Emergent metabolic dynamics in microbial communities

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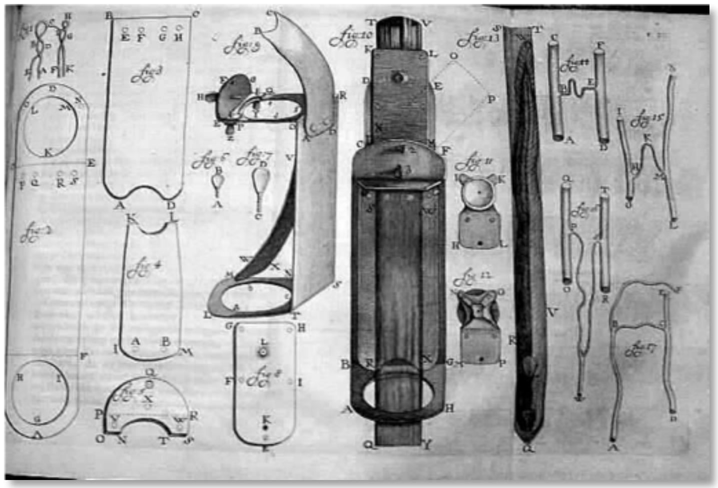
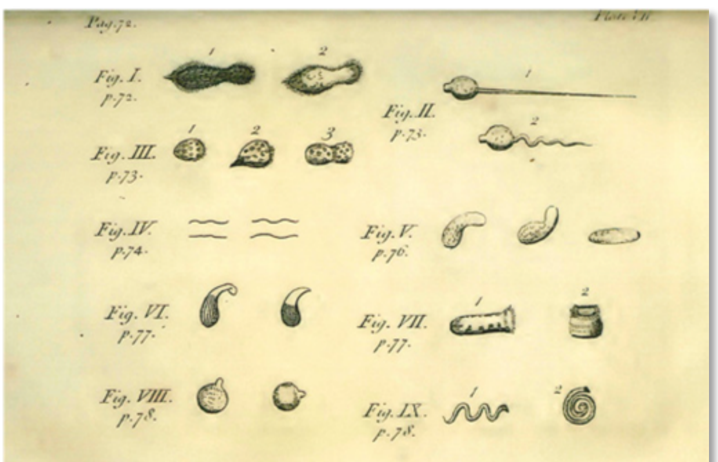
Bacteria have been studied for a long time ...





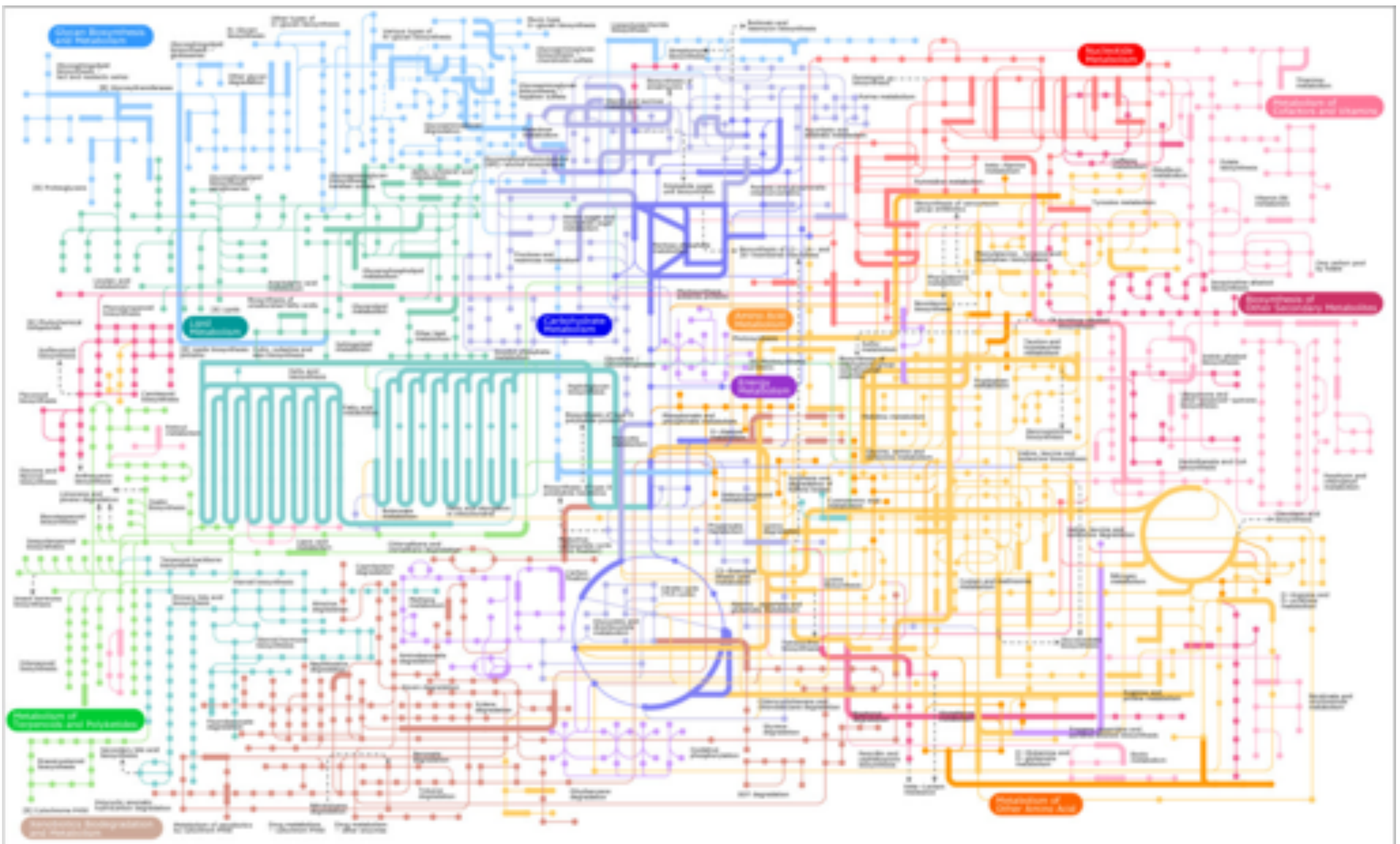
Antonie van Leeuwenhoek
1632 - 1723

Much of our understanding of biology is based on bacterial studies

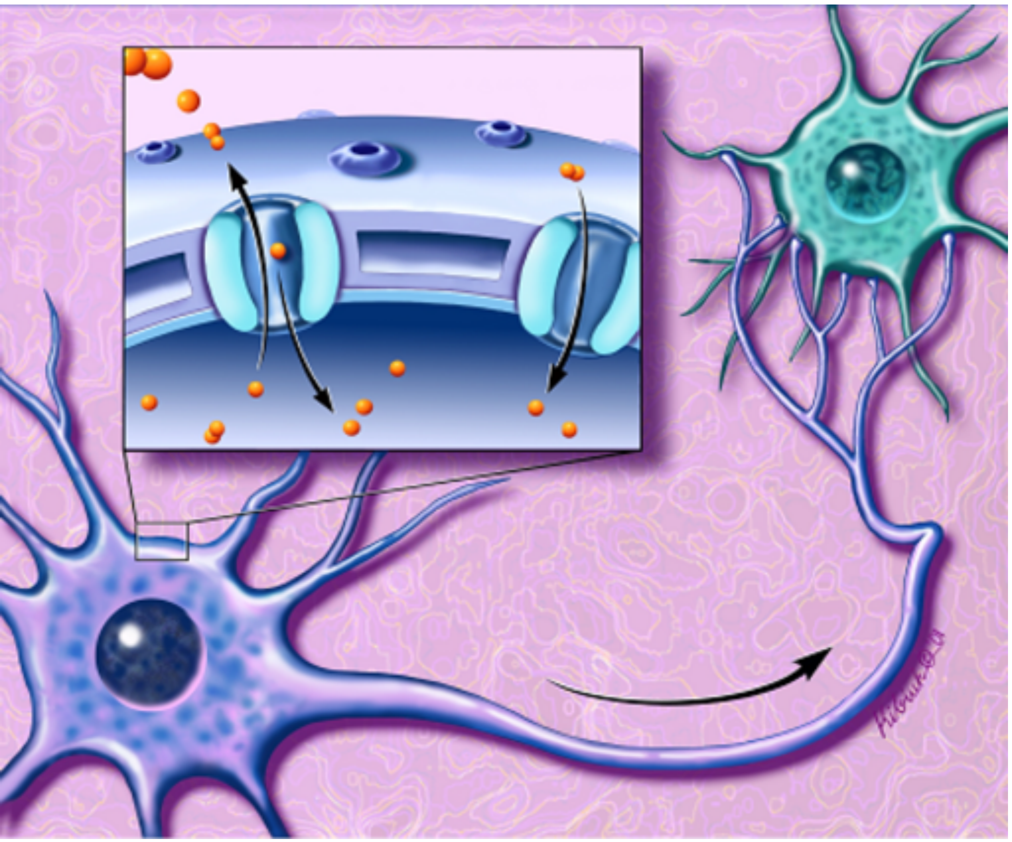
… for example:

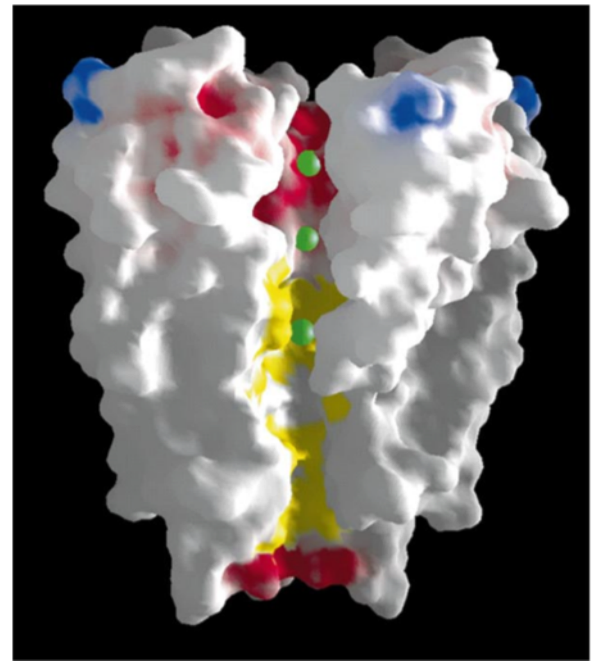
Identification and characterization of metabolic pathways

Metabolism



Bacteria even provided structural insight into the basis of brain activity





Potassium Ion Channel

Streptomyces lividans
(Gram positive soil bacteria)

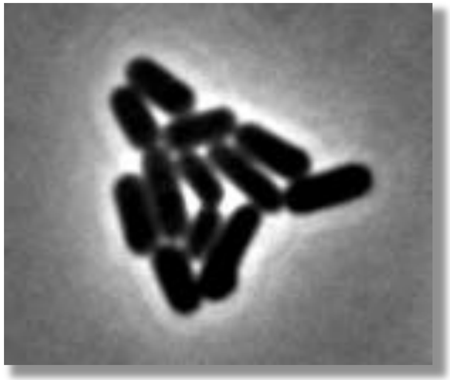
Doyle etal, (1998) Science 280/69

We have learned much from bacterial studies

… but there is a problem…

Bacteria are single celled organisms …

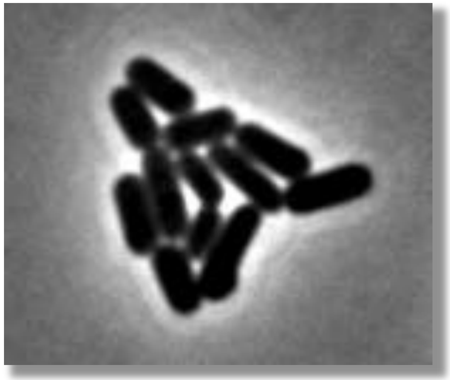
Our model system:



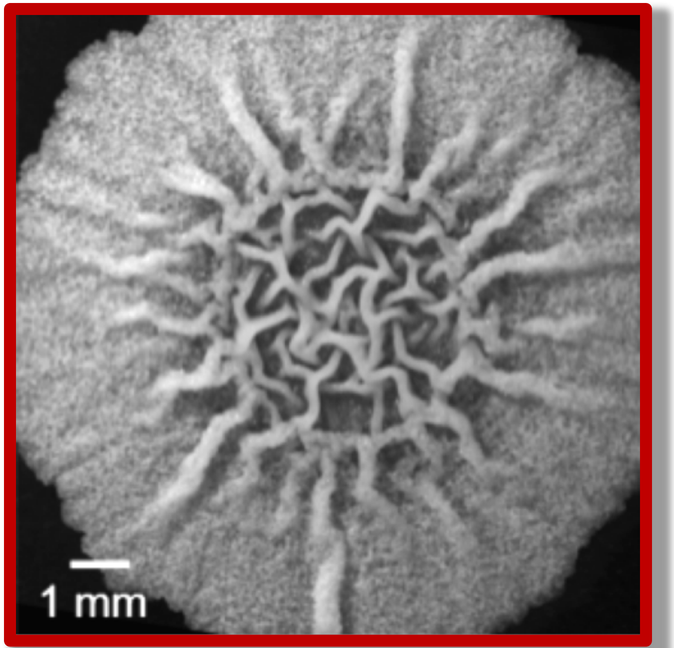
Bacillus subtilis

… but most bacteria reside in *communities:* Biofilms

Our model system:

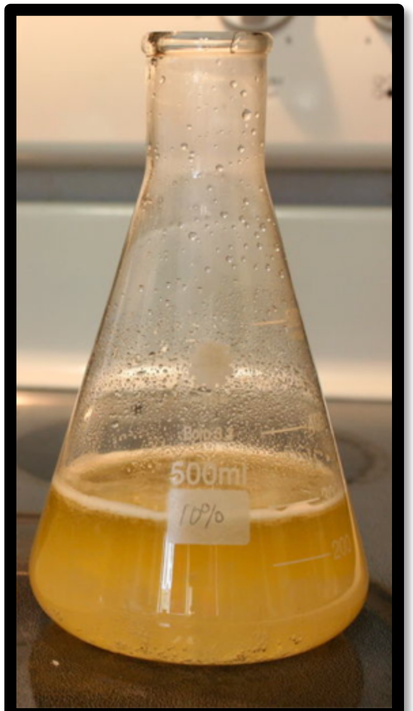


Bacillus subtilis

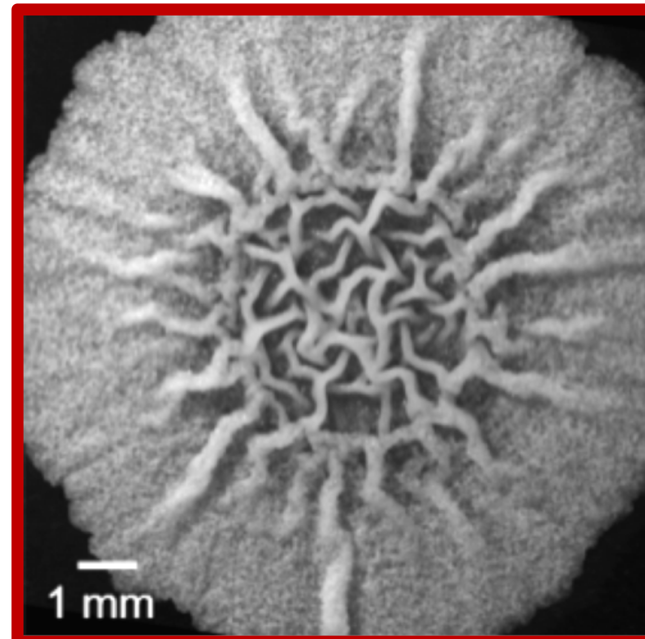


Bacillus subtilis **biofilm**

Bacterial behavior in biofilms remains unclear



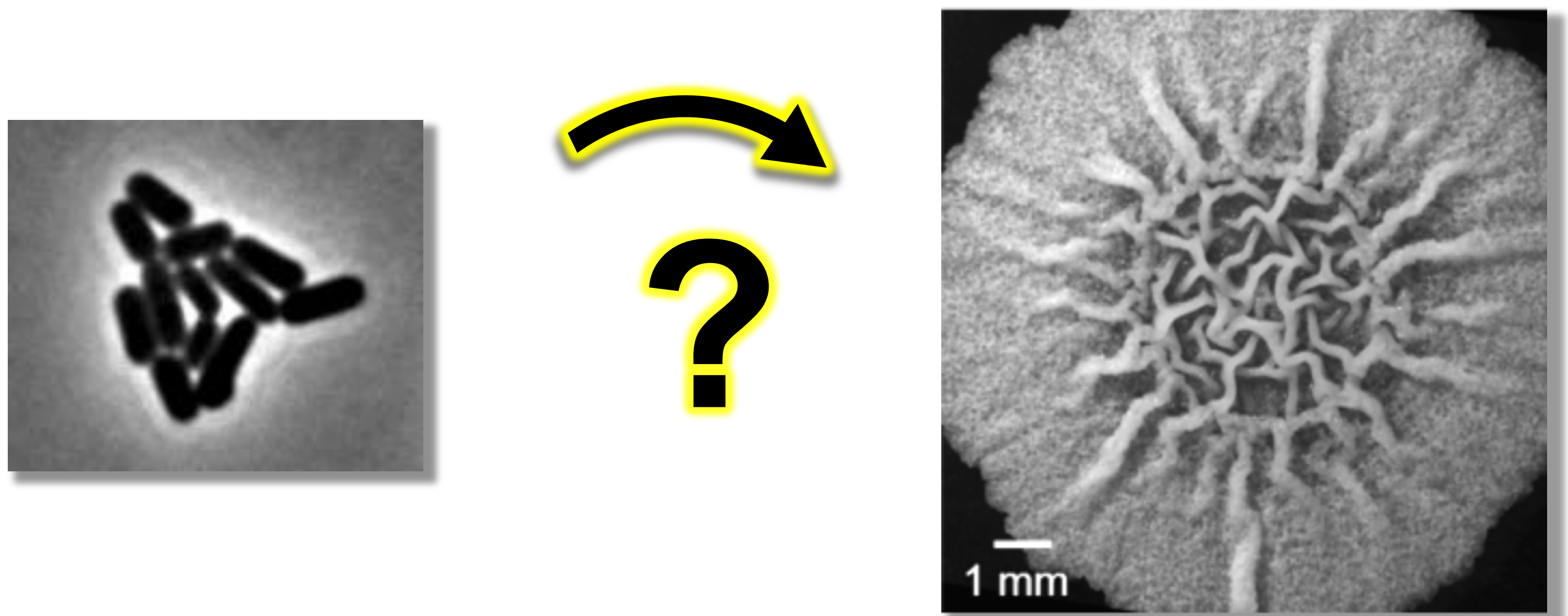
Liquid culture



Biofilm

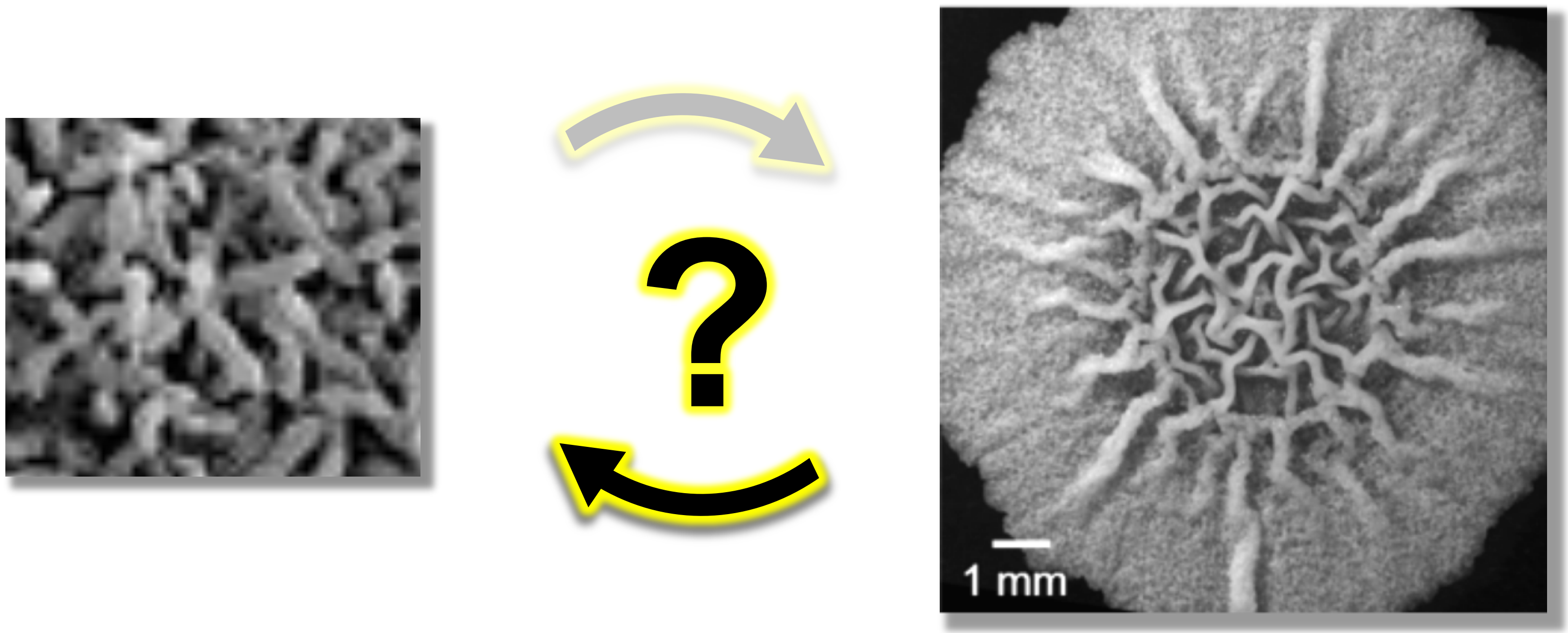
Fundamental questions:

Emergence of collective behavior

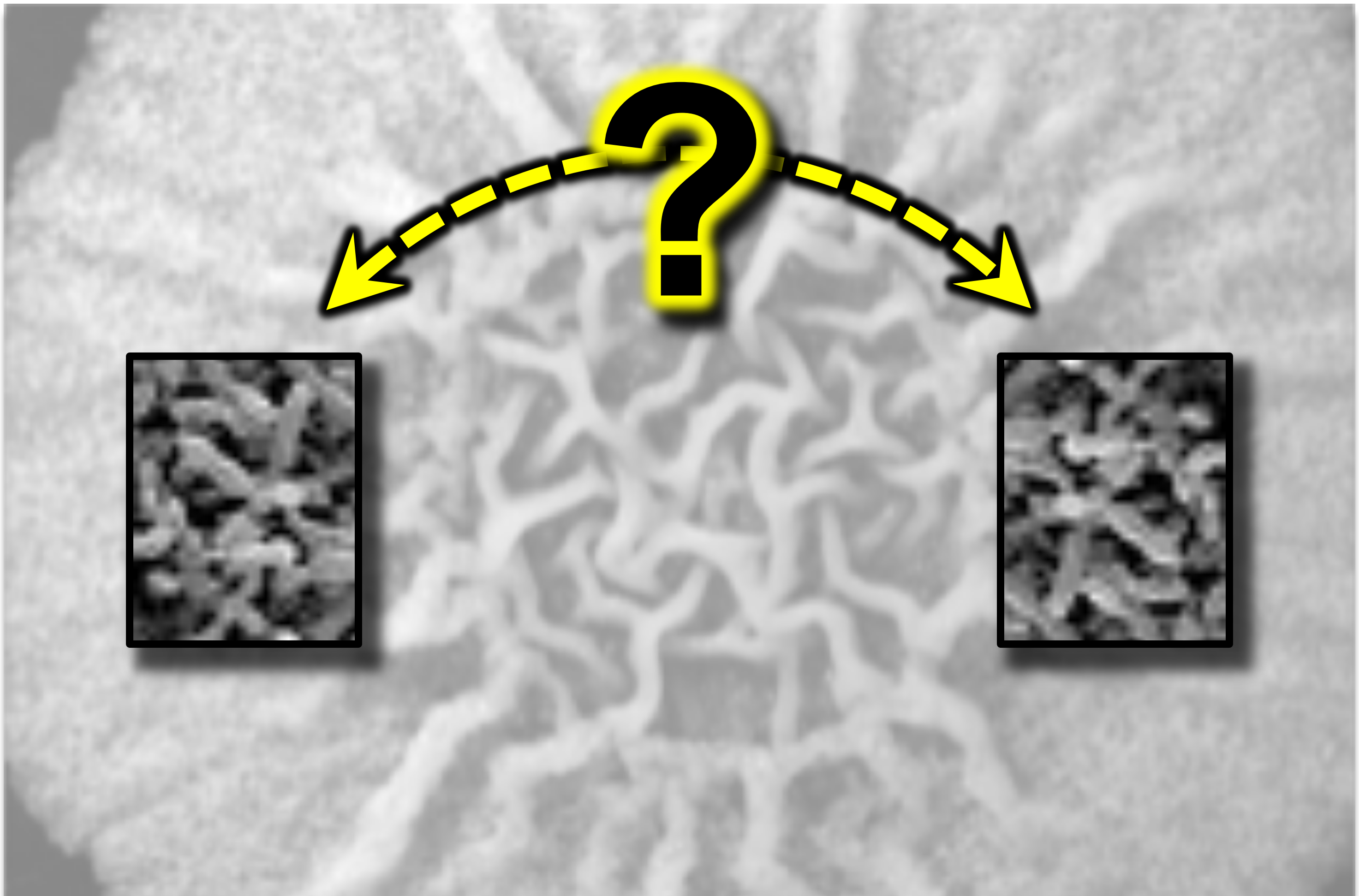


Fundamental questions:

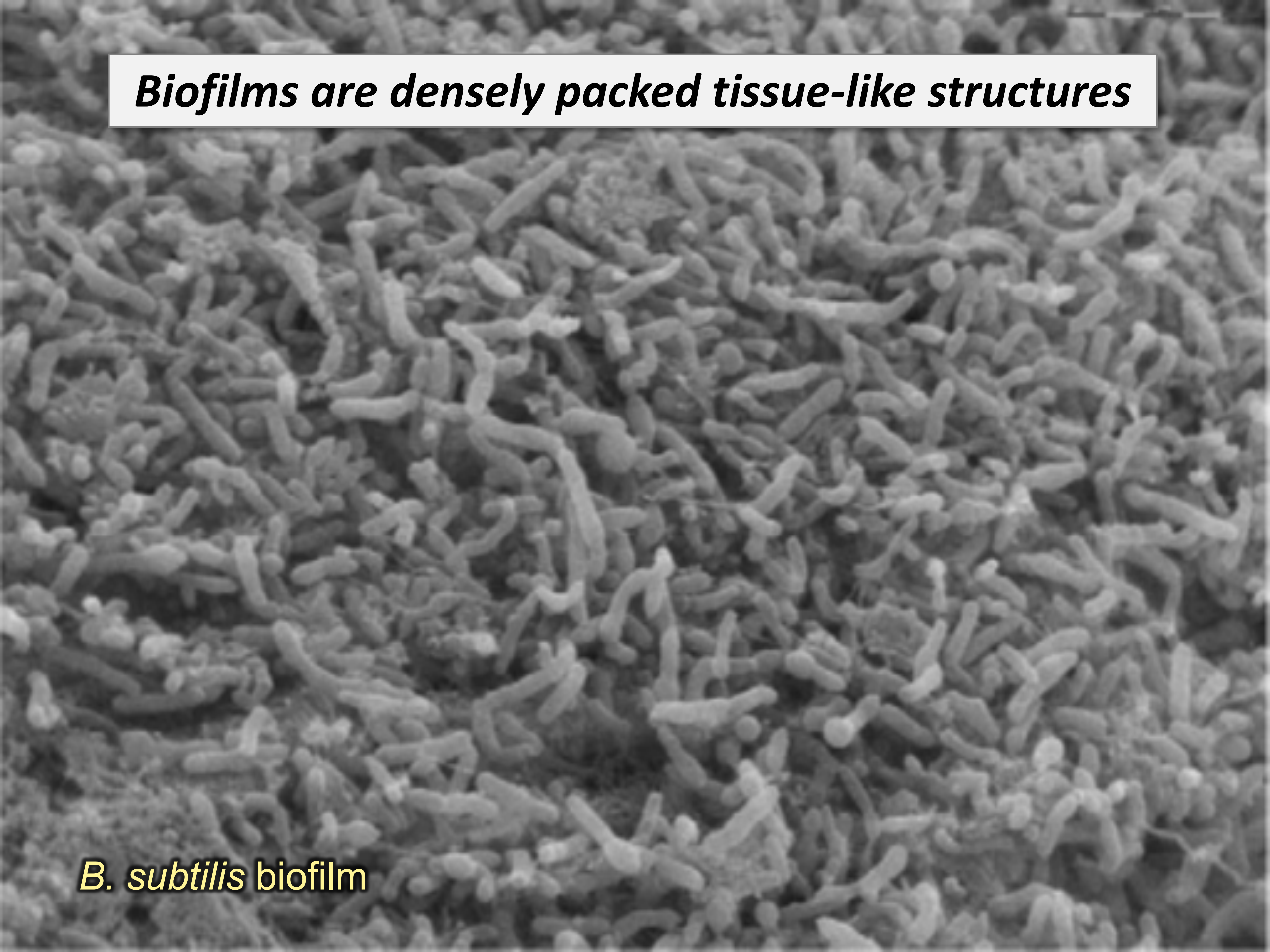
Behavior of single cells in the community



Communication and coordination within the biofilm



Biofilms are densely packed tissue-like structures



B. subtilis biofilm

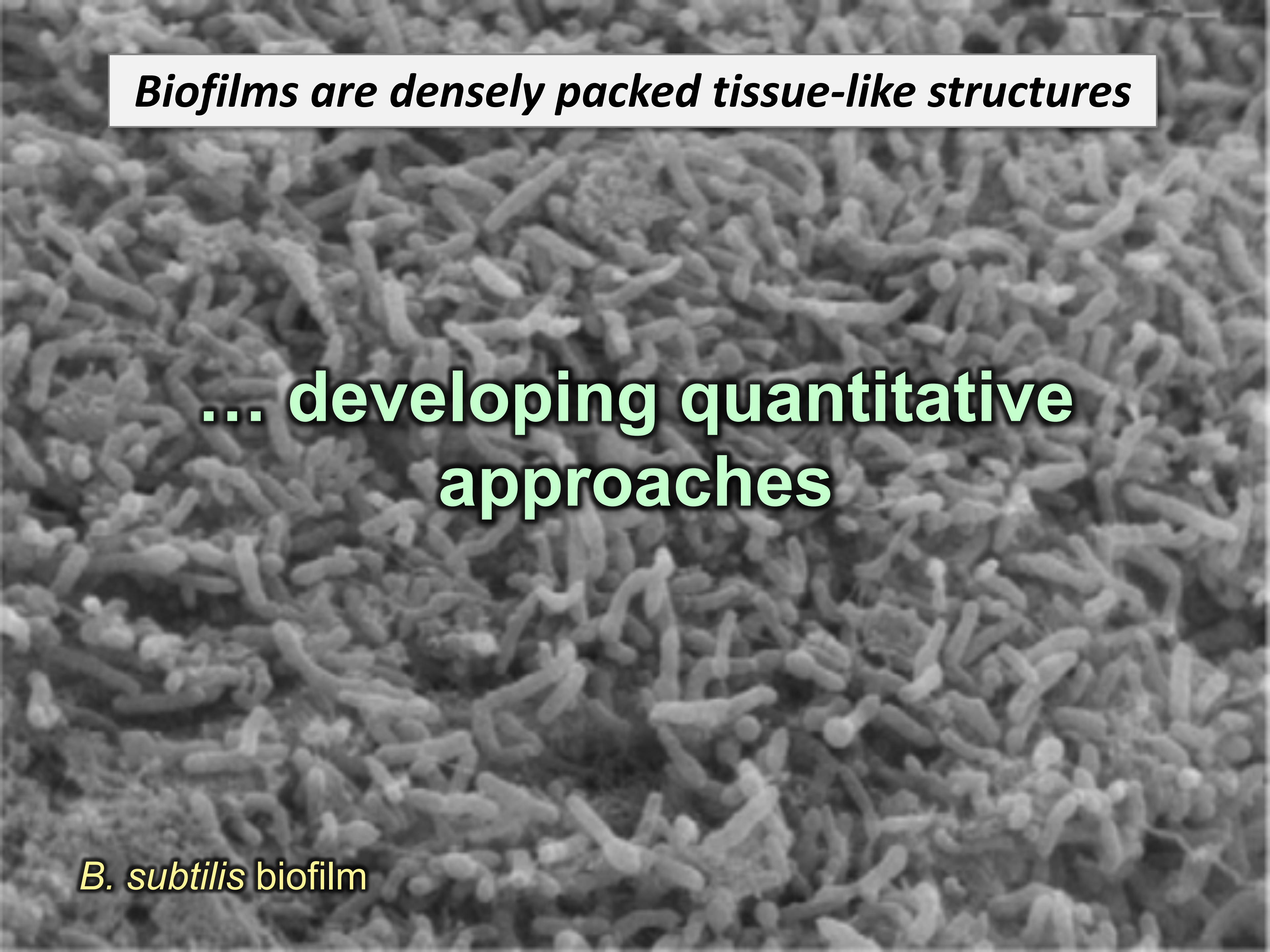
Biofilms are densely packed tissue-like structures

What goes on inside biofilms?

Is there new biology to be discovered?

B. subtilis biofilm

Biofilms are densely packed tissue-like structures

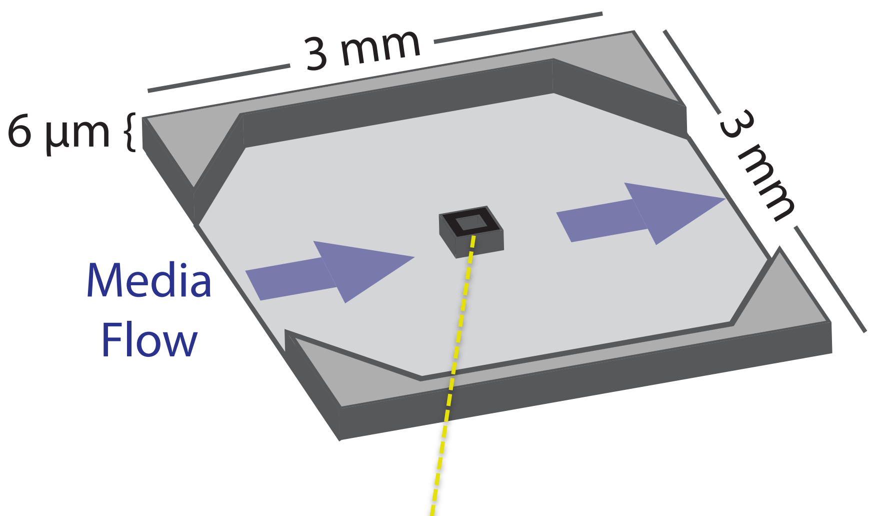


B. subtilis biofilm

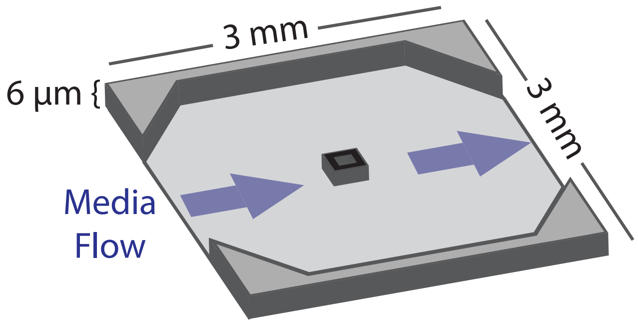
Simplification by restricting growth to 2D

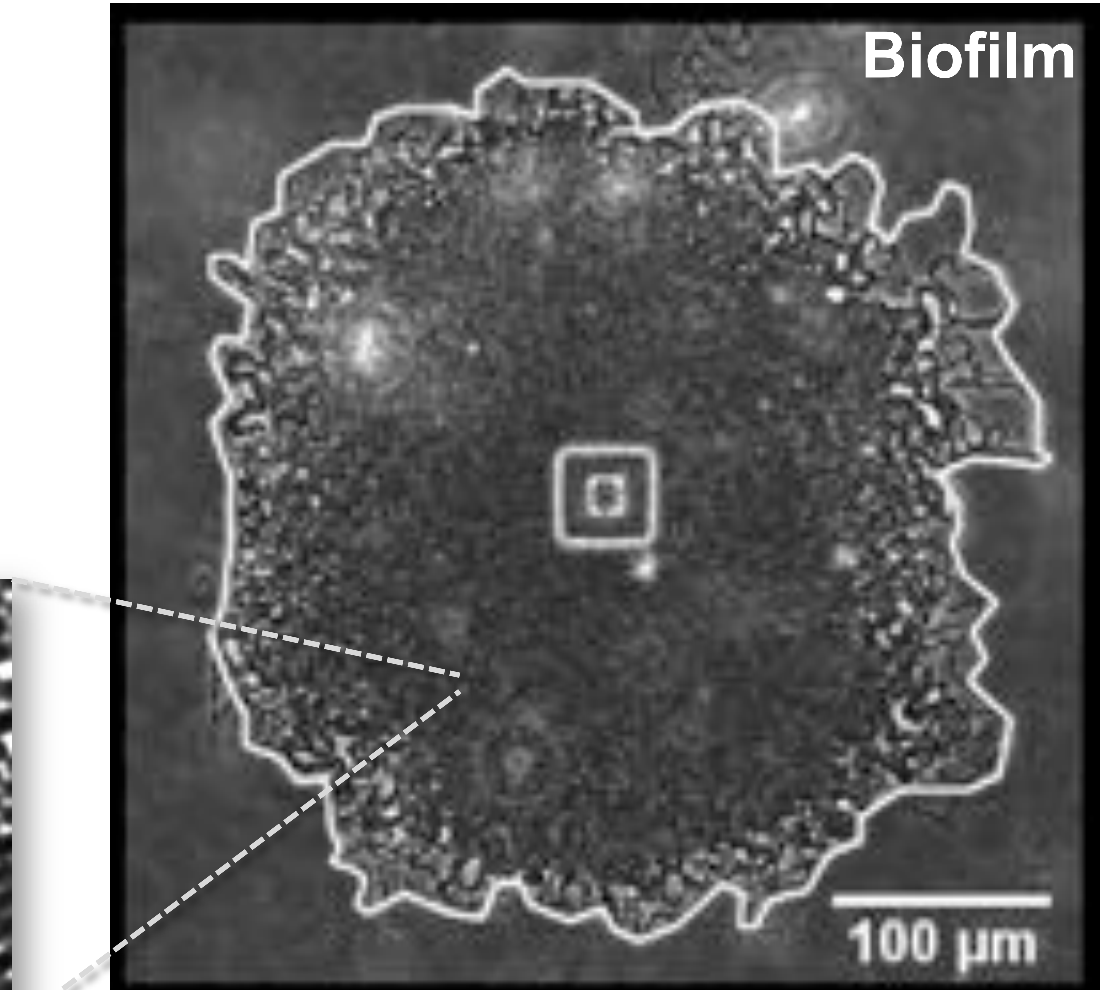
Utilizing unconventionally large microfluidic devices:

