Changes in the maintenance energy of non-growing microbes and the consequences it has on cell survival

Teuta Pilizota
University of Edinburgh, UK

KITP, October, 2022

The maintenance energy of bacteria in growing cultures

By S. J. Pirt

Department of Microbiology, Queen Elizabeth College, University of London, W.8

(*Communicated by Sir Cyril Hinshelwood, F.R.S.—Received* 22 *March* 1965)

The variation, with growth rate, of the yield of organism from the substrate used as energy source is attributed to consumption of energy at a constant rate for cell maintenance. From the laws of growth, a simple relation between the maintenance requirement, the growth yield and the growth rate is derived. The relation is shown to be in good agreement with the available data. A distinction is made between 'observed' yield and 'true' yield of organisms. Values for maintenance energies and 'true growth yields' have been calculated from the data.

Introduction

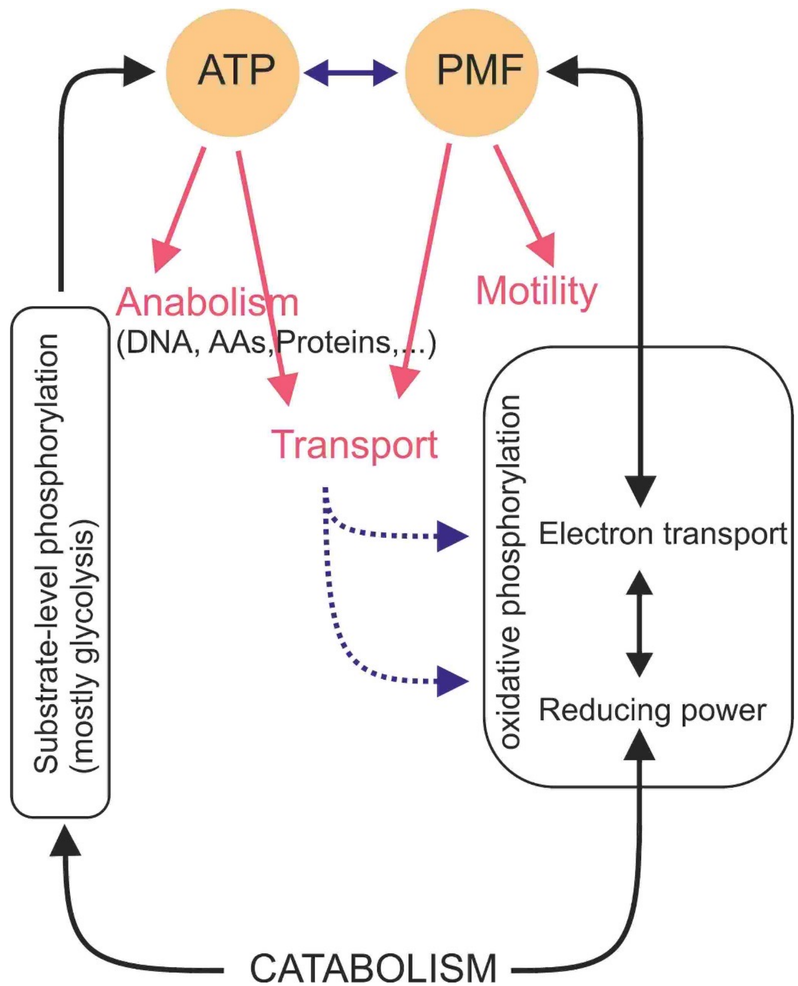
The term 'energy of maintenance' of bacteria refers to energy consumed for functions other than production of new cell material. But the existence of an 'energy of maintenance' for growing bacteria has been problematical. The subject has been recently considered in a symposium edited by Lamanna (1963) and in a review by Dawes & Ribbons (1964). Duclaux (1898) was probably the earliest microbiologist to distinguish between energy for growth and energy of maintenance of cells. He wrote for the substrate utilization in a culture,

$$ds = A dx + mx dt,$$

The work of Monod (1942) indicated that the energy of maintenance of *Escherichia coli* during growth was virtually zero. The question was re-opened by the development of continuous-flow culture which required a maintenance energy to be postulated to account for a deviation from the original theory of the process (Dawes & Ribbons 1964). There is a need for further investigation of the problem. A possible cause of confusion is that two different mathematical expressions for the maintenance requirement have emerged, and so far have not been critically compared. The present paper compares these expressions and further develops the theoretical relations of maintenance energy to growth. The measurements of maintenance energy so far reported are few and show a wide variation. Additional data and a comparison of all available measurements are given in this paper. From these data it can be seen that maintenance energy is a large and important factor at low growth rates.

