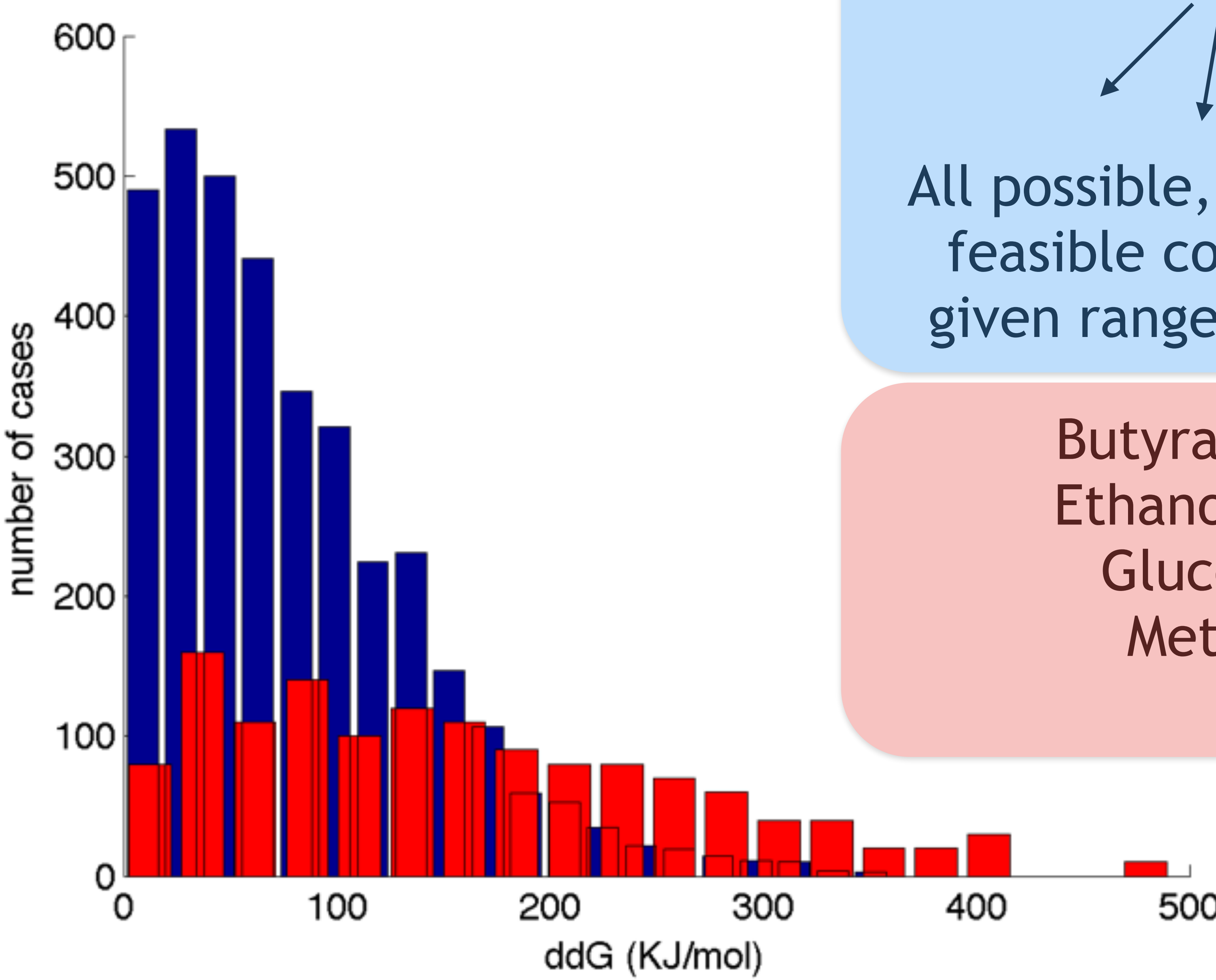


Thermodynamic inhibition as a general principle for explaining microbial communities / diversity



Significance of co-existence will depend on free energy levels of individual reactions.

Relevance of low-energy reactions:



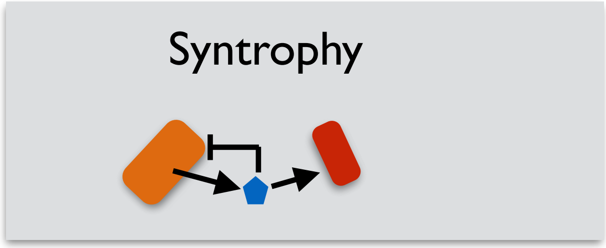
Glucose

All possible, thermodynamically feasible conversions within a given range of stoichiometries

Butyrate degradation
Ethanol fermentation
Glucose oxidation
Methanogenesis
....

Grosskopf T and Soyer OS, *ISME journal* (2016)

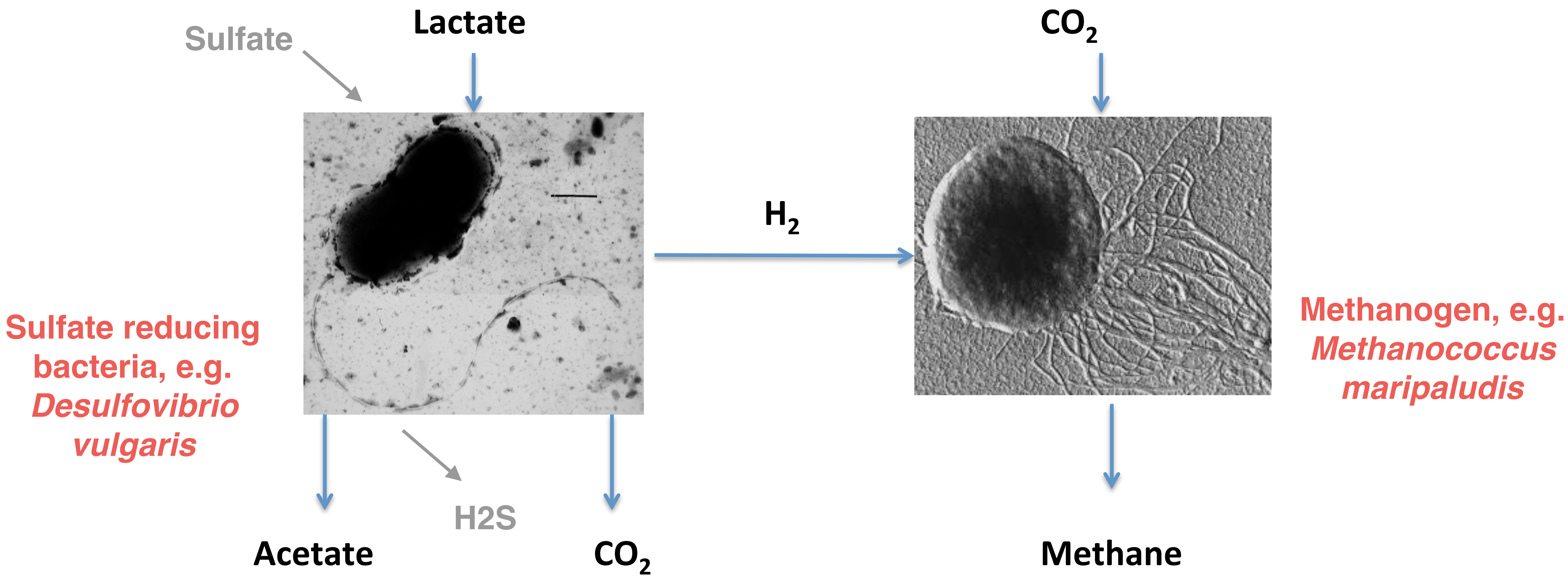
Metabolic cooperation from thermodynamic inhibition?



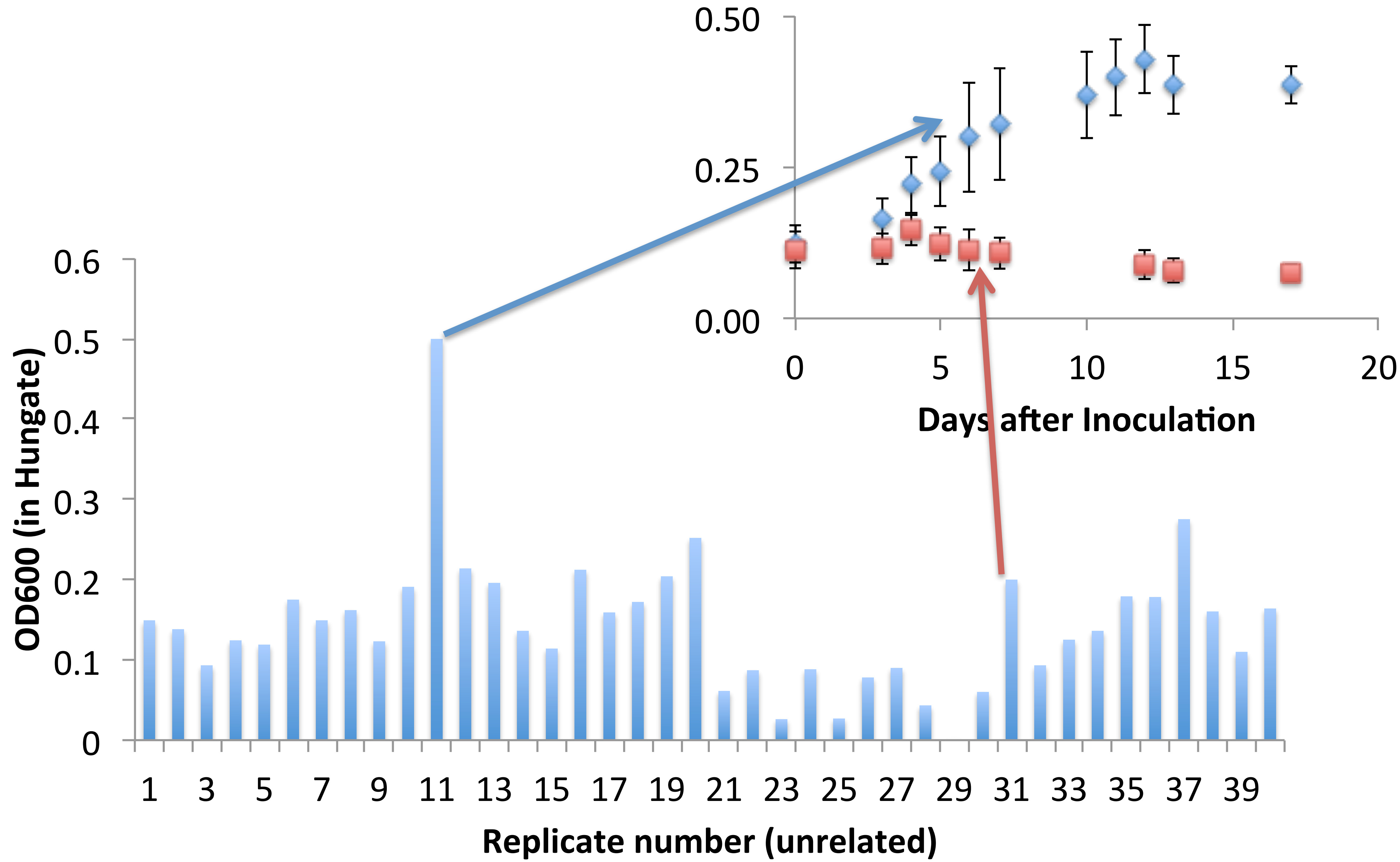
A syntrophy at the heart of anaerobic digestion

$2Lactate^{-} + Sulfate^{2-} \longrightarrow 2Acetate^{-} + 2HCO_3^{-} + H_2S$ DG = -160.4 kJ/mol

