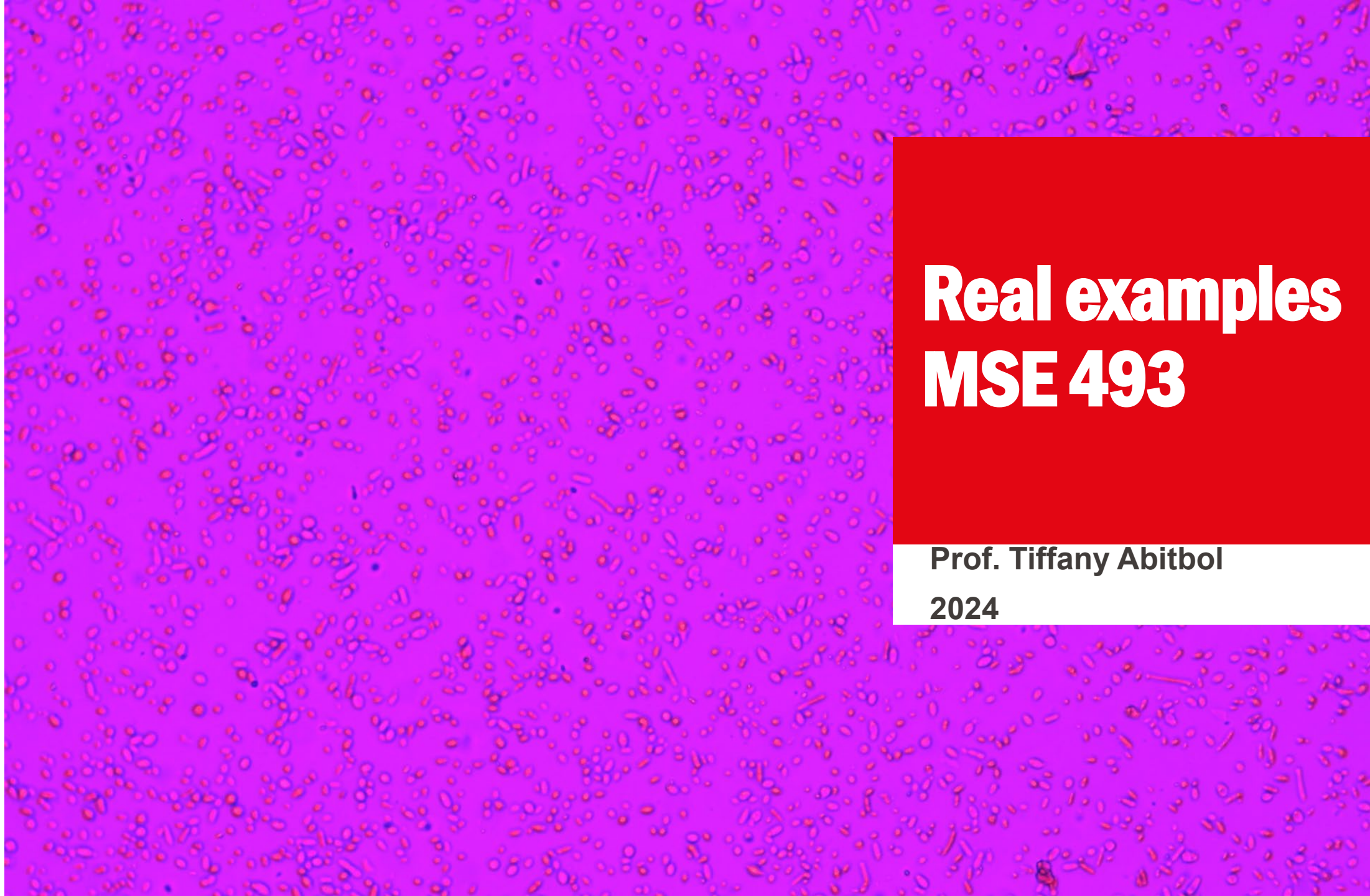


Real examples MSE 493

Prof. Tiffany Abitbol

2024

Journal Metrics - Matter

Performance metrics

6 days
Submission to first editorial decision ⓘ

42 days
Submission to decision after review ⓘ

84 days
Submission to acceptance ⓘ

30 days
Accept to online publication ⓘ

Editorial speed metrics reflect median values calculated over a calendar year. They are updated annually.

Impact metrics

17.3
Impact Factor ⓘ

3.6
Immediacy Index ⓘ

26.3
CiteScore ⓘ

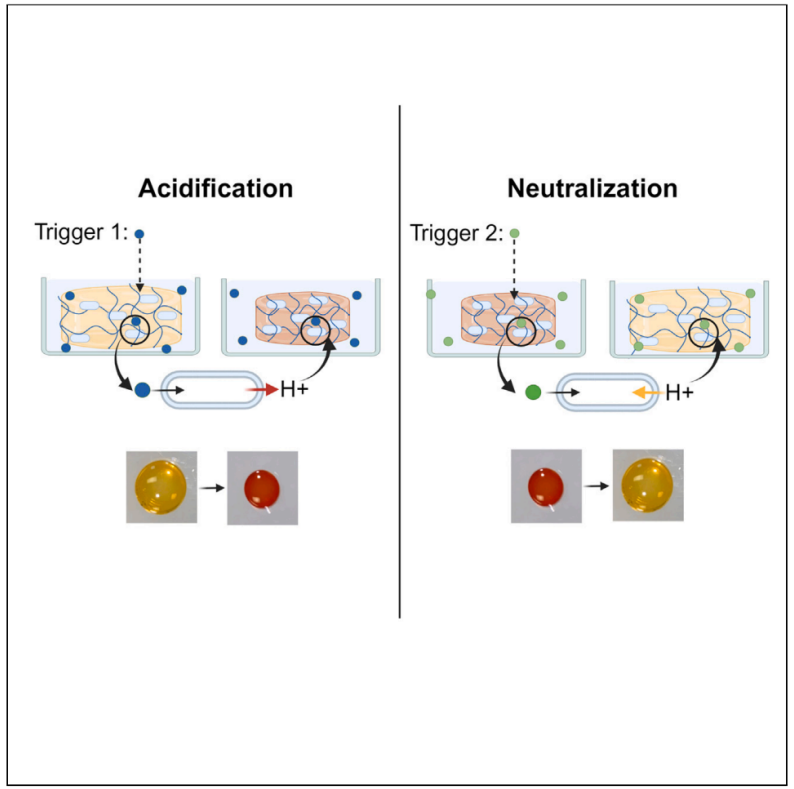
> Submit article ↗

- One way but not the only way to gauge article quality
- Impact factor: average number of citations received in a particular year by papers published in the journal during the two preceding years

https://www.cell.com/matter/home

Article

Microbially driven reversible size- and color-changing materials



A challenge of engineered living materials is employing living components to drive changes in their environment. This work demonstrates the alteration of pH driven by *Escherichia coli*, resulting in reversible size and color change of a pH-responsive hydrogel. This validates a new concept with potential applications in more sophisticated hydrogel-based shape-changing materials.

