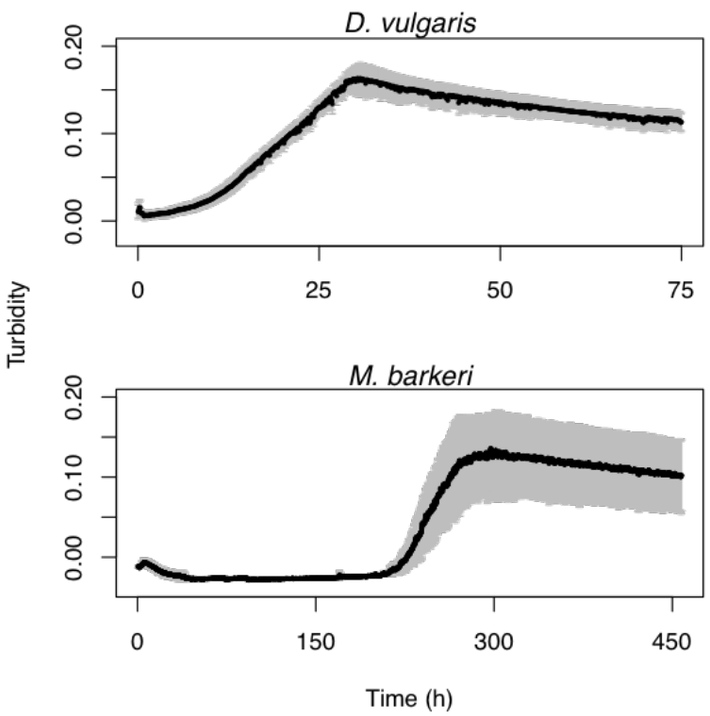
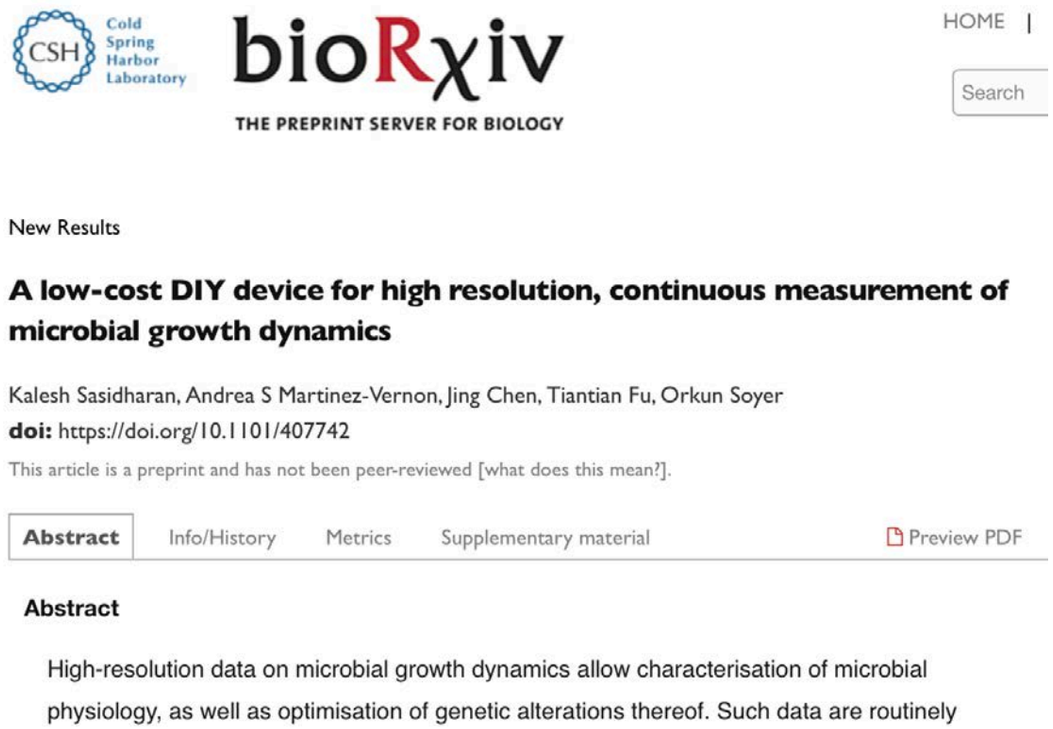
Enabling open source experimentation in life sciences research and education



MicrobeMeter



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New Results

A low-cost DIY device for high resolution, continuous measurement of microbial growth dynamics

Kalesh Sasidharan, Andrea S Martinez-Vernon, Jing Chen, Tiantian Fu, Orkun Soyer

doi: https://doi.org/10.1101/407742

This article is a preprint and has not been peer-reviewed [what does this mean?].

Abstract | Info/History | Metrics | Supplementary material | Preview PDF

Abstract

High-resolution data on microbial growth dynamics allow characterisation of microbial physiology, as well as optimisation of genetic alterations thereof. Such data are routinely

available to download at:
Follow progress at:

www.humanetechnologies.co.uk

@humanetechltd

An electrical and thermodynamical view of metabolism

Orkun S Soyer

ARM Workshop
Cambridge, 17 Sept 2018

OSS LAB

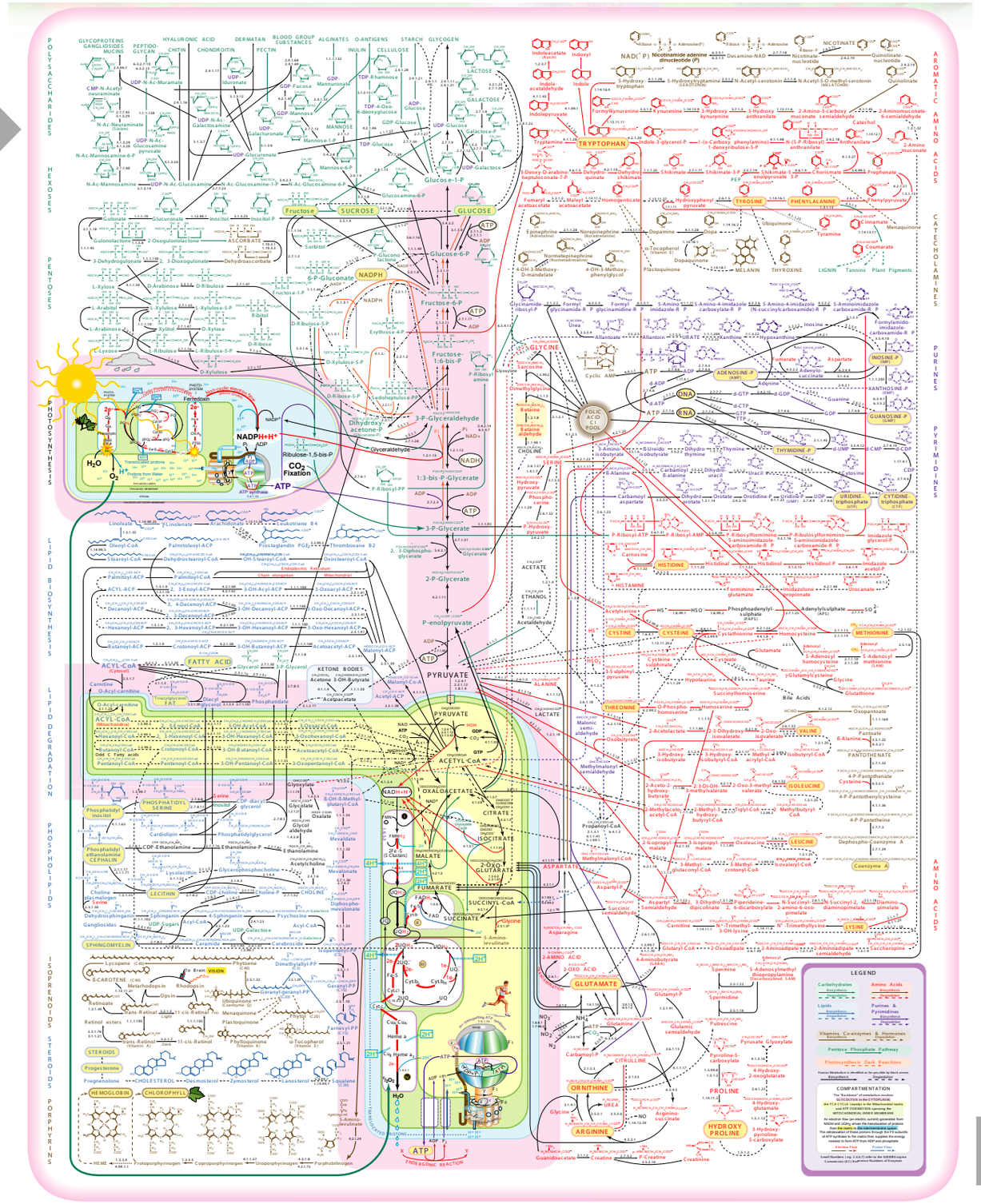
Bio Electrical Engineering
Innovation Hub @ Warwick

WARWICK
THE UNIVERSITY OF WARWICK

Metabolism as a set of pathways

Self sufficient
'energy extractor'

