An inside-out mechanism initiates apicobasal polarity in a single epithelial cell

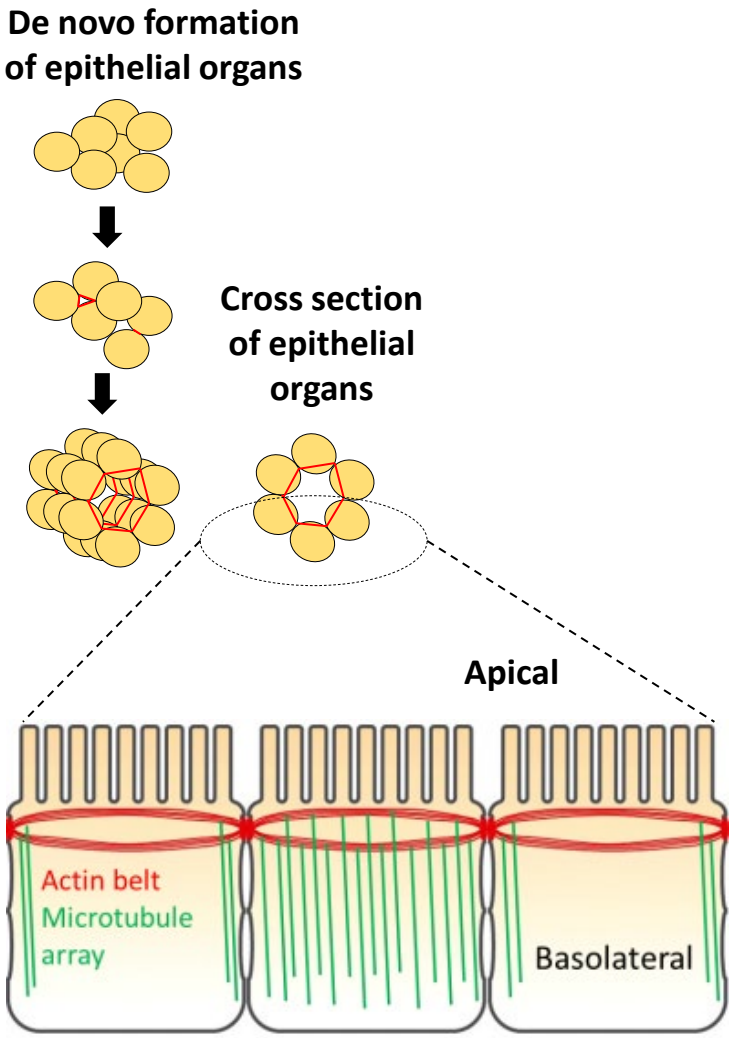
Chin-Lin Guo, MD PhD

Acknowledgement: Eddie Yang[1], Chiao-Yu Tseng[1], Yi-Wen Hung[1], Cherry Huang [1], S-Ting Lin[1], Ren-Yu Hu[1], Bi-Chang Chen[2], and Yi-Kuo Yu[3]

Fund Support: Academia Sinica, National Science & Technology Council, NIH

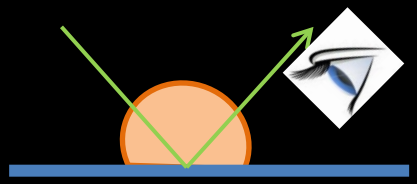
[1]Institute of Physics, Academia Sinica
[2]Research Center of Applied Sciences, Academia Sinica
[3]NIH/NCBI/NLM

The classical outside-in mechanism for collective apicobasal polarity formation in the epithelium

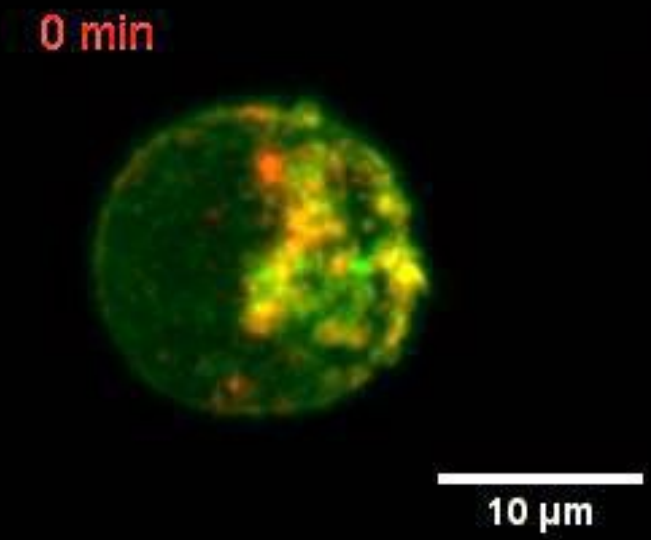


Formation of an actin belt-mimetic band-like structure in a single epithelial cell