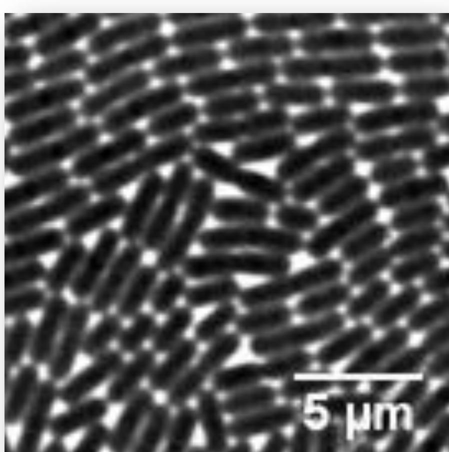
Growth chamber



Ability to measure single cells in a biofilm with over two million cells

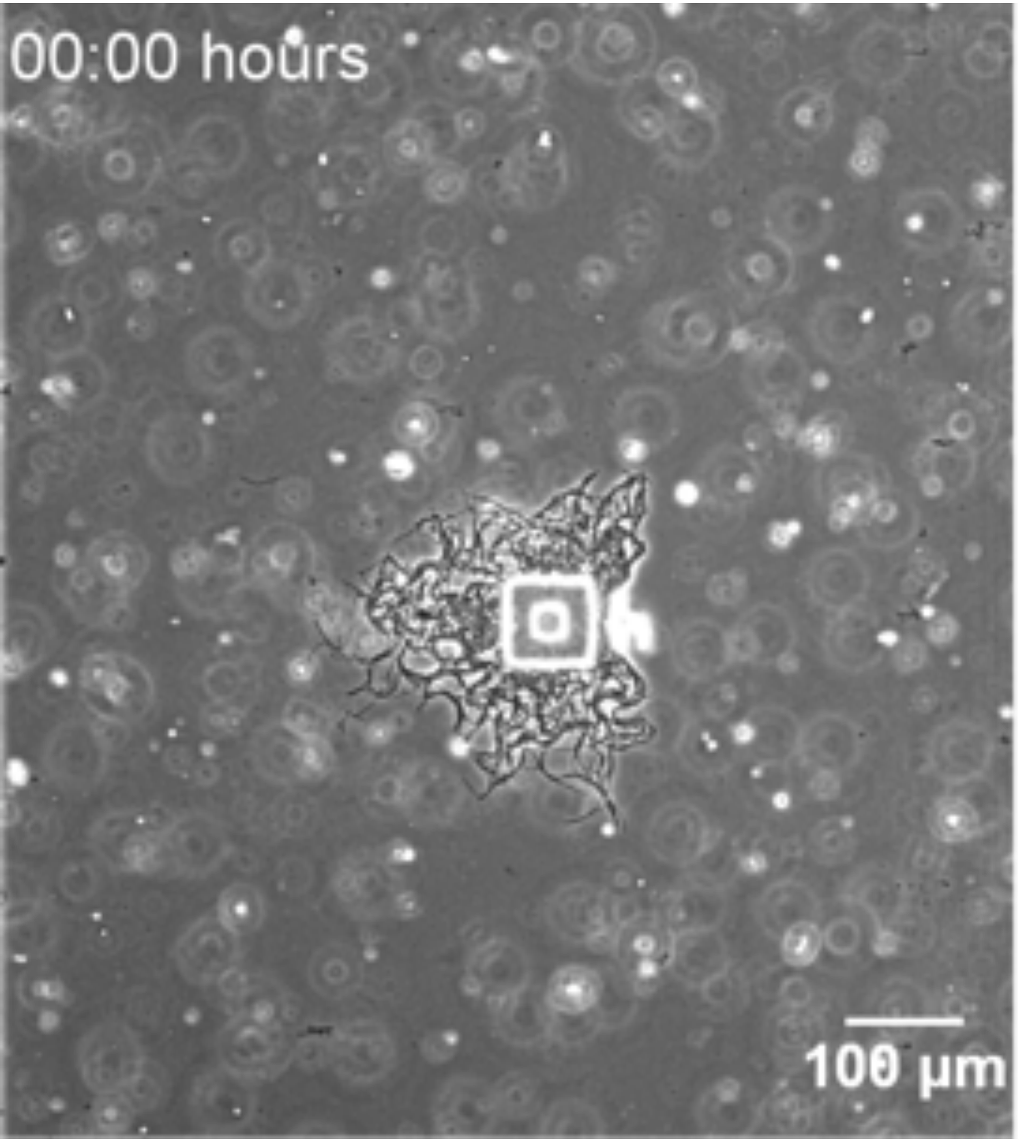


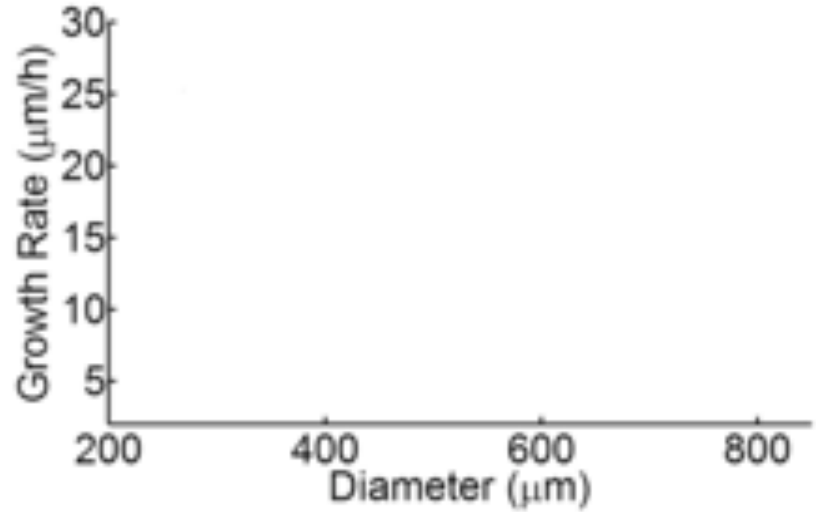




Bacillus subtilis

Unexpected biofilm growth dynamics

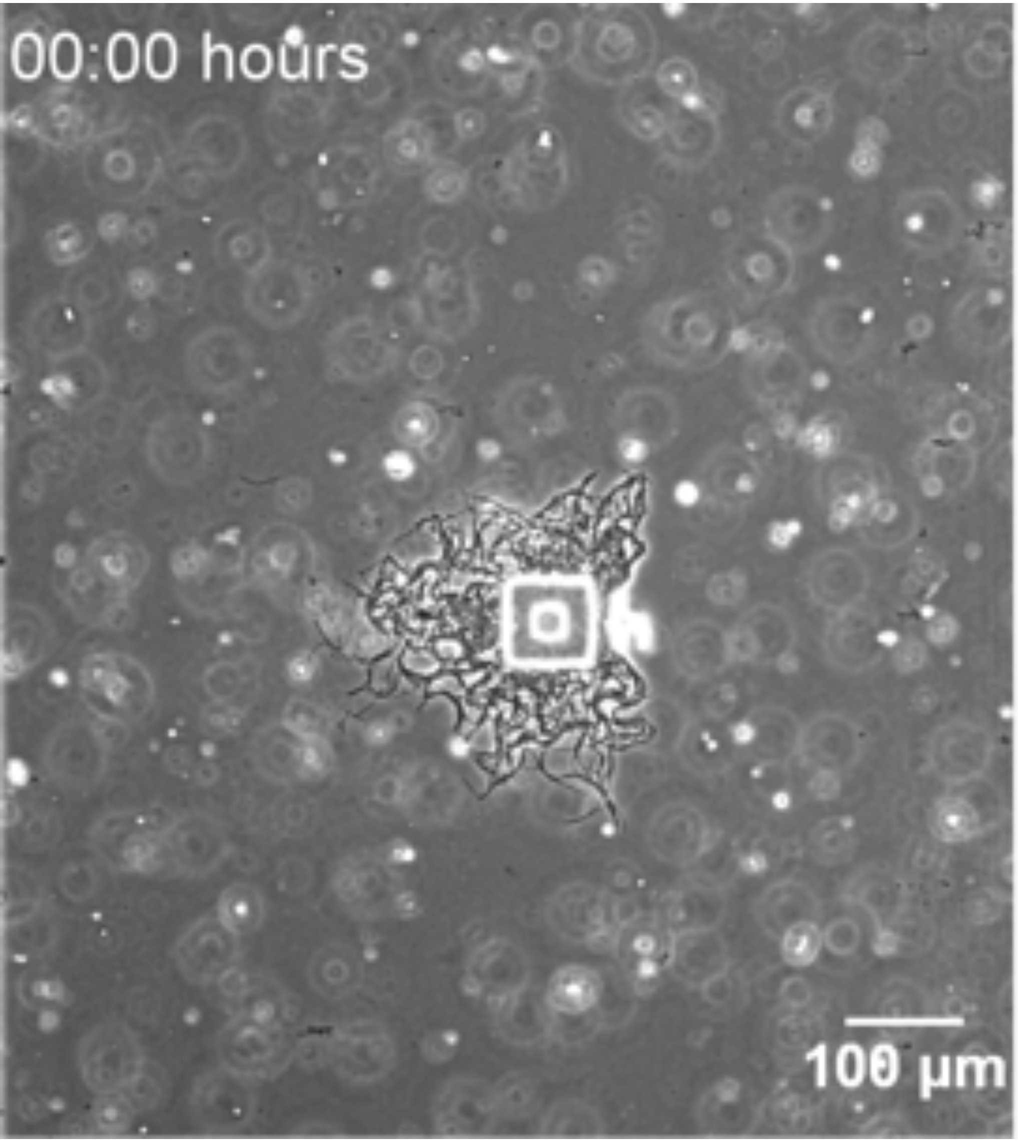


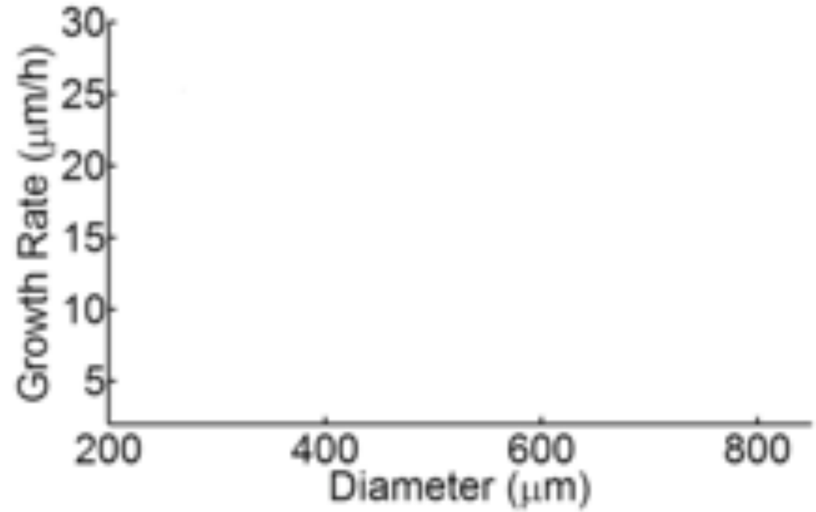


Bacillus subtilis

Liu *etal,* Nature, **523**, 550–554

Unexpected biofilm growth dynamics

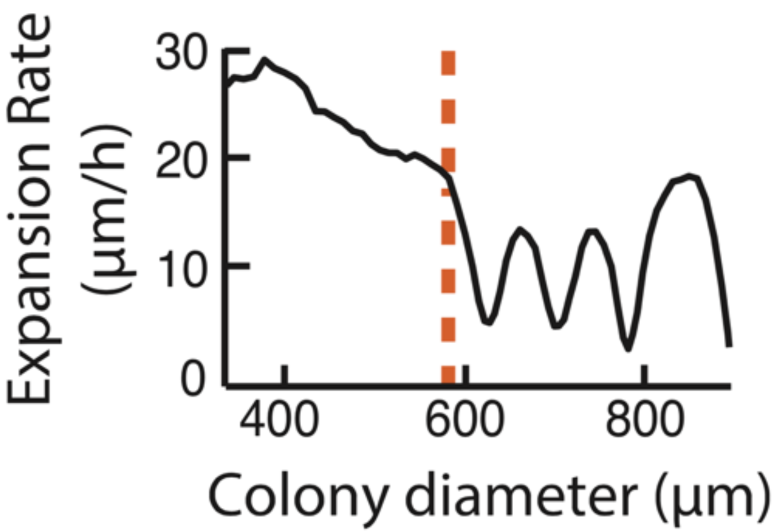


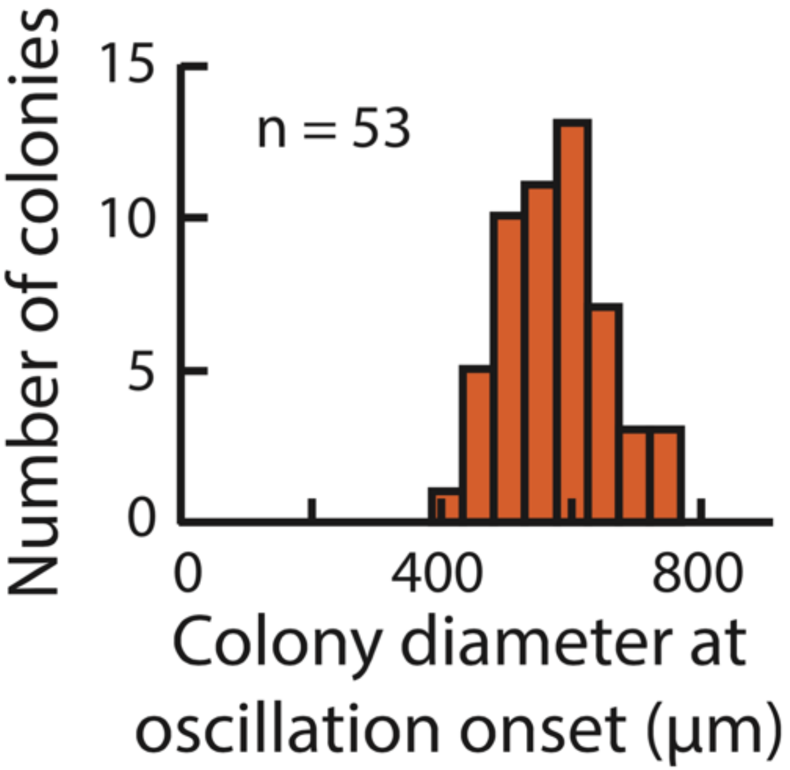


Bacillus subtilis

Liu *etal,* Nature, **523**, 550–554

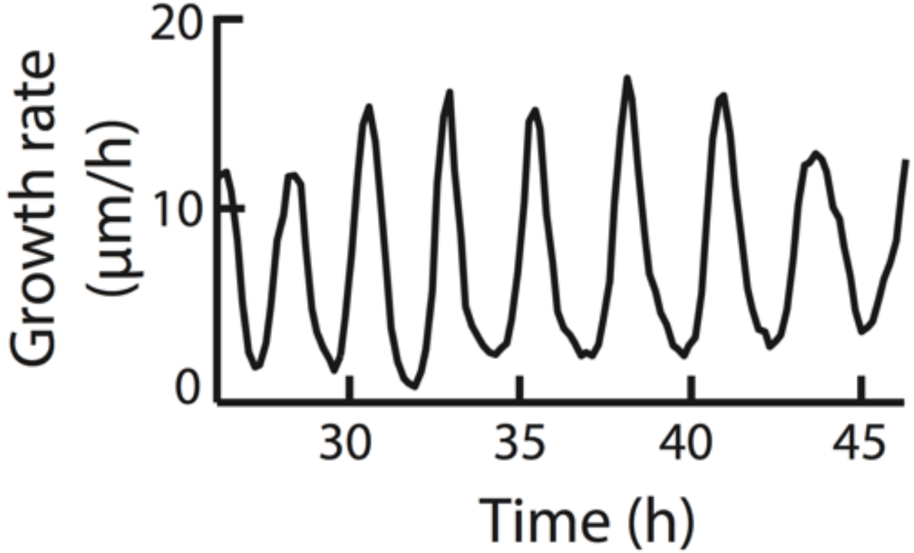
Oscillations start at a defined colony size

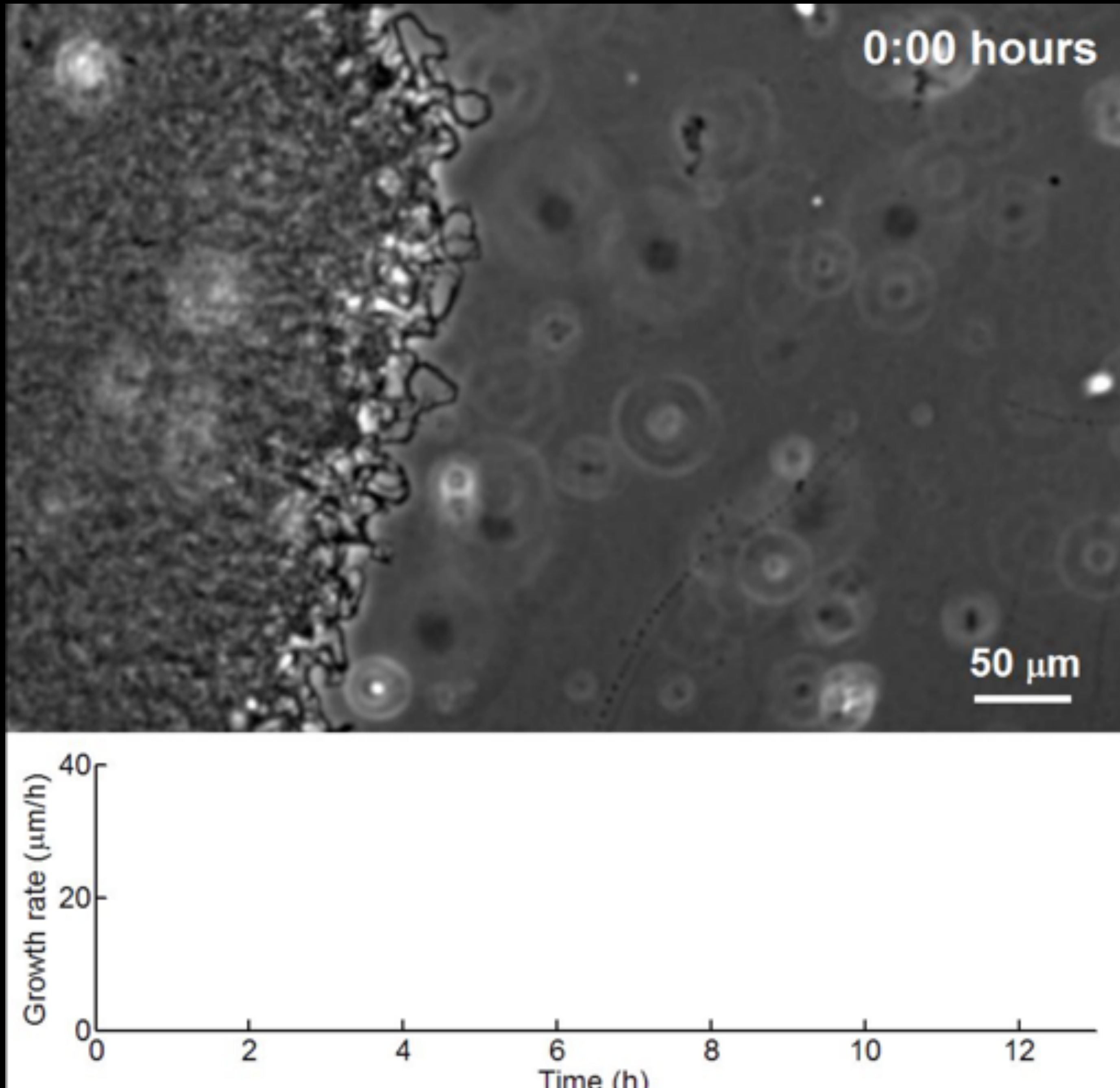




- A colony with a diameter of 600µm contains ~1 500 000 cells.

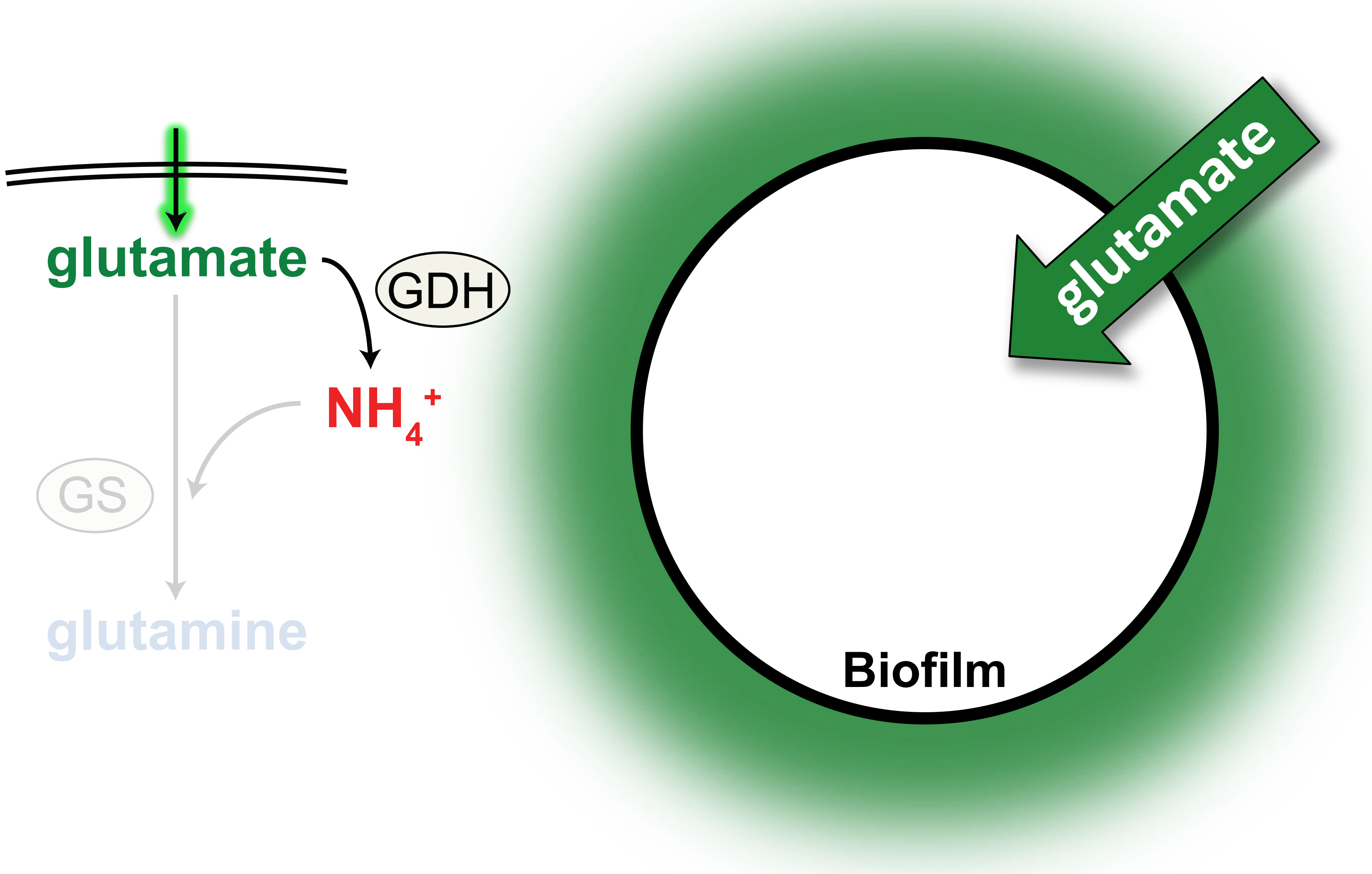
Oscillations in expansion rate persist for many hours



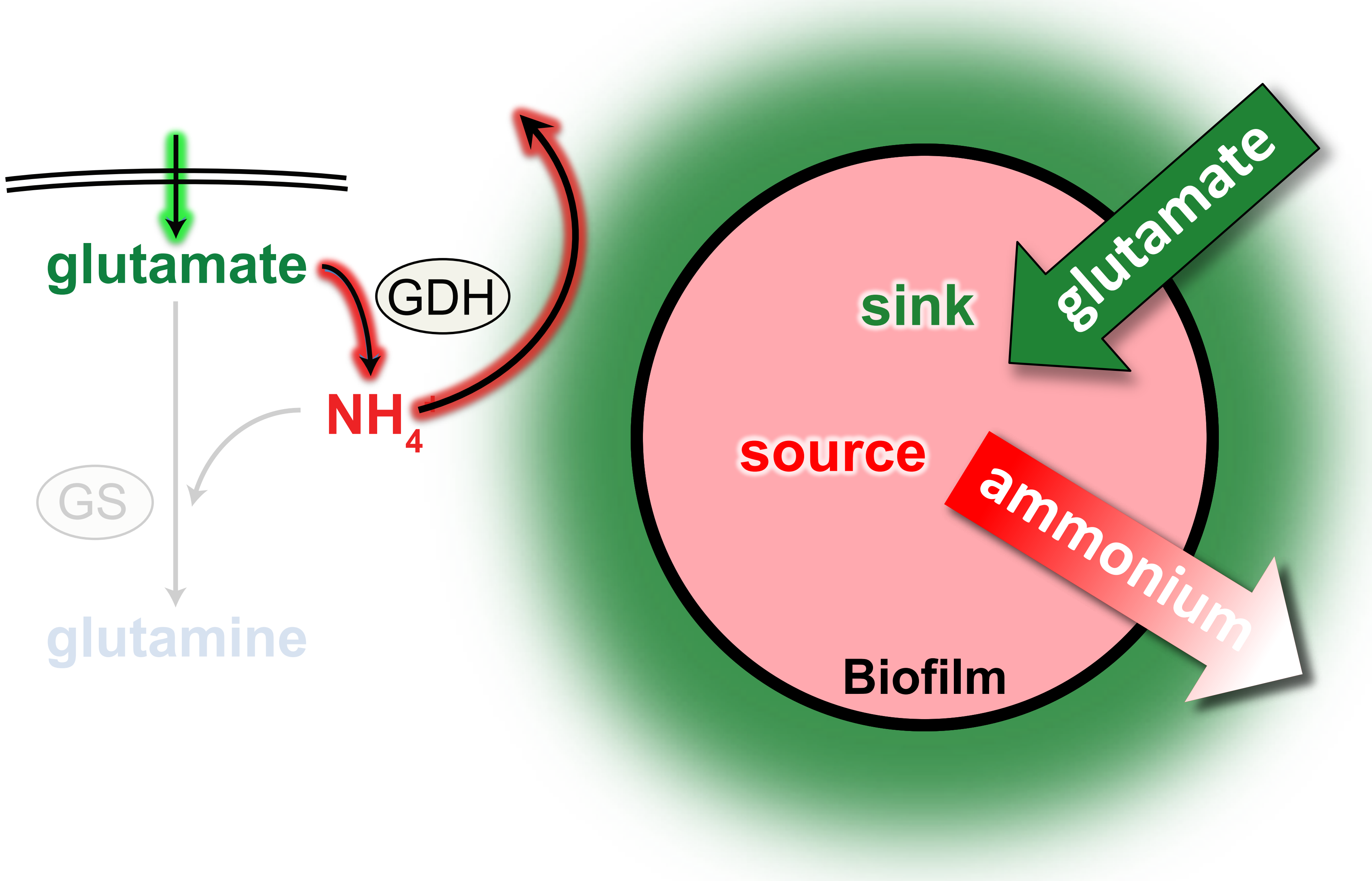

0:00 hours
50 μm
Growth rate (μm/h)
40
20
0
0
2
4
6
8
10
12
Time (h)

What could periodically pause biofilm growth ?

Glutamate is provided in the media

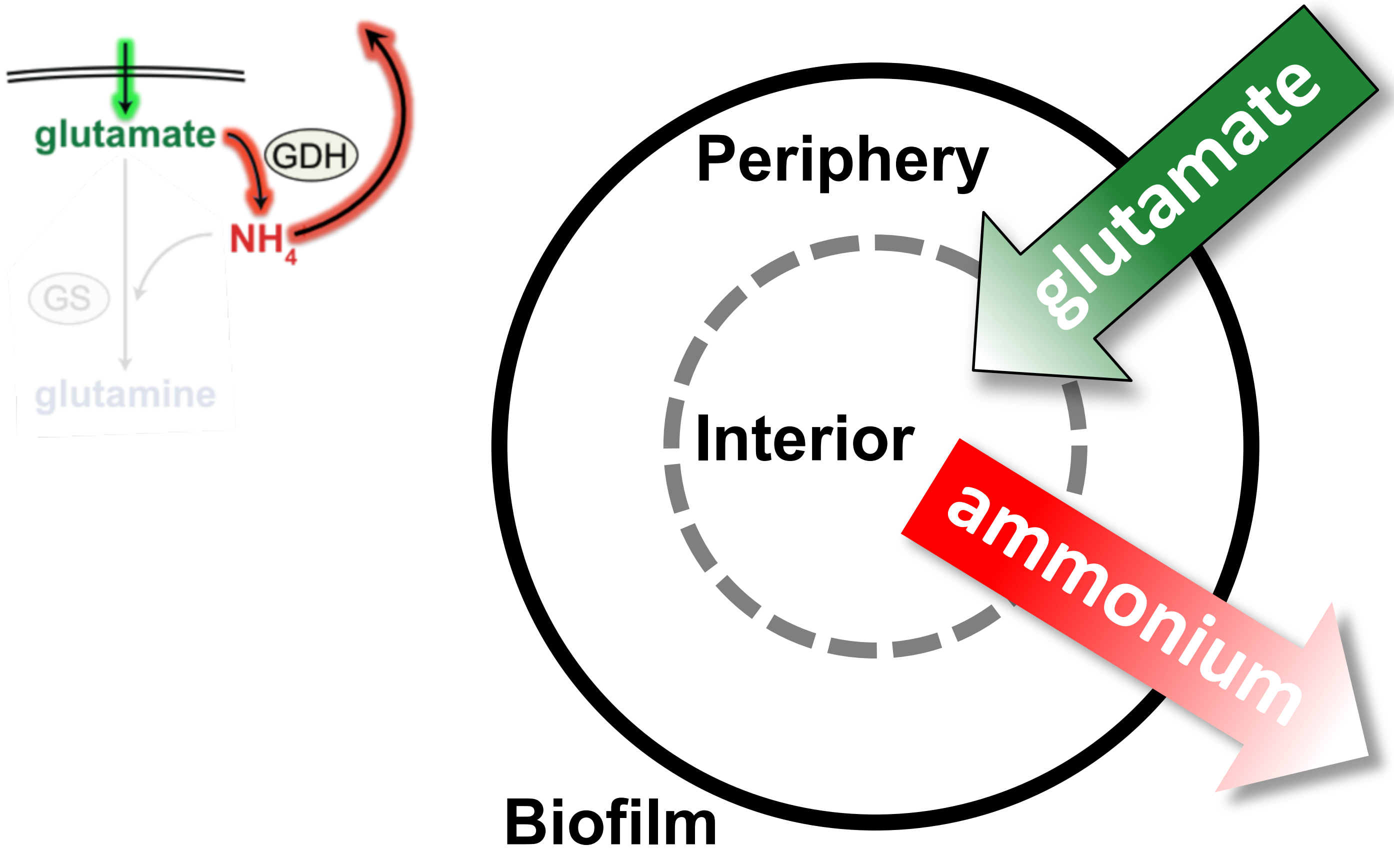


But the biofilm is the only source of ammonium

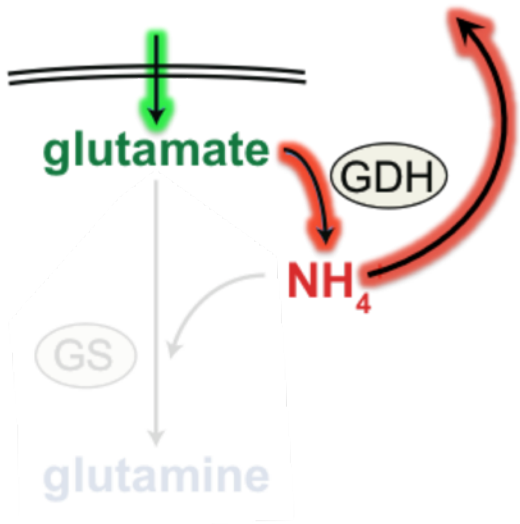


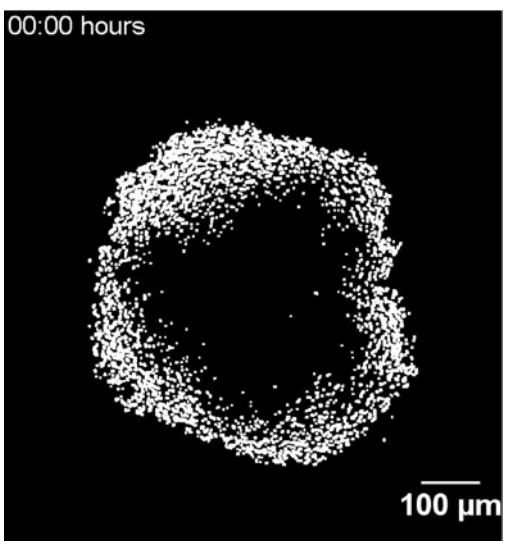
Glutamate is limited in the **interior**

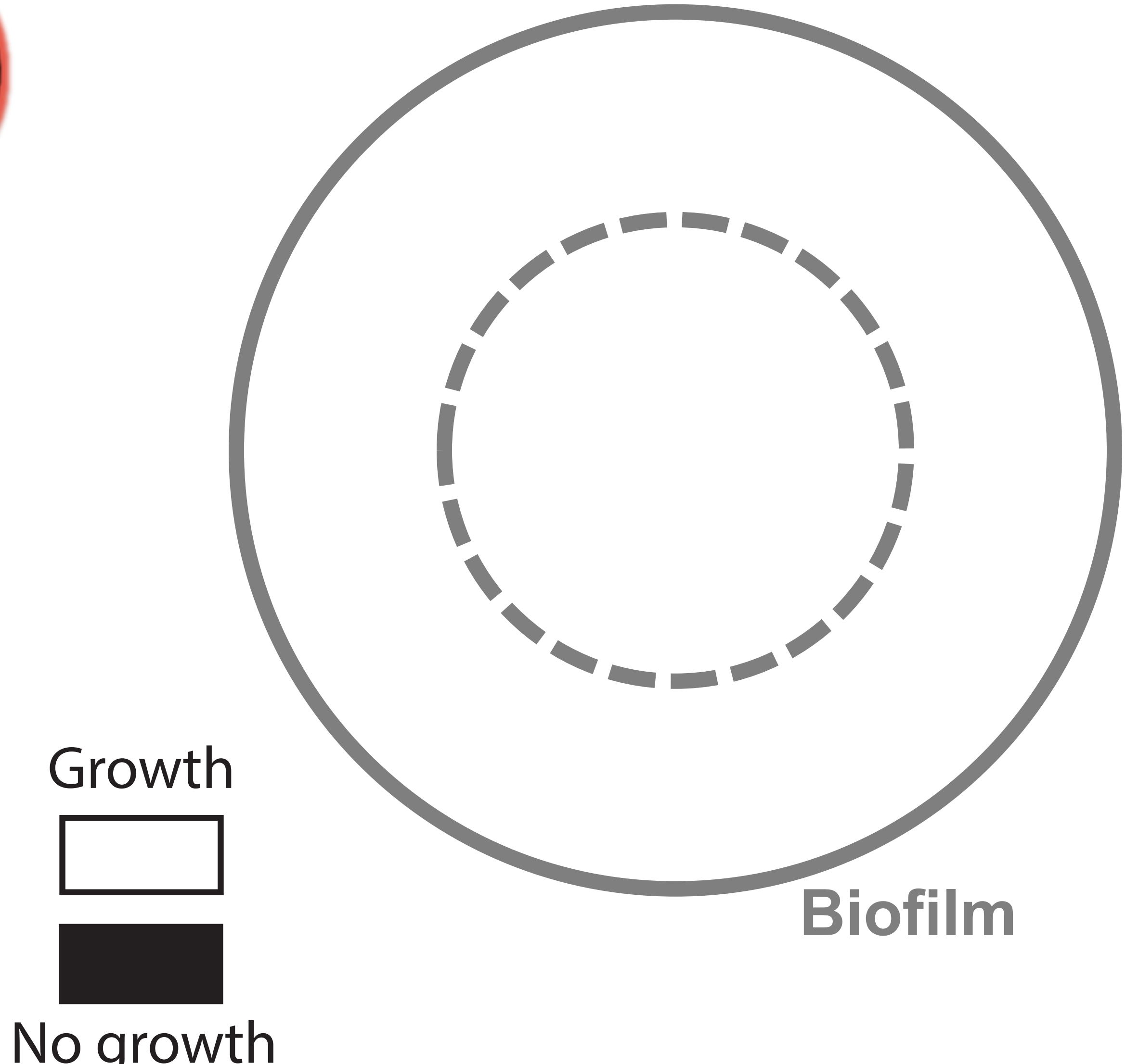
Ammonium is limited in the **periphery**



Metabolic feedback between periphery and interior







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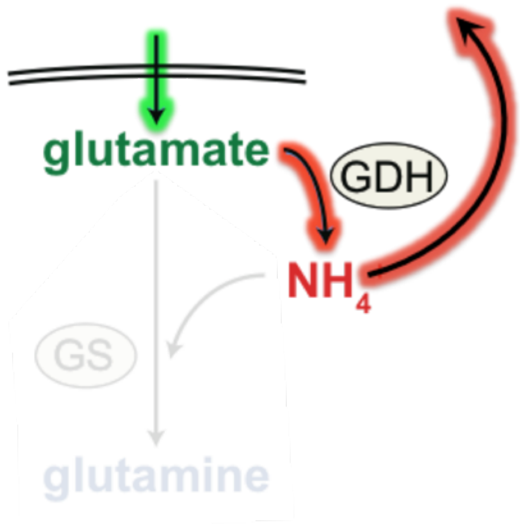
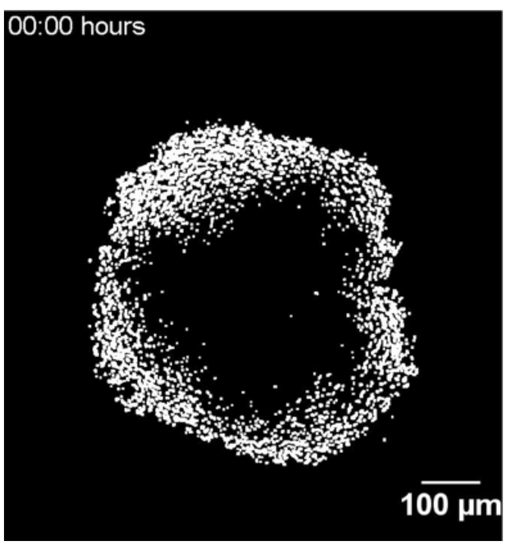
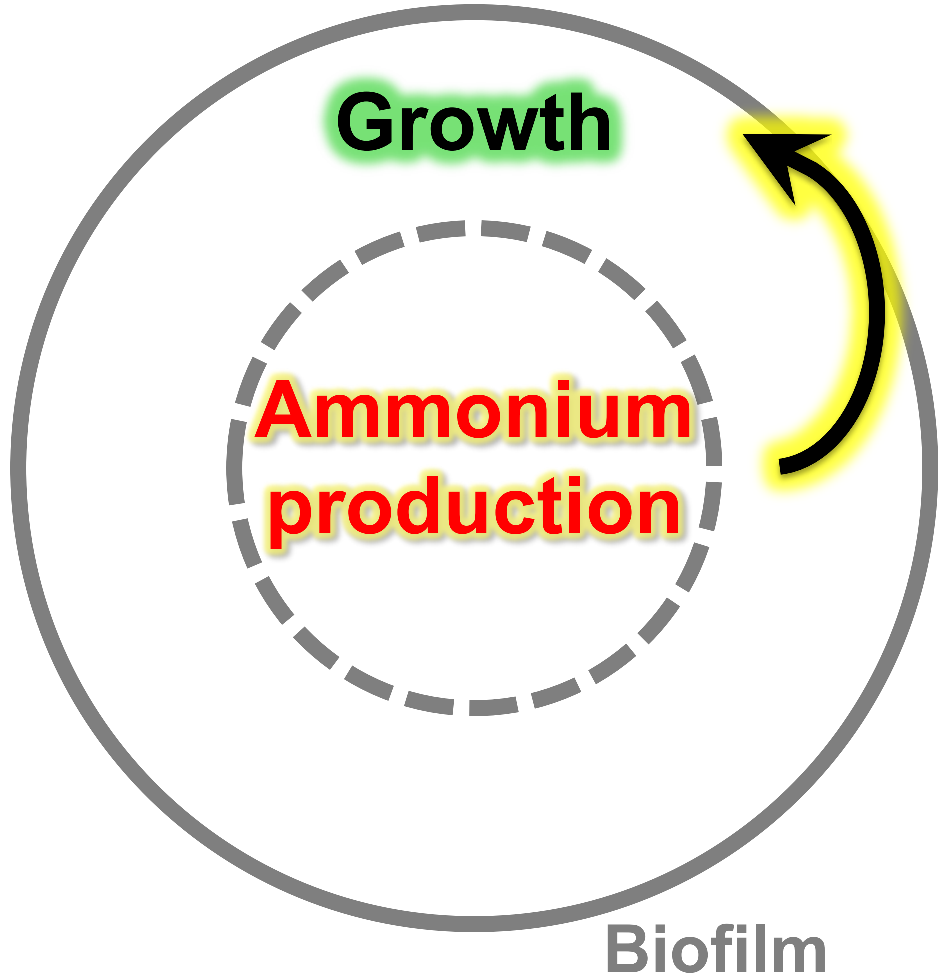
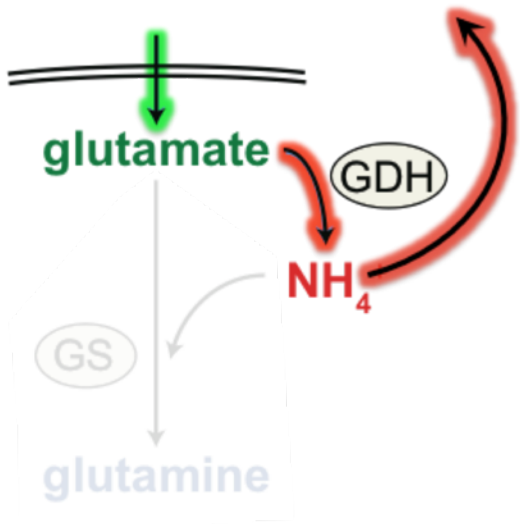
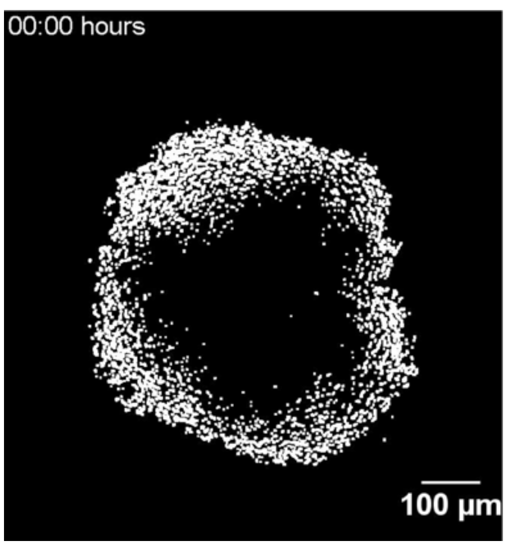


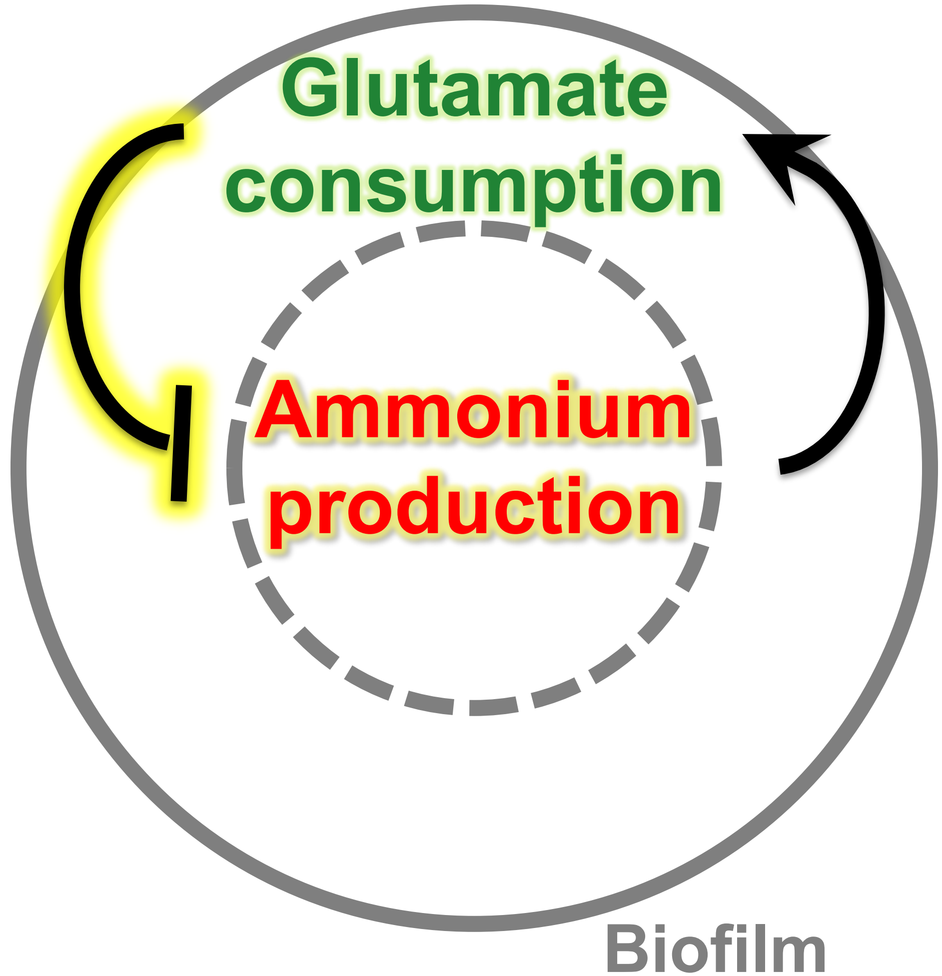




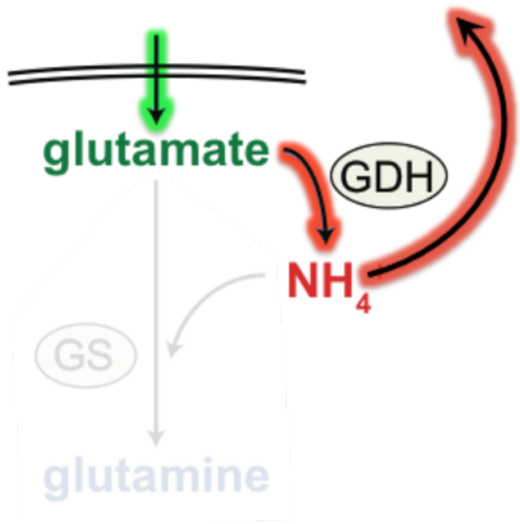
Metabolic feedback between periphery and interior