Jenevieve Kuang, Shanna Bonanno, Wei-Ting Chang, ..., Daniel J. Wilson, Leila F. Deravi, Neel S. Joshi

ne.joshi@northeastern.edu

Highlights

E. coli alters pH with varied carbon sources in a reversible and predictable manner

E. coli enables changes in size and color of a pH-responsive hydrogel

The living material displays reversible appearance changes inspired by nature

- Published May 2024
- DOI: 10.1016/j.matt.2024.03.009
- Color & size (3 mentions)
- We can understand that it is something that the bacteria is doing that is driving a change in color and size in a material

JOURNAL OF THE ROYAL SOCIETY INTERFACE

(IF = 3.7; cross disciplinary research at the interface of physics and life sciences)

Articles

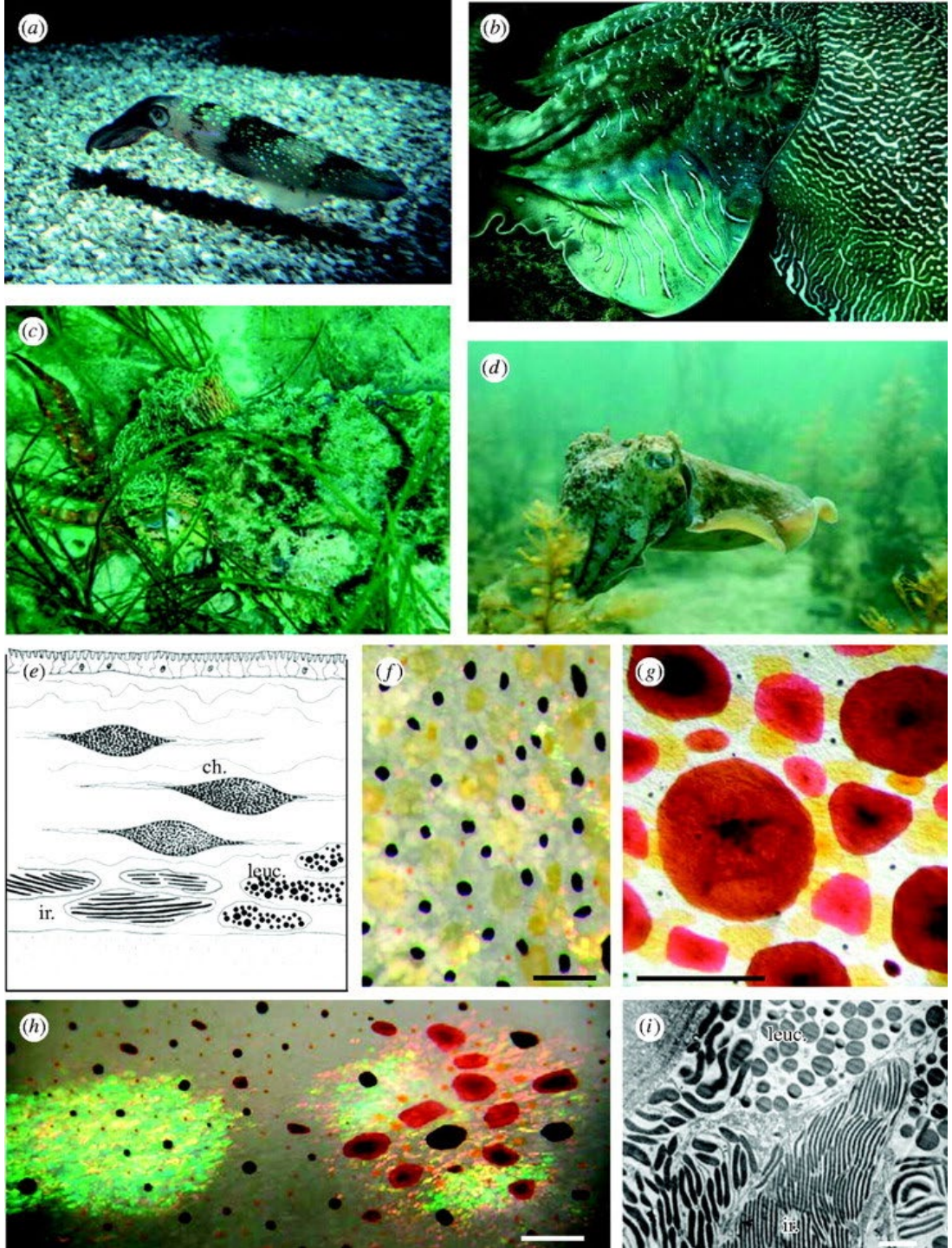
Mechanisms and behavioural functions of structural coloration in cephalopods

Lydia M Mäthger ✉, Eric J Denton,
N. Justin Marshall and Roger T Hanlon

Published: 15 December 2008
https://doi.org/10.1098/rsif.2008.0366.focus

- A natural example mentioned in the article: cephalopods

Cephalopod



- Although apparently, colorblind cephalopods show a wide range of body patterns for **camouflage** and **signaling/communication**
- Patterns ***change dramatically with the direction and movement***, can be used to communicate the movement of individuals in a school

https://doi.org/10.1098/rsif.2008.0366.focus

SUMMARY

Features of natural living systems underexplored in engineered living materials (ELMs) are macroscale appearance changes driven by active cellular processes. To overcome this technological gap, we demonstrate an ELM wherein the natural metabolism of *Escherichia coli* is used to drive reversible changes in pH-responsive hydrogels through the production or consumption of acidic metabolites. A color-changing function of the hydrogels relies on the custom design, synthesis, and coupling of a synthetic pH indicator dye into the polymer network. Manipulation of the starting pH conditions and the identity of the primary carbon source leads *E. coli* to alter pH, resulting in reversible size and color changes in the gels. Arrayed arrangements of multiple responsive hydrogels can mimic dynamic pixels that respond to changes in cell metabolism. Here, we expand the tool kit of ELMs to include size and color change as functional performance features that can be driven by active cellular processes.