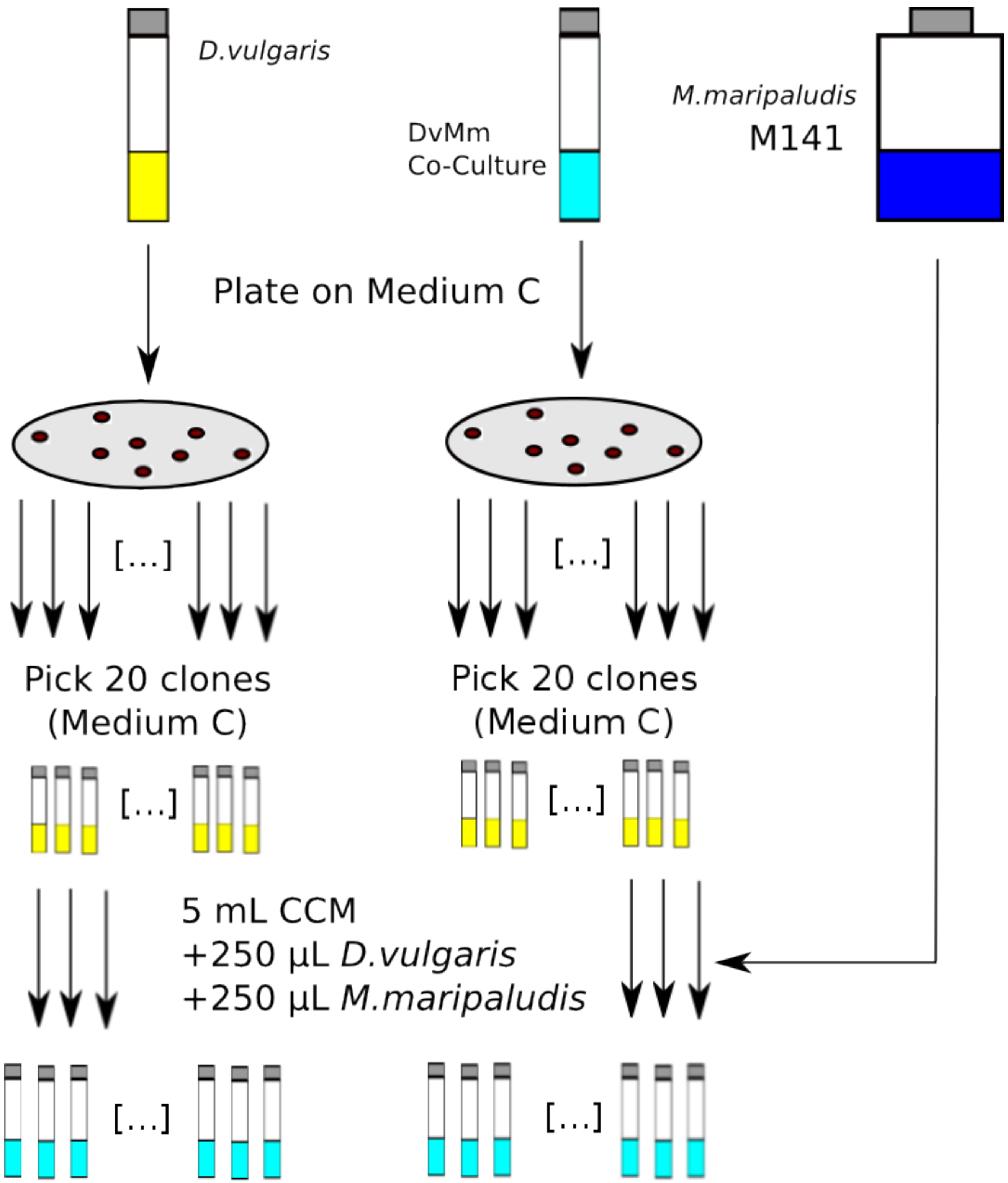
$Lactate^{-} + 2H_2O \longrightarrow Acetate^{-} + HCO_3^{-} + H^{+} + 2H_2$ DG = -4.2 kJ/mol

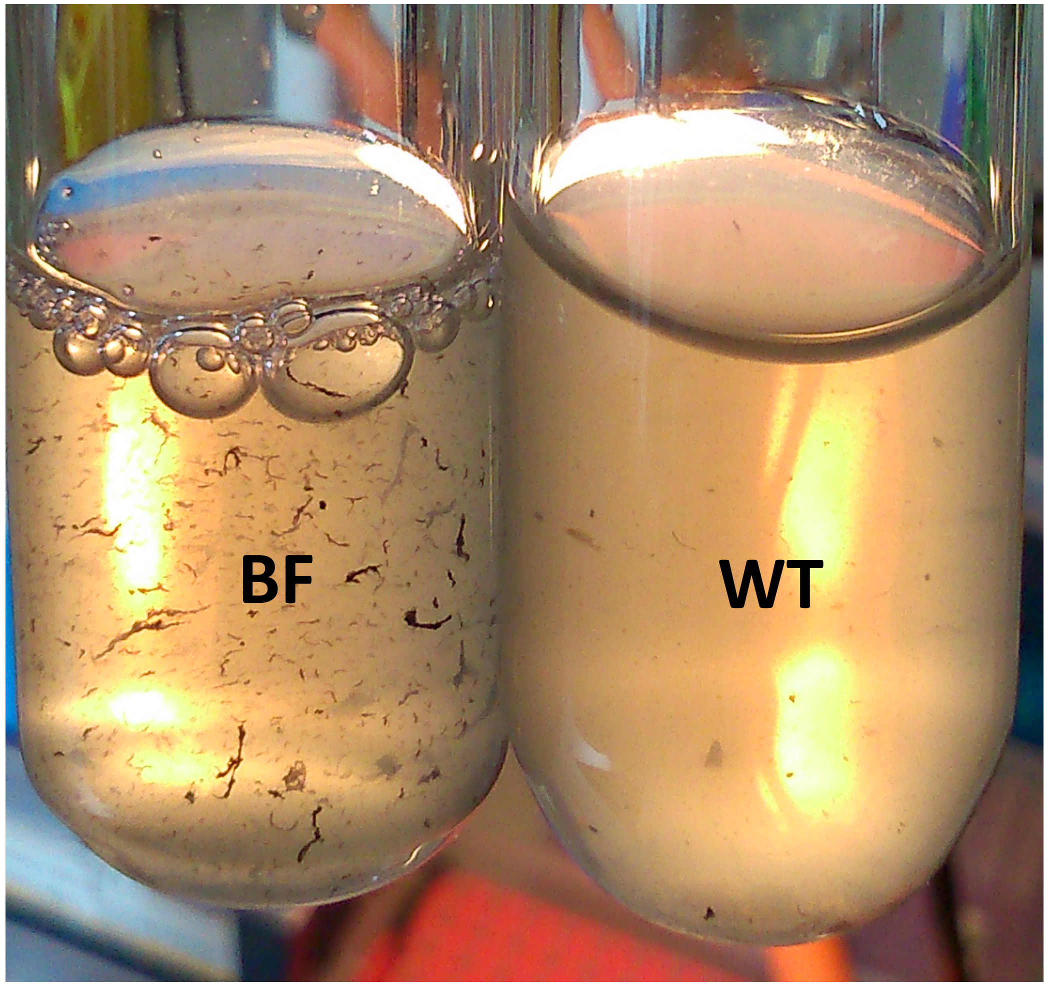


Initiating a co-culture…



A social clone?



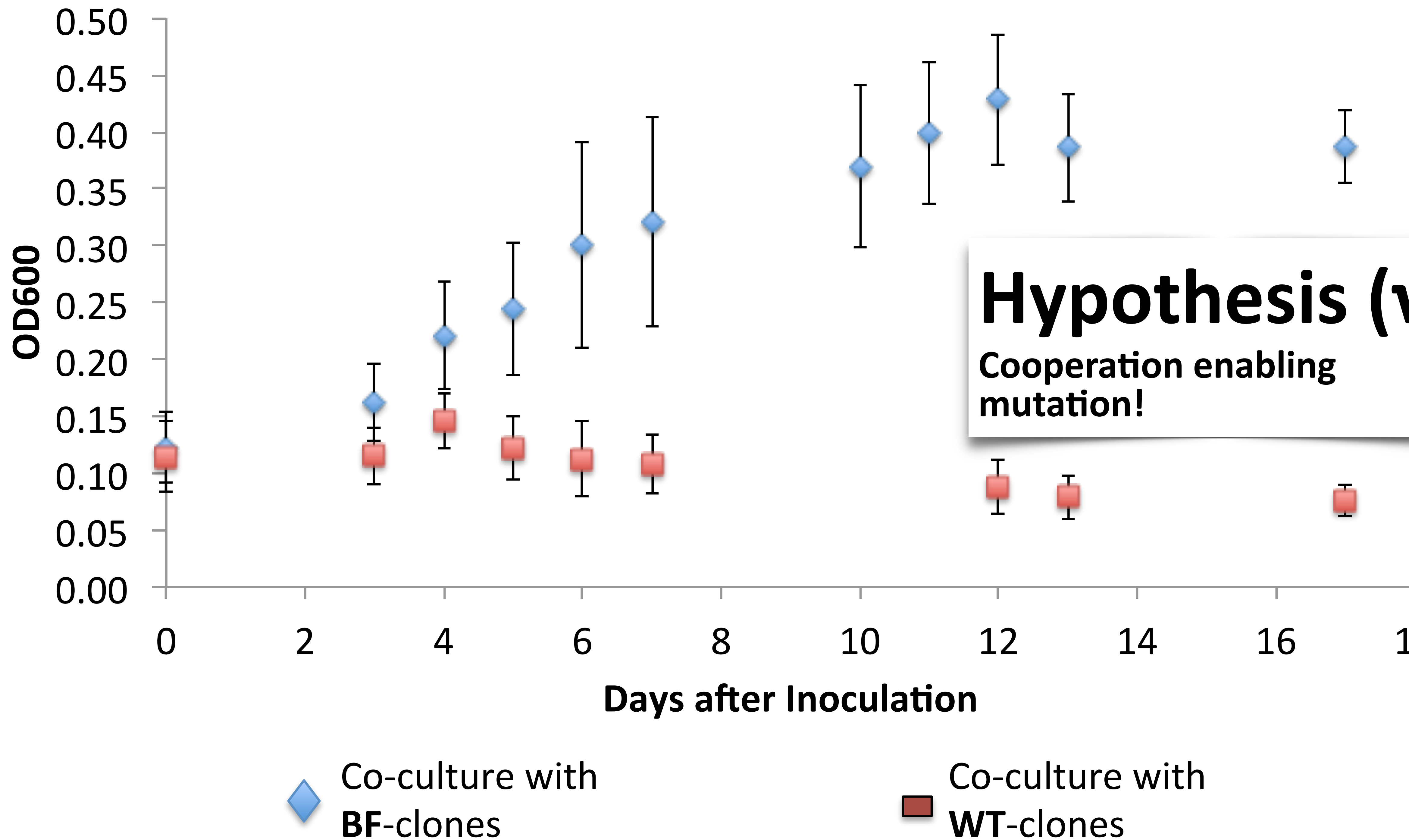


From
Co-Culture

From
Mono-Culture

A social clone?

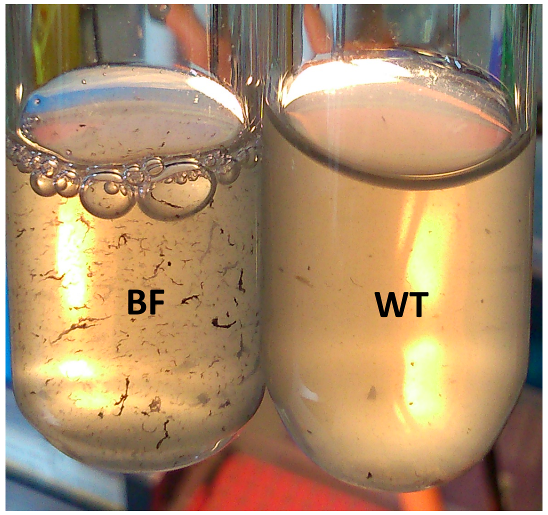
Mean values + standard deviation, 20 replicates each



Hypothesis (v)

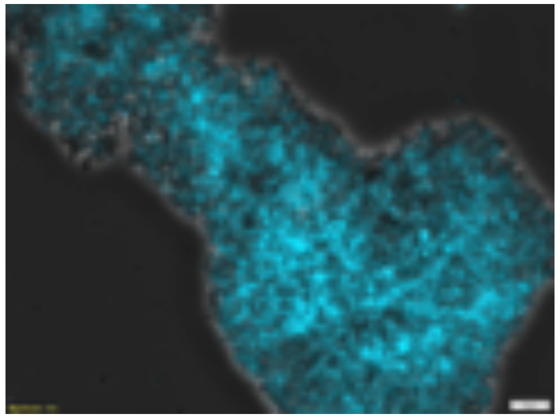
Cooperation enabling mutation!

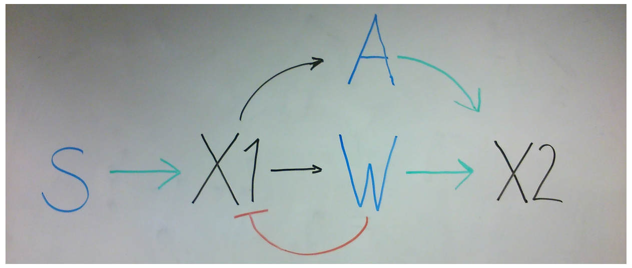
Molecular basis of metabolic cooperation?