ATP and PMF are main free energy sources in Escherichia coli

A coarse-grain view of free energy coordination in *E.coli*



ATP and PMF are main free energy sources in Escherichia coli

ATP is loved by life because

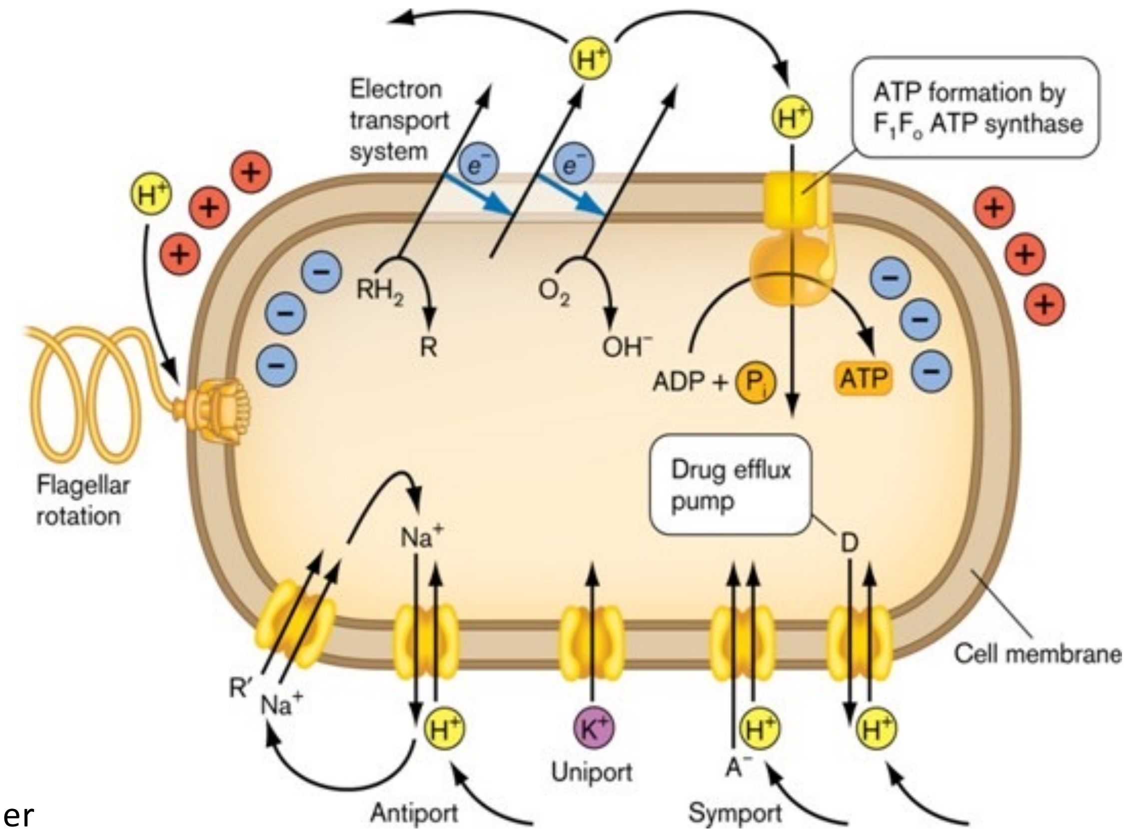
$$\mathrm{ATP} \rightarrow \mathrm{ADP} + \mathrm{P_i}$$

gives 30-70 kJ/mol

Proton Motive Force (PMF) is an electrochemical gradient of protons:

$$\mathrm{PMF} = \frac{kT}{e}\Delta\mathrm{pH} + \Delta\psi$$

- powers F_1F_o ATPase
- drives the transport of sugars, amino acids and other substrates across biological membranes
- powers bacterial flagella motor



$$\mathrm{PMF} = \frac{kT}{e}\Delta\mathrm{pH} + \Delta\psi$$

PMF is coupled to osmotic pressure and other IMFs

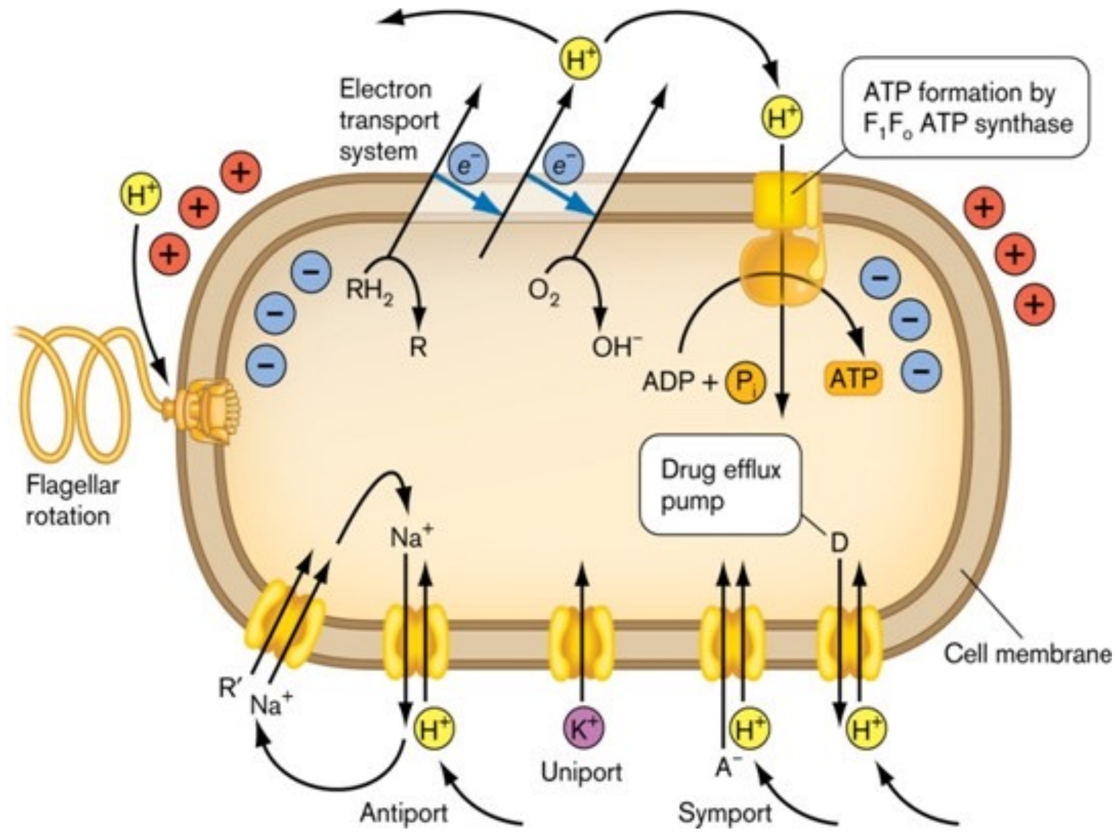
$$PMF = \Delta G_H = \frac{kT}{e}\Delta \text{pH} + \Delta\psi$$