Substrate

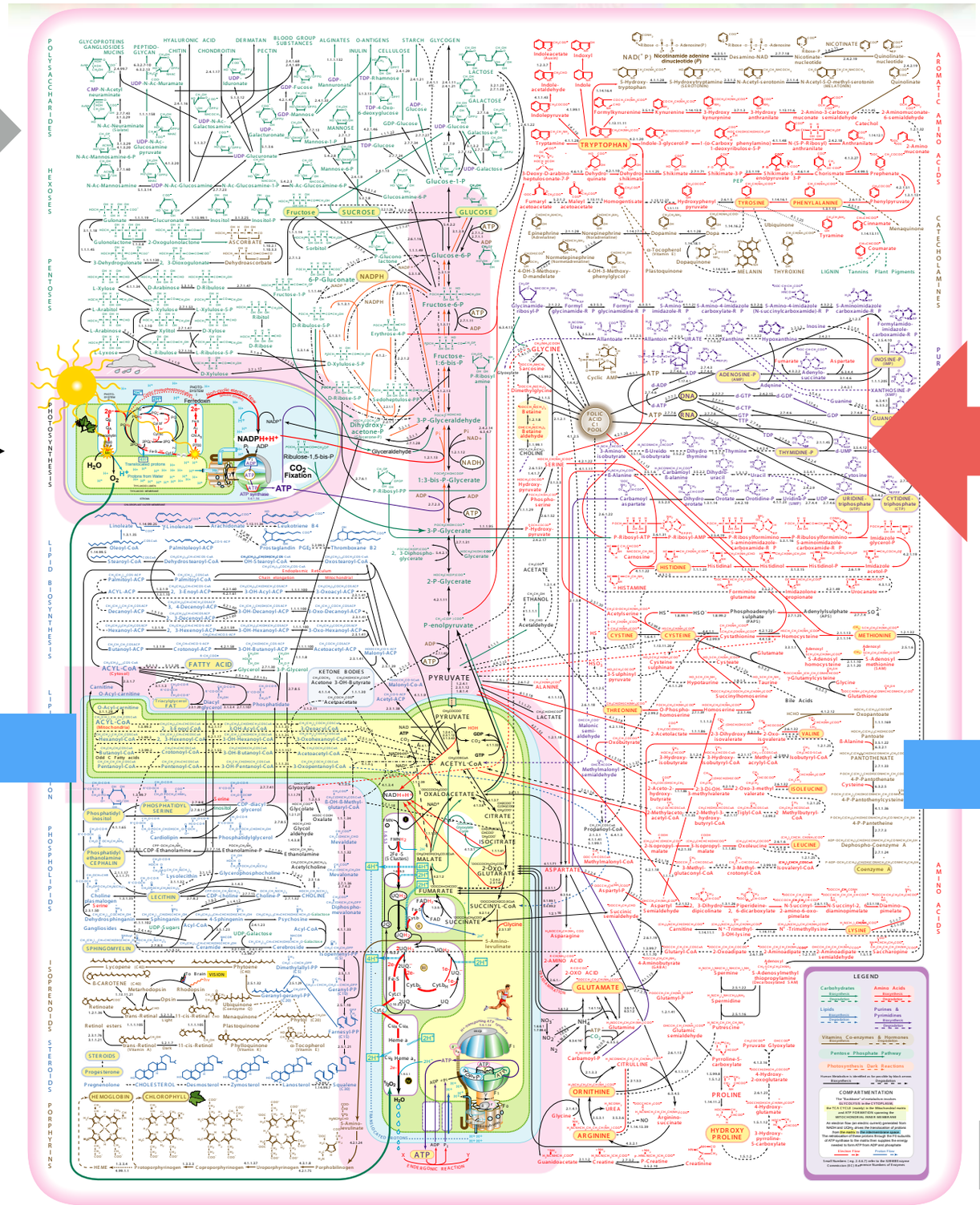


Waste

Metabolism as a set of pathways

~~Self sufficient~~
~~'energy extractor'~~

Substrate



Auxotrophs

Syntrophs

'Essential' co-factors

Energy rich byproducts

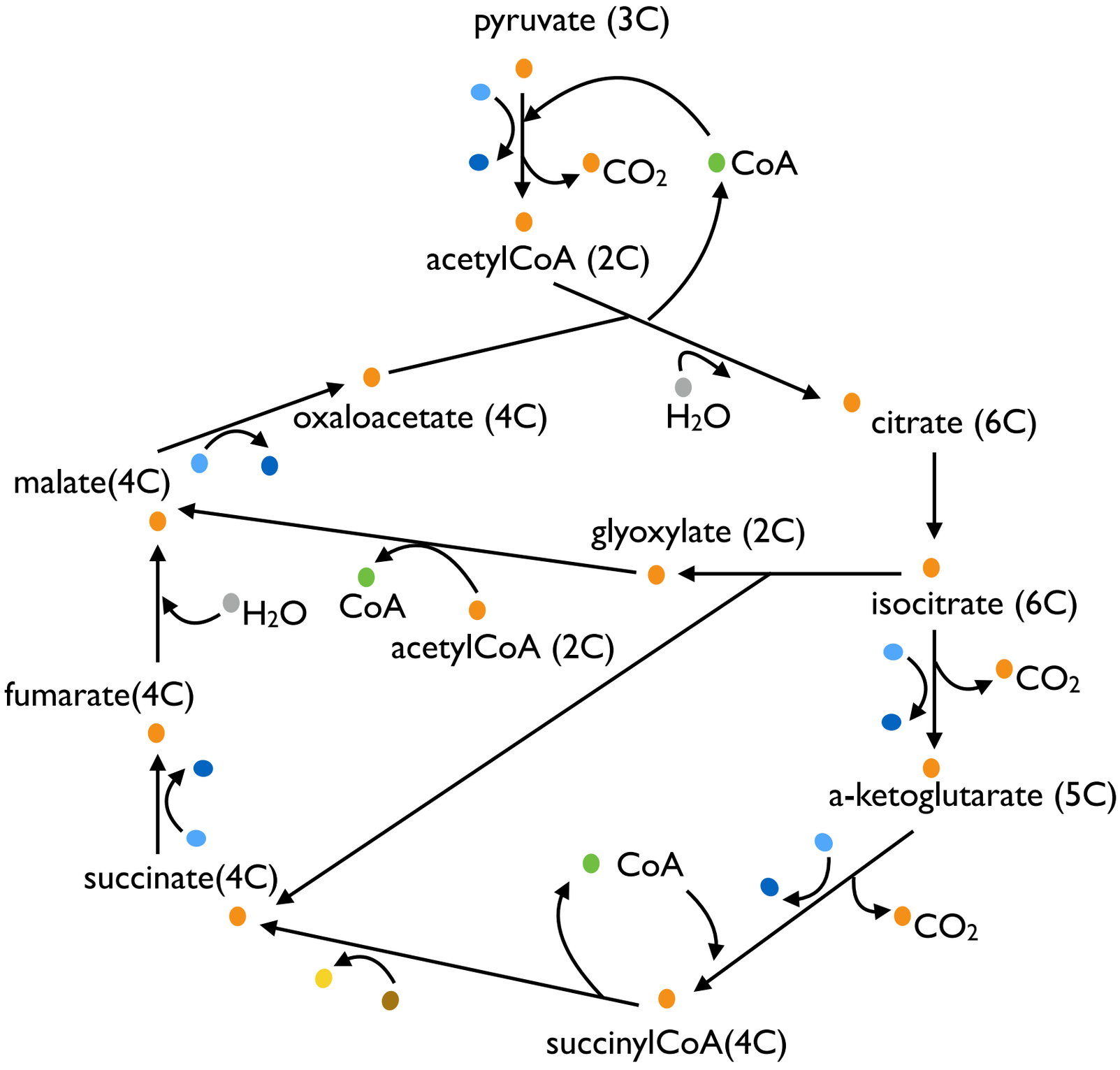
Overflow

Cross-feeders

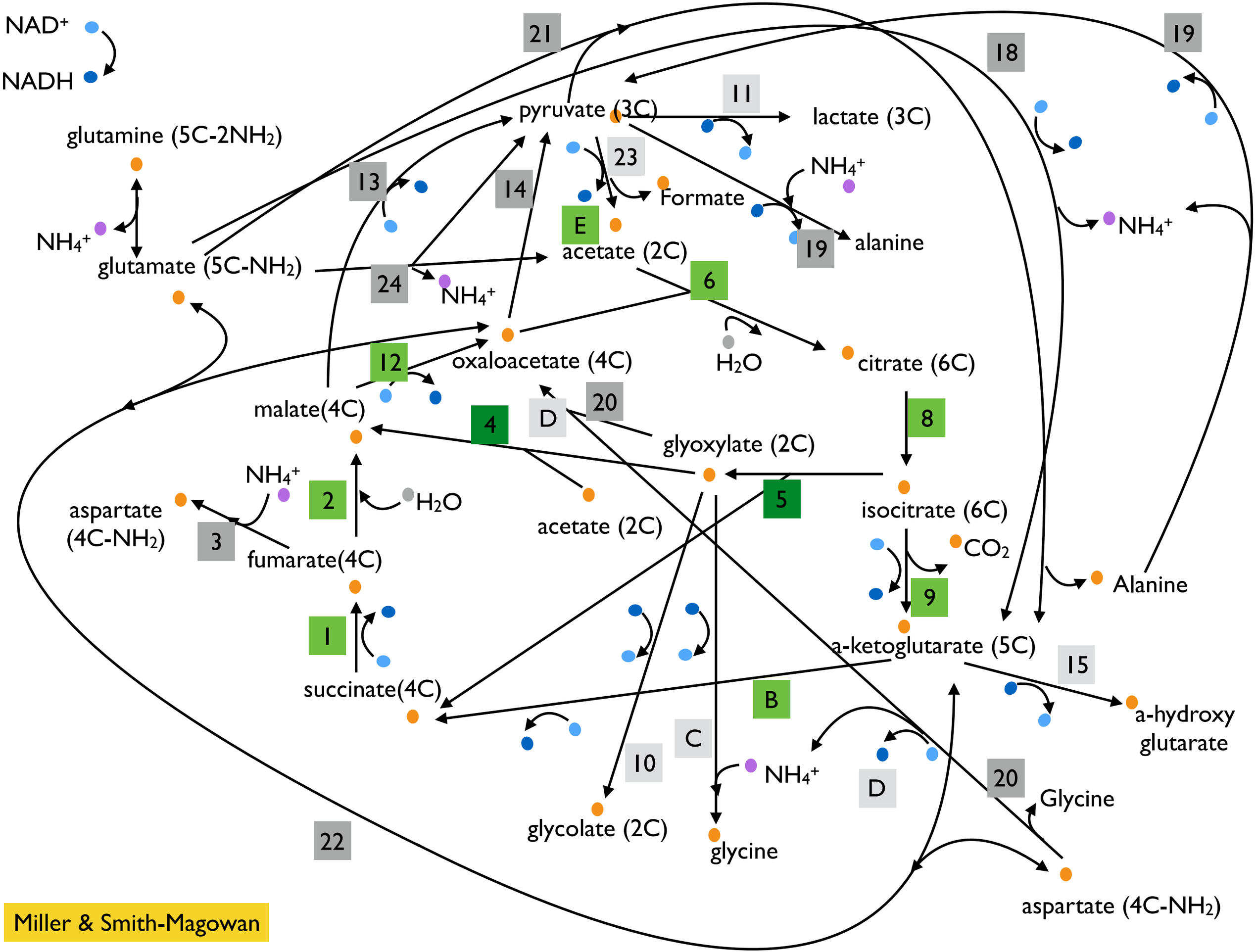
Waste

NAD+

NADH



Textbook TCA

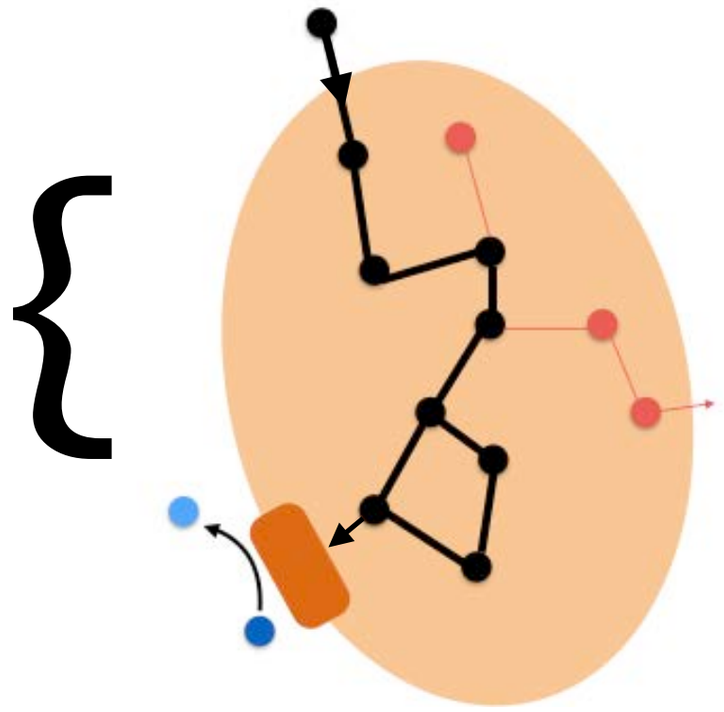

NAD+
NADH
glutamine (5C-2NH2)
NH4+
glutamate (5C-NH2)
pyruvate (3C)
lactate (3C)
11
21
18
19
13
14
23
Formate
E
acetate (2C)
NH4+
alanine
19
24
NH4+
6
H2O
citrate (6C)
12
oxaloacetate (4C)
malate(4C)
4
D
20
glyoxylate (2C)
8
NH4+
aspartate
(4C-NH2)
3
2
H2O
fumarate(4C)
acetate (2C)
5
isocitrate (6C)
CO2
9
Alanine
1
succinate(4C)
a-ketoglutarate (5C)
15
a-hydroxy
glutarate
B
C
10
NH4+
D
20
Glycine
glycolate (2C)
glycine
22
aspartate (4C-NH2)
Miller & Smith-Magowan

Metabolism as an electron flow system

Electron provision

Substrate Uptake

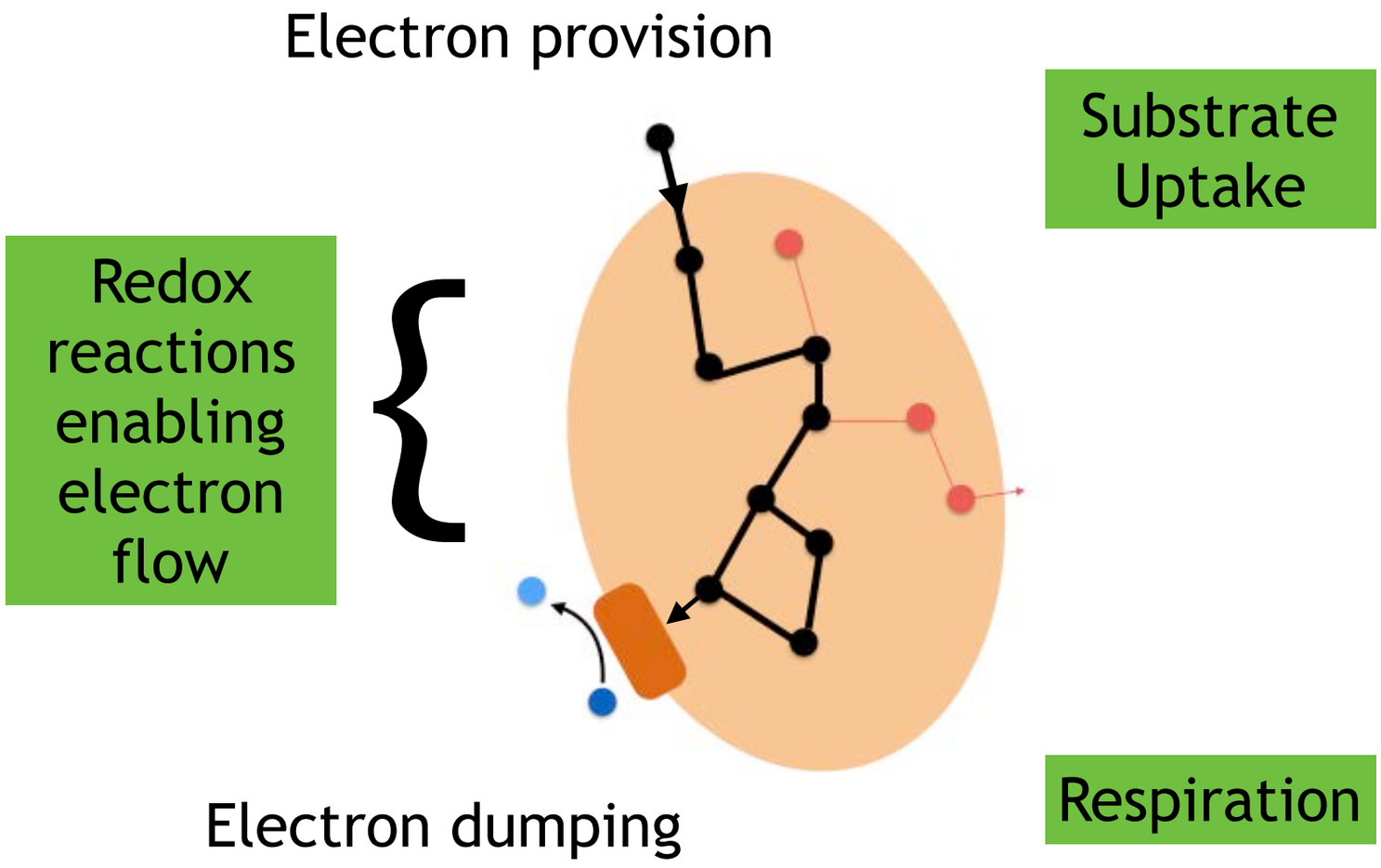
Redox reactions enabling electron flow



Electron dumping

Respiration

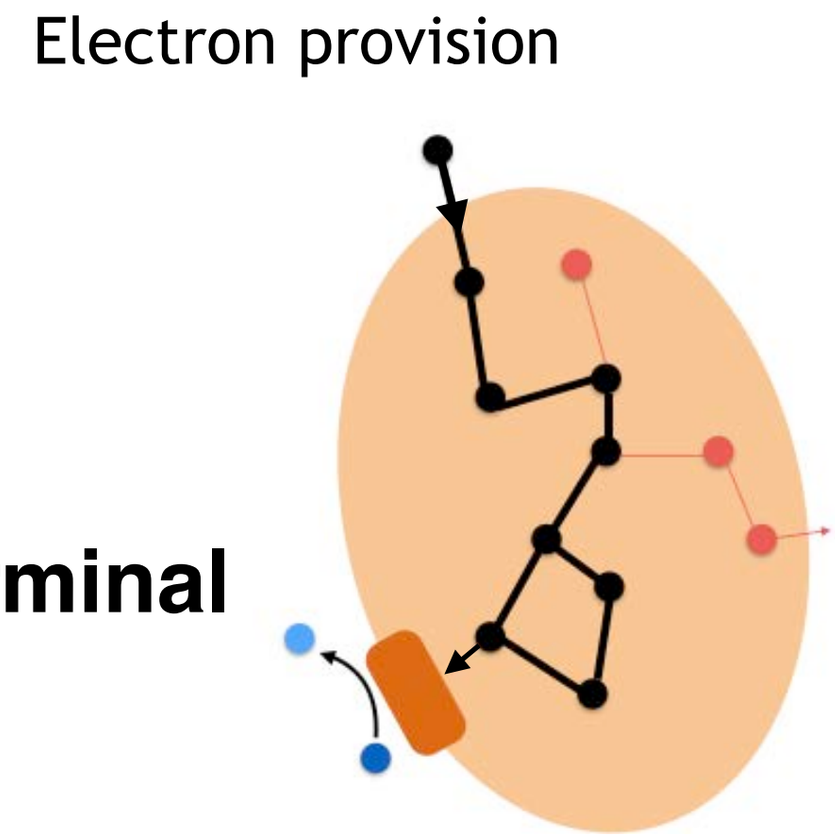
Metabolism as an electron flow system



Limitations ?

Physical/environmental limits on respiration

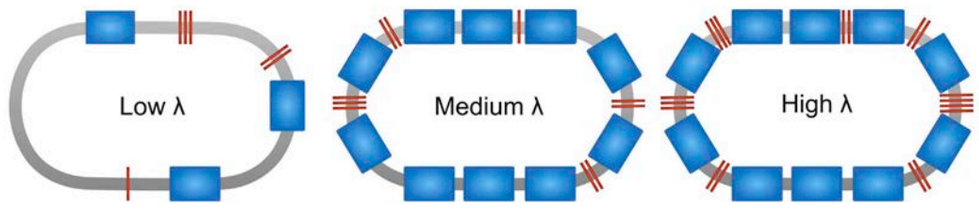
Managing the terminal electron sinks



Trade-offs in space/enzyme allocation

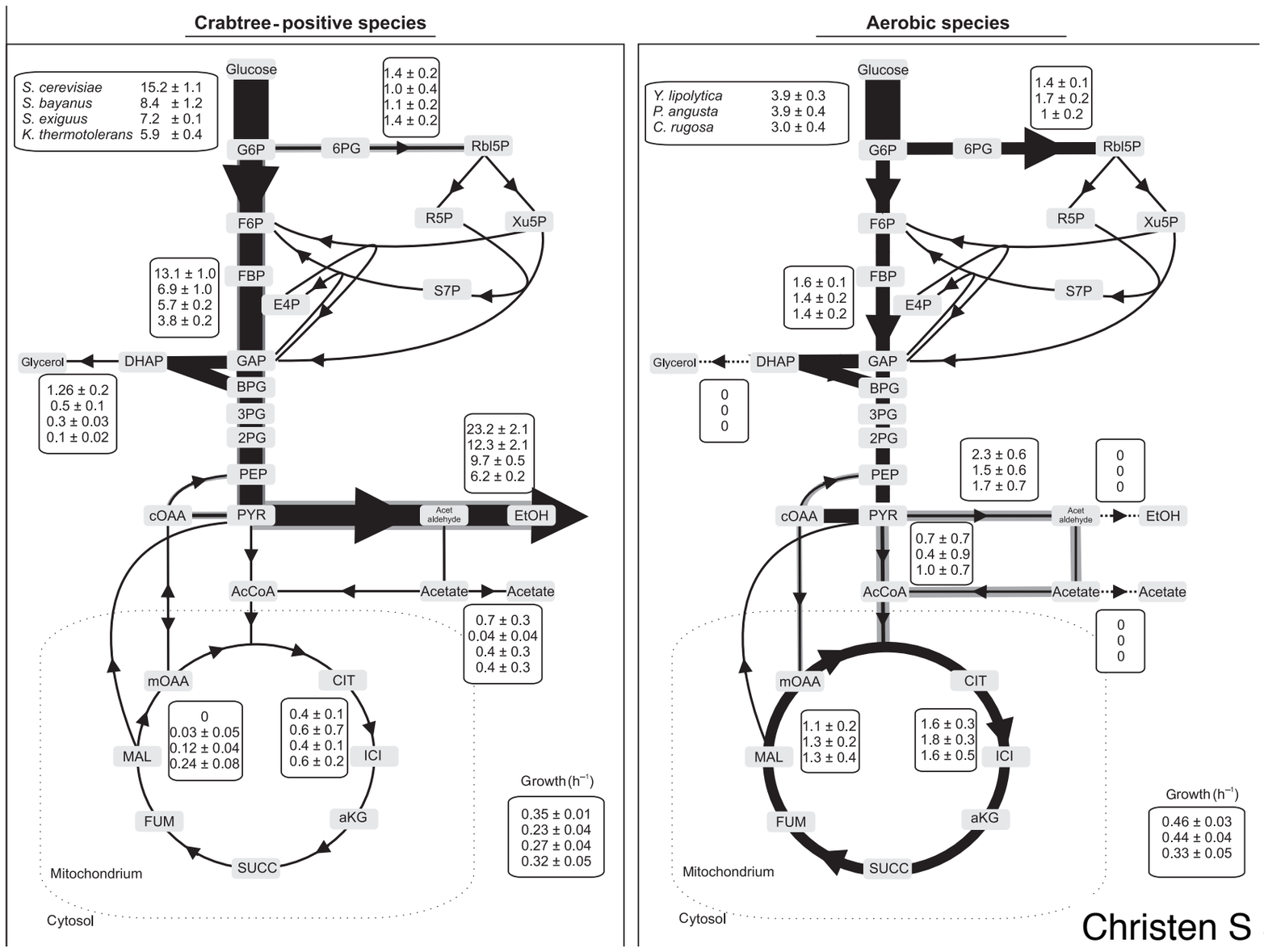
Szenk M, Dill KA, de Graff AMR, *Cell Systems 5* (2017)

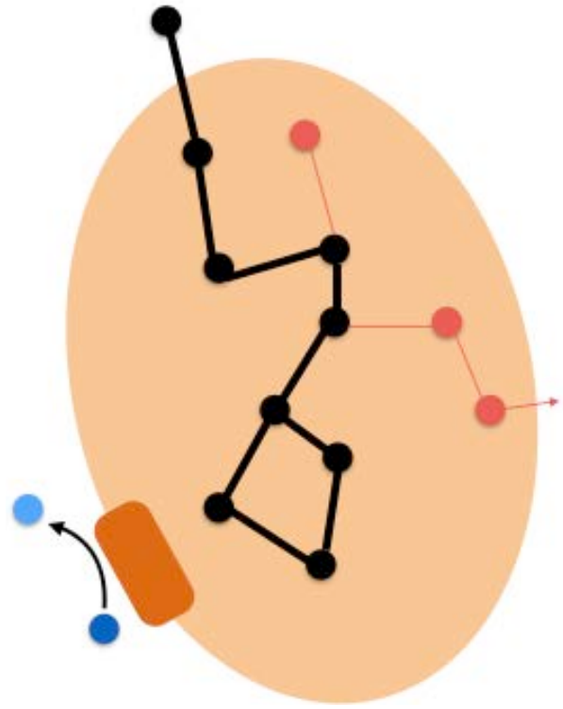
Basan M, et al. *Nature* 528 (2015)



Overcoming respiratory limits with metabolic overflow

‘overflow metabolism’ (a.k.a Warburg effect and Crabtree effect in cancer and yeast cells) seems to be present in all cells where it is studied.