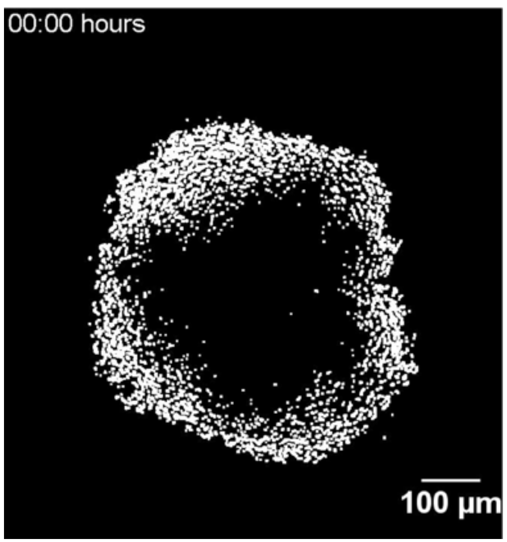


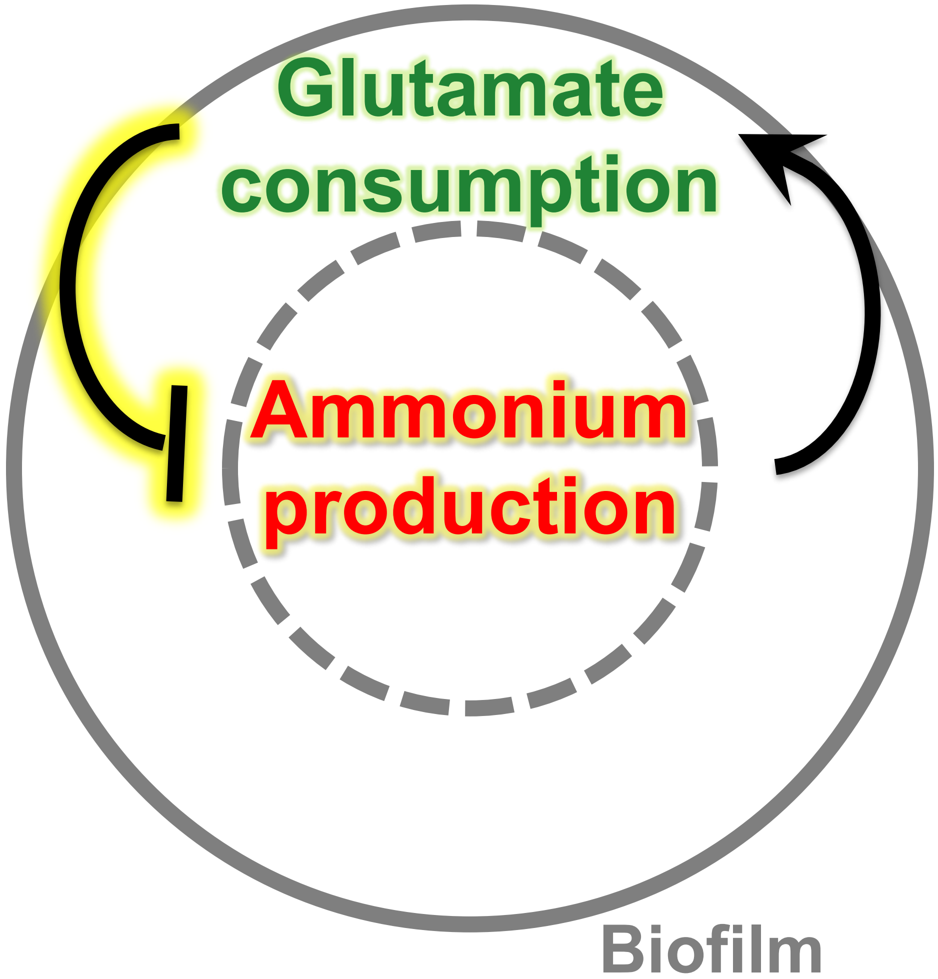




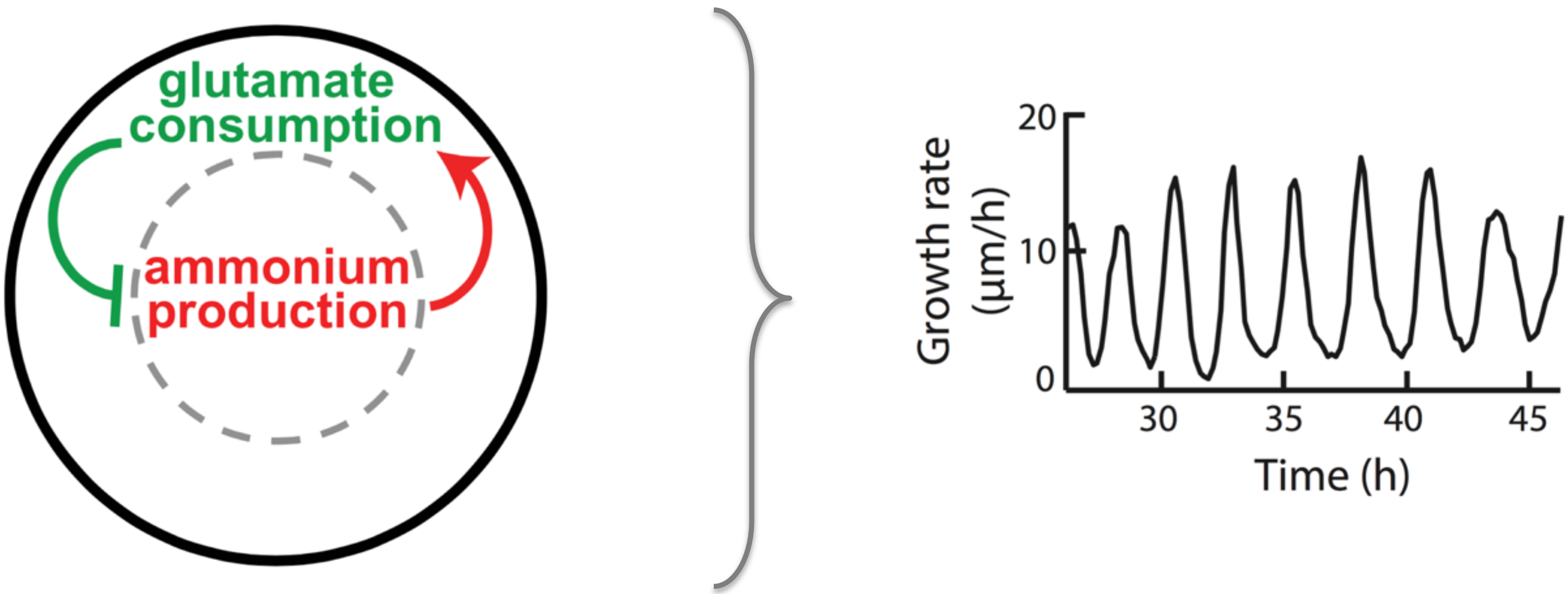
Metabolic feedback between periphery and interior



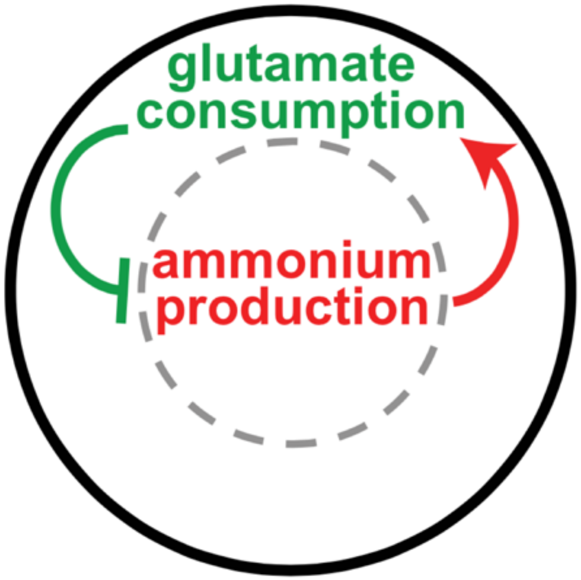


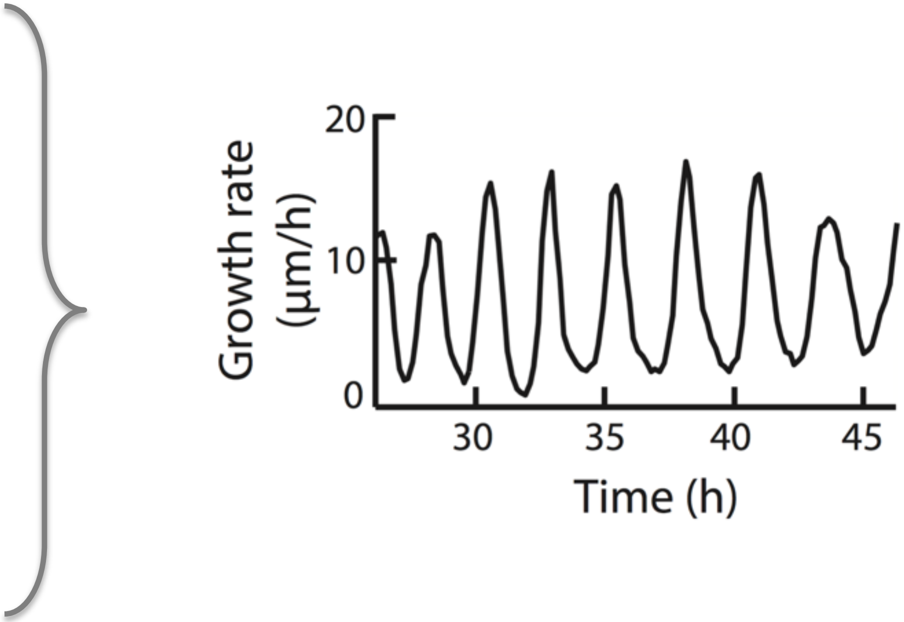


Metabolic codependence gives rise to oscillations



Do oscillations provide a biological benefit?

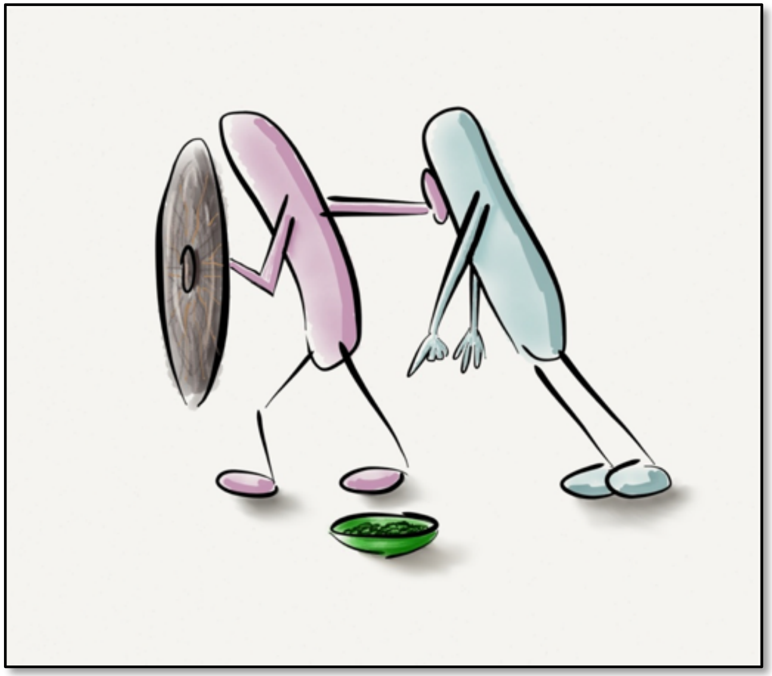


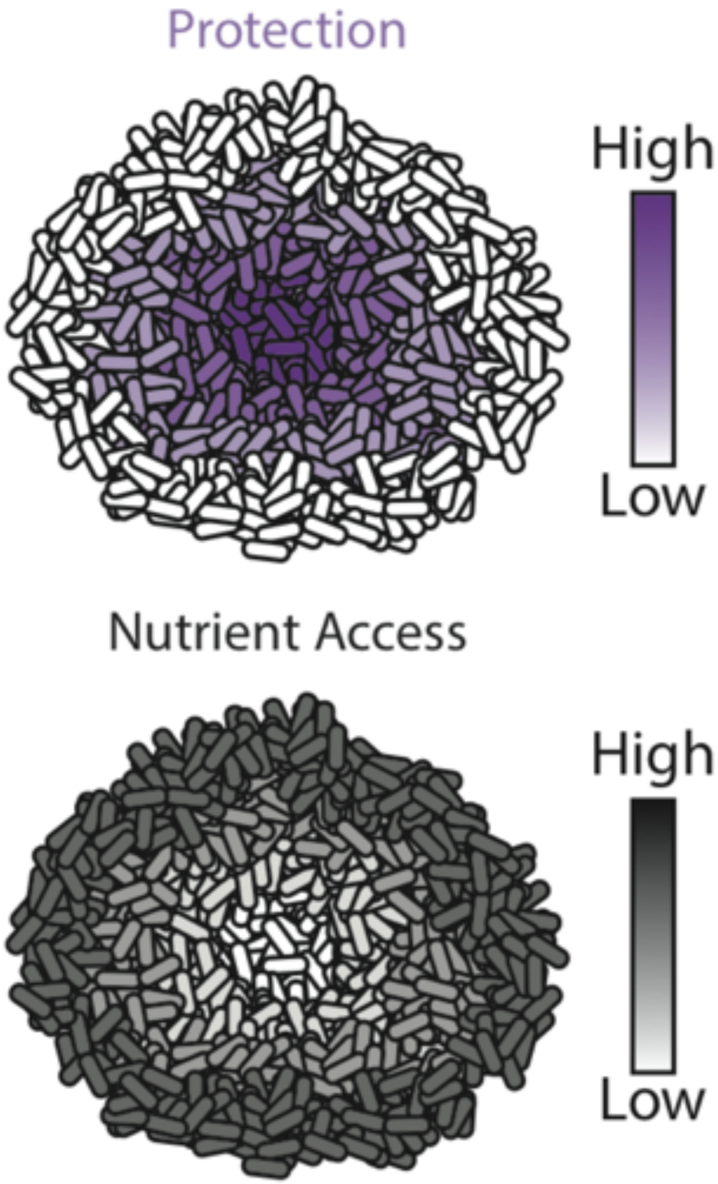


Internal conflict !

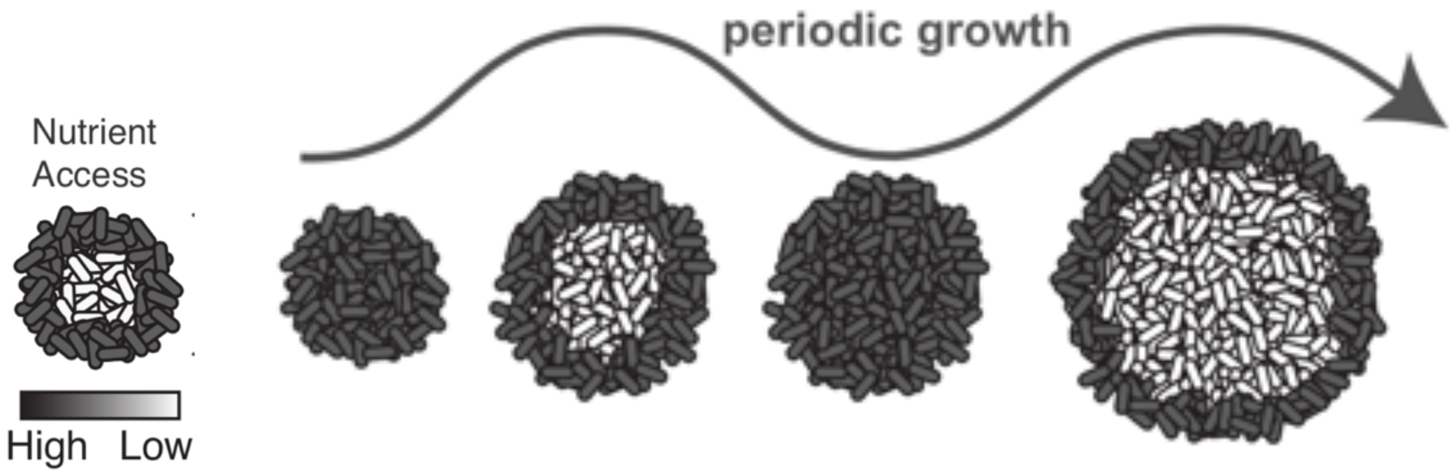
The same cells are both cooperating and competing

Protection *vs* Starvation

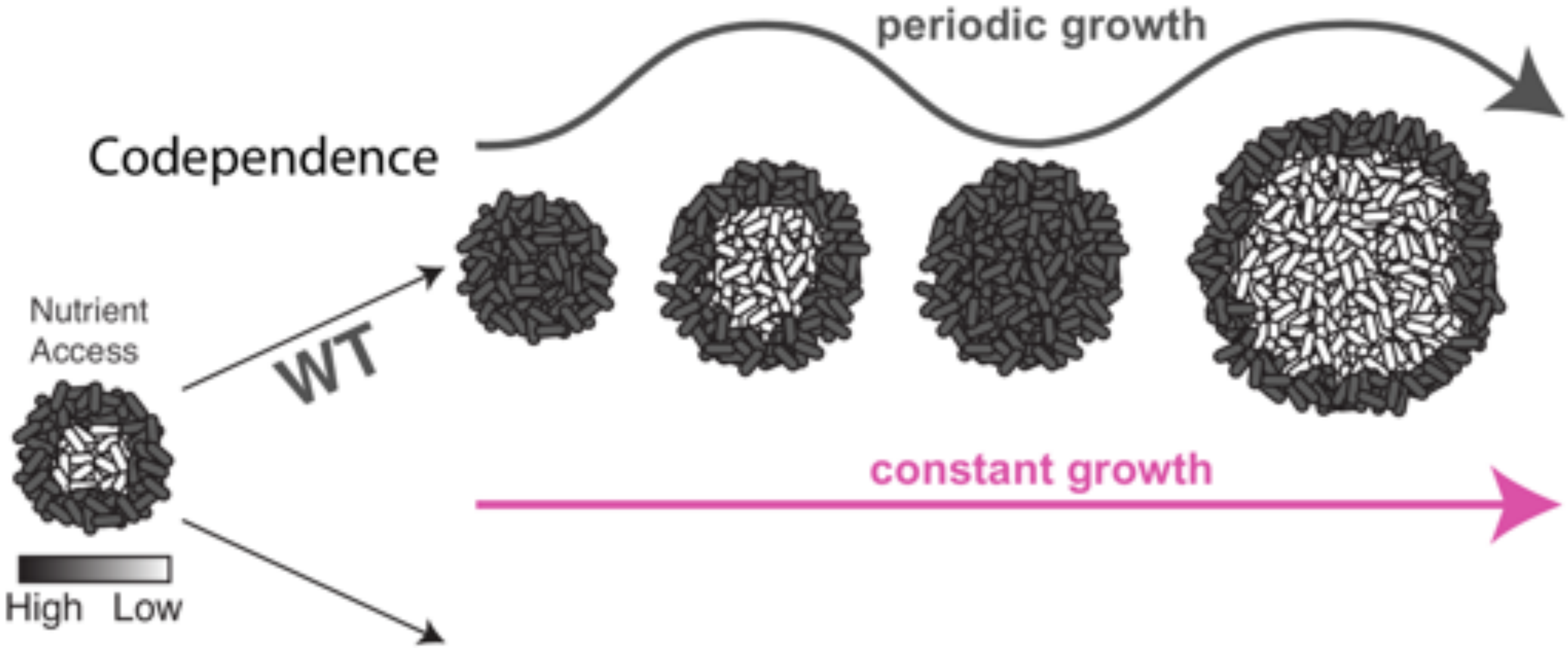




Hypothesis:
Metabolic Codependence resolves the conflict

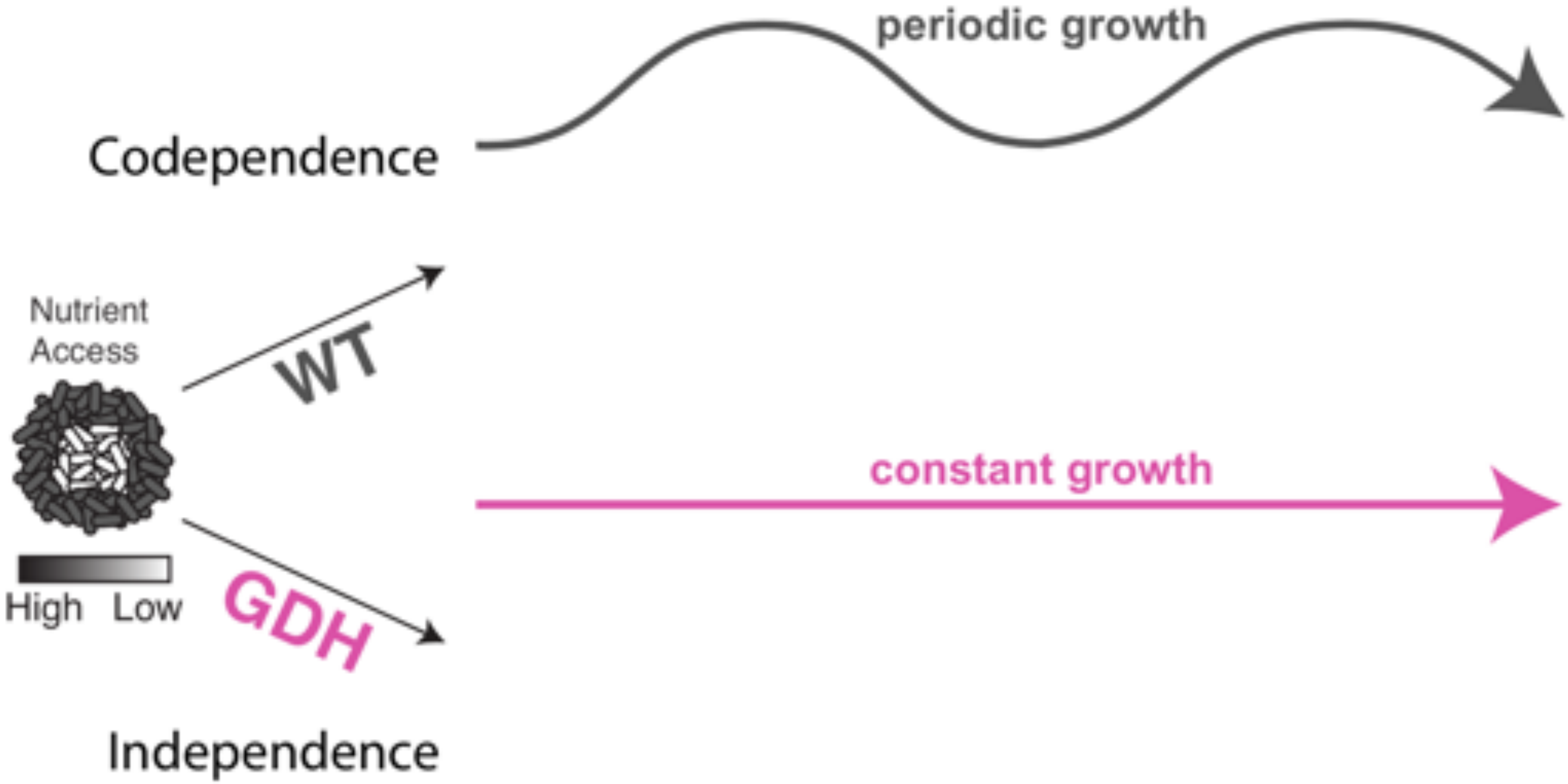


What about metabolic *Independence*?

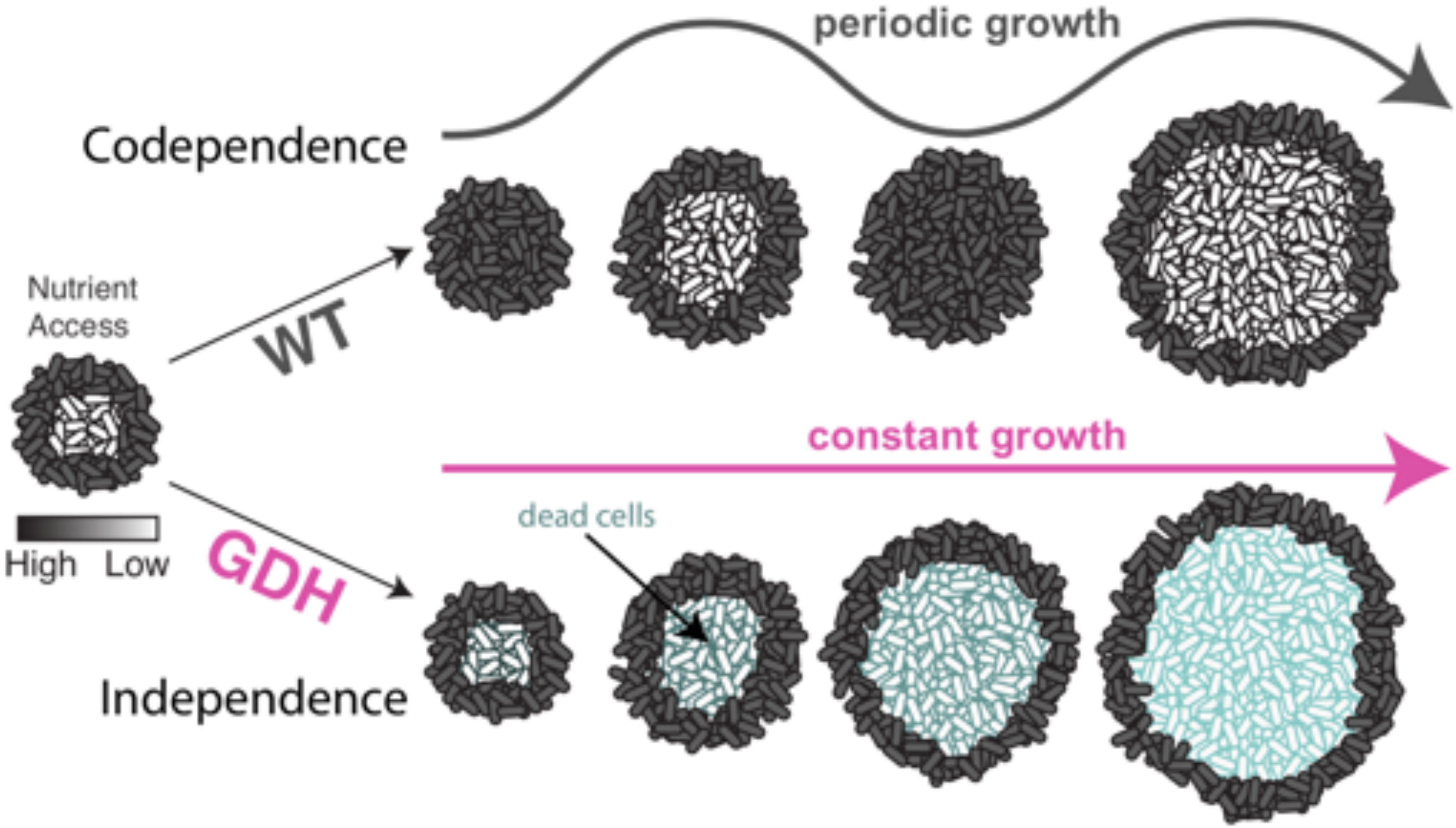


Independence

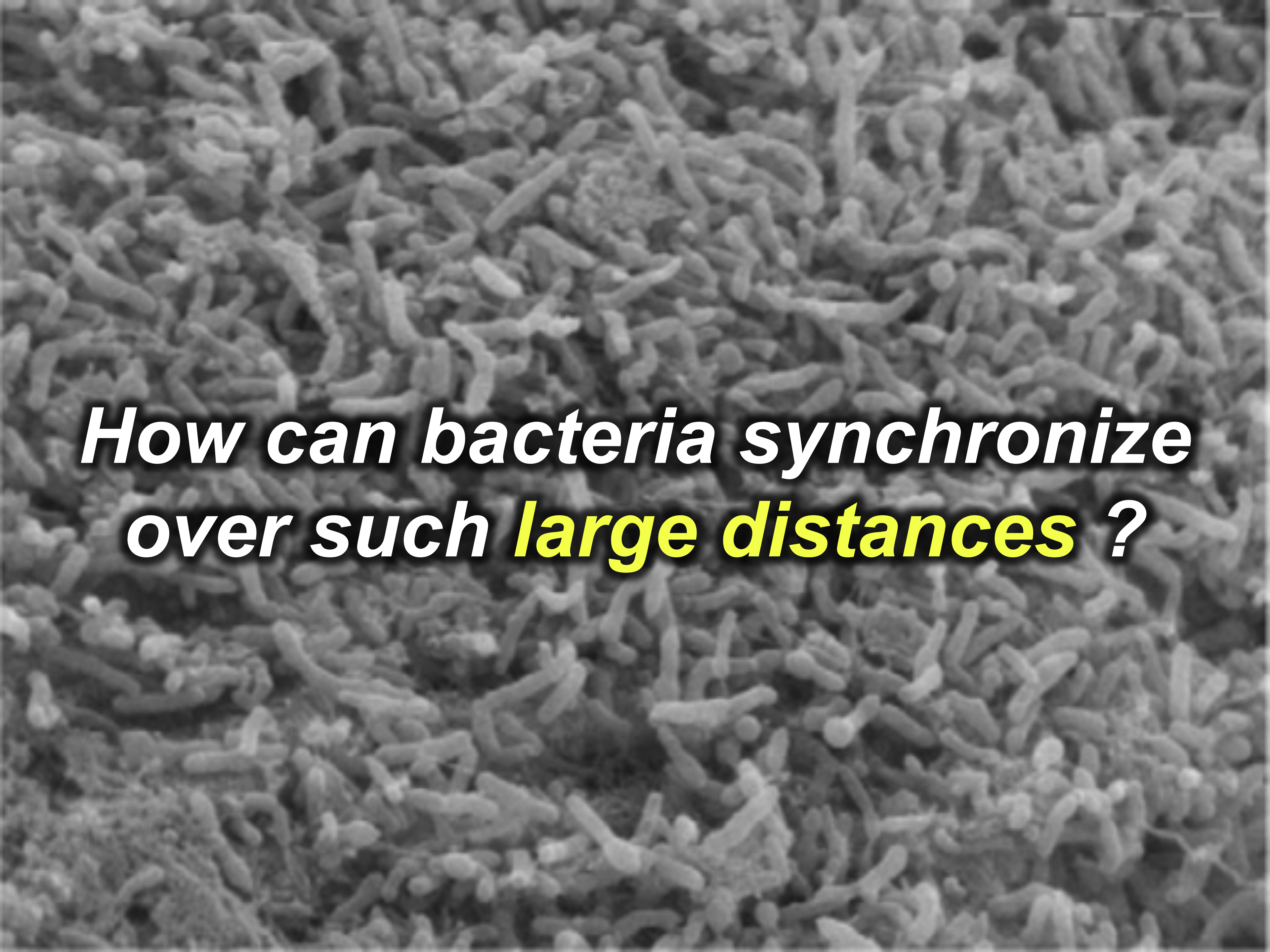
Let’s compare these two strategies



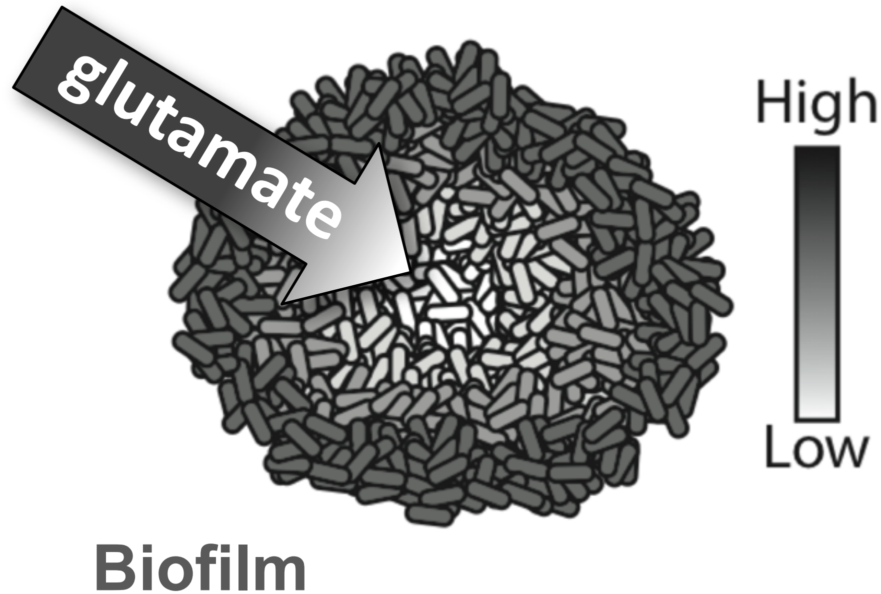
Metabolic Codependence is a beneficial strategy



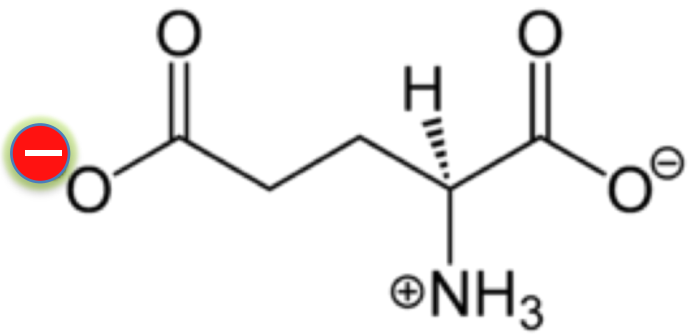

Do bacteria in biofilms
coordinate their behavior ?
YES !
Collective oscillations
that increase fitness


How can bacteria synchronize
over such large distances ?

… again glutamate plays a critical role

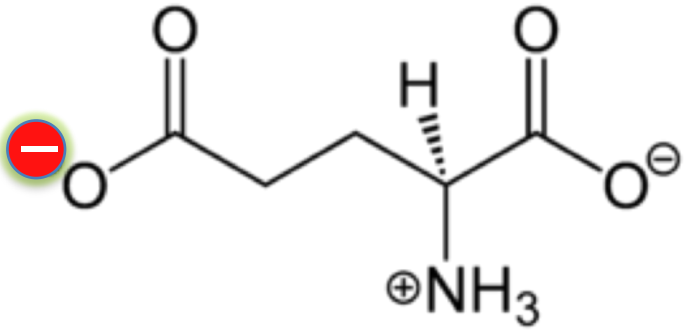


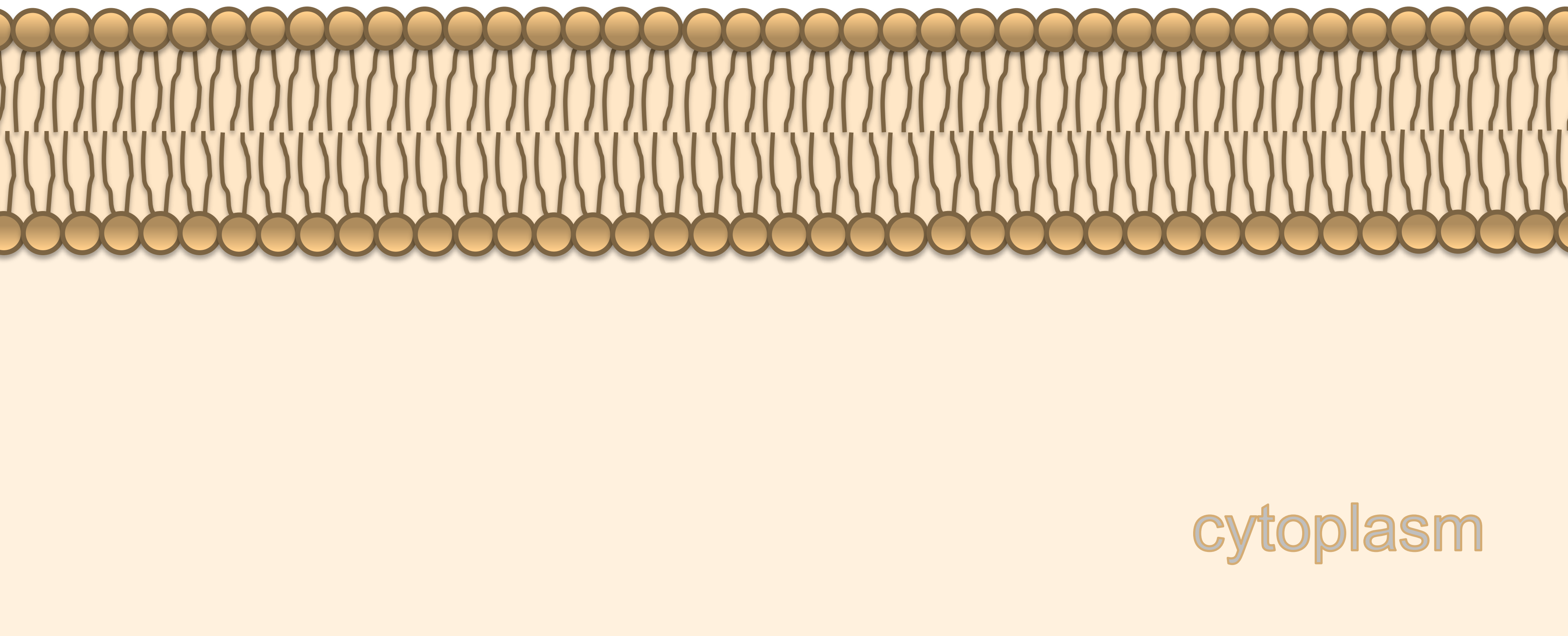
Glutamate is a charged amino acid



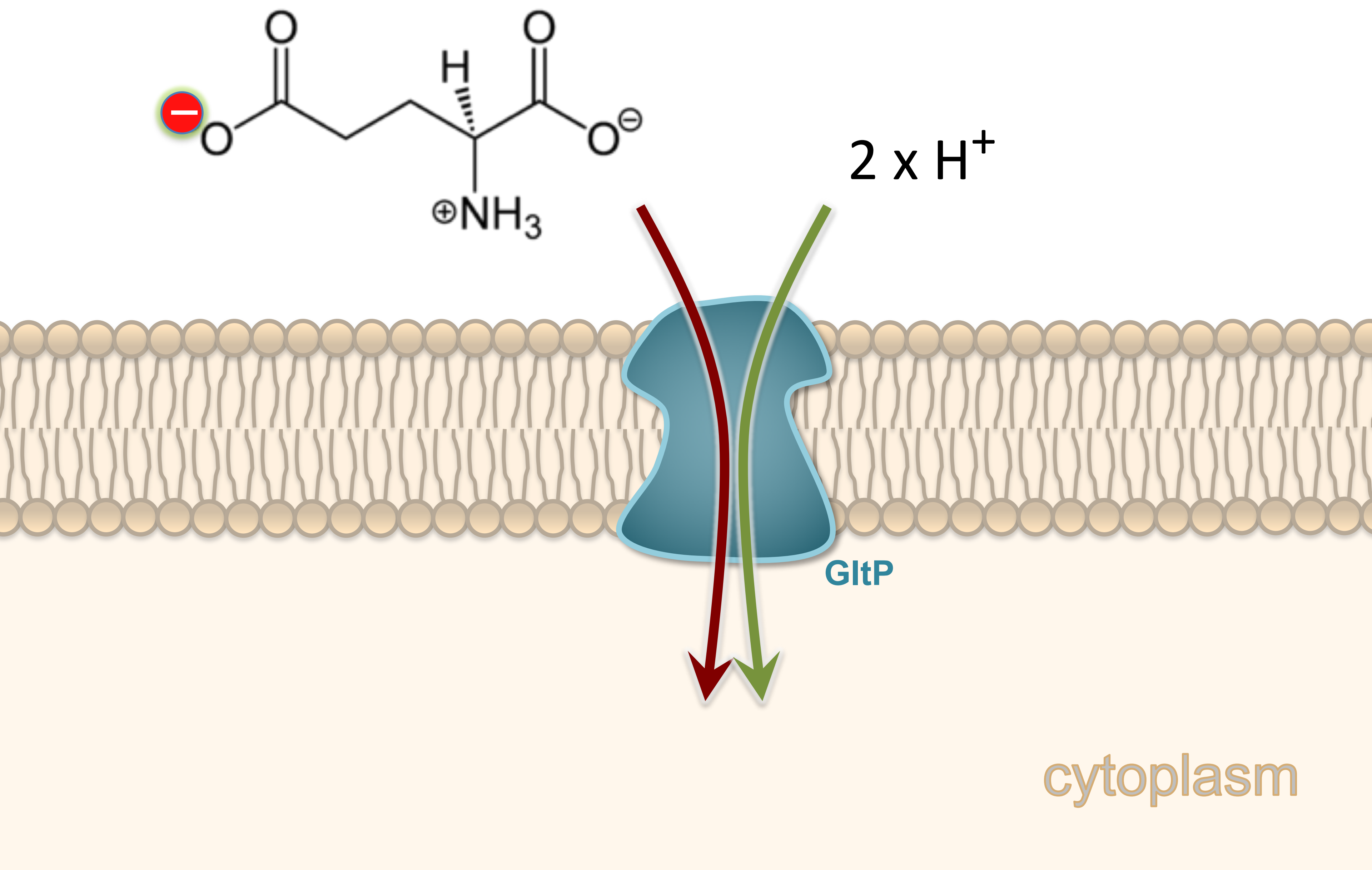
Biofilm

Glutamate cannot diffuse through the membrane

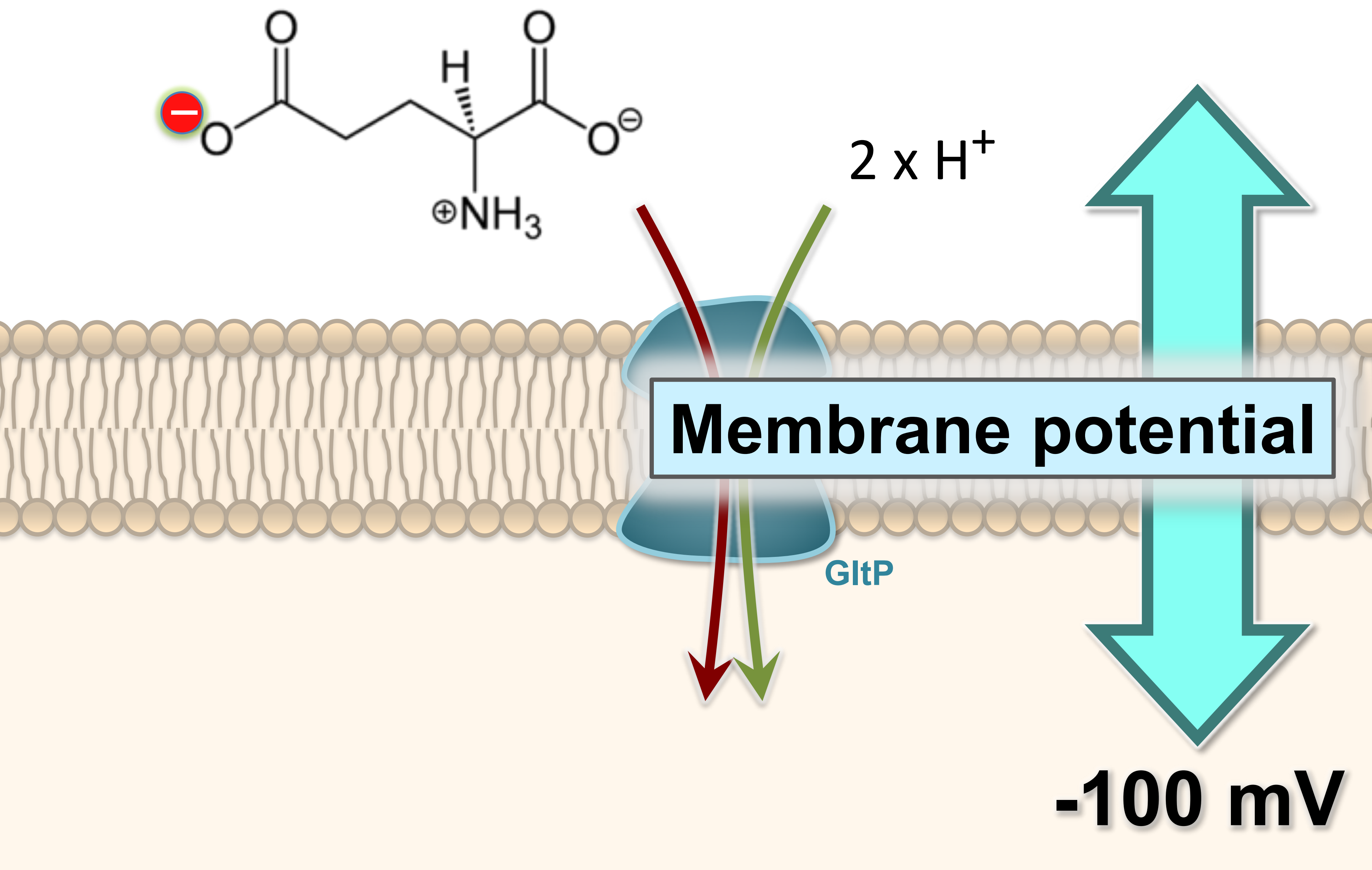




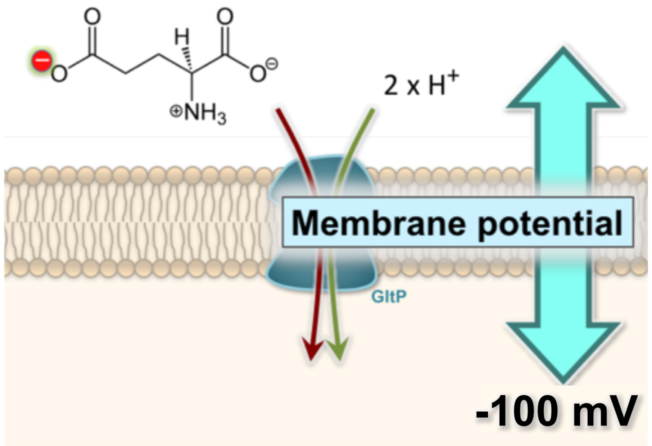
Proton Motive Force drives glutamate uptake

