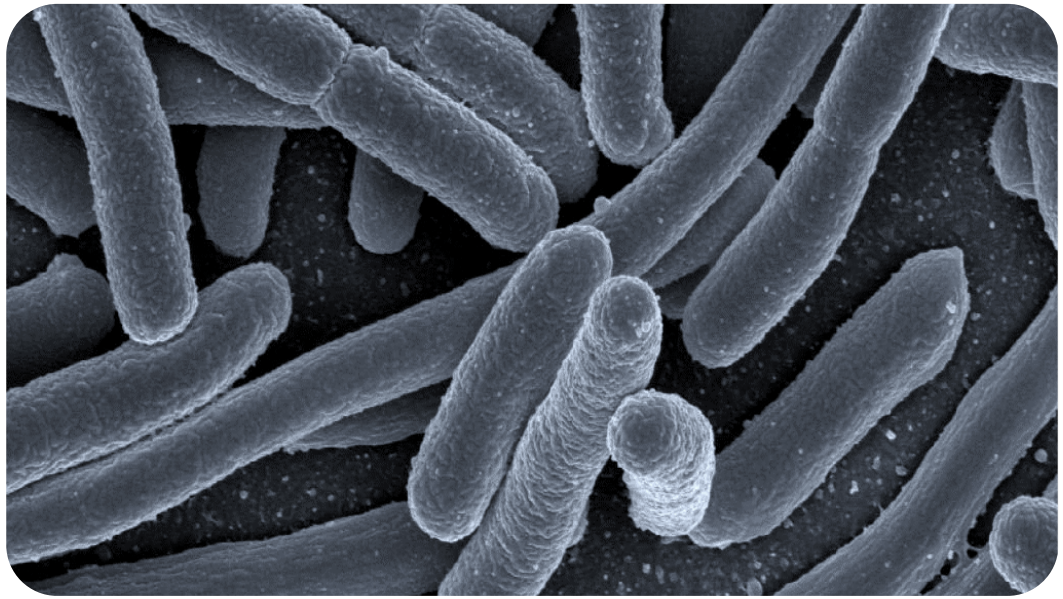
Electrochemical control of bacterial permeability



Santiago Caño-Muñiz

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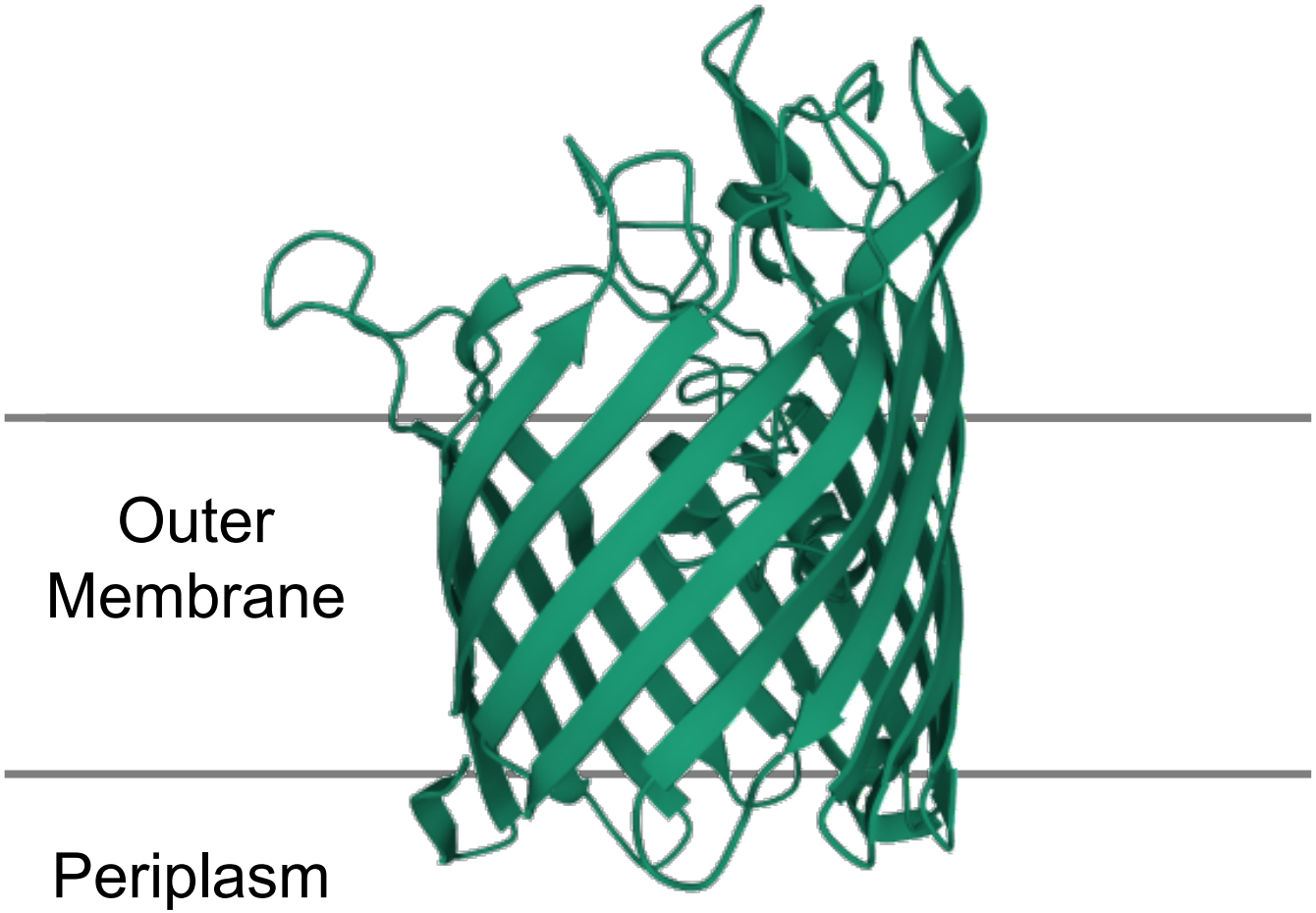
Floto Group
University Molecular Immunity Unit

Thesis outline

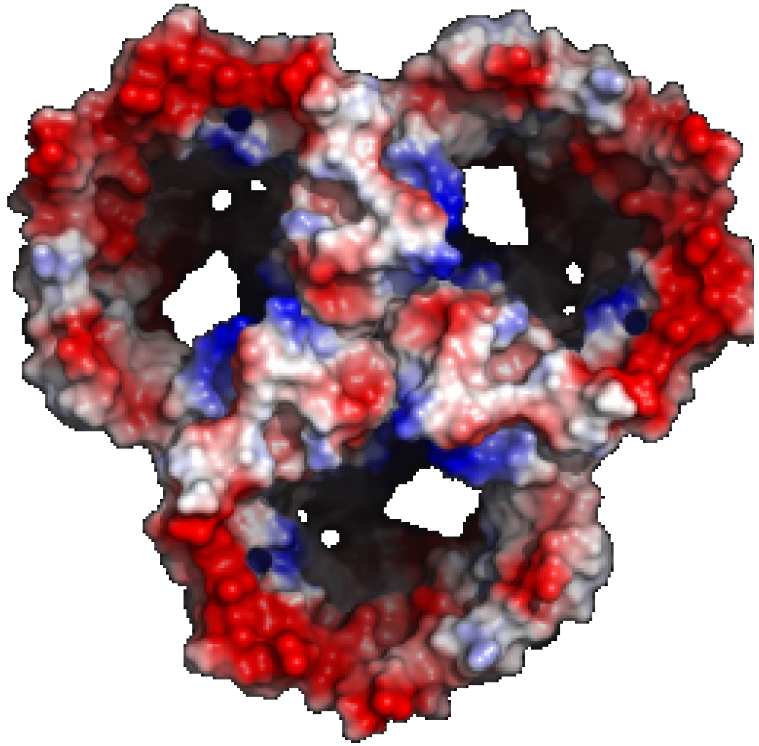
1. Periplasmic ions and porin permeability
    - Increase in intracellular K^+ increases porin permeability
    - Increase in intracellular H^+ decreases porin permeability
    - Optogenetics acidification of the periplasm abolish porin permeability
    - Molecular dynamics simulation indicates protonation periplasmic residues constrains pore size
2. Intracellular ion oscillations
    - Periplasmic pH is buffered from the external medium
    - Oscillation in pH, K and periplasmic pH but not Ca^{2+}
    - Membrane depolarization correlates with 2NBDG uptake
    - Rich carbon sources trigger membrane potential spikes
3. Ciprofloxacin uptake
    - Glucose increase ciprofloxacin uptake compared to lipids
    - Forcing E. coli to use lipid media reduces ciprofloxacin susceptibility
    - Metabolic KO only increase resistance in glucose medium
4. Indole effect on membrane potential
    - Gluconeogenic carbon metabolism drives indole production
    - Indole synthesis depolarizes the bacterial membrane

Porins mediate outer membrane permeability in Gram-negative bacteria

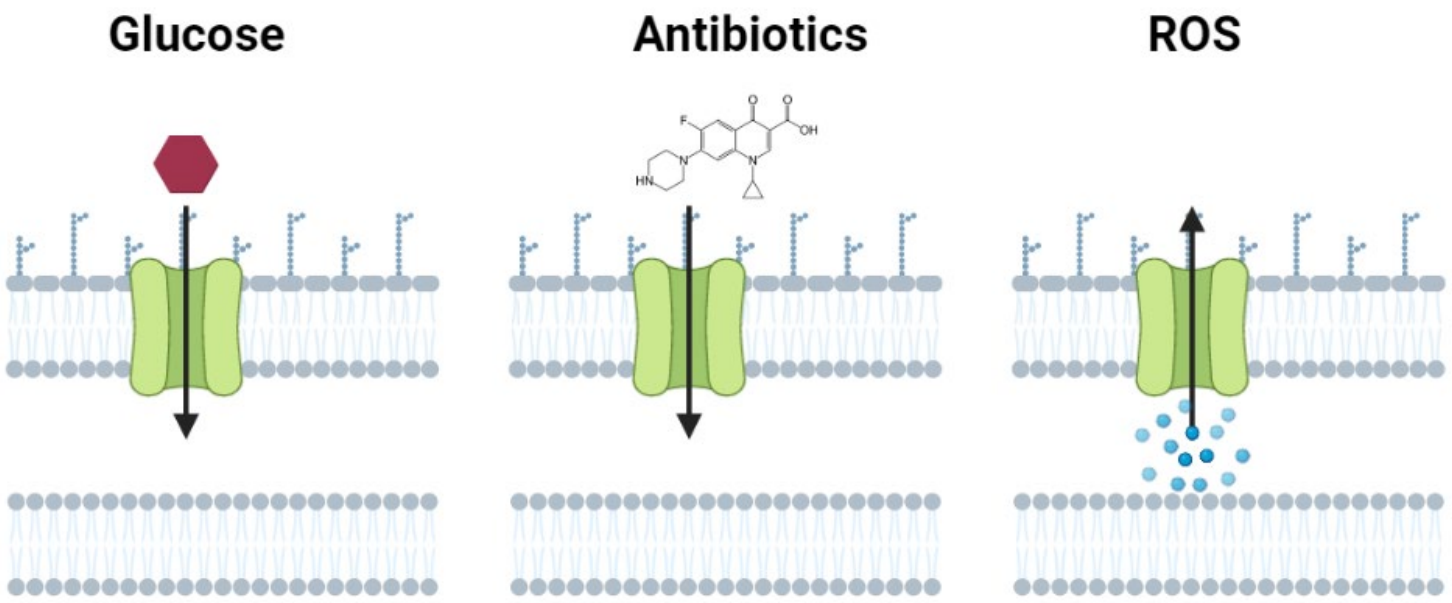
OmpC



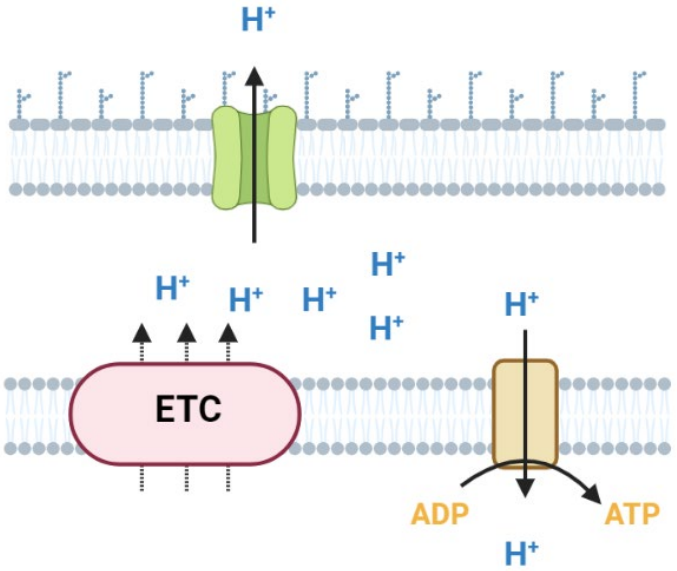
Homotrimers



Porins mediate outer membrane permeability in Gram-negative bacteria



but short-circuit respiration!