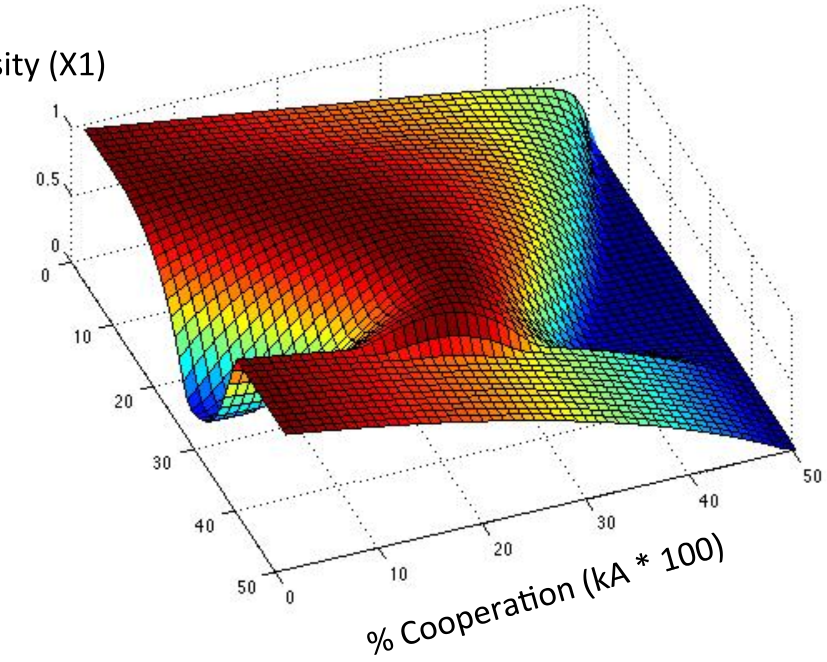


Alanine provision?

Biofilm formation?

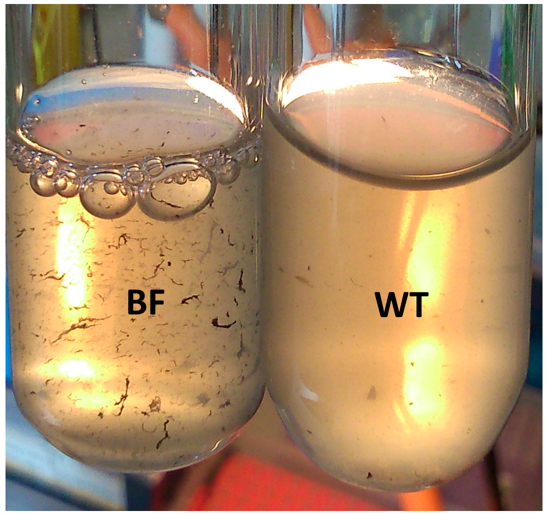




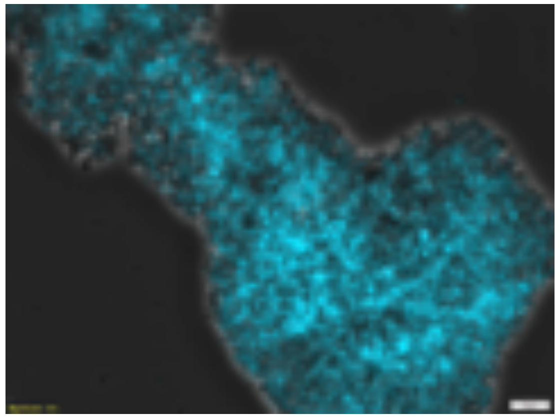


Thermodynamics?

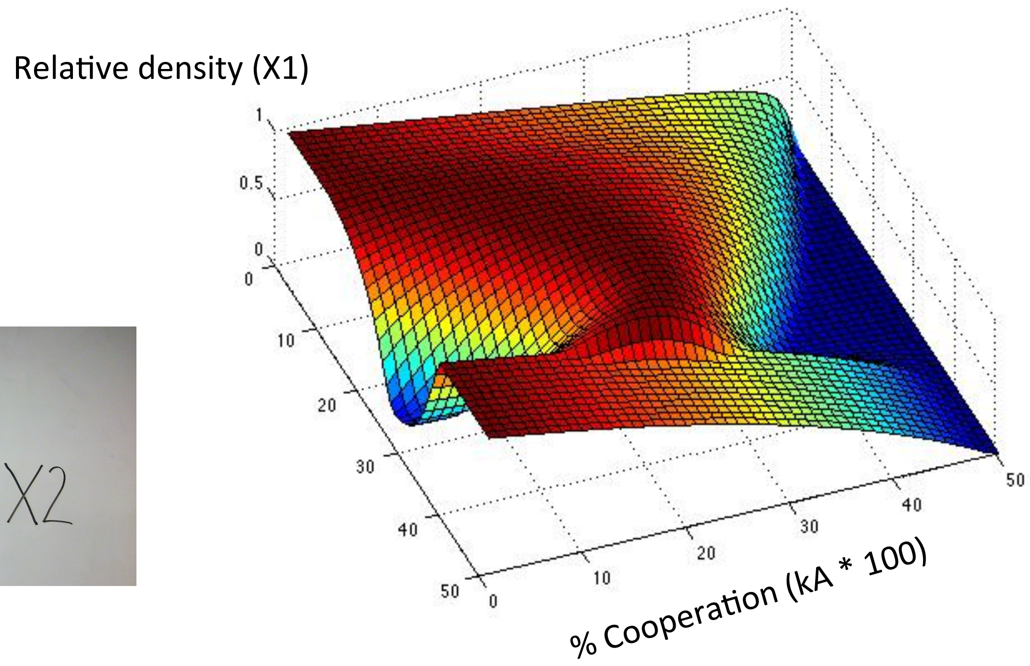
Molecular basis of metabolic cooperation?

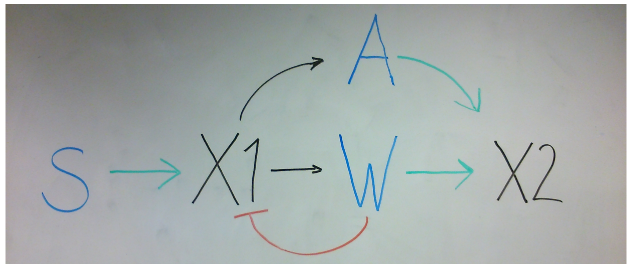


Biofilm formation?



Alanine provision?





Thermodynamics?

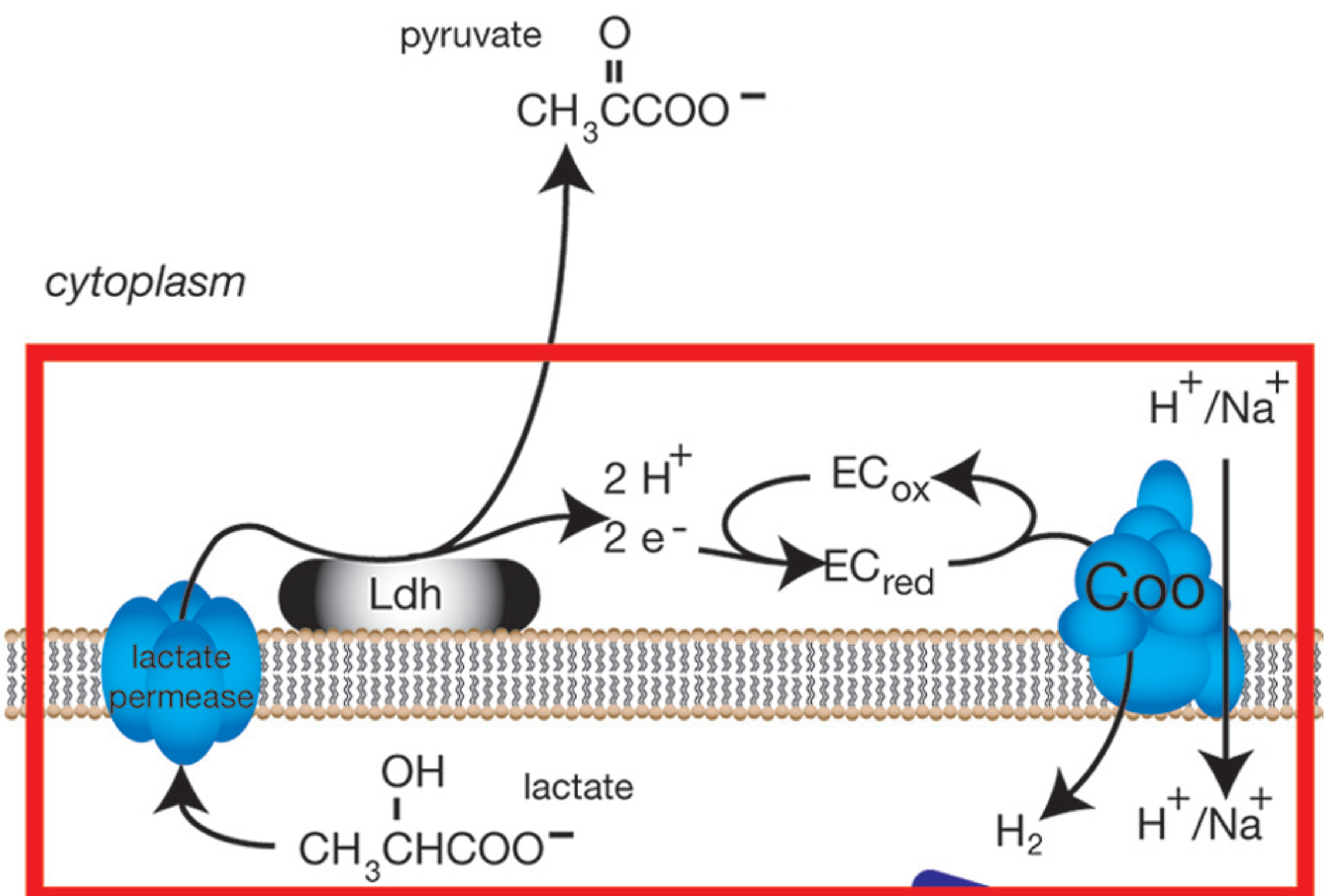
How to overcome thermodynamic limits?

Lactate$^-$ + $2H_20$ —> Acetate- + HCO_3^- + H^+ + $2H_2$ DG = -4.2 kJ/mol

Lactate —> Pyruvate + H_2 DG = +43 kJ/mol

Investment of energy into metabolic cooperation.

Lactate —> Pyruvate + $2H^+$ + 2e-	DG = -37 kJ/mol
$2H^+$ + 2e- —> H_2	DG = +81 kJ/mol
Na^+(out) —> Na+ (in)	DG = ? kJ/mol



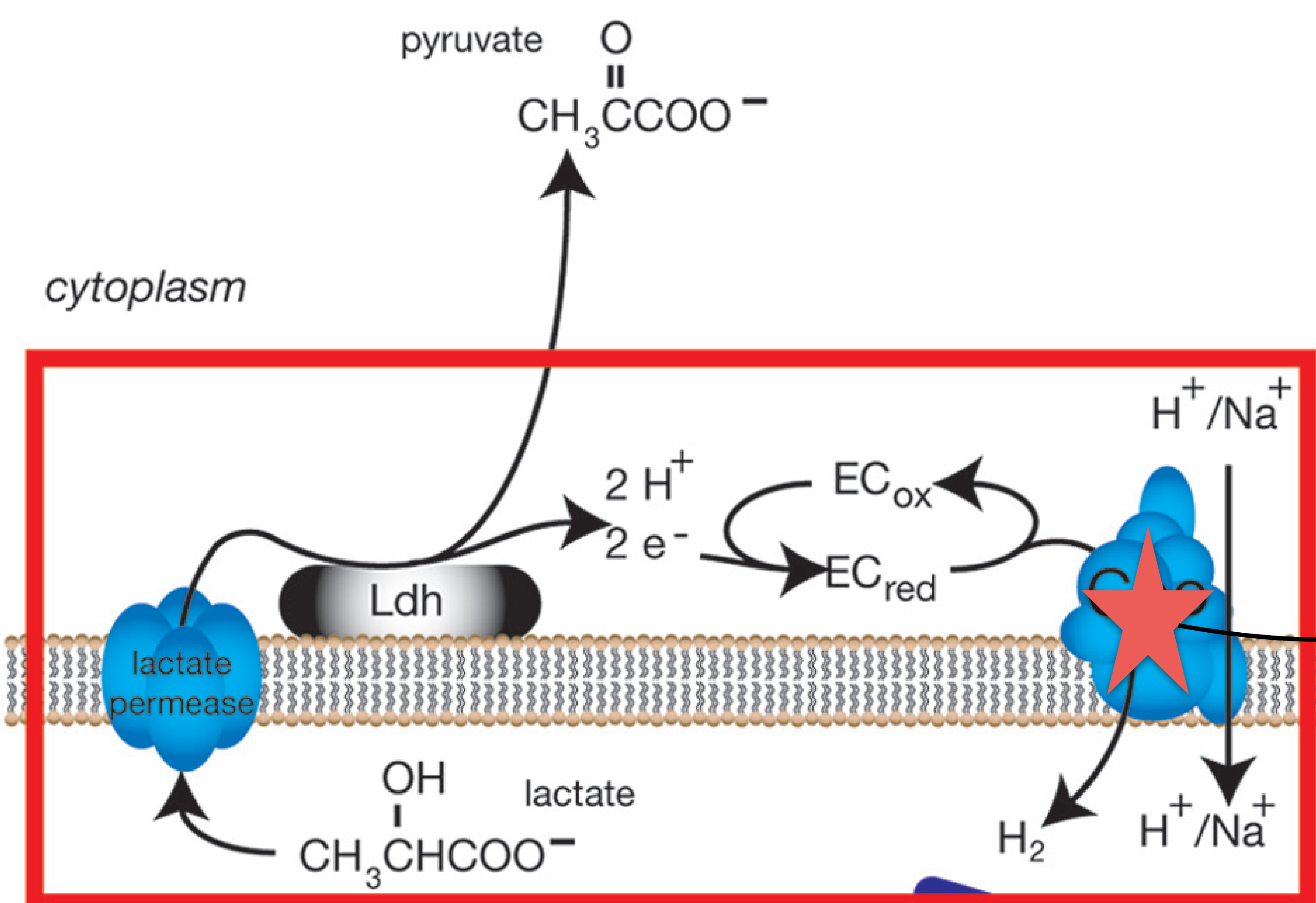
Walker *et al.*, *J. of Bact.* (2009)

Investment of energy into metabolic cooperation.