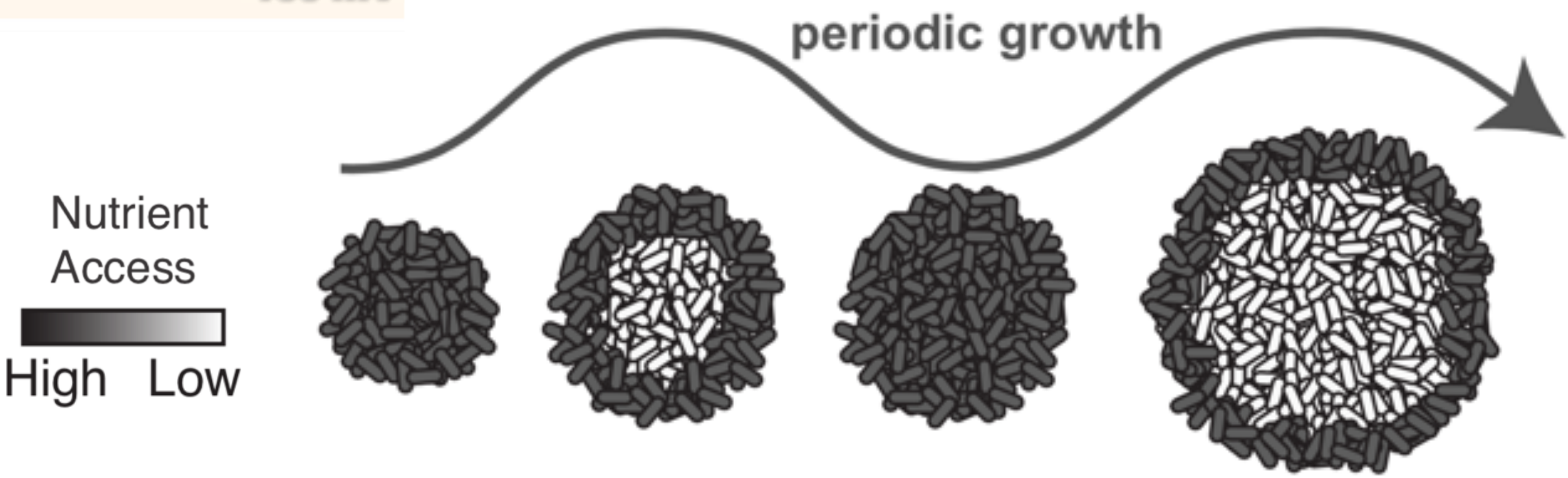


PMF depends on the membrane potential



Prediction:
Membrane potential regulation during biofilm growth





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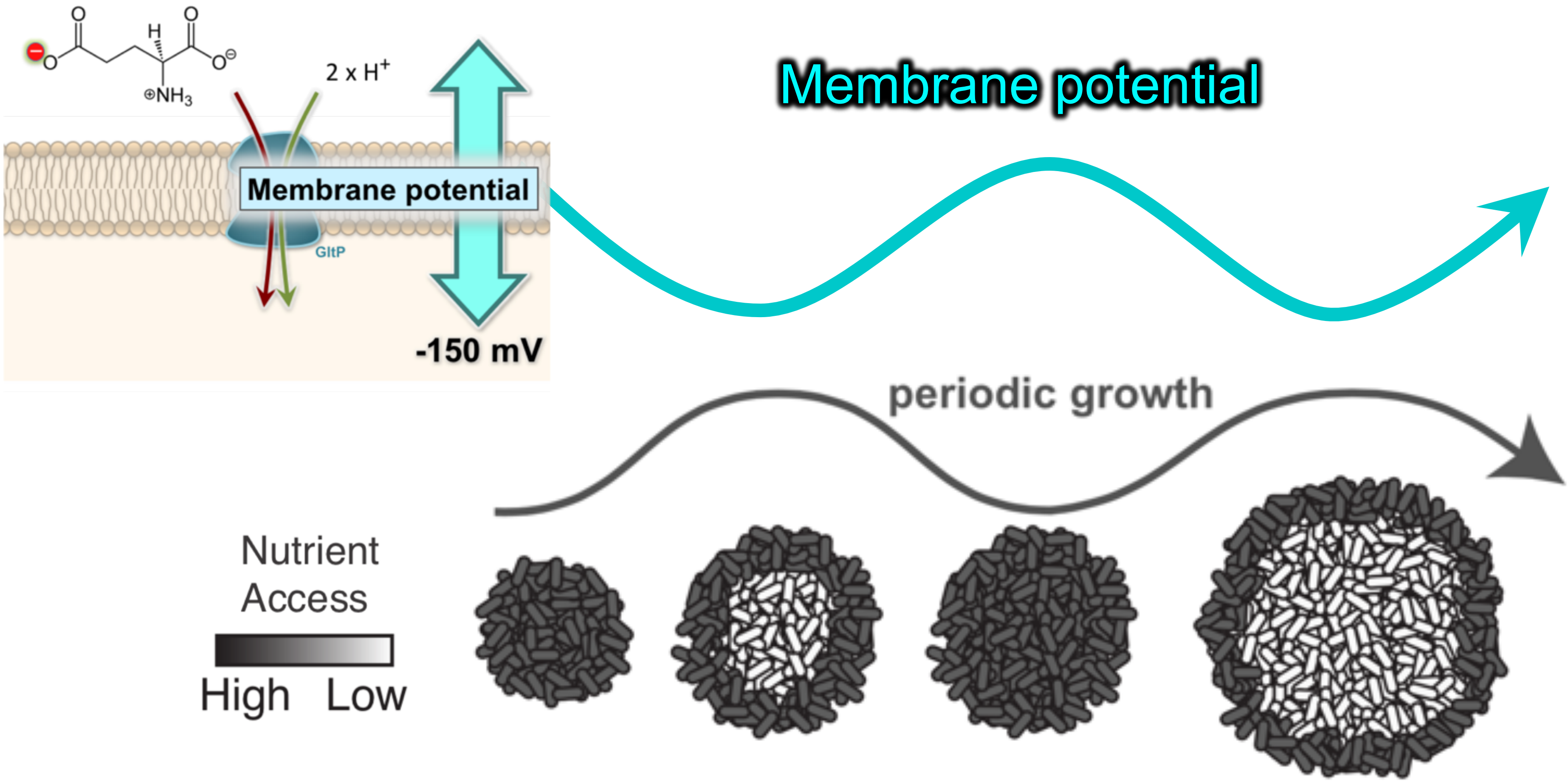
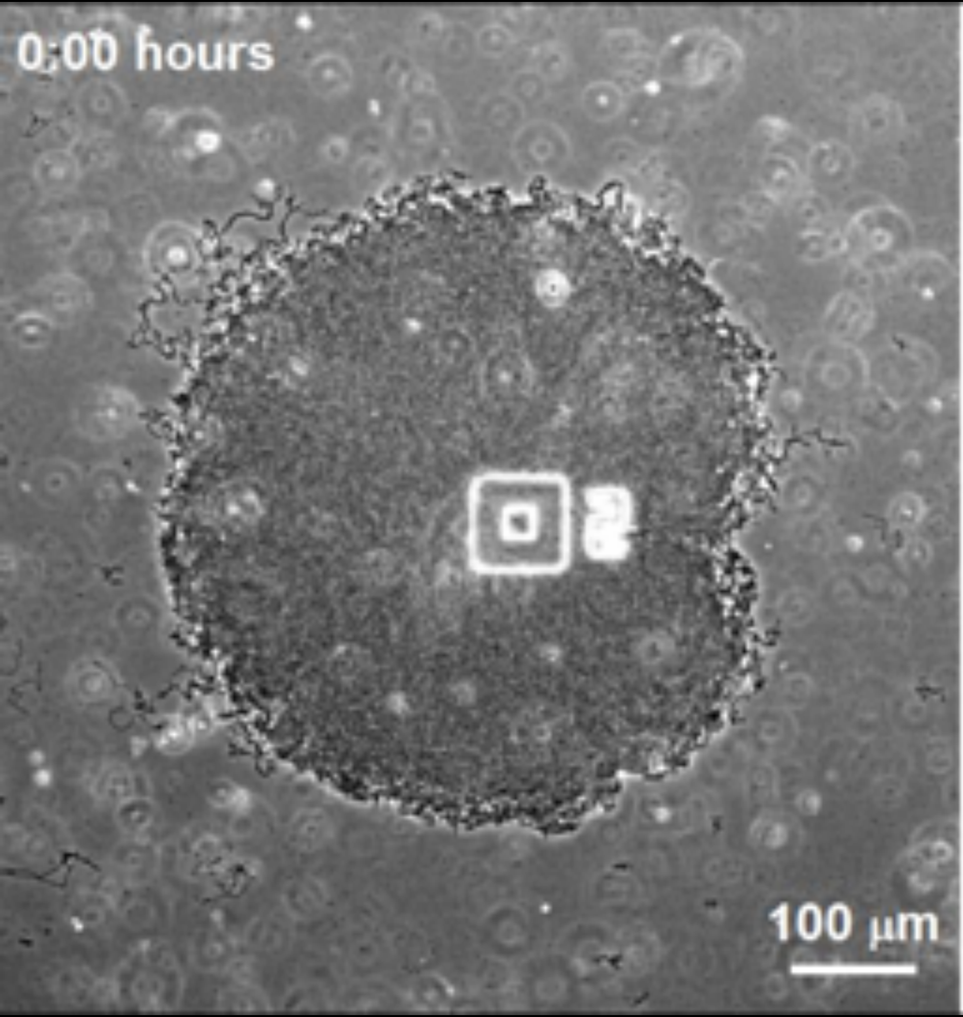
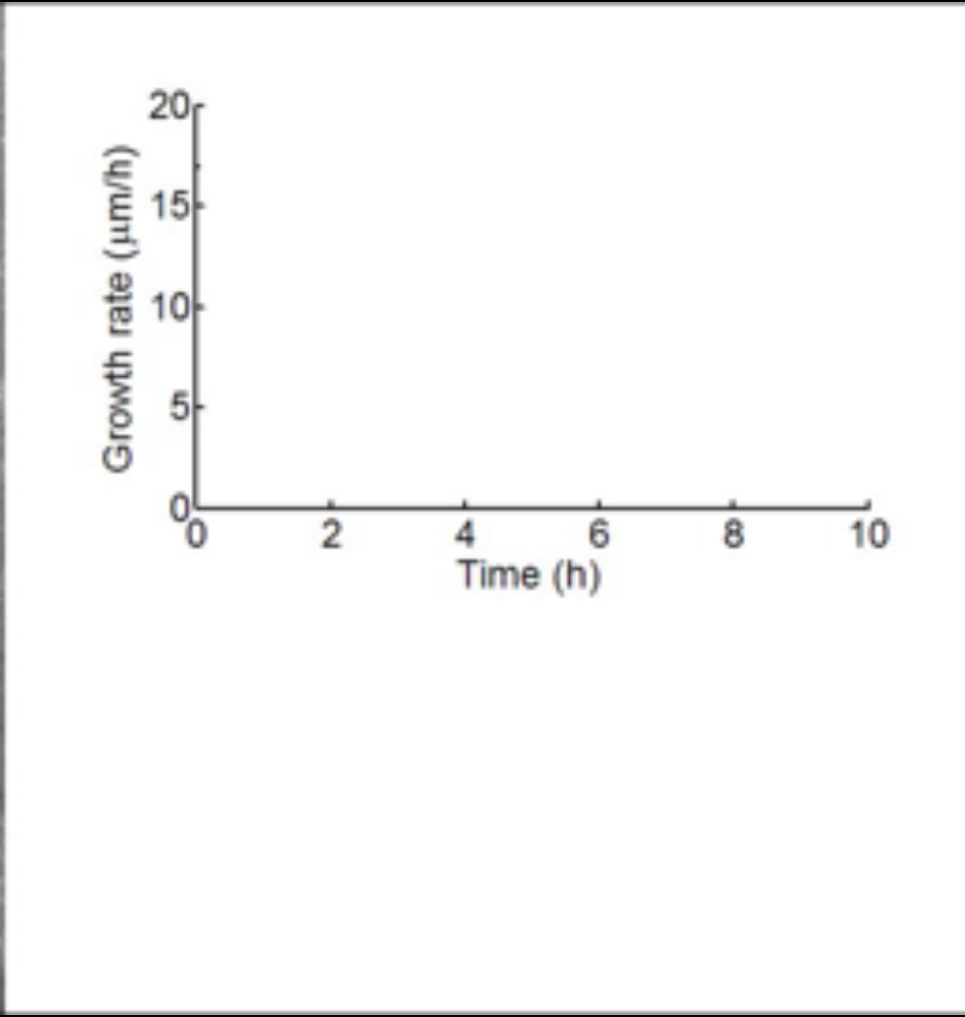
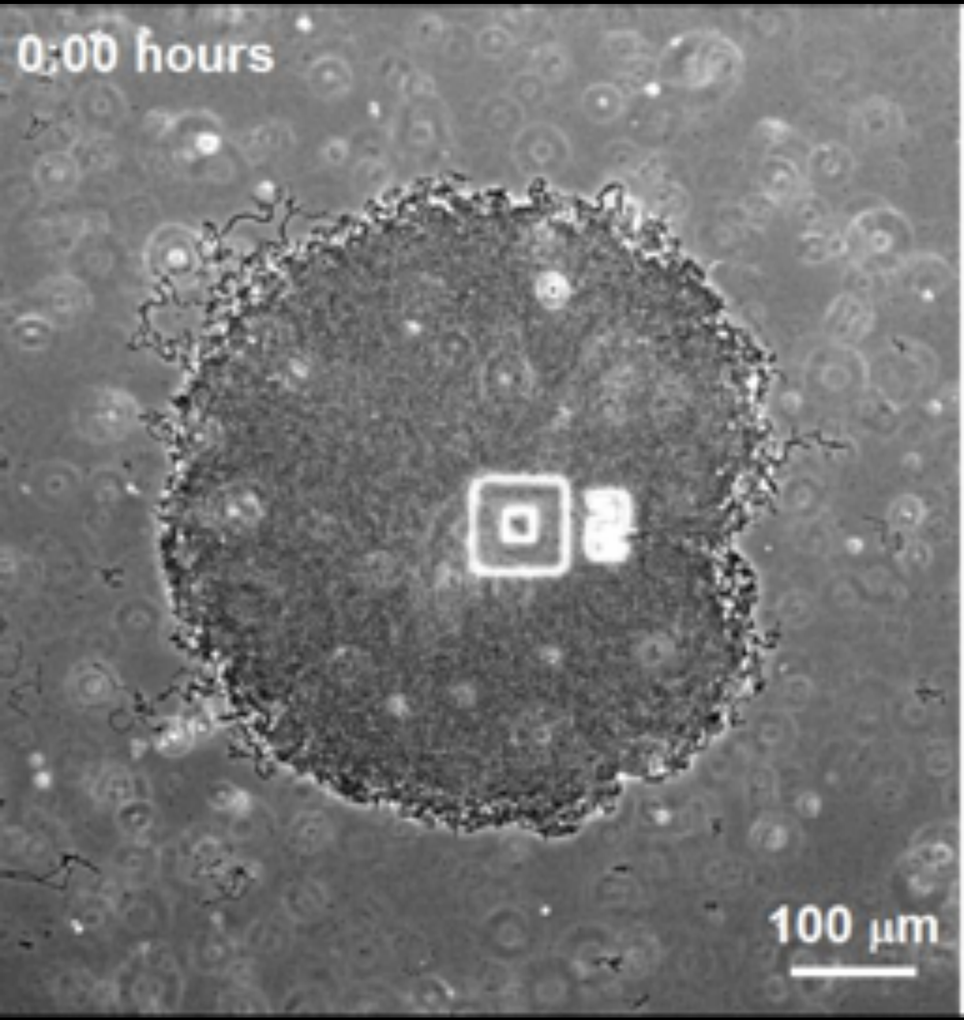


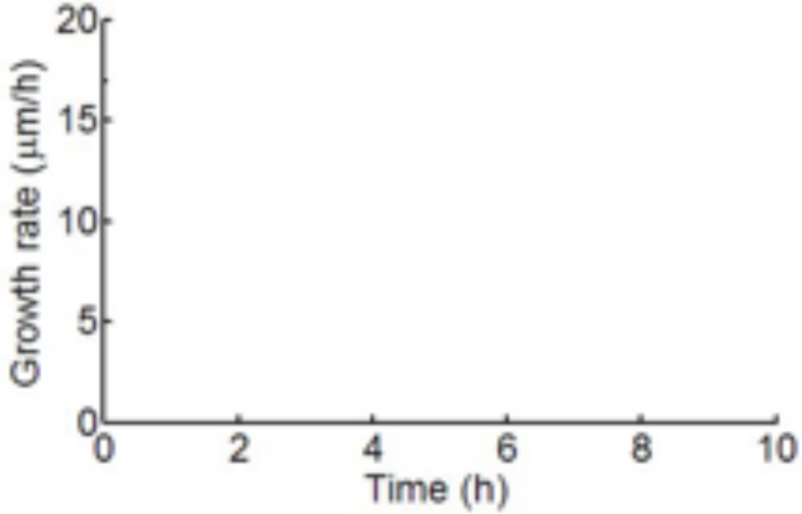
Metabolically driven growth oscillations



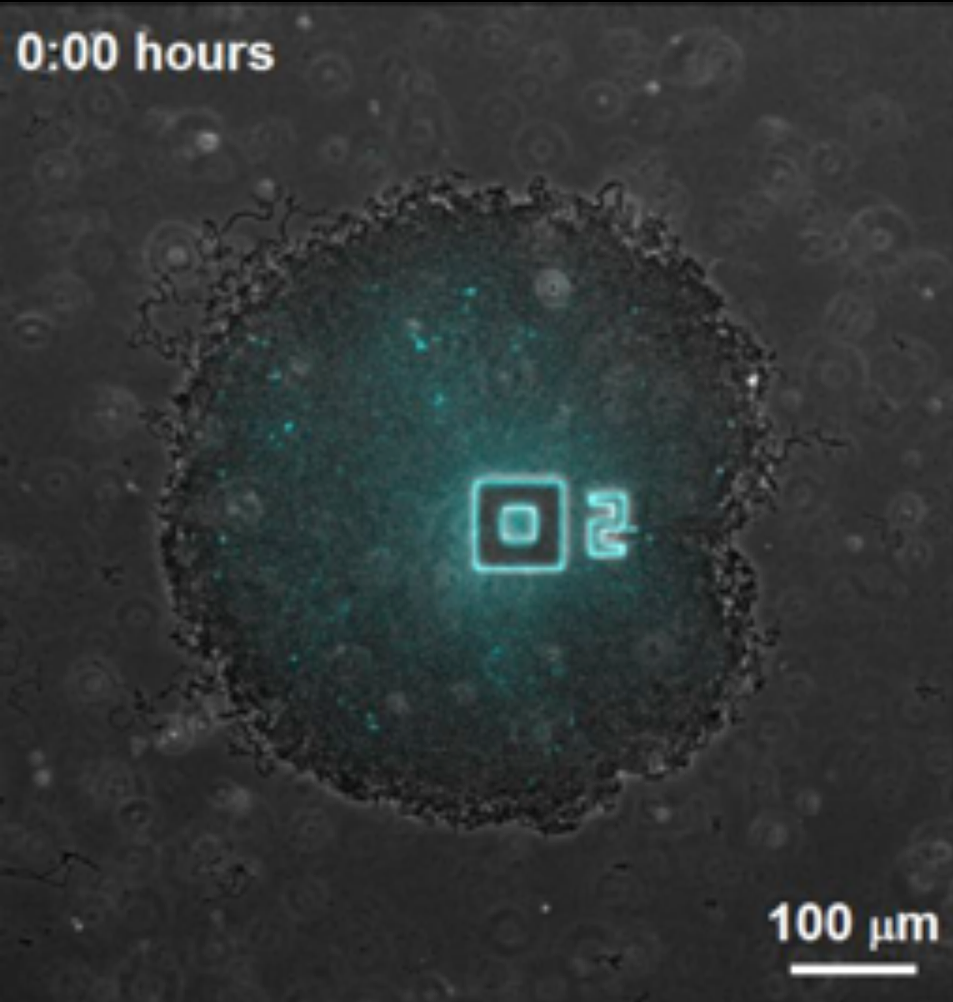


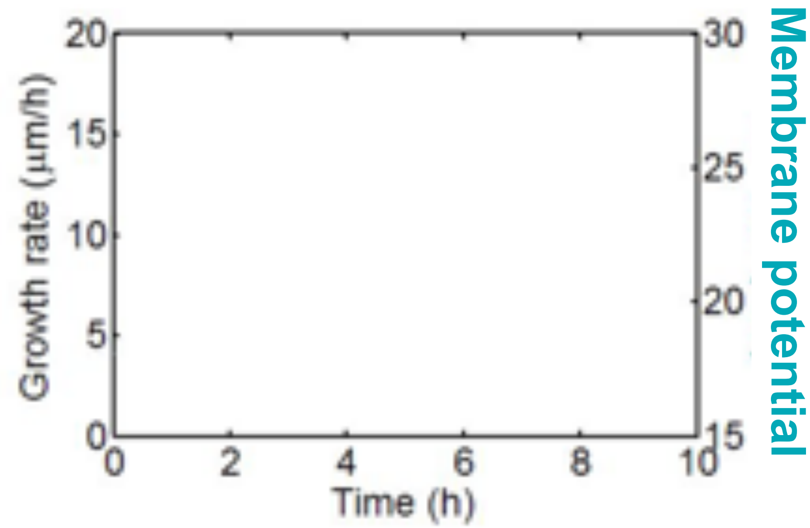
Metabolically driven growth oscillations



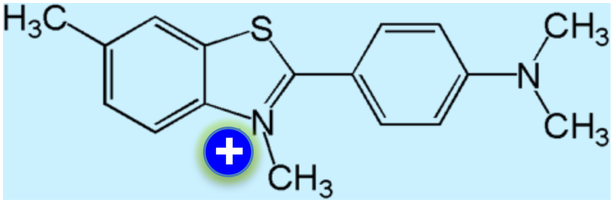


Measuring membrane potential dynamics





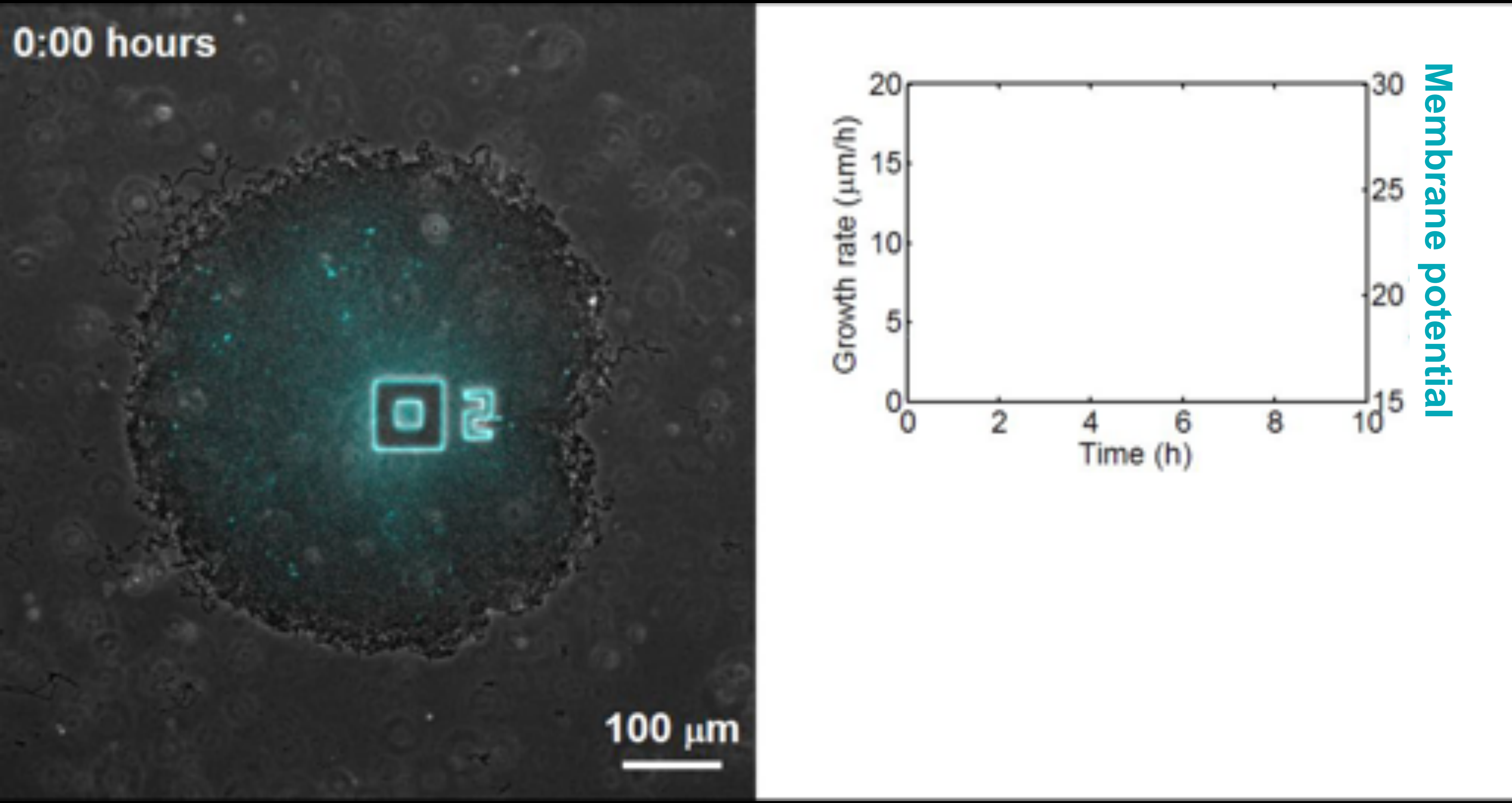
Cationic dye



Thioflavin-T

Prindle *et al.*, Nature, **527**, 59-63 (2015)

Measuring membrane potential dynamics



Thioflavin-T

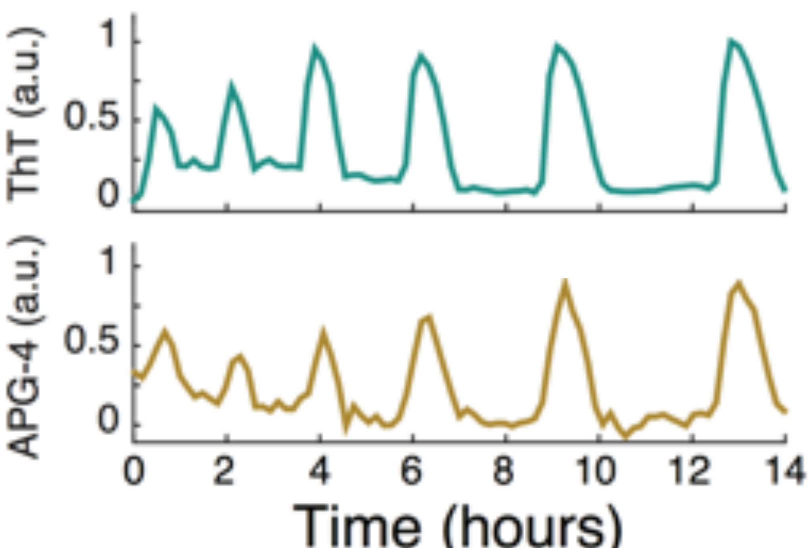
Prindle *et al*., Nature, **527**, 59-63 (2015)

Which ions are involved in membrane potential oscillations?

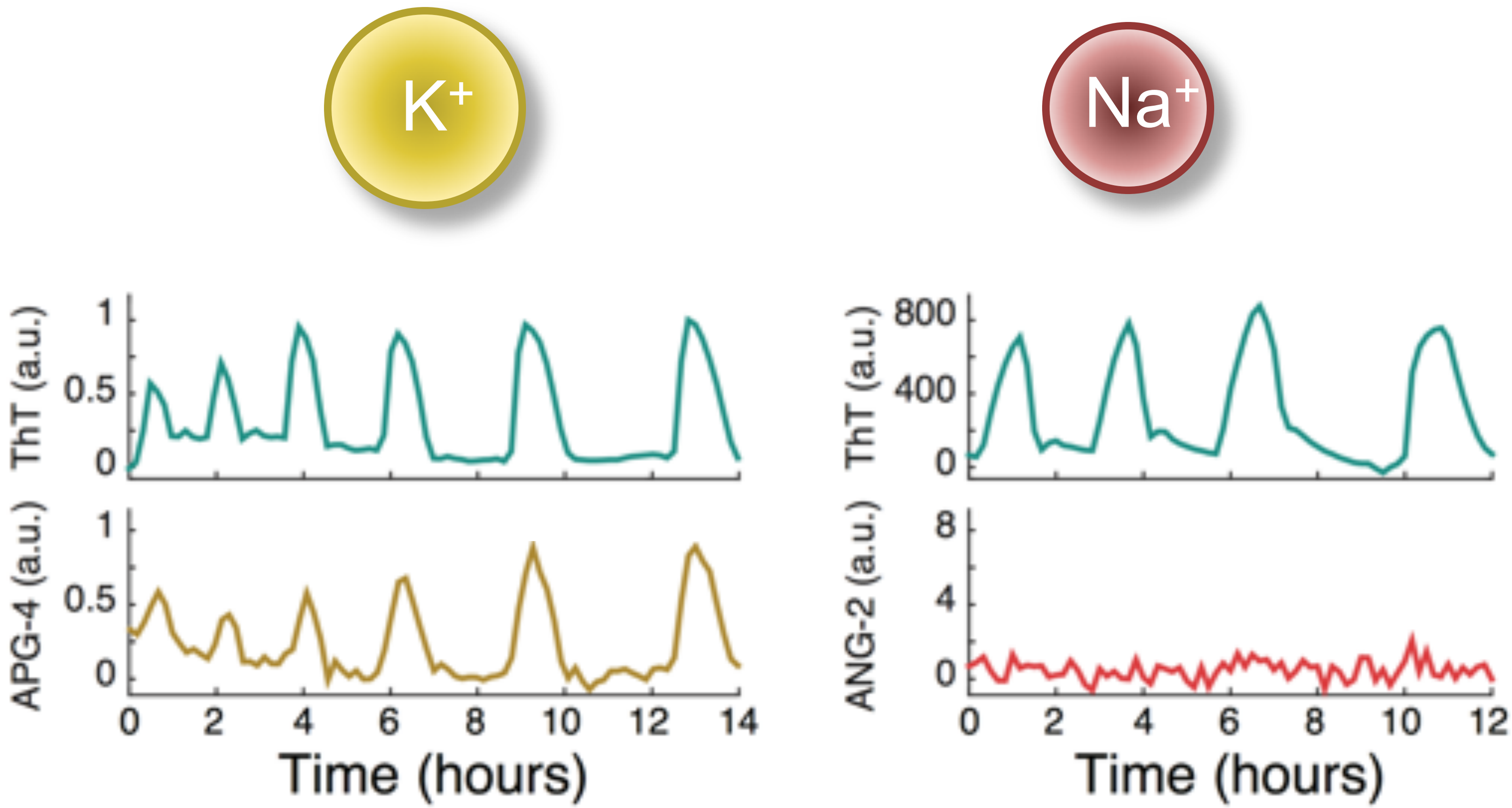
Which ions are involved in membrane potential oscillations?

K^+

Na^+



Which ions are involved in membrane potential oscillations?



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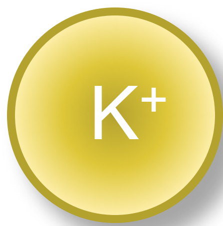
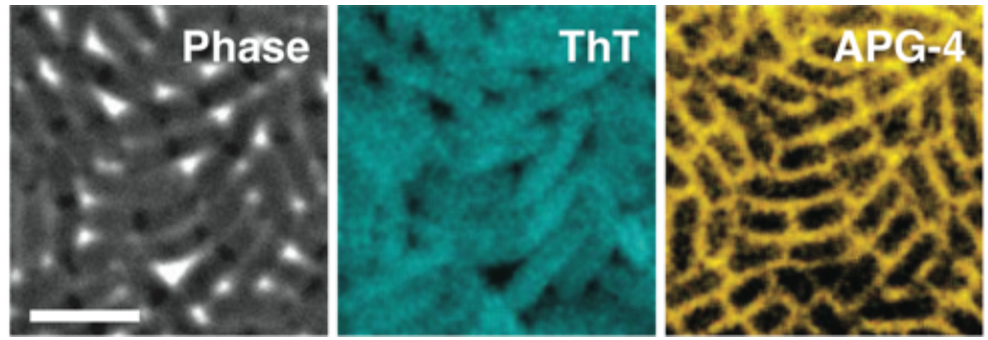
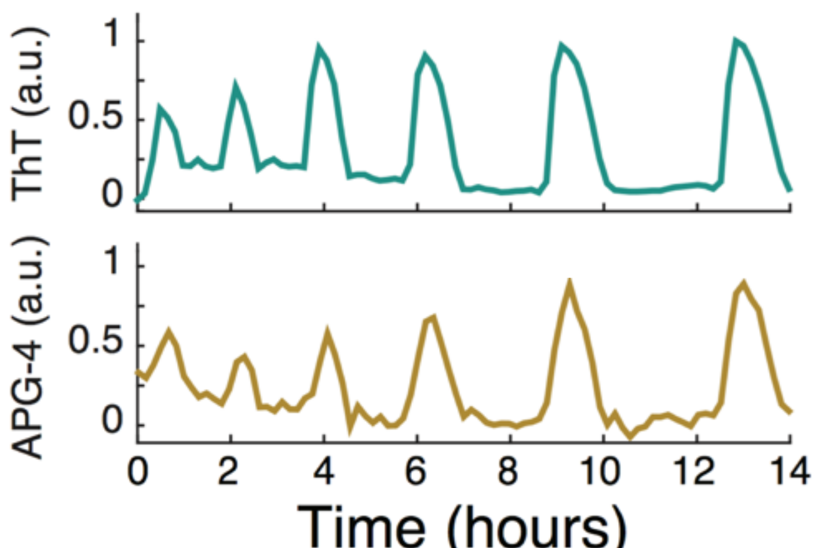
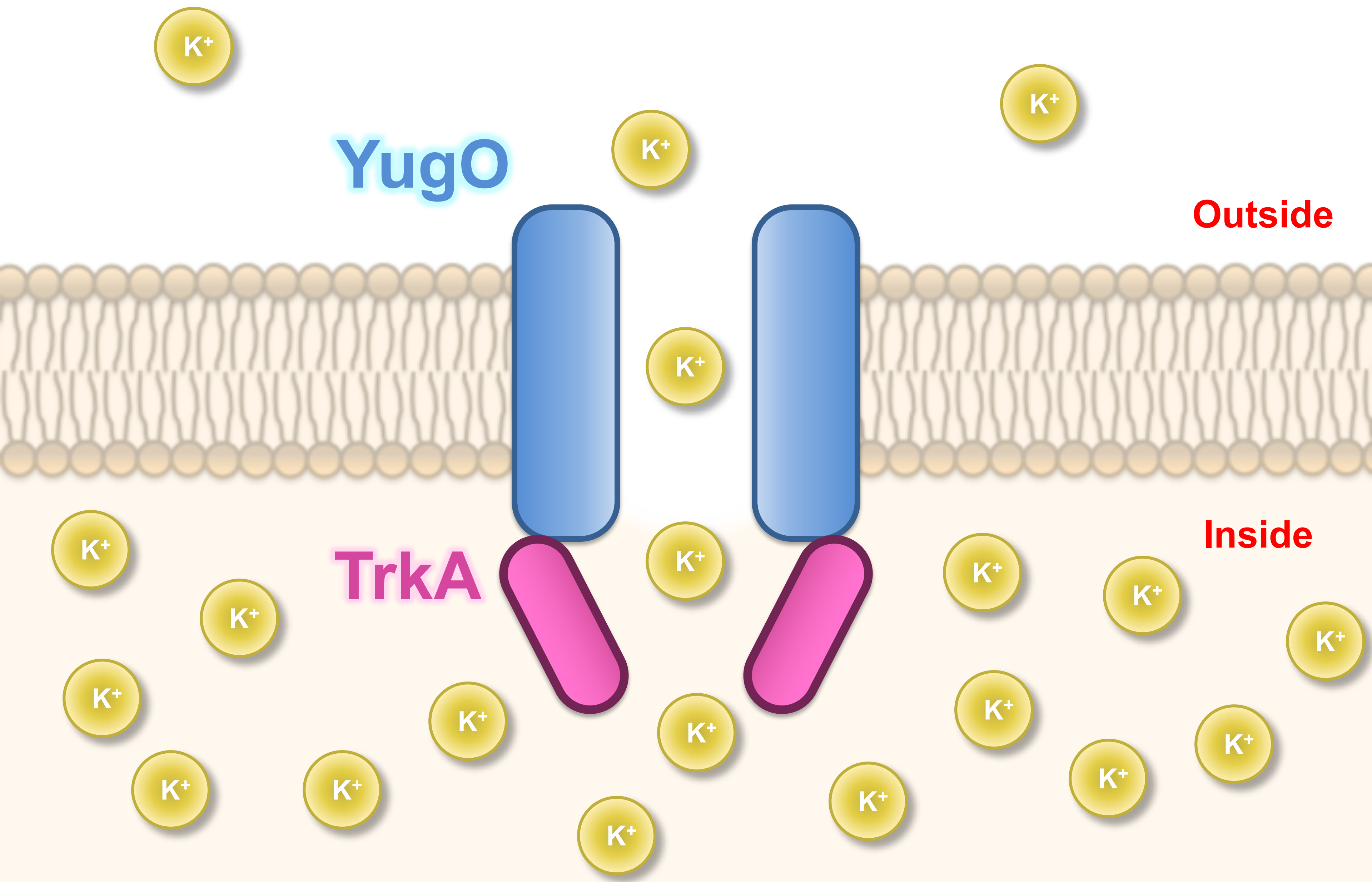




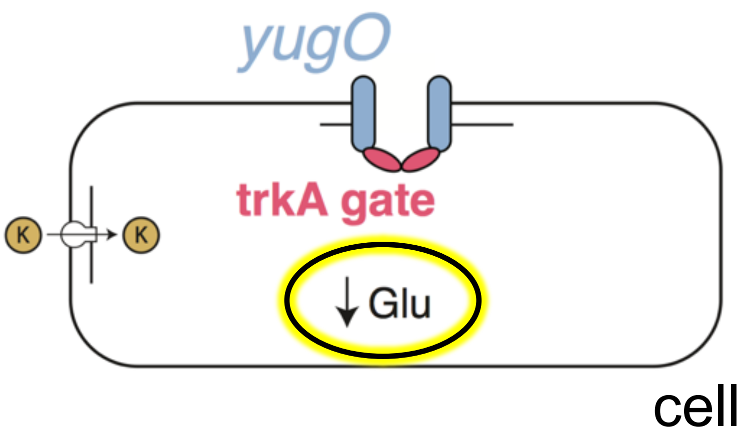


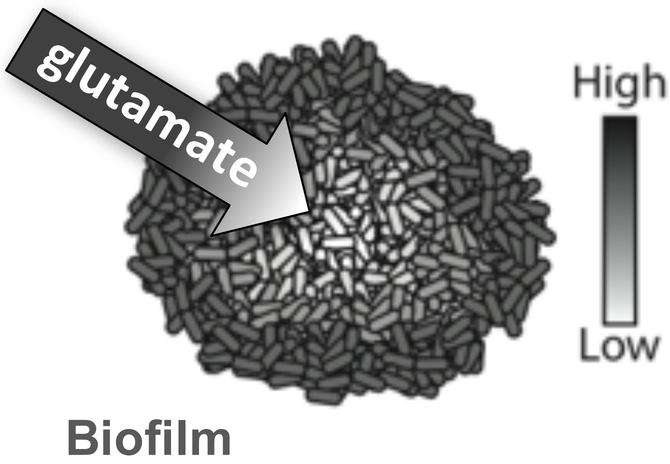
YugO, the *B. subtilis* K-channel



Mechanism for electrical signaling in biofilms

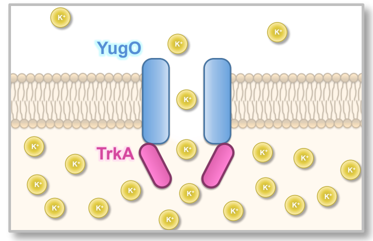
The trigger:

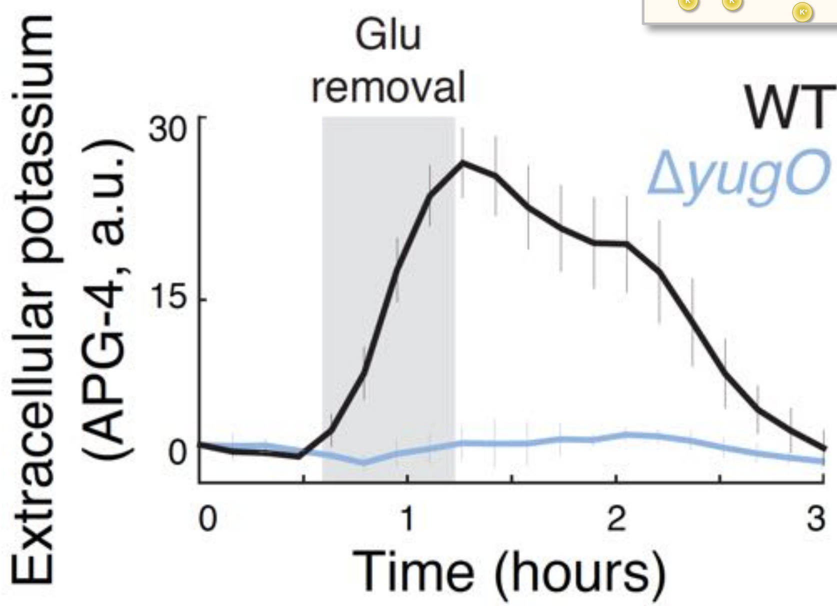


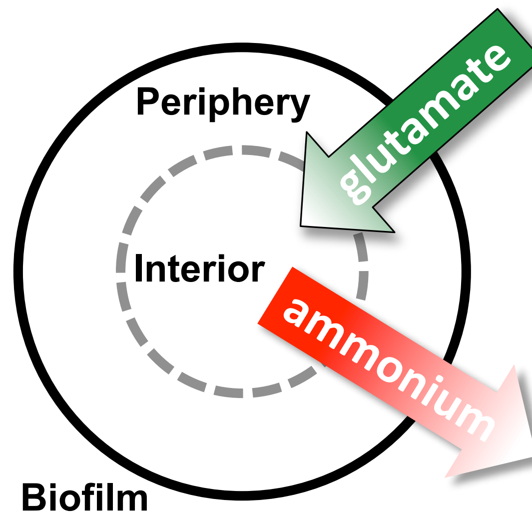


Glutamate limitation
for interior cells

Trigger for K^+ release: Glutamate starvation

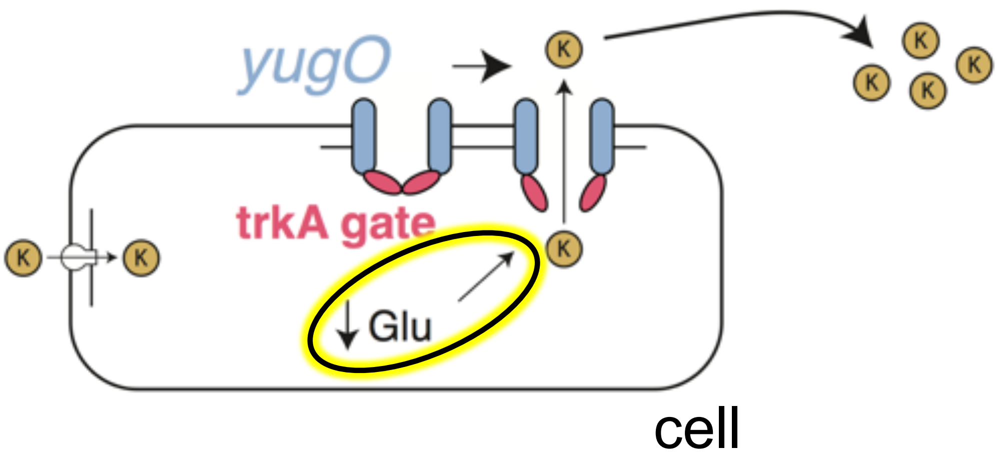






Mechanism for electrical signaling in biofilms

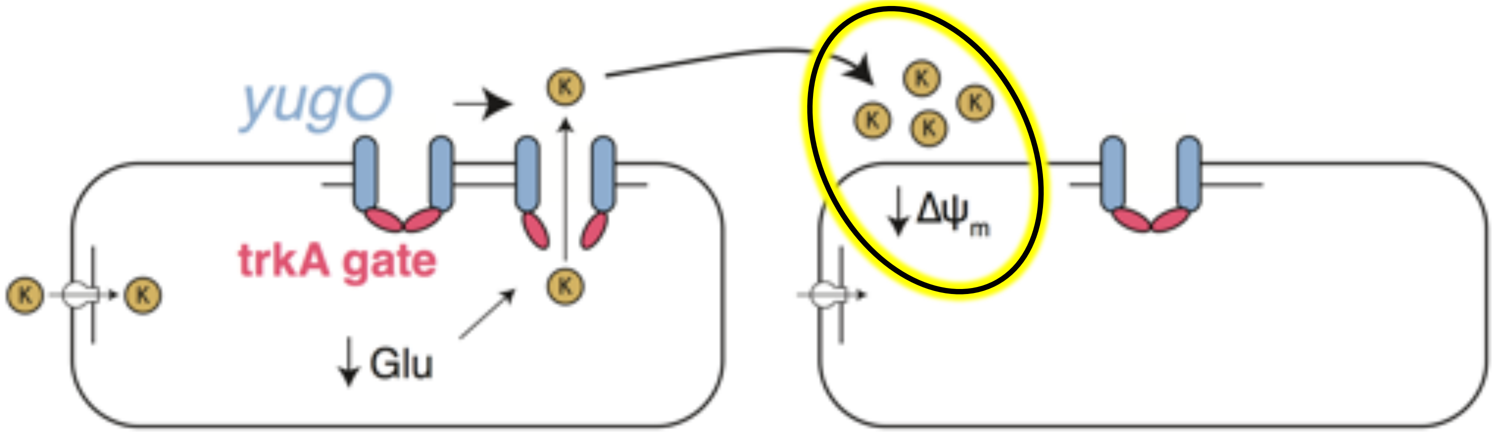
The trigger:



Opening of YugO channels

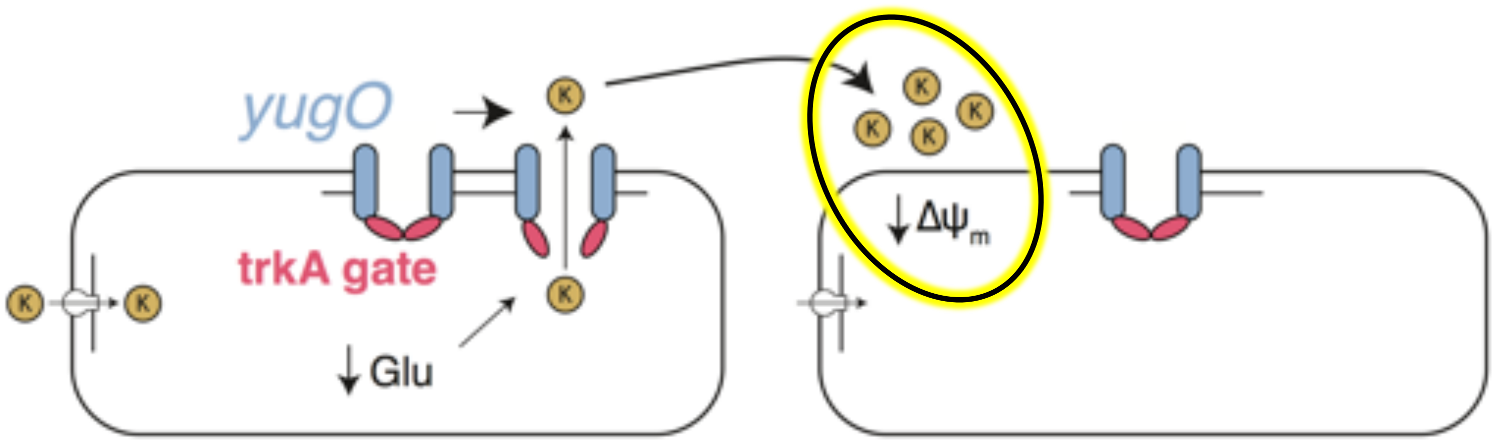
Mechanism for electrical signaling in biofilms

Signal relay:

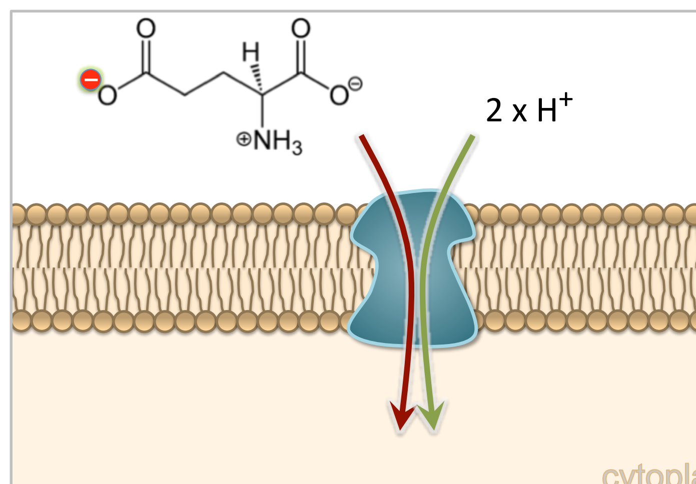


Depolarization of neighboring cells

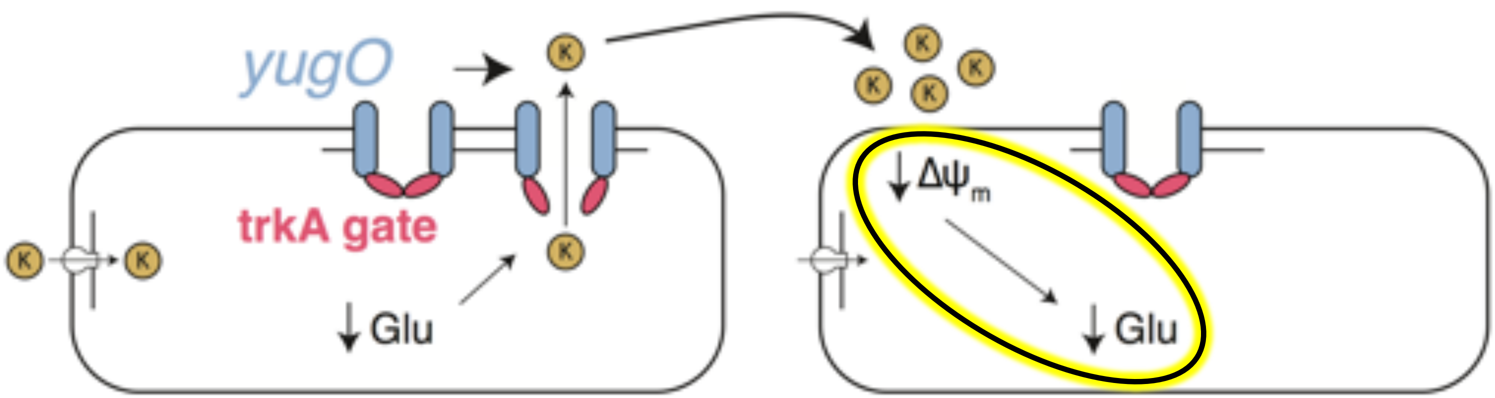
Mechanism for electrical signaling in biofilms
Signal relay:



Reduced glutamate uptake

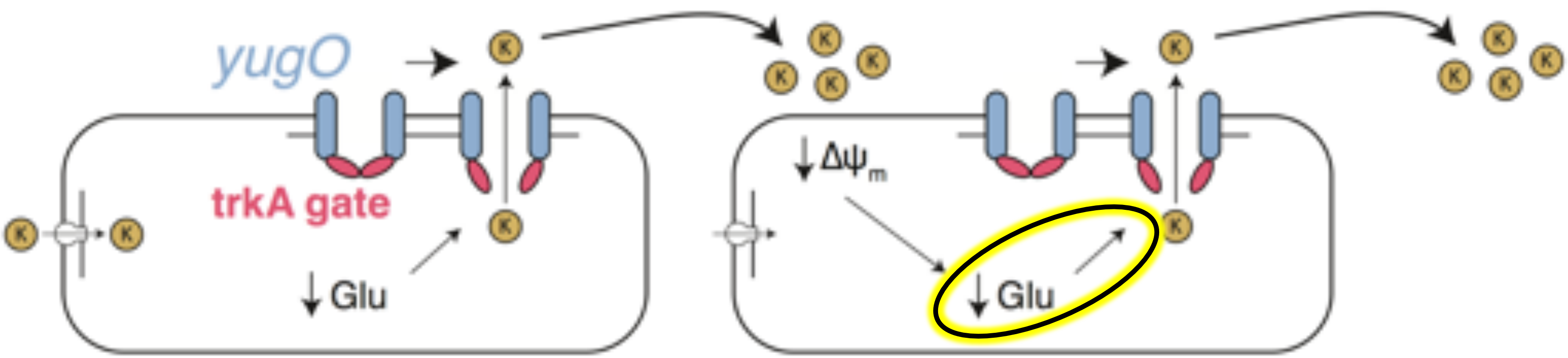


Mechanism for electrical signaling in biofilms
Signal relay:



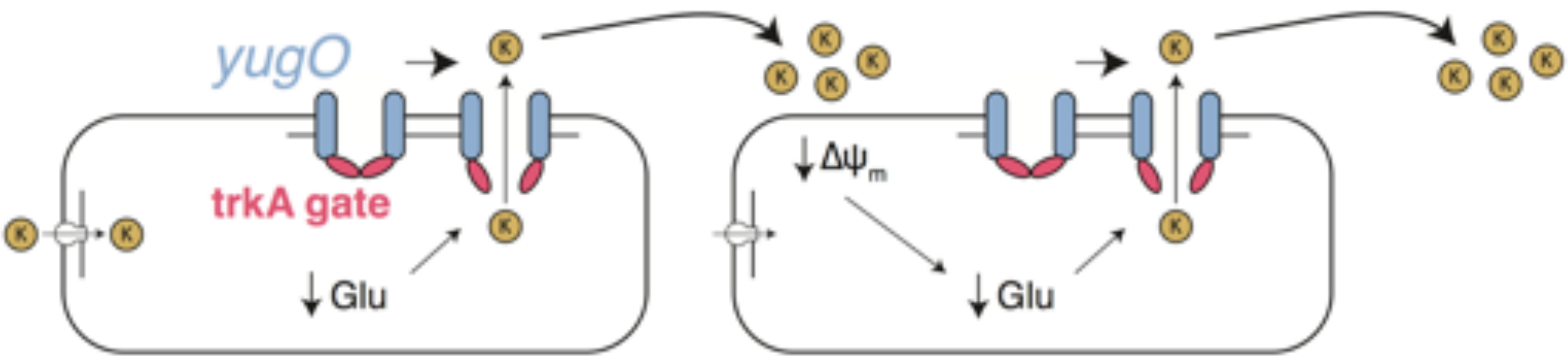
Glutamate limitation

Mechanism for electrical signaling in biofilms
Signal relay:

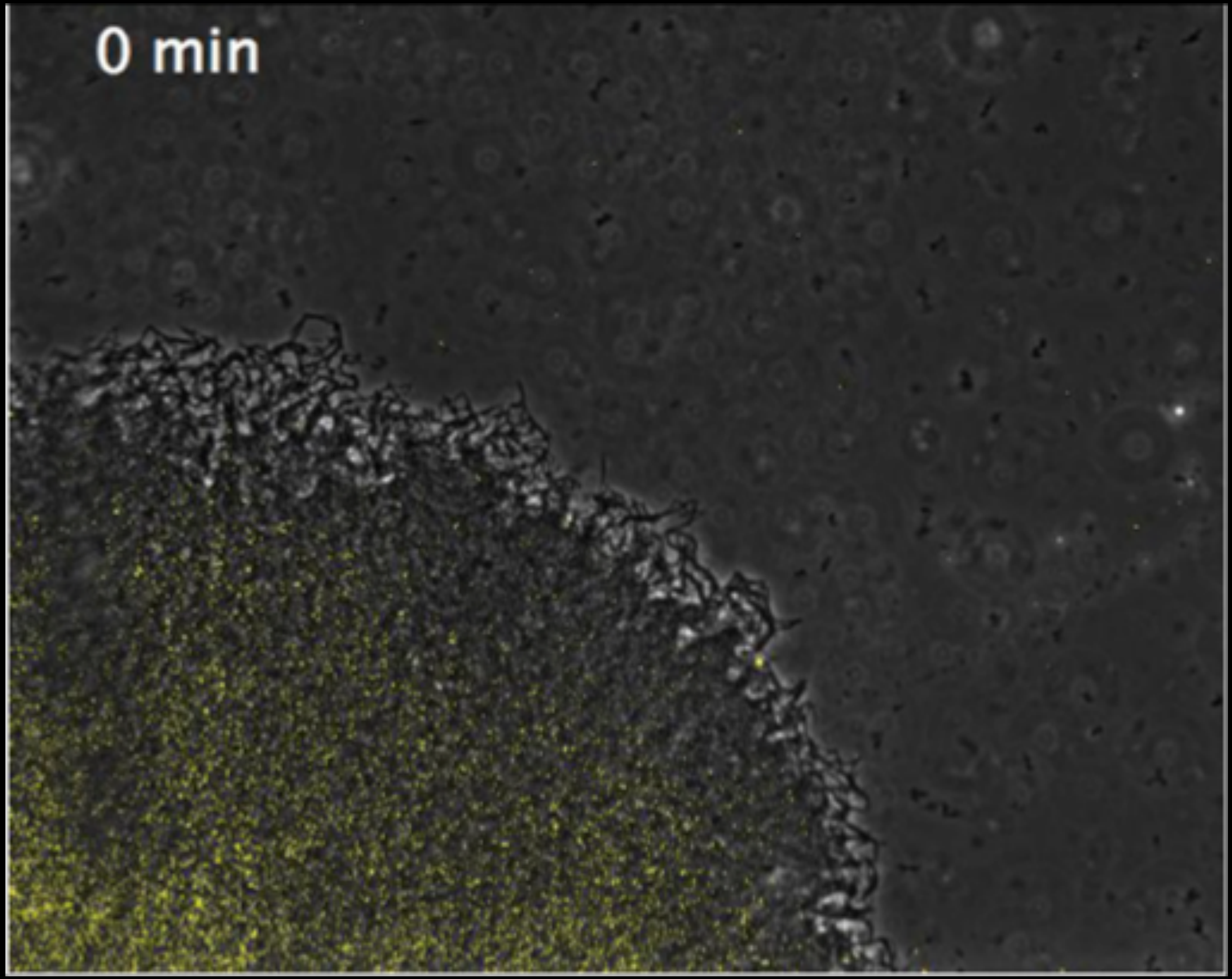


Opening of YugO channels

Should generate an actively relayed extracellular potassium wave



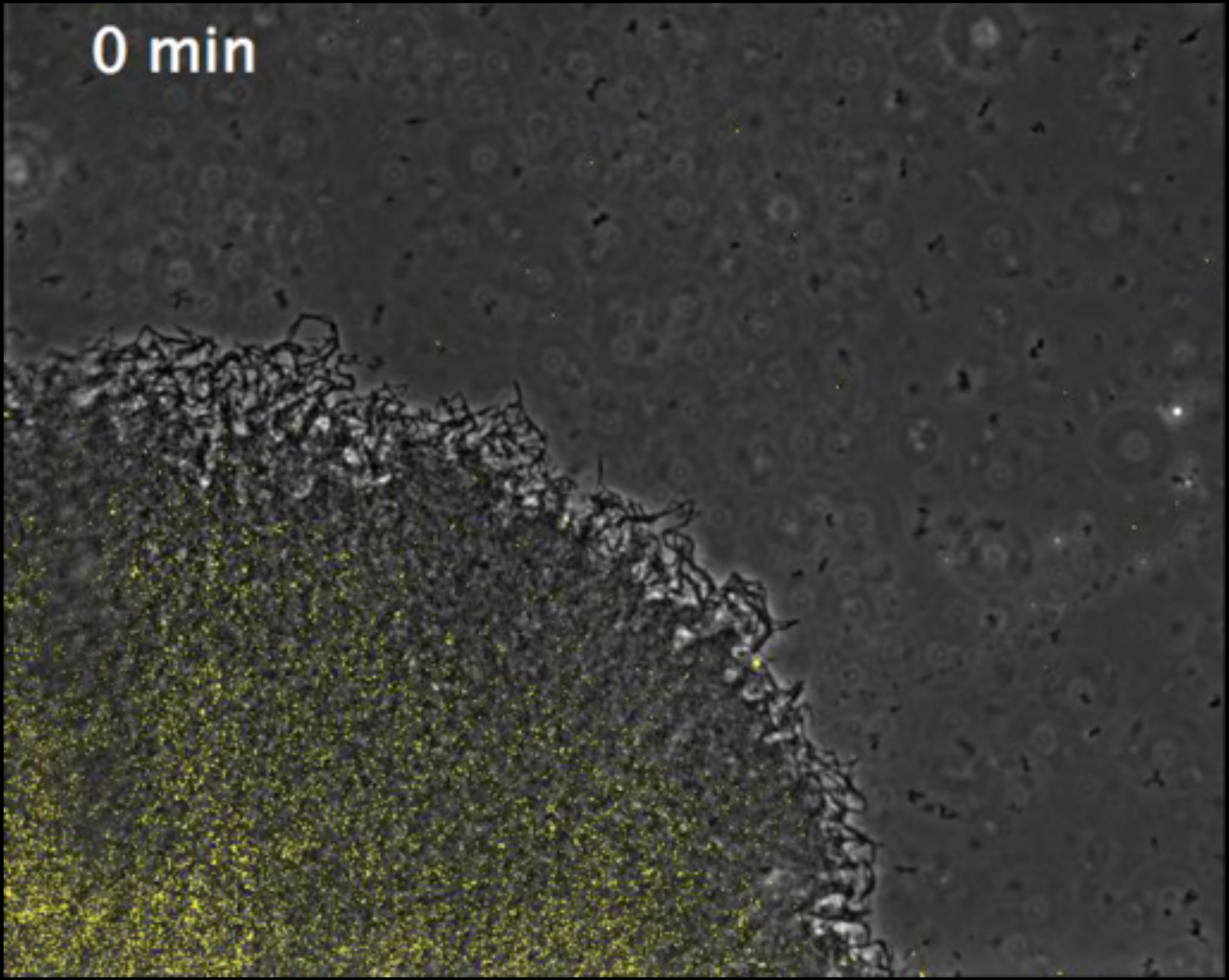
Extracellular potassium waves



APG-4

Prindle *et al*., Nature, **527**, 59-63 (2015)

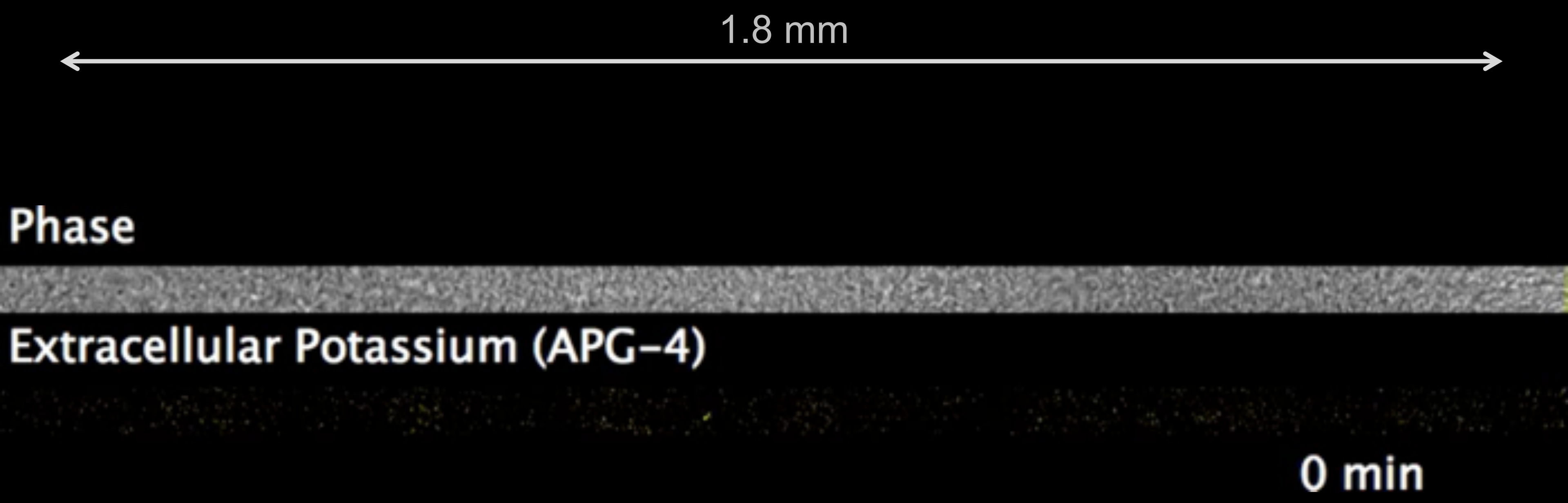
Extracellular potassium waves



APG-4

Prindle *et al*., Nature, **527**, 59-63 (2015)

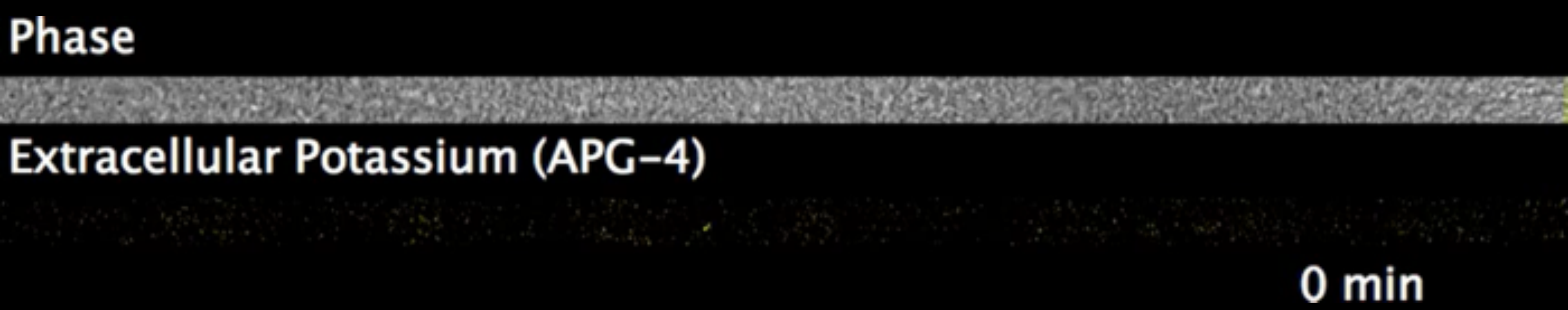
Extracellular potassium waves



Prindle *et al.*, Nature, **527**, 59-63 (2015)

Extracellular potassium waves

1.8 mm



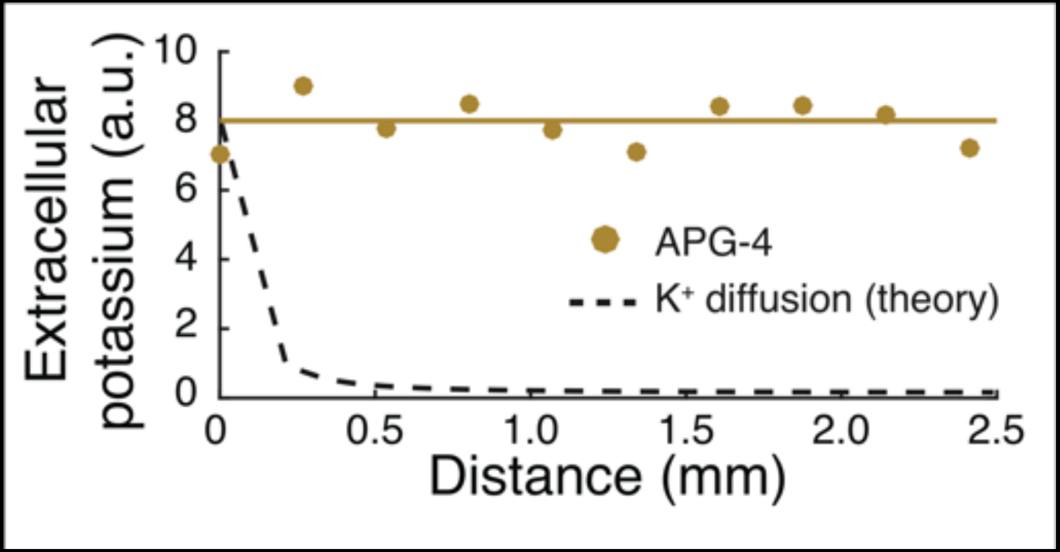
Prindle *et al.*, Nature, **527**, 59-63 (2015)

The potassium signal is actively propagated

Phase

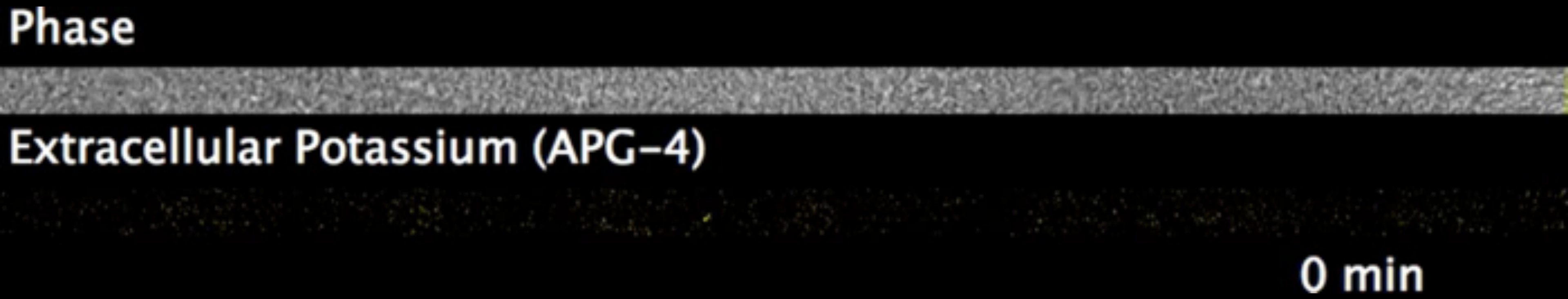
Extracellular Potassium (APG-4)

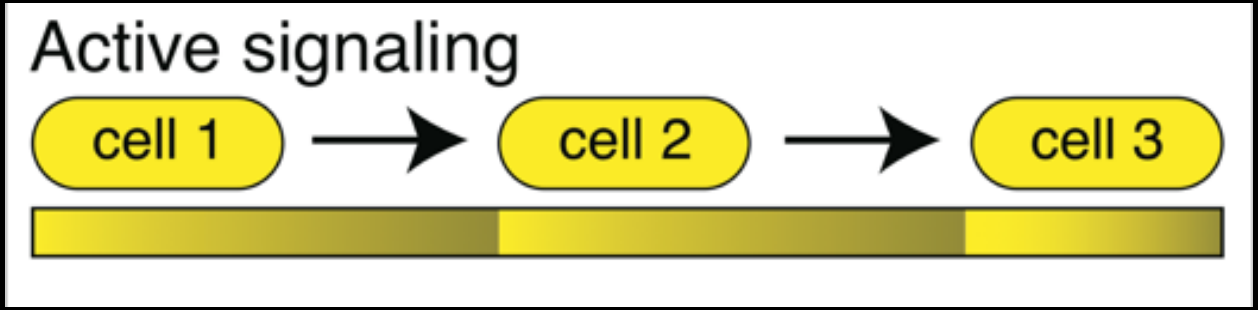
0 min



Prindle *et al.*, Nature, **527**, 59-63 (2015)

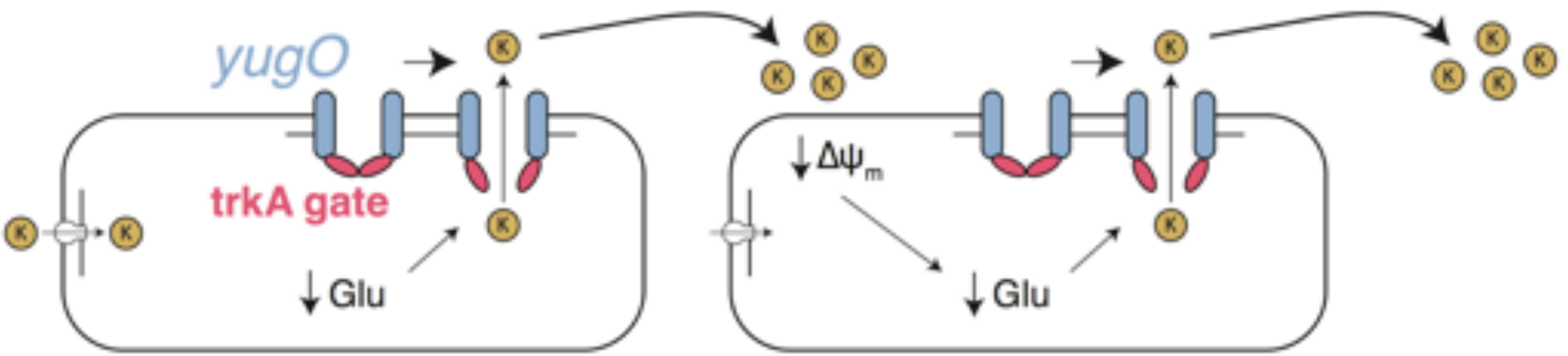
The potassium signal is actively propagated



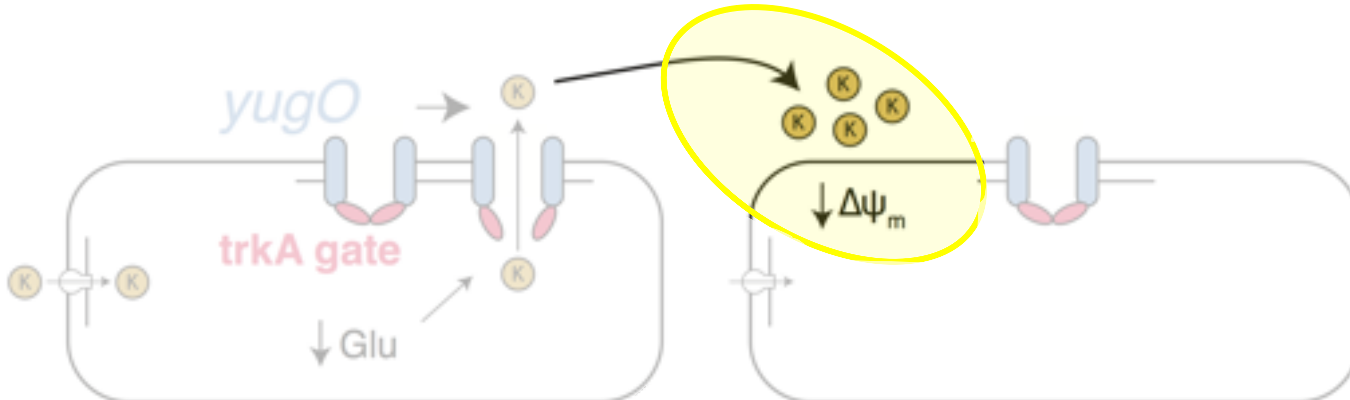


Prindle *et al*., Nature, **527**, 59-63 (2015)

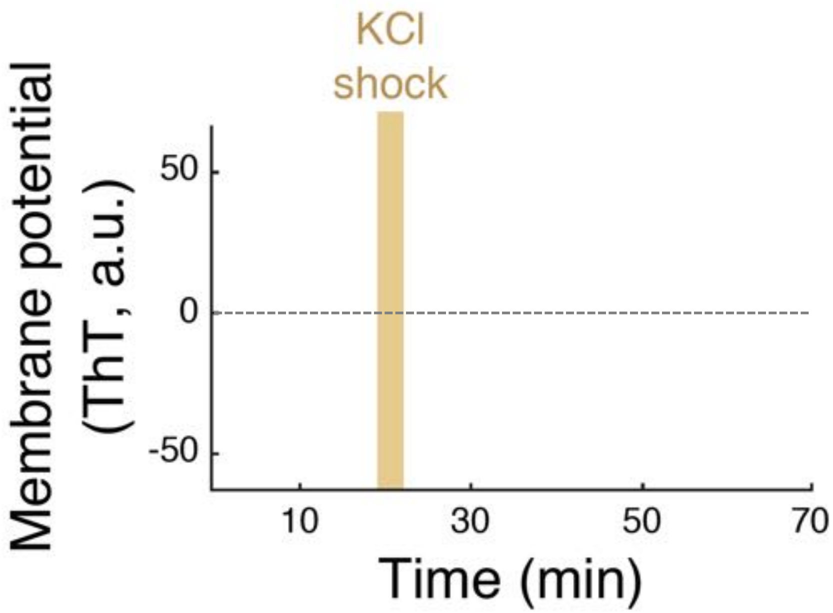
Let’s test our model further



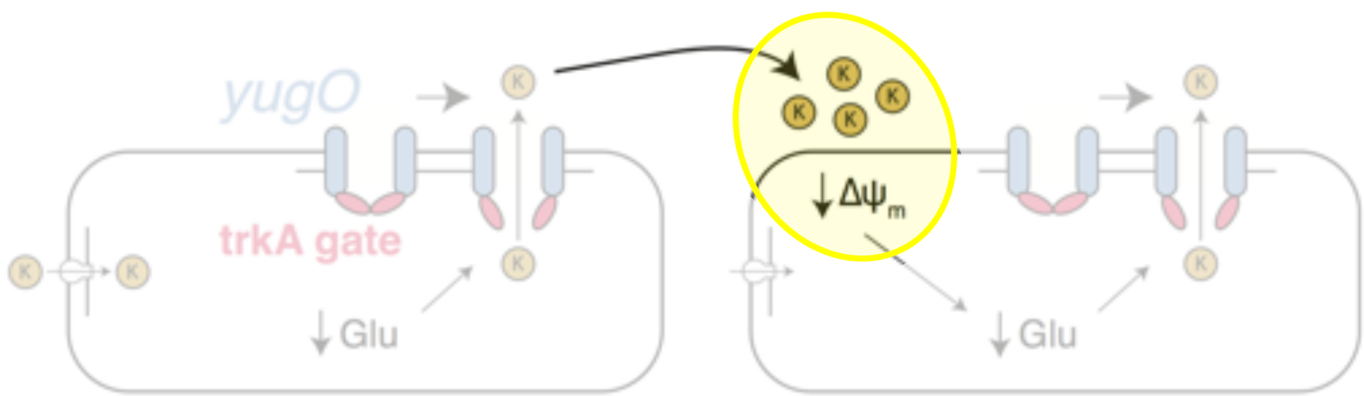
Let's test the propagation mechanism



Propagation: Potassium mediated depolarization



↓ De-polarization



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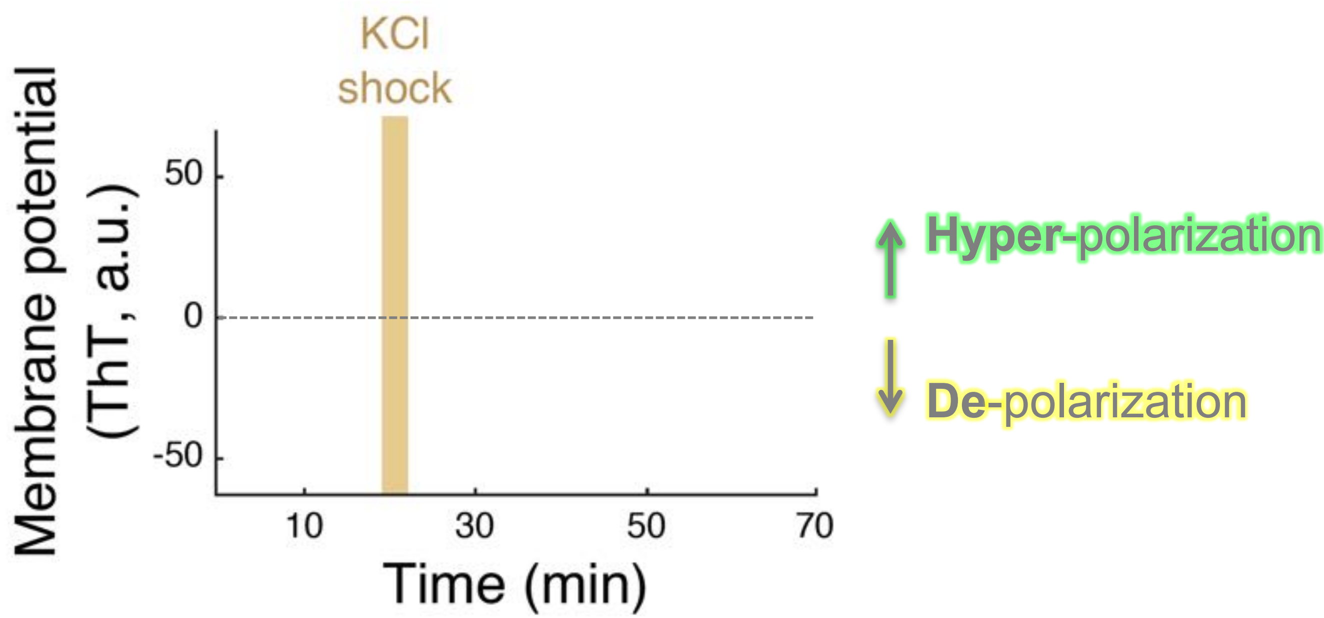
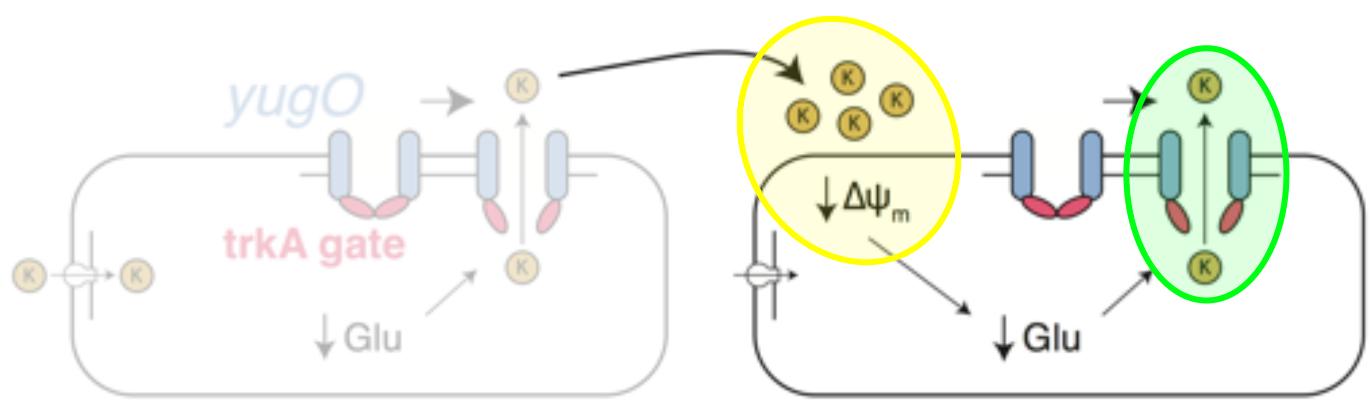
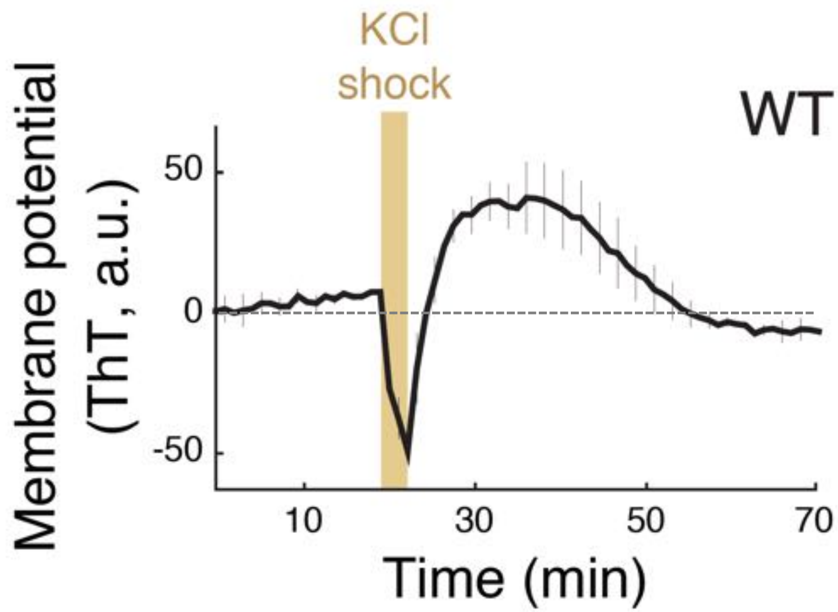
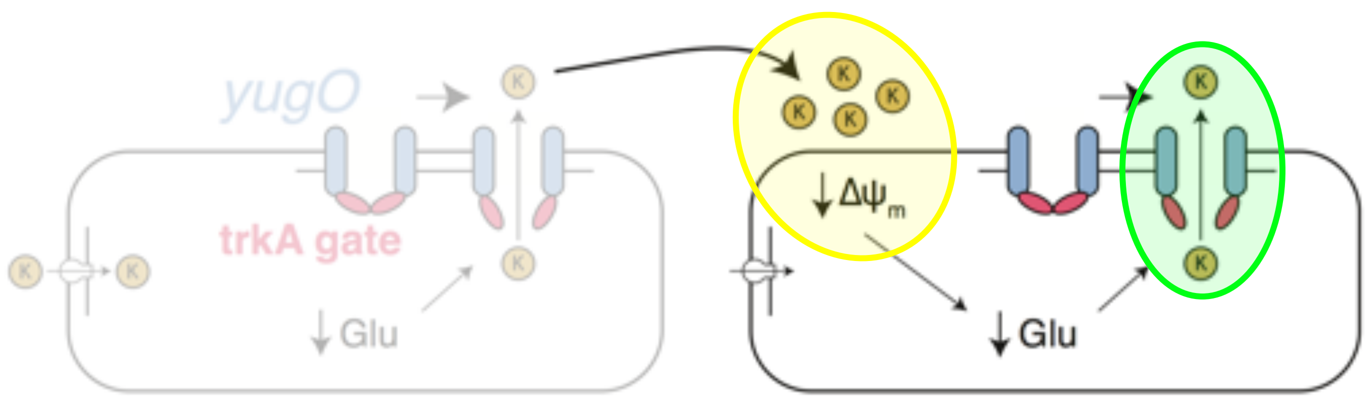