(How) Is porin permeability regulated?

in order to potentially

1) Balance **nutrient uptake** and **energy generation**

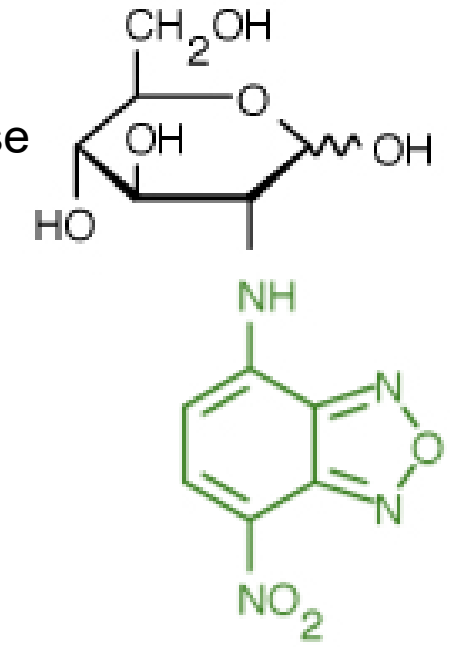
and

2) Protect bacteria from **natural** and **therapeutic antibiotics**

Measuring porin permeability using a fluorescent glucose analogue

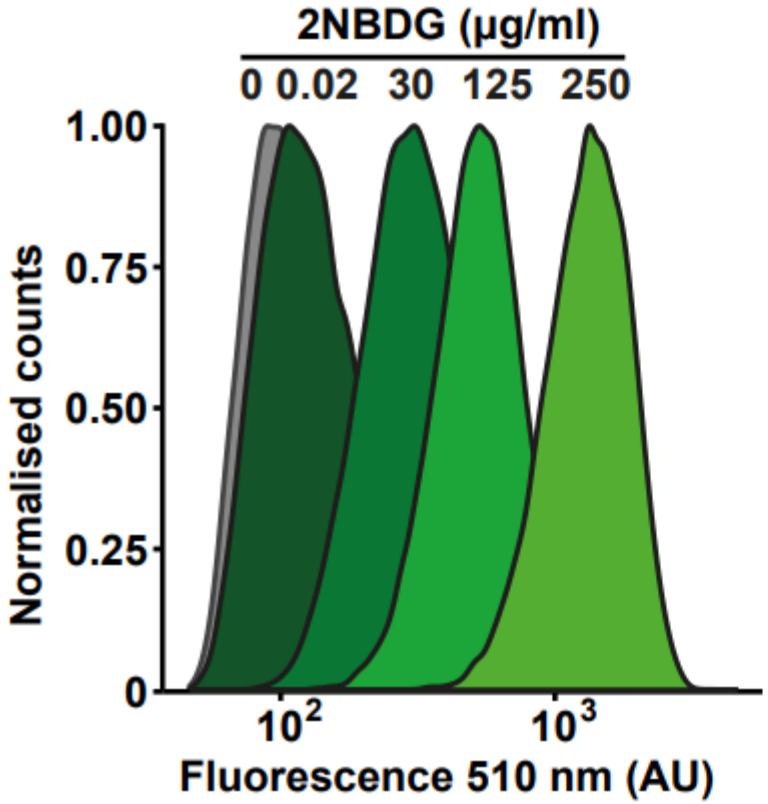
2-NBDG

Glucose

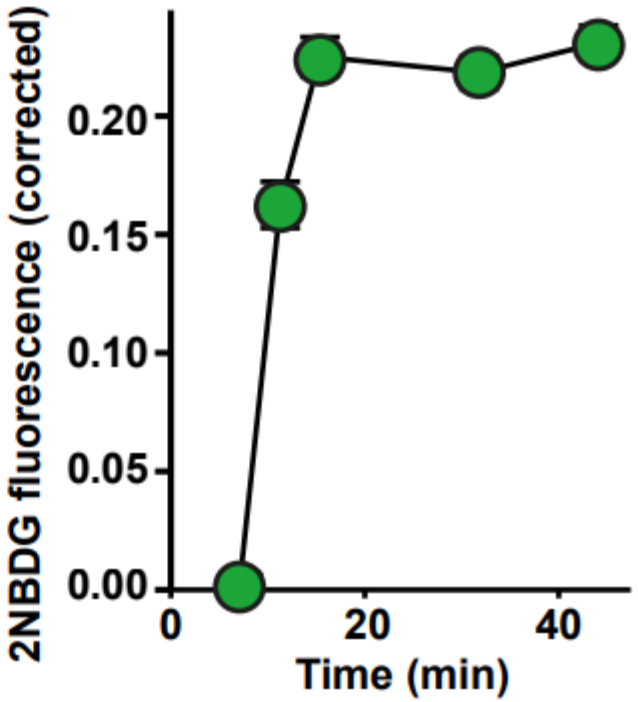


7-nitrobenzofurazan

