Single-cell bacterial electrophysiology

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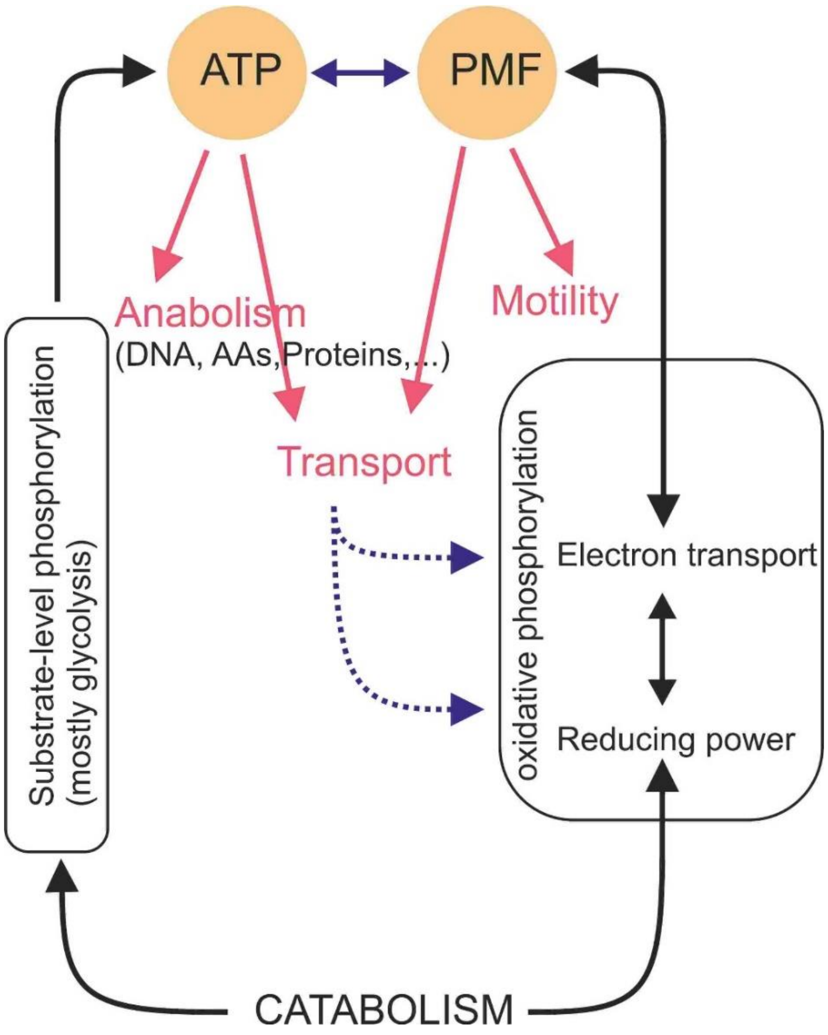
BEE Workshop, March 2019

ATP and PMF are main free energy sources in *Escherichia coli*

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

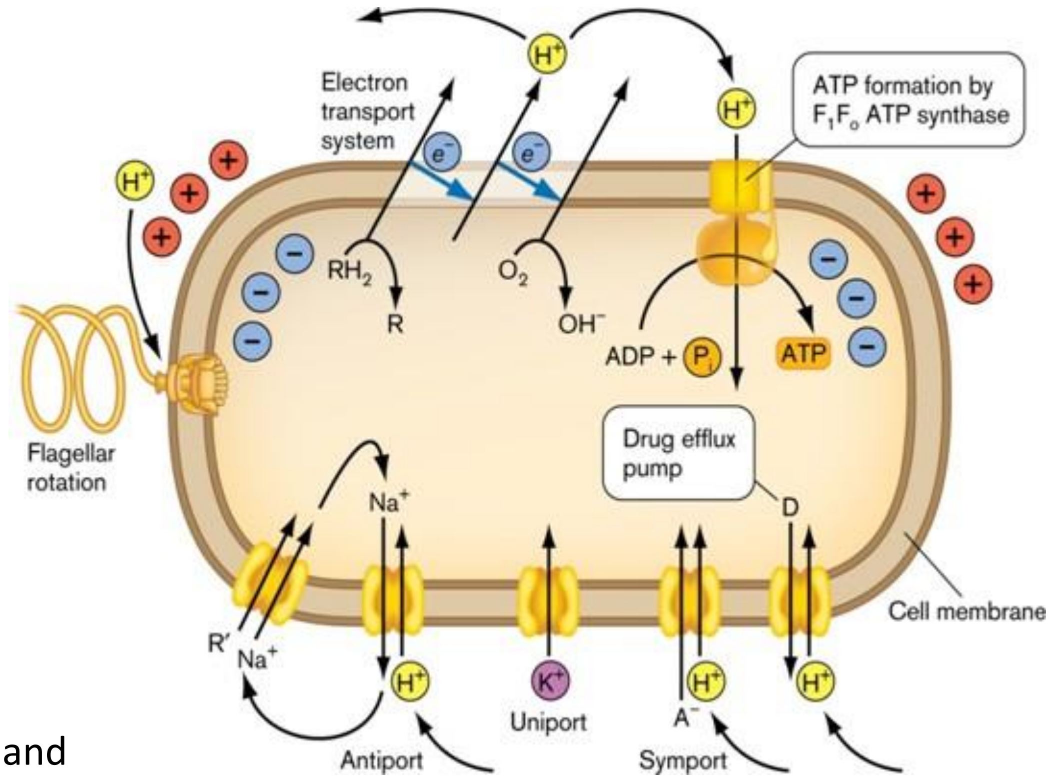


ATP and PMF are main free energy sources in *Escherichia coli*

ATP is loved by life because

$$ATP \rightarrow ADP + P_i$$

gives 30-70 kJ/mol



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

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

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

PMF dynamics can be modelled with a simple electric circuit

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

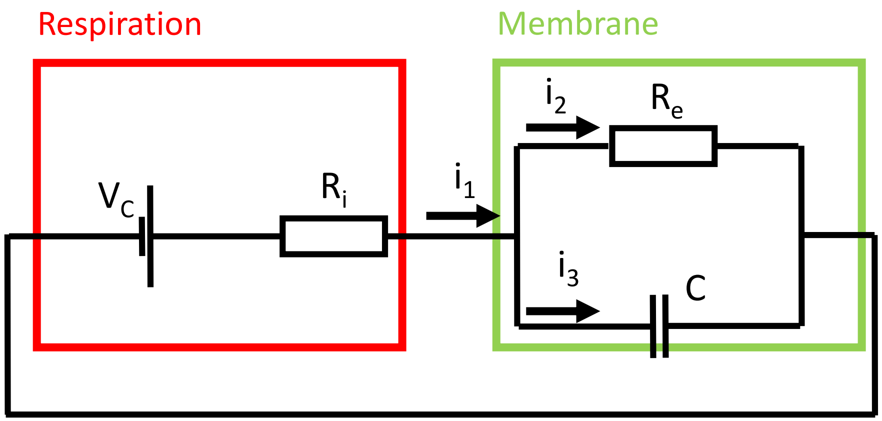
$\Delta pH = 0$

when $pH_{in} \sim pH_{out}$

PMF dynamics can be modelled with a simple electric circuit

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

$\Delta pH \approx 0$ when $pH_{in} \sim pH_{out}$



V_C – battery, respiration
R_i – internal battery resistance
R_e – membrane resistance (electron sink)
C – membrane capacitance