XY View

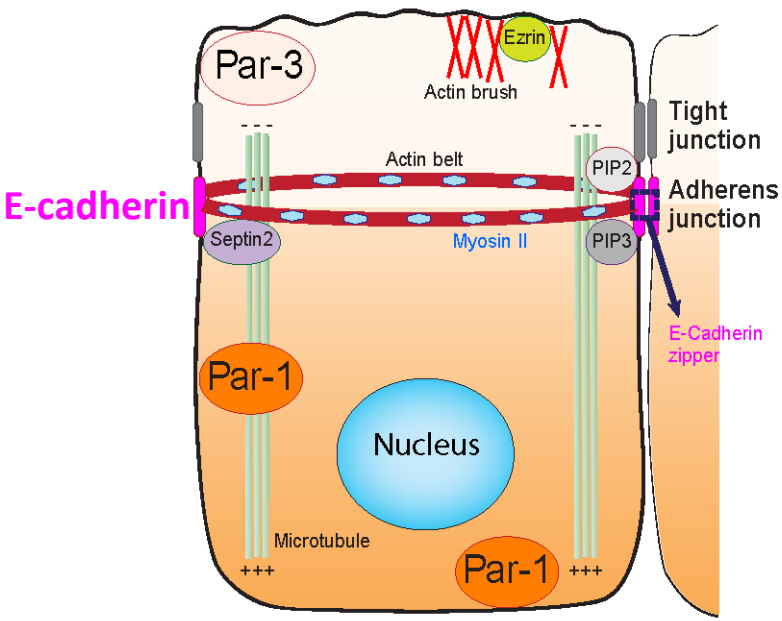


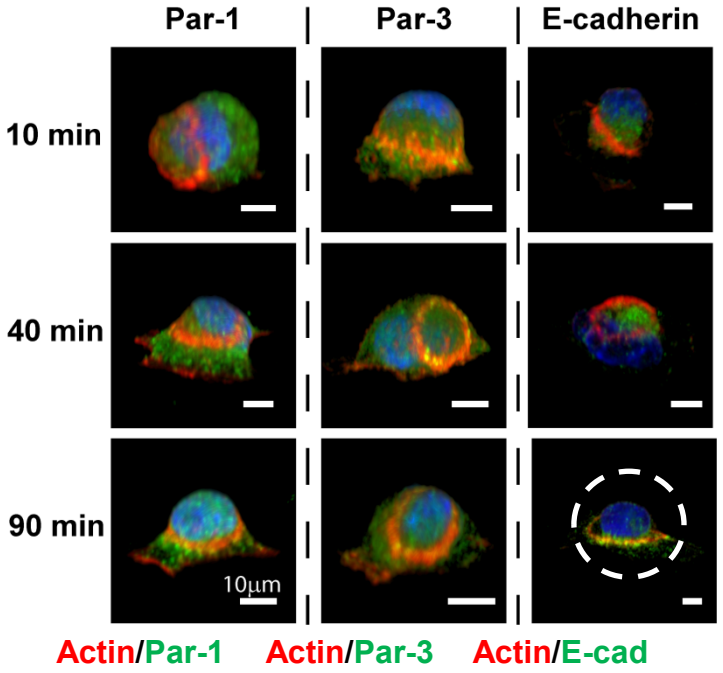
62.36±10.84% at 40mins

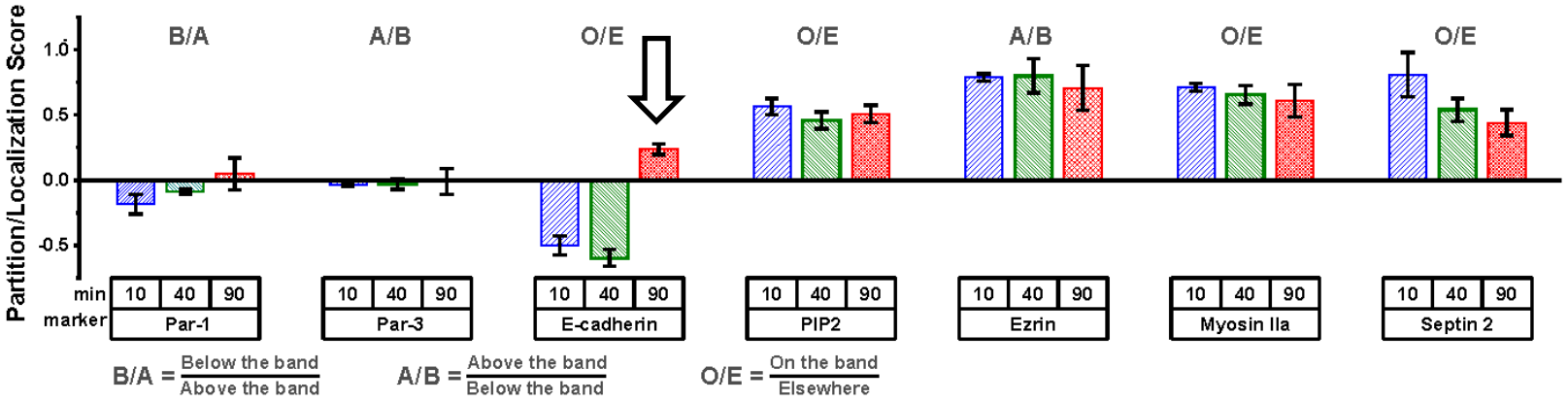


Actin/PIP_2

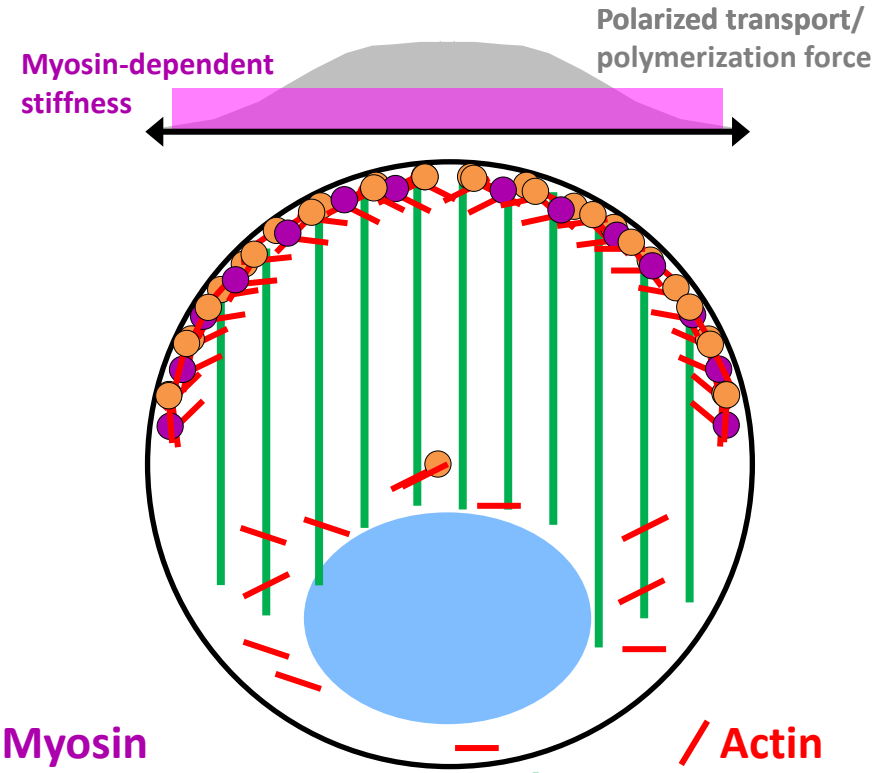
Pre-partitioning of Par complexes is not involved in the actin band formation

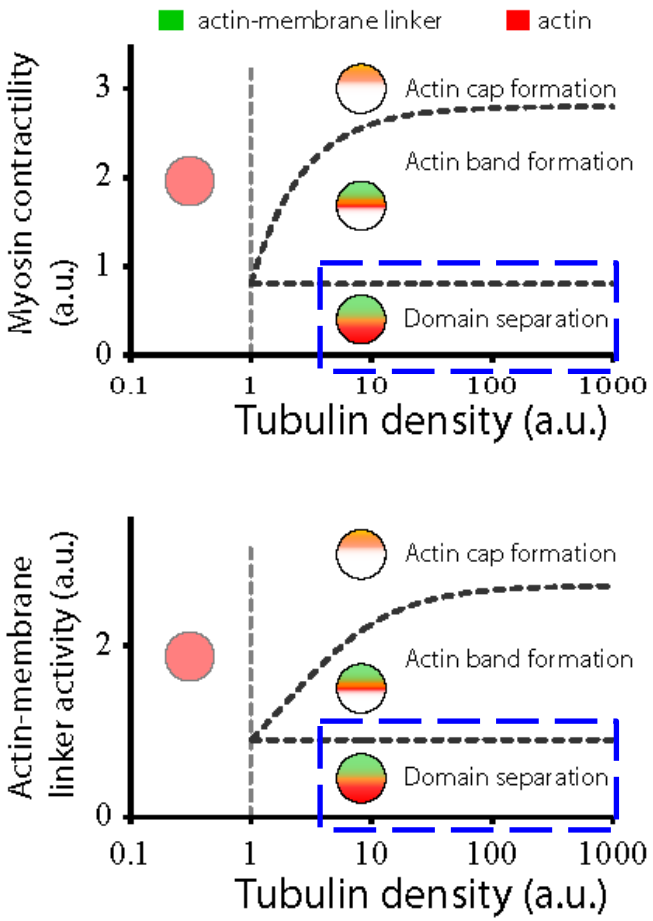






A hypothetical inside-out mechanism



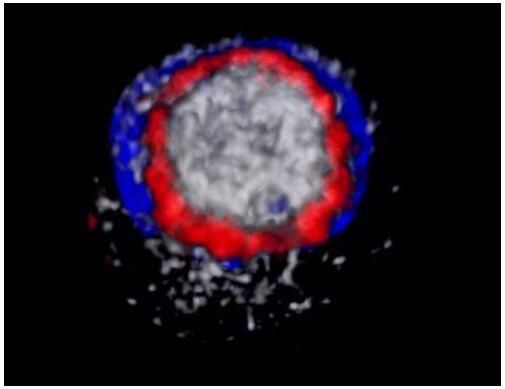


Hamant et al., Nat Comm 10, 2360 (2019)
Mirabet et al., PLoS Comp Biol 14, e1006011 (2018)
Kuo and Howard, Trends in Cell Biol 31, 50 (2021)
K. Kimura et al., Nat Cell Biol 19, 399 (2017)
Mani et al., Elife 10 (2021)
Farias et al.,Neuron 102, 184 (2019)
Mukherjee et al., Elife 9 (2020)
Huang et al. Nature 397, 267 (1999)
Saltini & Mulder, R Soc Open Sci 7, 201730 (2020)
Alkemade et al., Proc Natl Acad Sci U S A 119, e2112799119 (2022)

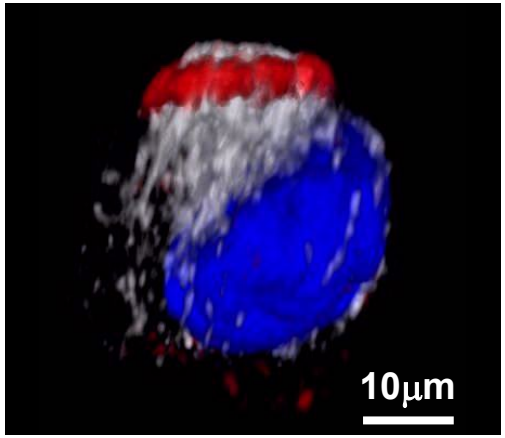
Kojima et al., Proc Natl Acad Sci U S A 91, 12962 (1994)
Inoue et al., EMBO J 38 (2019)
Eddy et al., Mol Biol Cell 13, 4470 (2002)
Dogterom & Koenderink, Nat Rev Mol Cell Biol 20, 38 (2019)
Zenker et al., Cell 173, 776 (2018)