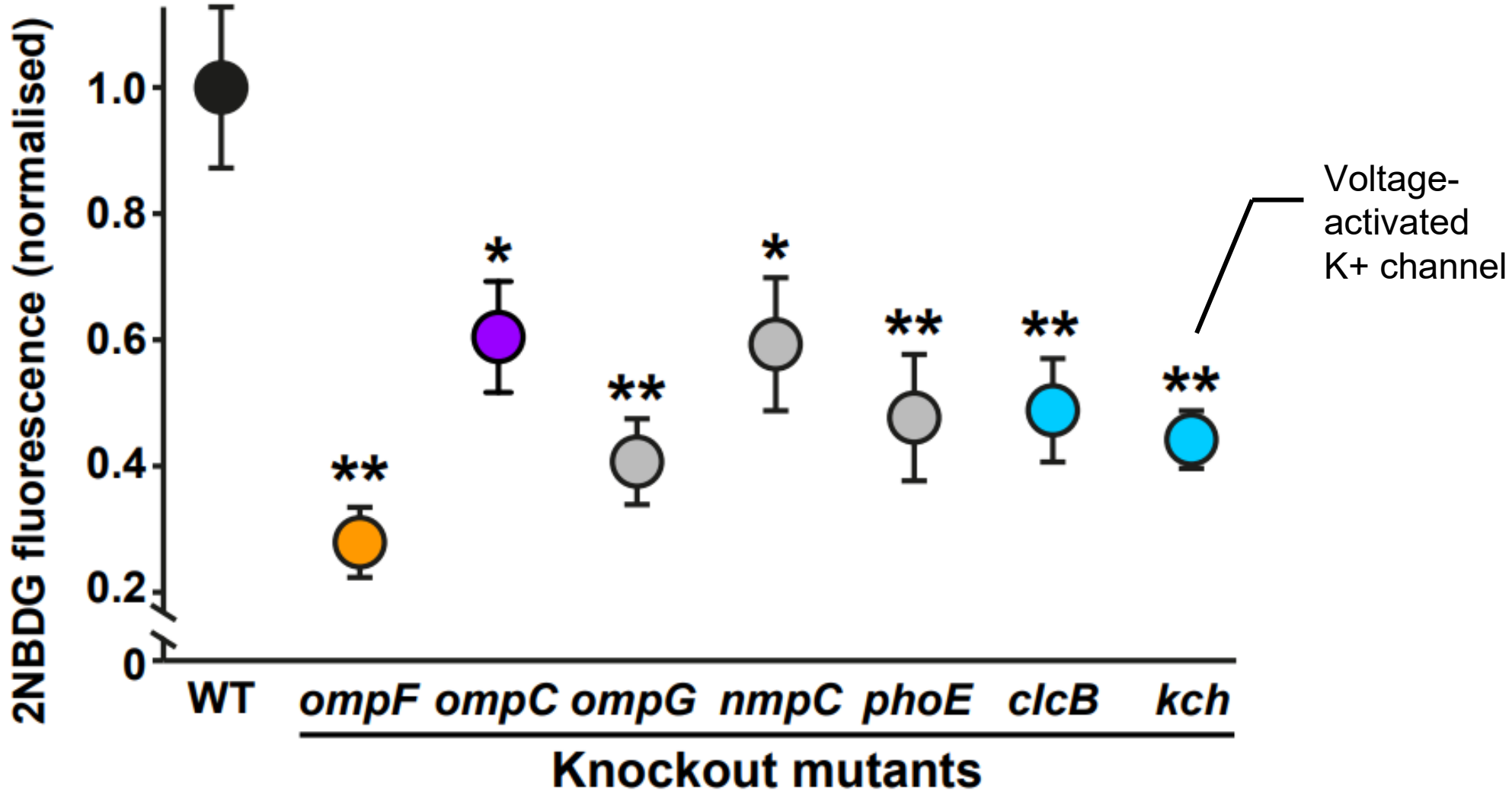
Over different concentrations



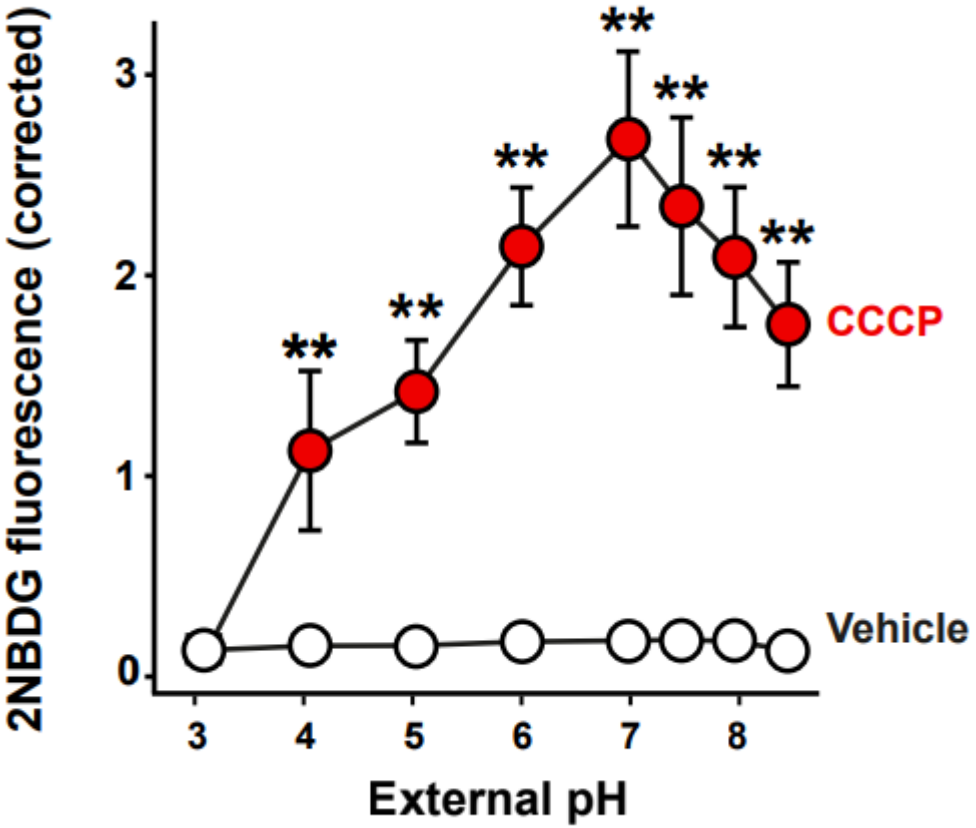
Over time

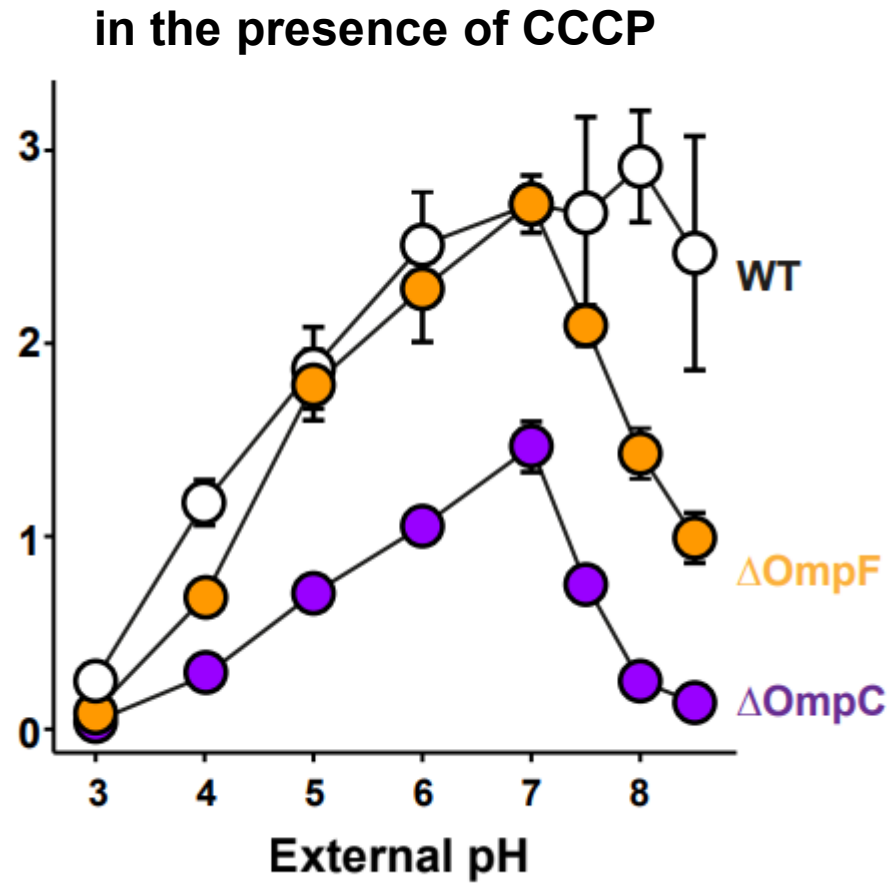


Porin permeability is regulated by ion channels



Porin permeability is regulated by internal H+ and K+





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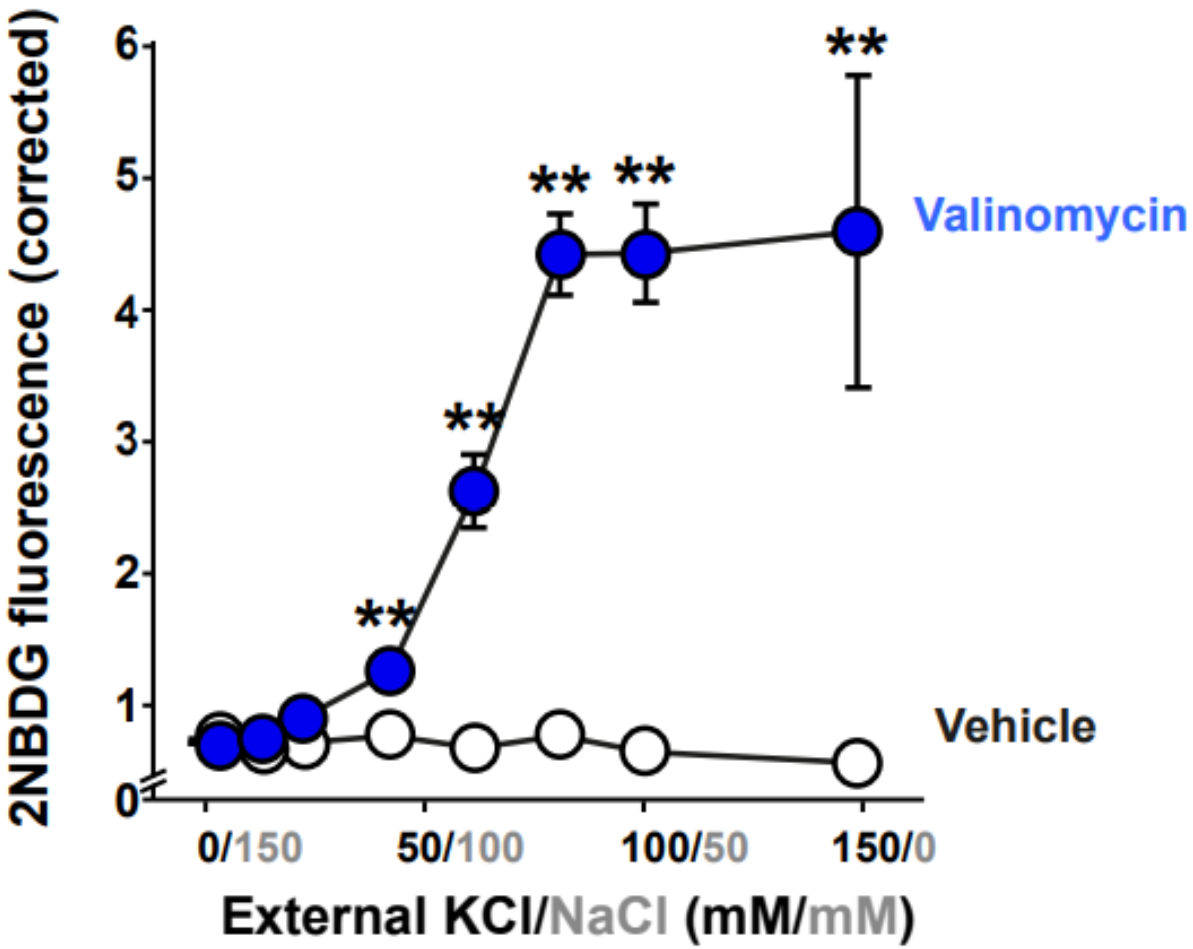
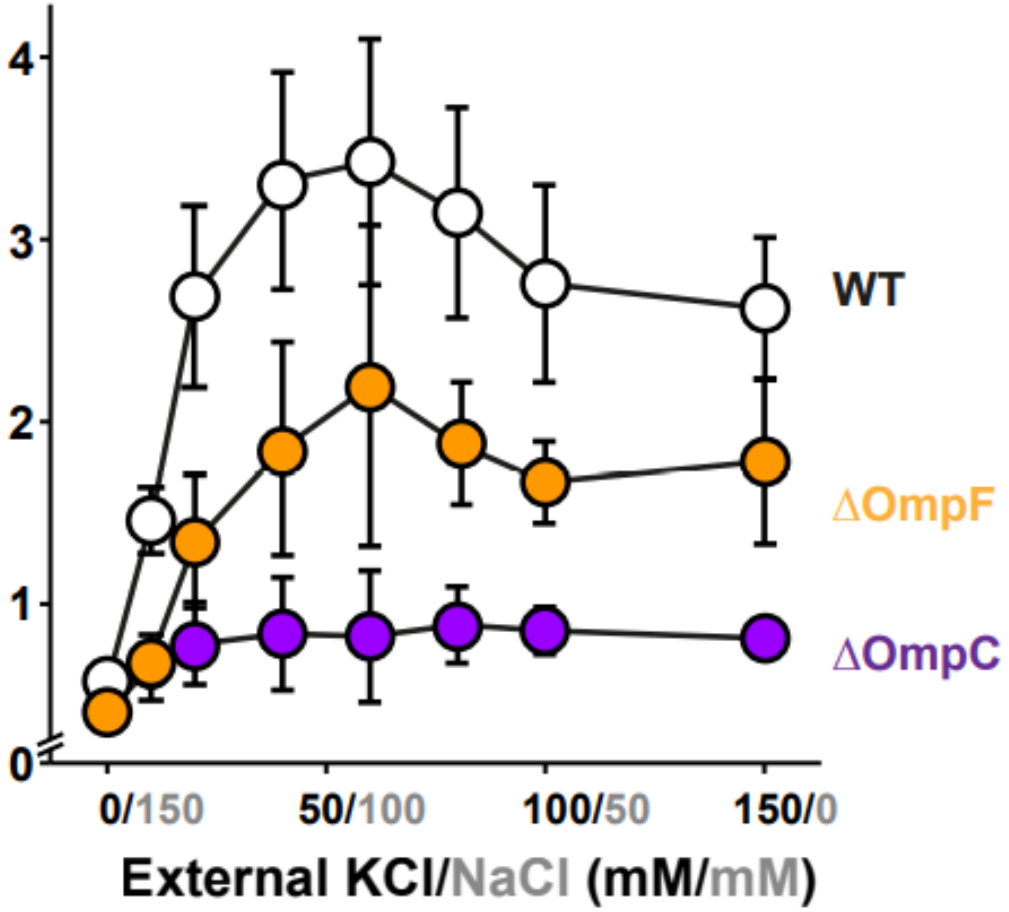
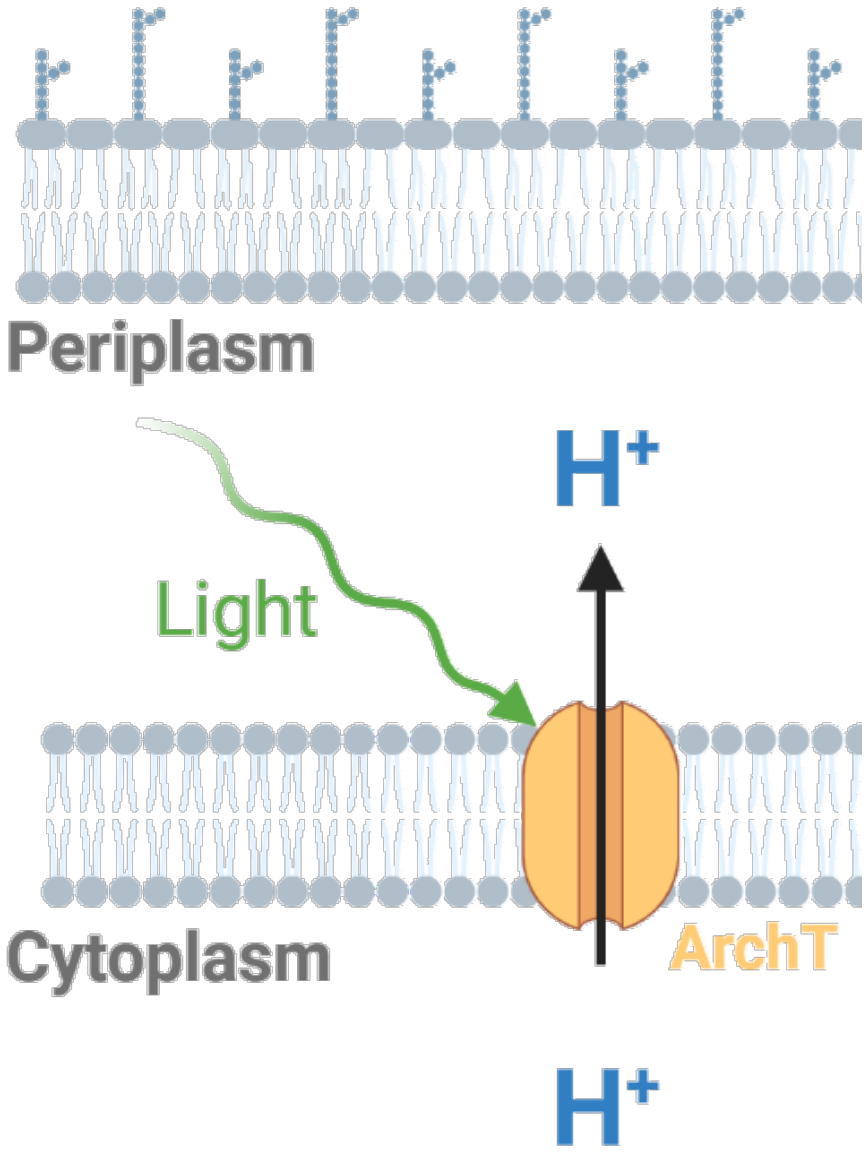