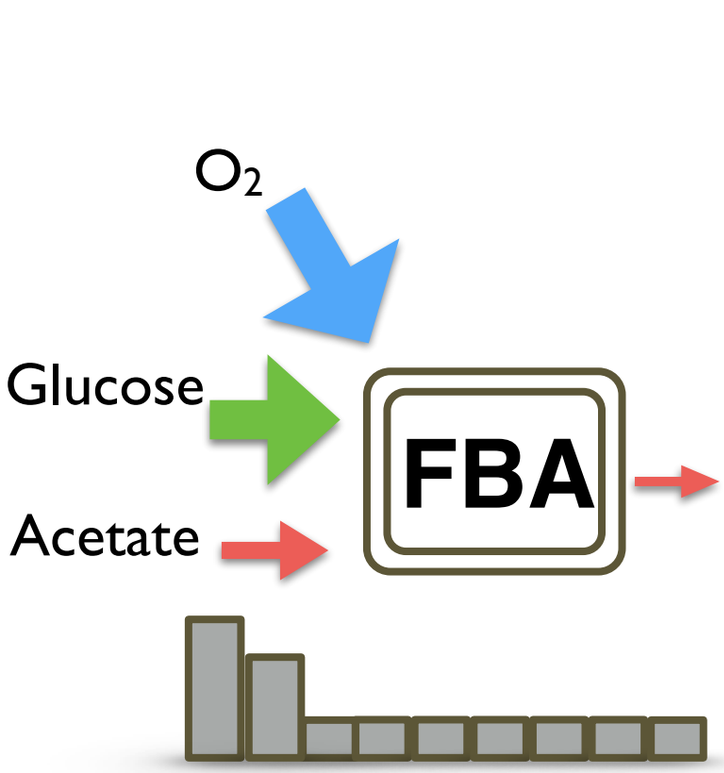




Christen S & Sauer U, *FEMS* (2011)

Vemuri G, et al. *Appl Env Microbial* (2006)

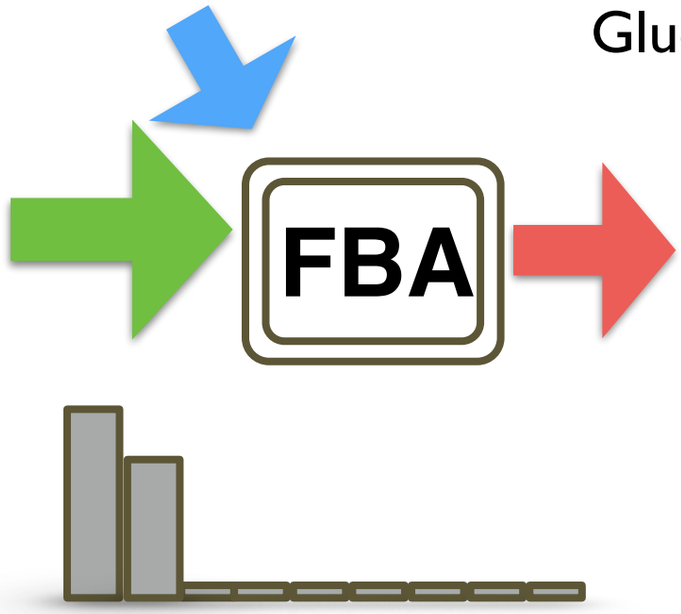
Overflow can emerge from trade-offs and can lead to cross-feeding



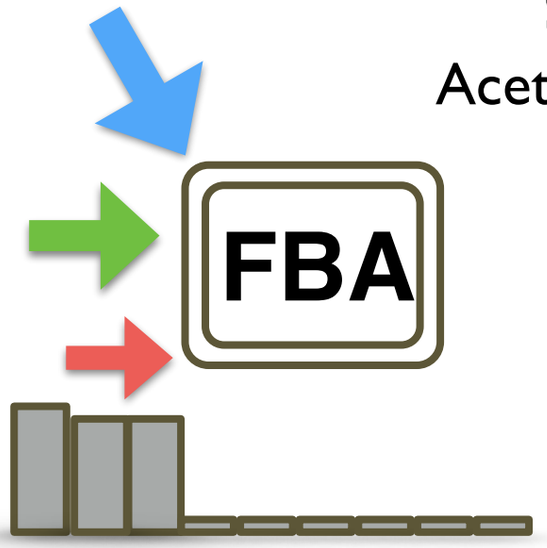
evoFBA

Eco-Evolution simulations

Species A
Glucose specialist



Species B
Acetate "specialist"

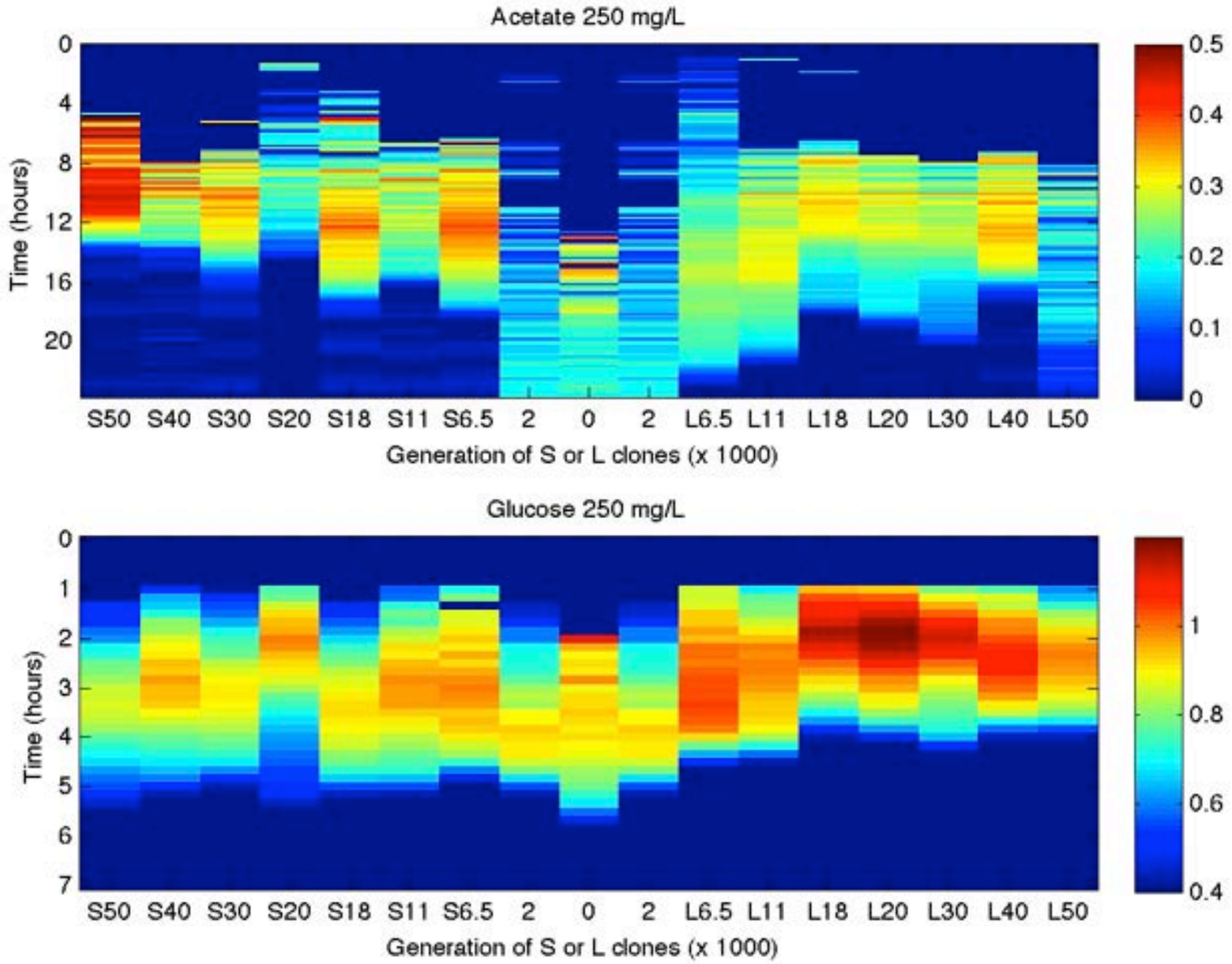


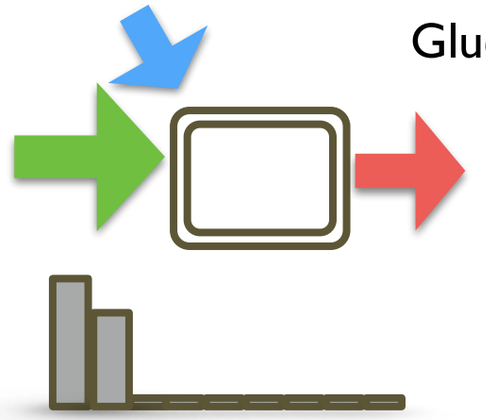
Grosskopf T, et al *BMC Evol Biology* 16(1):163 (2016)

Overflow can lead to cross-feeding

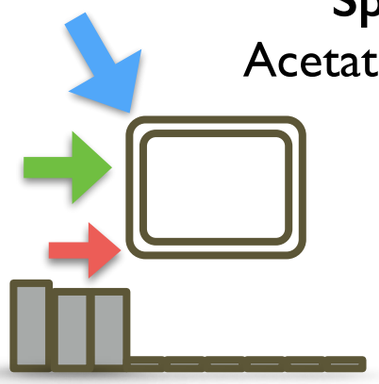
Ecological and evolutionary dynamics of coexisting lineages during a long-term experiment with *Escherichia coli*

Mickaël Le Gac[a,b], Jessica Plucain[a,b], Thomas Hindré[a,b], Richard E. Lenski[c,d,1], and Dominique Schneider[a,b,1]



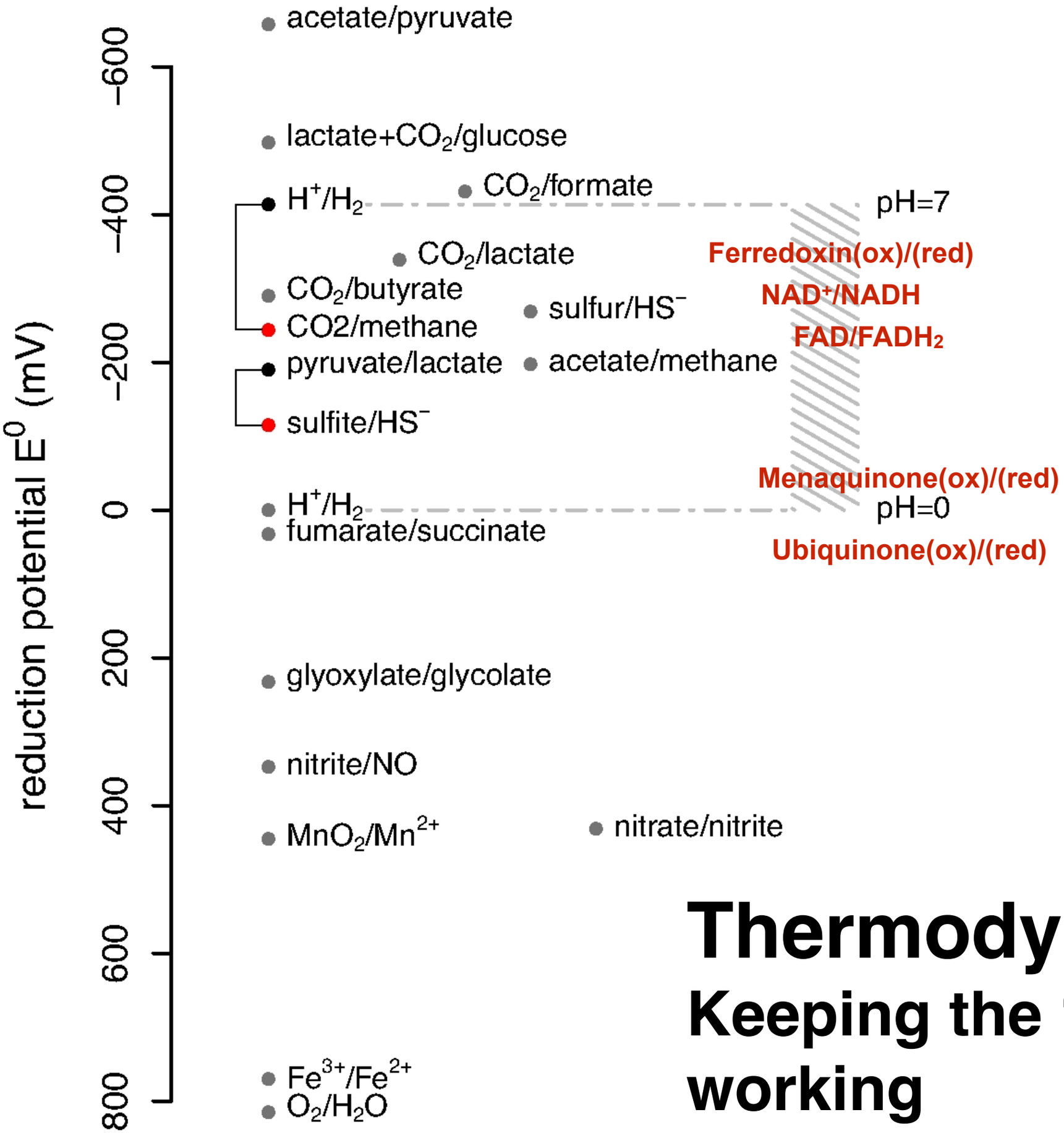


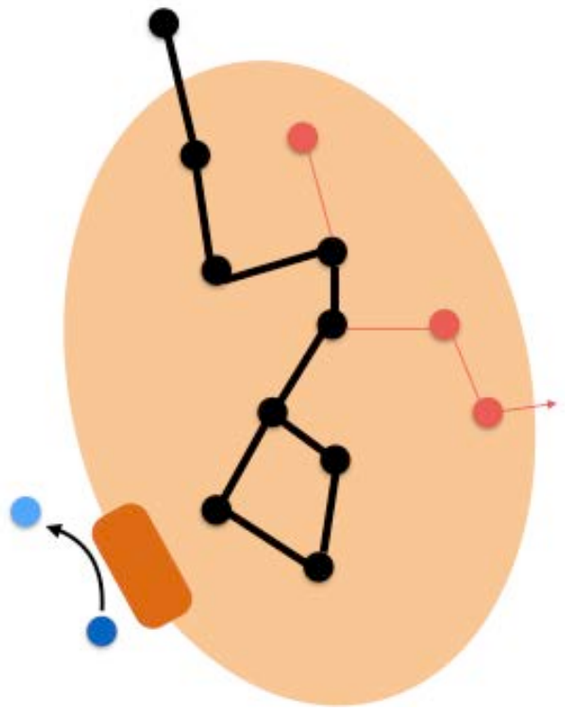
Species A
Glucose specialist



Species B
Acetate "specialist"