Polarization of microtubule networks precedes the formation of actin band

Top view



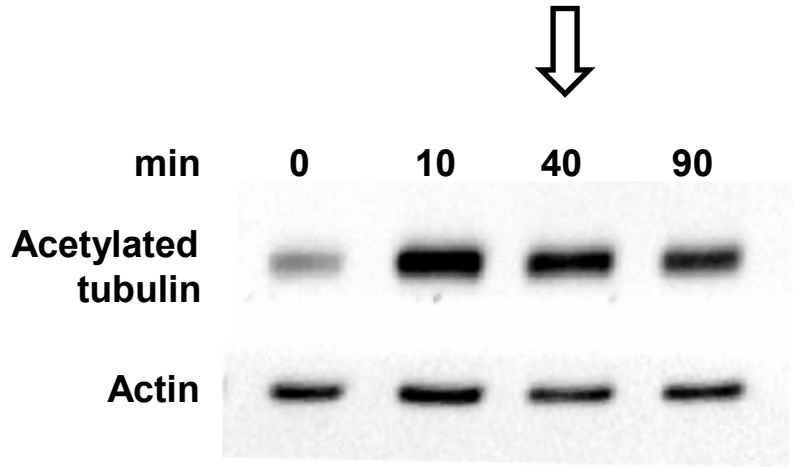
Lateral view

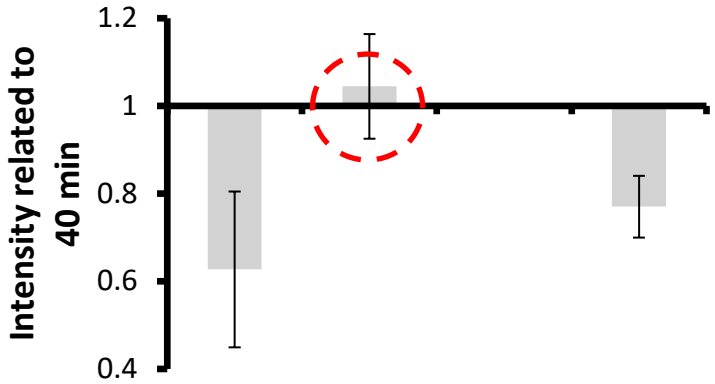


Acetylated tubulin

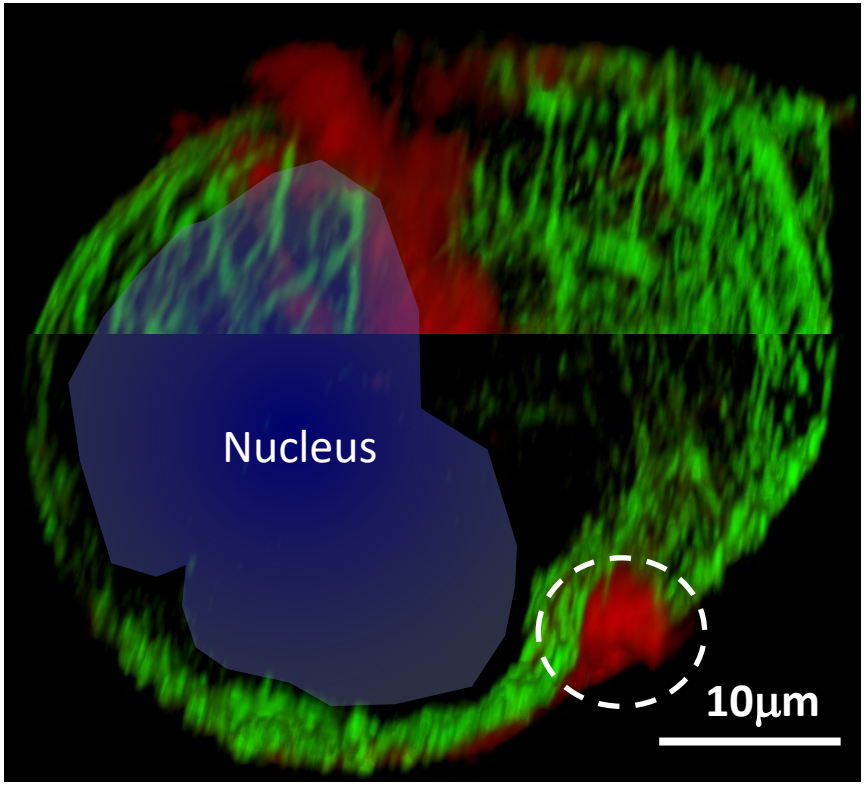
Actin/Nucleus (40mins)

Representative time for
actin band formation (62.36±10.84%)

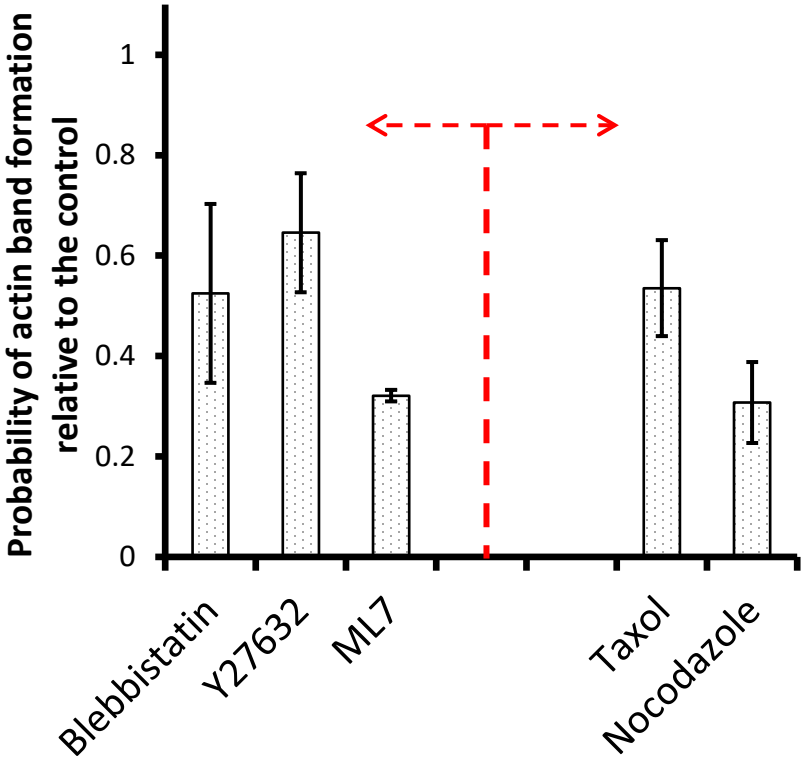




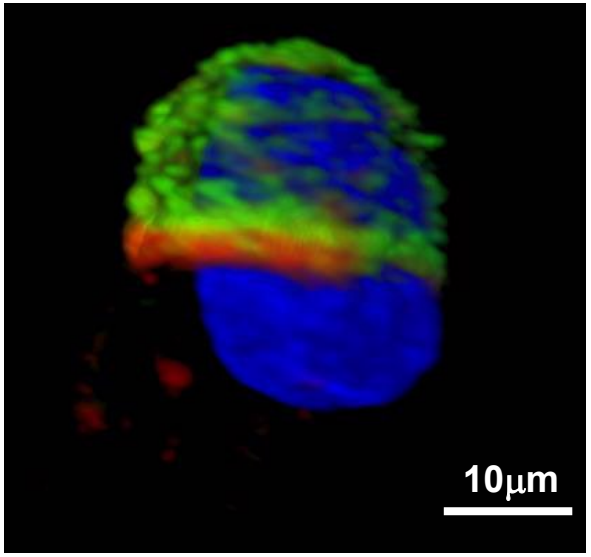
Evidence of steric repulsion between actin filaments and microtubule networks