in the presence of Valinomycin

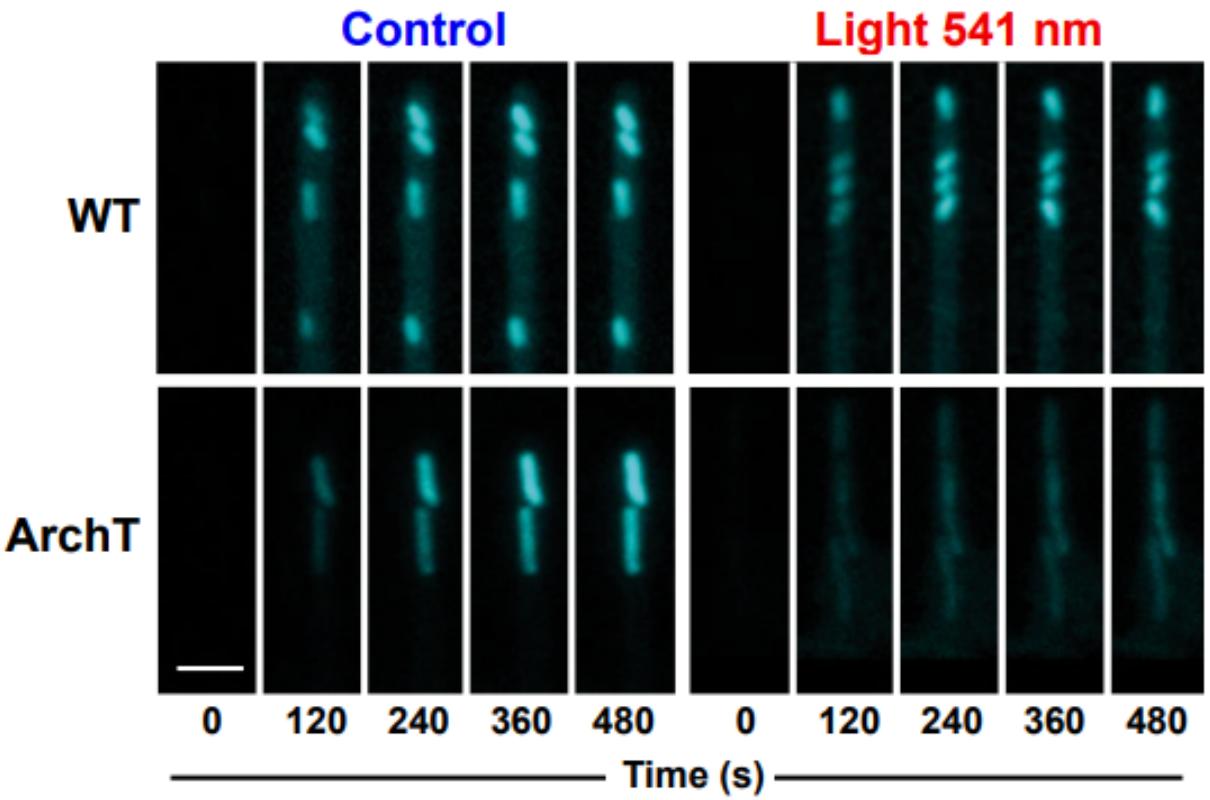


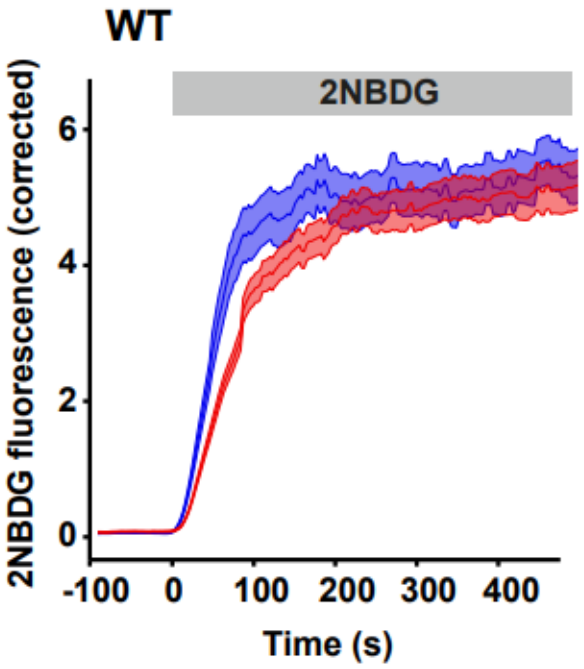
Direct manipulation of periplasmic H^+ affects porin permeability

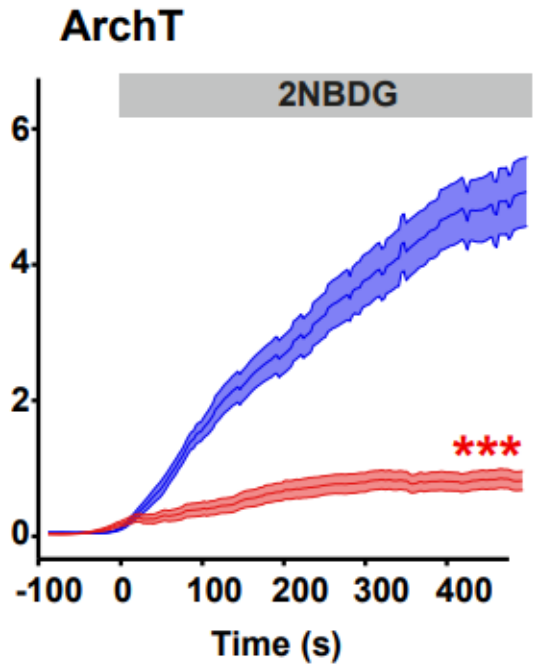


Increasing periplasmic H^+ reduces porin permeability

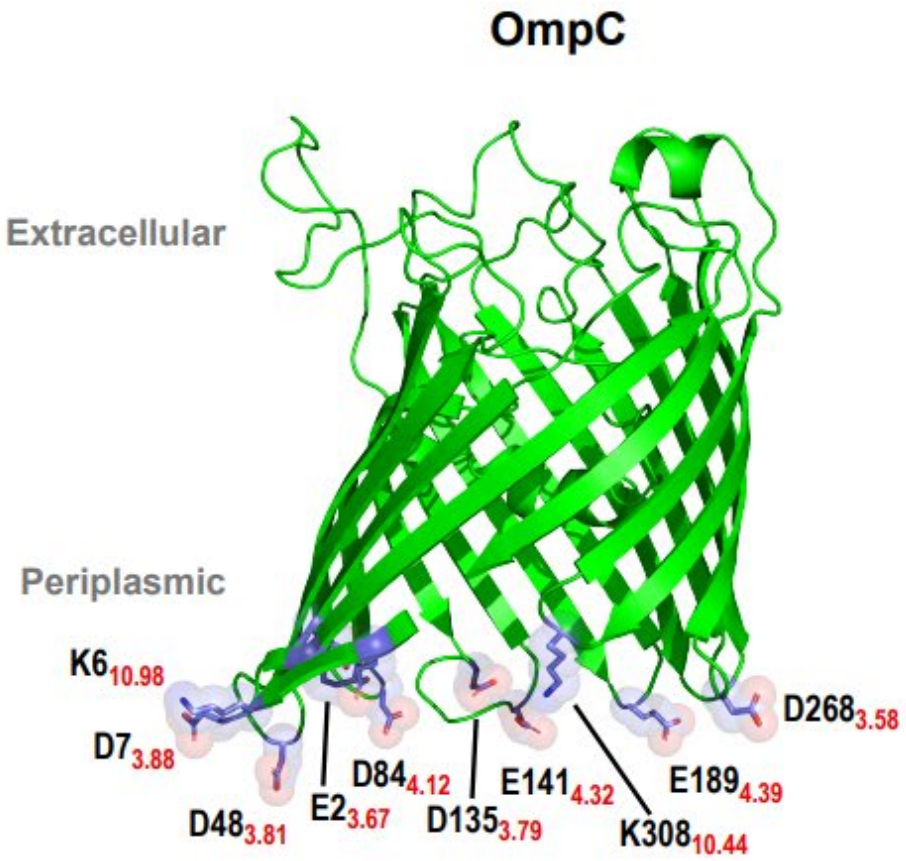
2NBDG uptake

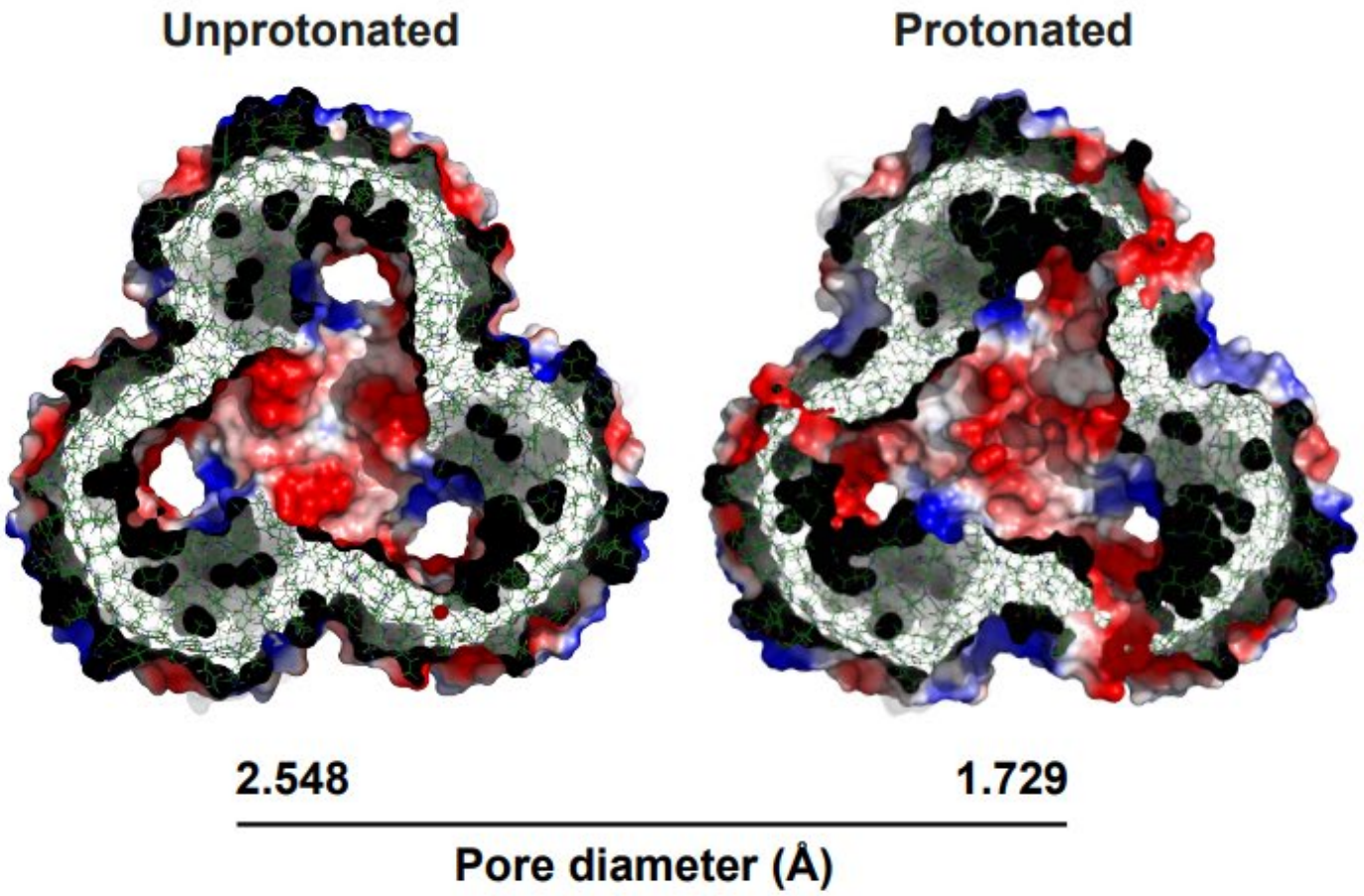






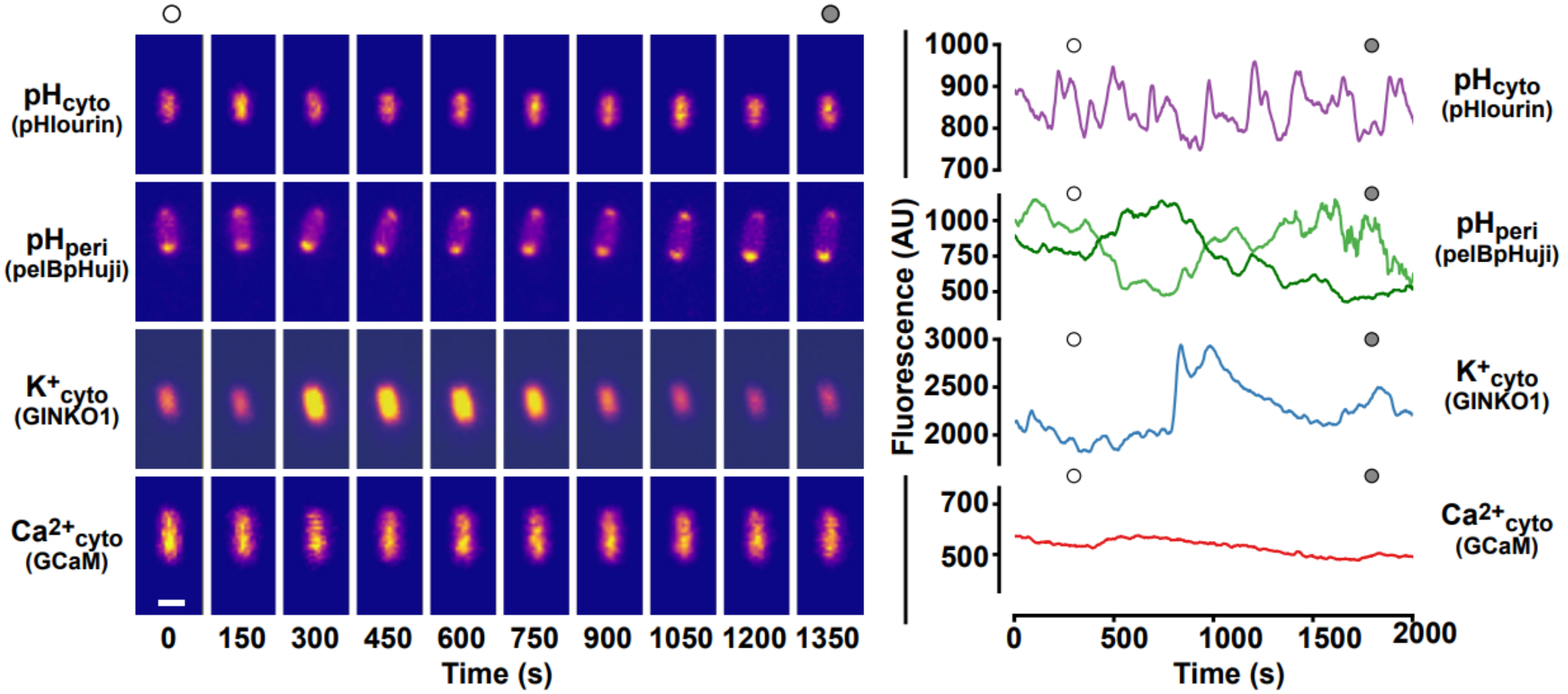
MD simulations indicate intrinsic permeability regulation by periplasmic H^+