Rotterdam et al. (2001)

PMF dynamics can be modelled with a simple electric circuit

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$= 0$

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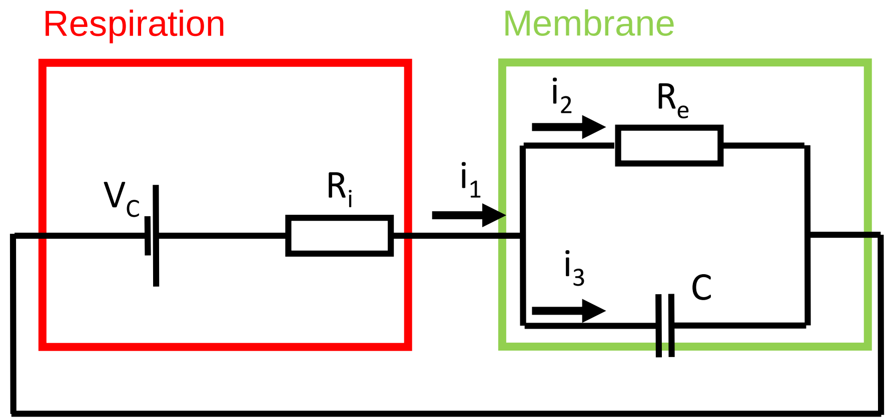

Kirchhoff's current law:

$$i_1(t) = i_2(t) + i_3(t)$$

Parallel voltages:

$$\frac{di_2}{dt}R_e(t) + \frac{dR_e}{dt}\mathrm{i}_2 = \frac{1}{\mathrm{C}}i_3$$

Total voltage:

$$V_C = i_1(t)R_i + i_2(t)R_e$$

V_C – battery, respiration
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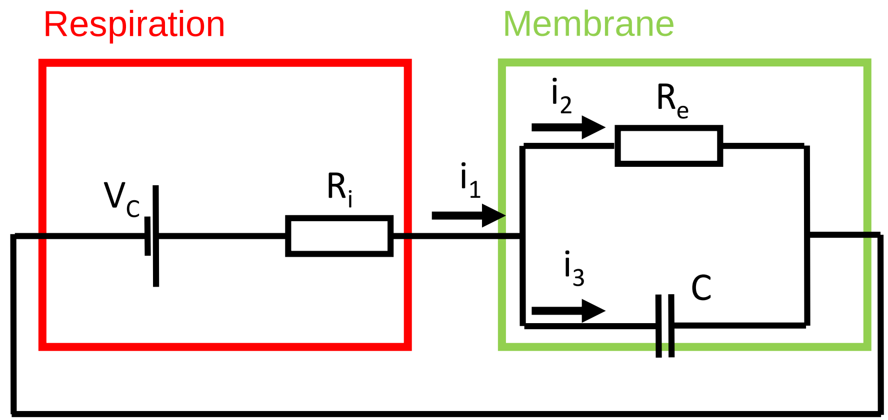


Electrical circuit analogy gives a frame work:

- predict what happens to PMF if we change the circuit parameters

or

- knowing the shape of PMF response, predict the mechanism and dynamics of the damage and assume functional dependency between circuit parameters and the amplitude of stress

- Also, we can envision measuring the resistance of the bacterial membrane in different conditions
- Or even better, try and measure I-V curves of different components

PMF dynamics can be modelled with a simple electric circuit

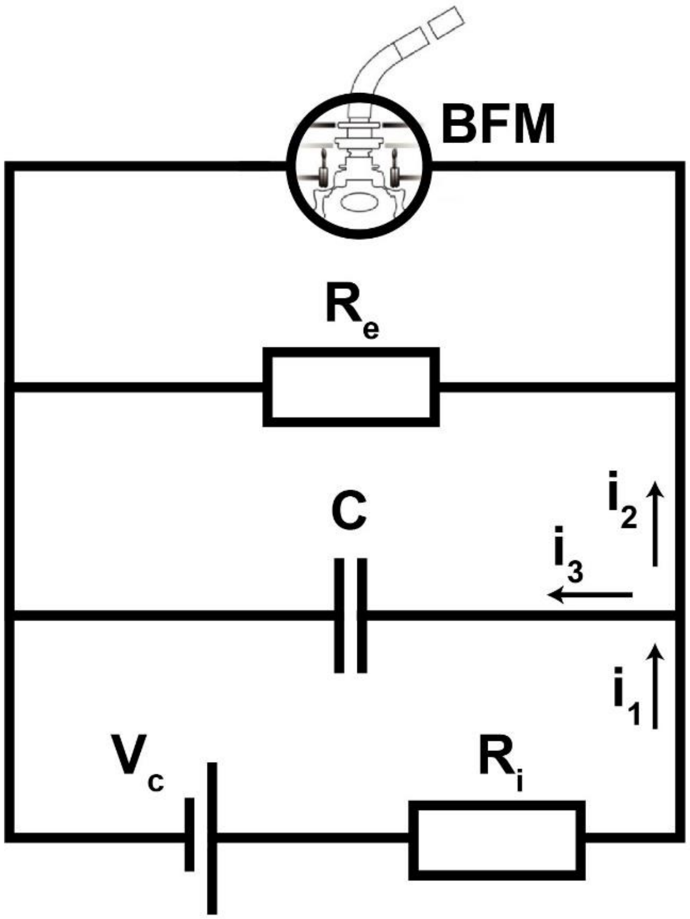
$$\mathrm{PMF} = \frac{kT}{e}\Delta pH + V_m$$