$$\Delta G_x = \frac{kT}{e} ln\left(\frac{[x]_i}{[x]_e}\right) + \Delta\psi$$

$$\Delta\psi = F\frac{Q_i}{C} = F\frac{V\sum_x z_x[x]_i}{SC_m}$$

$$\Delta\Pi = RT\sum_y \varphi_y([y]_i - [y]_e)$$

Terradot G, et al, bioarxiv, 2021

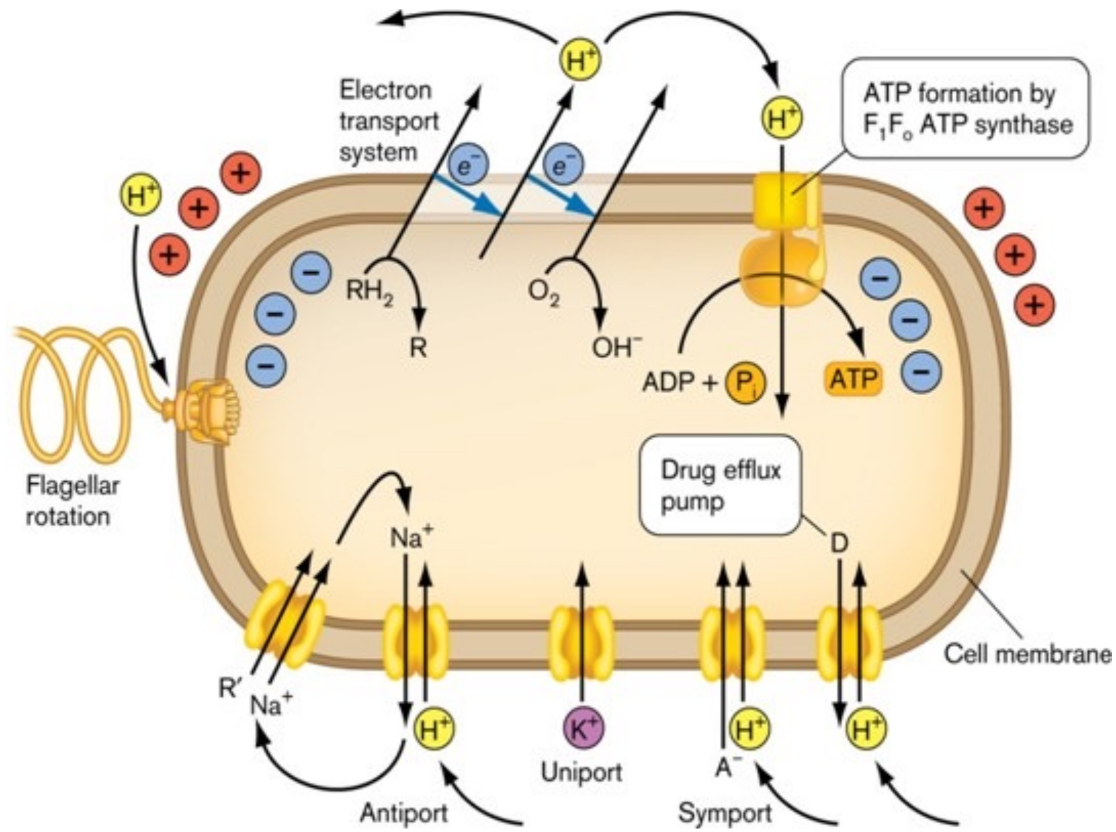


PMF is coupled to osmotic pressure and other IMFs

$$\frac{d[x]_i}{dt} = -\frac{d[x]_e}{dt} = j_L \pm \sigma \cdot j_P$$

$$j_P = k\left(1 - e^{\frac{\Delta G}{RT}}\right)$$

$$j_L = \frac{S}{V} P_x \Delta x f(\Delta\psi)$$



Terradot G, et al, bioarxiv, 2021

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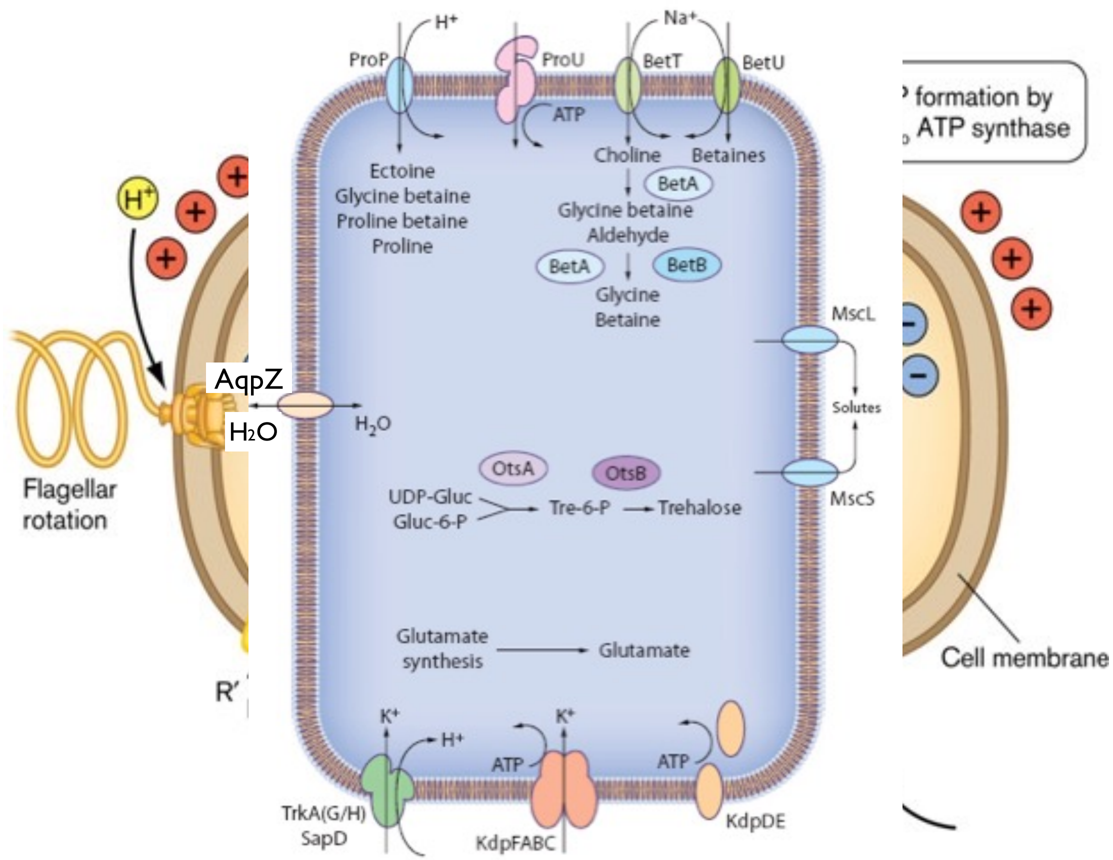
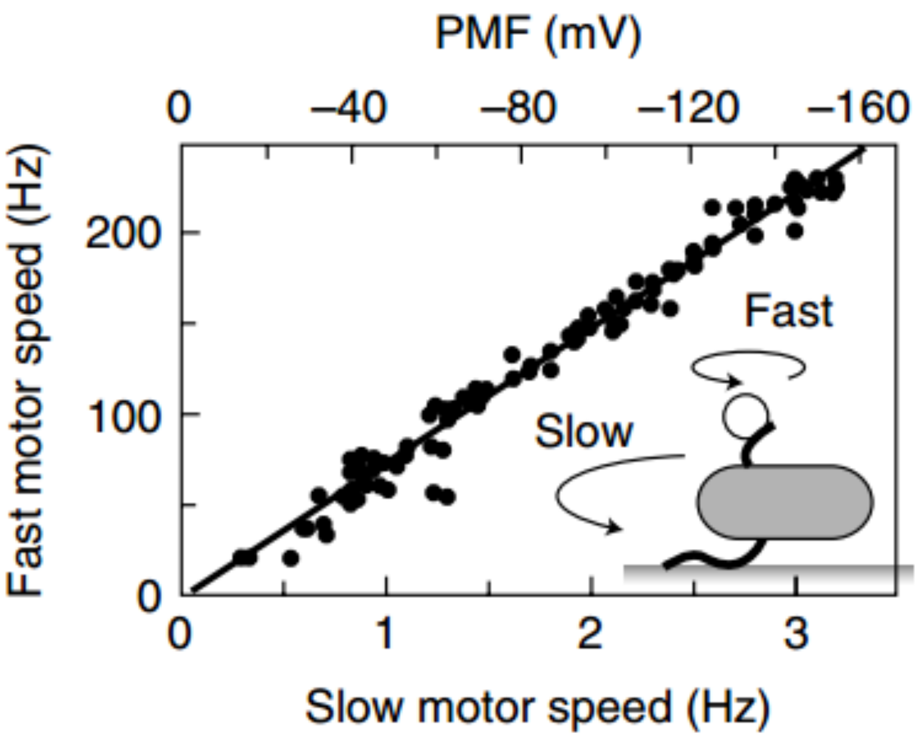