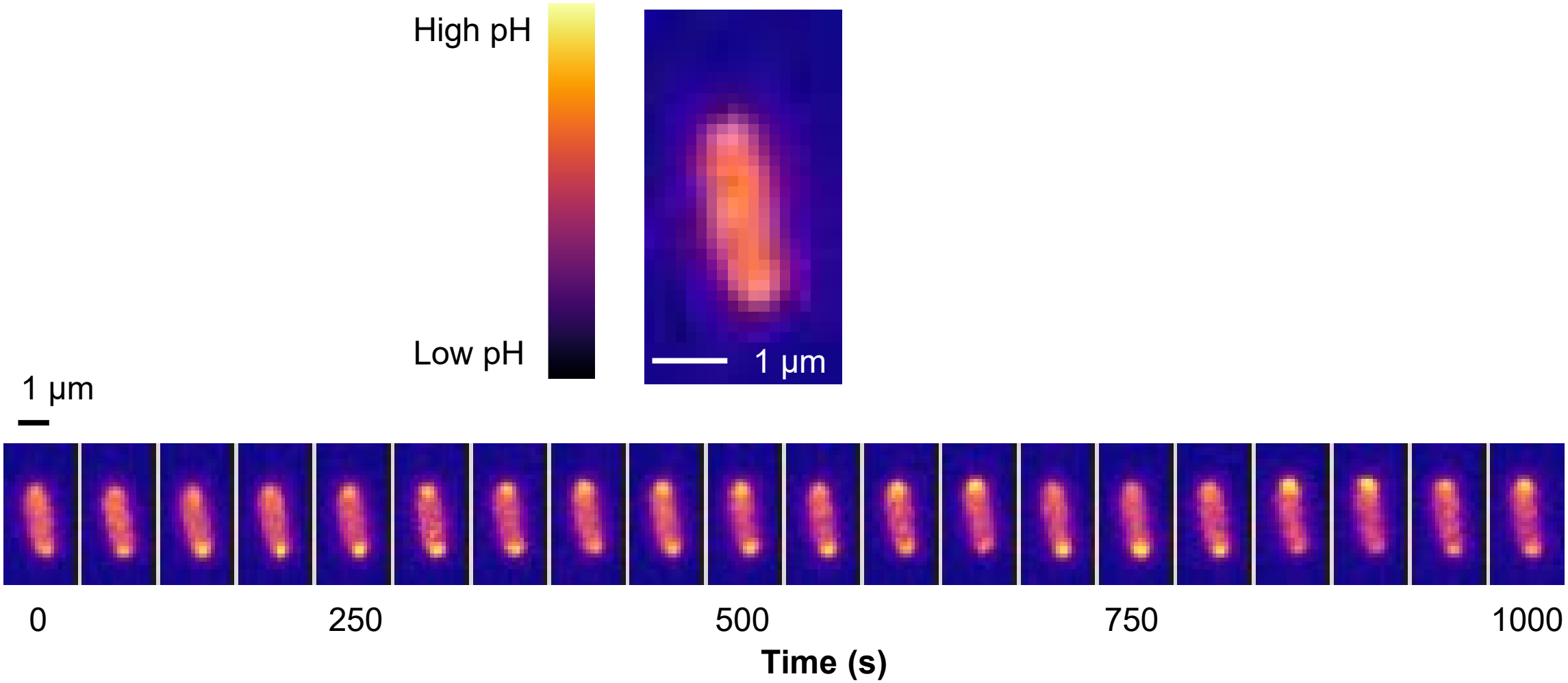


Ali Alsulami
Tom Blundell

Dynamic fluctuation of internal ions within *E. coli*

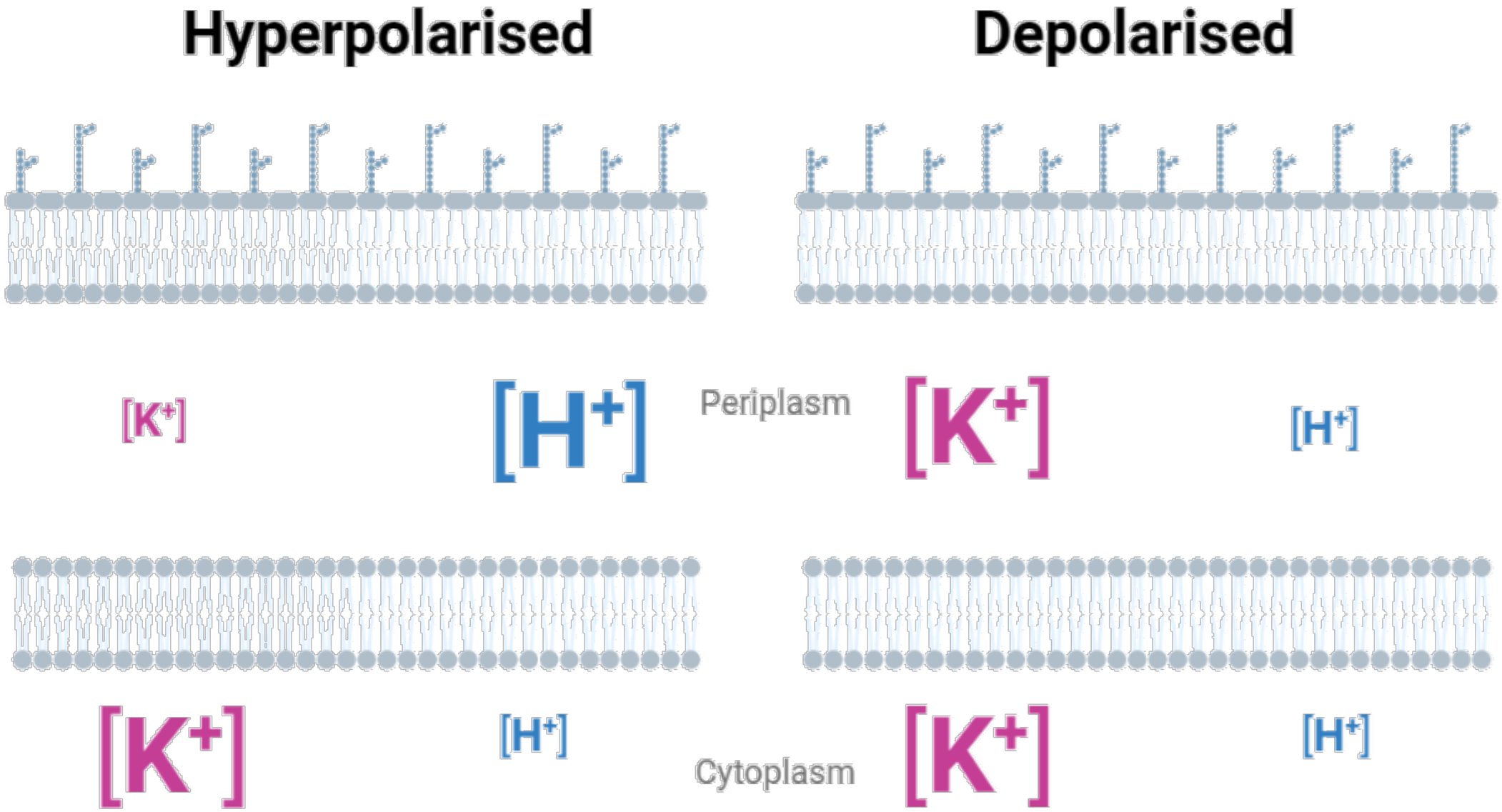


Oscillations in periplasmic H^+ within bacteria

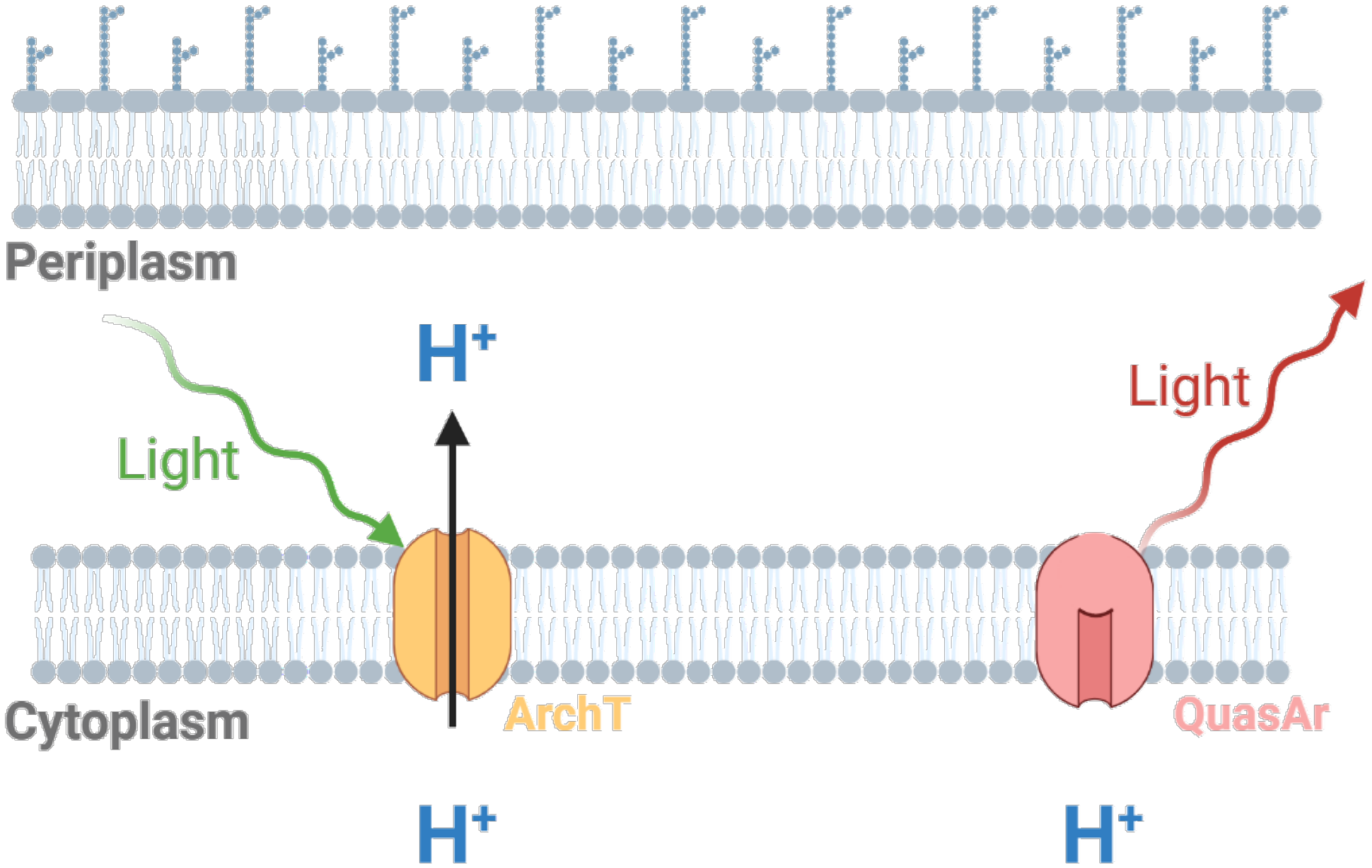


Can we use inner **membrane voltage** to monitor **periplasmic ions?**