$= 0$

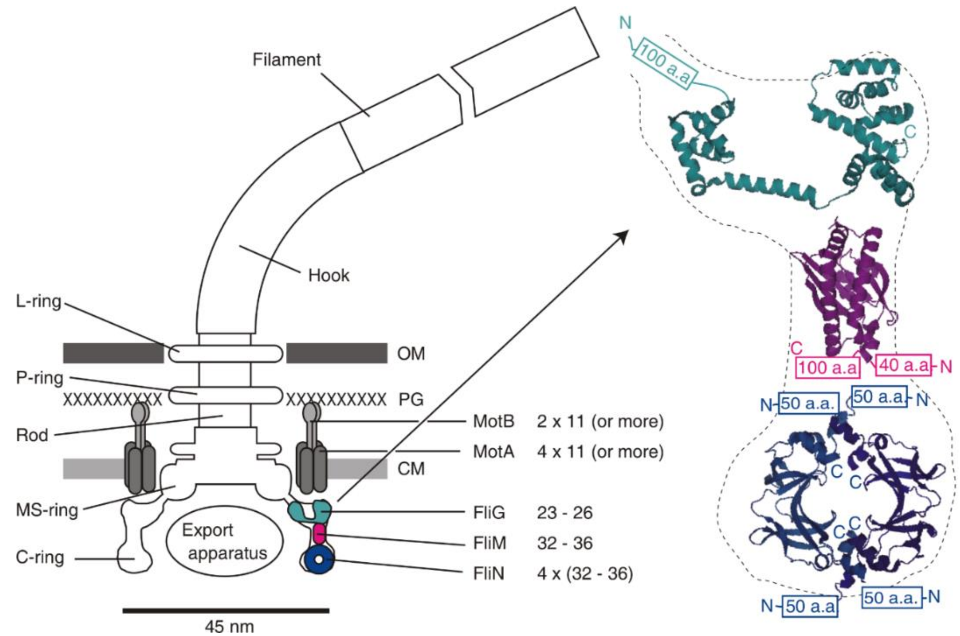
when $pH_{in} \sim pH_{out}$

Electrical circuit analogy gives a frame work:

$$\frac{V_m}{V_{m,0}} = f(S,t)$$



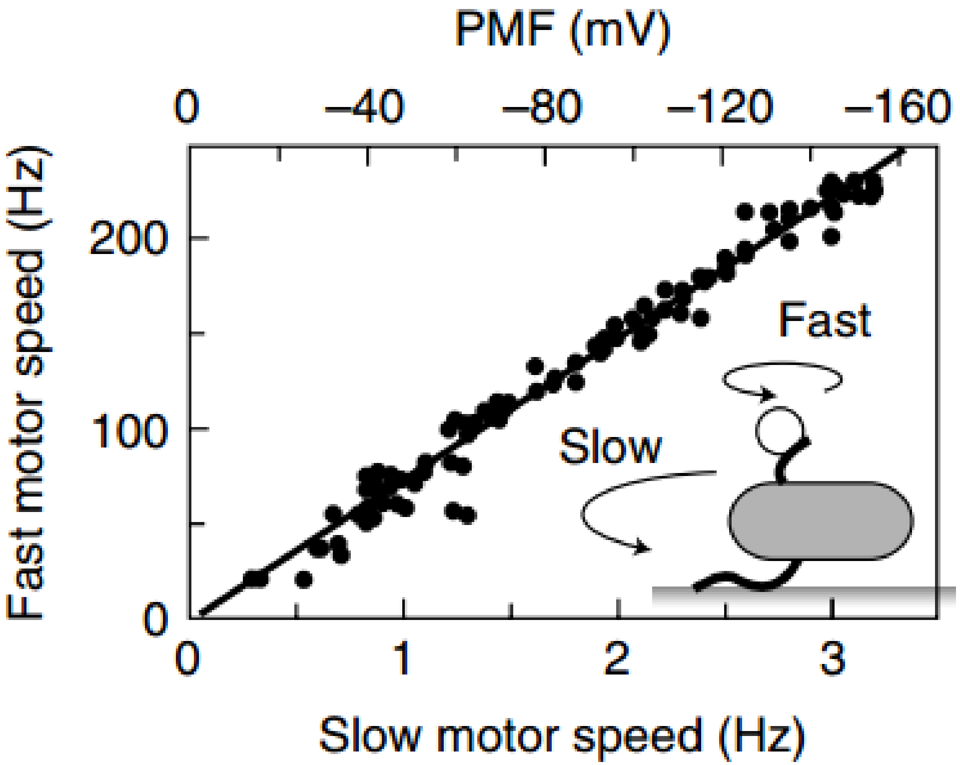
Bacterial flagellar motor can be used as a sensor for PMF



Bacterial flagellar motor can be used as a sensor for PMF

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

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



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

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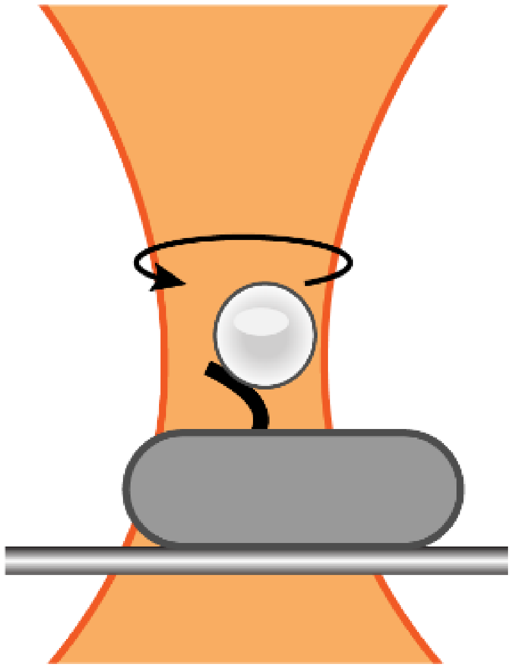
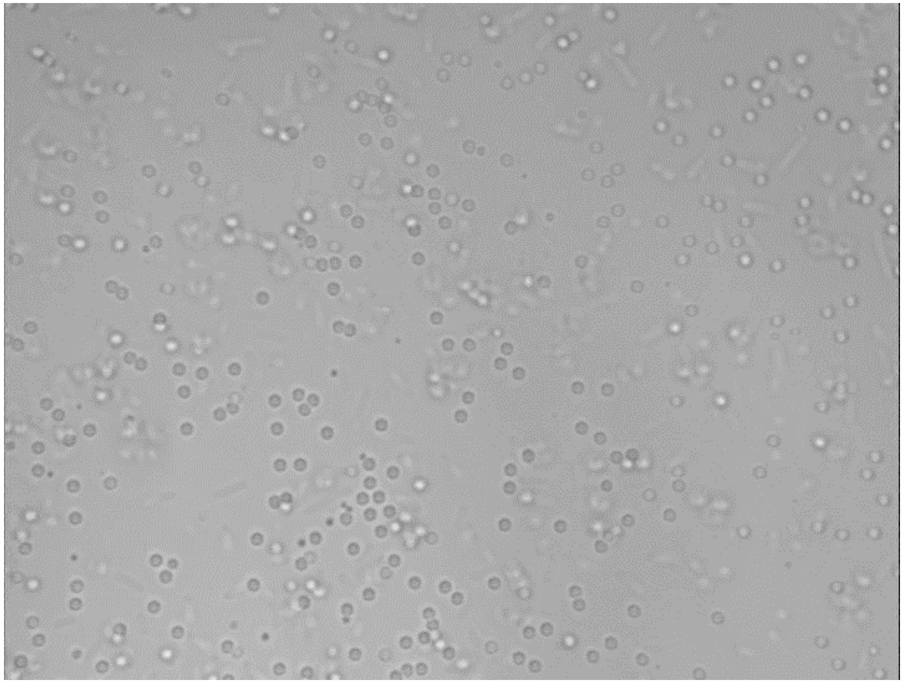