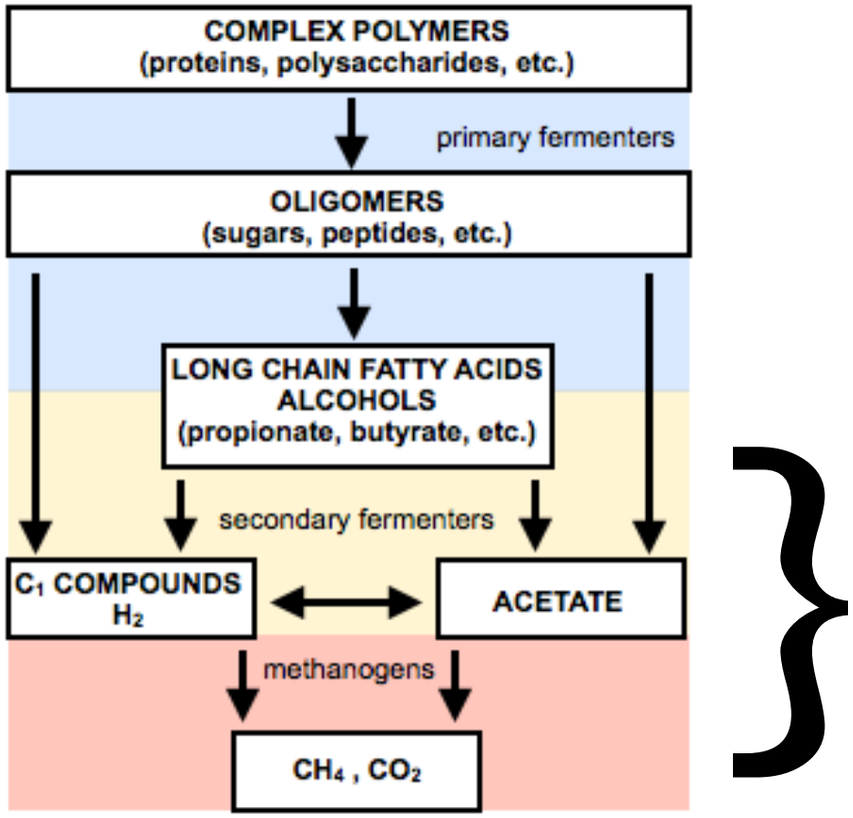
Grosskopf T, et al *BMC Evol Biology* 16(1):163 (2016)



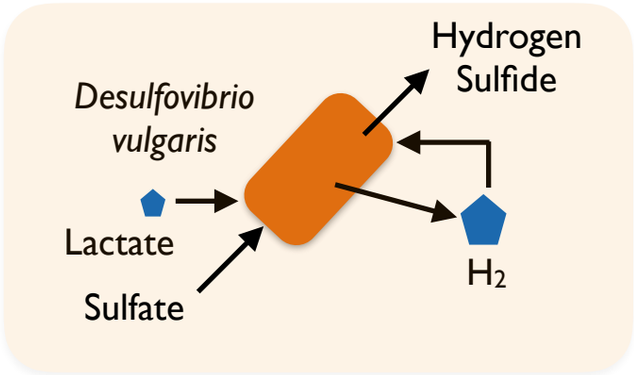


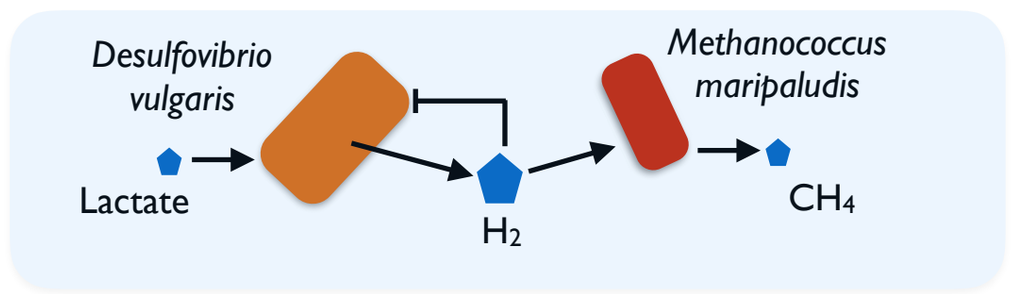
Thermodynamic limits
Keeping the ‘redox ladder’ working

Overcoming respiratory & thermodynamics limits with syntrophy



Schink B *Microbiol Mol Biol Rev* 61:2 (1997)





$$Lactate + 2Sulfate + 3H^+ \longrightarrow 3CO_2 + 2HS^- + 3H_2O \qquad \Delta G_0 = -259.09 \text{ kJ/mol}$$

$$Lactate + H_2O \longrightarrow Acetate + CO_2 + 2H_2 \qquad \Delta G_0 = -8.79 \text{ kJ/mol}$$

$$Lactate + H_2O \longrightarrow Acetate + 0.5CO_2 + 0.5CH_4 \qquad \Delta G_0 = -74.19 \text{ kJ/mol}$$

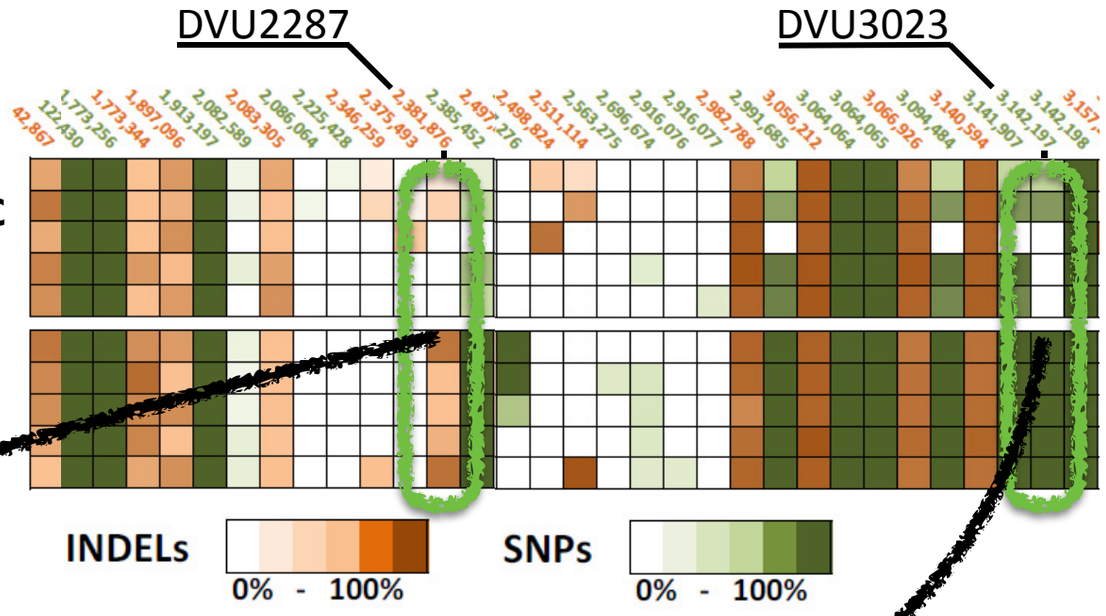
Syntrophy formation requires energy investment to overcome thermodynamic bottlenecks

$$\text{Lactate} + H_2O \longrightarrow \text{Acetate} + CO_2 + 2H_2 \qquad \Delta G_0 = -8.79 \text{ kJ/mol}$$

?

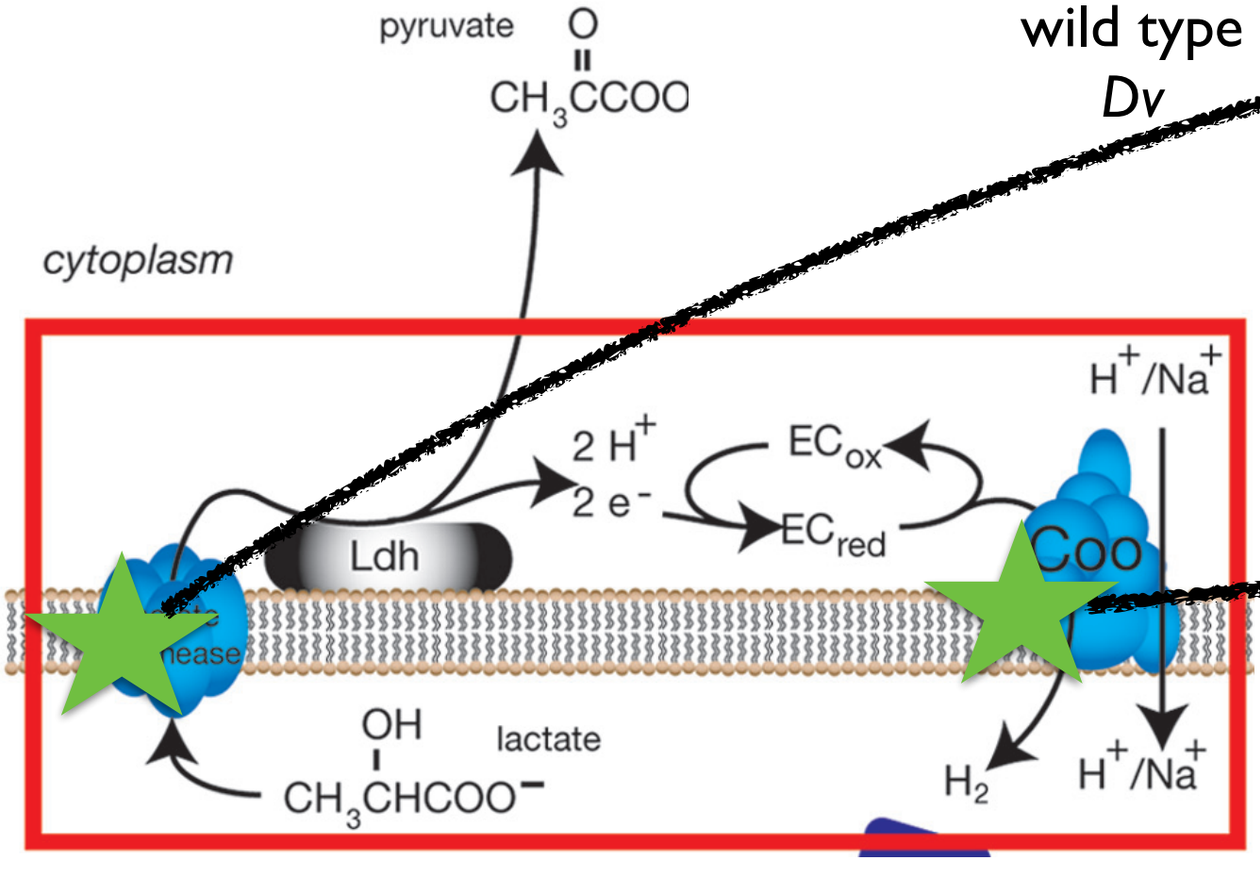
$$\text{Lactate} \longrightarrow \text{Pyruvate} + H_2$$

$$Na^+ \text{ (out)} \longrightarrow Na^+\text{(in)}$$



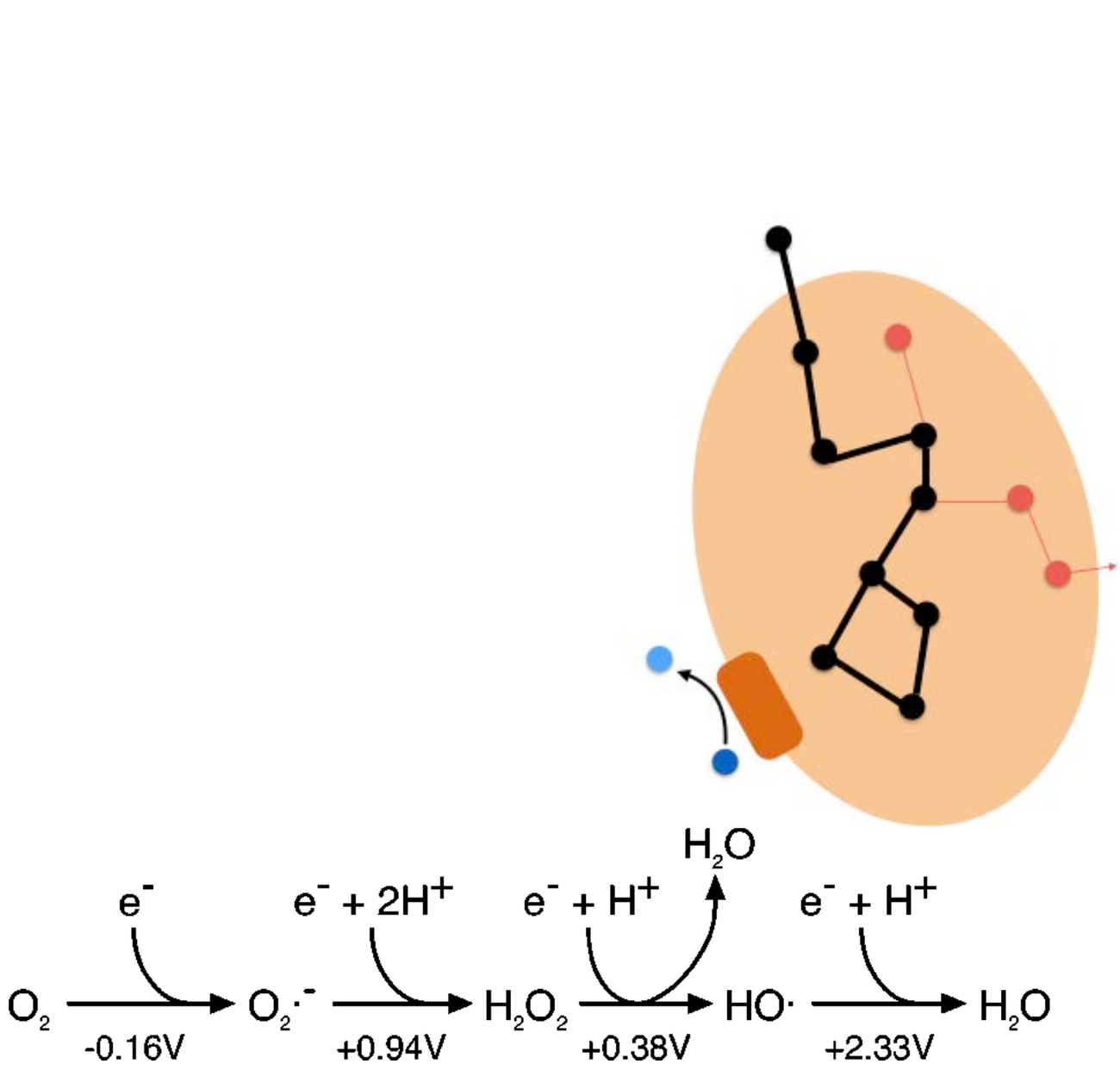
syntrophic *Dv*

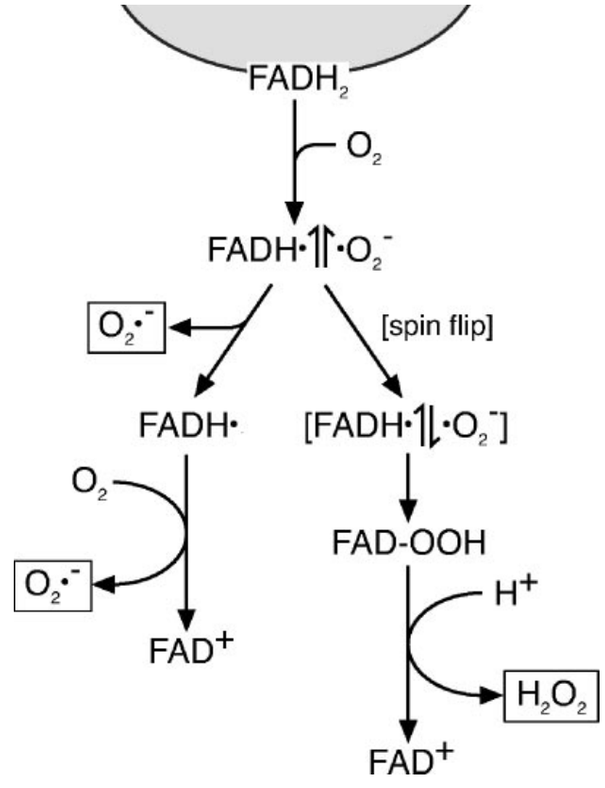
wild type *Dv*



Grosskopf T. et al. ***ISME J.*** (2016).