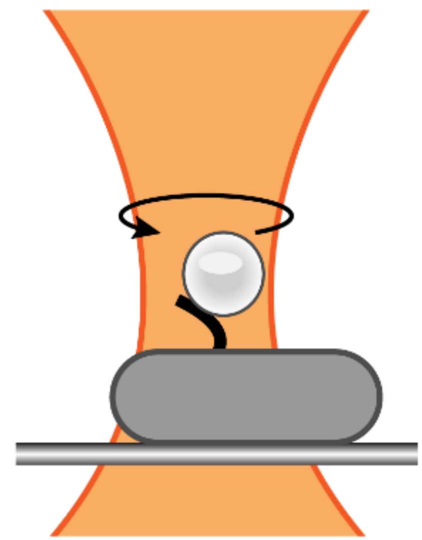
Bacterial flagellar motor can be used as a sensor for PMF

Speed of the motor has been shown to vary linearly with PMF



Fung, Berg, Nature, 375 (1995)
Gabel, Berg, PNAs, 100 (2003)

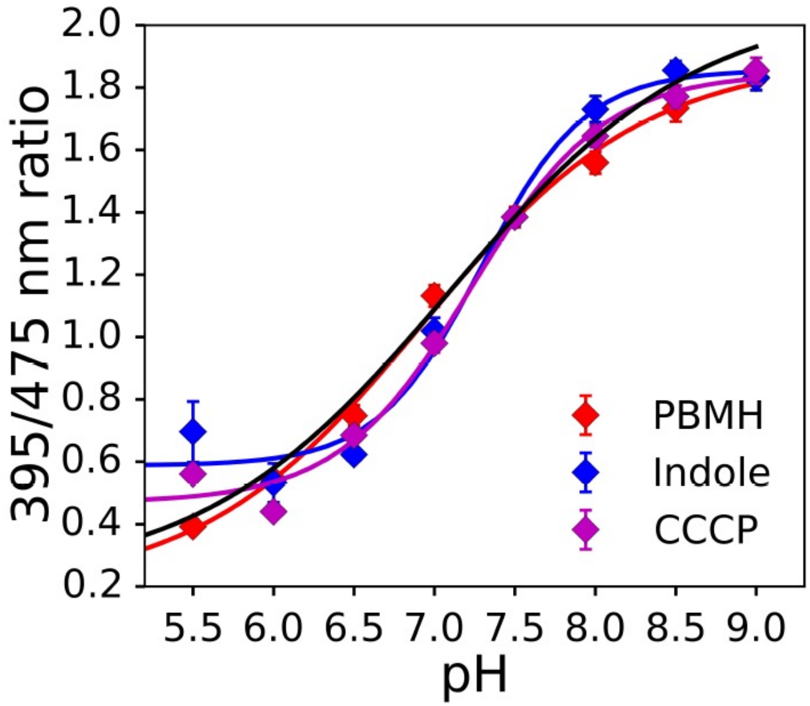
Speed of an individual flagellar motor can be measured by back focal plane interferometry

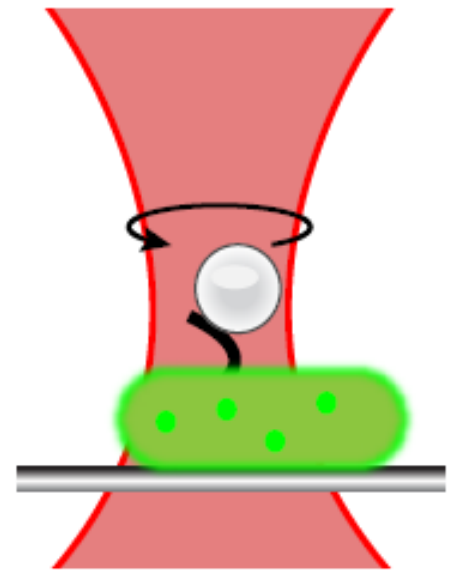


Bai, Branch, Nicolau, Pilizota, Steel, Maini, Berry, Science 2010
Pilizota, Brown, Leake, Berry, Armitage, PNAS 2009
Rosko J, Martinez V, Poon W, Pilizota T, PNAS, 2017