Bai, Branch, Nicolau, Pilizota, Steel, Maini, Berry, Science 2010
Pilizota, Brown, Leake, Berry, Armitage, PNAS 2009
Rosko J, Martinez V, Poon W, Pilizota T, PNAS, 2017

Bacterial flagellar motor can be used as a sensor for PMF

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

Video a gift from Keiichi Namba

Bead assay experiment

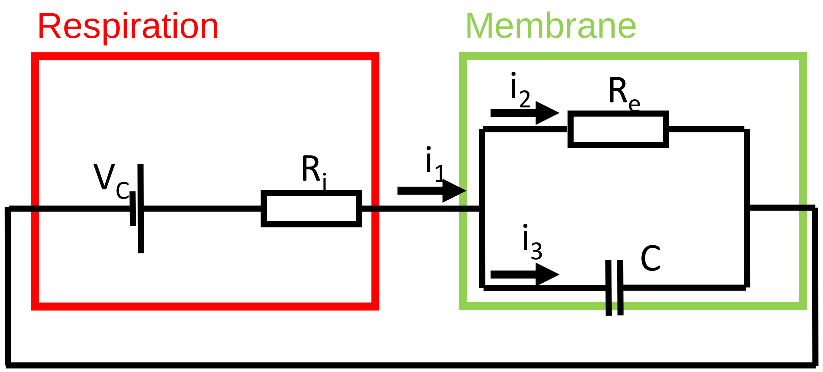
University of Edinburgh

SynthSys
Centre for Synthetic and Systems Biology

PMF dynamics can be modelled with a simple electric circuit

$$\mathrm{PMF} = \Delta pH + V_m$$

$\Delta pH = 0$ when $pH_{in} \sim pH_{out}$



Electrical circuit analogy gives a frame work:

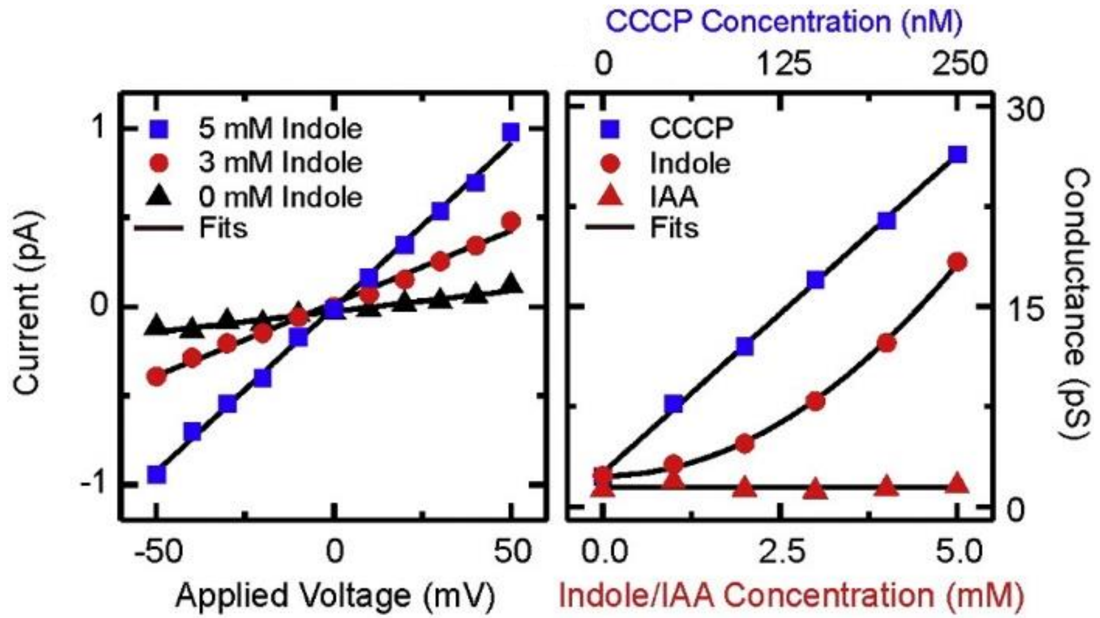
- knowing the shape of PMF response, predict the mechanism and dynamics of the damage and give functional dependency between circuit parameters and the amplitude of stress

$$\frac{V_m}{V_{m,0}} = \frac{\omega}{\omega_0} = f(S, t)$$

We are starting with a known stress

Indole is a protonophore at high concentrations

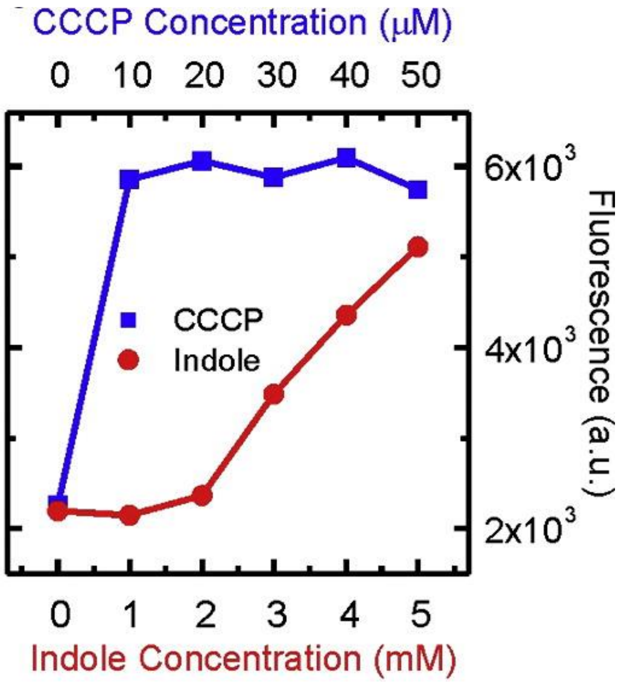
- Like CCCP, indol is a protonophore
- Dependence on indol concentration is quadratic (due to the carrier dimer formation)
- The effect of indol on bilayer conductance is reversible



Chimerel C, et al, Biochem. Biophys. Acta, 1818 (2012)