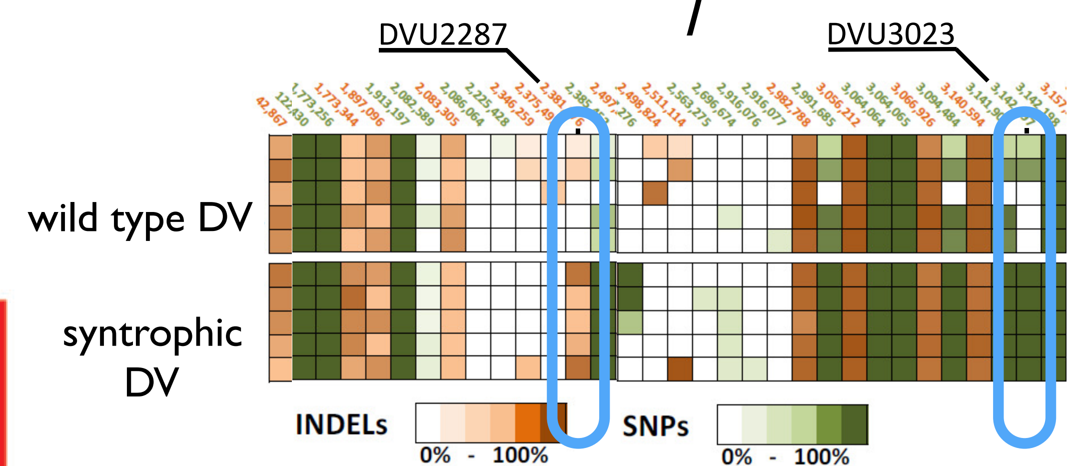
Lactate —> Pyruvate + $2H^+$ + 2e- DG = -37 kJ/mol

$2H^+$ + 2e- —> H_2 DG = +81 kJ/mol

Na^+(out) —> Na+ (in) DG = ? kJ/mol



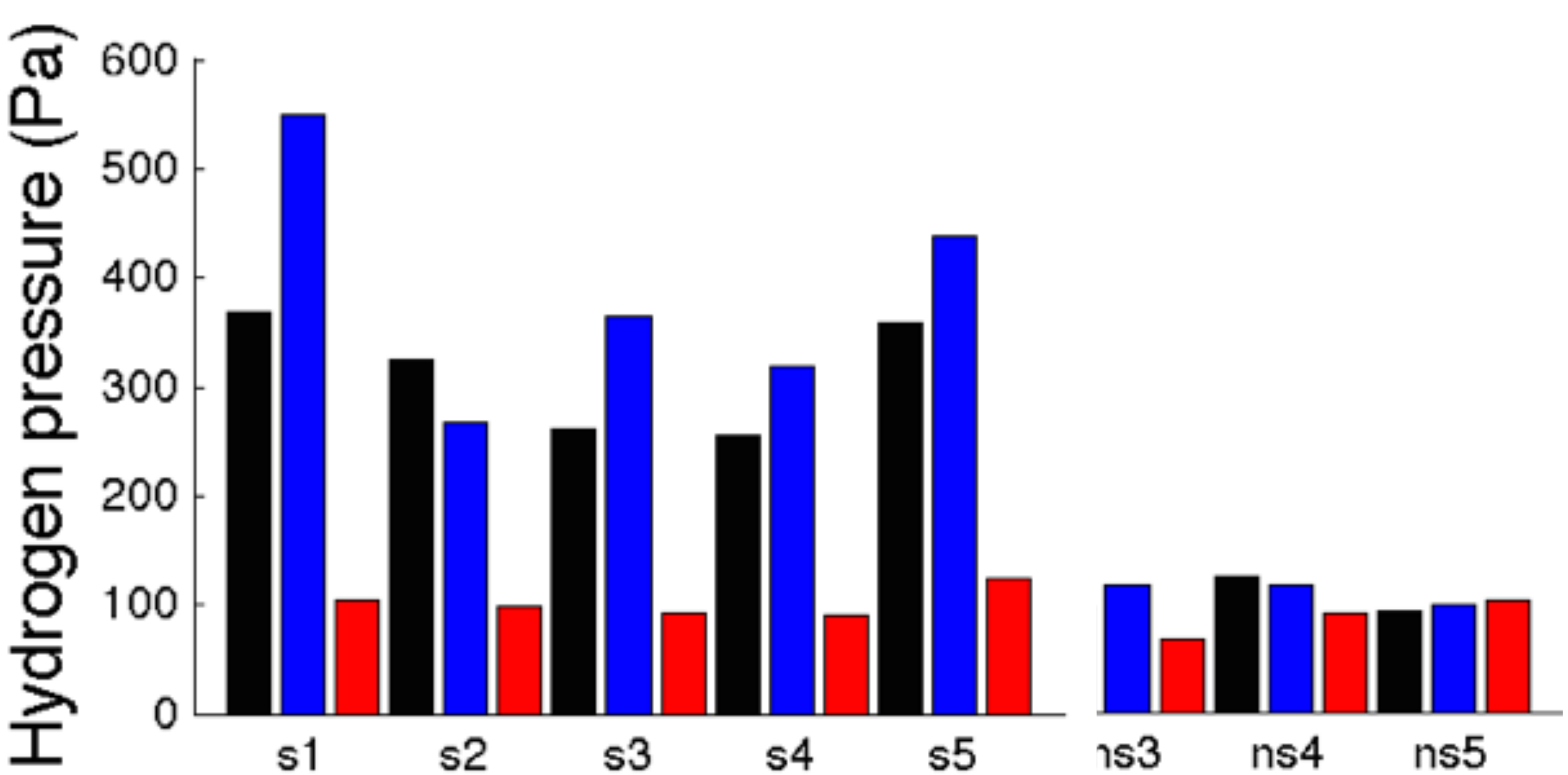
Walker *et al.*, *J. of Bact.* (2009)



One mutation present in all five "syntrophic" clones!

Thermodynamic basis of metabolic cooperation

Syntrophic clone produces more H_2....

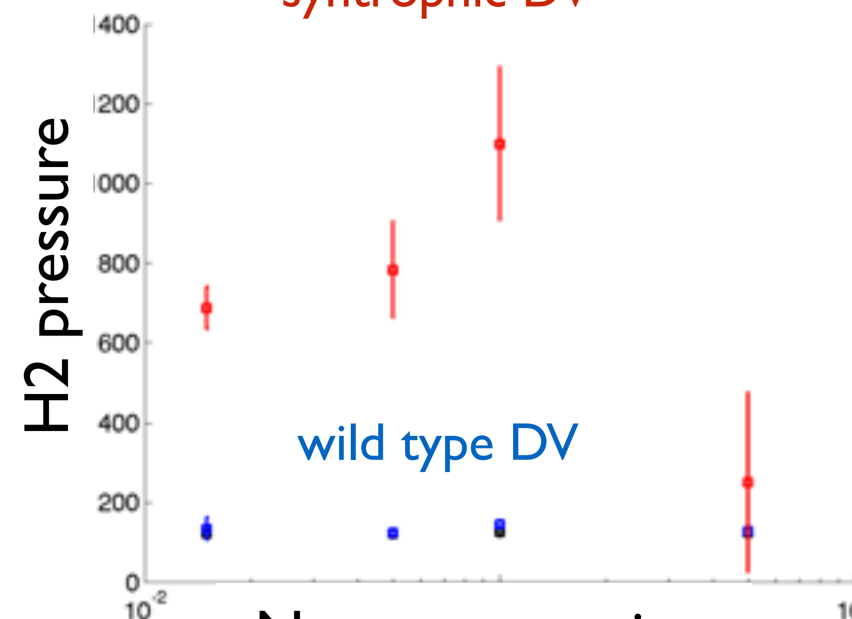


syntrophic DV

wild type DV

… and production level depends on external Na concentration

syntrophic DV



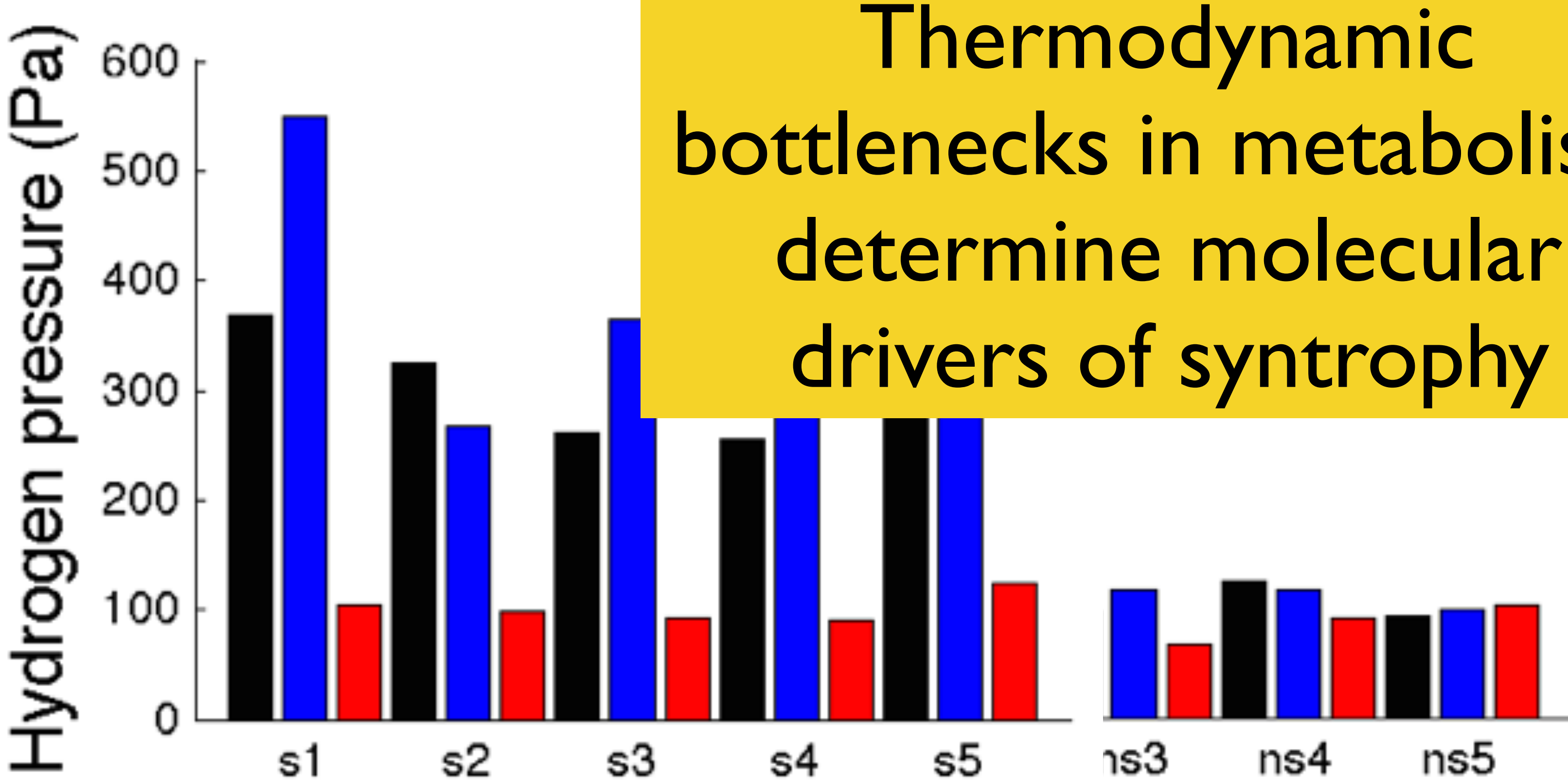
Na concentration

Grosskopf T, et al, *ISME Journal* (2016)