We use ratiometric pHlurin as a sensor for cytoplasmic pH

in vivo





Krasnopeeva E, Lo CJ and Pilizota T, Biophys J, 116(12), 2019

Wang YK, et al Sci. Reports, 9 (2019)

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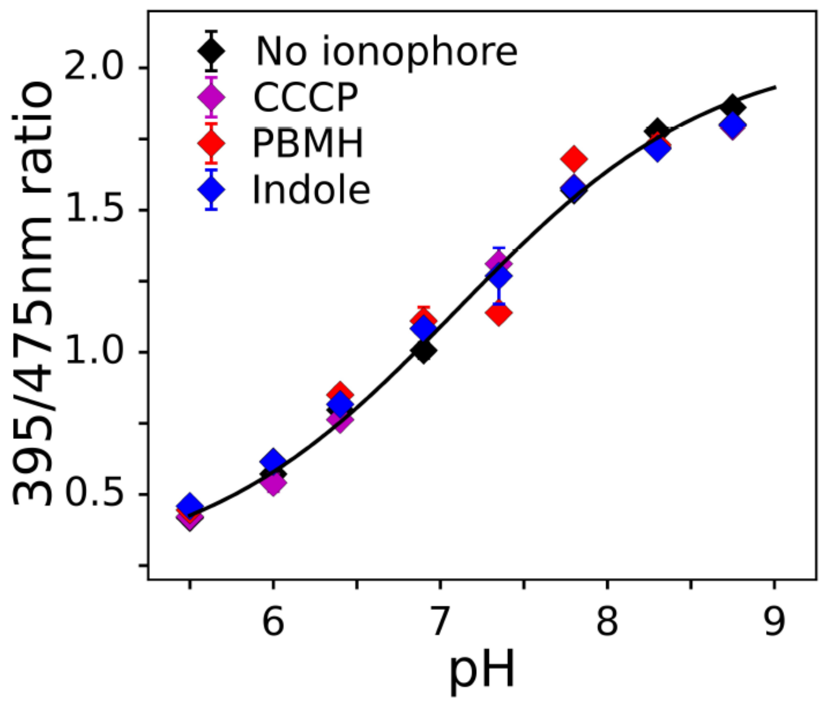
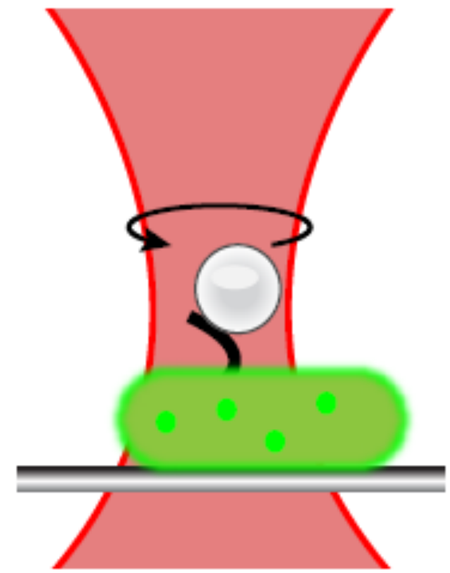
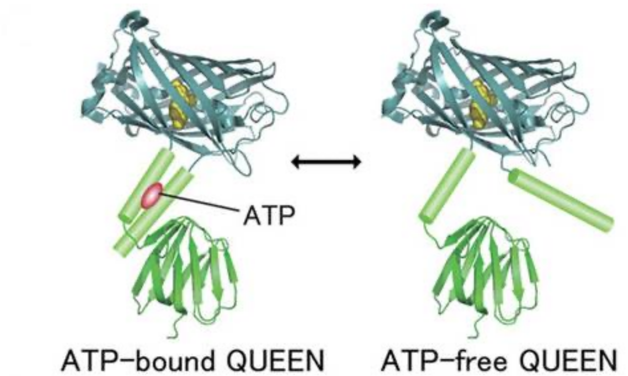


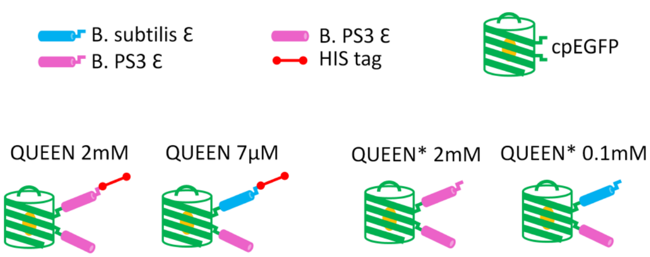


Krasnopeeva E, Lo CJ and Pilizota T, Biophys J, 116(12), 2019

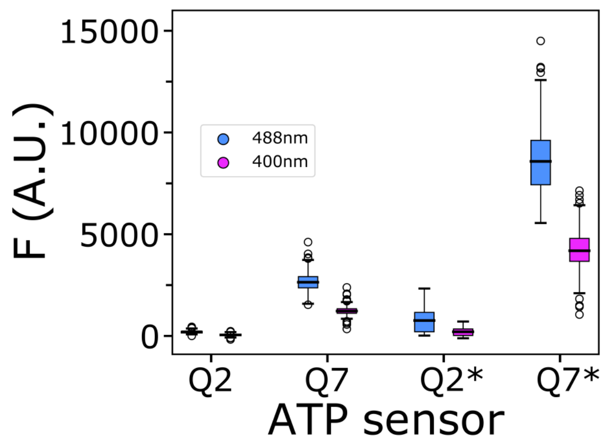
Wang YK, et al Sci. Reports, 9 (2019)