Indole is a protonophore at high concentrations

Oxonol VI, anionic dye that binds to lipid membranes



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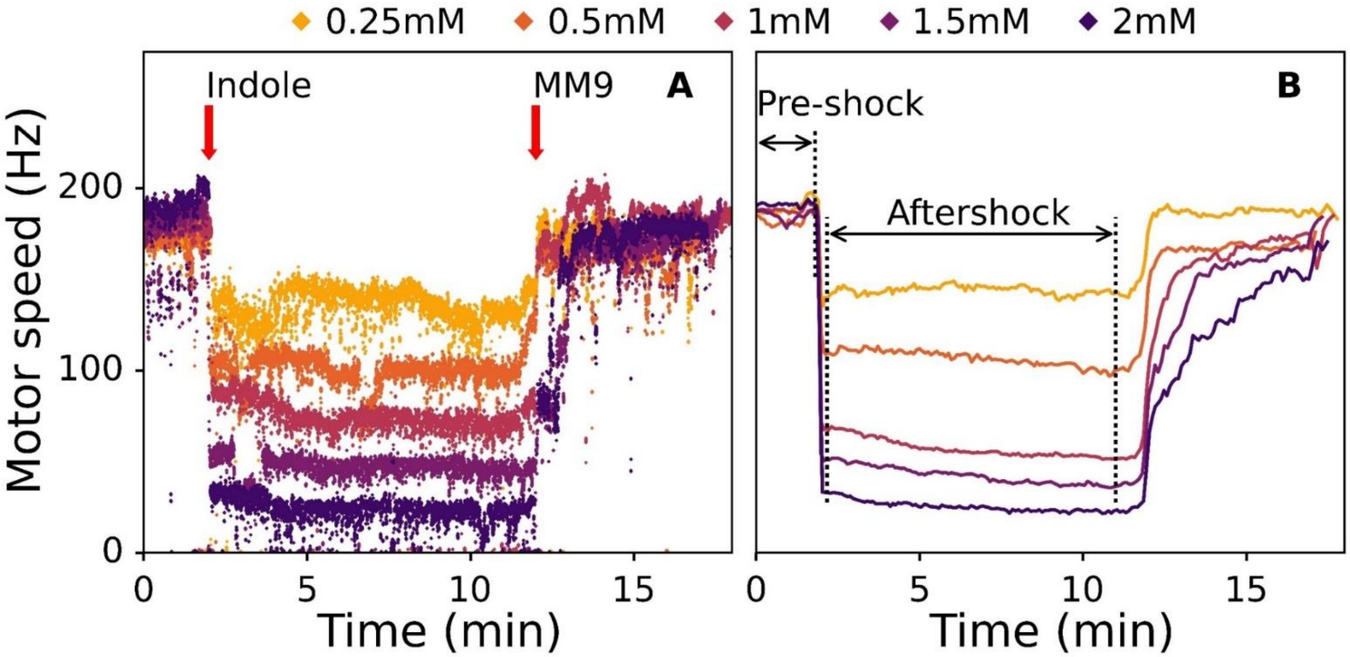
SynthSys Centre for Synthetic and Systems Biology