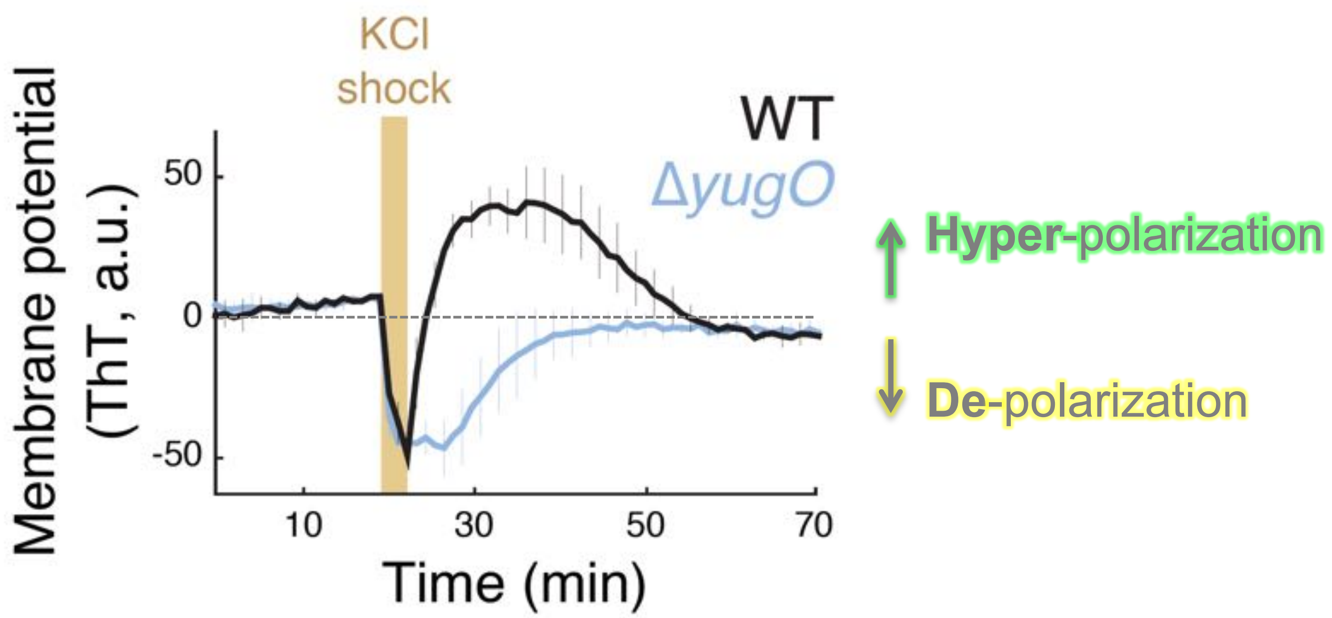


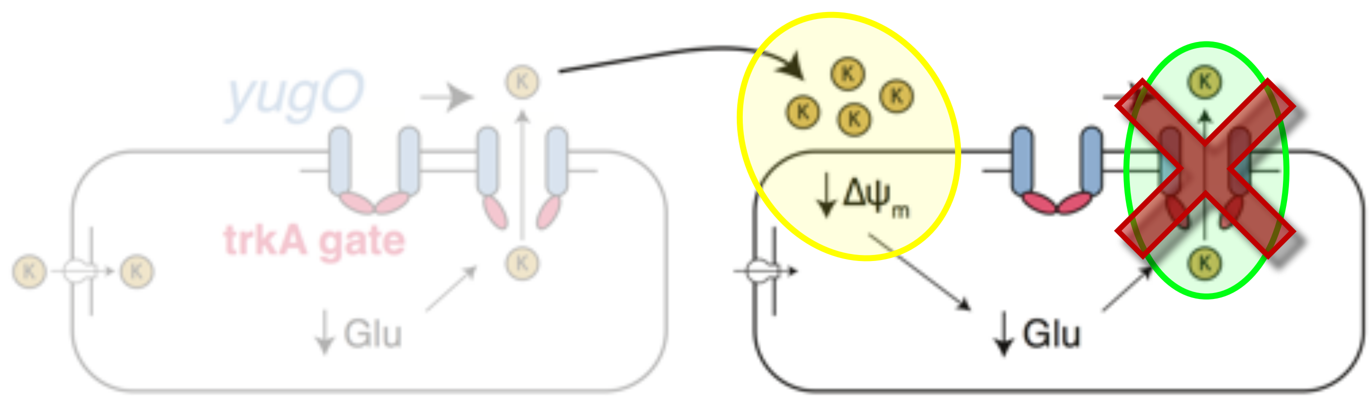
Propagation: Potassium mediated depolarization



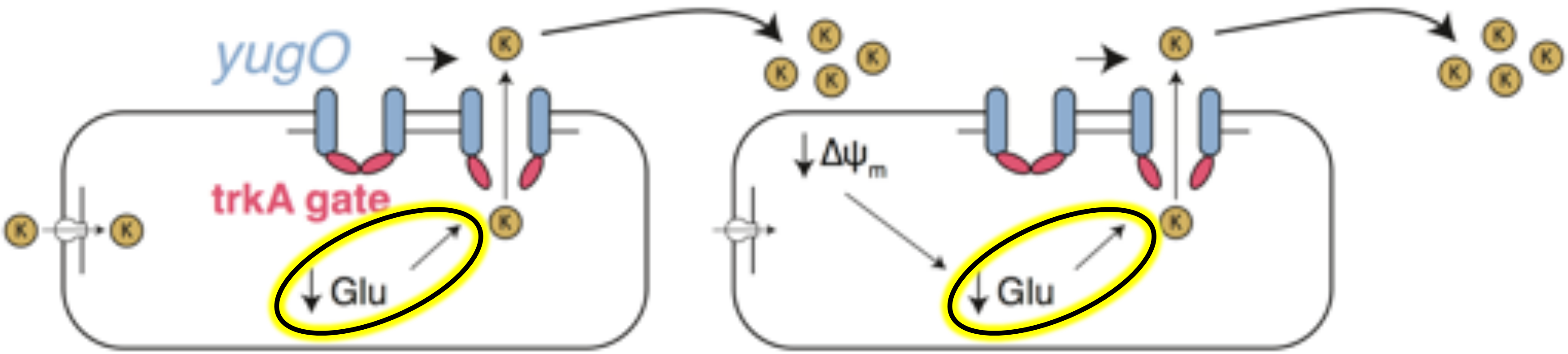


Propagation: Potassium mediated depolarization





Is the response Glutamate specific ?



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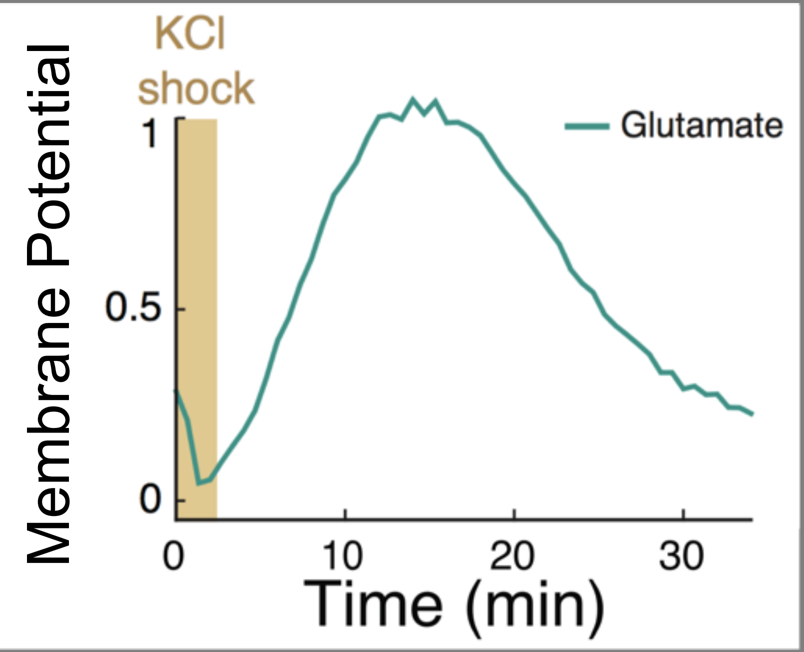
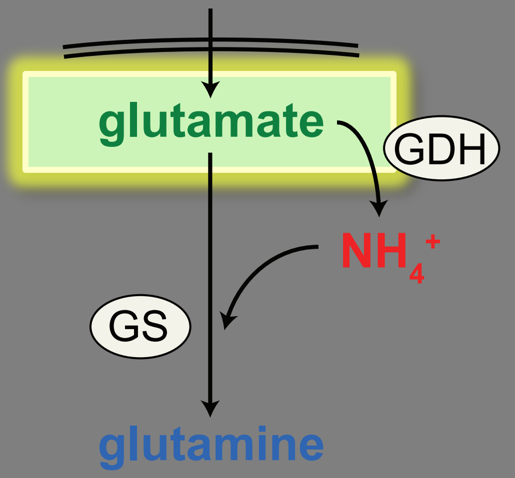
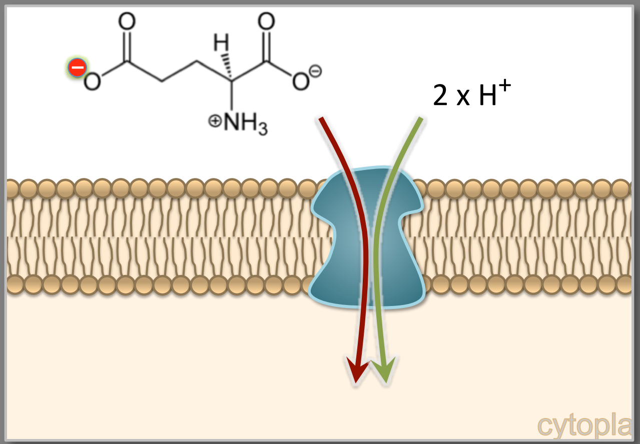
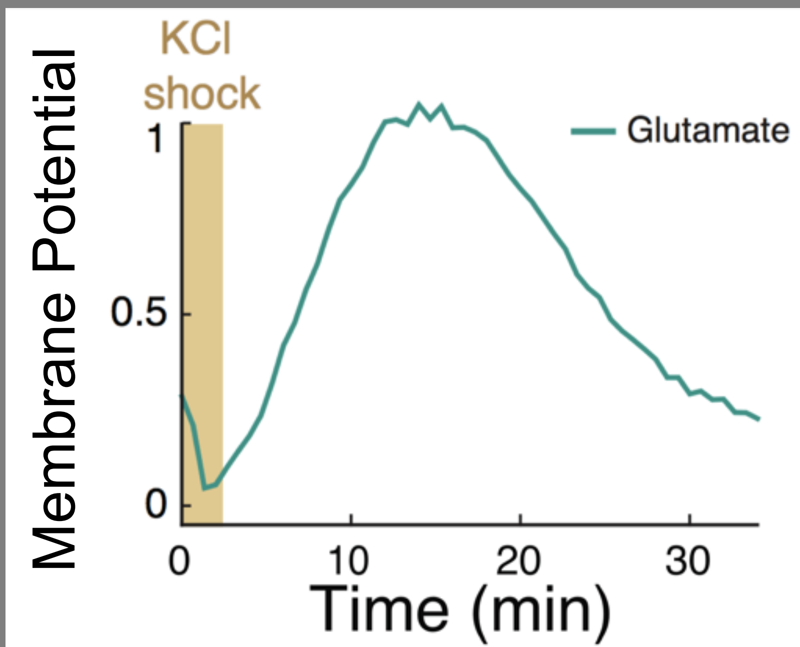
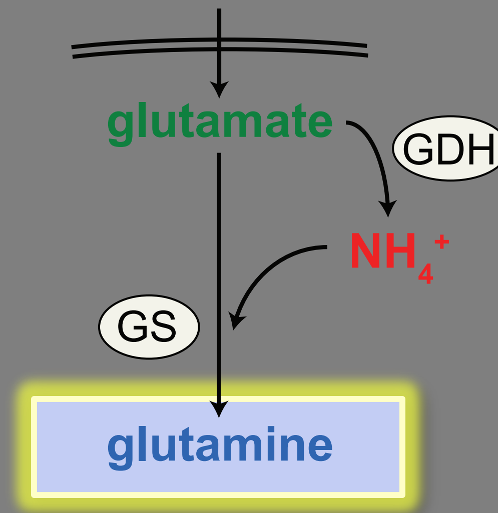


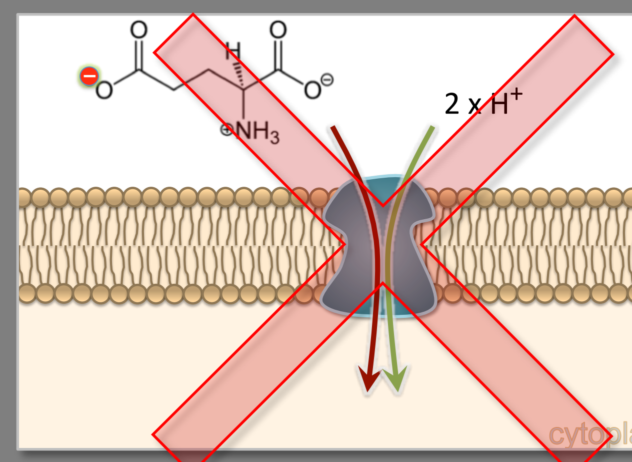




What if we bypass the need for Glutamate ?







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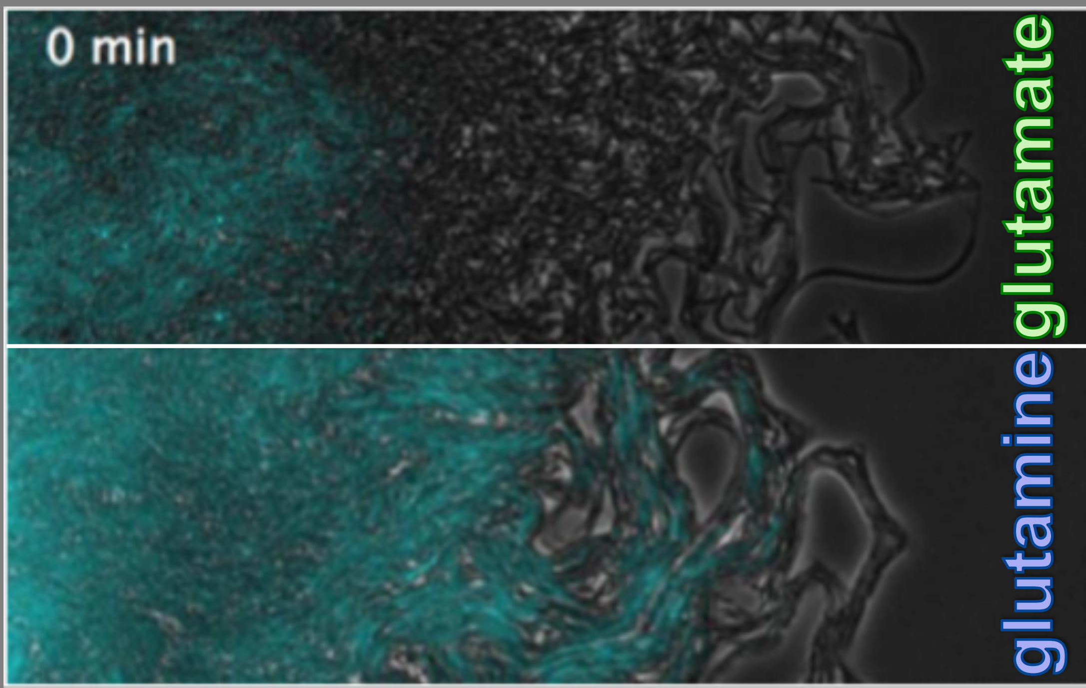

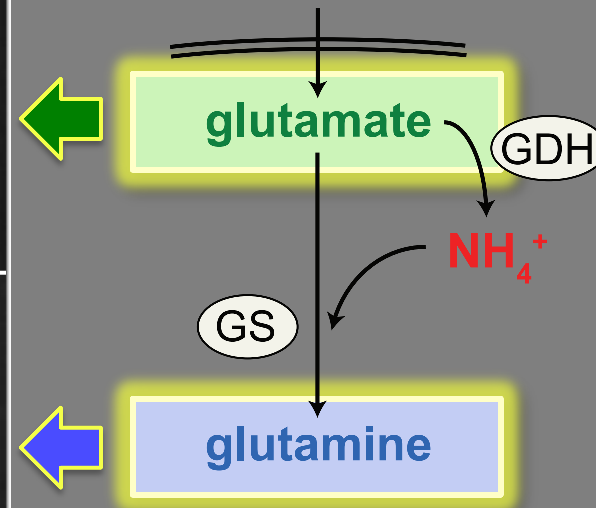

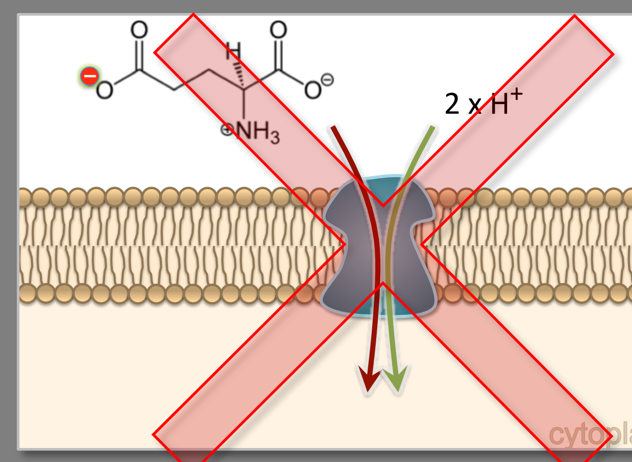

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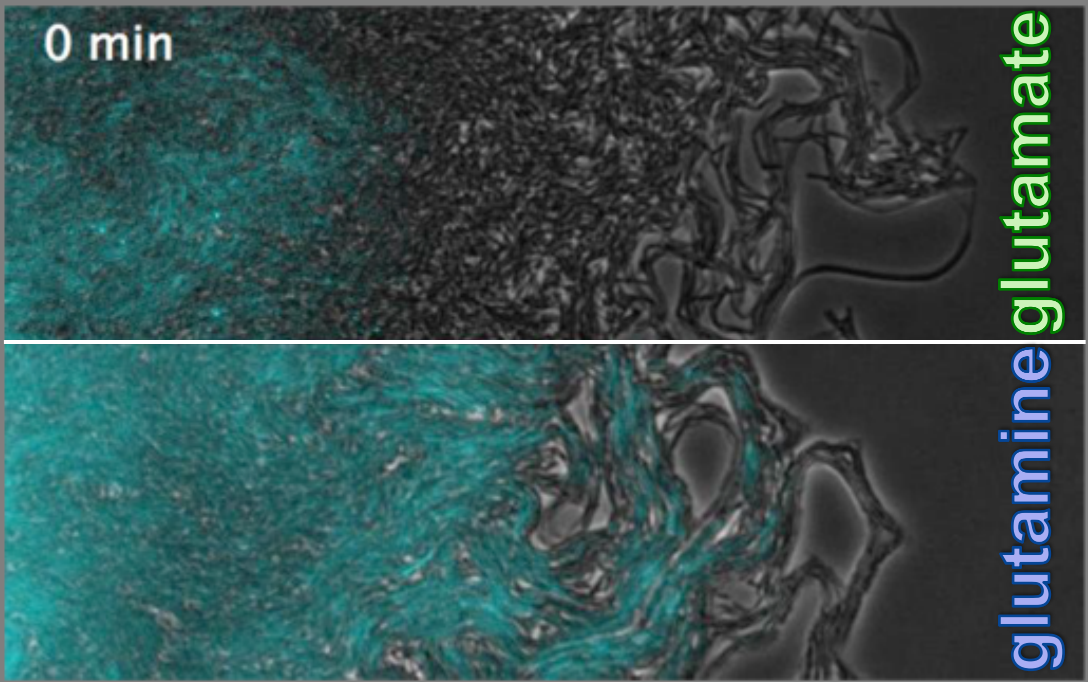
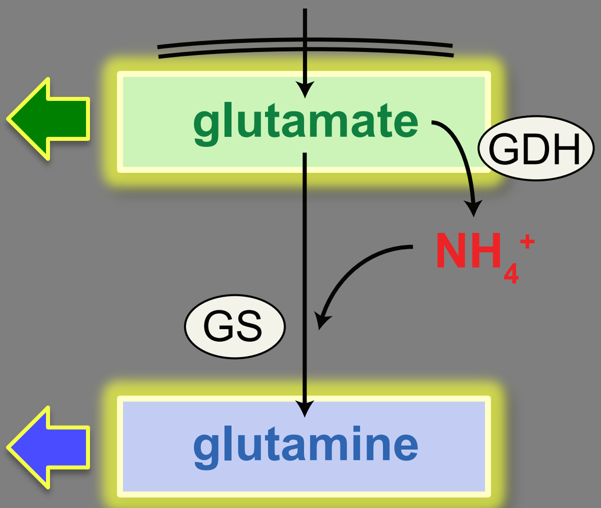
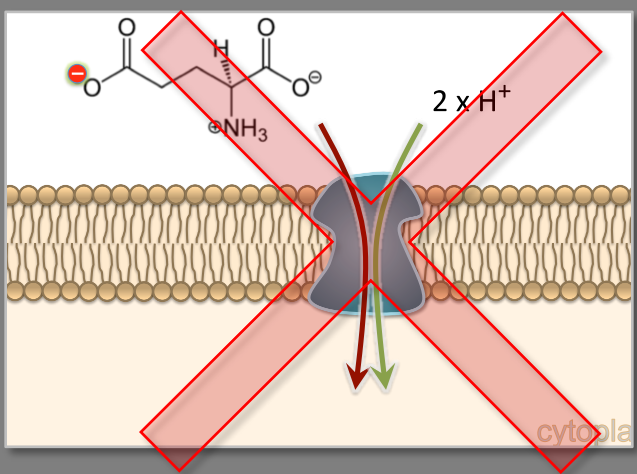
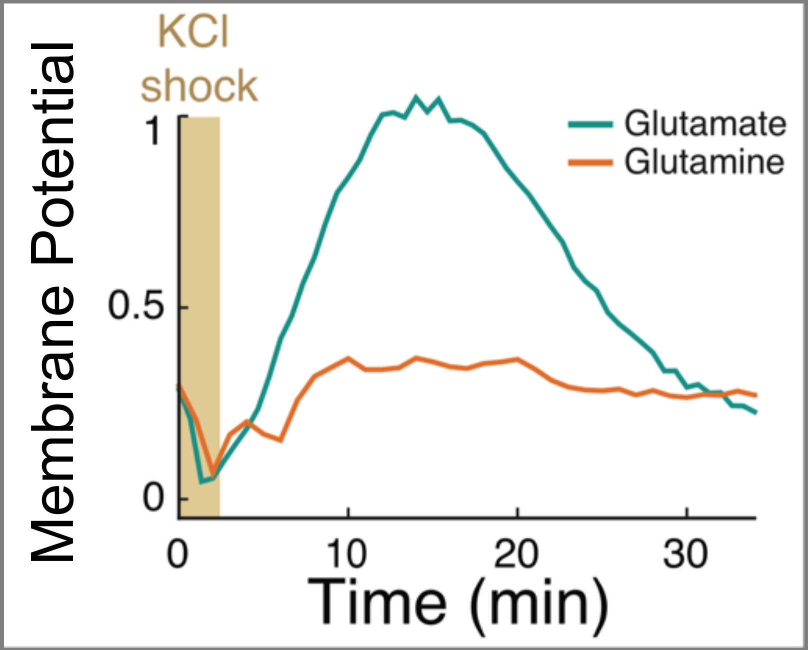
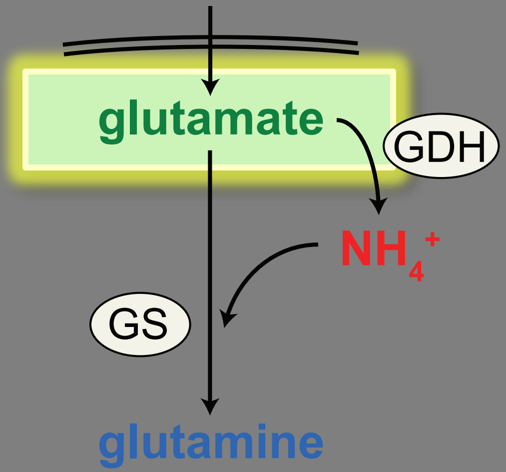


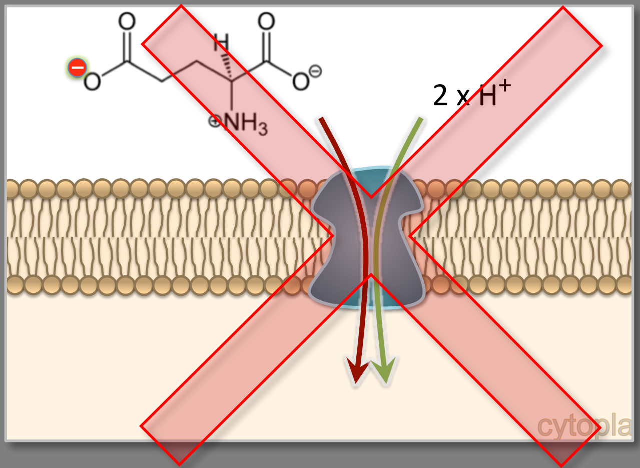




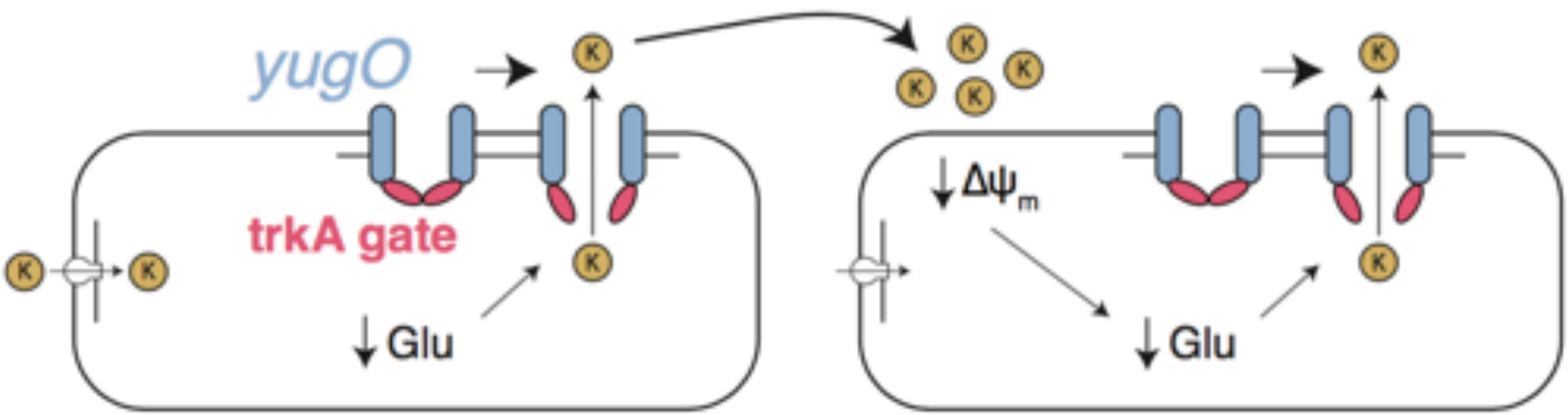
Membrane potential response is glutamate specific







Model for electrical signaling in biofilms



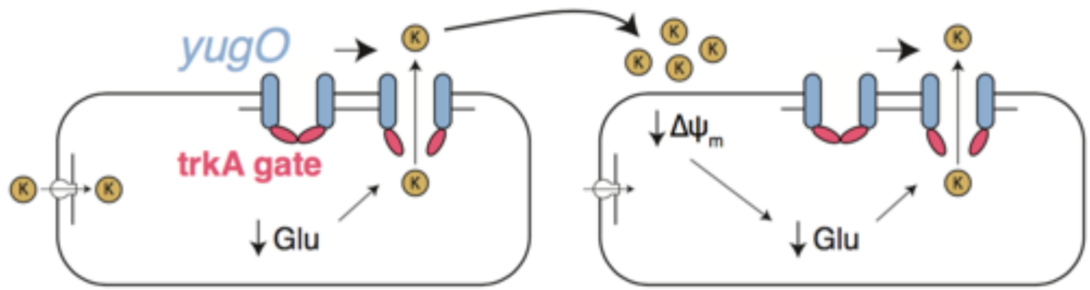
Collaborators

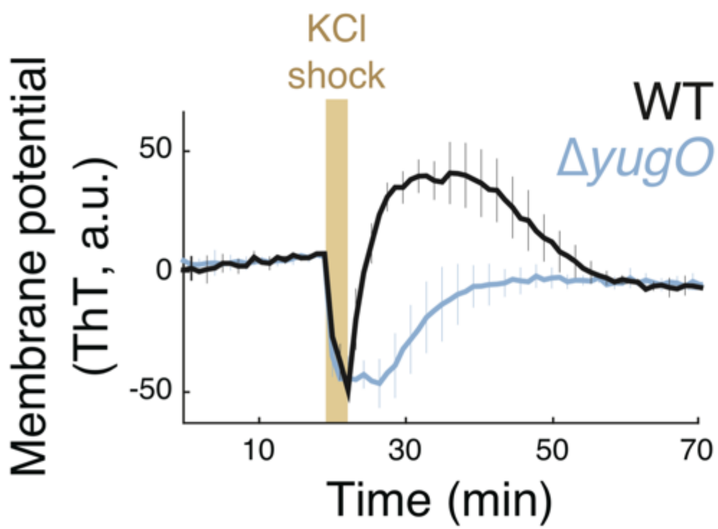
Jordi Garcia-Ojalvo

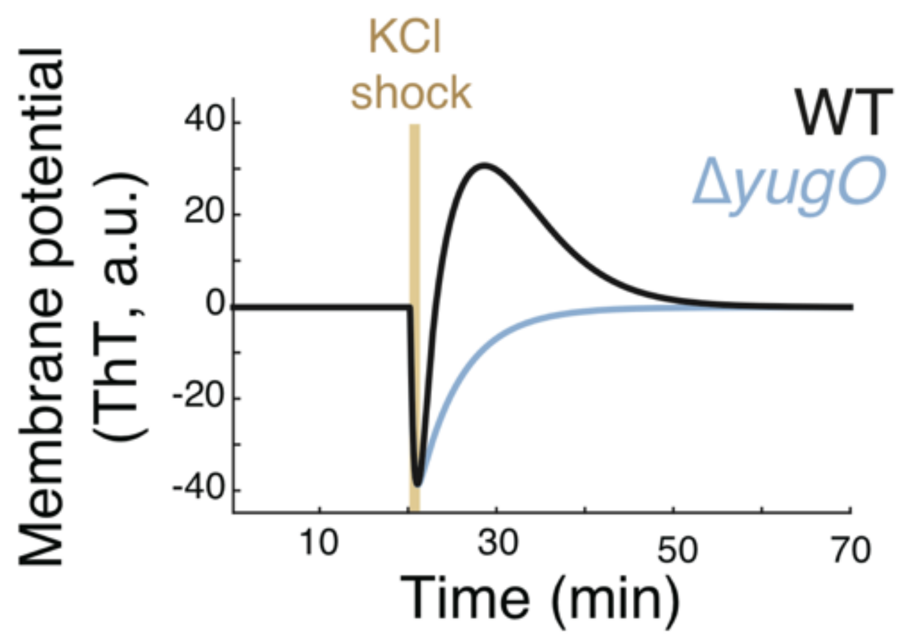
Marcal Gabalda
Rosa Martinez-Corral

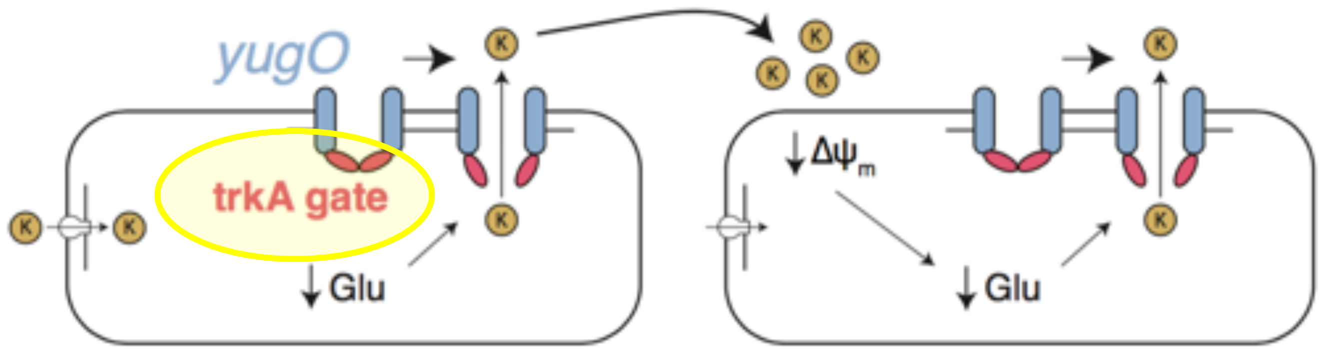
CRG
Centre for Genomic Regulation

Mathematical model accounts for observations

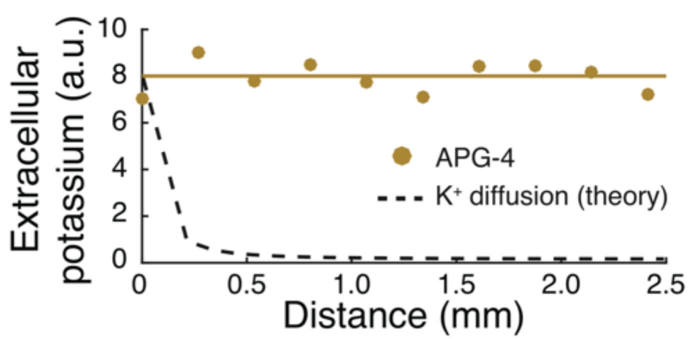


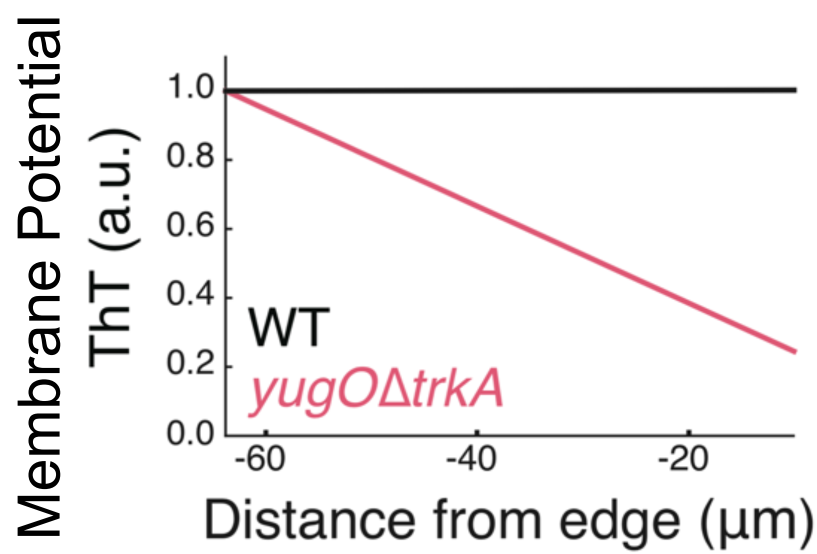




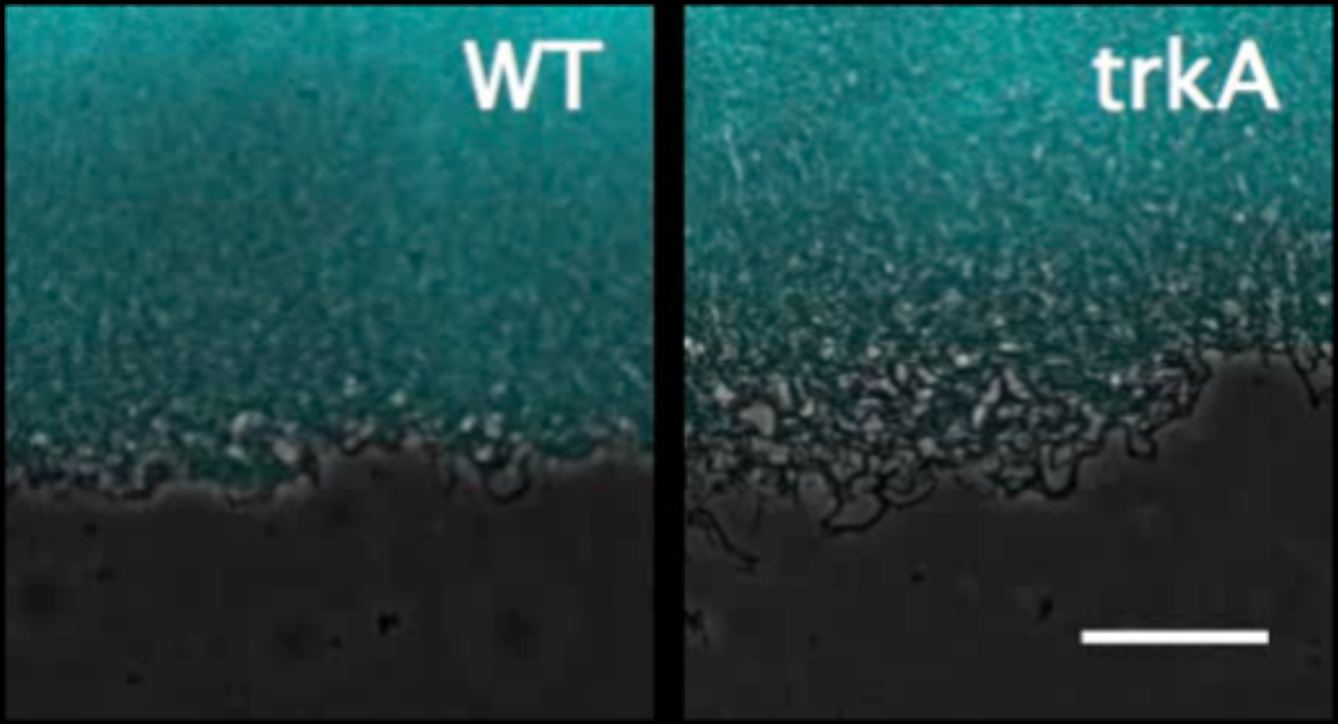


Modeling prediction:





Reduced signal propagation efficiency



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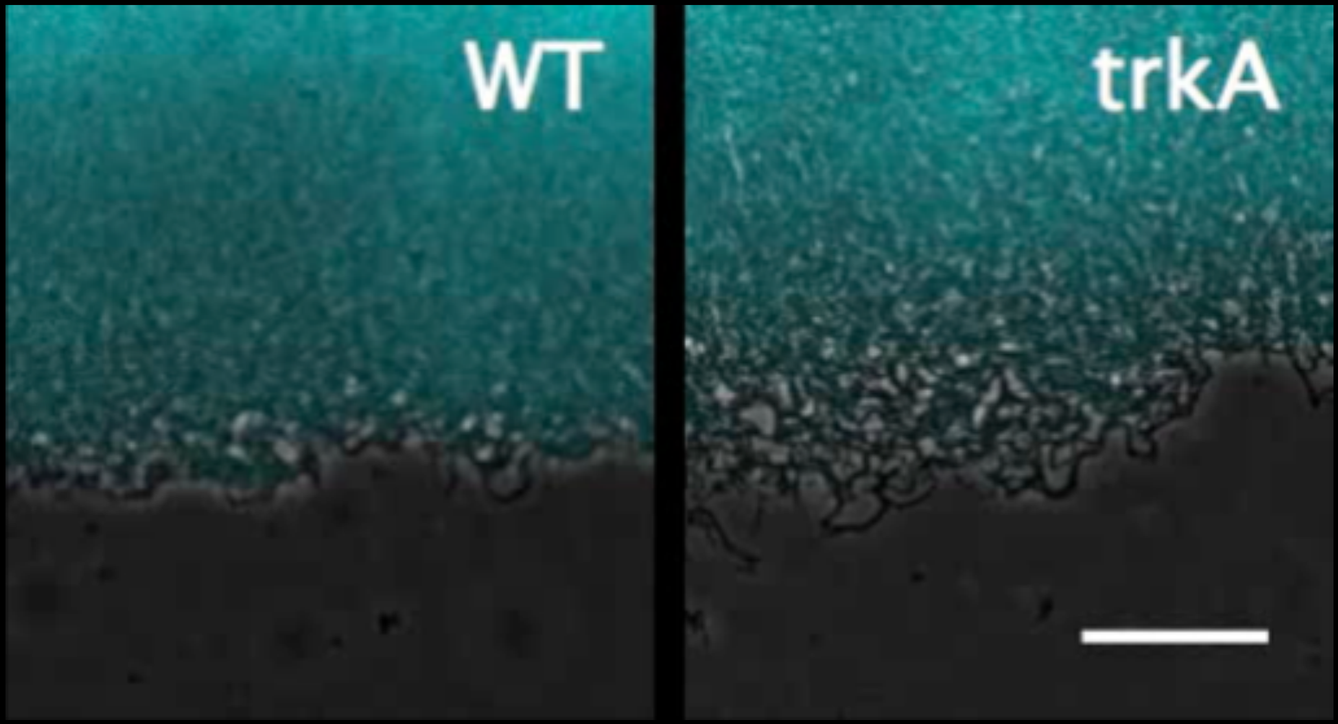
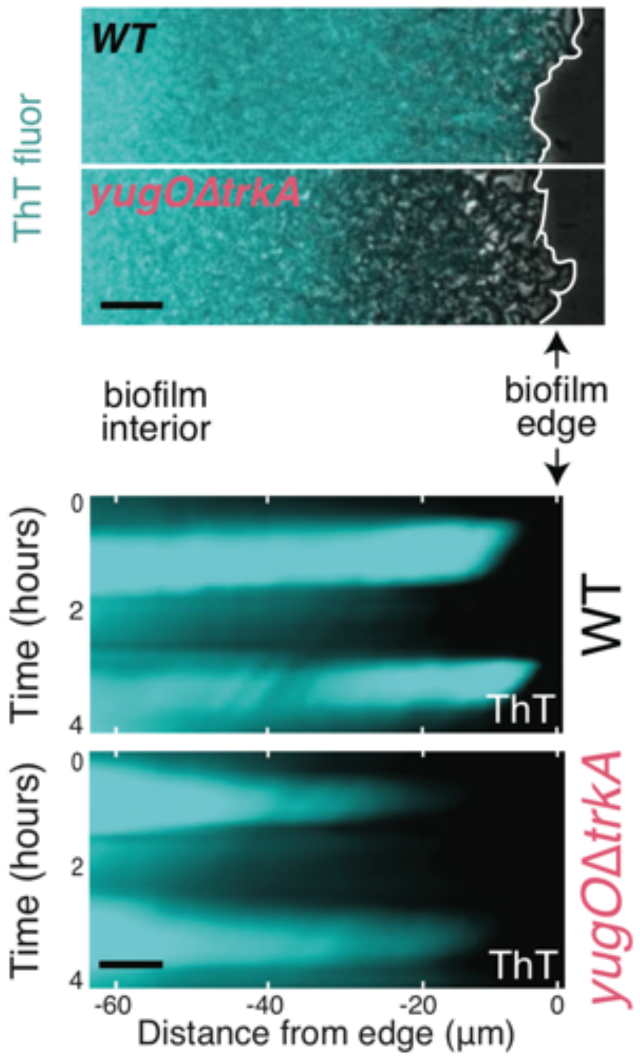
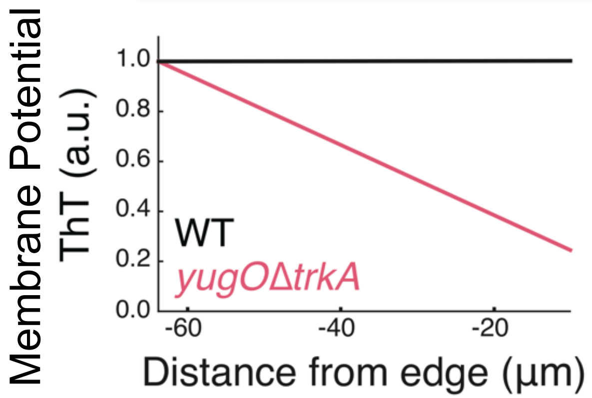


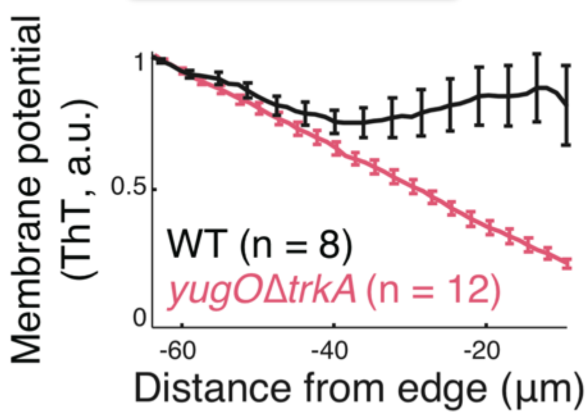
Ion channel gating promotes long-range signaling



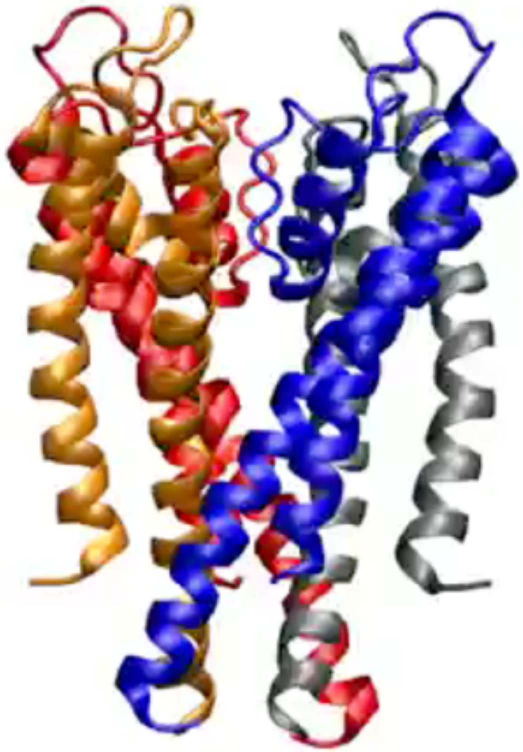
Modeling prediction :



Observation :



A long-standing question:
What is the function for bacterial ion channels?