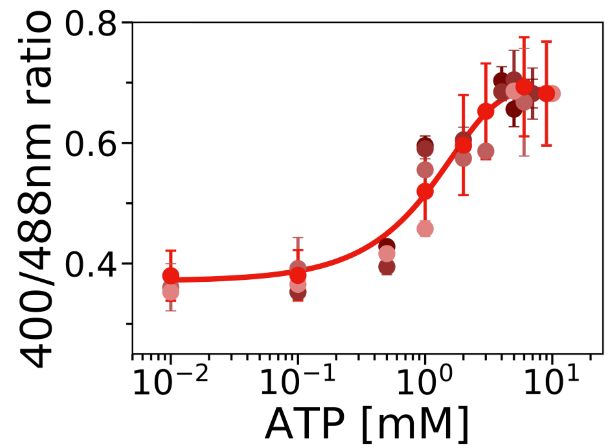
We improved the expression of QUEEN ATP sensor





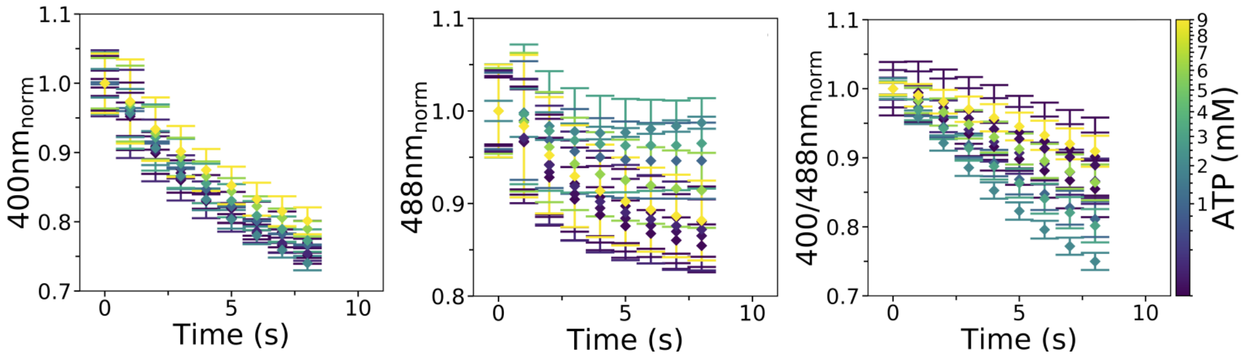
Yaginuma H, et al, Nature Scientific Reports, 2014





Mancini L and Pilizota T, Bioarxiv

The new QUEEN sensor is suitable only for snapshot imaging



$PMF = PMF_0 e^{-\alpha t}$
$\alpha = a * lnP + P_0$

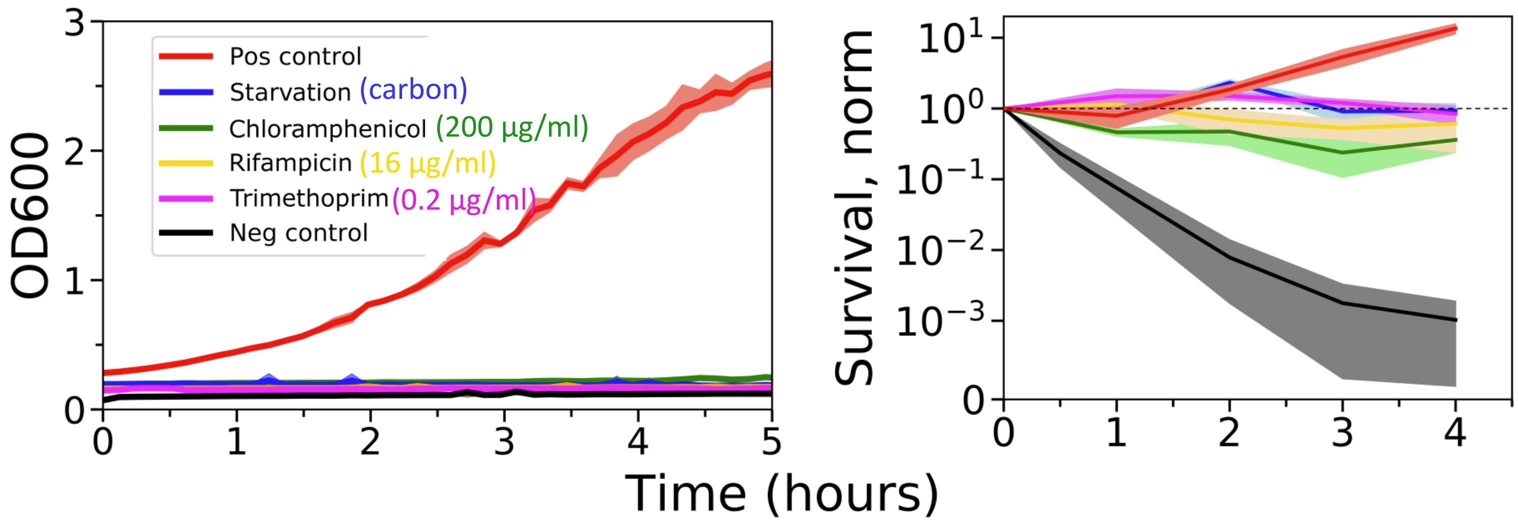
Mancini L and Pilizota T, Bioarxiv

Krasnopeeva E, Lo CJ and Pilizota T, Biophys J, 116(12), 2019

Carbon starvation and bacteriostatic drugs can induce instantaneous dormancy of a large population of cells