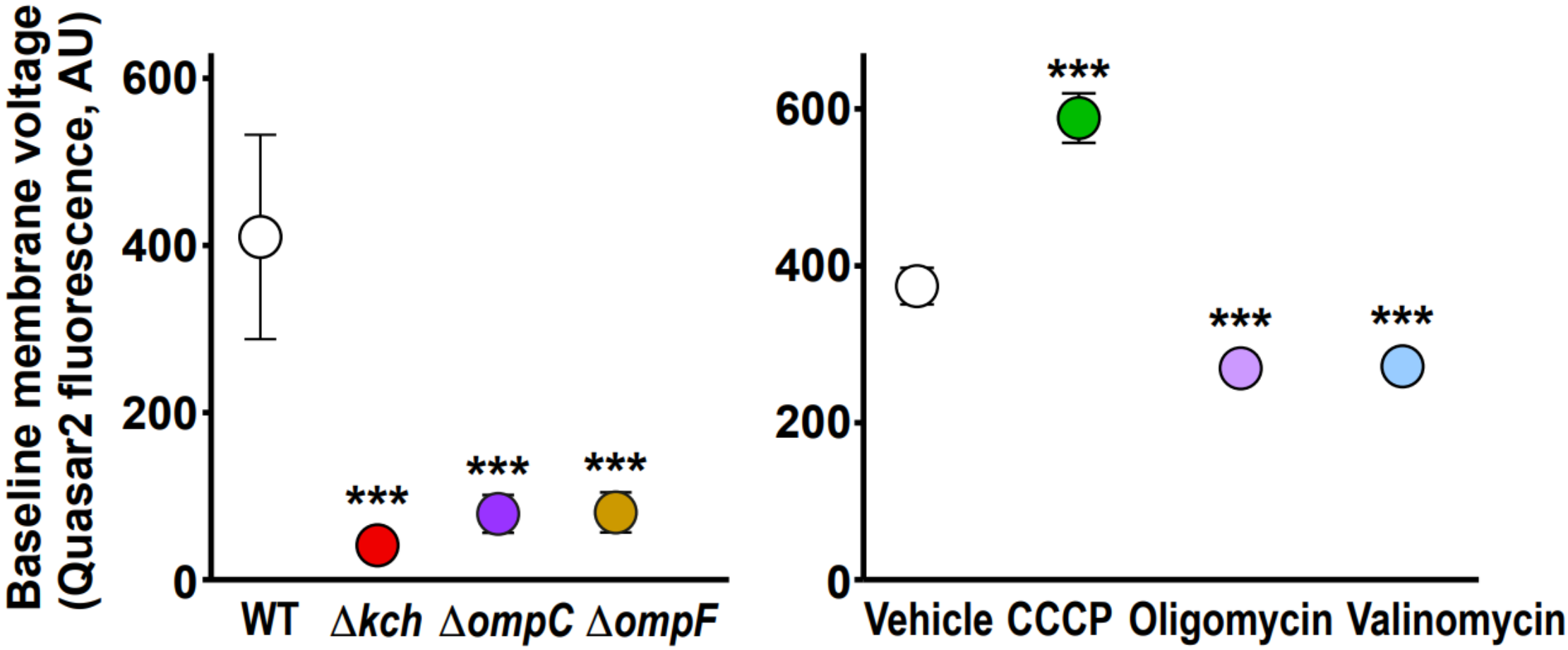
Ionic control of inner membrane voltage



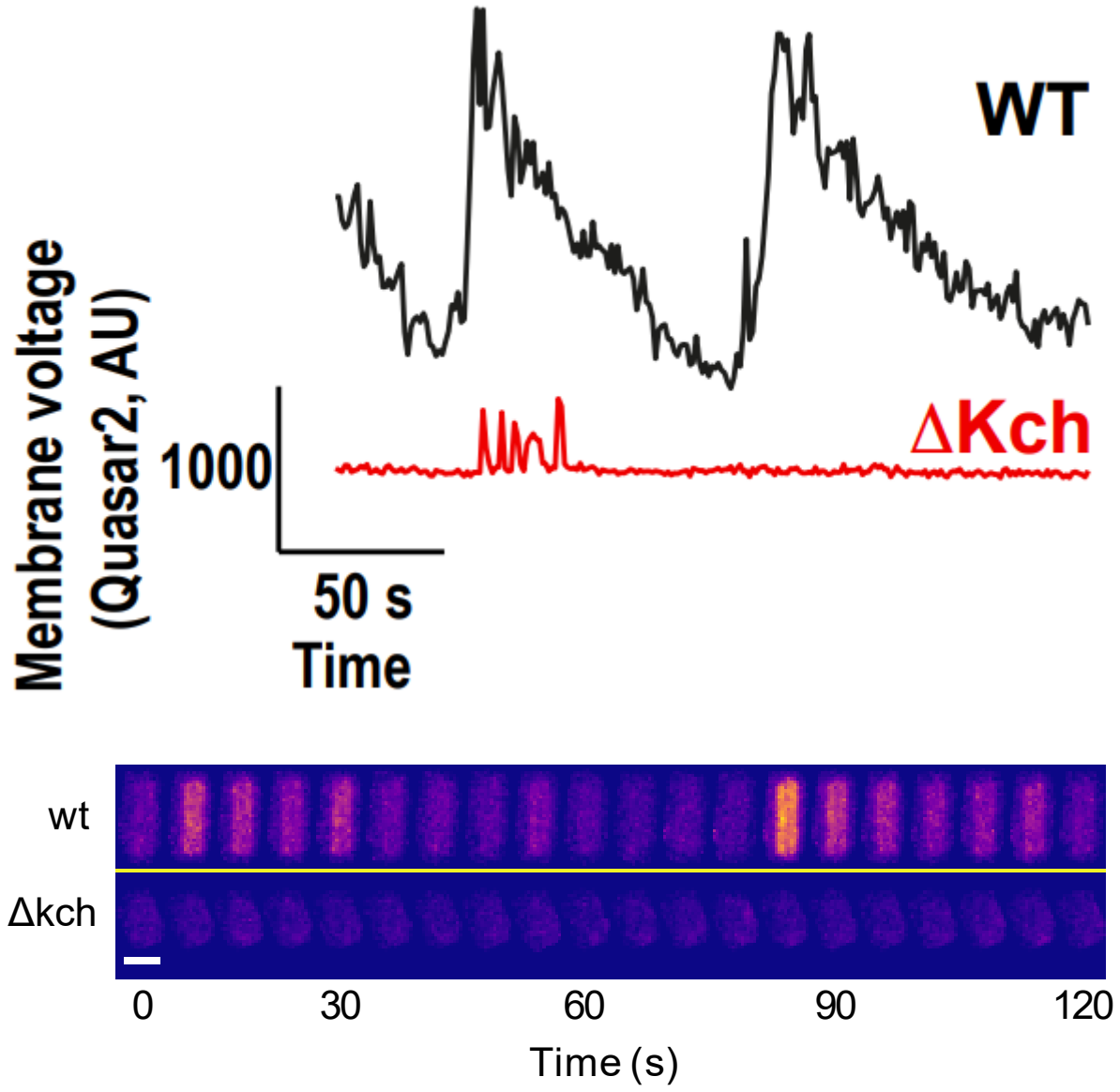
Monitoring inner membrane voltage



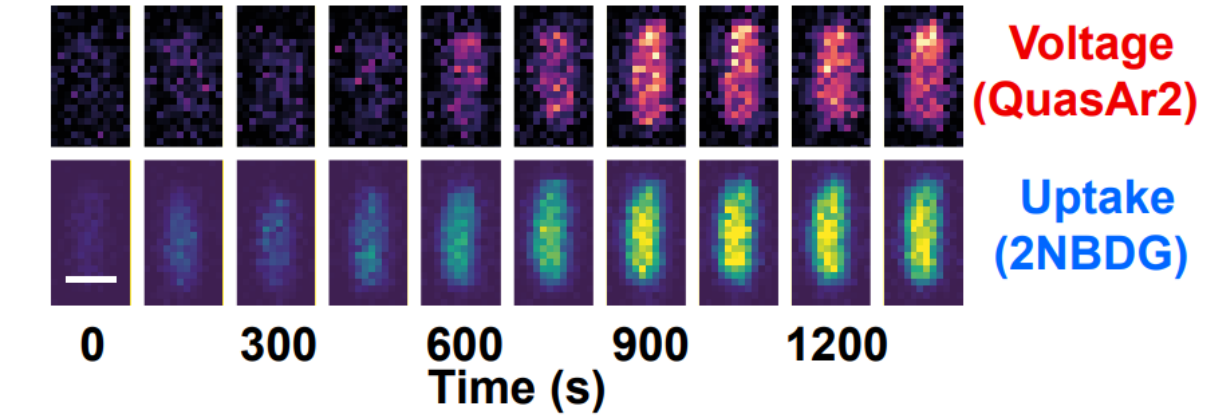
Inner membrane voltage can account for H+ and K+ conductance

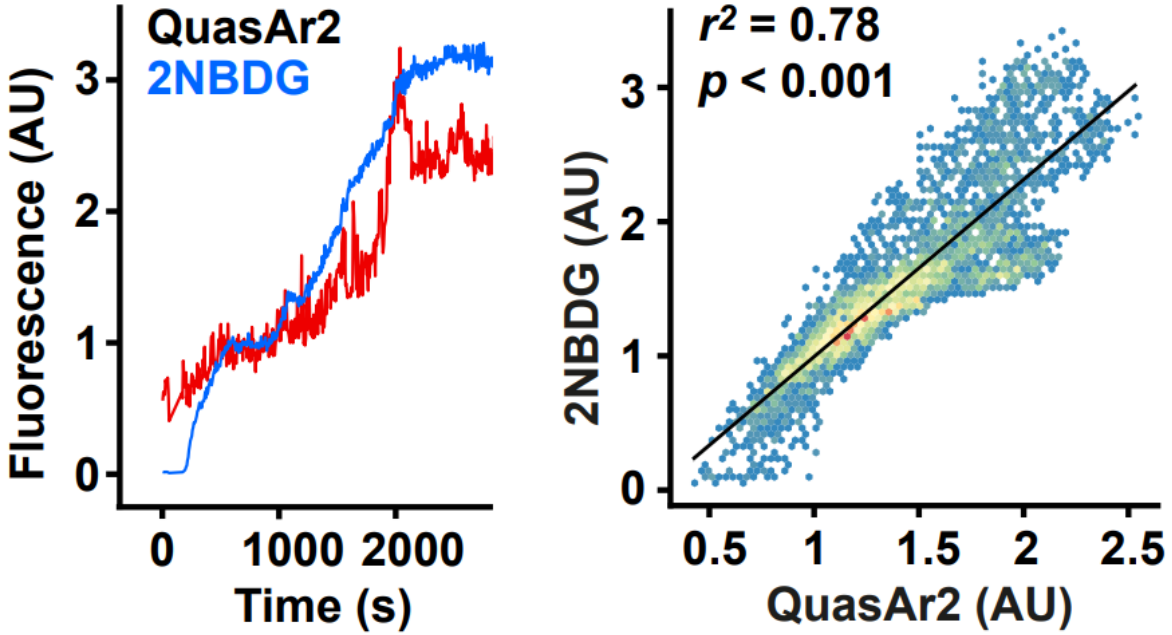


'Action potentials' driven by voltage-gated K^+ channel (Kch)