Upon indole treatment PMF sharply drops in reversible manner

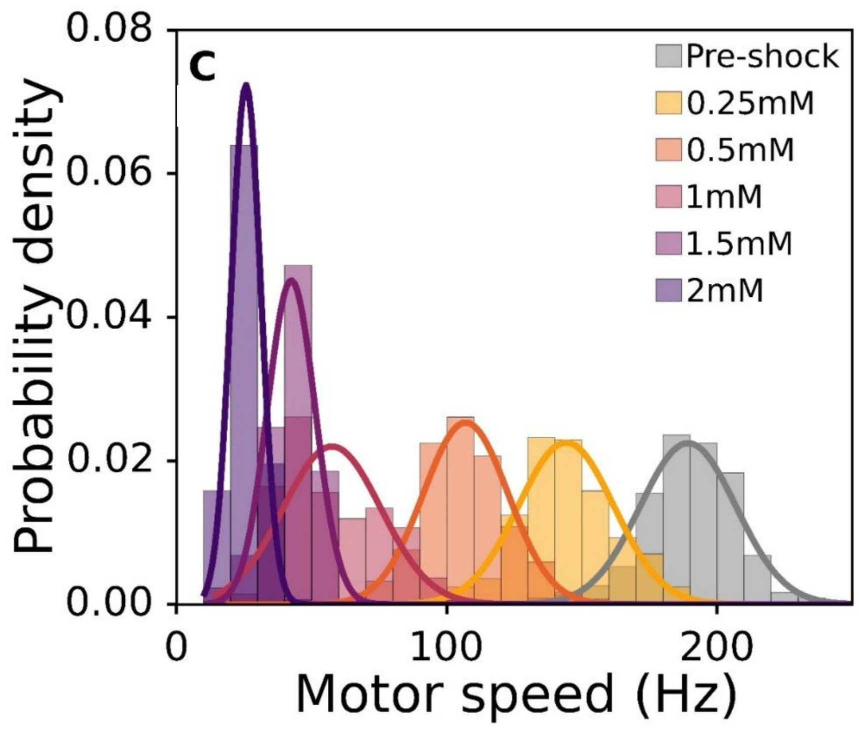
Katya

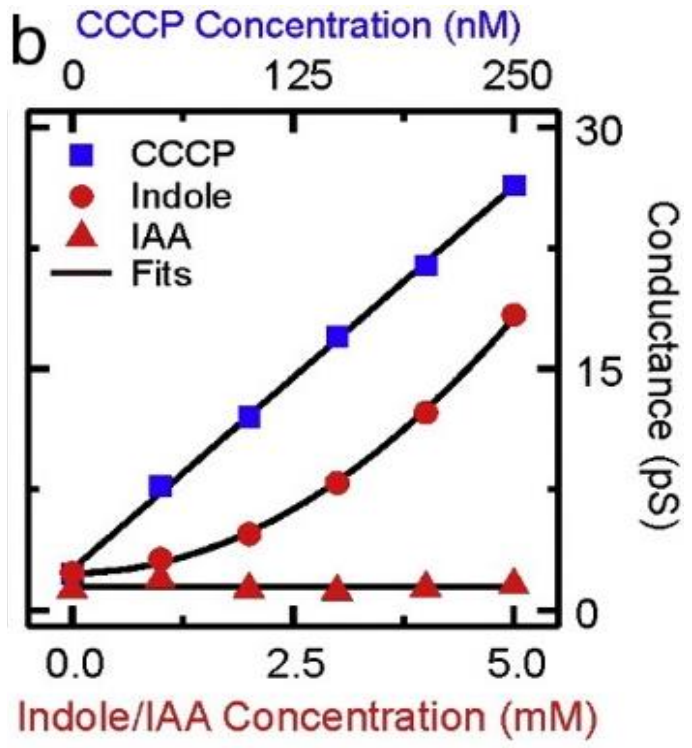
Experimental speed traces   Mean speeds



Krasnopeeva E, *et al* BiophysJ, In Revision

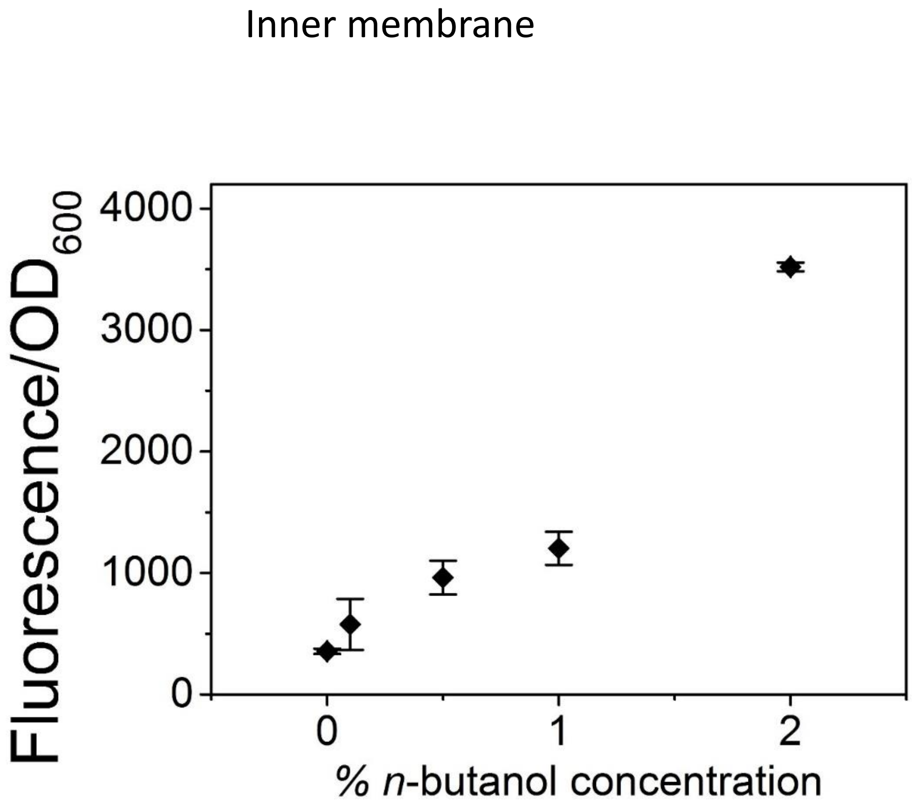
PMF is inversely proportional to the indol concentration

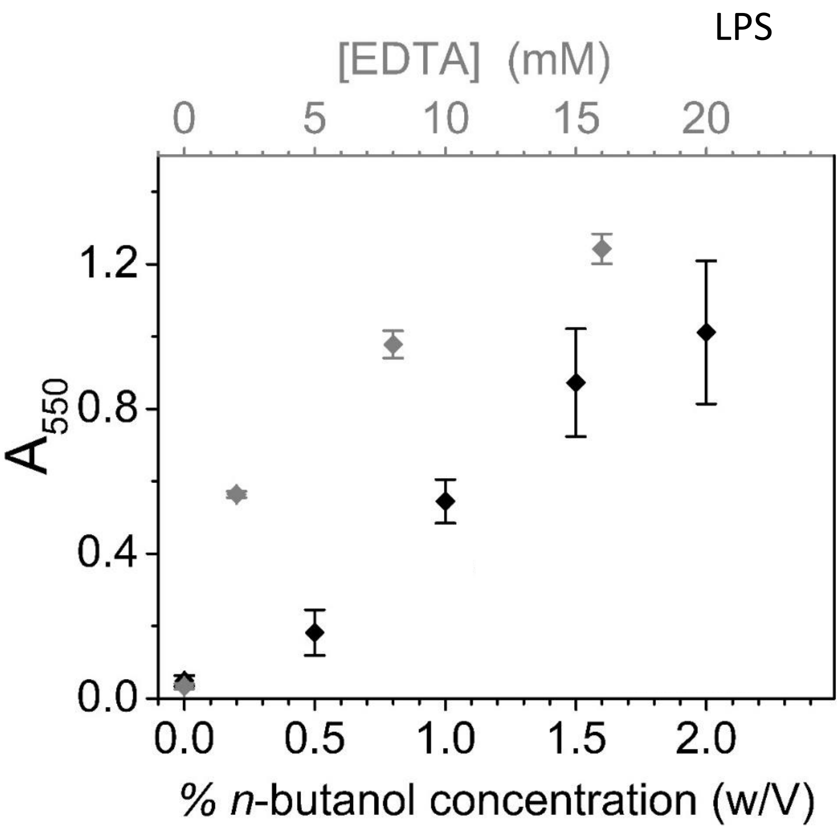




Krasnopeeva E, *et al* BiophysJ, In Revision

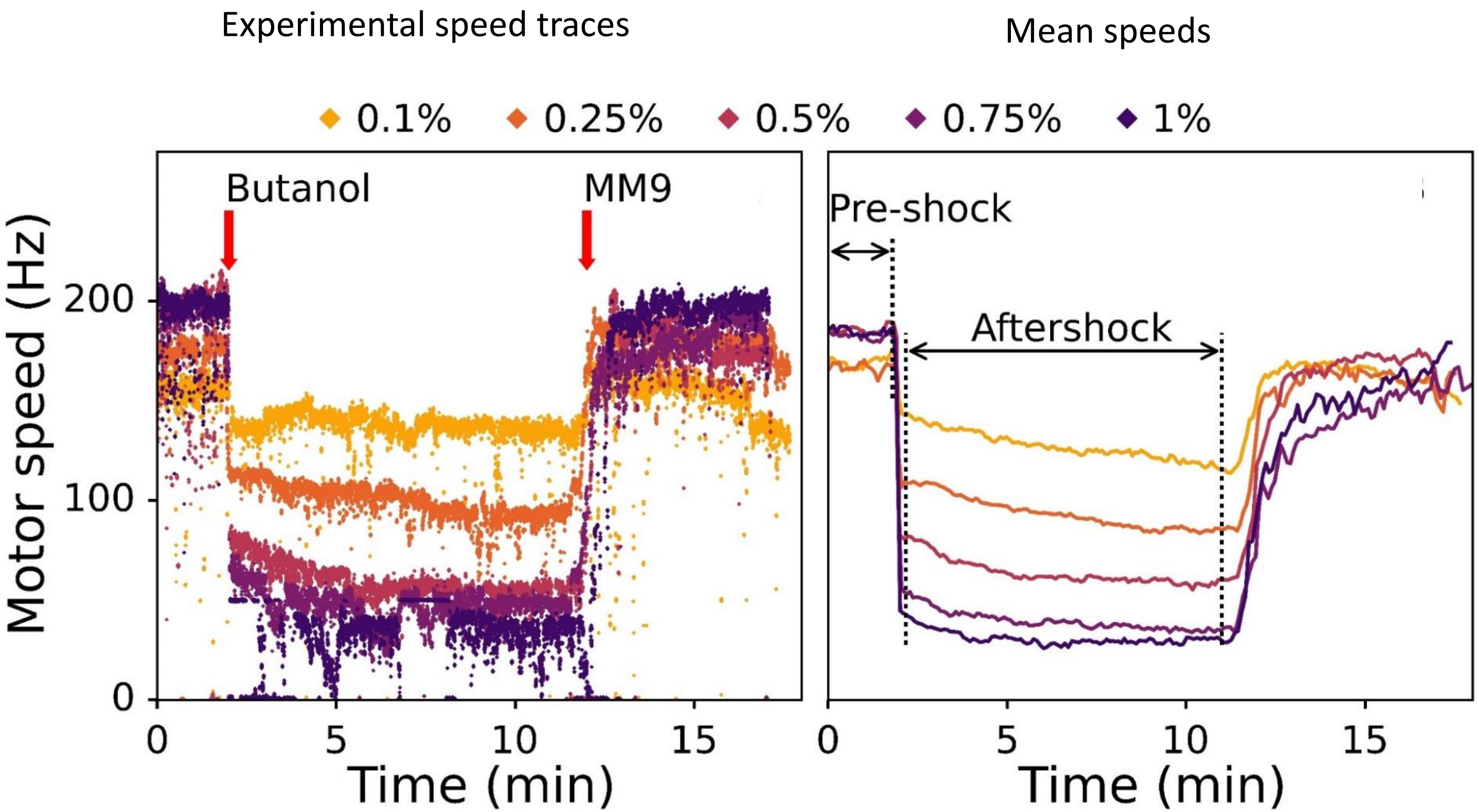
The effect of butanol on *E. coli* membrane is not know