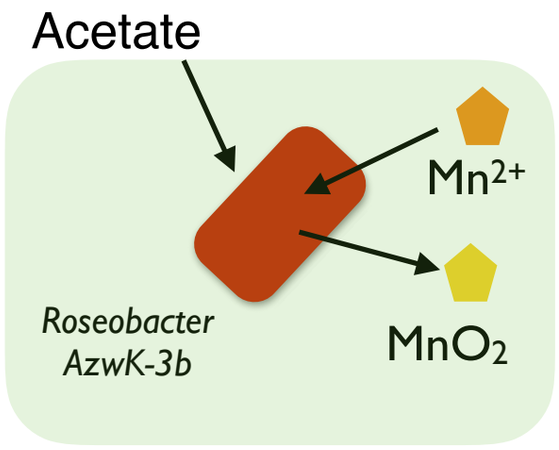
Limits due to toxic byproducts of redox chemistry





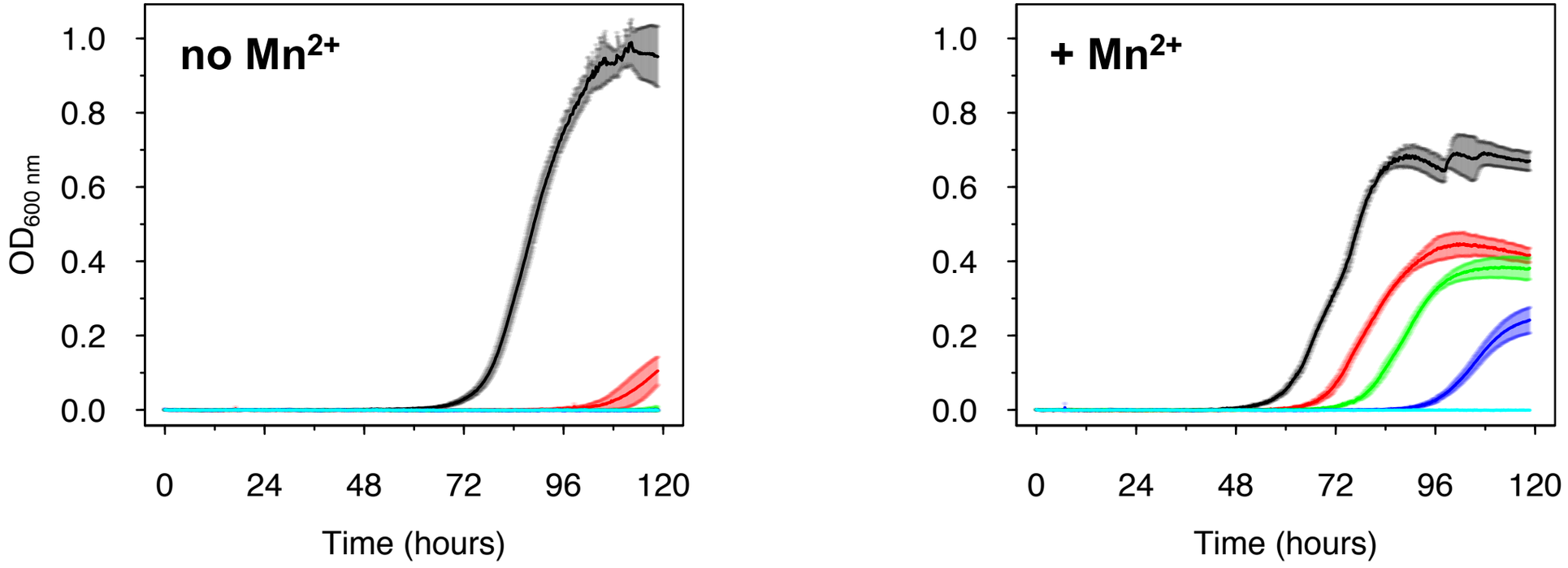
Imlay J, *An. Rev. Of Microbiol.* (2003)

Overcoming toxic effects of respiration with metal oxidation



An ocean-dwelling bacterium that displays strong Mn oxidation. Hansel et al *ASM* 72:5 (2006)

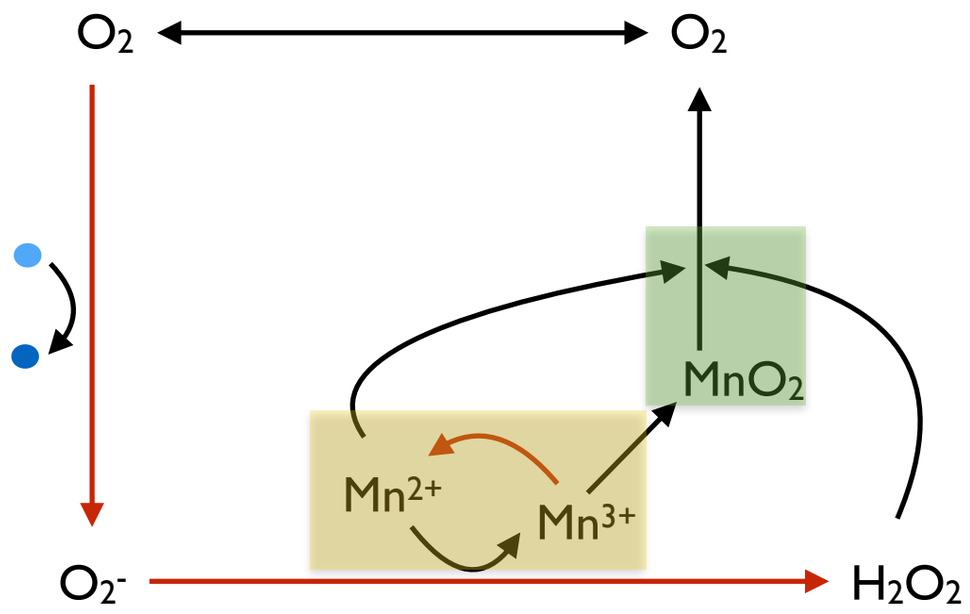
MnO_x revealed as mitigator of nitrite toxicity

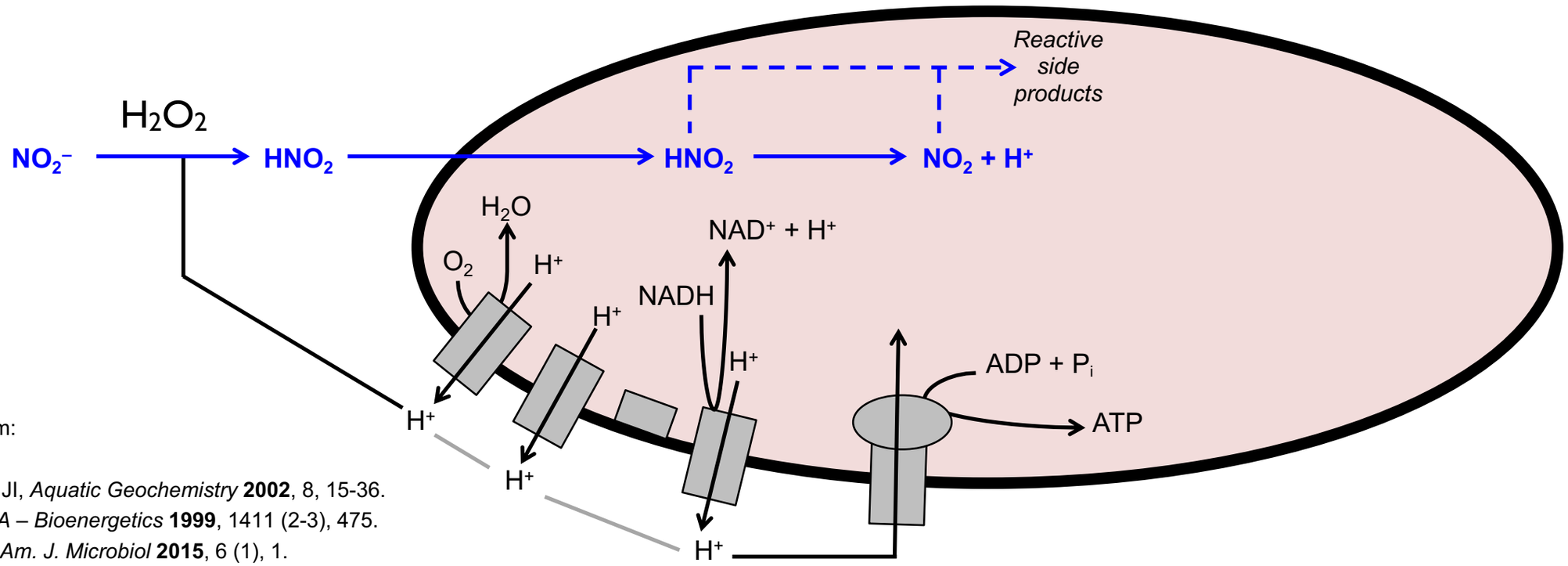


Zerfass et al
https://www.biorxiv.org/content/early/2018/04/04/294975

Biogenic MnOx as a strong redox agent

MnO_x mediated H_2O_2 scavenging





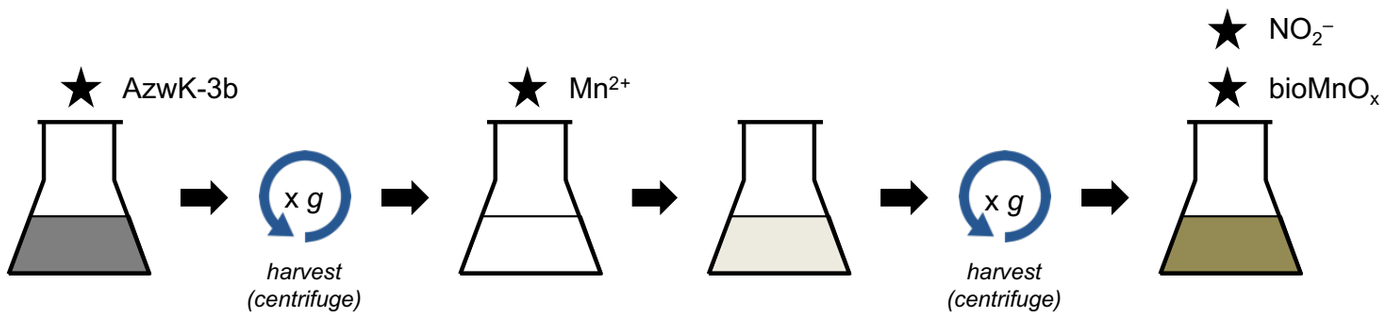
With information from:

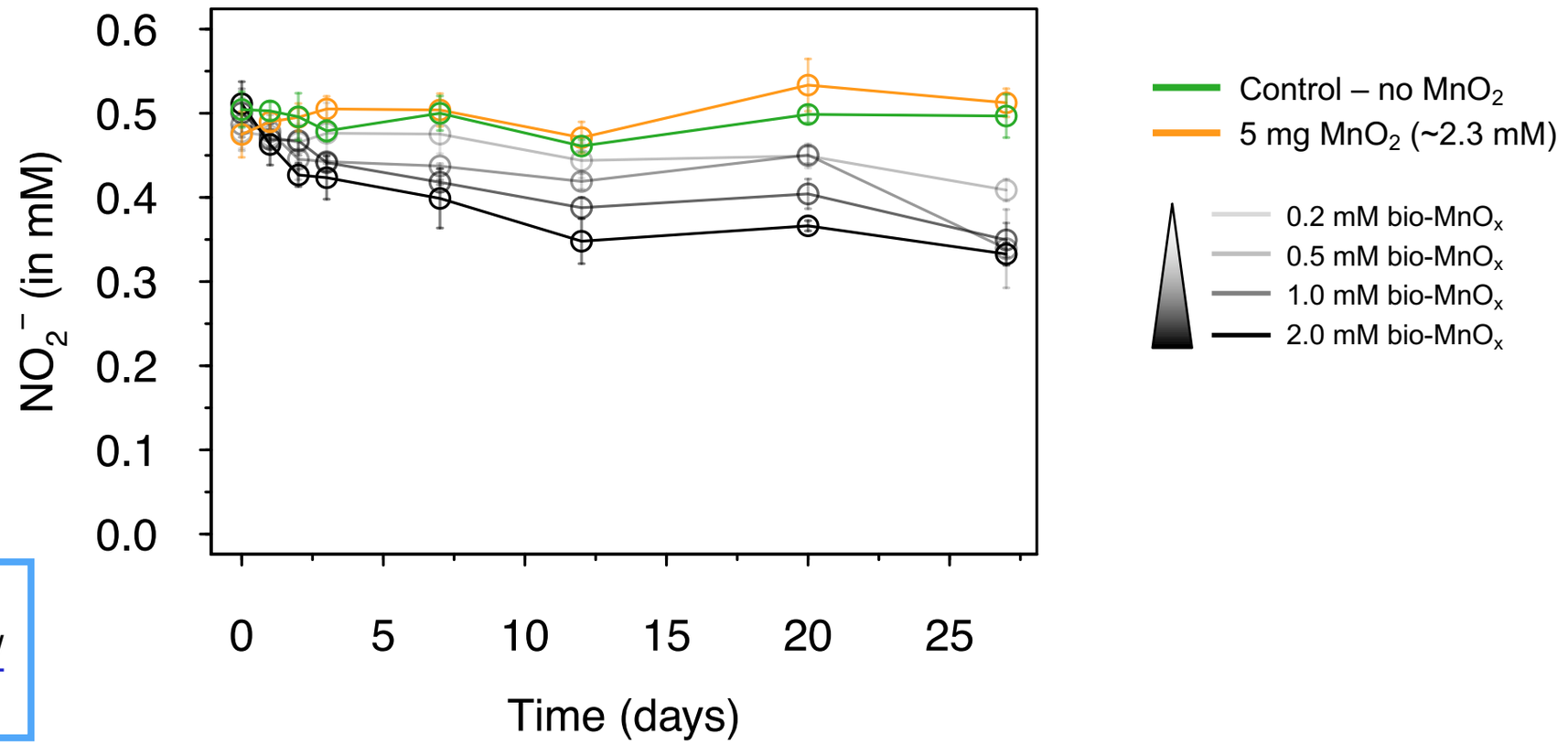
Luther III GW, Popp JI, *Aquatic Geochemistry* **2002**, 8, 15-36.
Cammack et al., *BBA – Bioenergetics* **1999**, 1411 (2-3), 475.
Müller-Herbst et al., *Am. J. Microbiol* **2015**, 6 (1), 1.
Michal G., Schomburg D., *Biochemical Pathways: An Atlas of Biochemistry and Molecular Biology*. 2nd edition, Wiley **2012**.