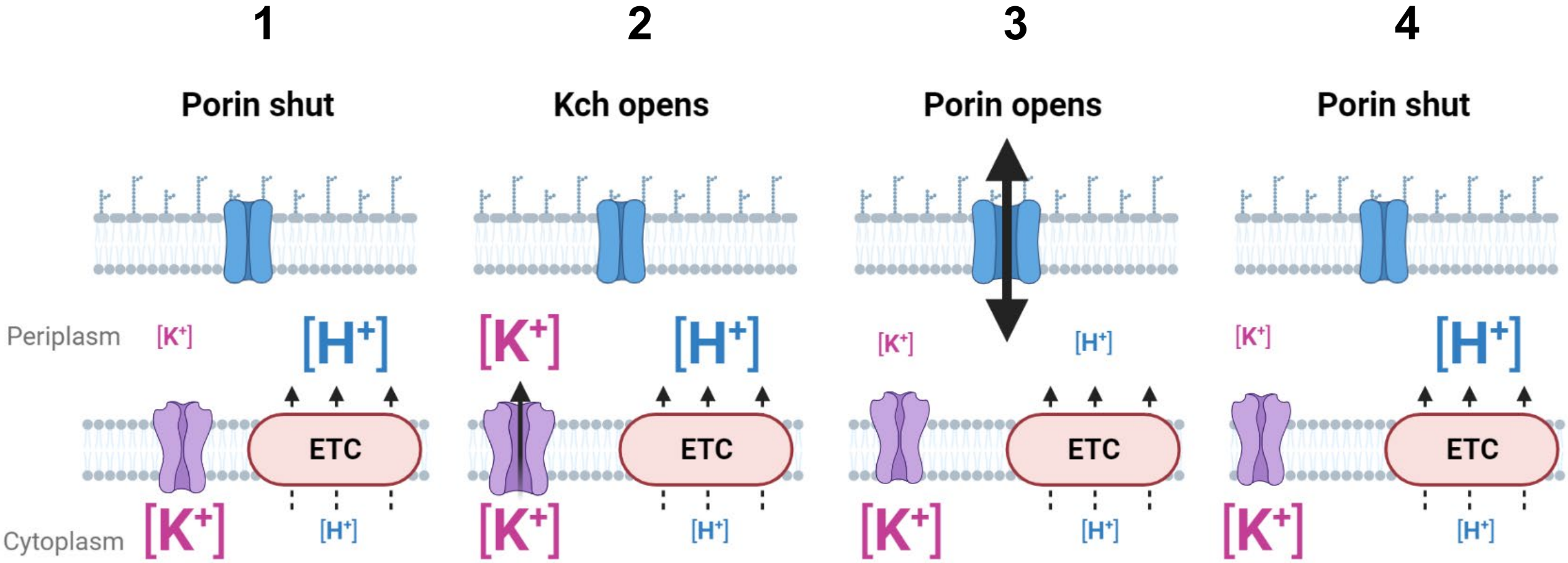


Membrane depolarisation correlates with 2NBDG uptake



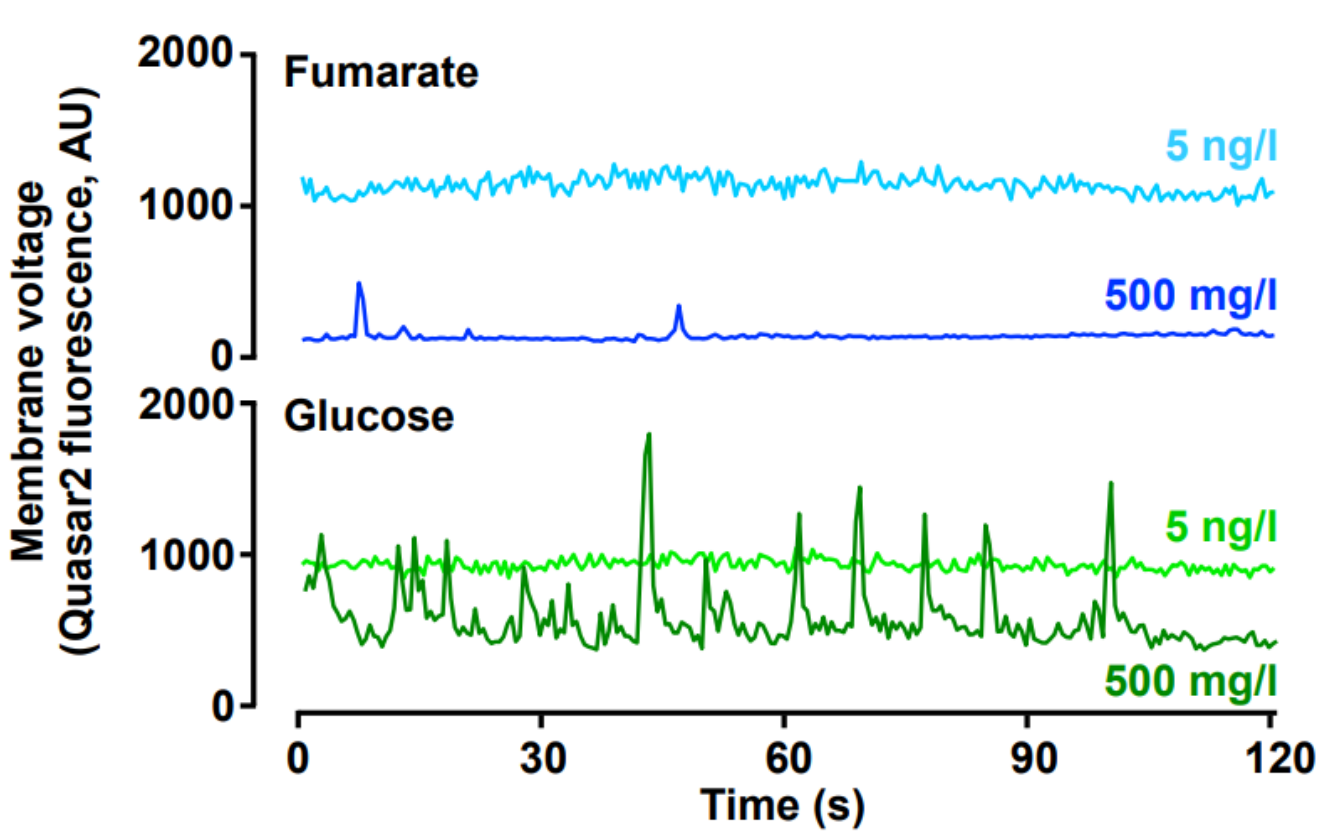


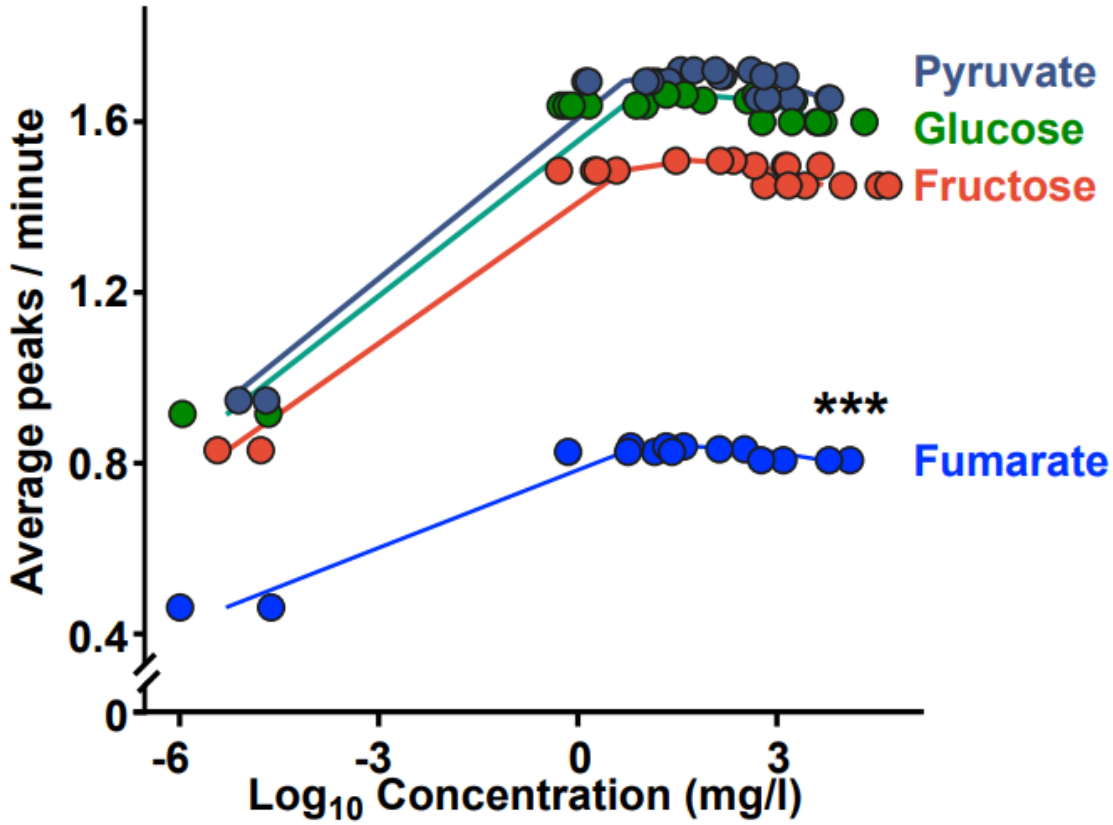
A model for how bacteria control porin opening



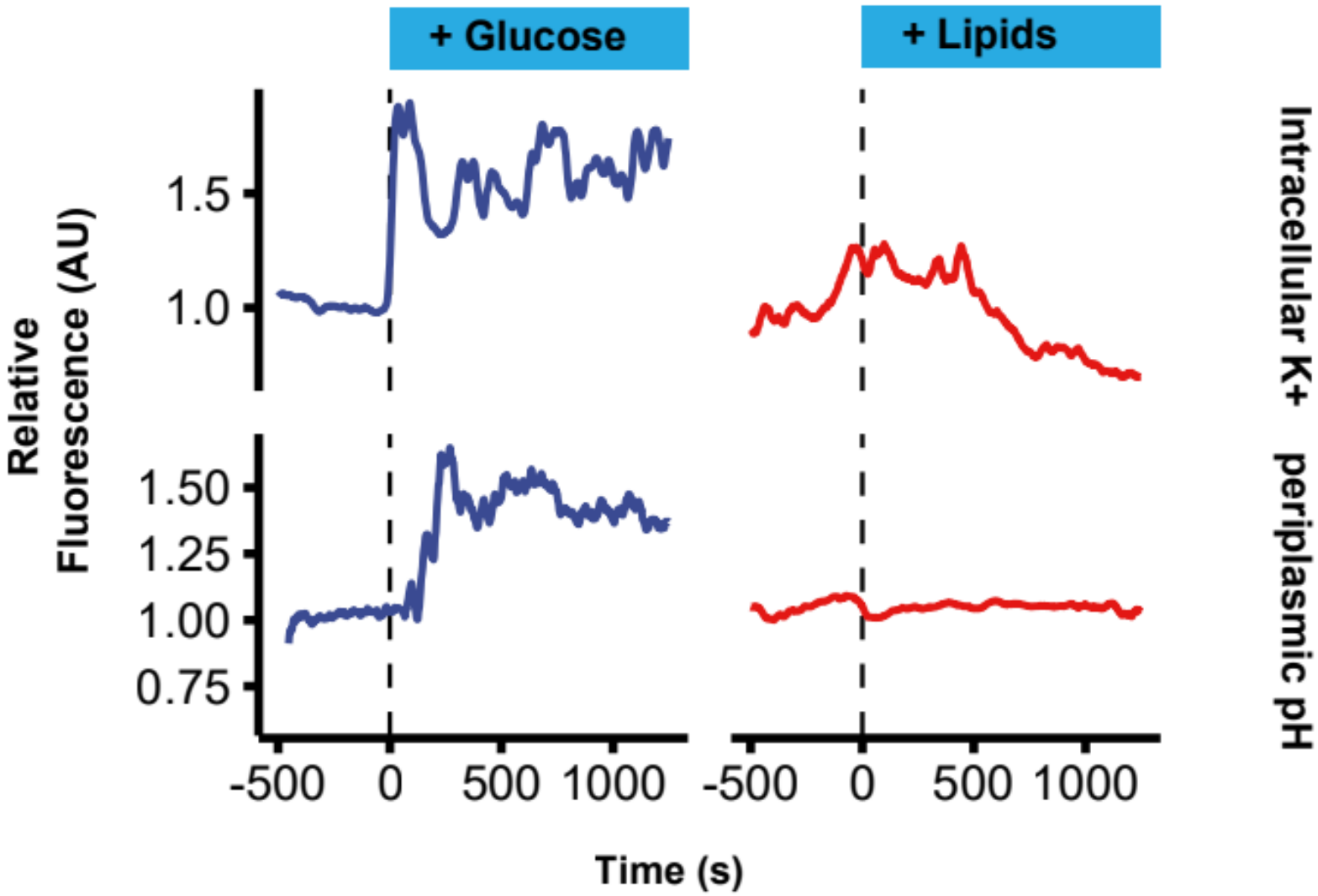
If the model is correct:
Action potentials should **increase with** the amount and quality of **carbon source**