Potassium Ion Channel
Streptomyces lividans
(Gram positive soil bacteria)

Doyle *et al*, (1998) Science 280/69

A long-standing question

Paul Blount
UT Southwestern

Channels in microbes: so many holes to fill

roscientists. However, their natural roles in microbial physiology remain largely unknown. The intellectual and technical schisms between 'neuro' and 'micro' biology must be bridged before we know how we became so smart, and whether microbes are just as smart.

Molecular Microbiology (2004) 53(2), 373–380

Chris Miller
Brandeis U - HHMI

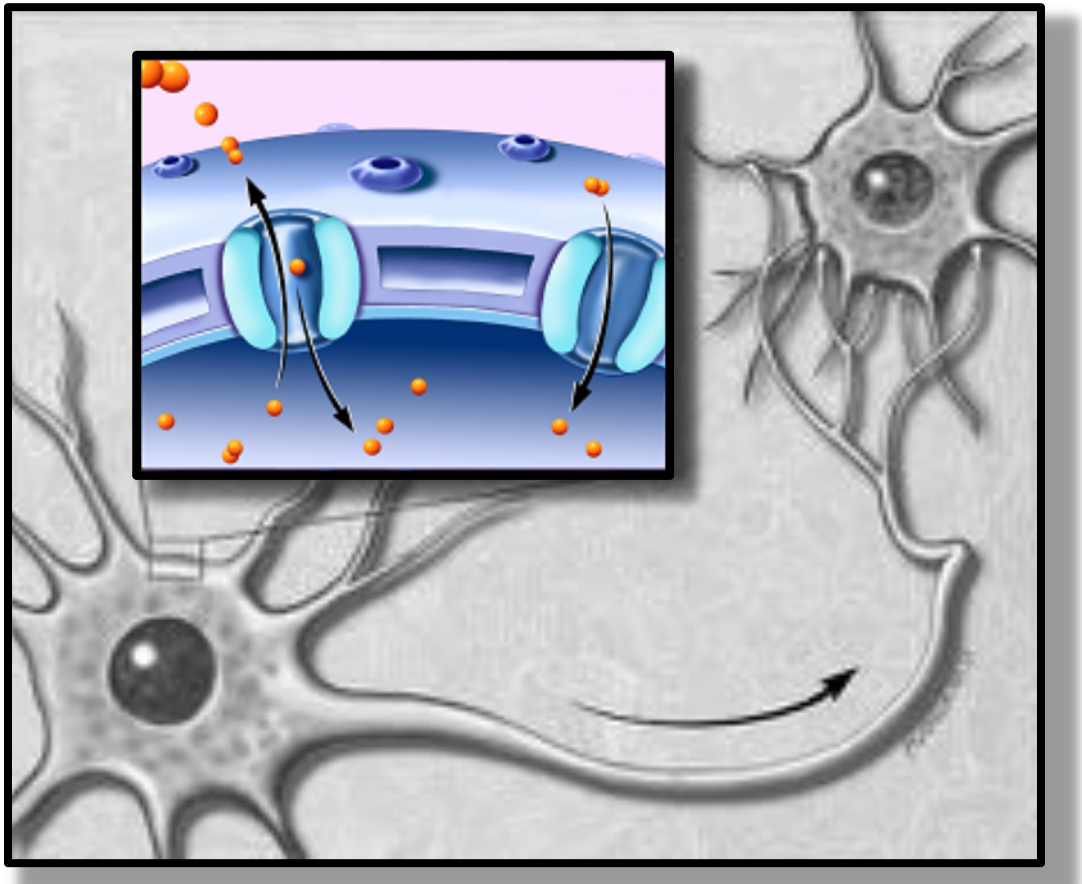
A biological role for prokaryotic ClC chloride channels

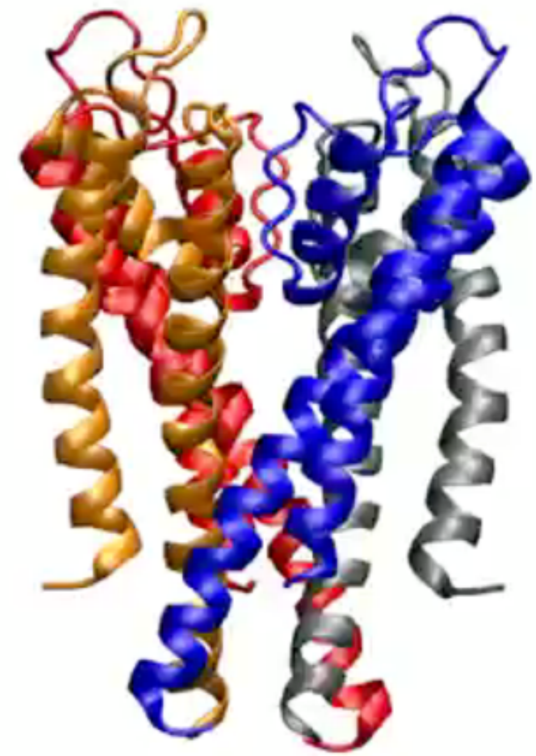
Ramkumar Iyer, Tina M. Iverson, Alessio Accardi & Christopher Miller

ion channels in bacteria are unknown. Strong conservation of functionally important sequences from bacteria to vertebrates, and of structure itself[10], suggests that prokaryotes use ion channels in roles more adaptive than providing high-quality protein to structural biologists. Here we show that *Escherichia*

NATURE | VOL 419 | 17 OCTOBER 2002

Bacterial ion channel structures have provided many insights





Potassium Ion Channel
Streptomyces lividans
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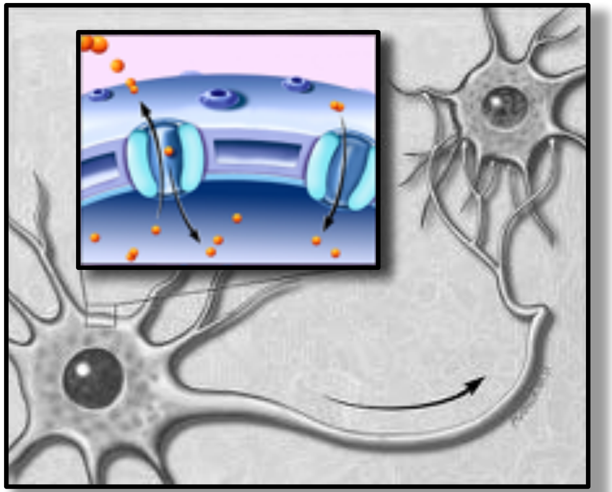
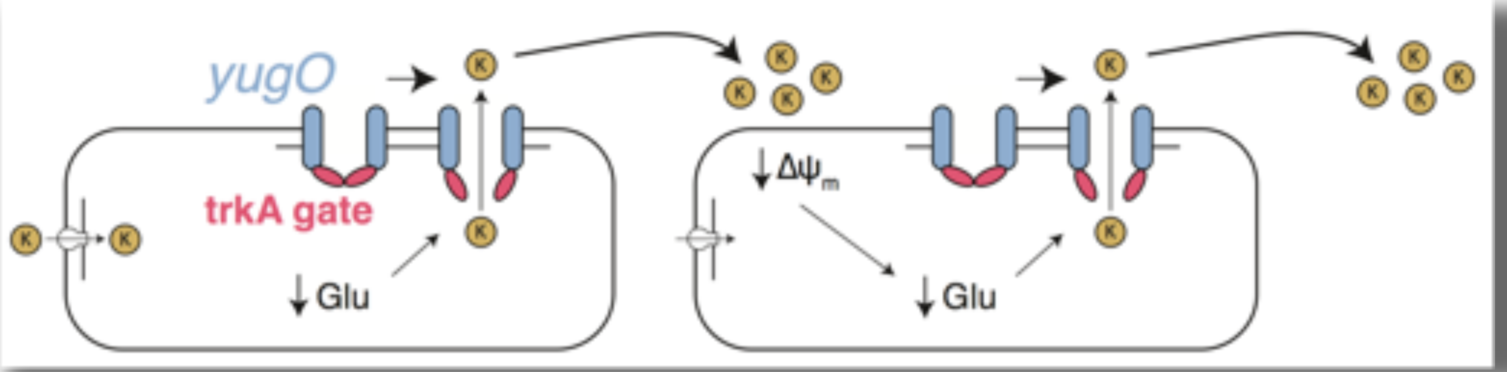
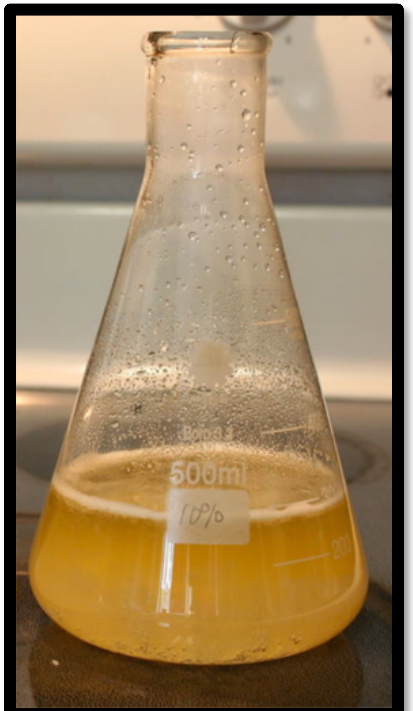
Functional similarity between mammalian and bacterial ion channels

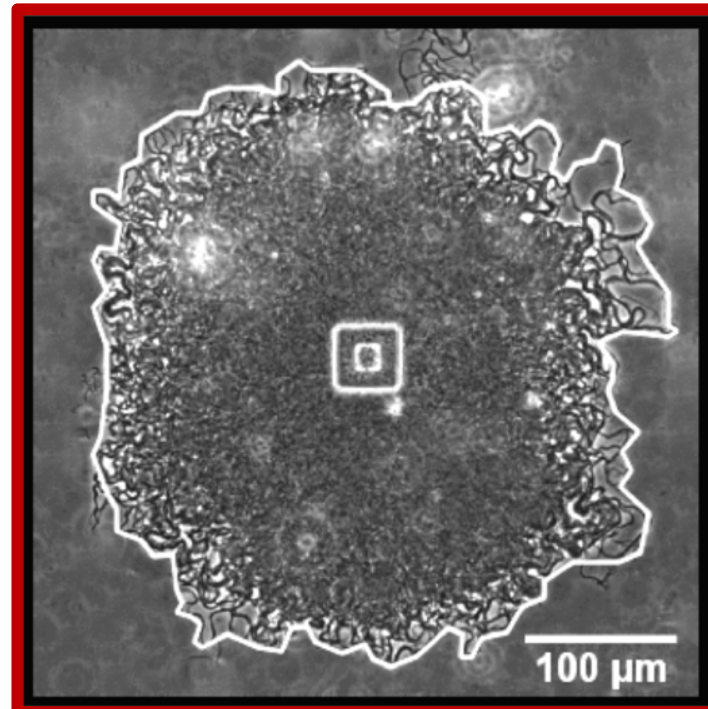




… the context of the community matters

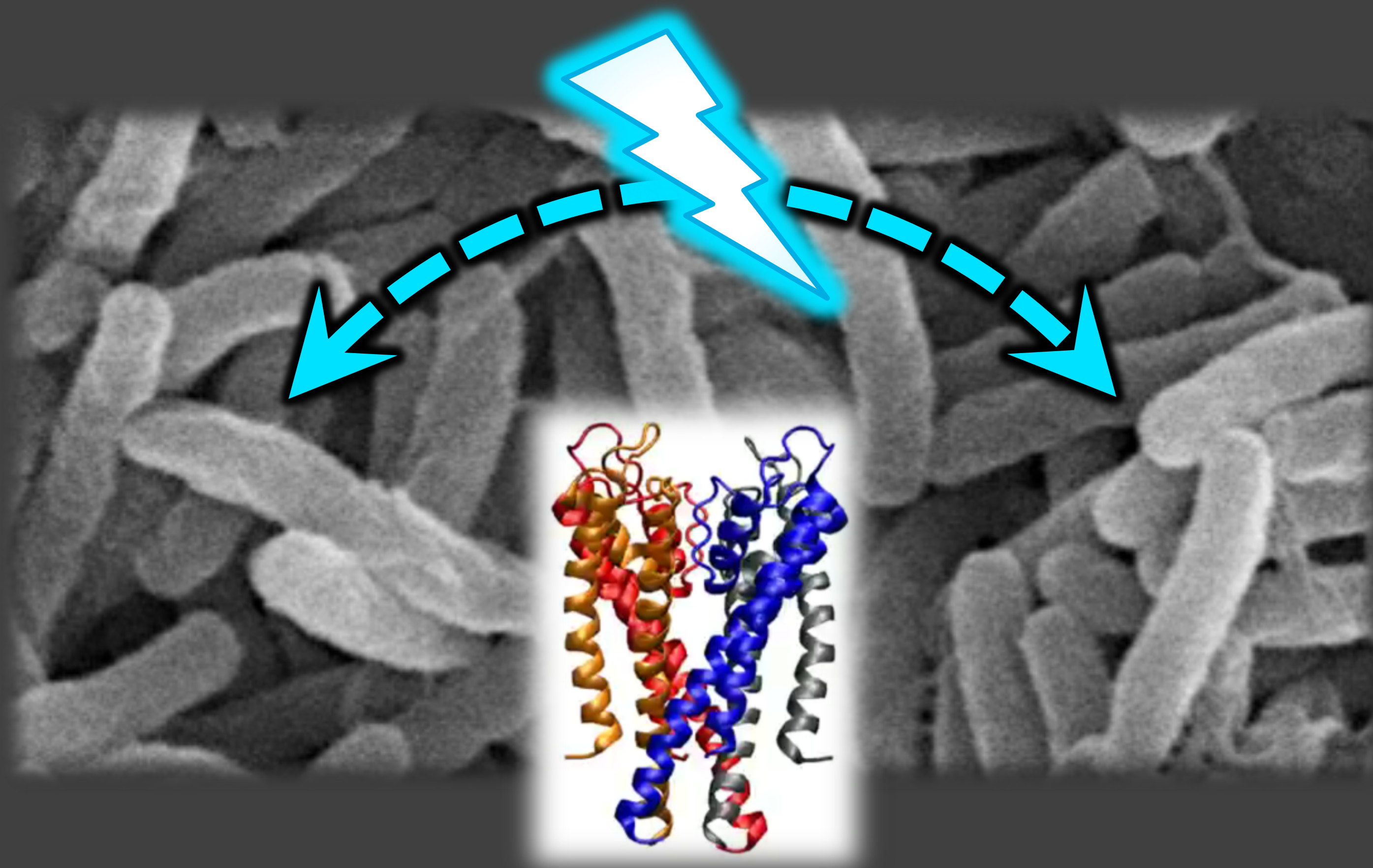


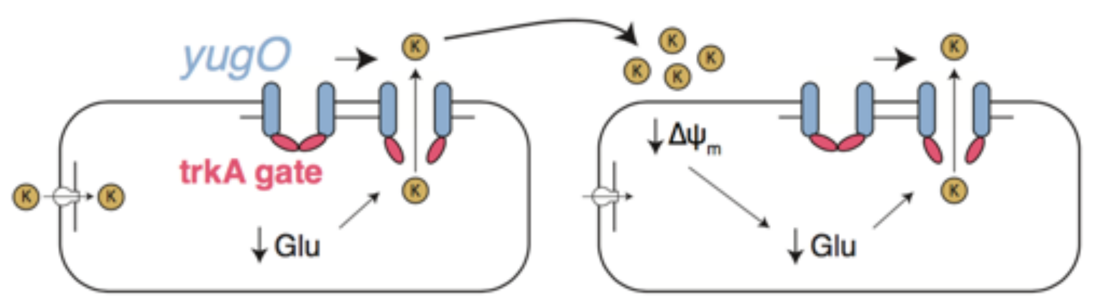
Liquid culture



Biofilm

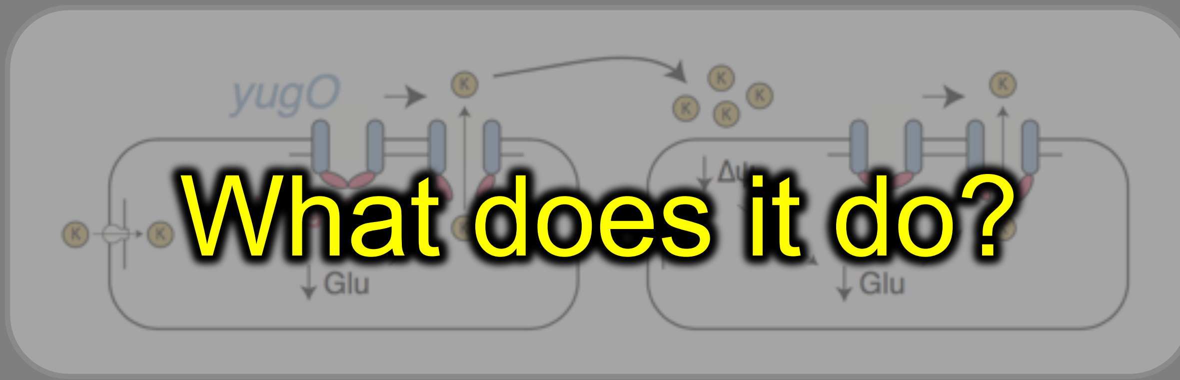
Ion channel-mediated electrical cell-to-cell signaling

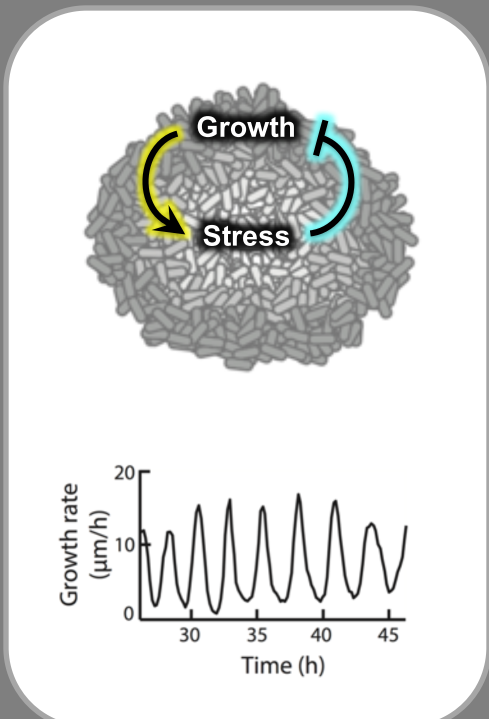


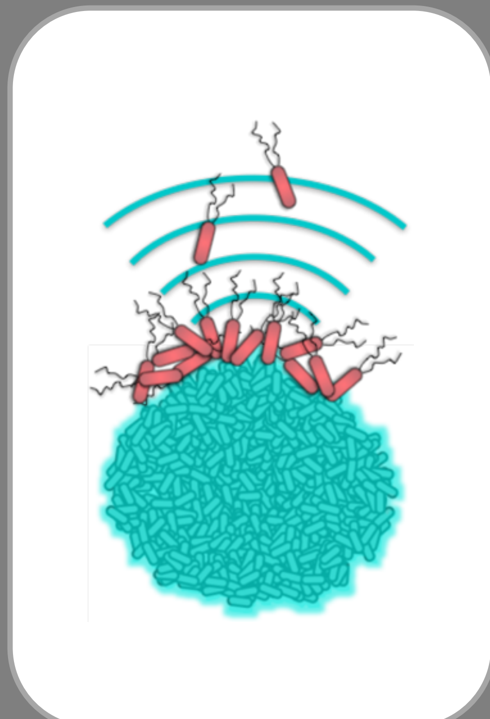


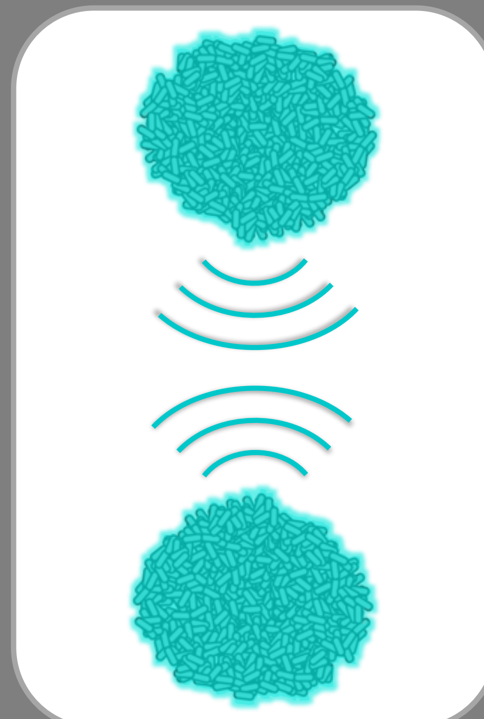
How does it work?

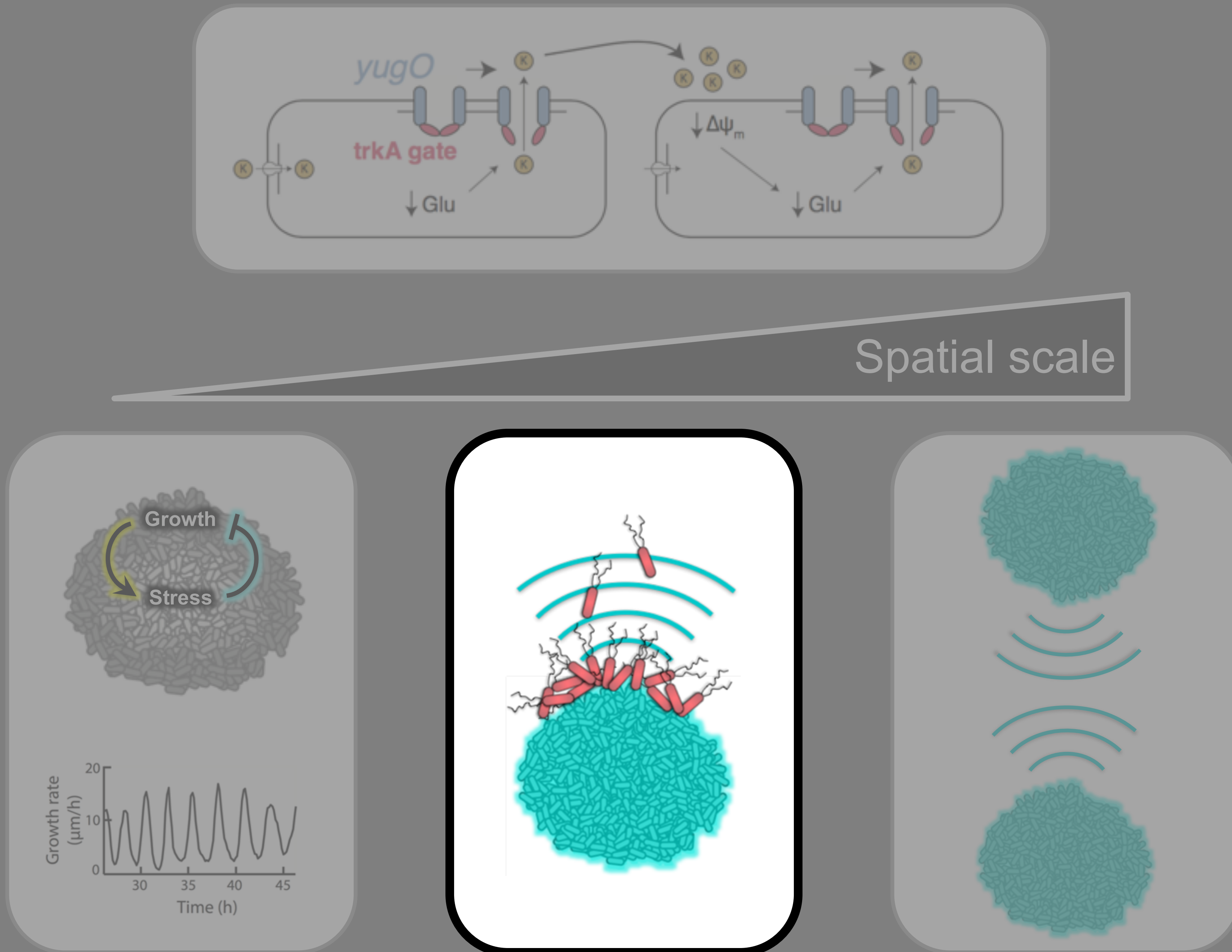
What does it do?





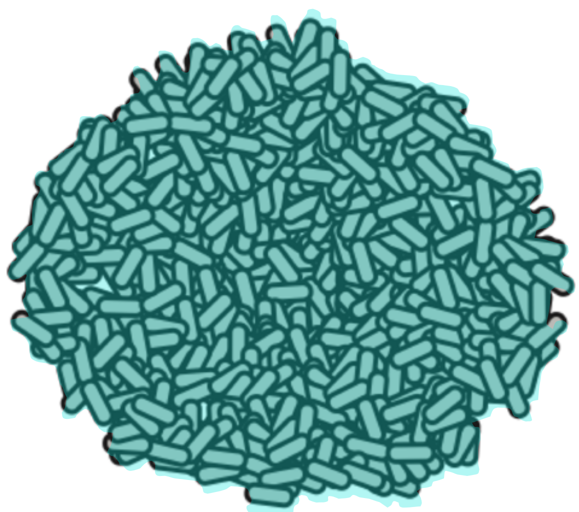




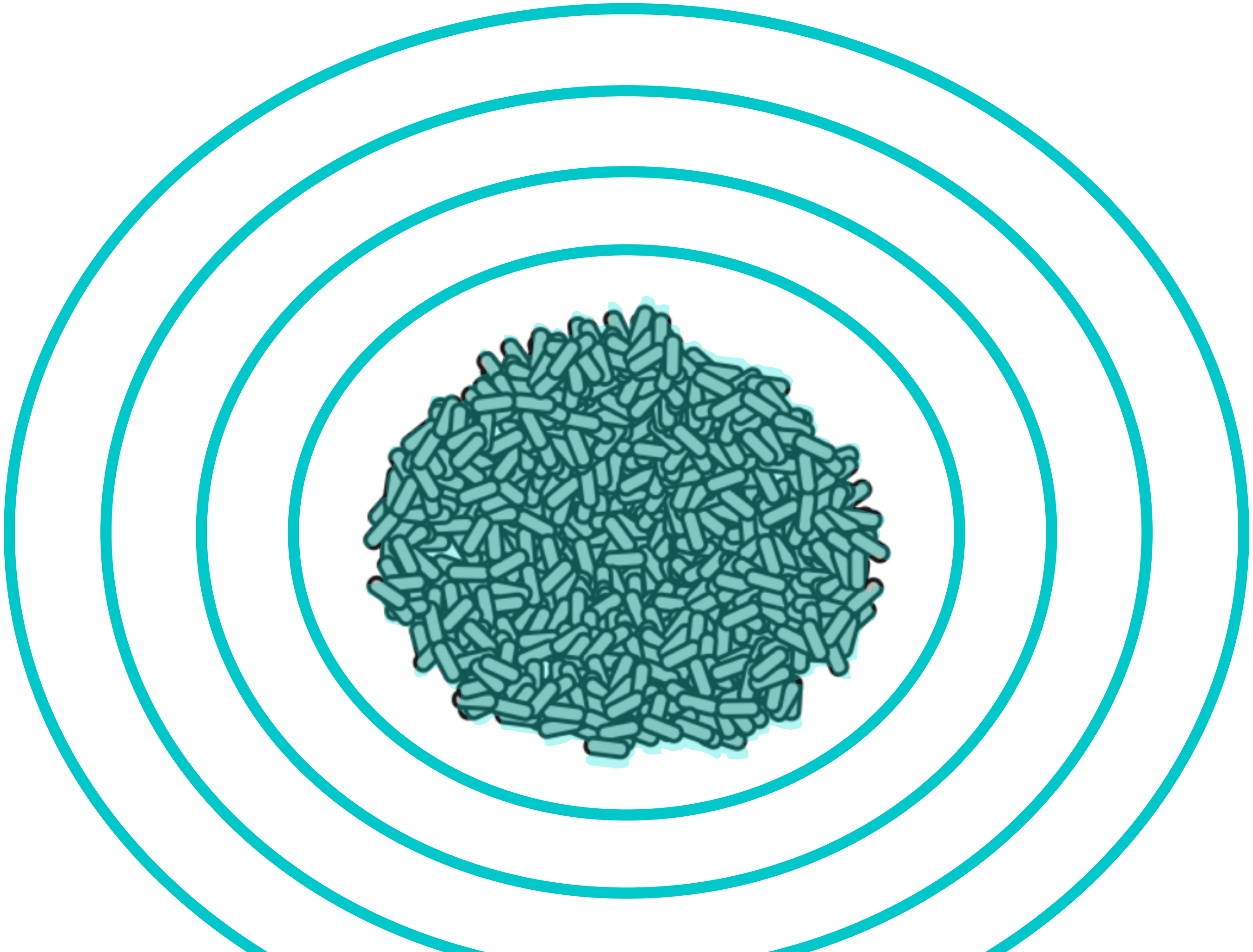


yugO
trkA gate
K
Glu
Δψm
Spatial scale
Growth
Stress
Growth rate (μm/h)
20
10
0
30
35
40
45
Time (h)

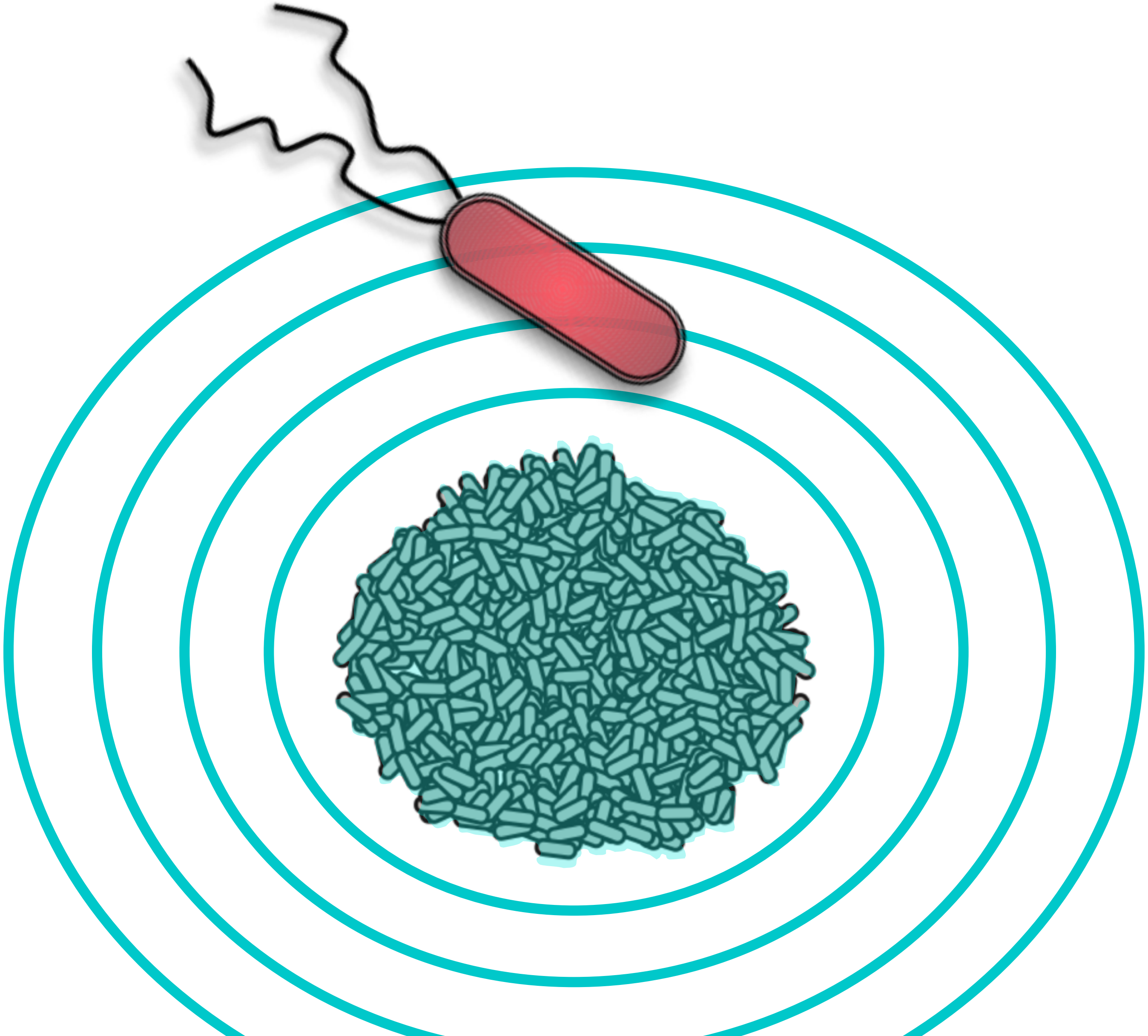
Question…



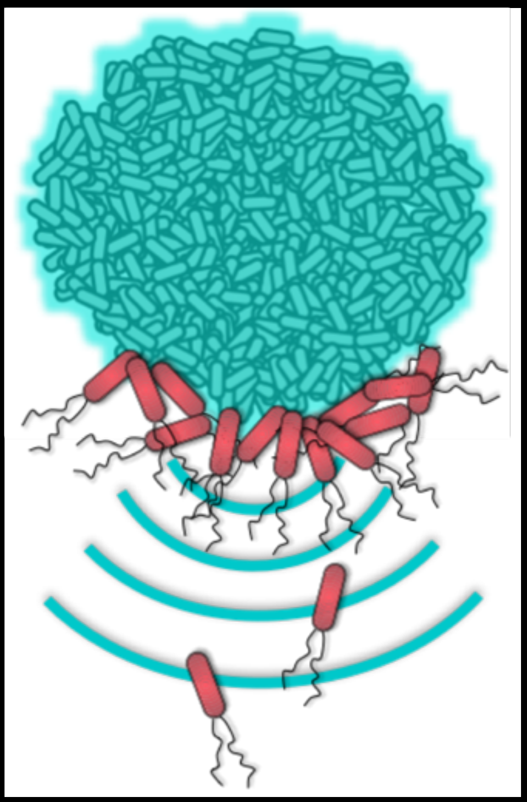
Question...