Positive control = no treatment

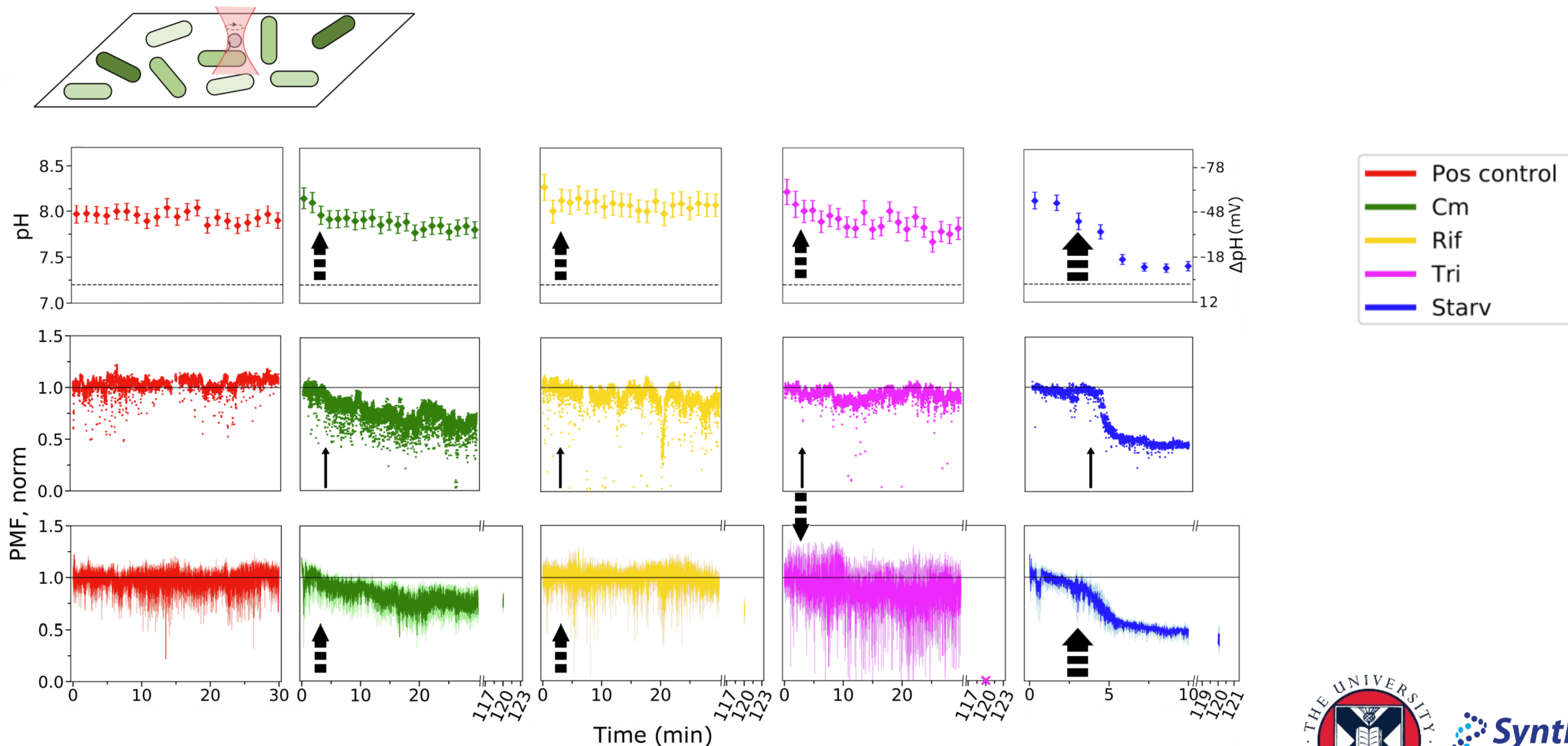
Negative control = 5xMIC ciprofloxacin



We also tried
N-starvation = no full growth arrest in the plate reader
Indole = full growth arrest, but significant viability loss

Mancini L and Pilizota T, Bioarxiv

The PMF of dormant cells varies in a condition dependent manner



Mancini L and Pilizota T, Bioarxiv

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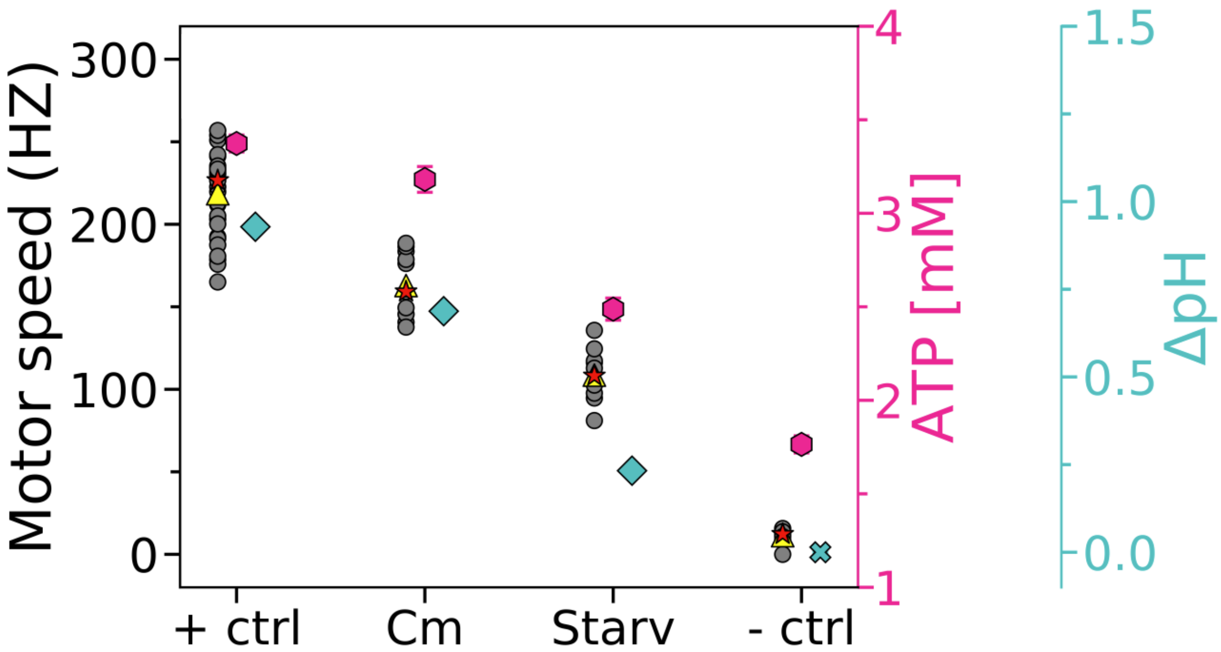
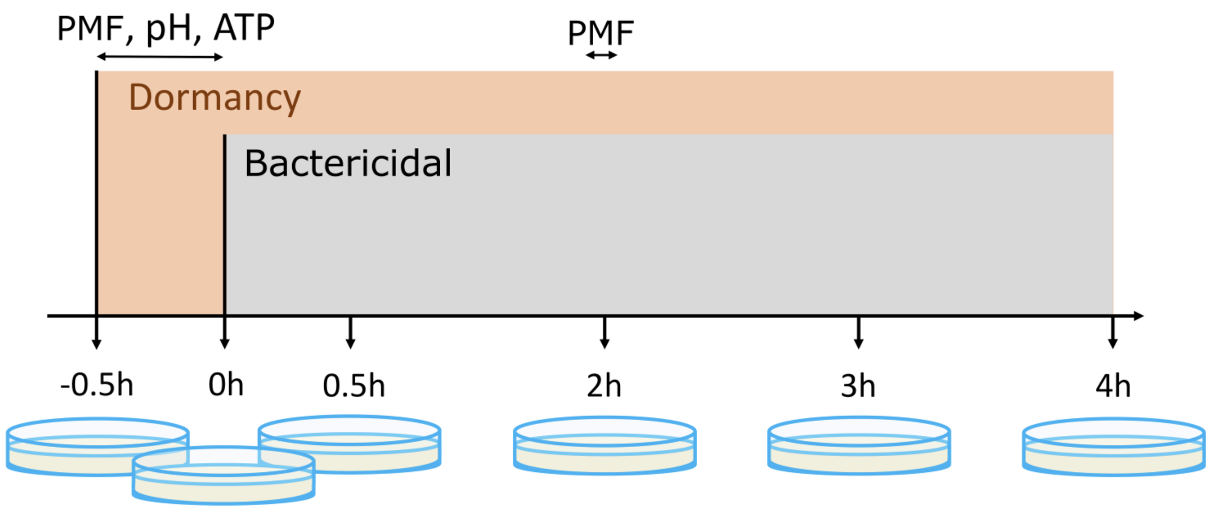
ATP levels of dormant cells are condition dependent