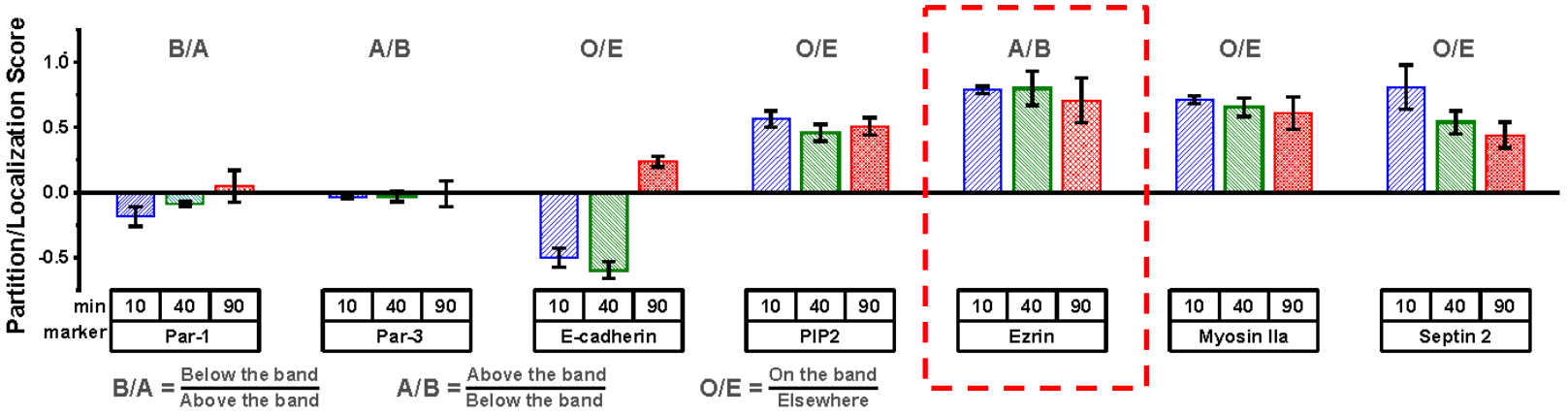
Actin/microtubule (Expansion microscopy)



Evidence of the actin-membrane linker for the actin band formation

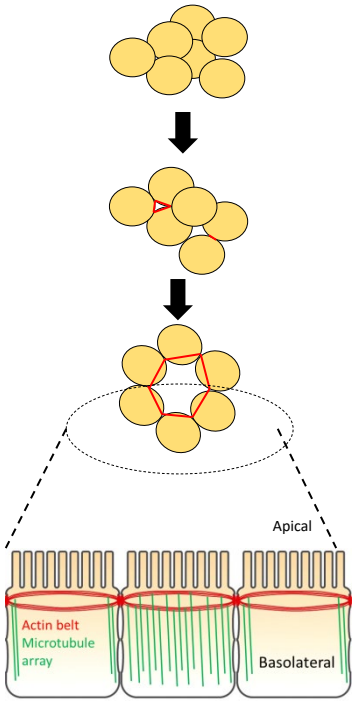


Actin/Ezrin/nucleus (40min)



Summary & Potential impacts

Classical outside-in mechanism



E-cadherin-mediated cell-cell adhesion

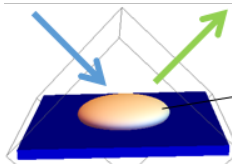
⬇

Segregation of apical & basal complexes

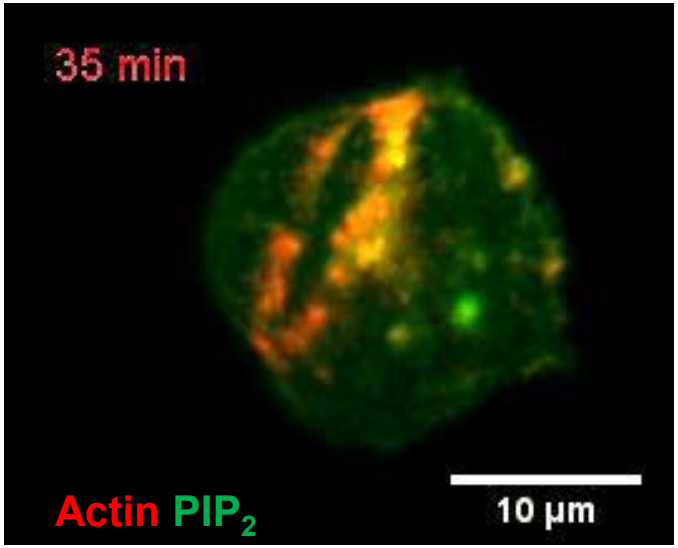
⬇

Formation of a circumferential actin belt

Our observation



Lattice light-sheet microscopy



Our inside-out mechanism

Organization of a polar microtubule network

Ezrin ⬇ | Myosin-dependent steric repulsion ⊣

Membrane anchorage of actin filaments

⬇

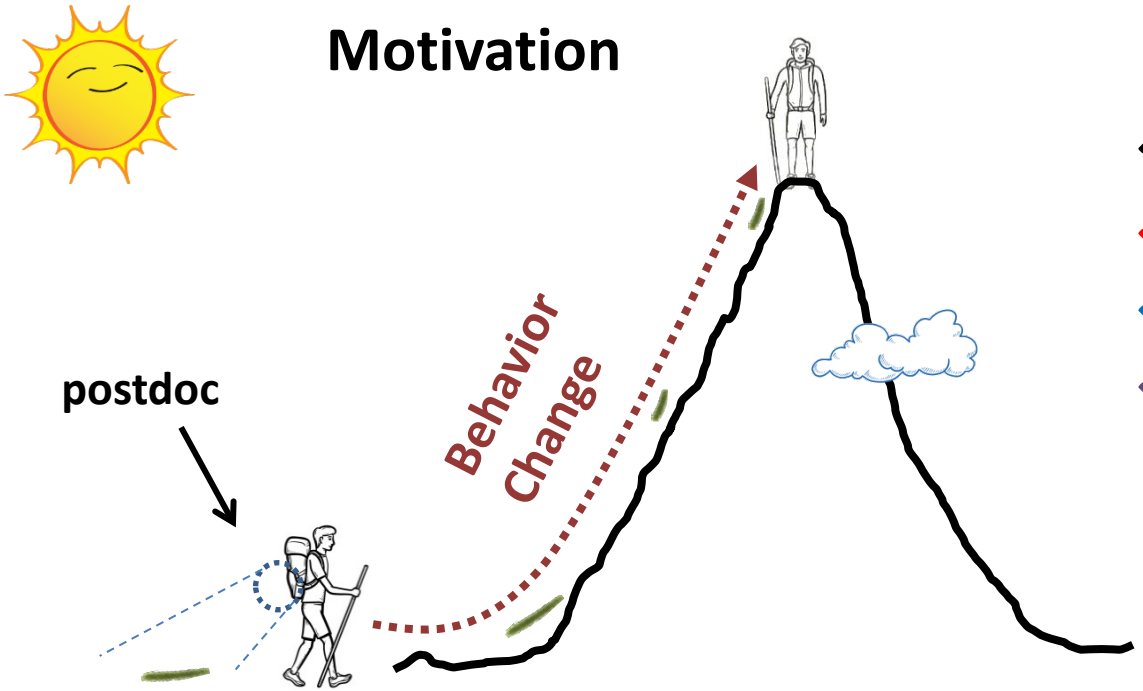
Formation of a circumferential actin band

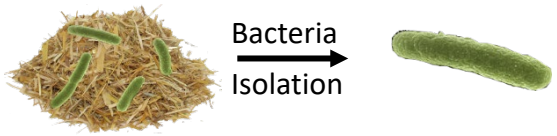
⬇

Recruitment of E-cadherin

1. Robustness (Turing reaction-diffusion vs microtubule polarization)
2. Therapeutic purpose (less targets)
3. Future work (immune synapse)