Fletcher E, et al, App. Microb, and Biotech, 100 (2016)

Upon butanol treatment PMF sharply drops, similarly to indol



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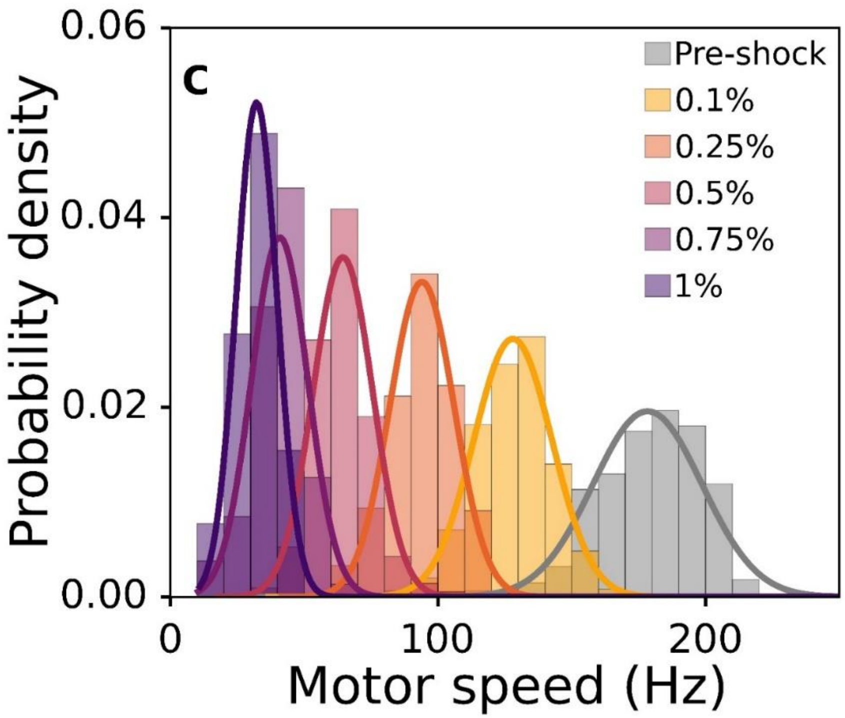
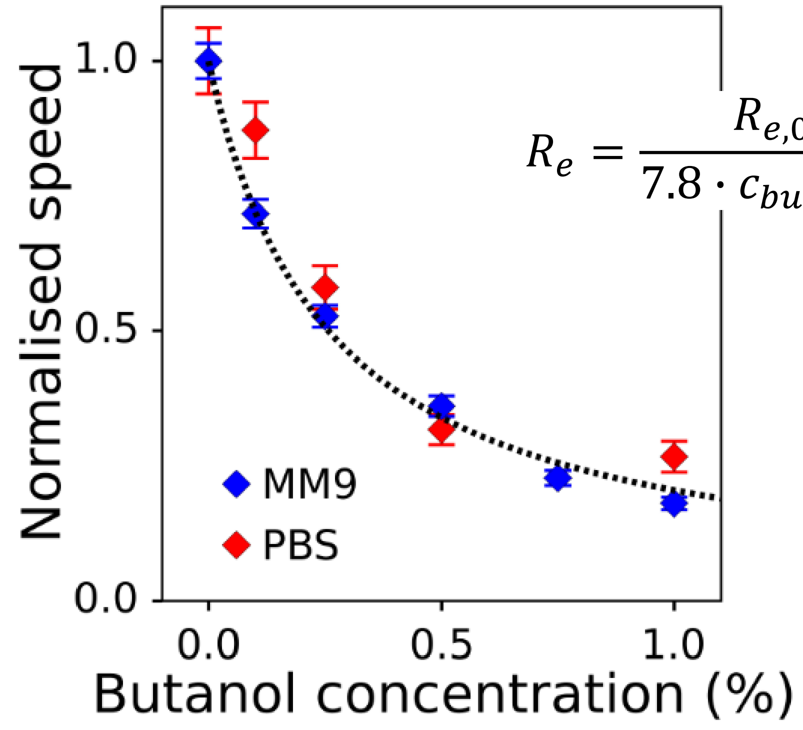
Krasnopeeva E, *et al* BiophysJ, In Revision