Membrane voltage spikes increase with carbon source





Periplasmic H+ and K+ oscillations depend on carbon source

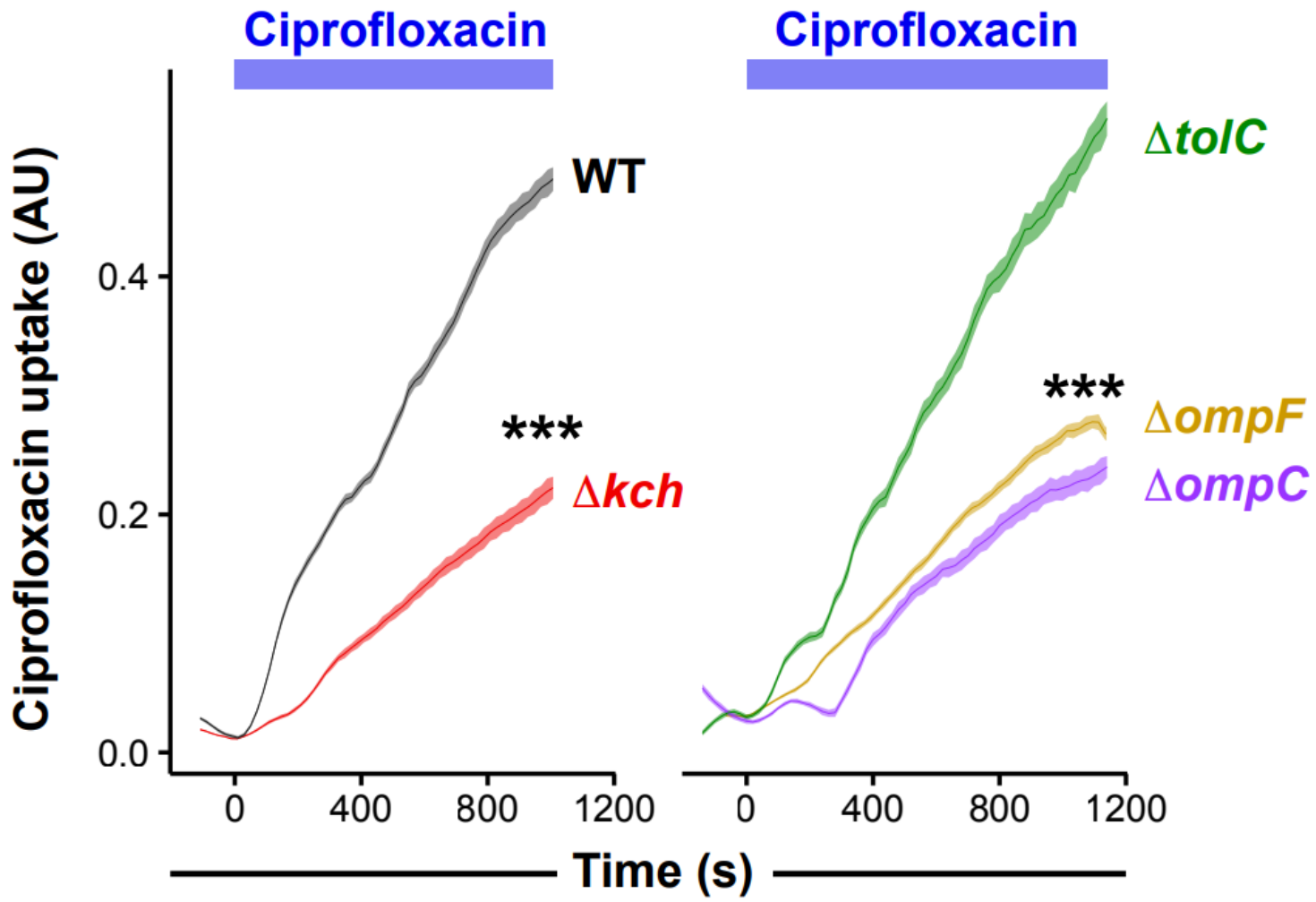


A model for metabolic control of porin opening

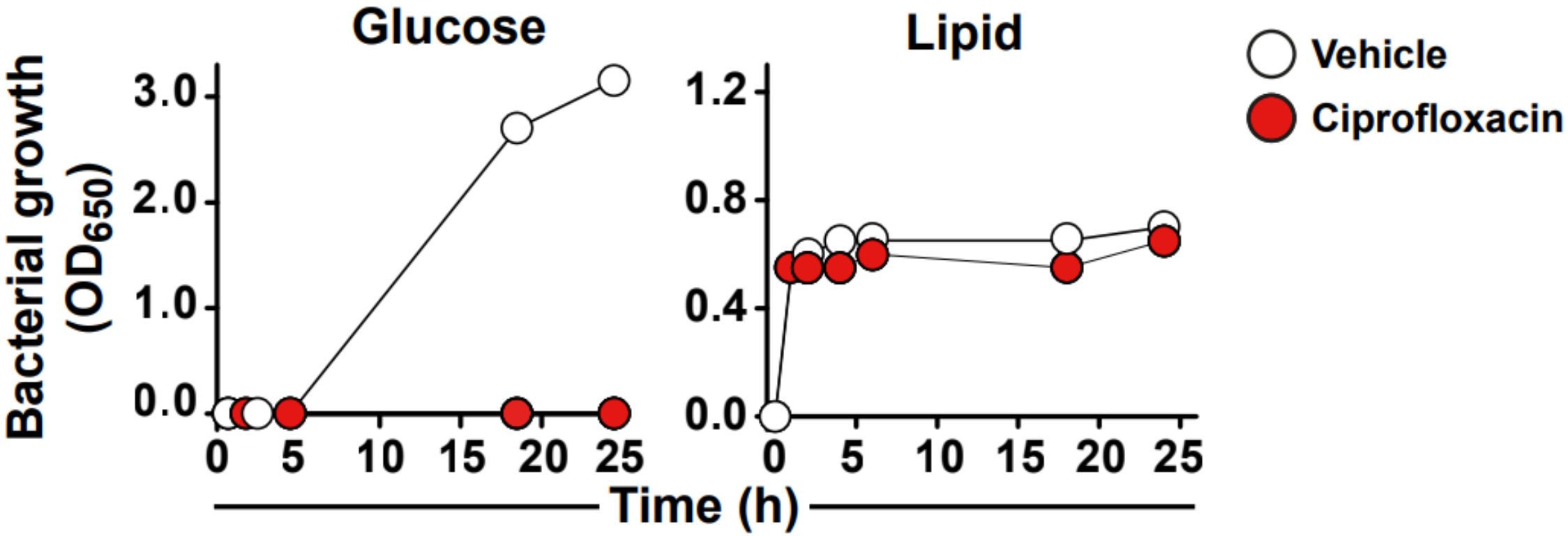
Metabolic state	ETC activity	Kch activation	Periplasmic ions	Porin permeability
Starvation	None	None	Low H+, Low K+	Open
Growth in lipid	Low	Low	**High H+**, Low K+	Closed
Growth in low glucose	Low	Low	**High H+**, Low K+	Closed
Growth in high glucose	High	High	Variable H+, **High K+**	Open

Does porin regulation explain antibiotic resistance?

Ciprofloxacin entry is mediated by porins

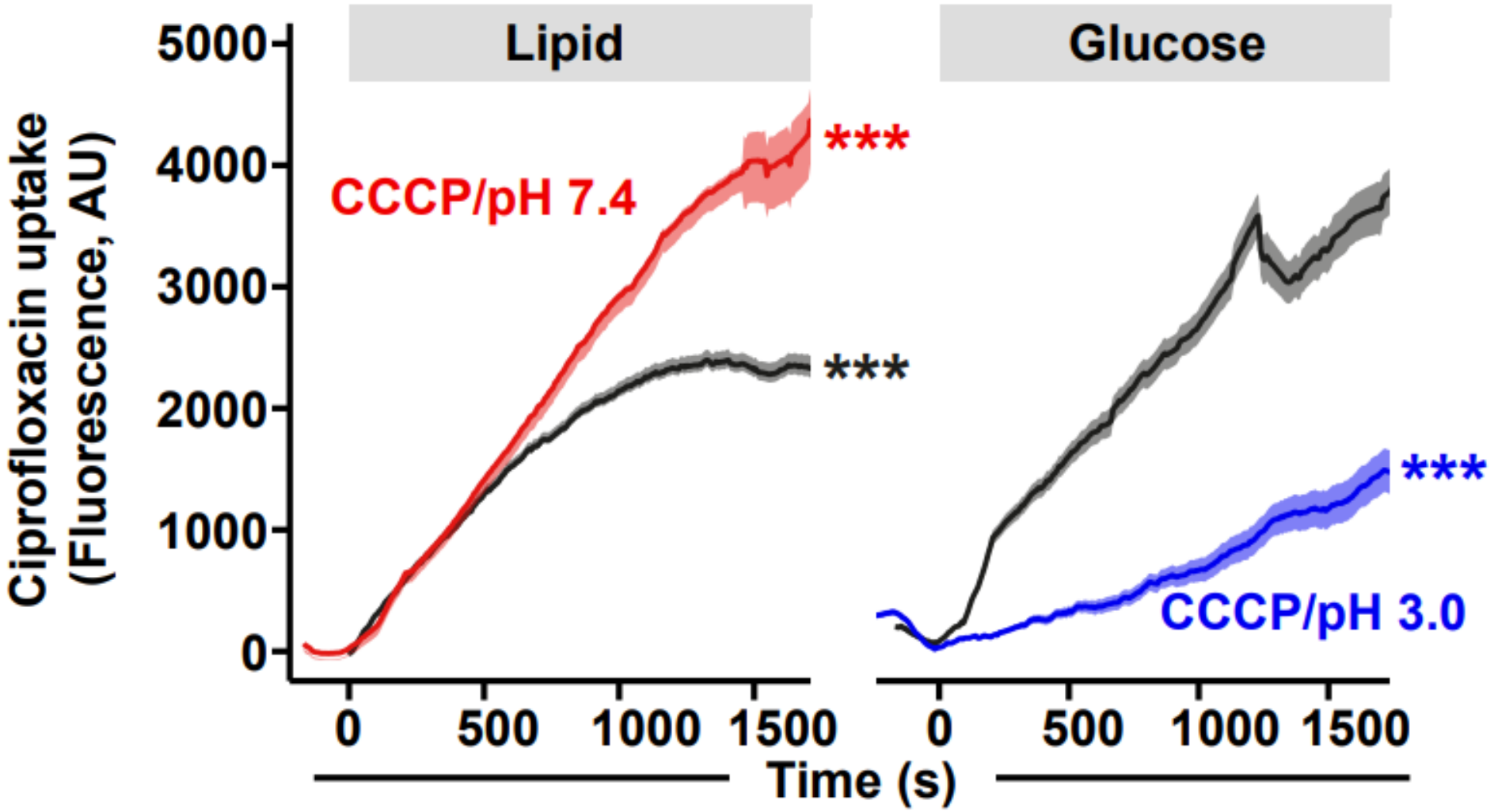


Lipid carbon source increases antibiotic resistance

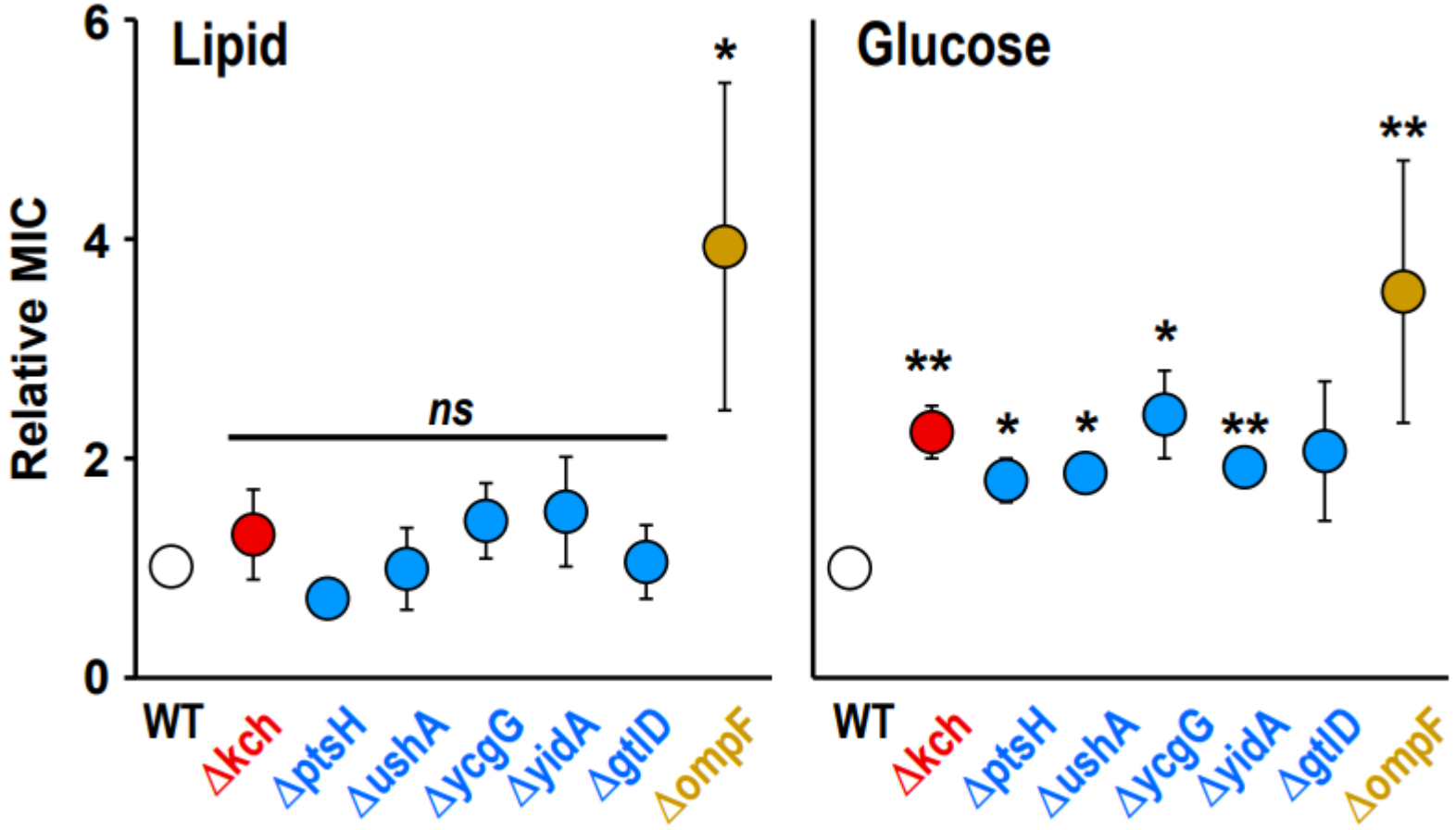


Dr Ieuan Evans

Lipid carbon source reduce porin permeability



Metabolic activity controls porin permeability and thus MIC



Dr Anja Hagting

Conclusions

1. Porin permeability is regulated by **periplasmic H^+ and K^+**

   Structural modelling suggests that regulation may be **porin-intrinsic**

2. Changes in periplasmic H^+ and K^+ may explain differential porin permeability during starvation and growth in different carbon sources

3. Porin regulation may underlie increases in antibiotic resistance during growth in lipids (and possibly in the phagosome) and the effect of AMR mutations in central metabolism genes

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wellcome

Cystic Fibrosis Trust

fondation BOTNAR