Metabolic reprogramming in response to progressive environmental changes





Bacteria Isolation

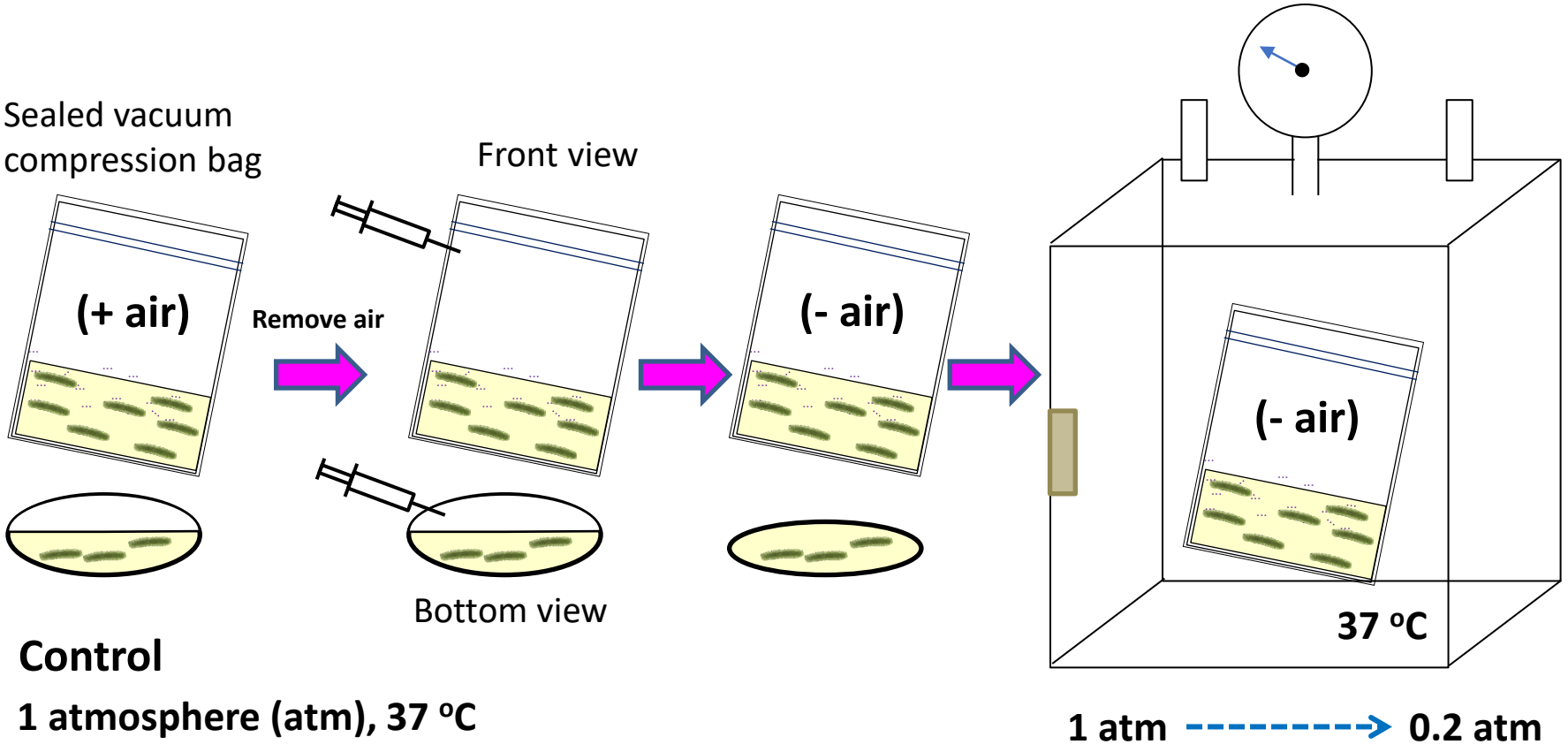
Nature Environment (Hay)

Bacterium YTG-1

Whole Genome Sequencing

≅ *E. coli* strain K-12 substrain MG1655

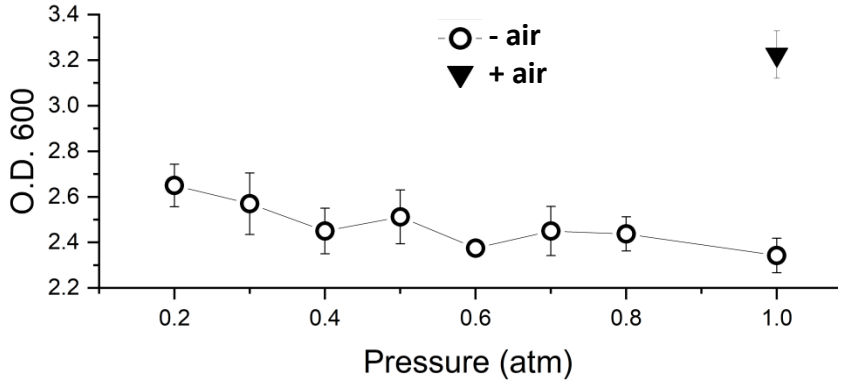
Experimental design: a semi-open culture system to maintain a constant pressure

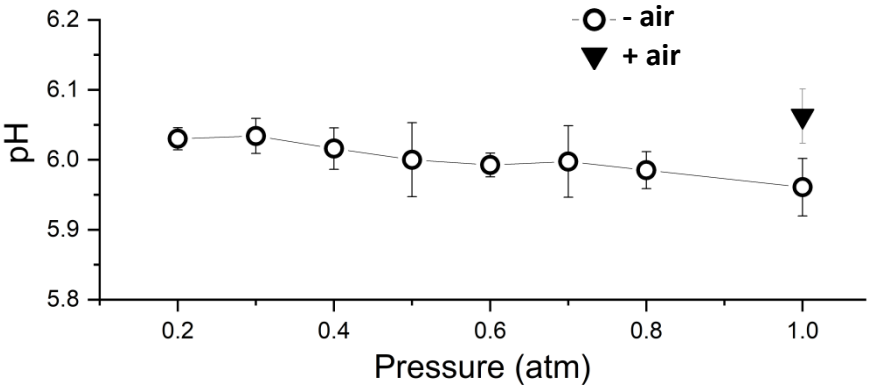


Medium (dextrose + peptone from junk food, pH = 7.4 adjusted by Na_2HPO_4)

Bacterial cell (with a fixed initial density, OD = 0.24 ± 0.01 measured by 600nm visible light)

6 hours later

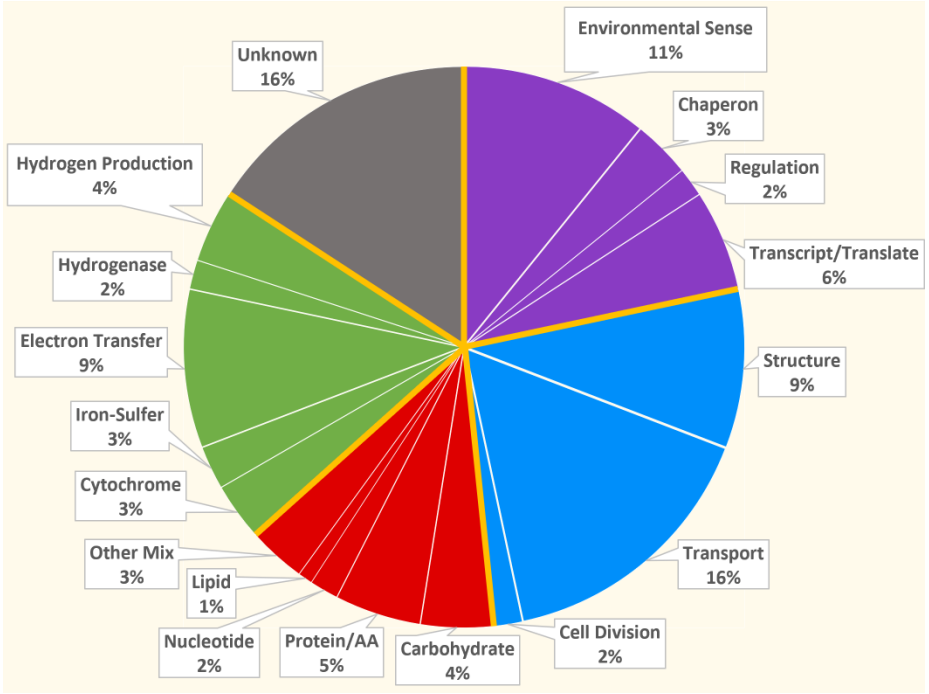




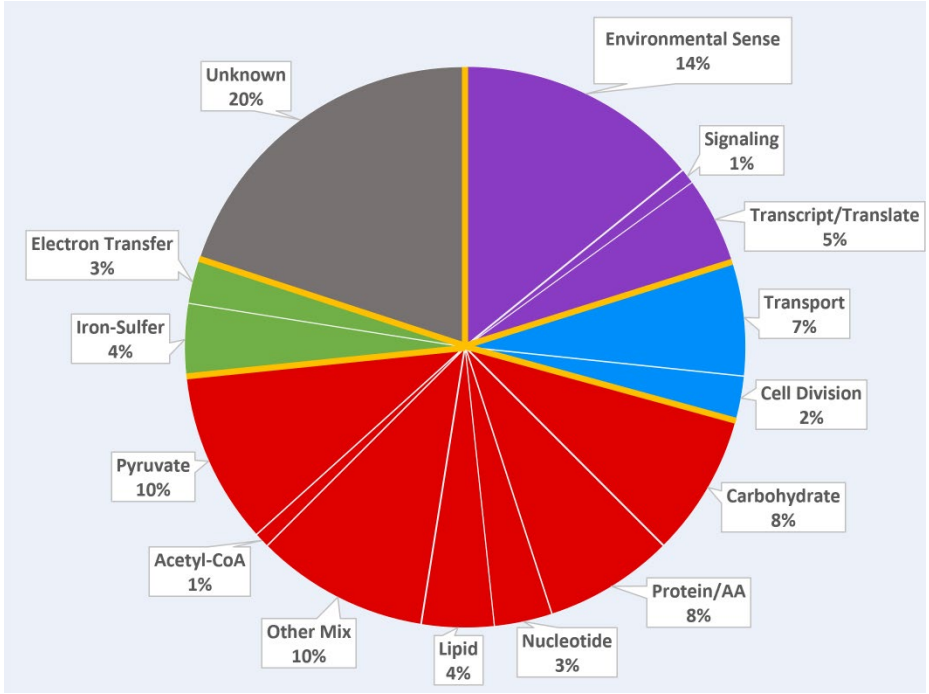
monotonic increase of cell number & pH

Comparison of the top 10% upregulated proteins

Air deprivation (+ air, 1 atm ⟶ - air, 1 atm)