- Addresses an "underexplored" gap – macroscopic changes driven by cellular processes
- Using the metabolism of ***E. coli*** to drive changes in a ***pH responsive hydrogel***
- Color change relies on a **pH sensitive color switching dye**
- Size change relies on whether the hydrogel is swollen or not, which depends on pH

3 main ingredients

- E. coli **bacteria** to generate and consume acid
- **Hydrogel**s that swell and de-swell depending on pH
- **Dye** that changes color with pH

+ 2 concepts

- pH
- pKa

pH & pKa (refresher)

$$K_a = \frac{[A^-][H^+]}{[HA]}$$

- **pH=−log[H+]**; determined by the concentration of protons in a solutions
- **pKa=−log(Ka);** Ka is the acid dissociation constant, describes the extent to which an acid dissociates in solution; higher Ka indicates more dissociation and a stronger acid, lower pKa = stronger acids, and *vice versa*
- Strong acids completely dissociate in water, pKa < 1, low pH
- Weak acids (like acrylic acid) partially dissociate, higher pKa 2-10, don’t lower the pH as much as strong acids do

Strong vs. weak acids

$$K_a = \frac{[A^-][H^+]}{[HA]}$$

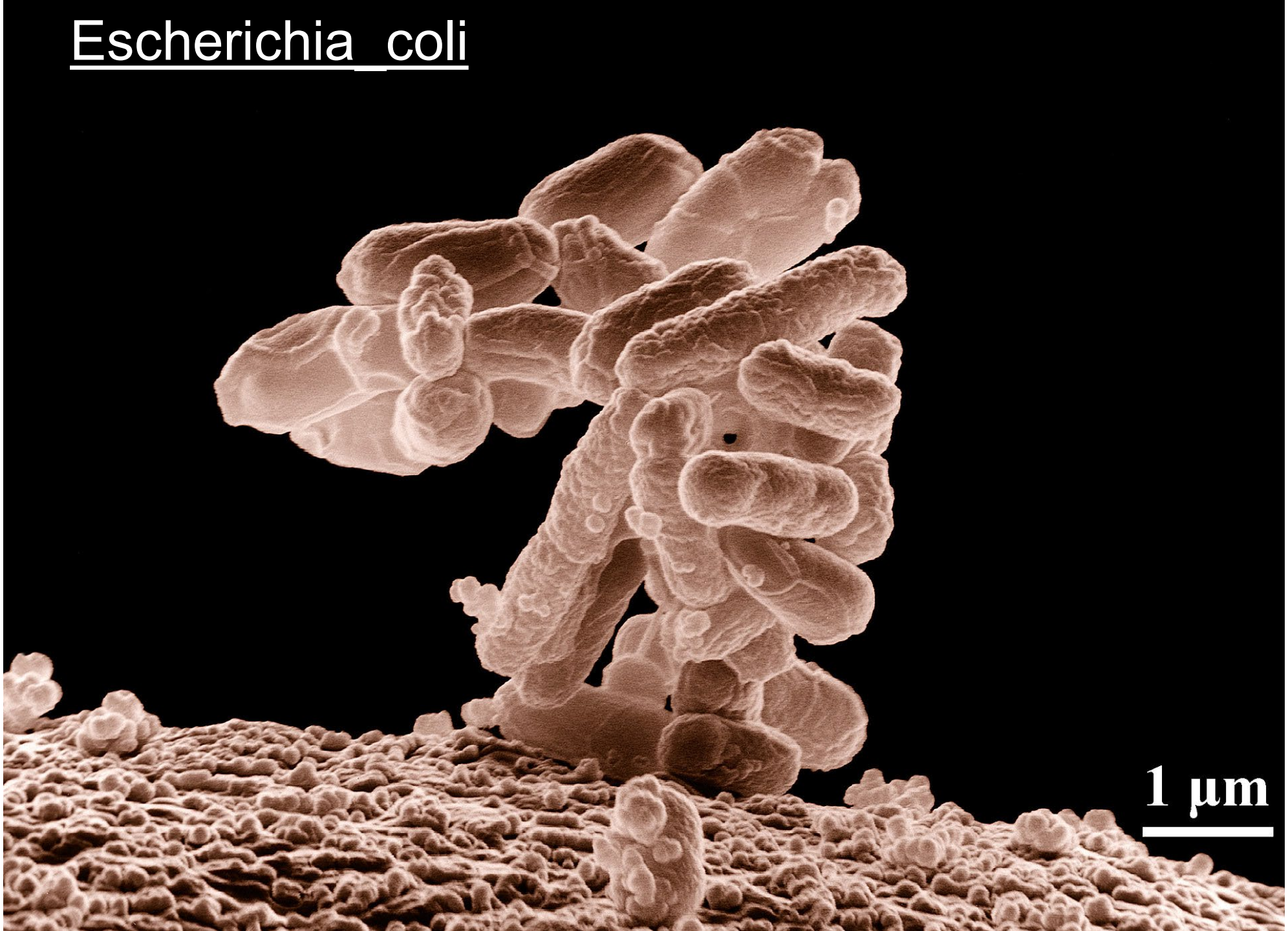
- Strong acids fully dissociate at almost any pH (HCl, H_2SO_4, HNO_3, etc.)
- The dissociation of weak acids is pH dependent:

  pH<pKa: protonated form, not dissociated

  pH>pKa: dissociated form

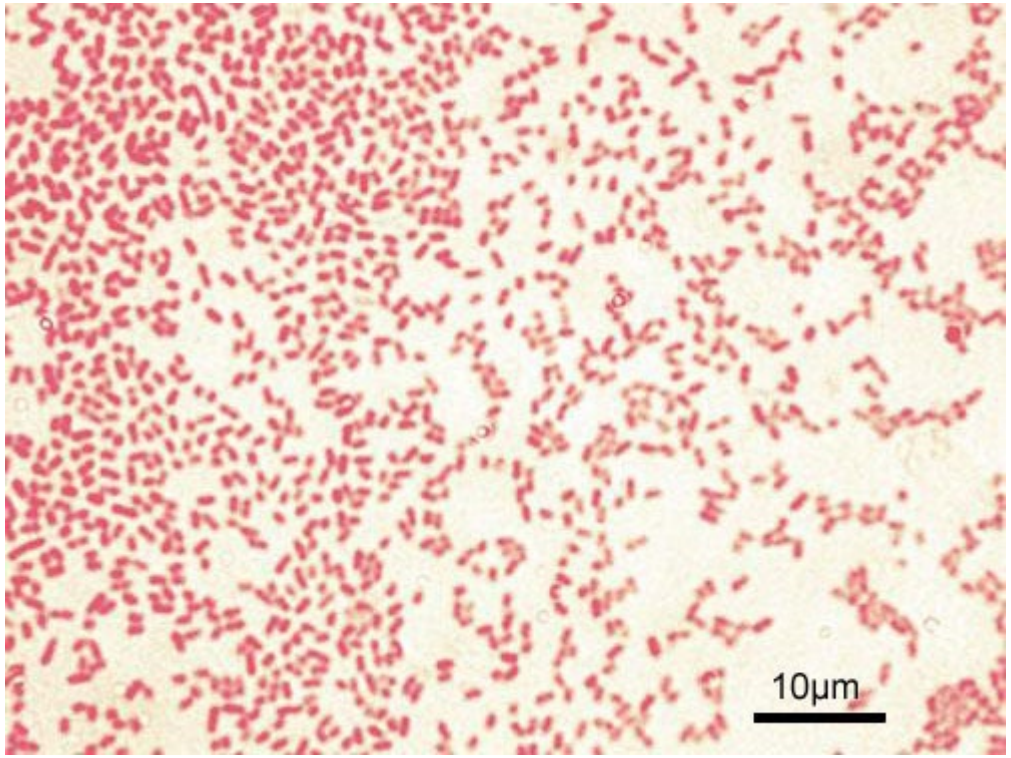
  pH = pKa: 50% protonated and 50% deprotonated