Biogenic MnOx as a strong redox agent

MnO_x mediated nitrite (NO_{2-}) scavenging

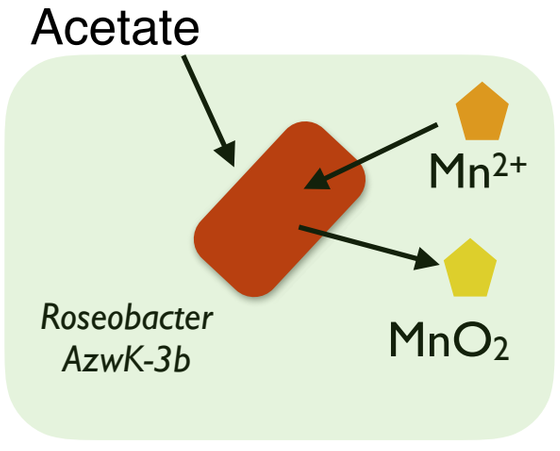
$$NO_2^- + MnO_2 + 2\,H^+ \rightarrow Mn^{2+} + NO_3^- + H_2O$$

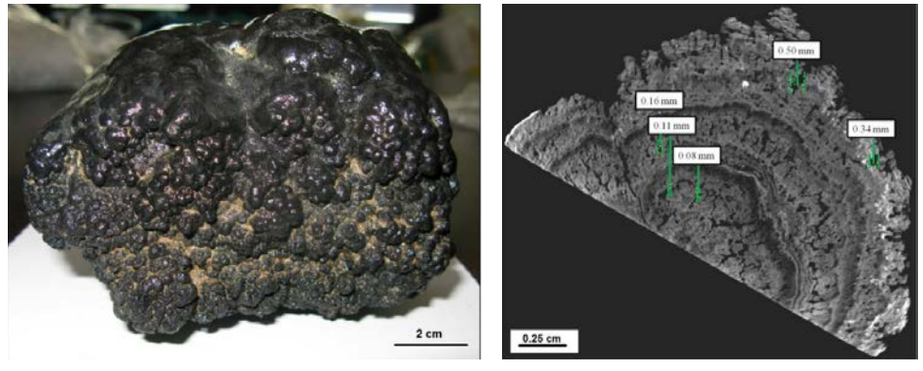




Zerfass et al
https://www.biorxiv.org/content/early/2018/04/04/294975

Biogenic MnOx as a strong redox agent

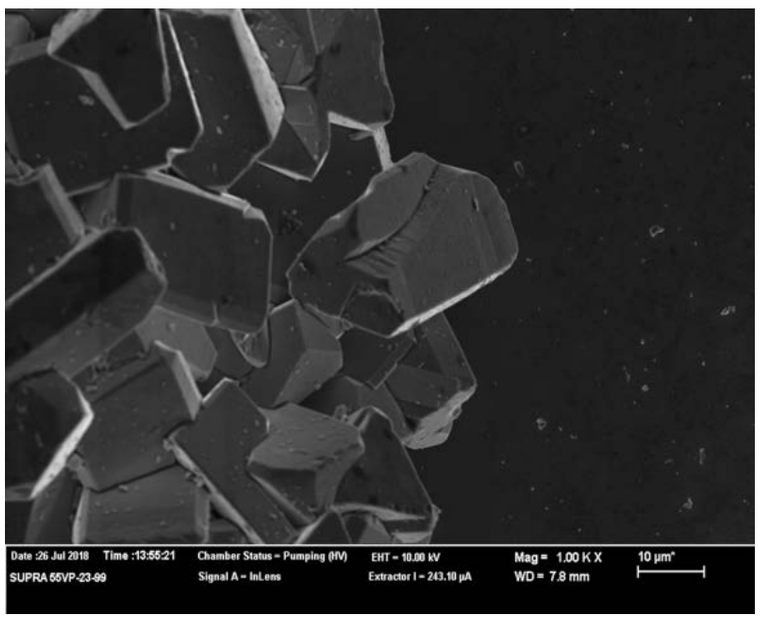


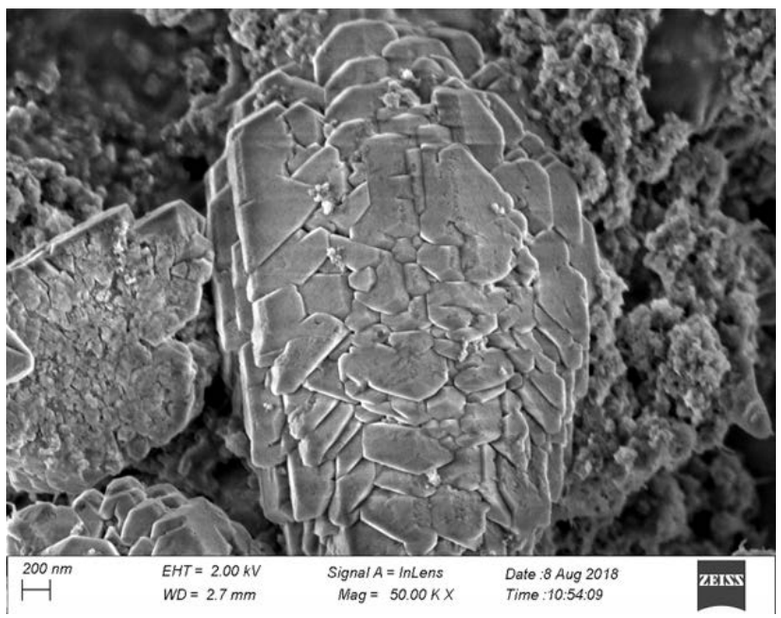


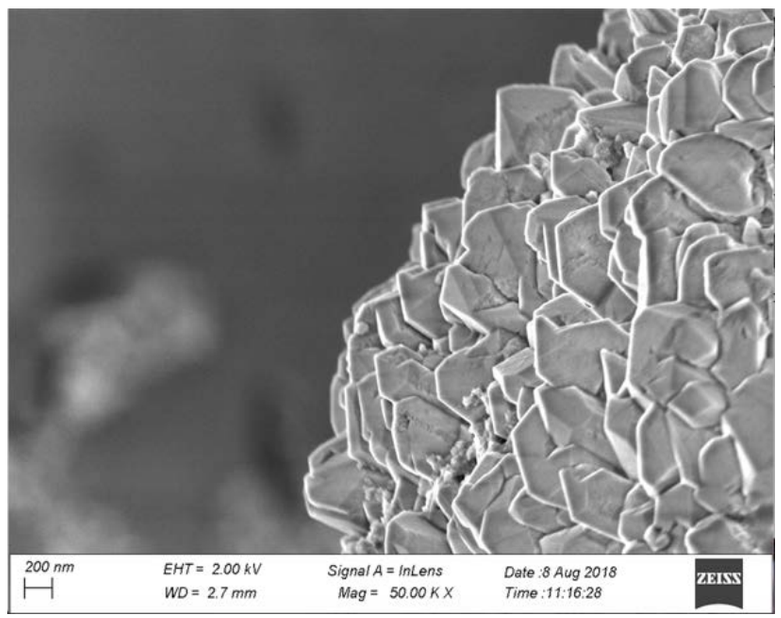
Polymetallic (Mn-rich) nodules of biogenic origin.

Blöthe et al., *Environ. Sci. Technol.* **2015,** 49, 7692.

MnO_x is a strong oxidizer that is used in batteries among other applications. Synthetic MnOx has low reactivity when 'aged' and readily dissolves upon oxidizing action







Kremin C and Zerfass C, Unpublished results

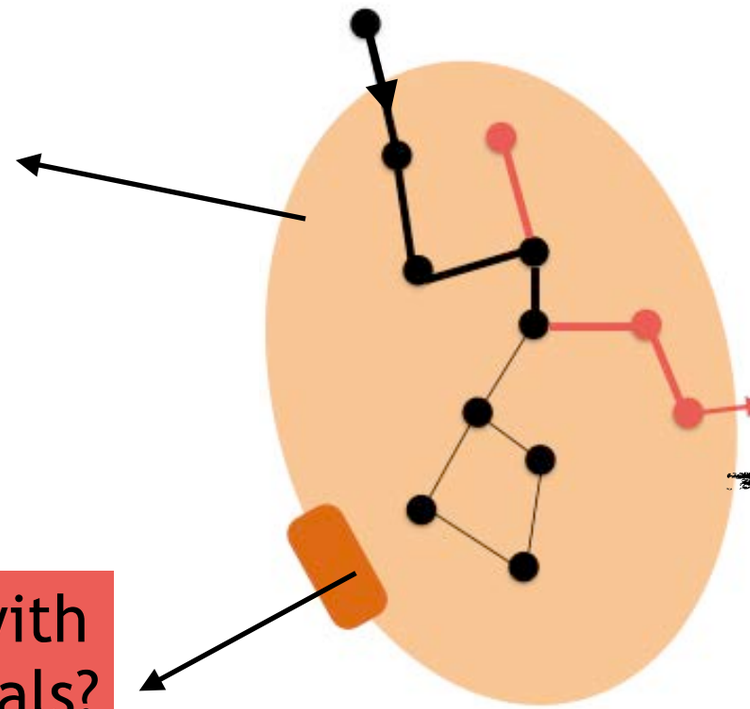
Metabolism as an electron flow system

Keeping up the electron flow

Electron-carrying compounds?

Electron-dense storage compounds?

Electron rich substrates



Diverse ‘overflow’ pathways

Organic acids, H_2, …

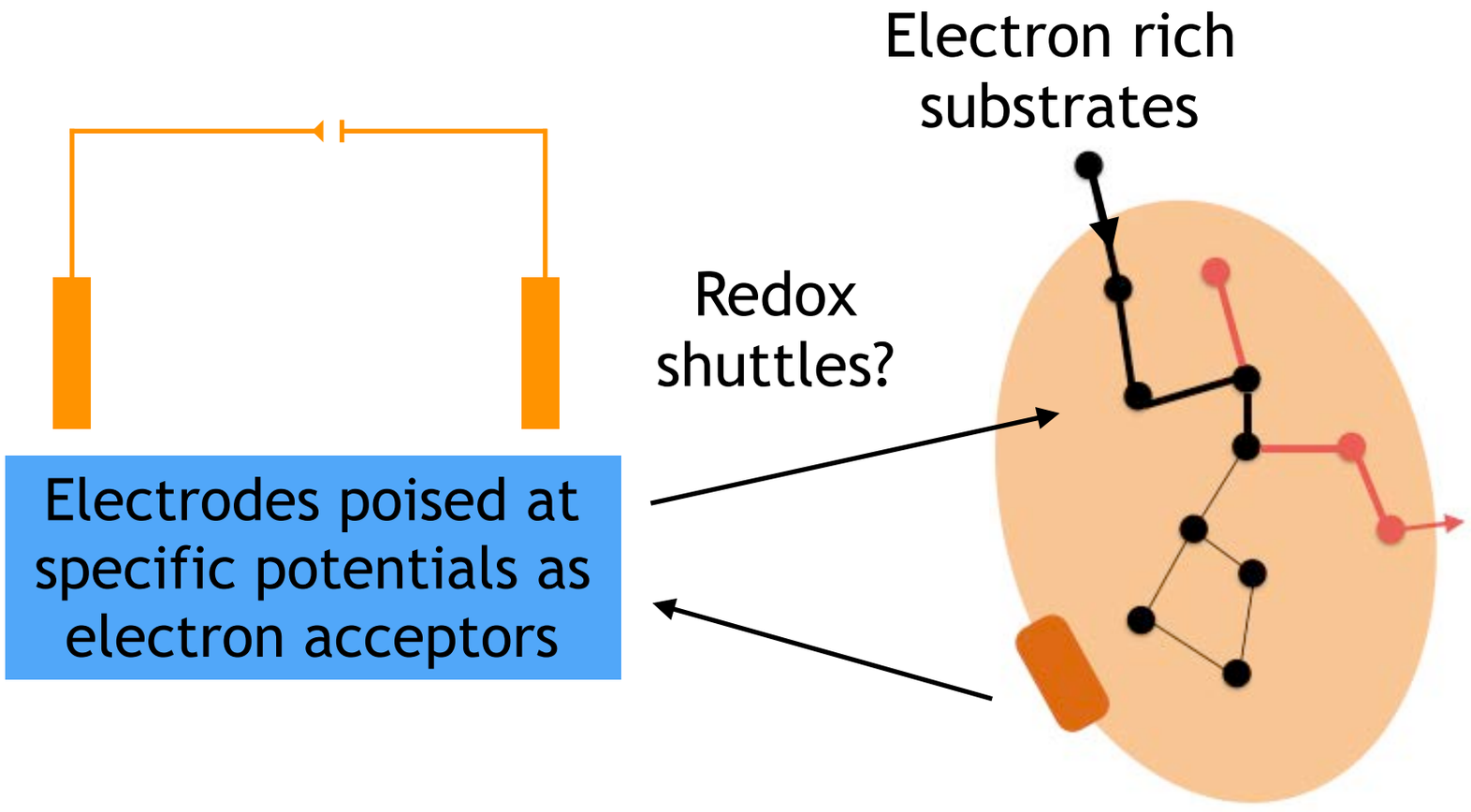
Interactions with transition metals?

Direct electron exchanges with other cells?

Cross-feeding
Syntrophy

Consequences for engineering and study of metabolism

Controlling/influencing the electron flow with external electrons (electronic metabolism)



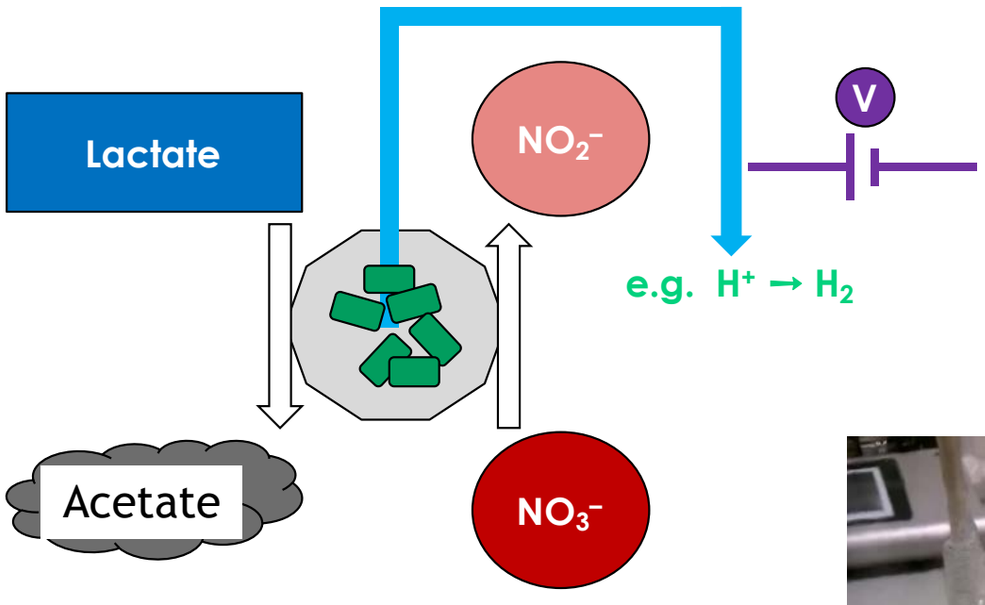
Electromicrobiology

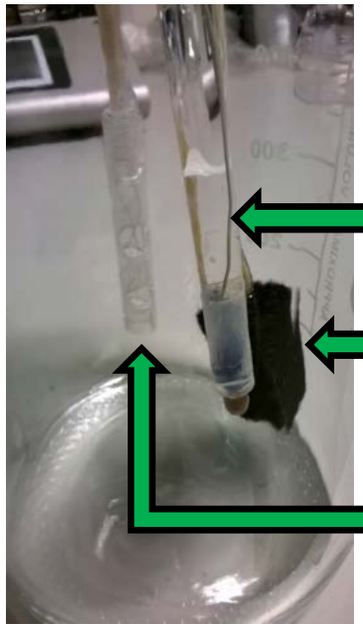
Lovley D, *An. Rev. Of Microbiology* (2012)

Kato S, *Microbes Environ* 30 (2015)

”Electronic control” of cell metabolism

Control of respiration in *Shewenalla oneidensis* using electrodes poised at specific potentials