Thermodynamic basis of metabolic cooperation

… and production level depends on external Na concentration

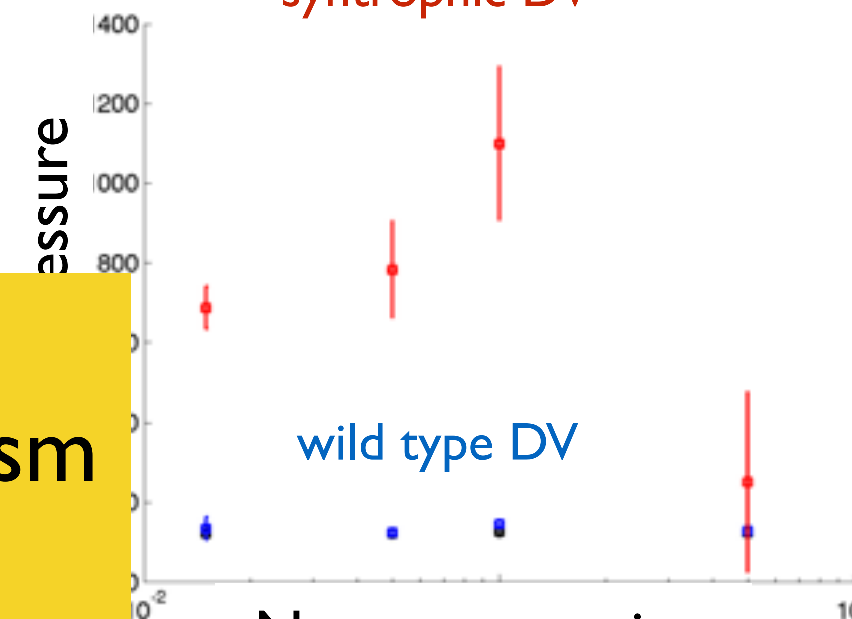
Syntrophic clone produces more H_2….



syntrophic DV

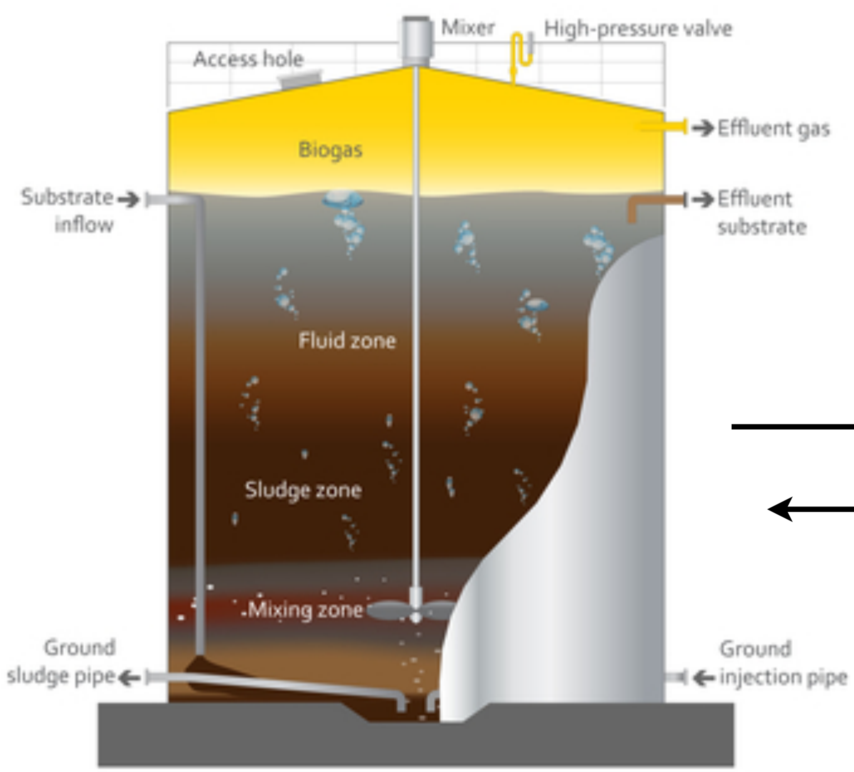
wild type DV

Thermodynamic bottlenecks in metabolism determine molecular drivers of syntrophy

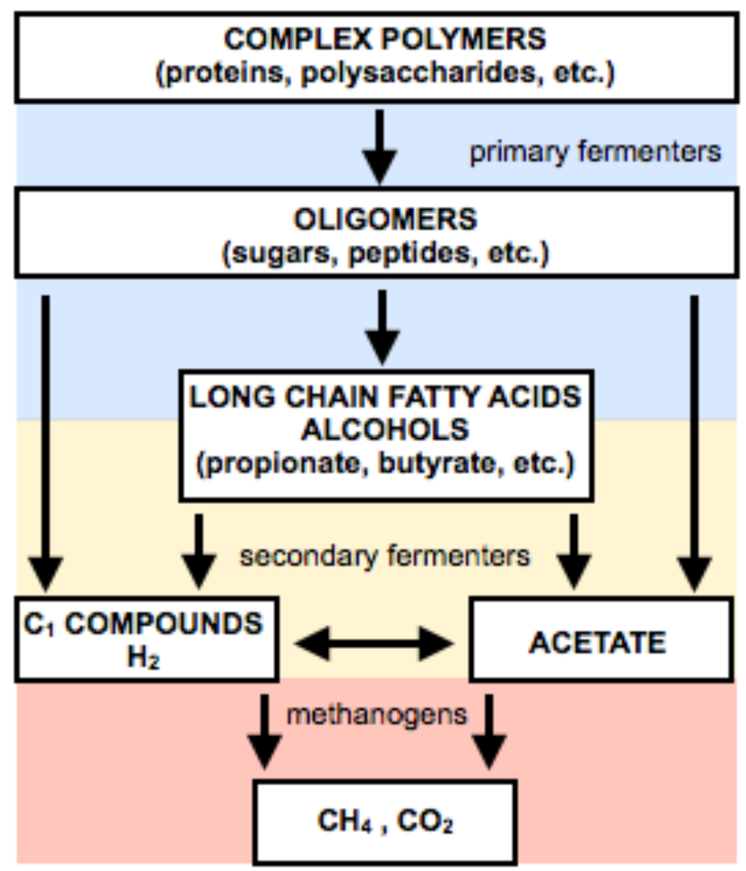


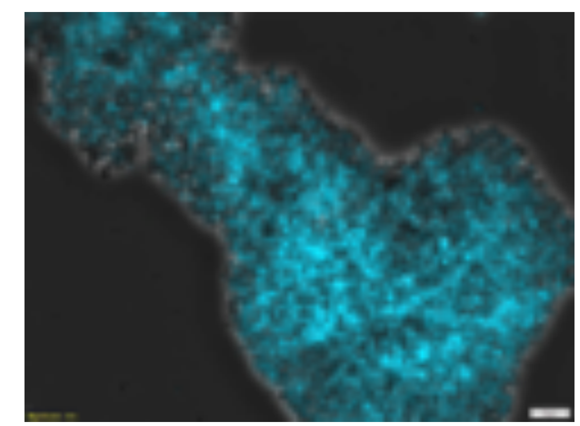
Grosskopf T, et al, *ISME Journal* (2016)

Thermodynamics as a design principle for multi-cellular ecosystems?

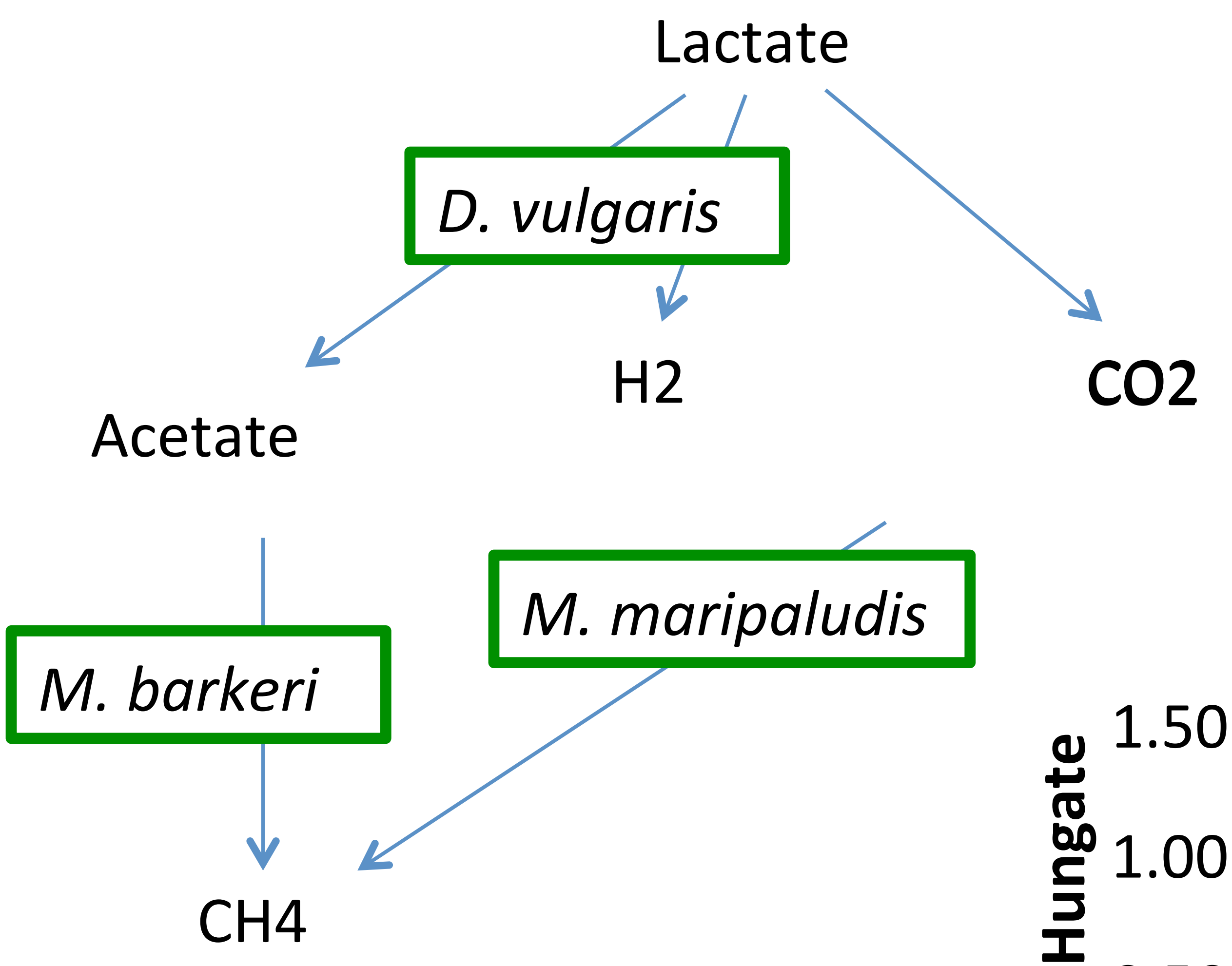


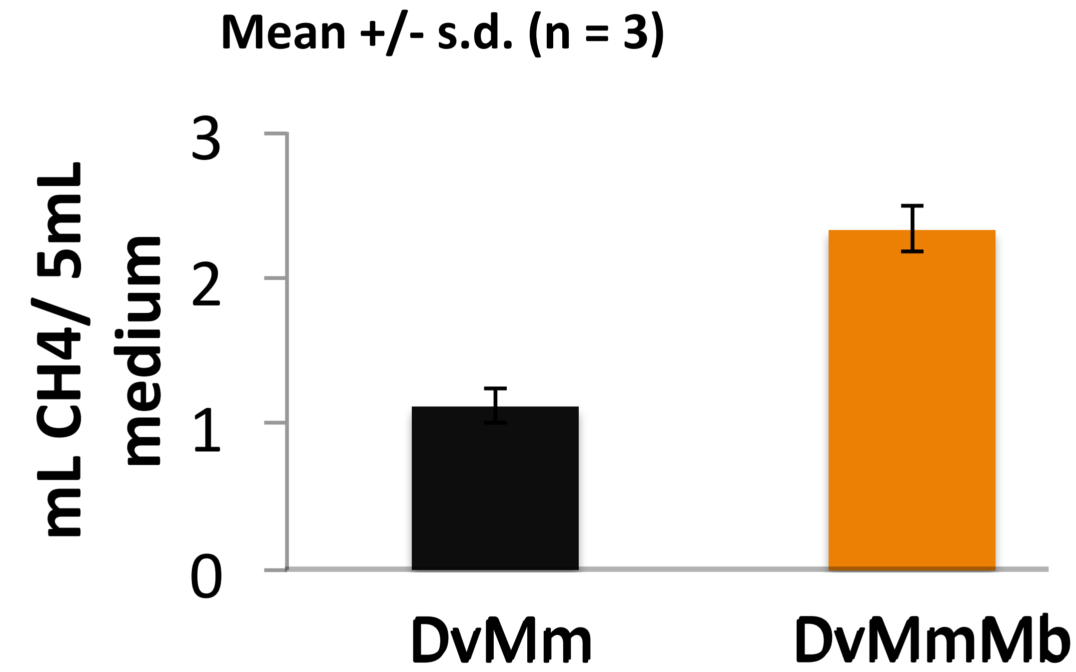
http://www.mannvit.com/Markets/UnitedKingdom/AnaerobicDigestion/