PMF is inversely proportional to the butanol concentration



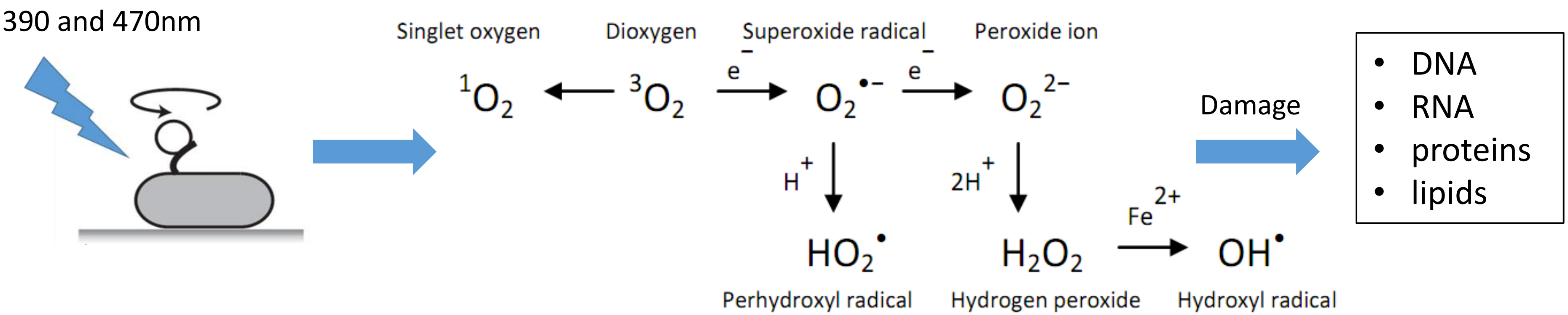


$$R_e = \frac{R_{e,0}}{7.8 \cdot c_{but} + 1}$$

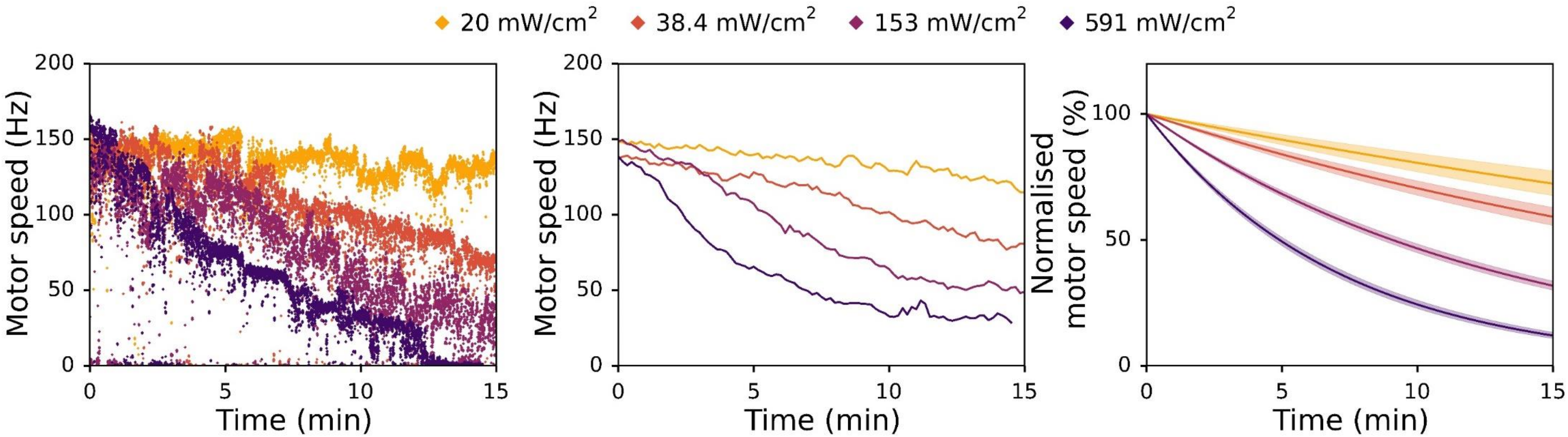
Krasnopeeva E, *et al* BiophysJ, In Revision

Exposure to light can lead to membrane damage

Cell exposure to light of a short wavelength induces reactive oxygen species (ROS) formation



Exposure to light causes gradual drop of PMF



Experimental speed traces

Mean speeds

Exponential fit: $\omega = \omega_0 e^{-\alpha t}$

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PMF decay function can be inferred

$$PMF = PMF_0\, e^{-(a*lnP+b)t}$$

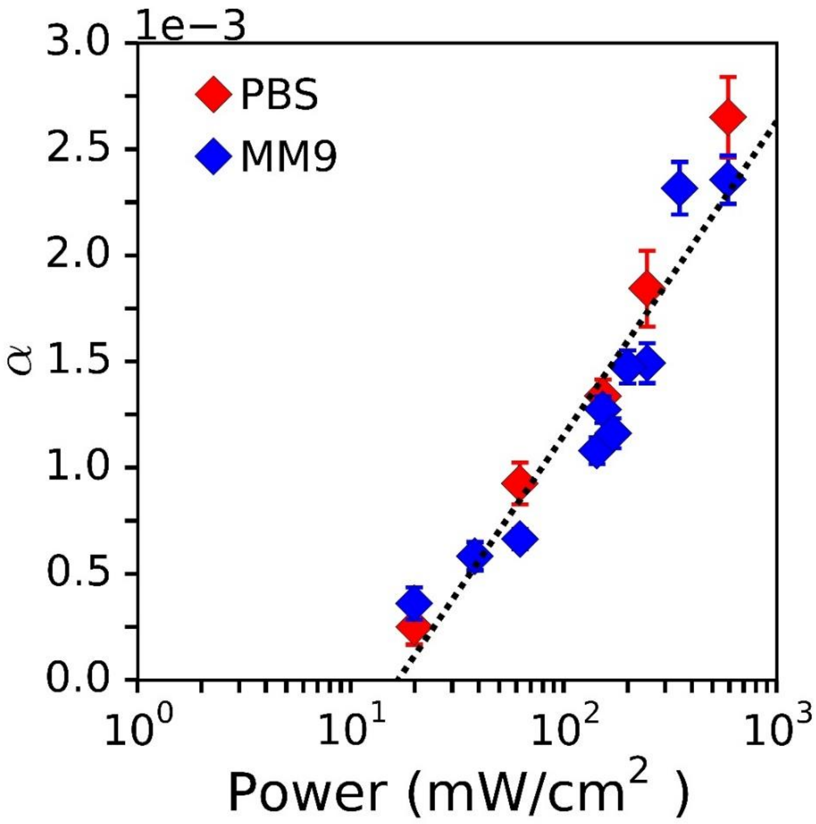
$$R_e = \frac{R_{e,0}}{2\cdot\left(\frac{P_{eff}}{P_{eff,0}}\right)+1}$$

$PMF = PMF_0\, e^{-\alpha t}$

$\alpha = a * lnP{+}P_0$

where *P* is an effective power

- The shape of the function does not depend on the energy source or on the way we apply light
- P_0 is a minimal power required for the damage to occur



Total: 277 cells in MM9 and 116 cells in PBS; 393 total

Krasnopeeva E, *et al* BiophysJ, In Revision

Conclusions

Analysing PMF during indol treatment confirms it as protonophore

Same analysis shows butanol is ionophore

We identified a functional dependency between the degree of photodamage and light intensity

Analysing PMF could be used further to probe other stresses, overall membrane resistance, and potentially I-V curve of different components within the membrane

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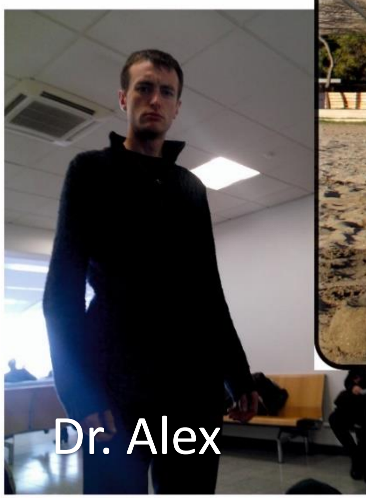

Guillaume

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