Escherichia_coli



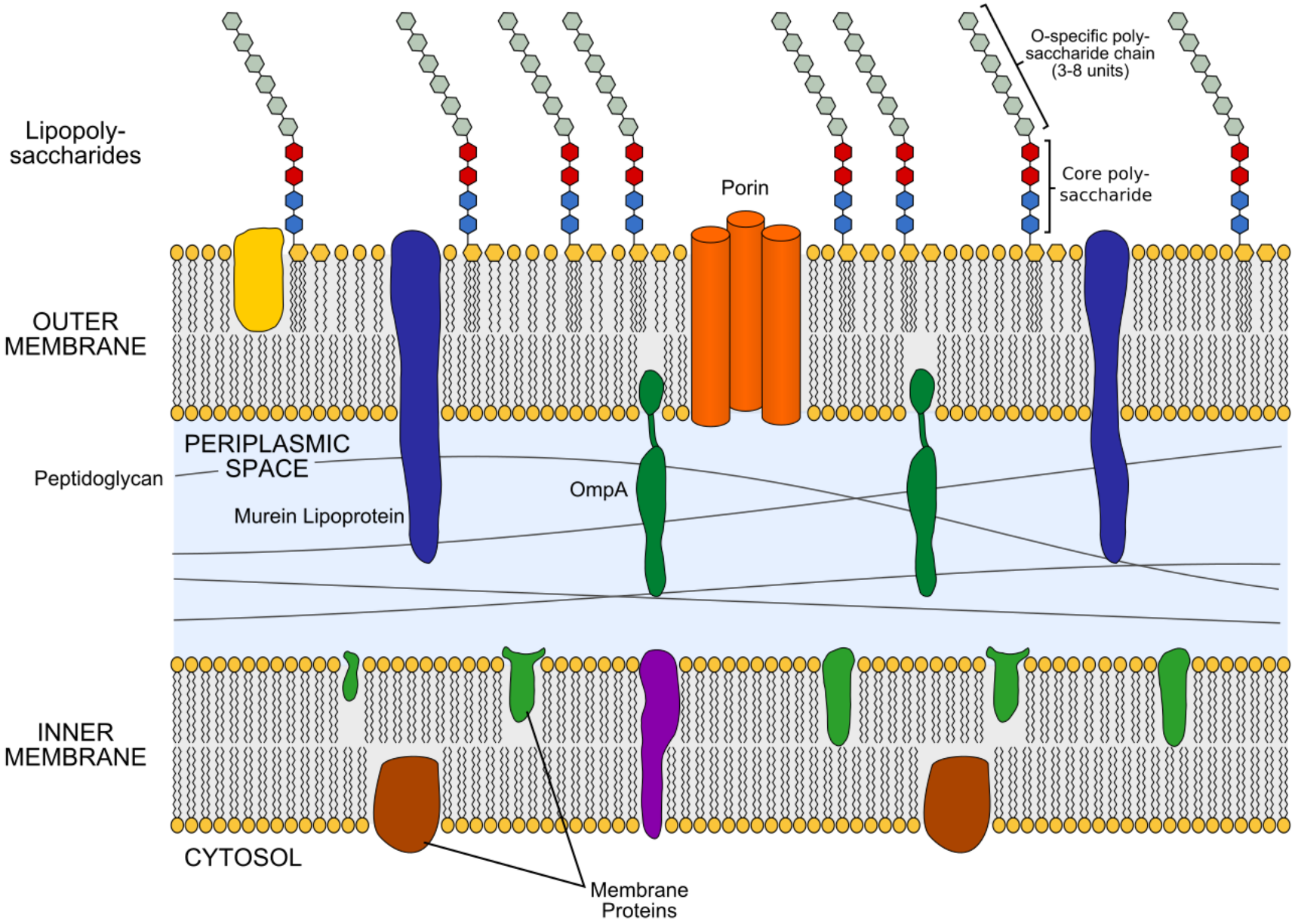
Gram-negative

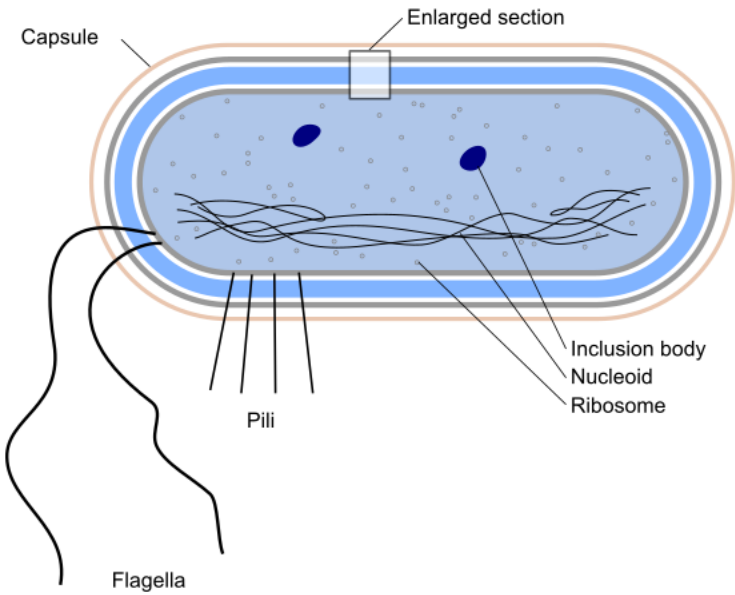
- E. coli is gram negative, some pathogenic, some not
- Gram negative refers to bacteria that do not retain the crystal violet stain used in Gram staining
- Defining characteristic is cell envelope, consisting of a thin peptidoglycan layer sandwiched between an inner and outer membrane



Gram negative bacteria

Gram negative bacteria

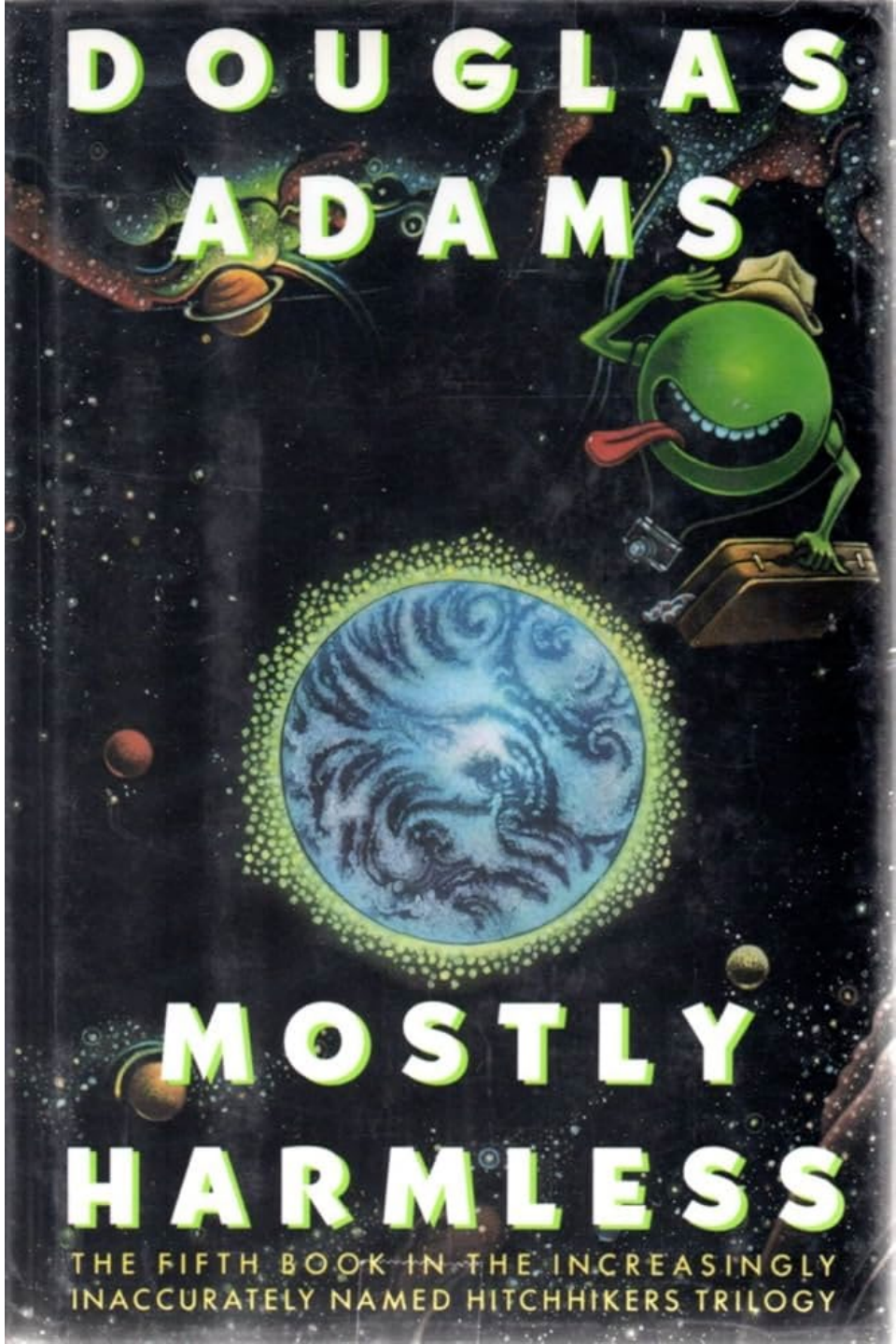




- Outer membrane acts as a barrier to many antibiotics

Escherichia coli (*E. coli*)

- Commonly found in intestines of humans and other animals
- Rod-shaped
- Mostly harmless
- Commonly used in laboratories for research and in biotech to produce recombinant proteins (e.g. human insulin)
- **BL21** is a strain of *E. coli* that is commonly used for protein expression as it has been genetically modified to optimize the production of recombinant protein