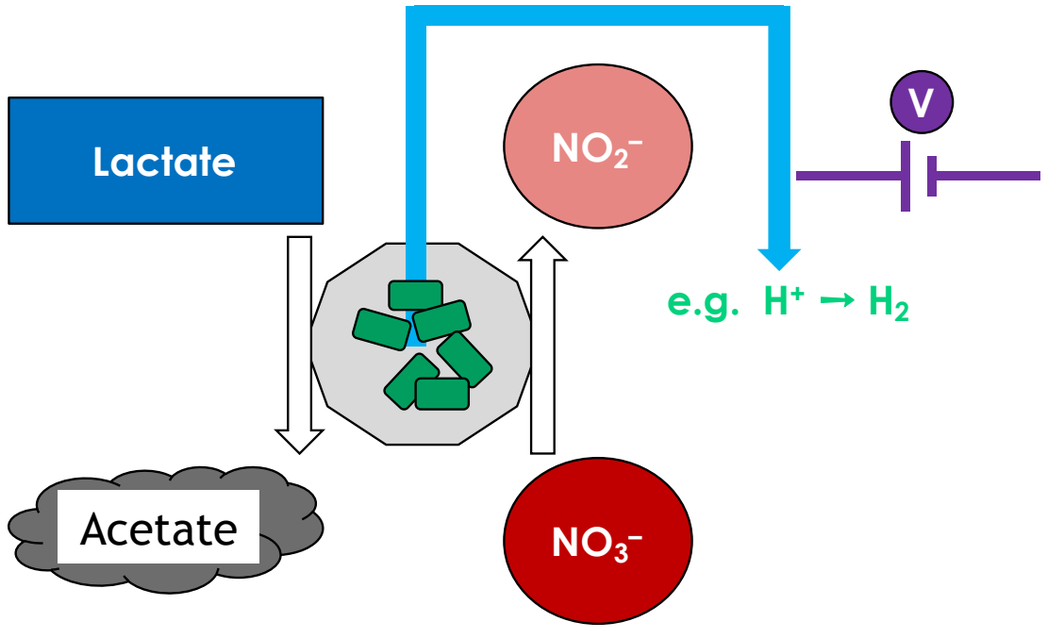




Christian Zerfass

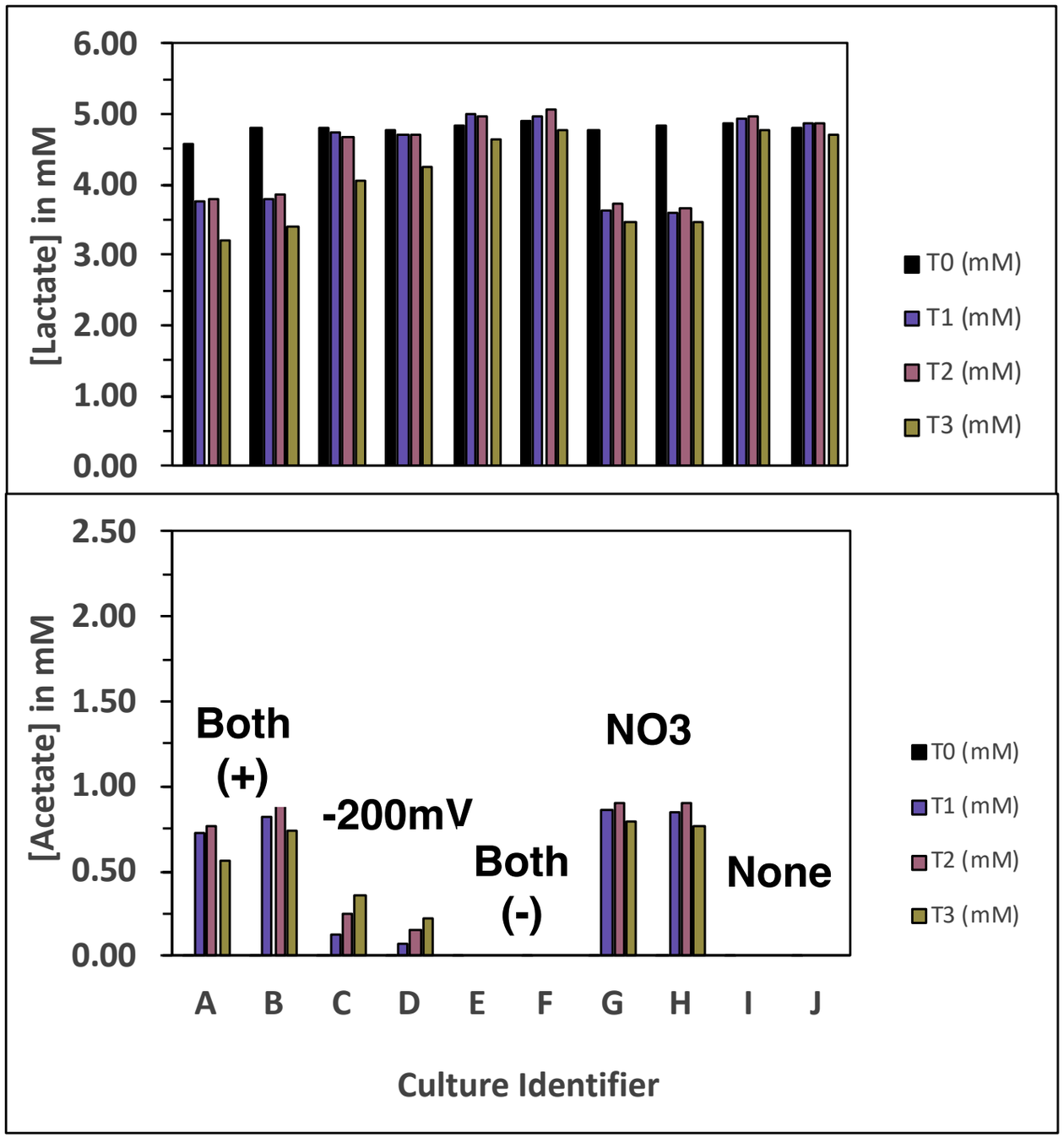
”Electronic control” of cell metabolism

Control of respiration in *Shewenalla oneidensis* using electrodes poised at specific potentials



Christian Zerfass

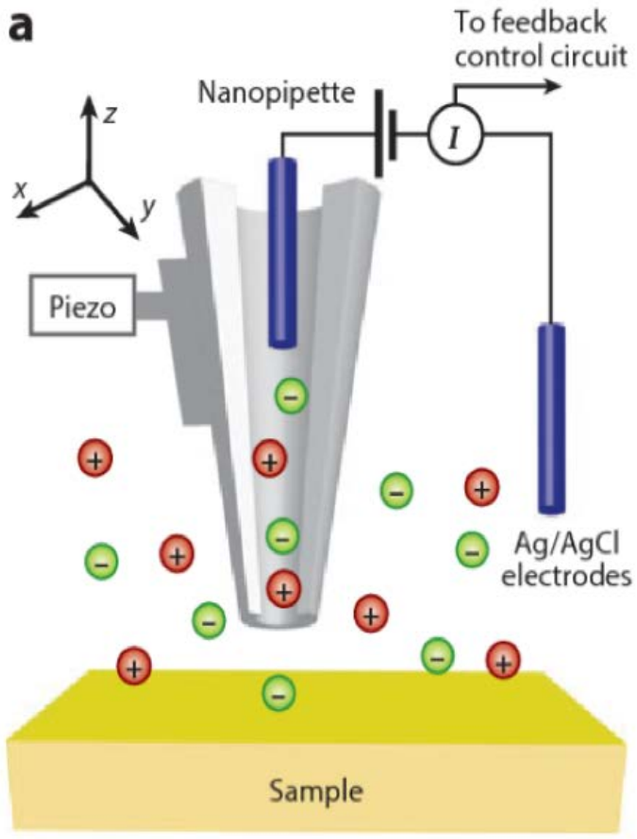
Unpublished data

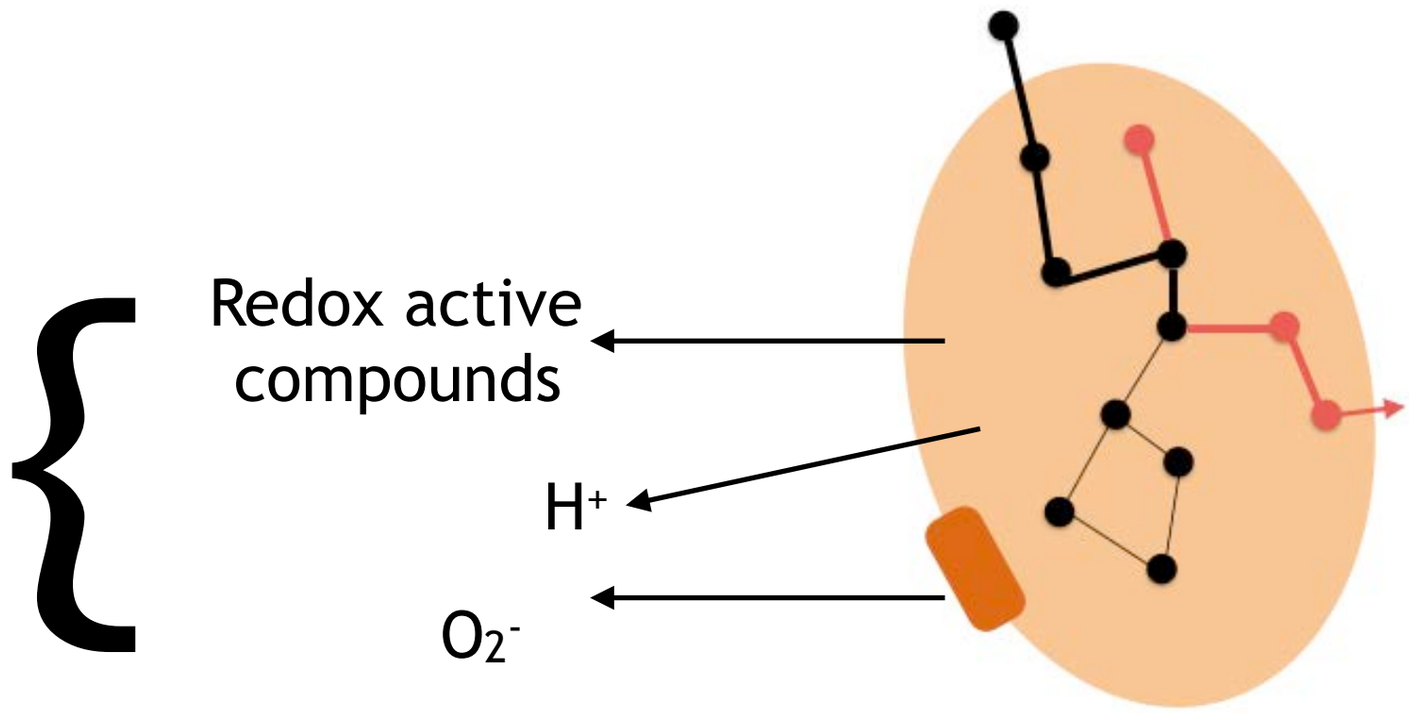


Electrical measurement of metabolism?

Scanning ion conductance measurements (SICM) and scanning electrochemical microscopy (SECM) at single cell level

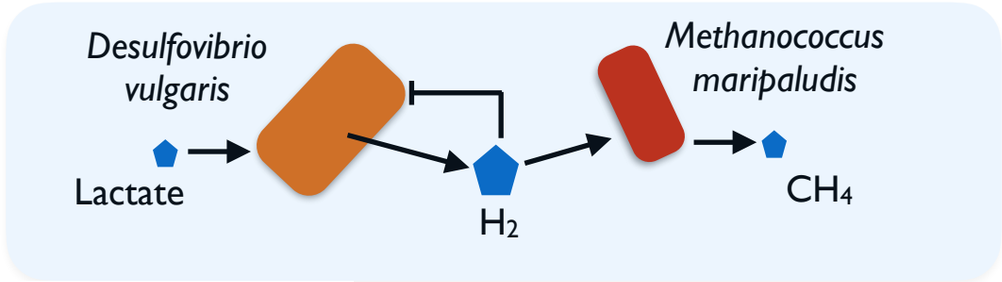
Kelsey Cremin





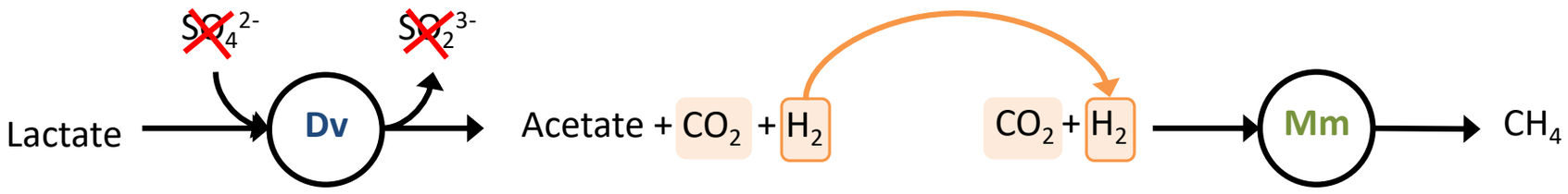
Page A, Perry D, Unwin PR. 2017 *Proc. R. Soc. A* **473**.

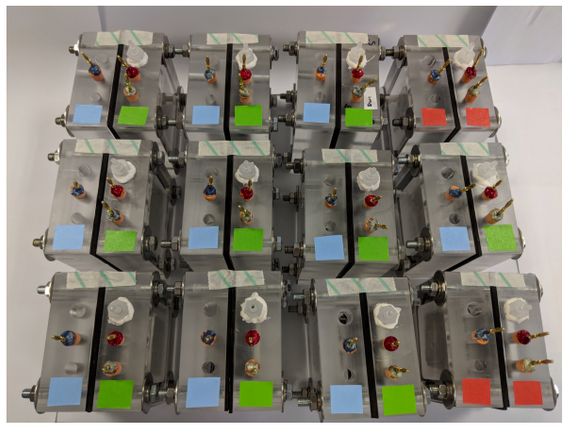
Electrical measurement of metabolism?

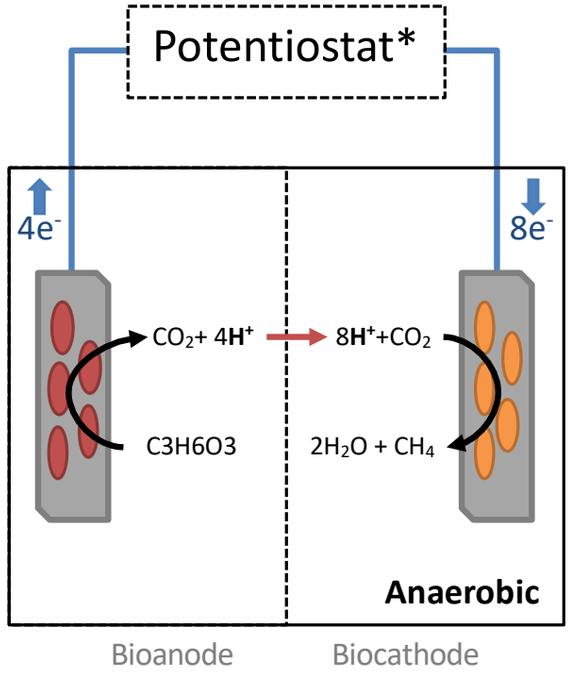


Andrea Martinez-Vernon

Control/study of syntrophy across wires!