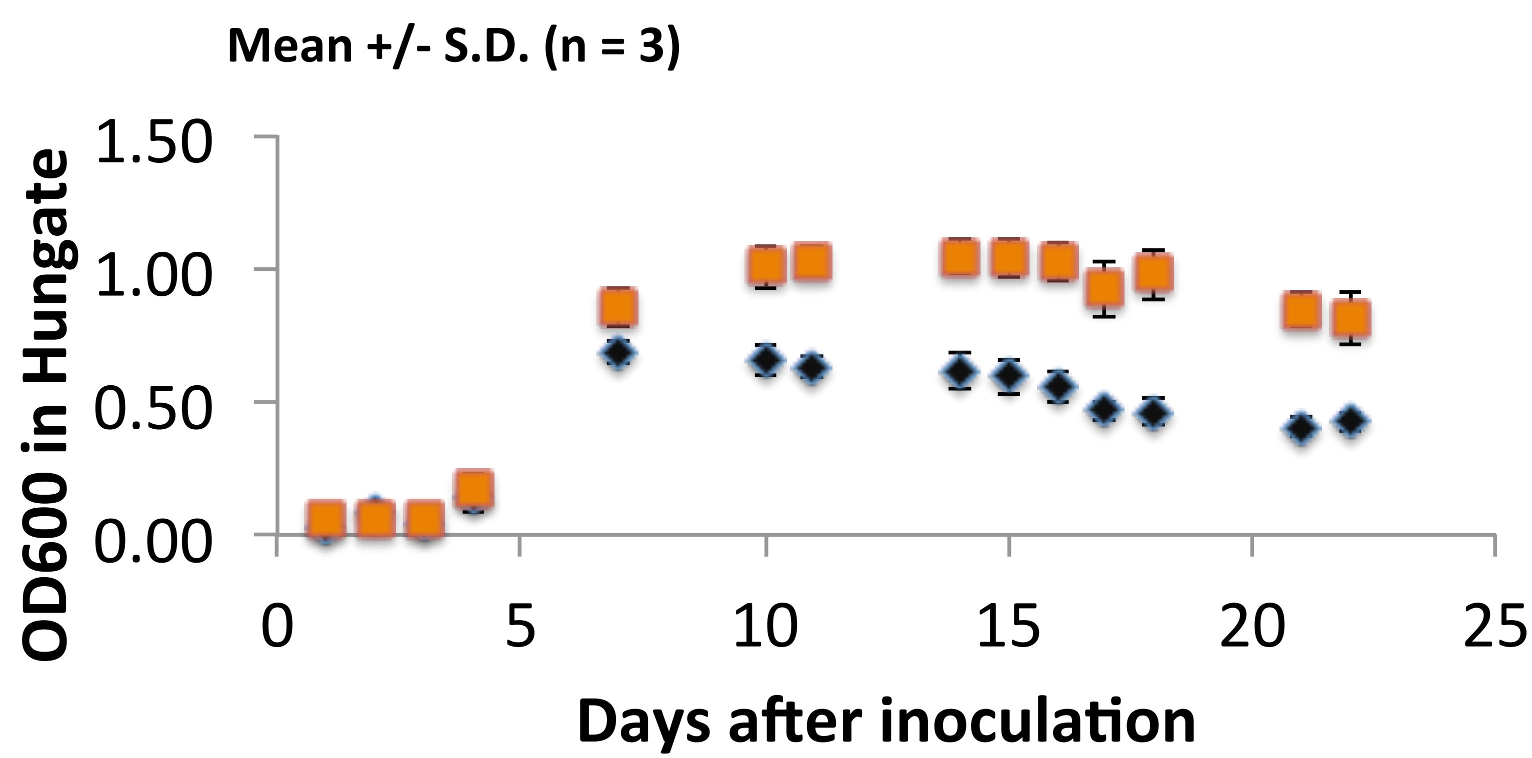




Exploiting thermodynamic inhibition for synthetic community design

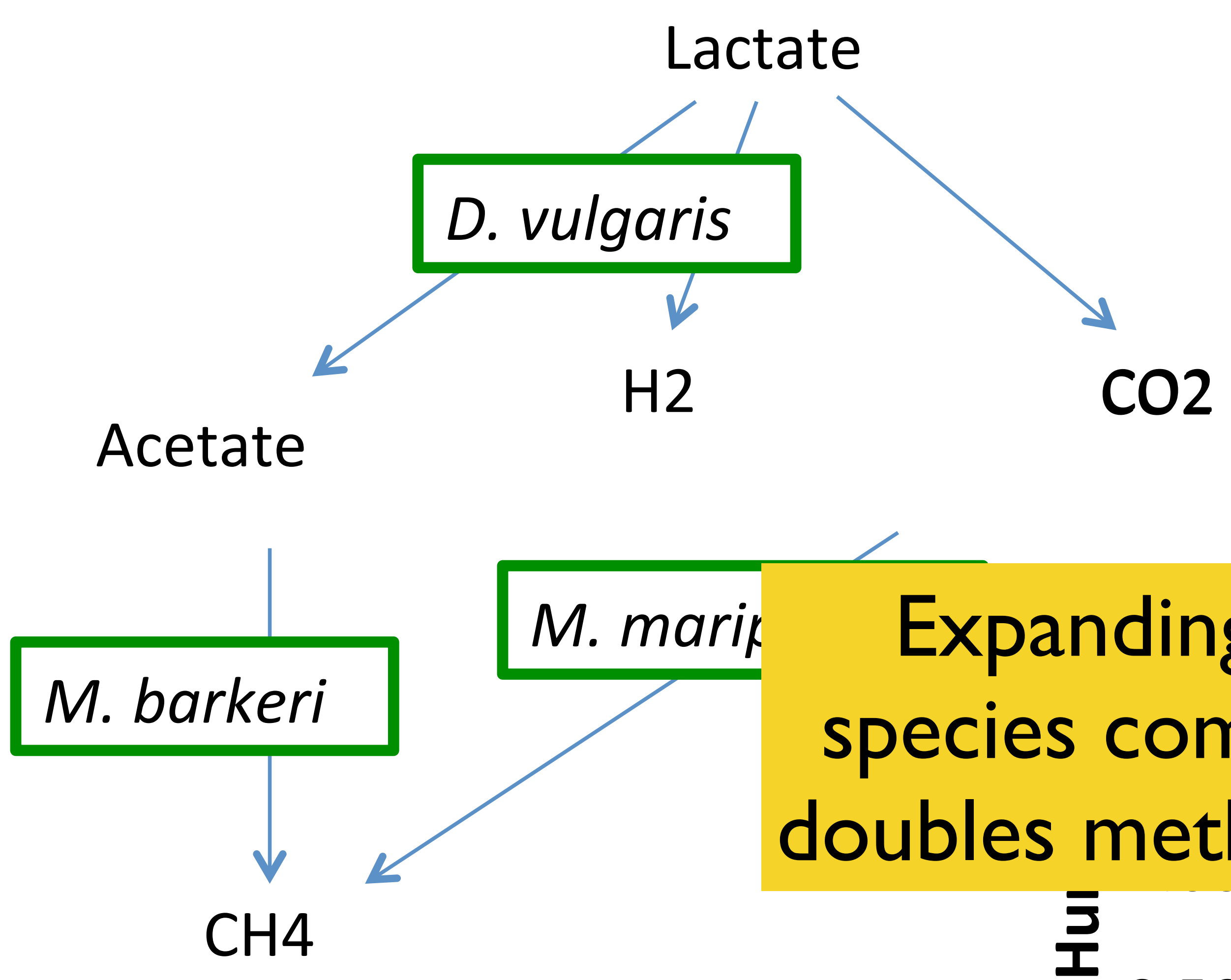


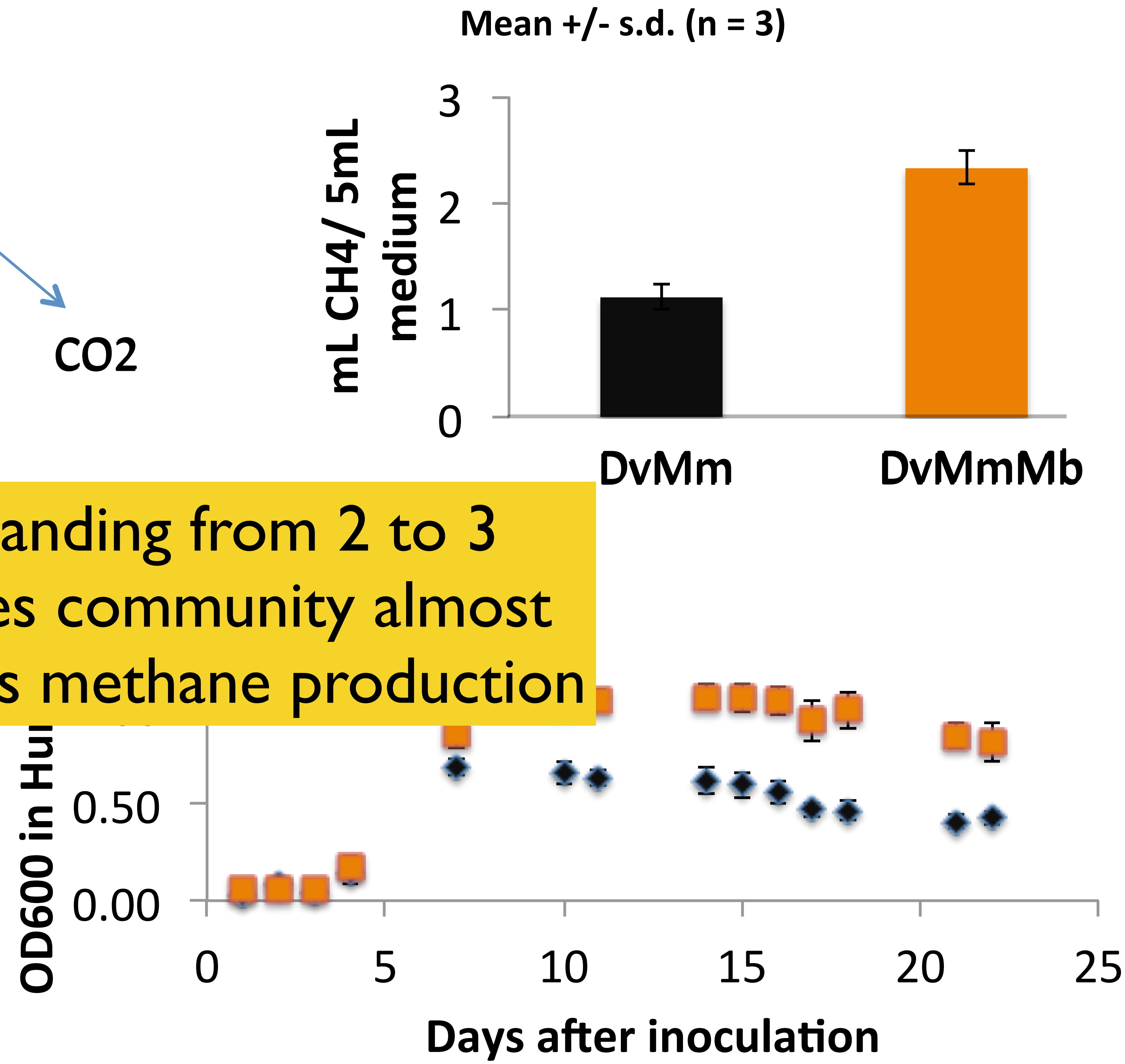




Grosskopf T, et al, *in preperation*

Exploiting thermodynamic inhibition for synthetic community design



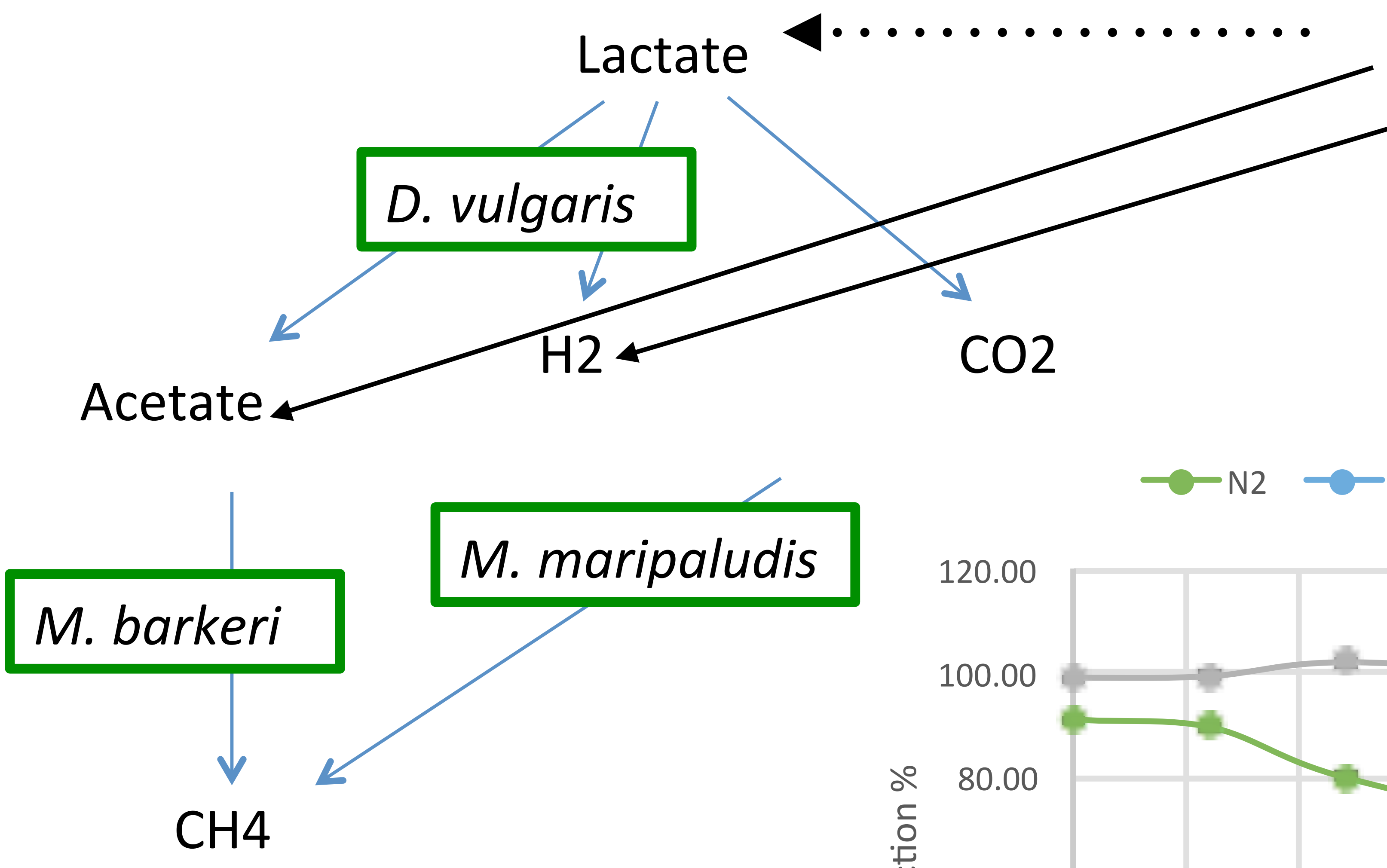


Expanding from 2 to 3 species community almost doubles methane production