Pressure reduction (- air, 1 atm ⟶ - air, 0.2 atm)

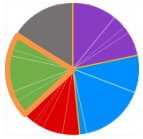


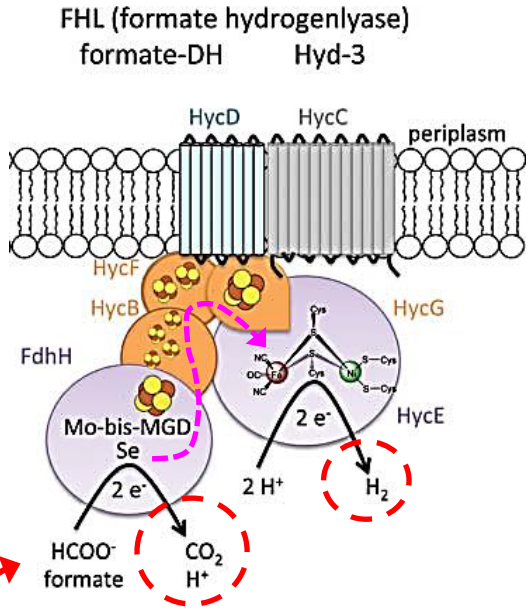
fermentation/catabolism /membrane/homeostasis/unknown

fermentation/catabolism /membrane/homeostasis/unknown

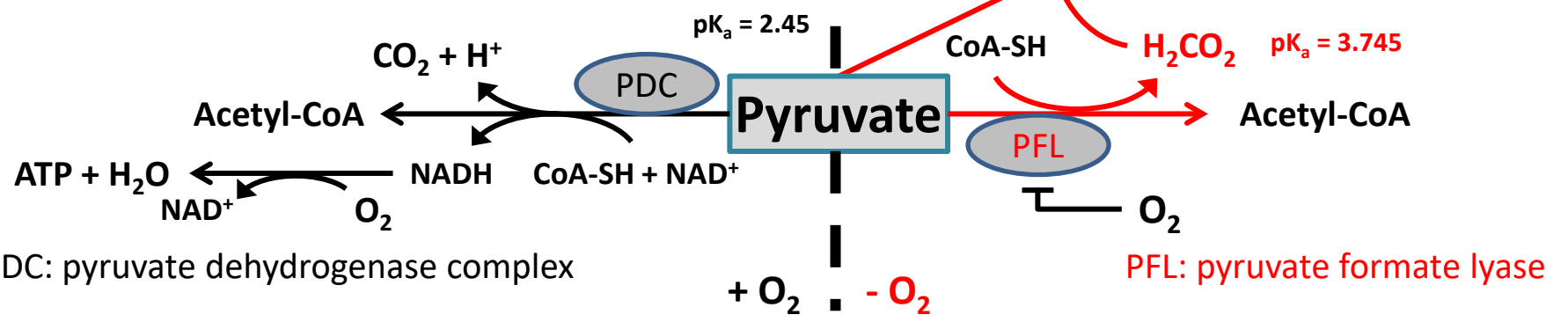
The top 10% upregulated proteins for fermentation

from (+ air, 1 atm) to (- air, 1 atm)