Mancini L and Pilizota T, Bioarxiv

Energy levels of dormant states show correlation with susceptibility to some antibiotics



Mancini L and Pilizota T, Bioarxiv

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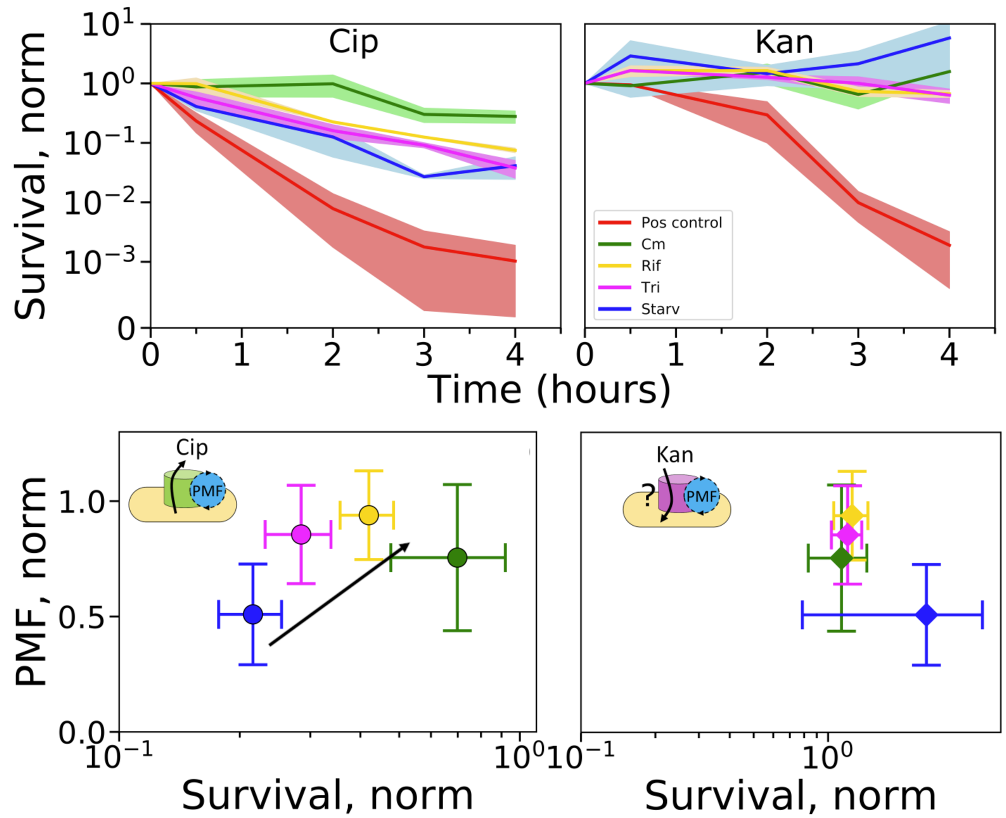

Mancini L and Pilizota T, Bioarxiv

Conclusions

Different growth arrests result in different energy profiles

Survival under bactericidal antibiotics correlates with energy levels for some antibiotics

The University of Edinburgh

SynthSys Centre for Synthetic and Systems Biology

PILIZOTA LAB

James Flewellen
Smitha Hegde
Amritha Janardanan

Mark Zurbruegg
Tommy Schmidlechner
Jack Hocking
Eric Chapman
Nicolo Tormena
Urte Puodziunaite
Keshav Todi
Diana Coroiu
Uriel Barboza Perez

COLLABORATORS:

Peter Swain, University of Edinburgh
Chien-Jung Lo, National Central University, Taiwan
Wilson Poon, University of Edinburgh
Michel Maharbiz, UC Berkeley

HFSP
Office of Naval Research Global
EPSRC
Engineering and Physical Sciences Research Council
The University of Edinburgh

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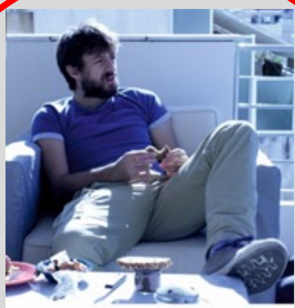
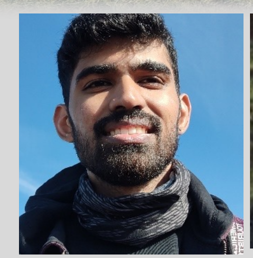


Leonardo

Lucas

The University of Edinburgh





PI dye permeates the membrane after loss of PMF