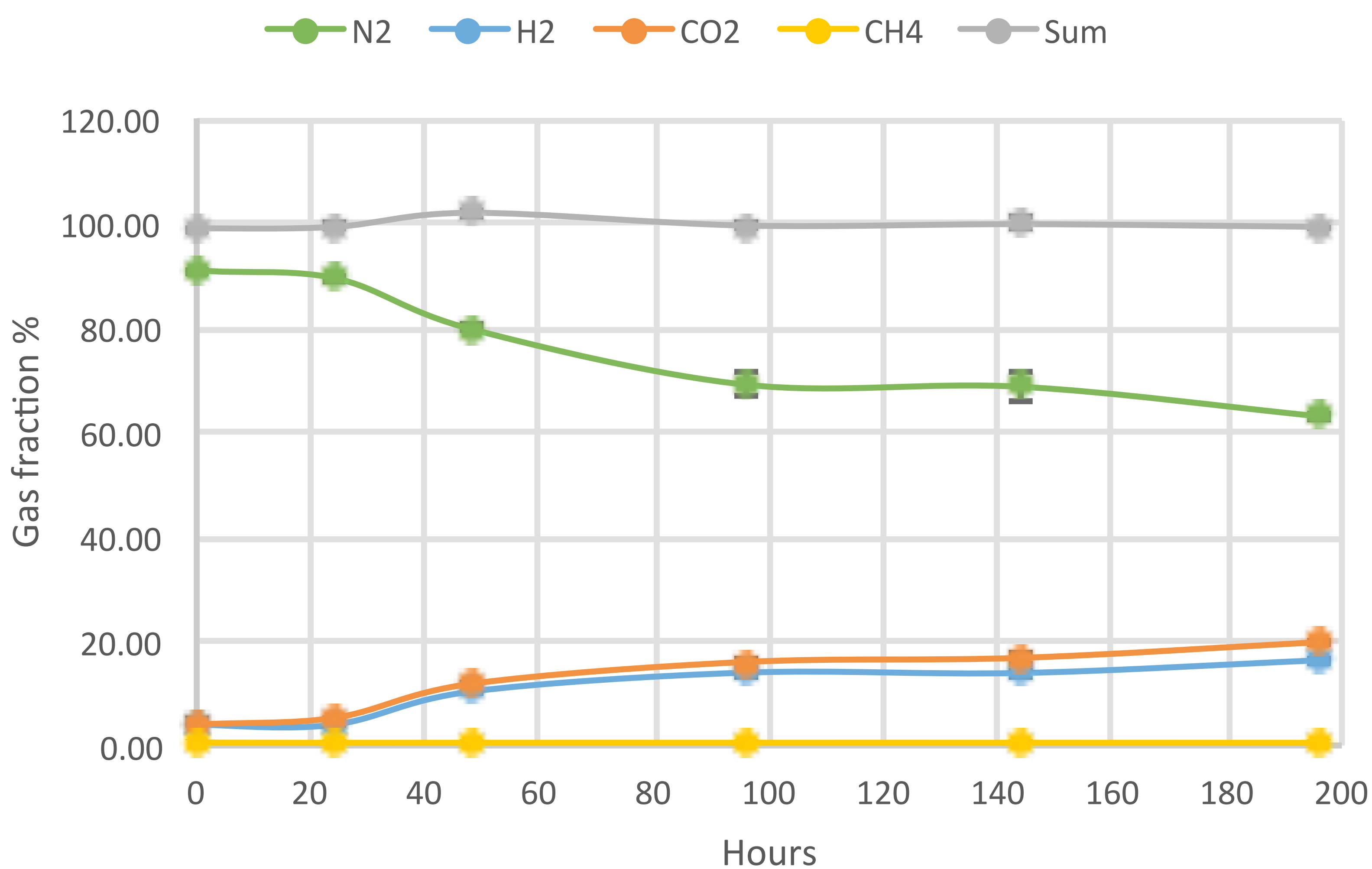
Grosskopf T, et al, *in preperation*

Expanding the synthetic community towards cellulose to methane conversion

Cellulose degrader(s)



Ca on defined media with cellulose



Jing Chen

Tobias Großkopf

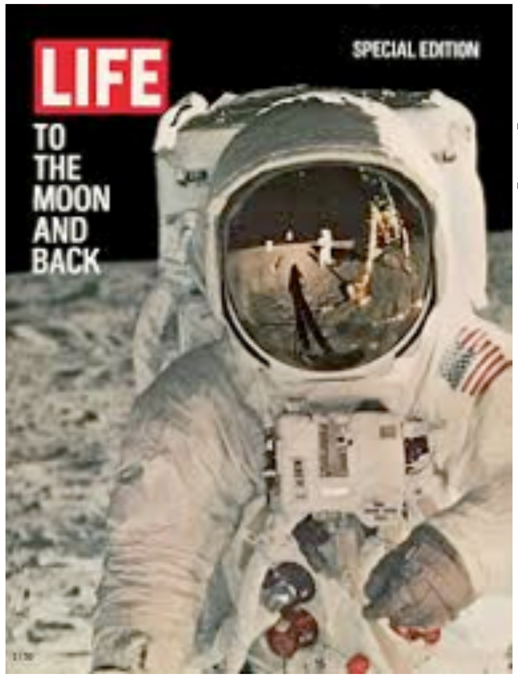
Matthew Wade

Synthetic microbial communities - Future

Community Building

Microbial Communities @ the Isaac Newton Institute:
http://www.newton.ac.uk/event/umc