Pinske & Sawers, Biomolecular Concepts 2014, 5: 55-70

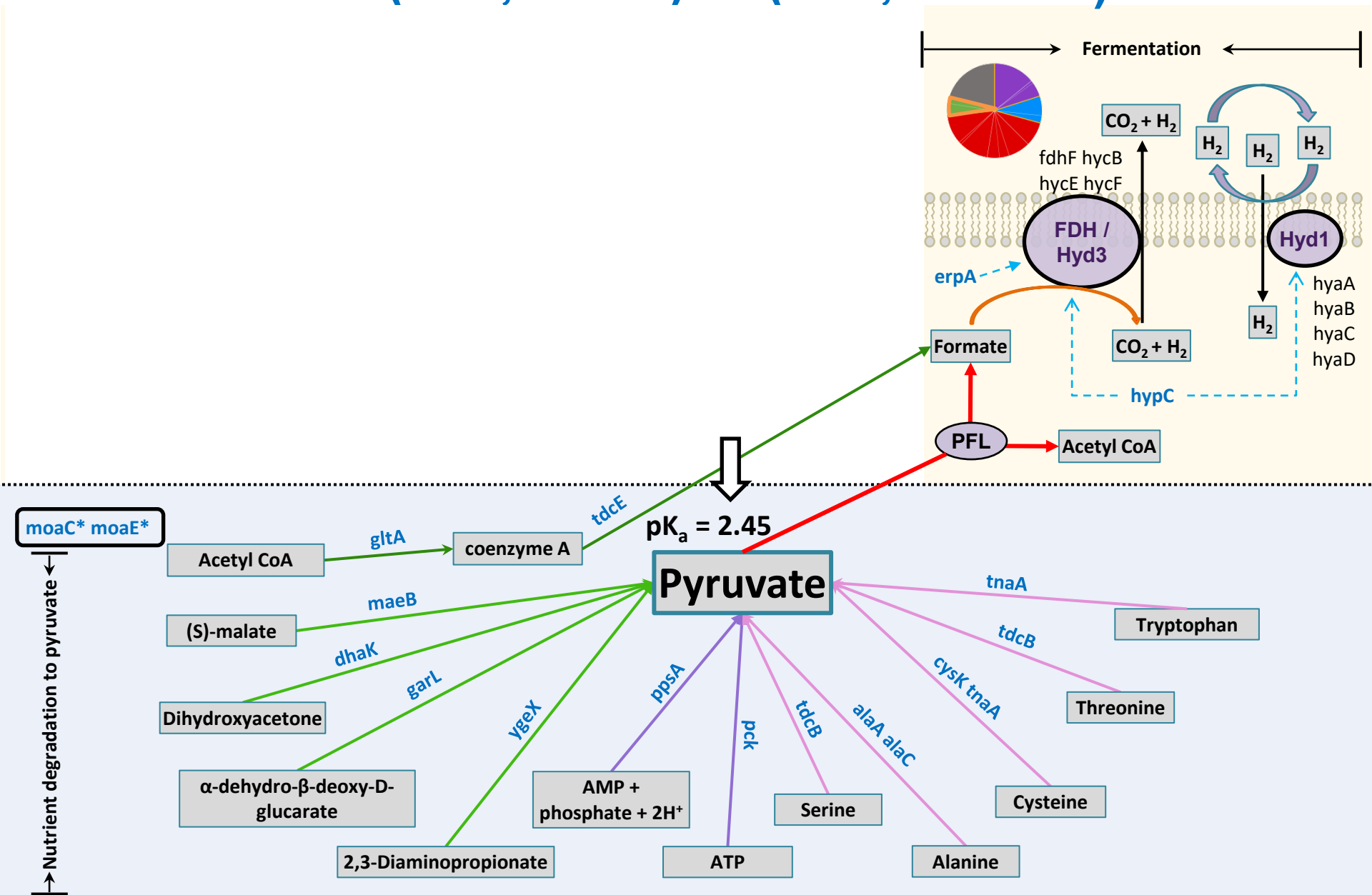


PDC: pyruvate dehydrogenase complex

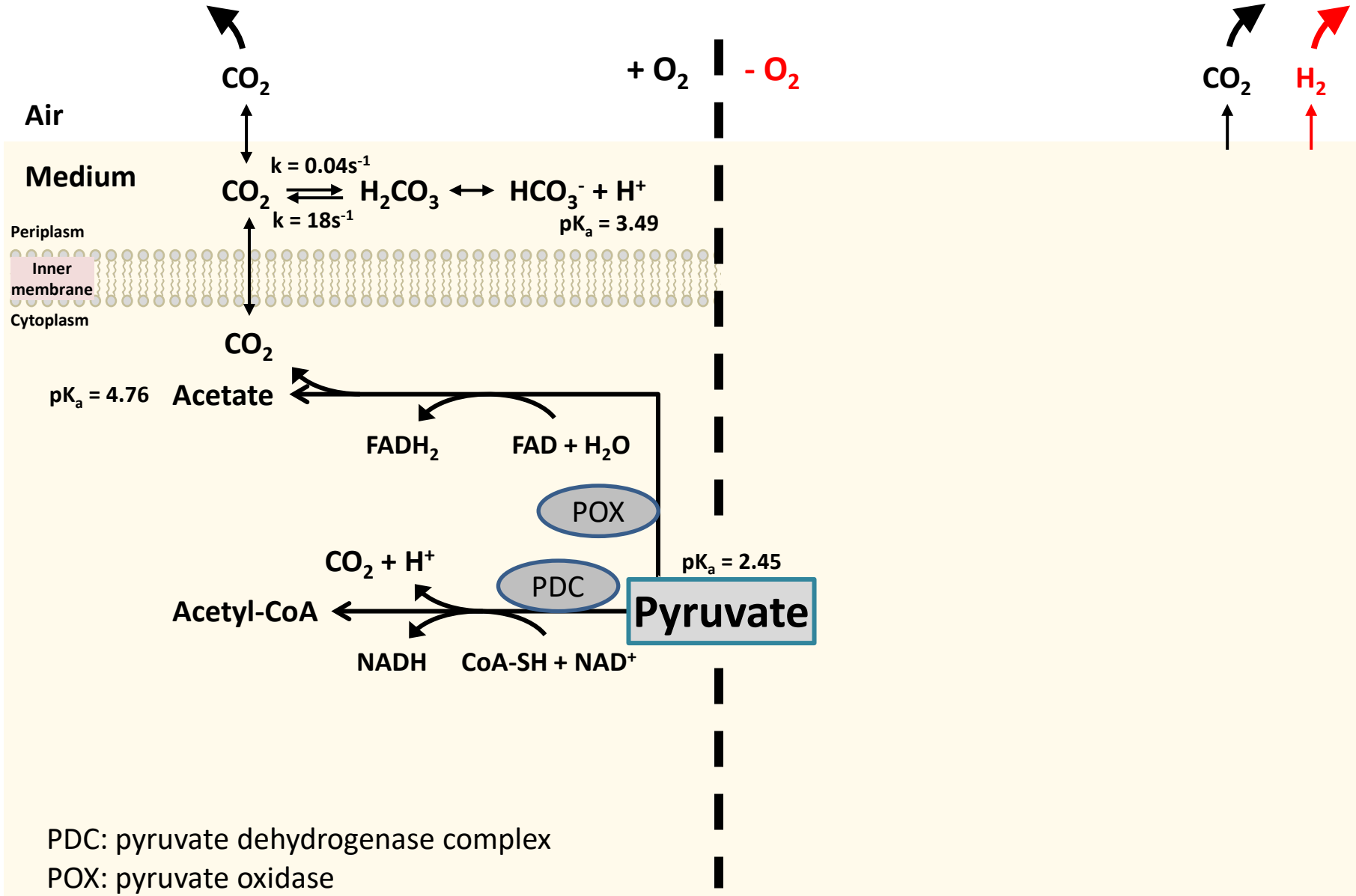
PFL: pyruvate formate lyase

The top 10% upregulated proteins for fermentation

from (- air, 1 atm) to (- air, 0.2 atm)



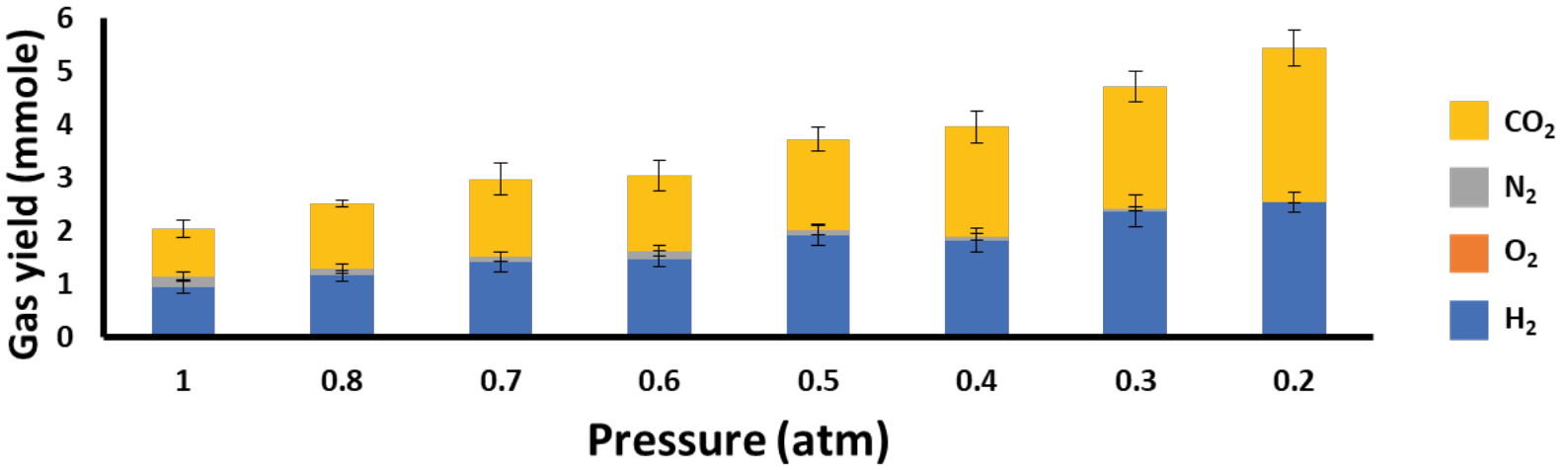
Formate decomposition and proton removal