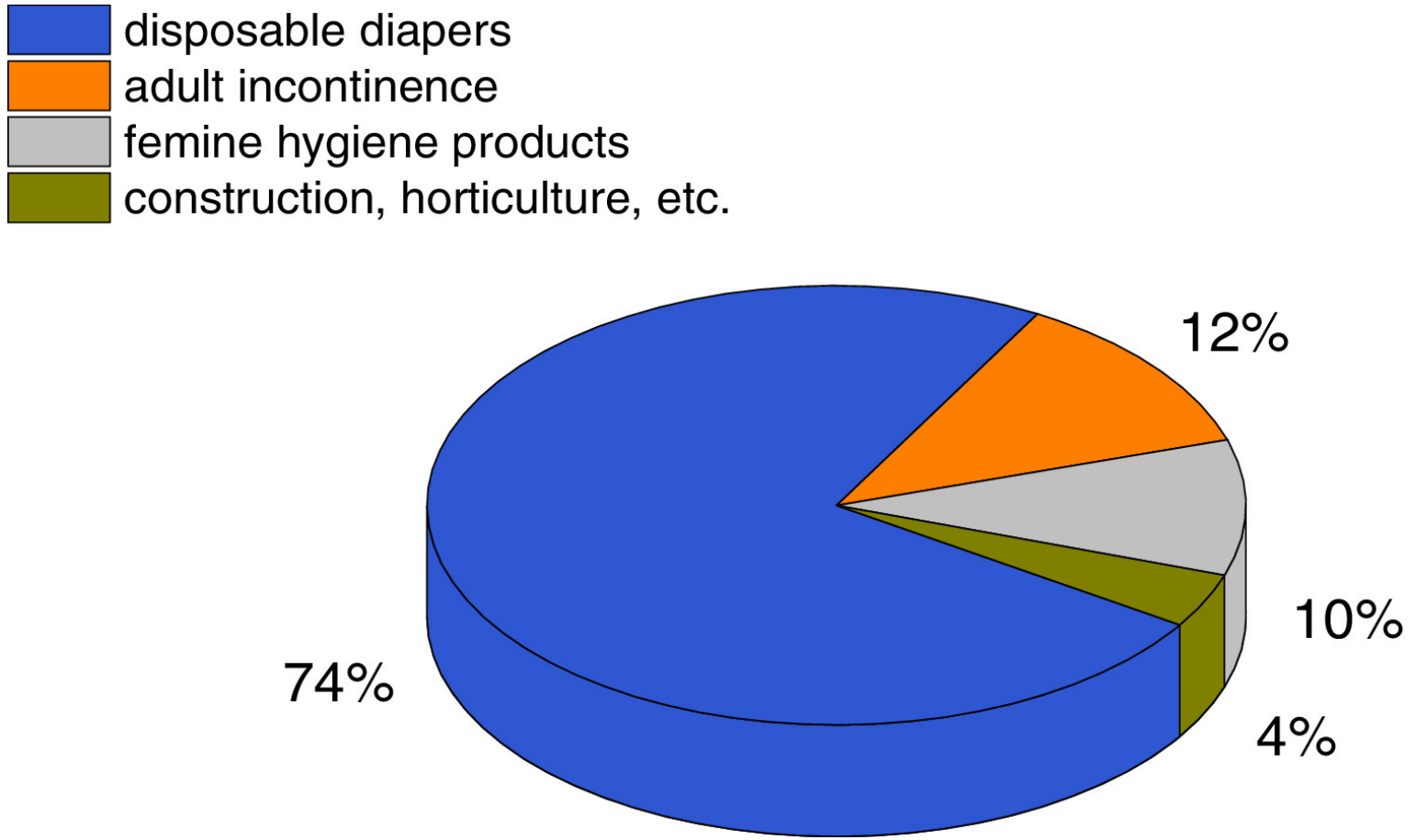


Hydrogel

- 3D network of hydrophilic polymers
- Can absorb and retain lots of water
- Soft, flexible, and hydrated (tissue-like)
- Moldable
- Often biocompatible
- Can respond to external stimuli
- Used in drug delivery, tissue engineering, wound dressings, diapers, agriculture, cosmetics, food, etc.,

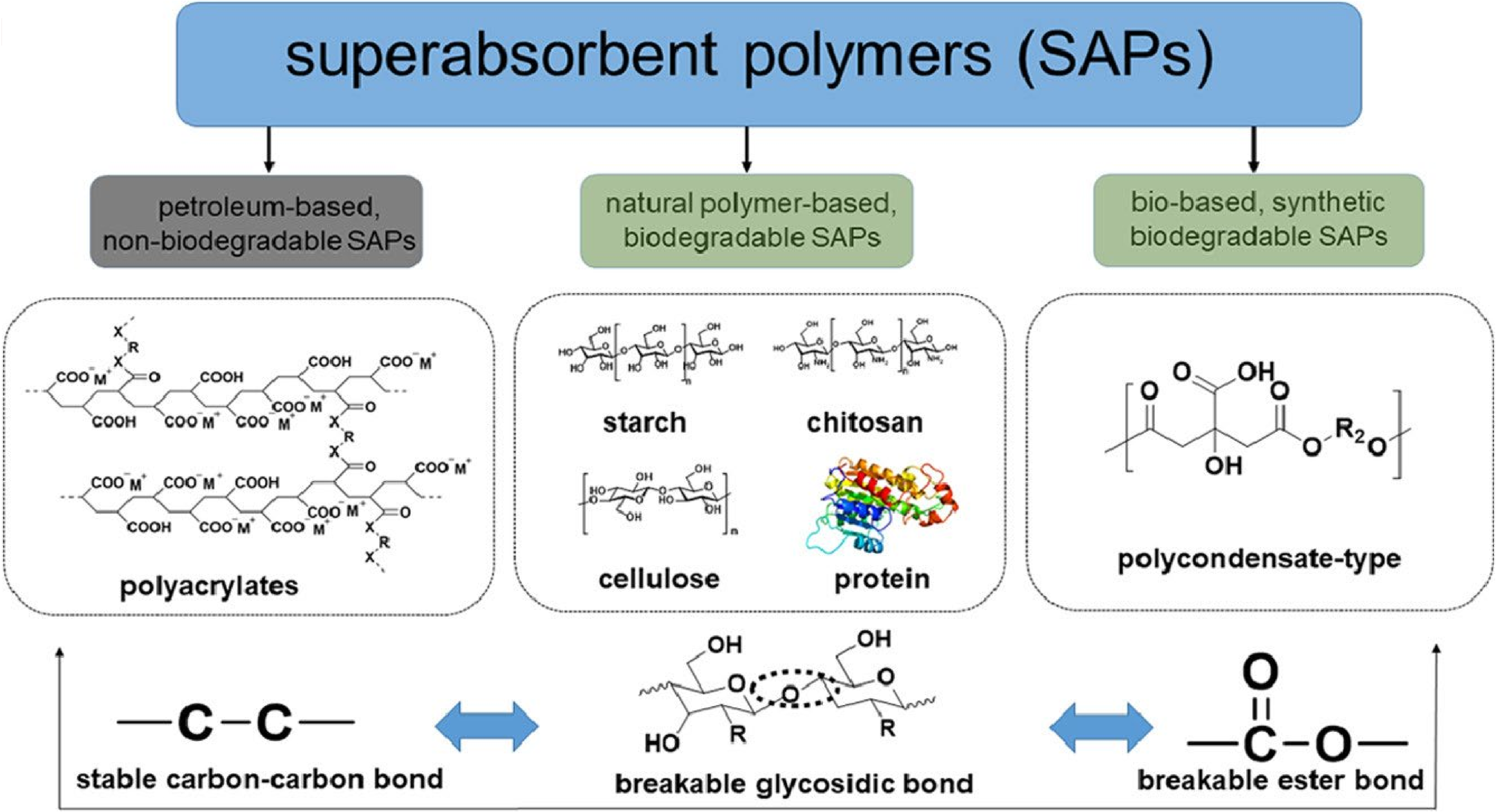
- cellulose nanofibers in water (natural fibers)
- this solid gel is 98% water (ca. 50 g/g)