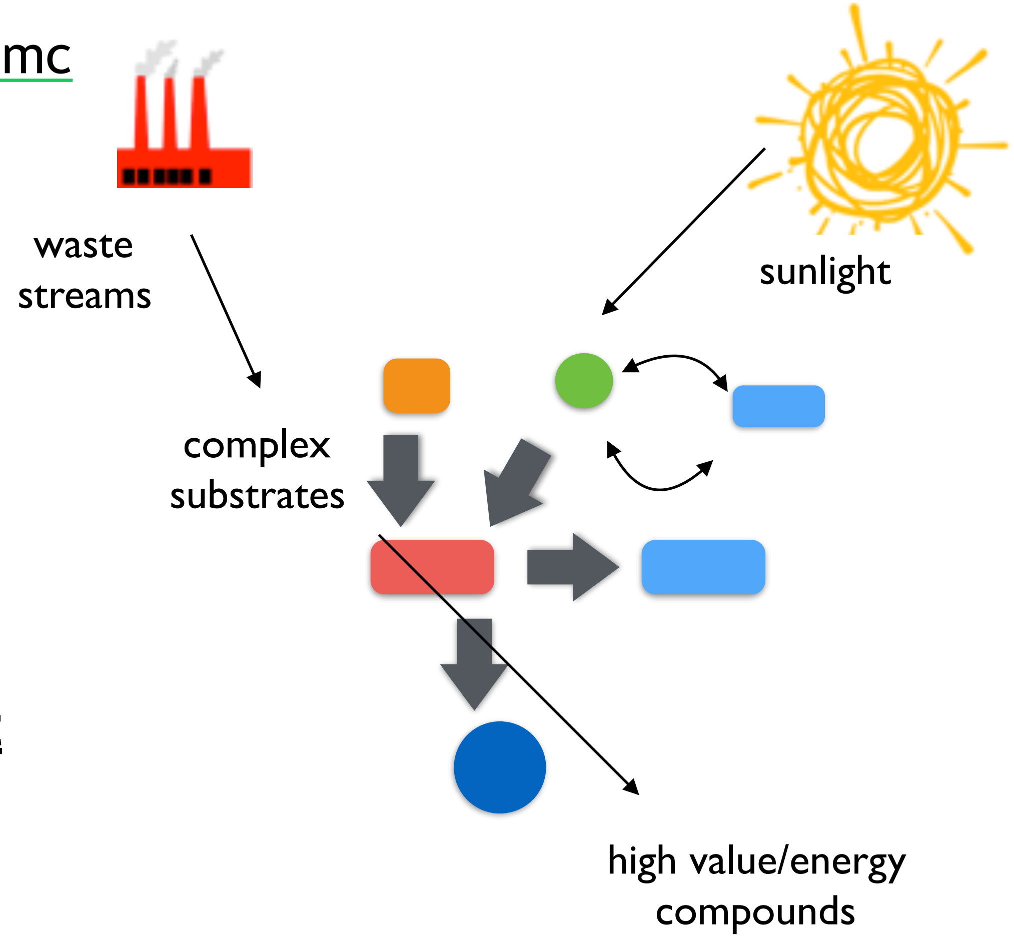
Research Theme at WISB



Tool Development

Non-invasive monitoring

Spatial measurement



Applications

Synthetic soil ecosystem

Model gut

Anaerobic Digestion

Biological Production

Fuel Cells

Basic Science

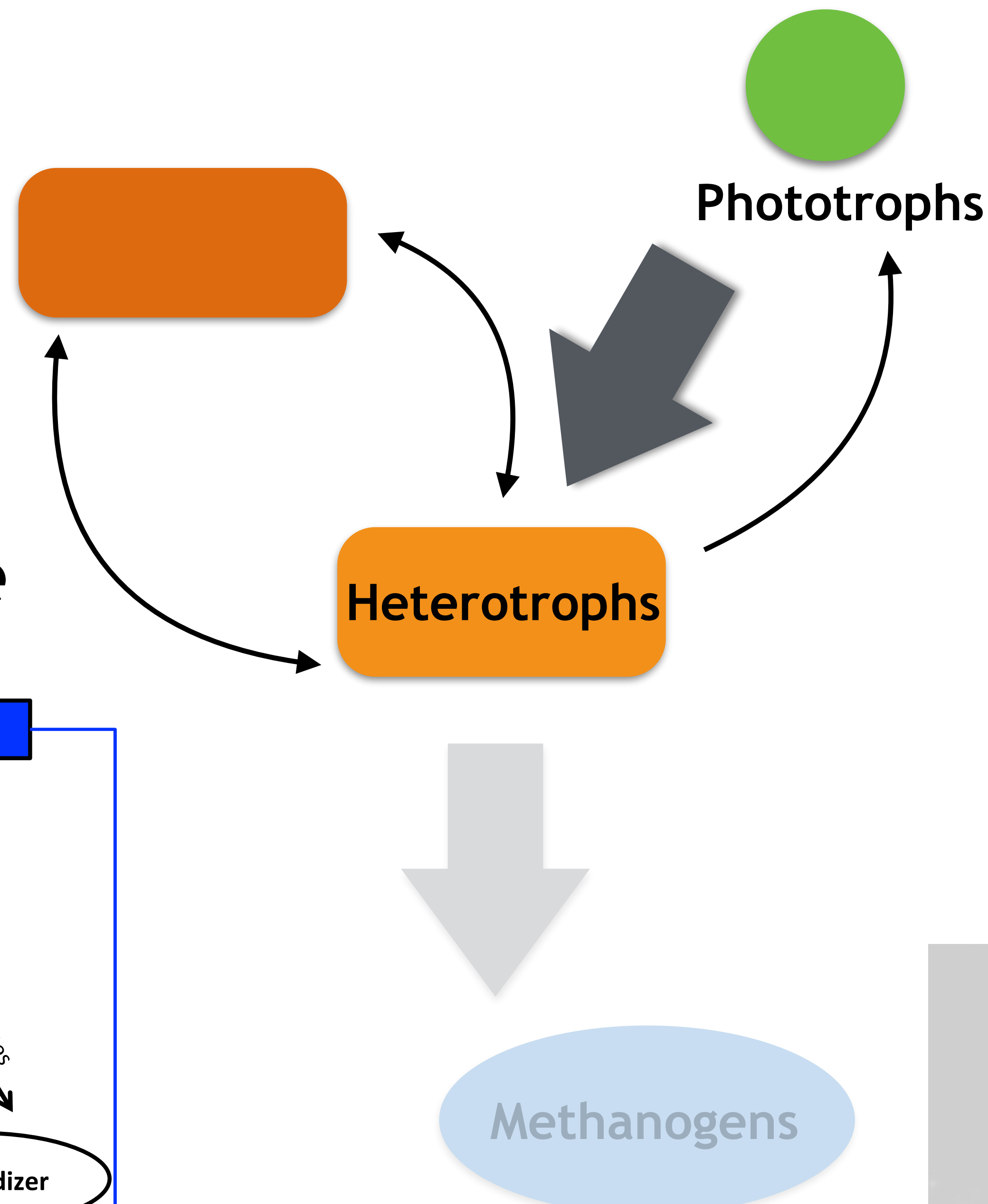
Community stability

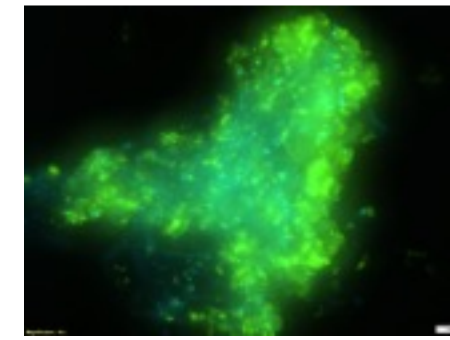
Multi-step metabolism

Multi-cellularity

Non-model organisms

Synthetic microbial communities - Immediate Future: Closed ecosystems

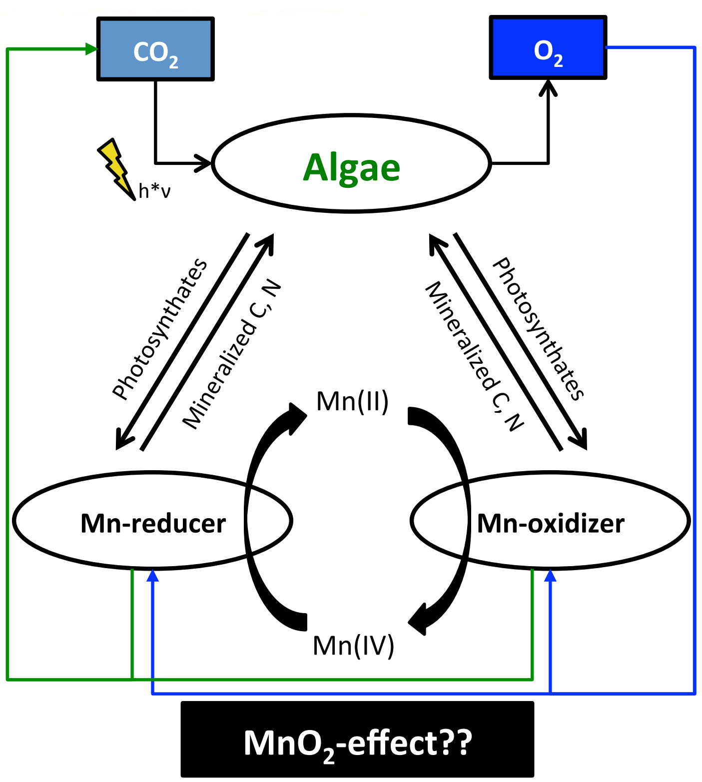




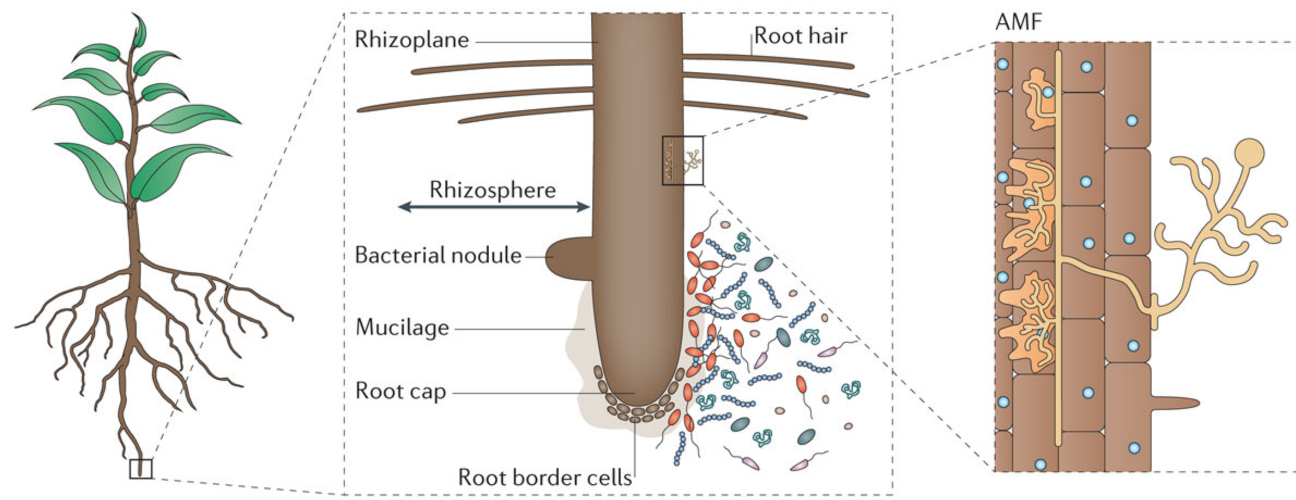
Re-Mineralization Cycle

Christian Zerfass

with Joseph Christie-Oleza

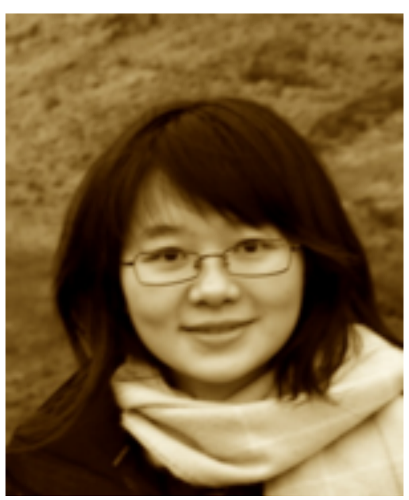


Synthetic microbial communities - Immediate Future: Model plant support ecosystem



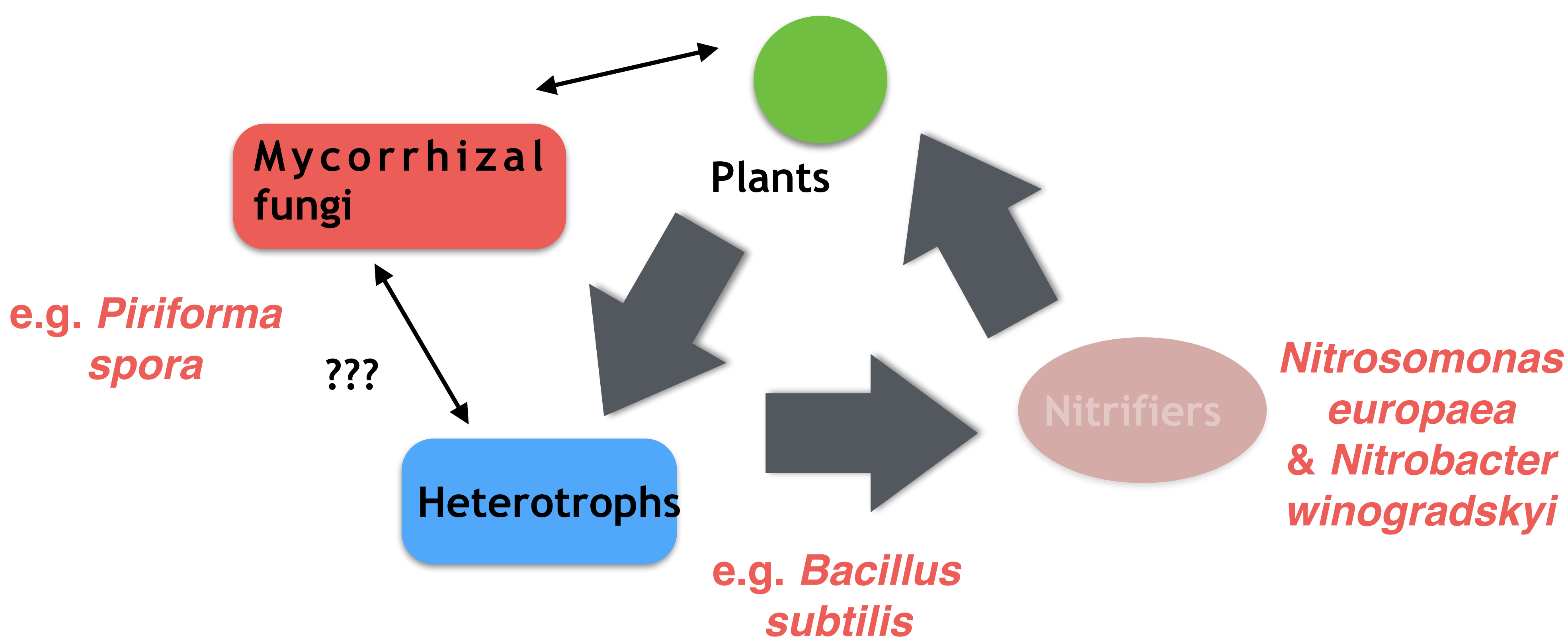
Philippo L, et al., 2013

Nature Reviews | Microbiology



Xue Jiang

with Patrick Schaefer



THANK YOU

OSS LAB http://osslab.lifesci.warwick.ac.uk

Collaborators

Signaling Systems:
Carsten Wiuf & Elisenda Feliu (Copenhagen University)
Steve Porter (University of Exeter)
Julien Olivier & Peter Swain (University of Edinburgh)

Metabolism Evolution:
Dominique Schneider (University of Grenoble)
Richard Lenski (Michigan State University)

Synthetic Communities:
David Swarbreck (TGAC)
Joseph Christie-Oleza (University of Warwick)
Patrick Schaefer (University of Warwick)

Noteworthy

Microbial Communities @ the Isaac Newton Institute:
http://www.newton.ac.uk/event/umc

Open Positions

Post-doc positions available @ OSS LAB

PhD studenthips available @ EPSRC & BBSRC Centre for Doctoral Training in Synthetic Biology SynBio CDT

Funders

BBSRC bioscience for the future
EPSRC Pioneering research and skills
dstl