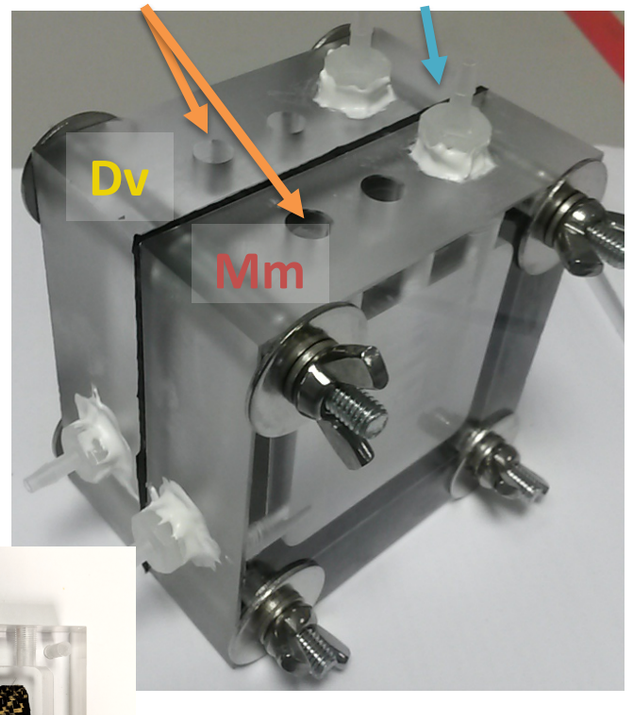




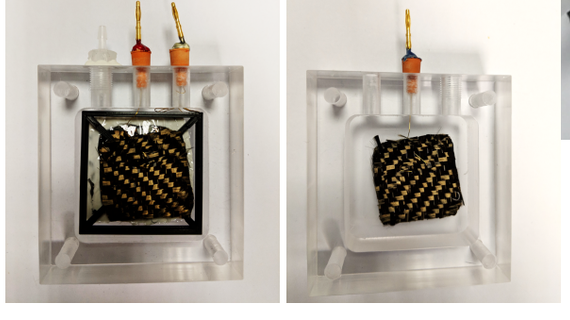


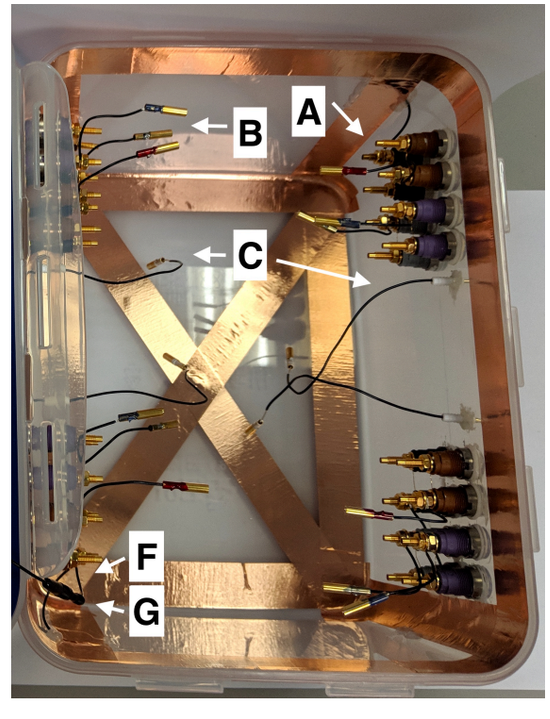
Electrochemical set up
*voltage control

ELECTRODES GAS COLLECTION



Electrochemical cell

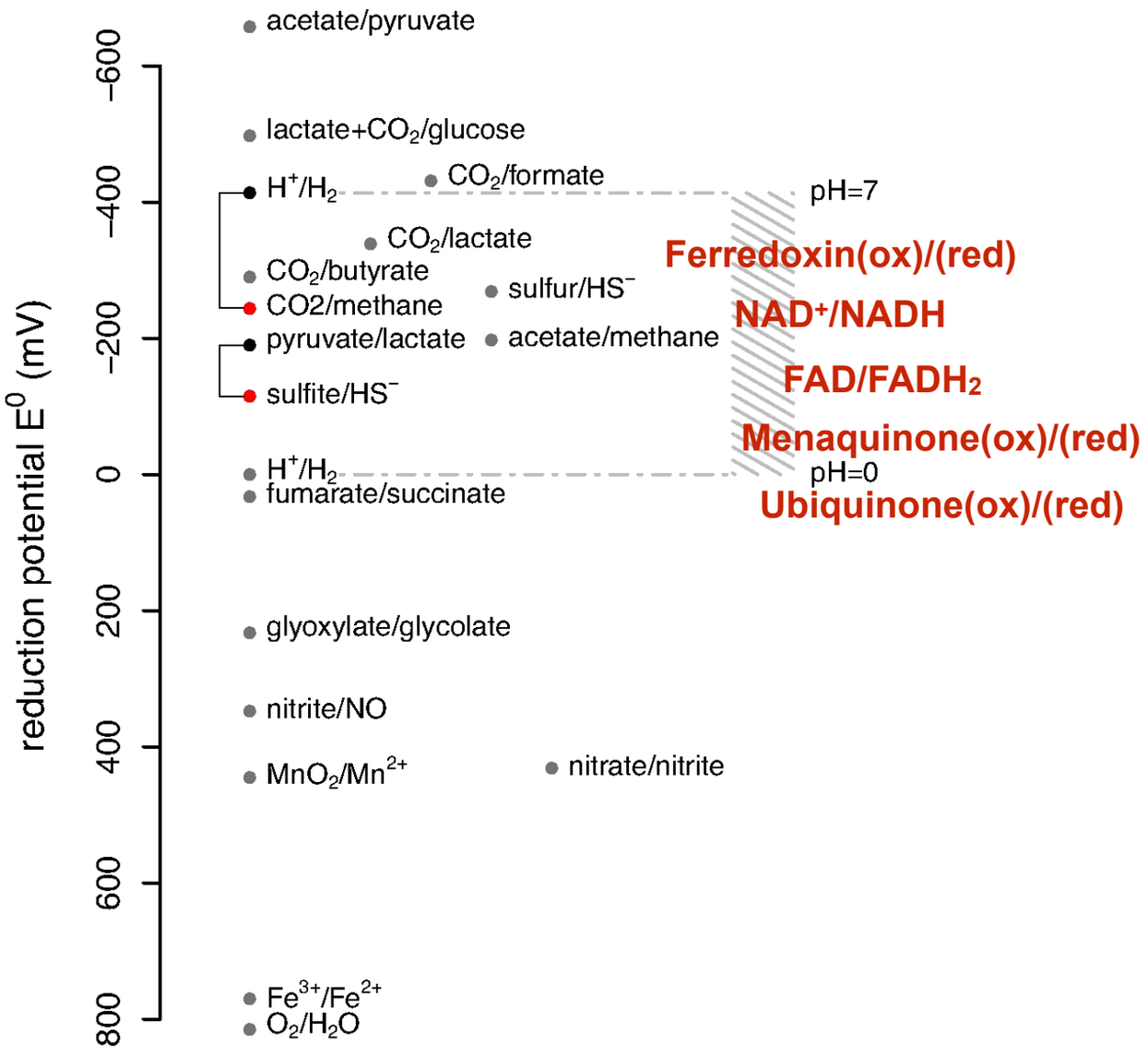


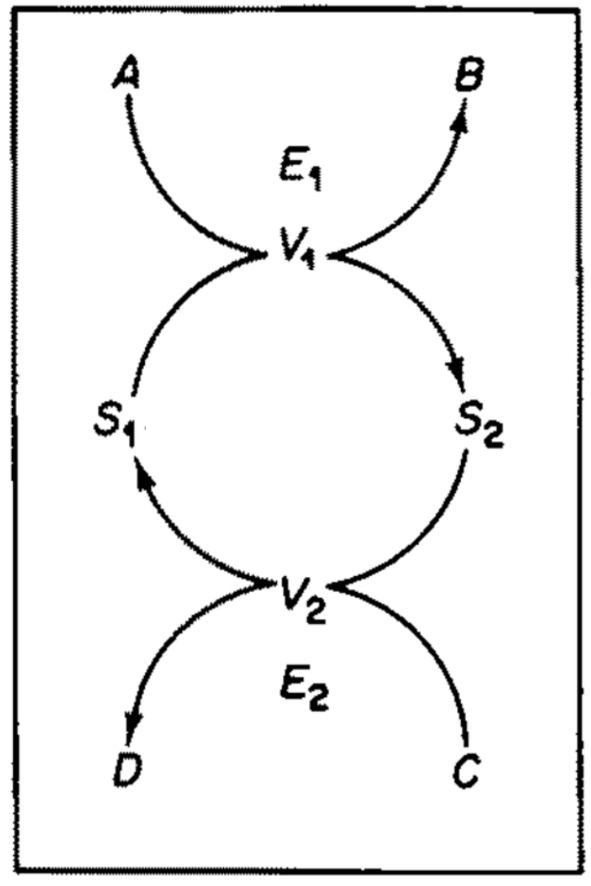


Modelling dynamics of coupled reactions

Coupled cycles, shared substrates/enzymes, and substrate feedbacks in redox systems

Clare Hayes



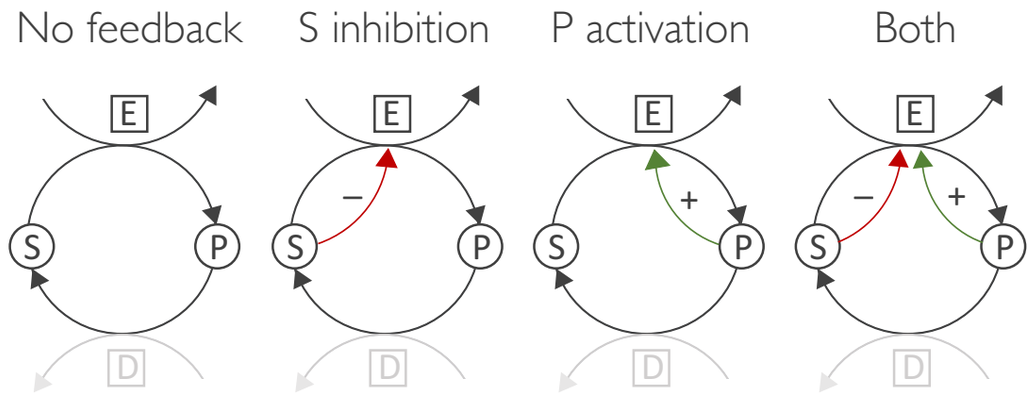


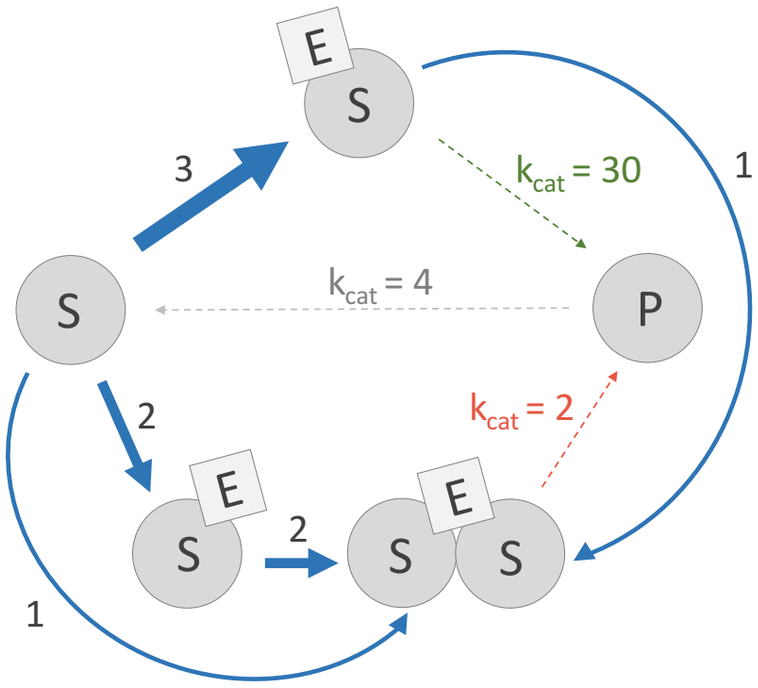
Hervagault & Canu. Journal of Theoretical Biology 127, 439-449 (1987).

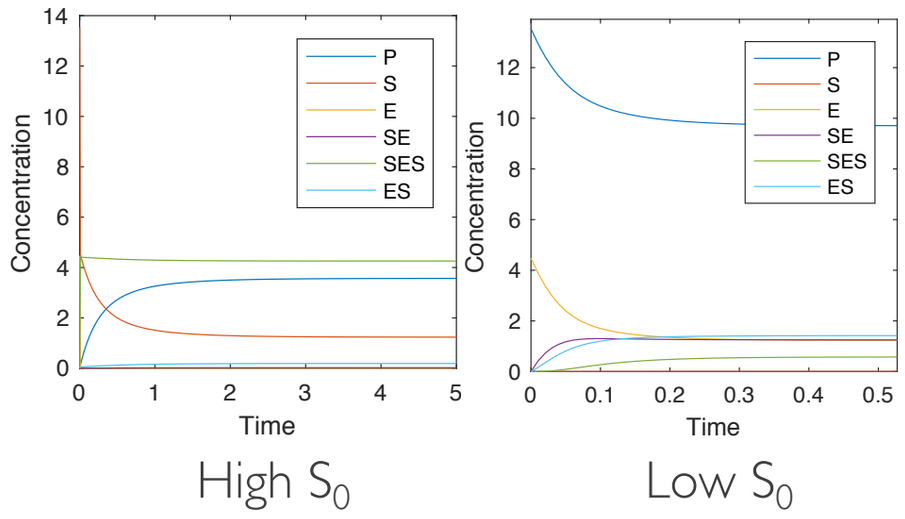
Dynamics of coupled redox reactions

Substrate feedback in a cyclic reaction can generate bistability and oscillation

Clare Hayes







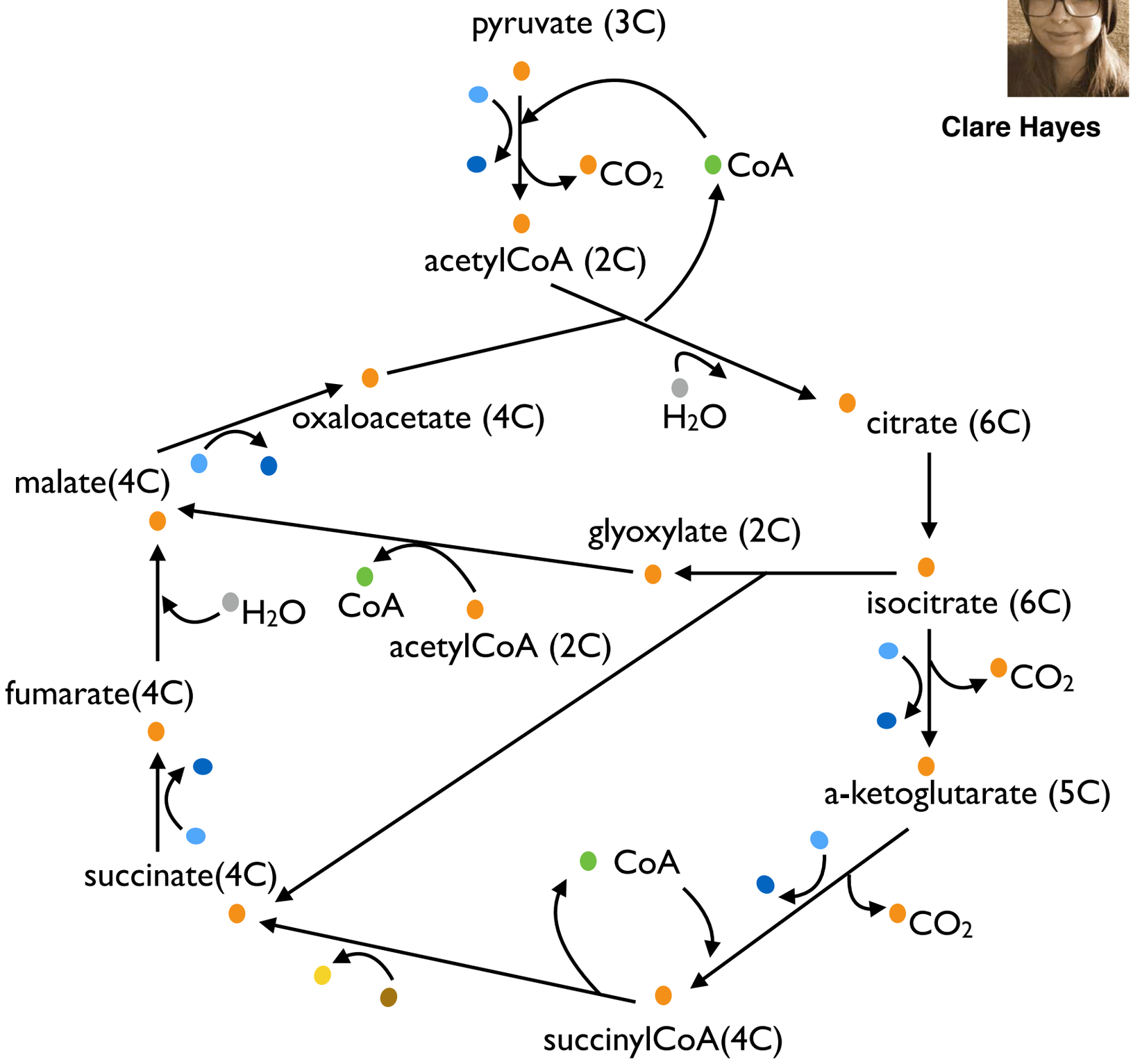
Unpublished results

Condition for bistability: $\frac{k3}{k8+k9} - 1 > K_m \frac{k10}{k11}$ $K_m = \frac{k7+k8}{k6}$

Electrochemically responsive bistable redox systems?

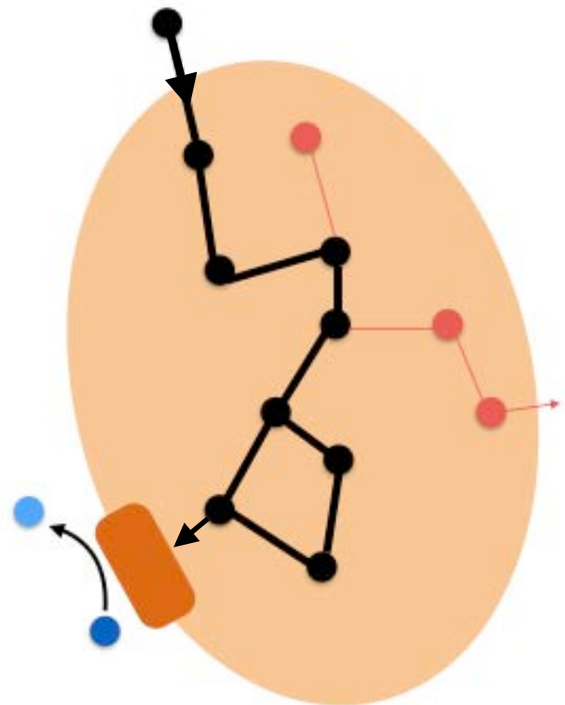
Electrodes poised at specific potentials as electron acceptors

Clare Hayes



PHYSIOLOGY IS ELECTRICITY!

Electron provision



Electron dumping

Metabolism ~ Electron Flow

Cell mechanics ~ Electrostatics

Motility

Division

Multi-cellular interactions

Spatial organisation

…

Bio Electrical Engineering Innovation Hub at Warwick

Innovator Club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Electrochemistry

Electrical imaging

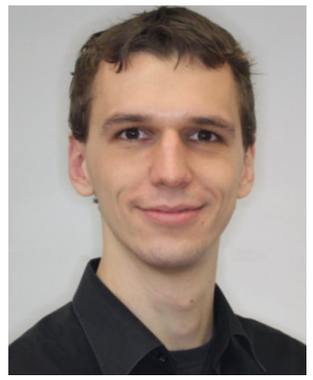

Murray Grant


Stefan Bon

Synthetic biology

Microbial ecology

Metabolic engineering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THANK YOU

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Jing Chen

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Allen Reed

Open PhD and PDRA positions

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BBSRC bioscience for the future

EPSRC Pioneering research and skills

CANCER RESEARCH UK PIONEER AWARD

WARWICK THE UNIVERSITY OF WARWICK

Collaborators

Munehiro Asally, Marco Polin, Pat Unwin, Chris Quince, Joseph Christie-Oleza, Patrick Schaefer (University of Warwick)
Angus Buckling (University of Exeter)
Dominique Schneider (University of Grenoble)

Initiatives

Bee

Bio Electrical Engineering Innovation Hub @ Warwick

WARWICK CENTRE FOR INTEGRATIVE SYNTHETIC BIOLOGY

EPSRC & BBSRC Centre for Doctoral Training in Synthetic Biology

CDT SynBio