SAPs (super absorbing polymers)



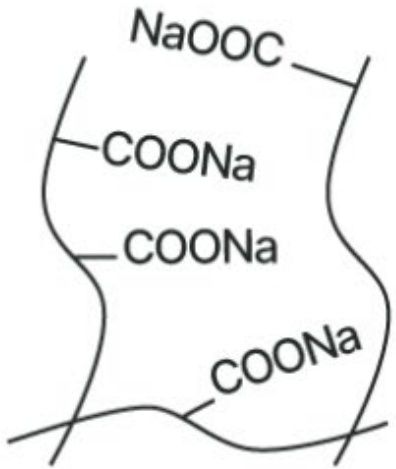
- Water absorption capacity = mass of absorbed water/dried weight of polymer (g/g)
- Typical hydrogel = 10 g/g
- Typical SAP = 100-1000g/g

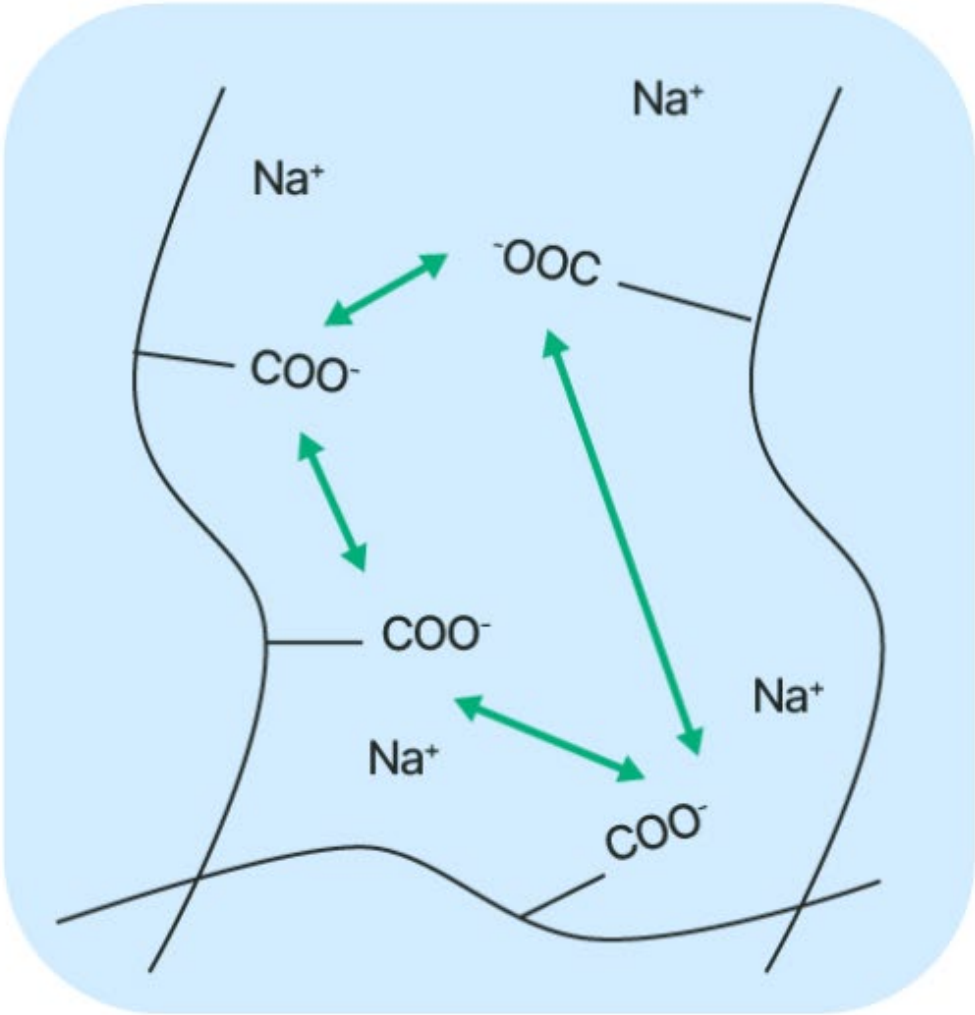
https://doi.org/10.1016/j.progpolymsci.2021.101475



https://doi.org/10.1016/j.progpolymsci.2021.101475

SAPs

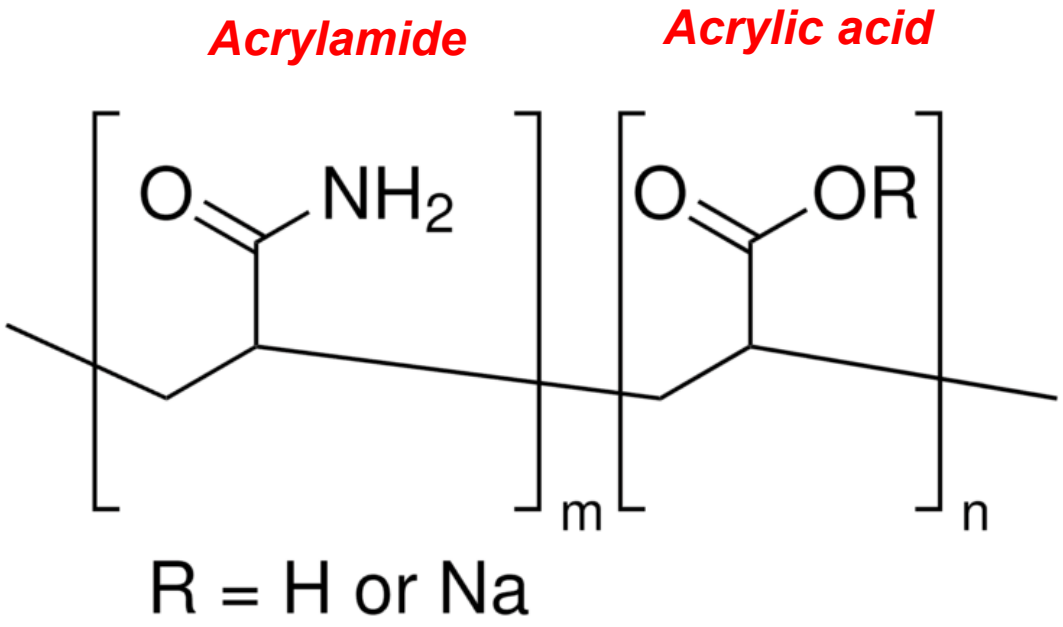




$$\text{Water Absorption} \propto \frac{\text{Osmotic pressure of inside and outside of SAP particles By ion concentration difference} + \text{Affinity with SAP aqueous solution}}{\text{Crosslink density}}$$