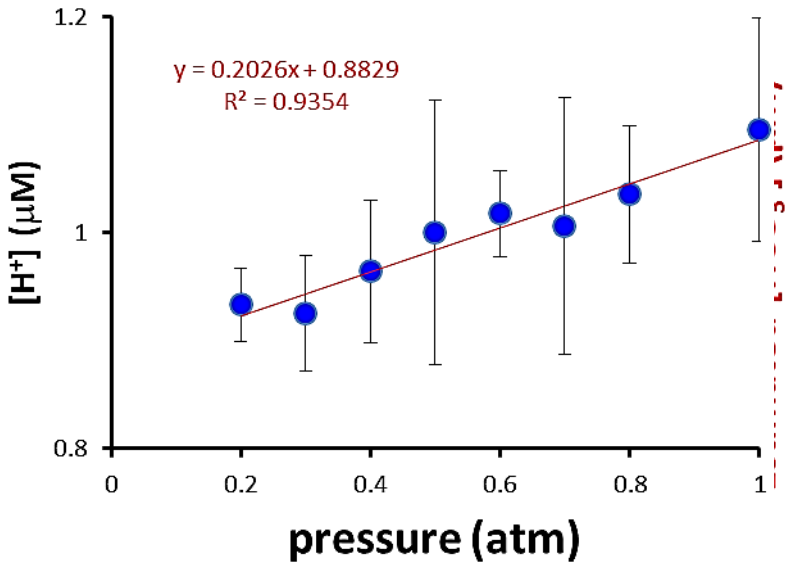


PDC: pyruvate dehydrogenase complex

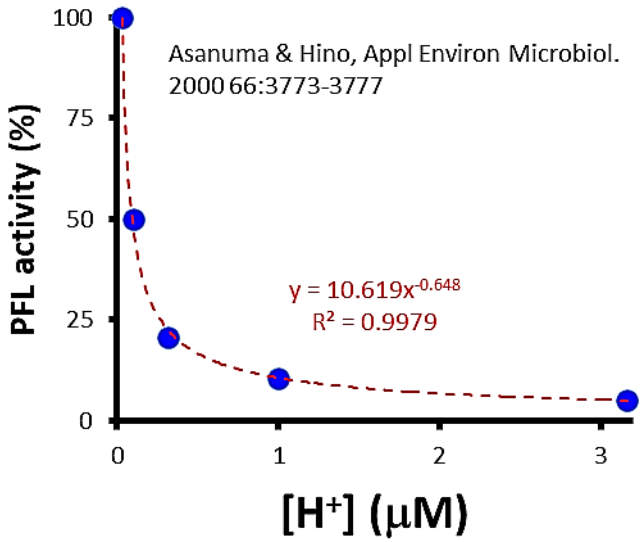
POX: pyruvate oxidase

Pressure-dependent production of H_2 and CO_2

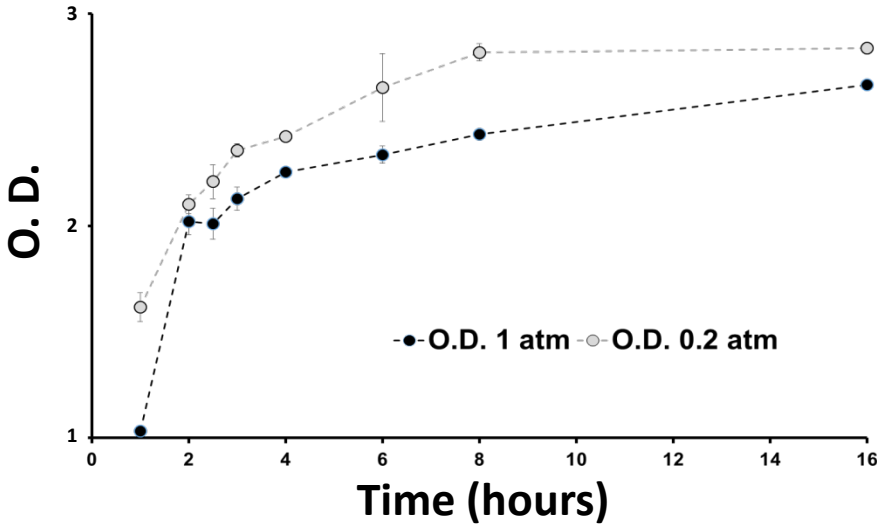


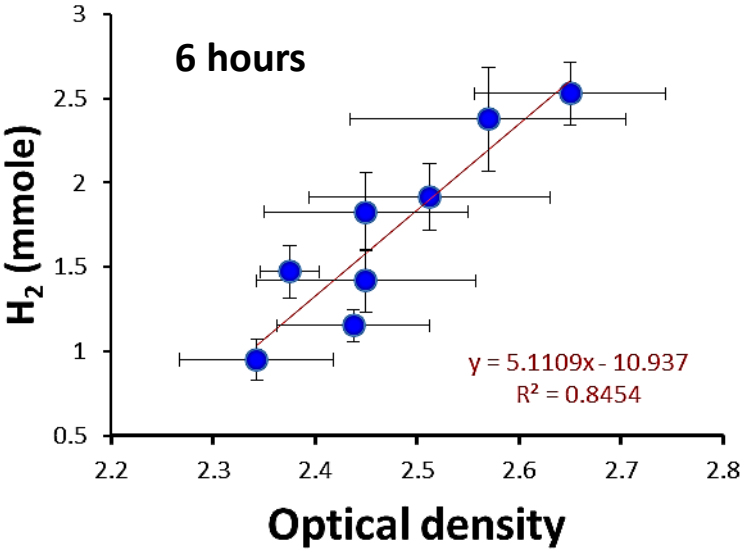


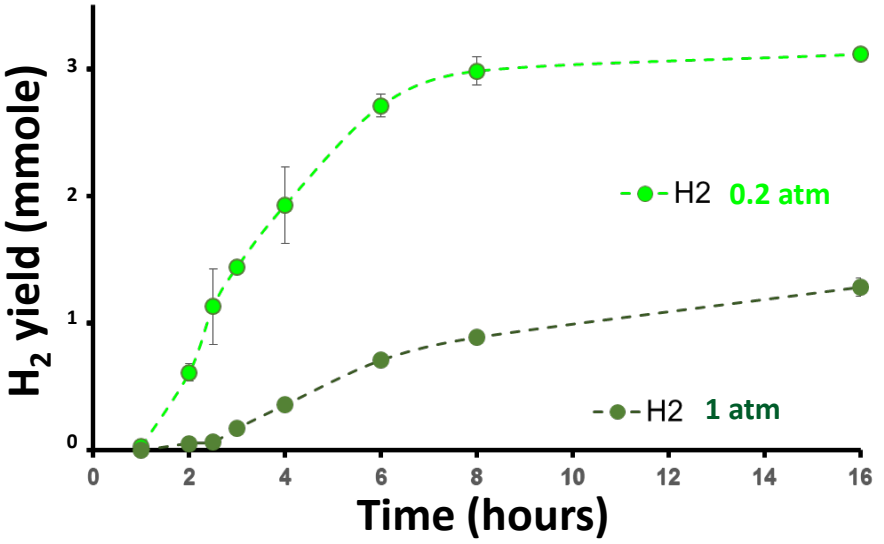
PFL: pyruvate formate lyase



Cell growth and H_2 production





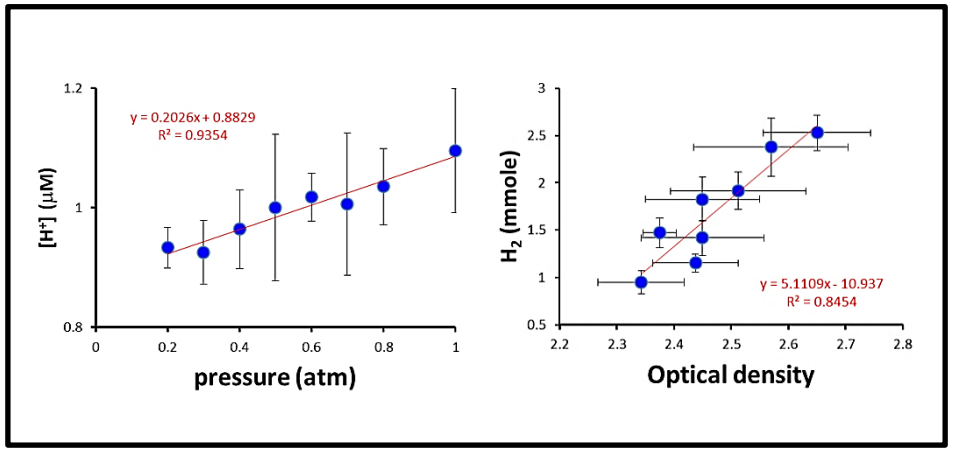


$$\frac{d\mathrm{H}_2(t)}{dt} = \beta N(t) \; ?$$

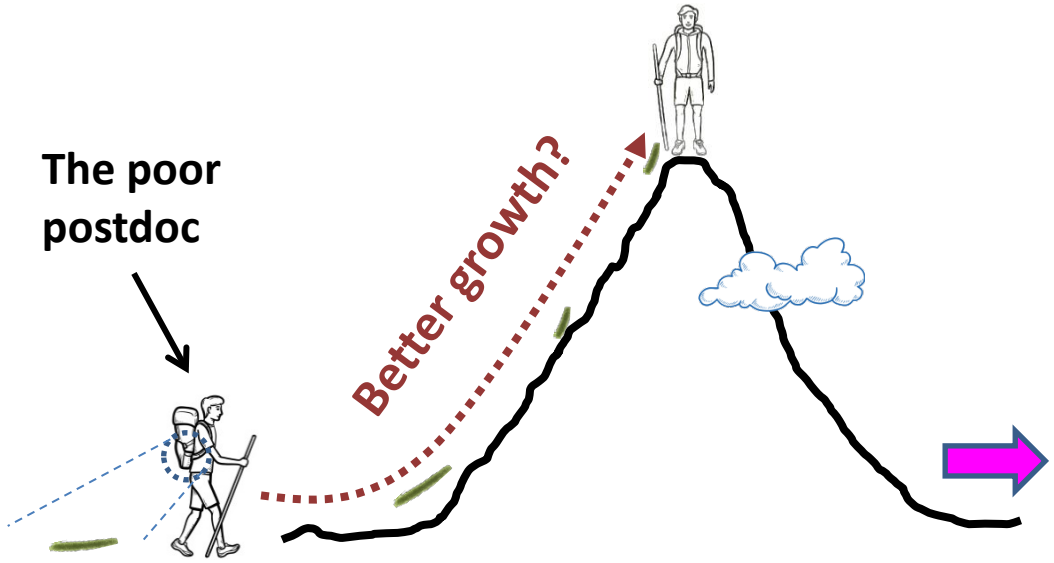
$$\frac{d\mathrm{H}_2(t)}{dt} = \beta \frac{dN(t)}{dt} \; ?$$

$$\mathrm{H}_2(t) \propto [N(t) - N_0]$$

Summary & Potential uses



Easy to get diarrhea?



Industrial H_2 production
a win-win strategy? (future work)