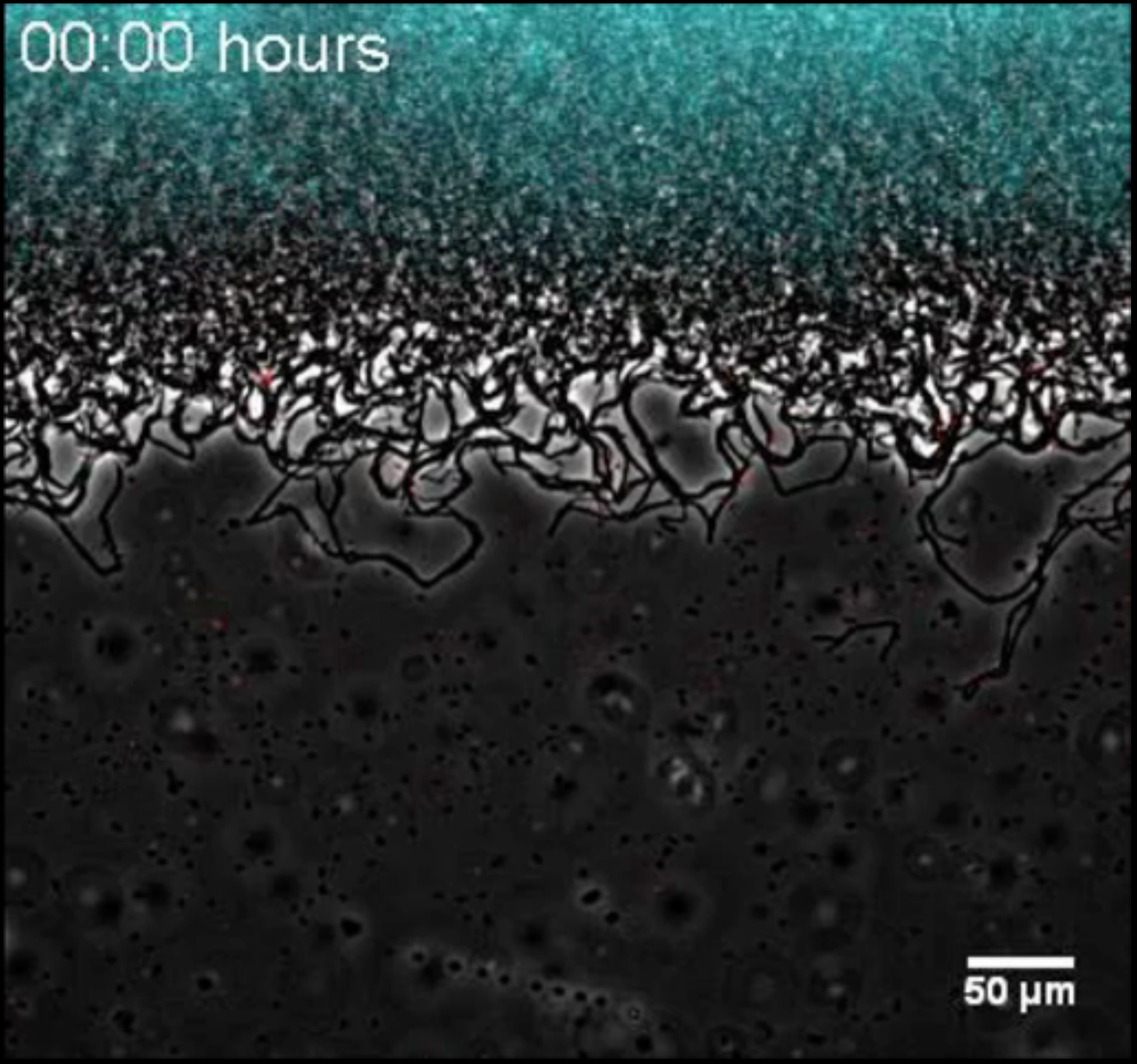


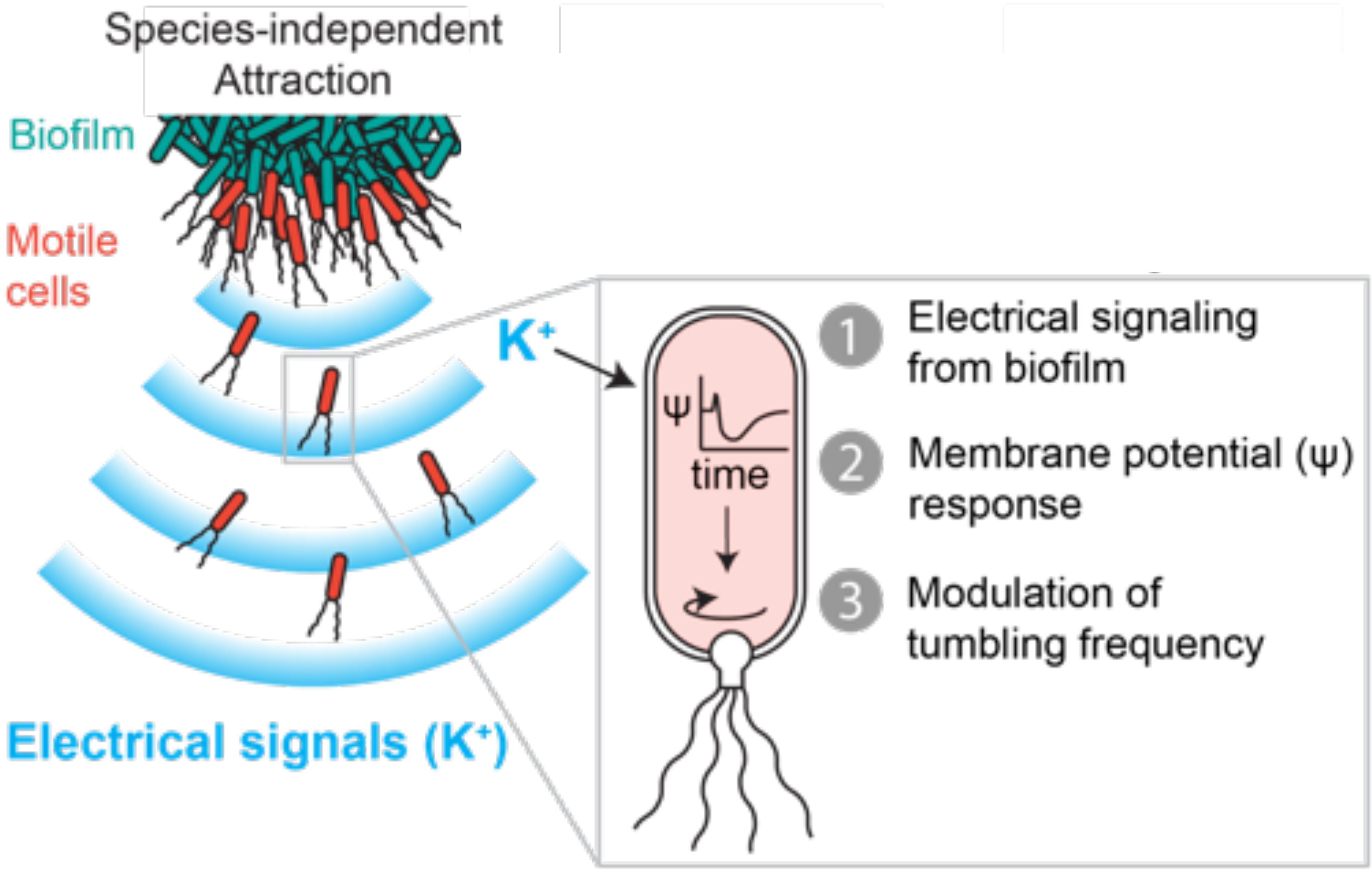
Question…



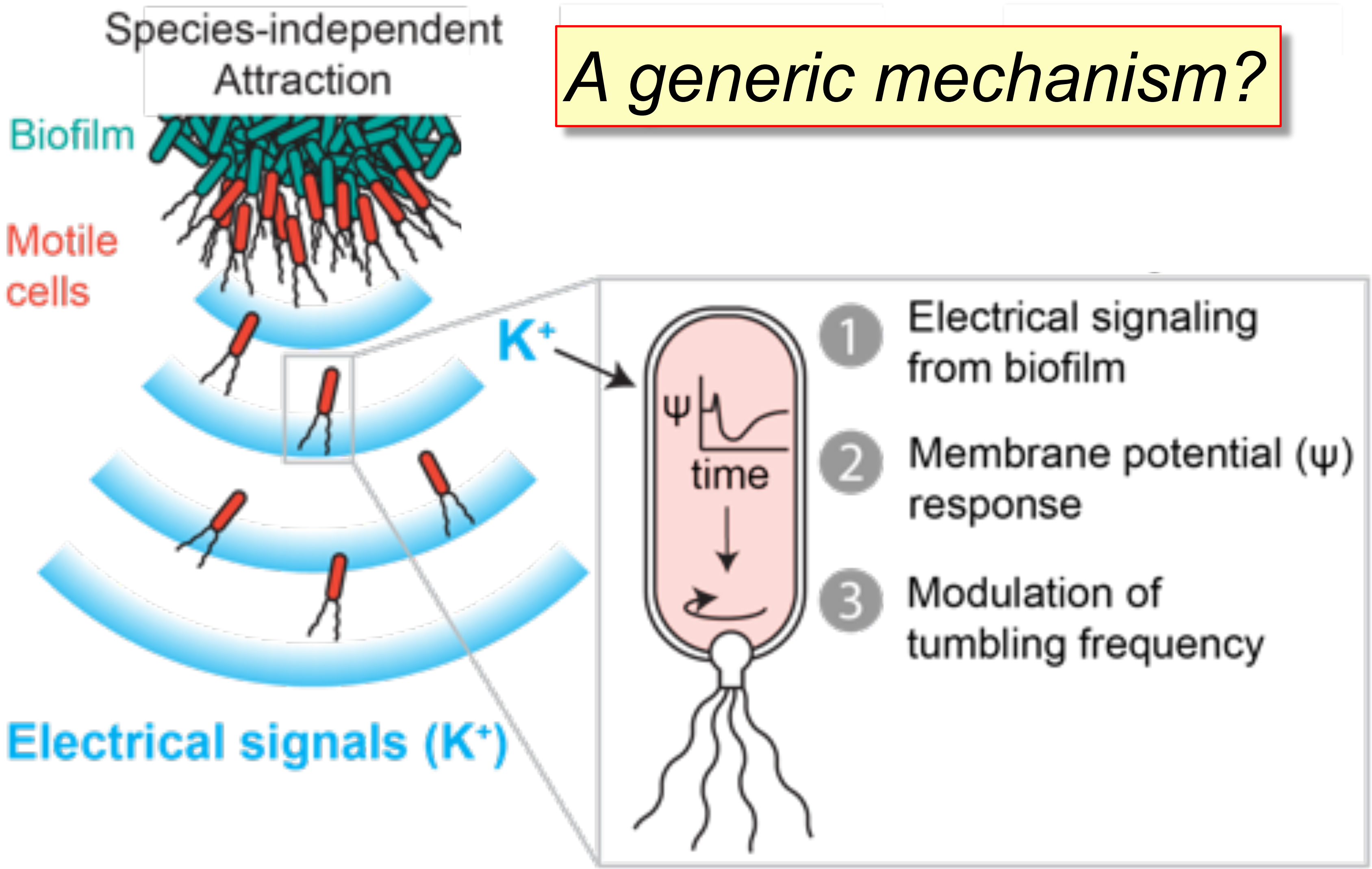
Electrical signaling-mediated attraction of motile cells to a biofilm

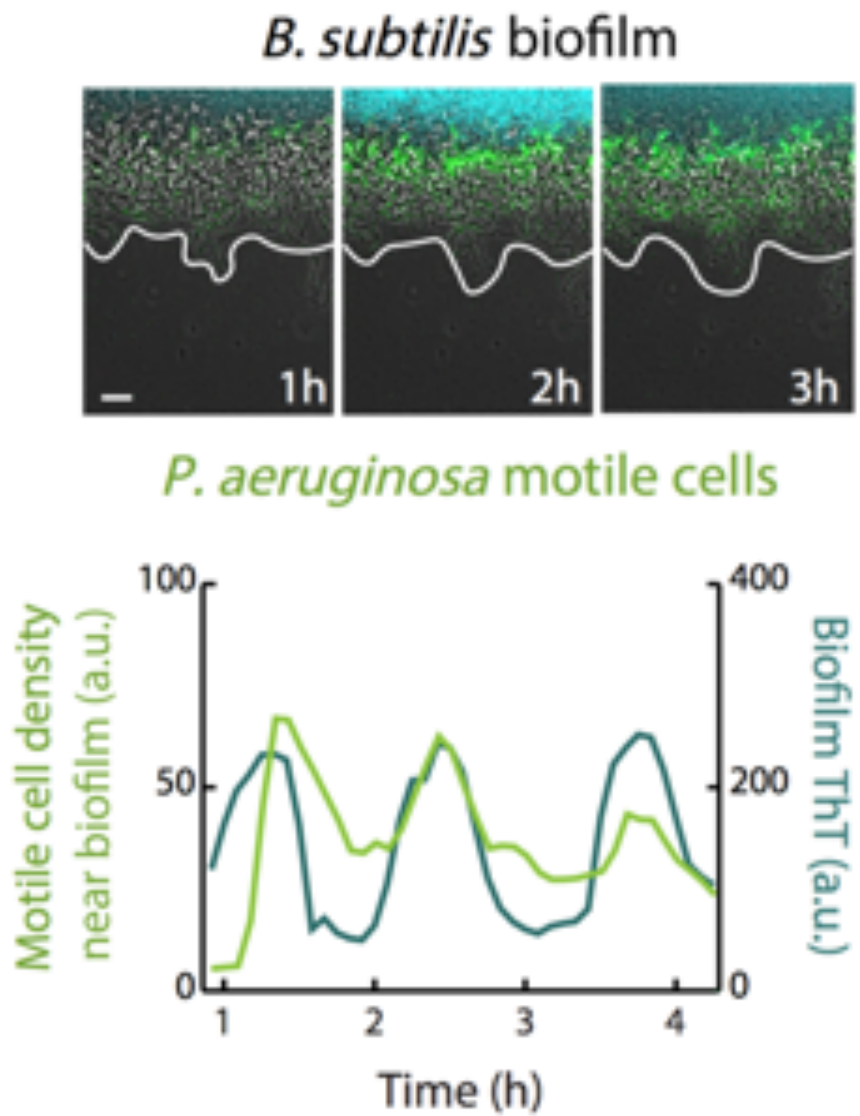





Species-independent Attraction
Biofilm
Motile cells
K+
Ψ
time
1 Electrical signaling from biofilm
2 Membrane potential (ψ) response
3 Modulation of tumbling frequency
Electrical signals (K+)

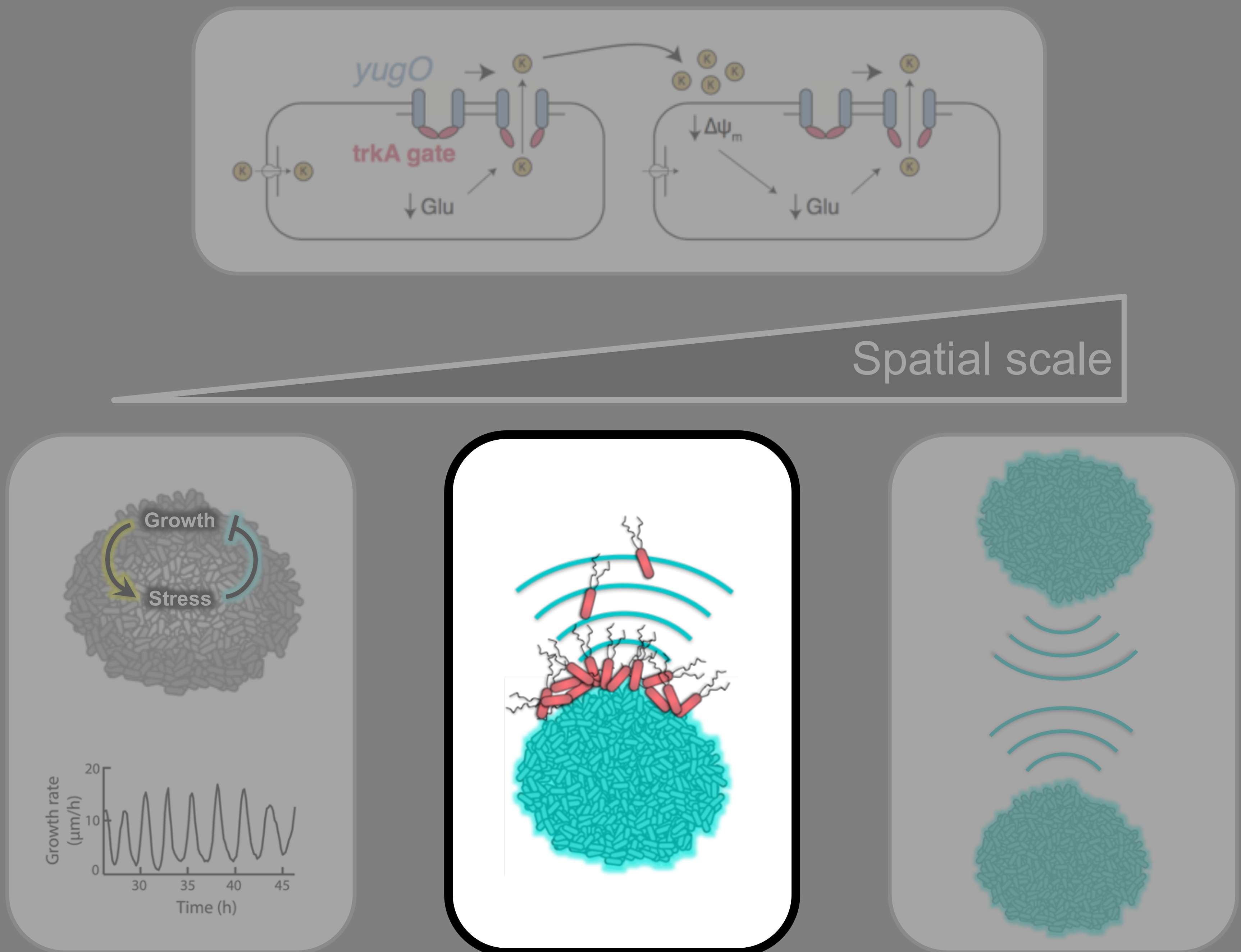
A generic mechanism?



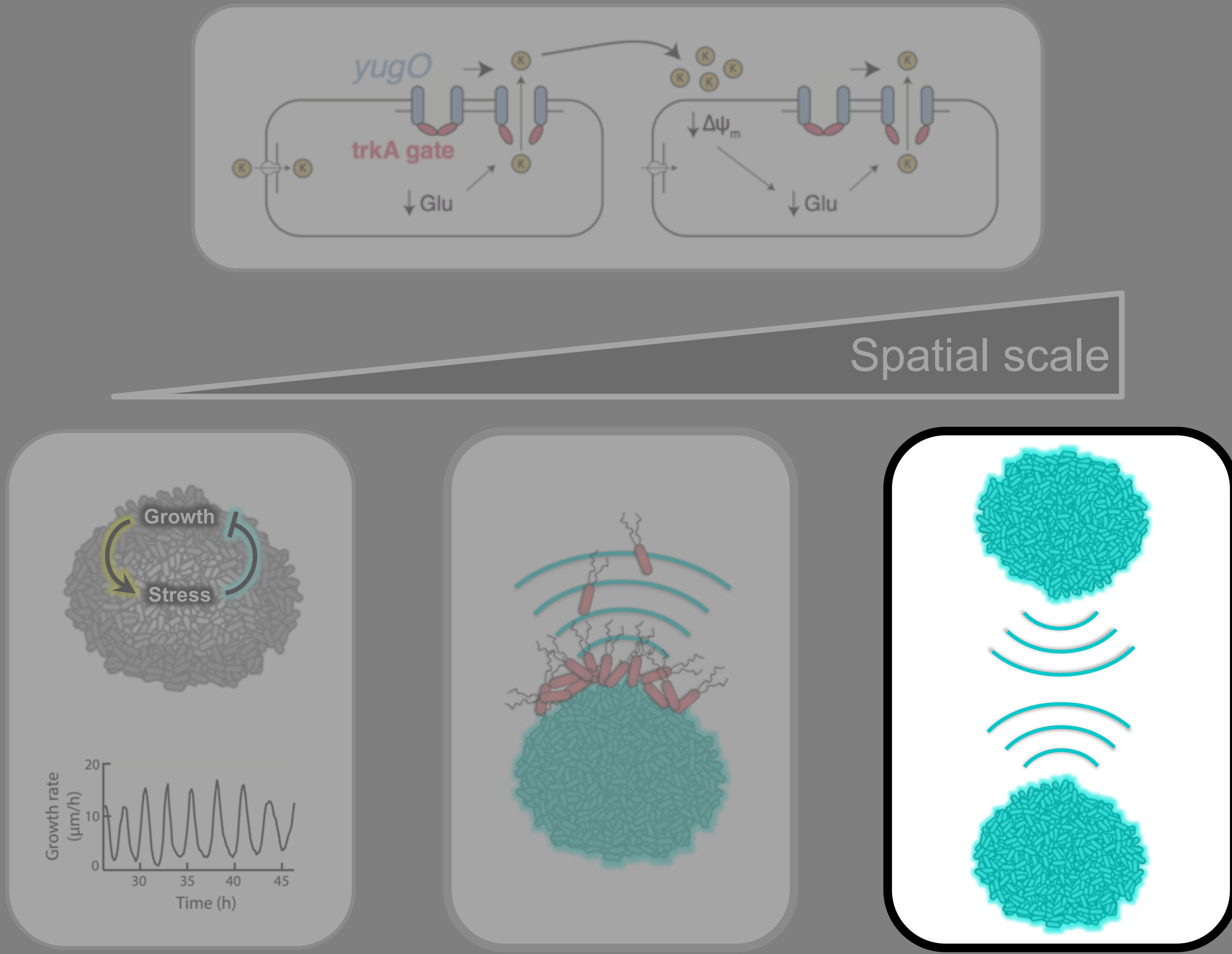


Cross species interaction

Mechanism for the formation of a mixed-species biofilm

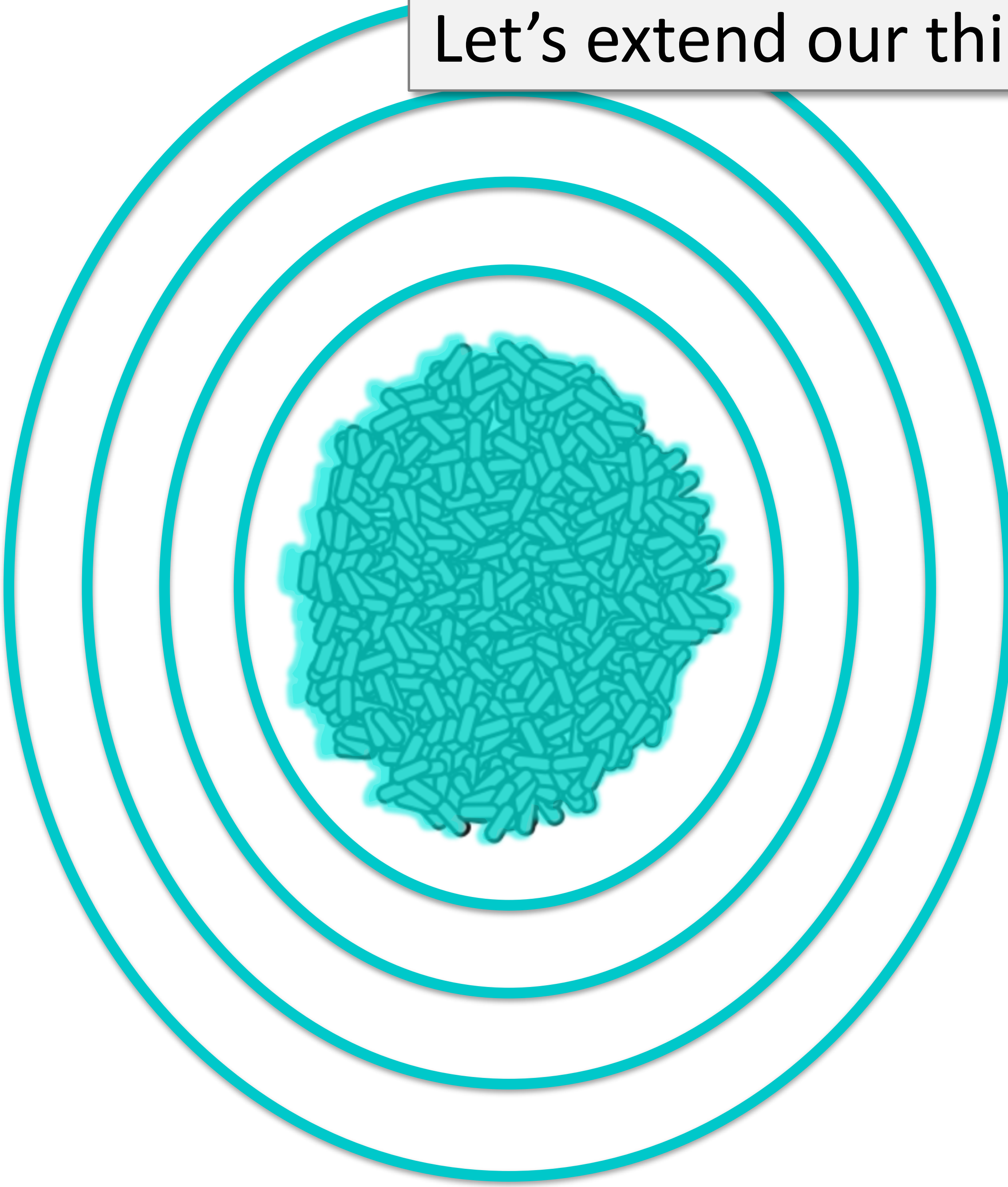


yugO
trkA gate
Glu
Δψm
Glu
Spatial scale
Growth
Stress
Growth rate (µm/h)
20
10
0
30
35
40
45
Time (h)

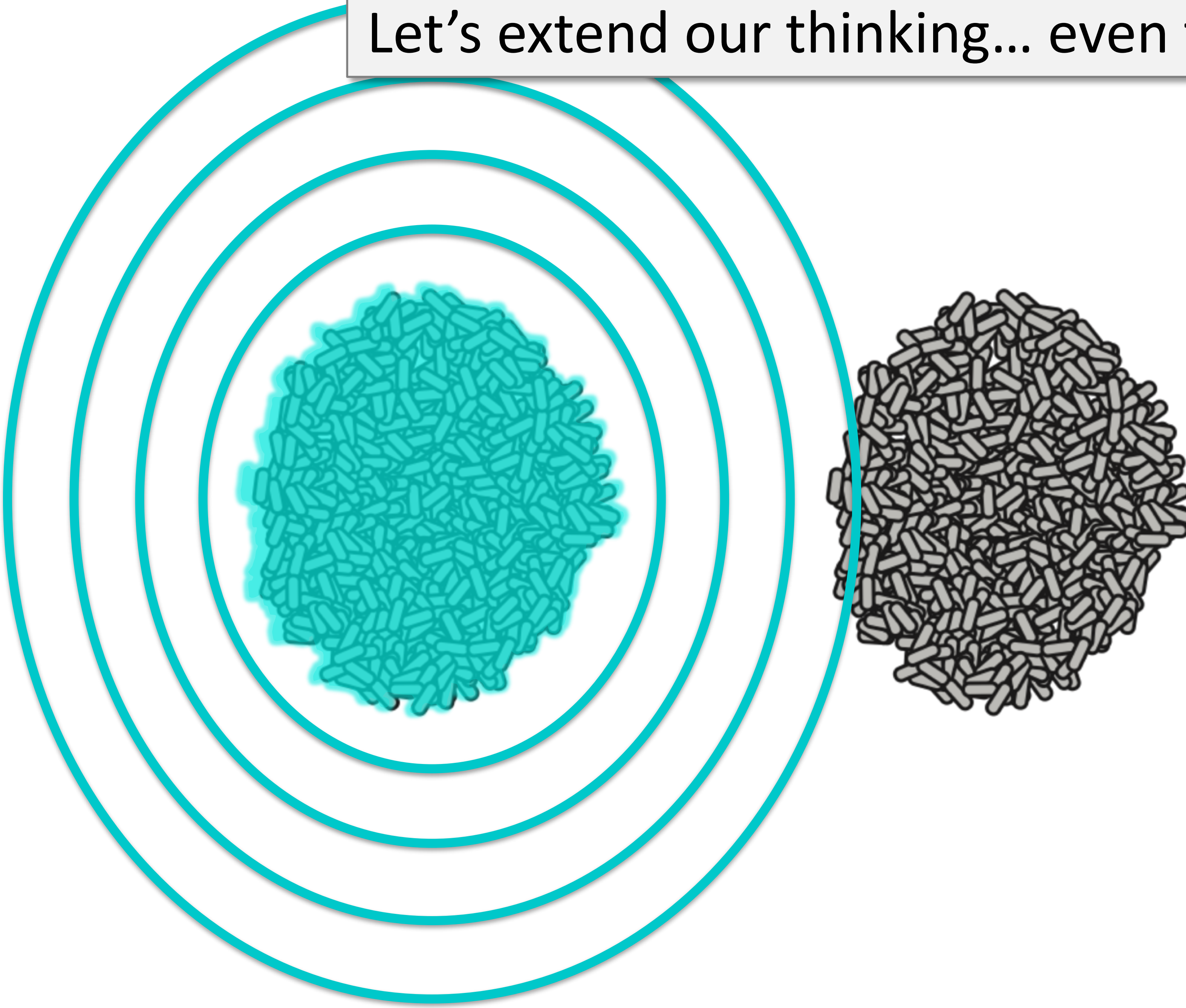


yugO
trkA gate
K
↓Glu
↓Δψ_m
Spatial scale
Growth
Stress
Growth rate (μm/h)
20
10
0
30
35
40
45
Time (h)

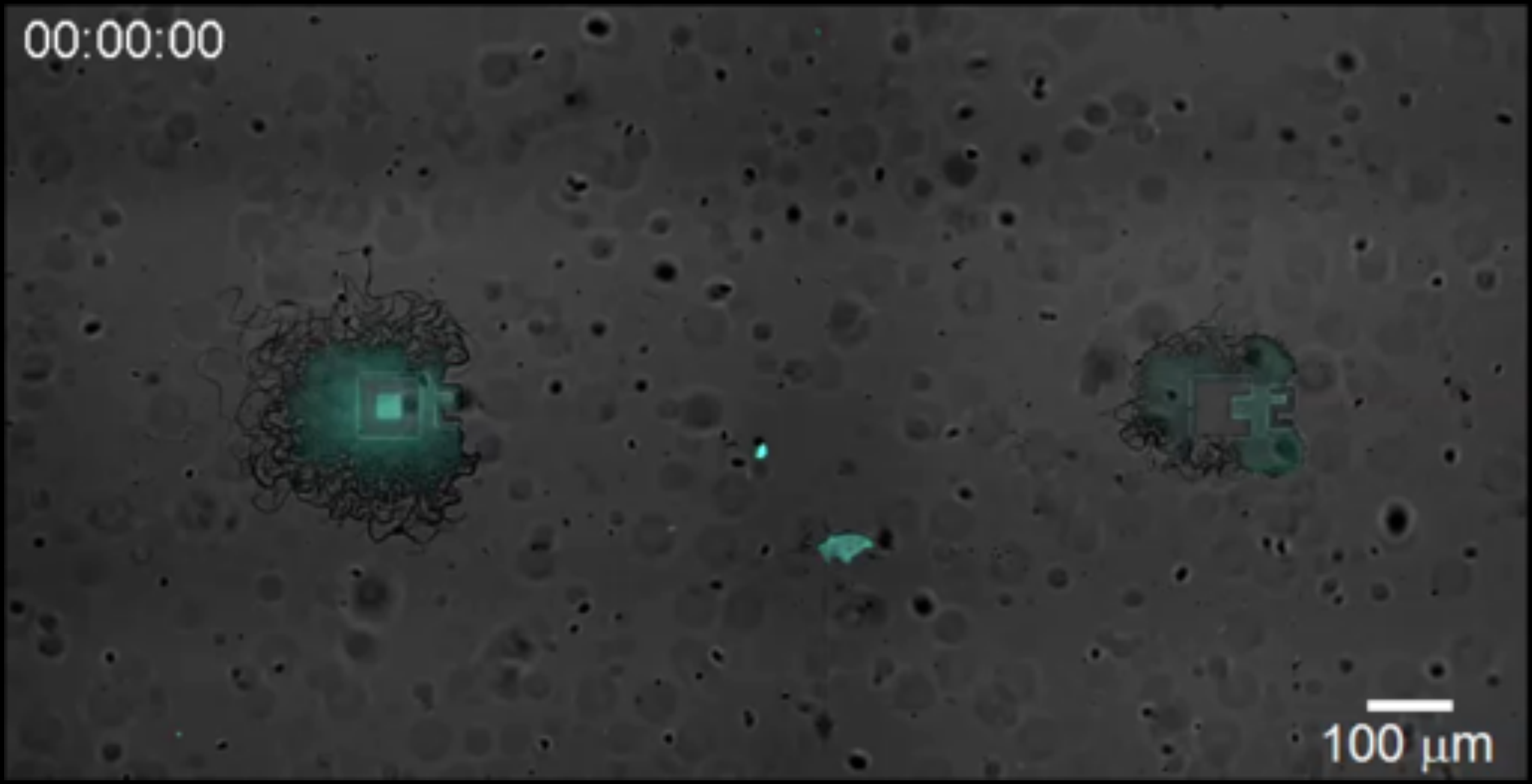
Let’s extend our thinking… even further



Let’s extend our thinking… even further

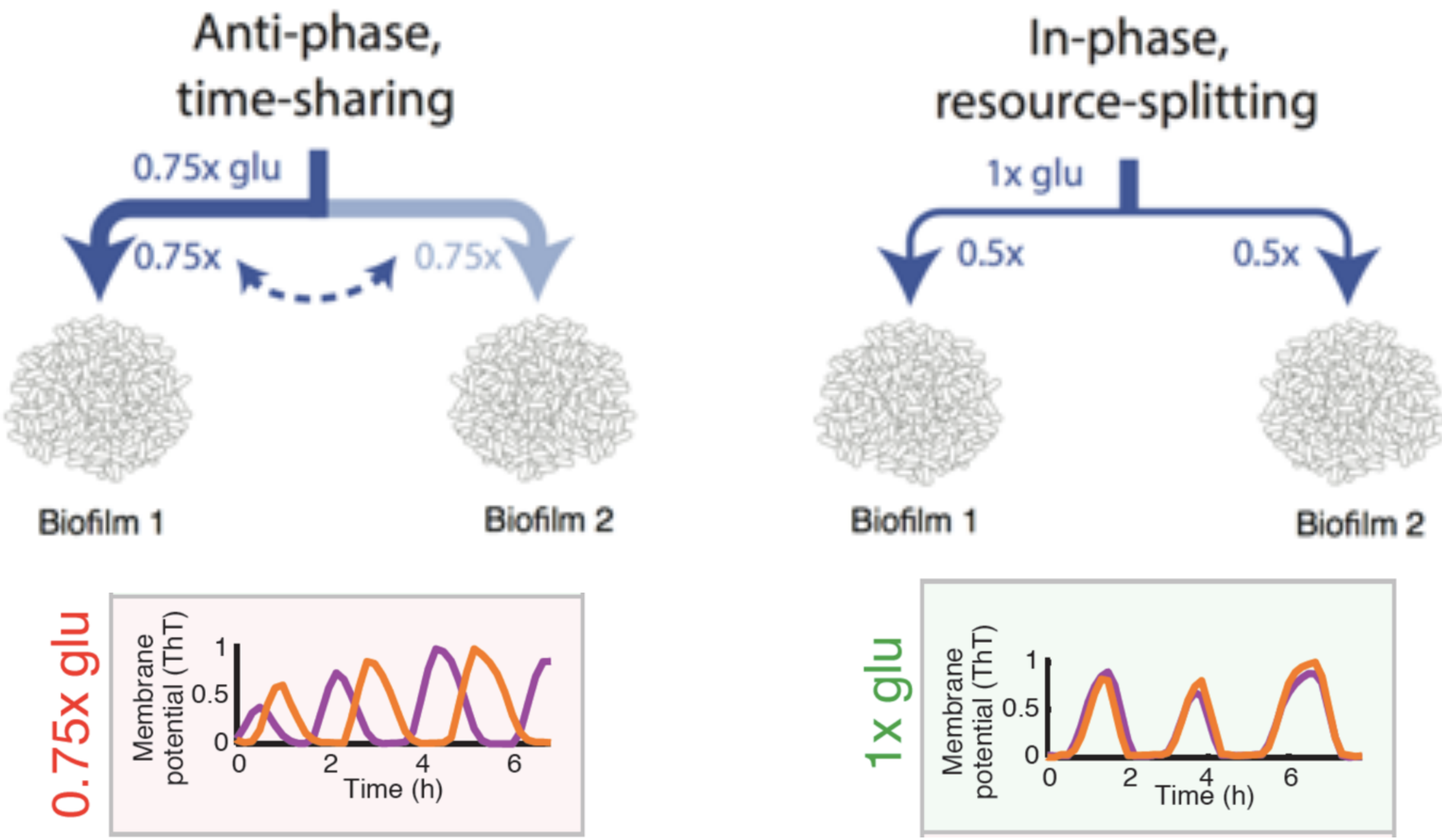


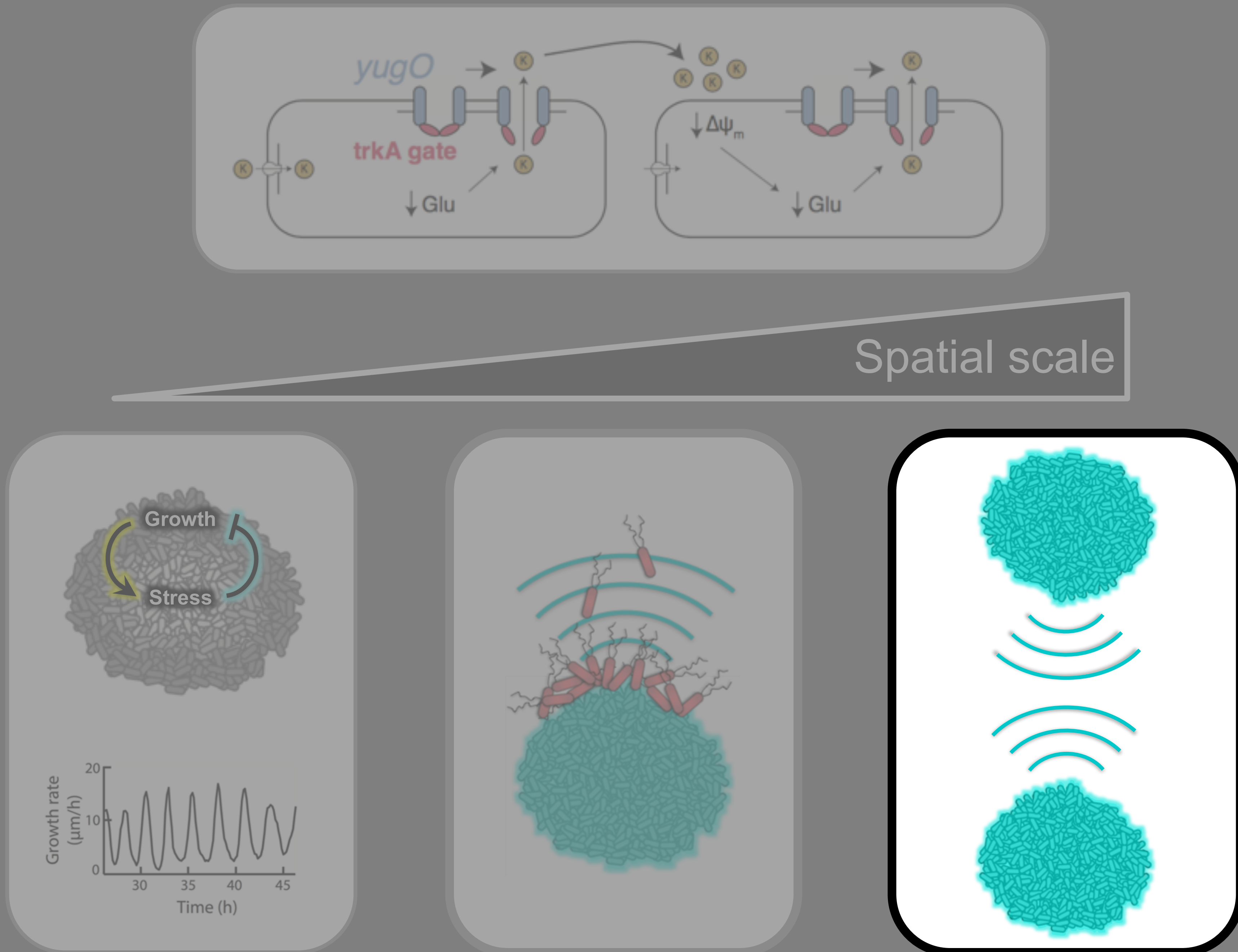
Coupling between two biofilms



Membrane potential
(Th-T)

A Timeshare hypothesis





yugO
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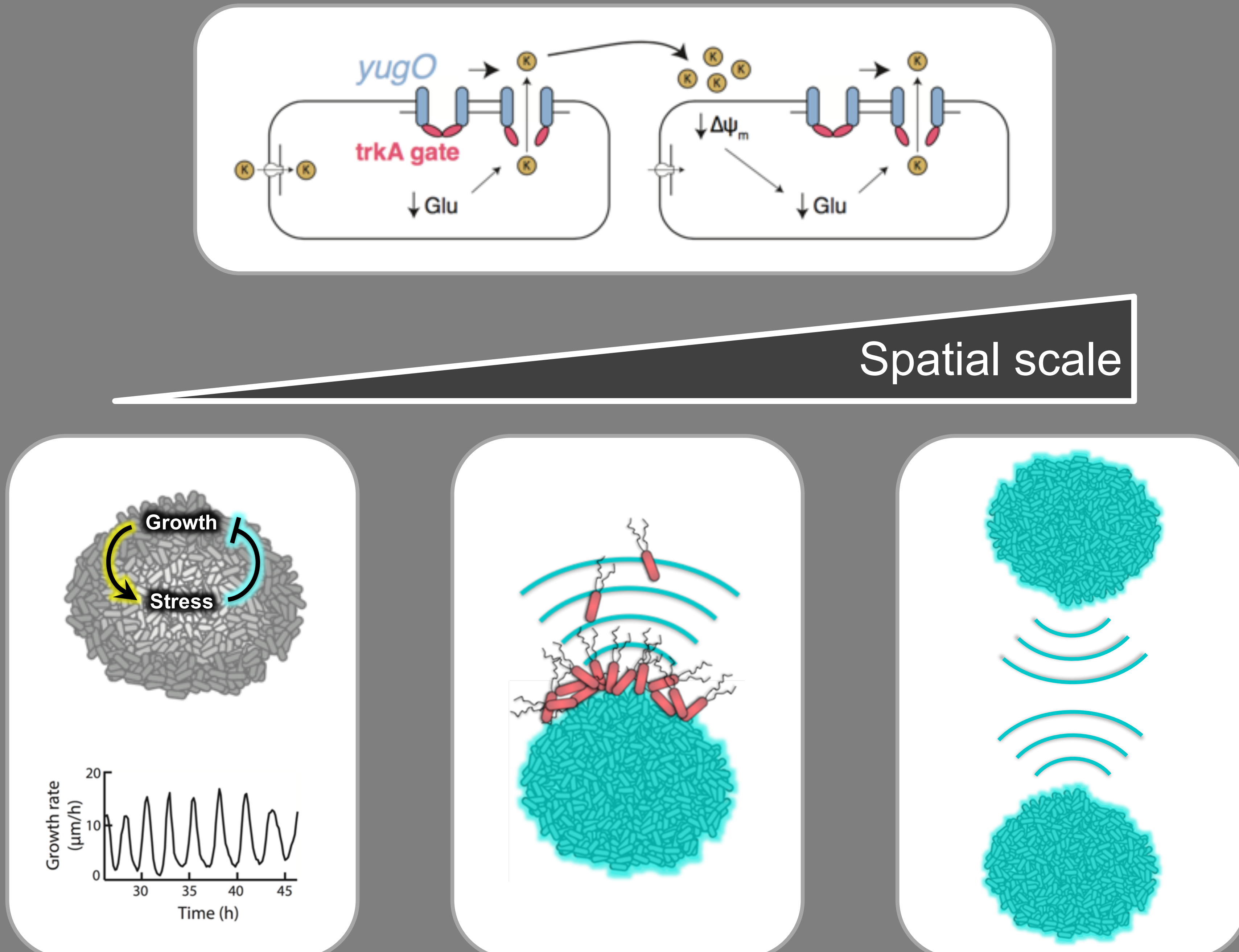
yugO
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Northwestern CENTER FOR SYNTHETIC BIOLOGY

NORTHWESTERN UNIVERSITY FEINBERG SCHOOL OF MEDICINE

Prindle Lab

Peter Tran

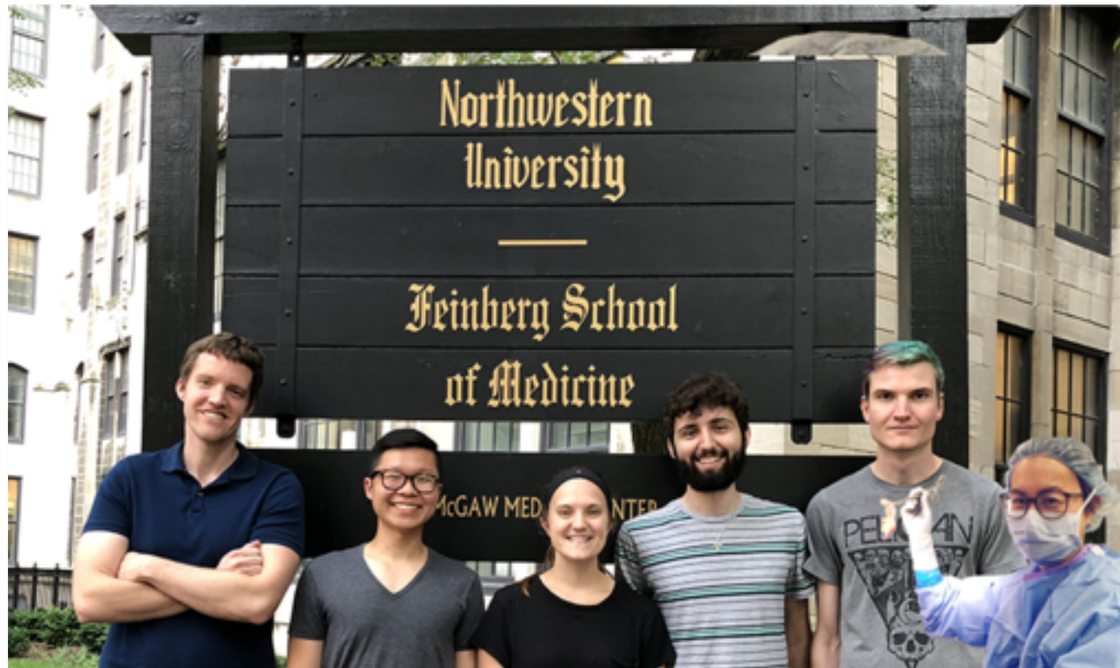
Kaila Smith

Corey Kennelly

Garth Fisher

Stephen Lander (not pictured)

Yi Liu (now Stanford)



Asally Lab

Suel Lab

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