SAP in article: PAAcAAM

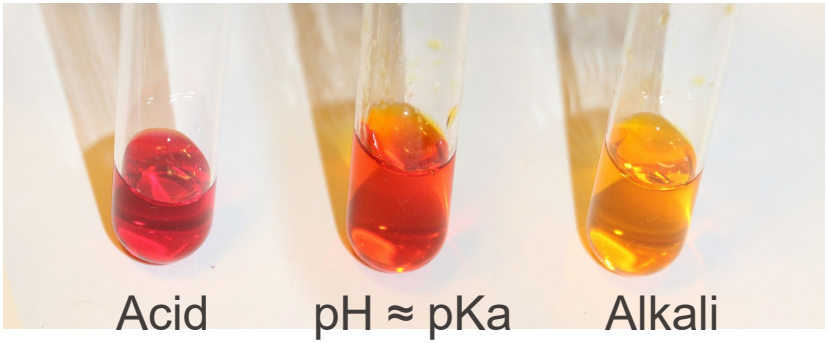
- Poly(acrylamide-co-acrylic acid)
- Selected for ease of fabrication & reversible pH swelling behavior, between pH 4 and 7
- pH range is compatible with the survival of *E. coli*
- Acrylic acid is the pH sensitive part



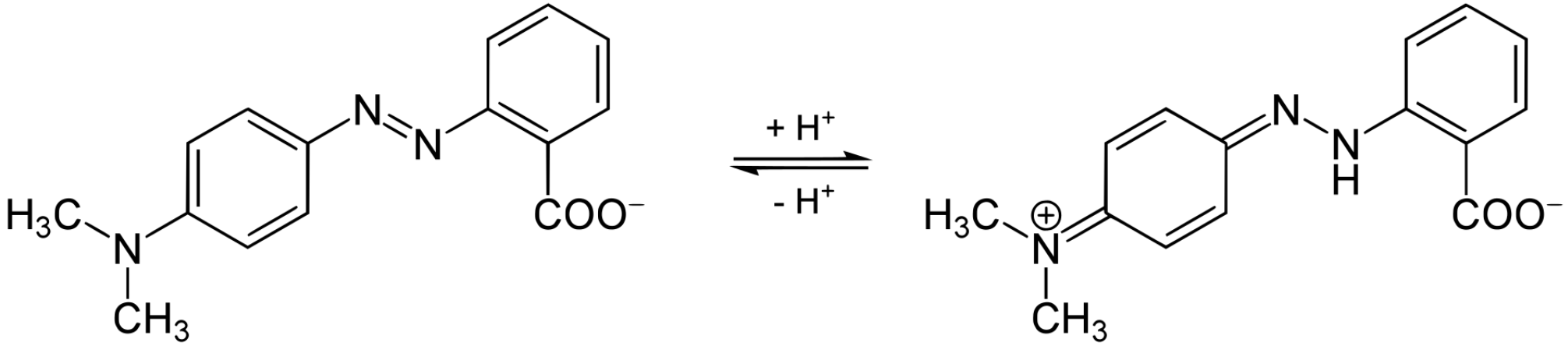
Do you know why?

pKa acrylic acid = 4.2
pH>pKa = deprotonated = swollen (alkaline state)
pH<pKa = protonated = de-swollen (acid state)

pH switching dye (Methyl Red)



Alkaline state = deprotonated



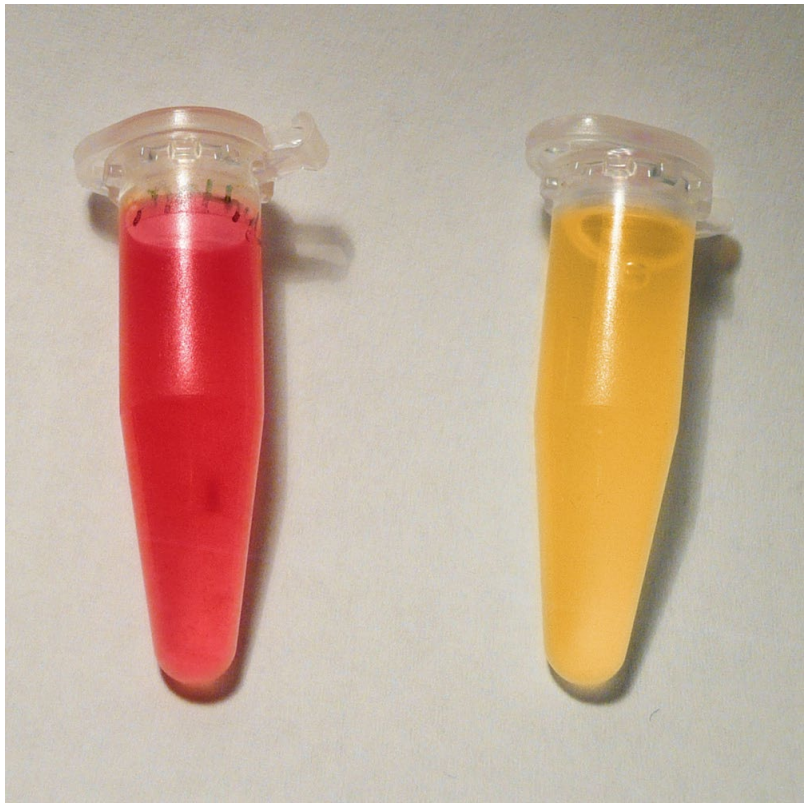
Acidic state = protonated

- (2-(N,N-dimethyl-4-aminophenyl) azobenzenecarboxylic acid)
- Color switching at pH range accessible to *E. coli*

Methyl_red

Used as indicator in microbiology

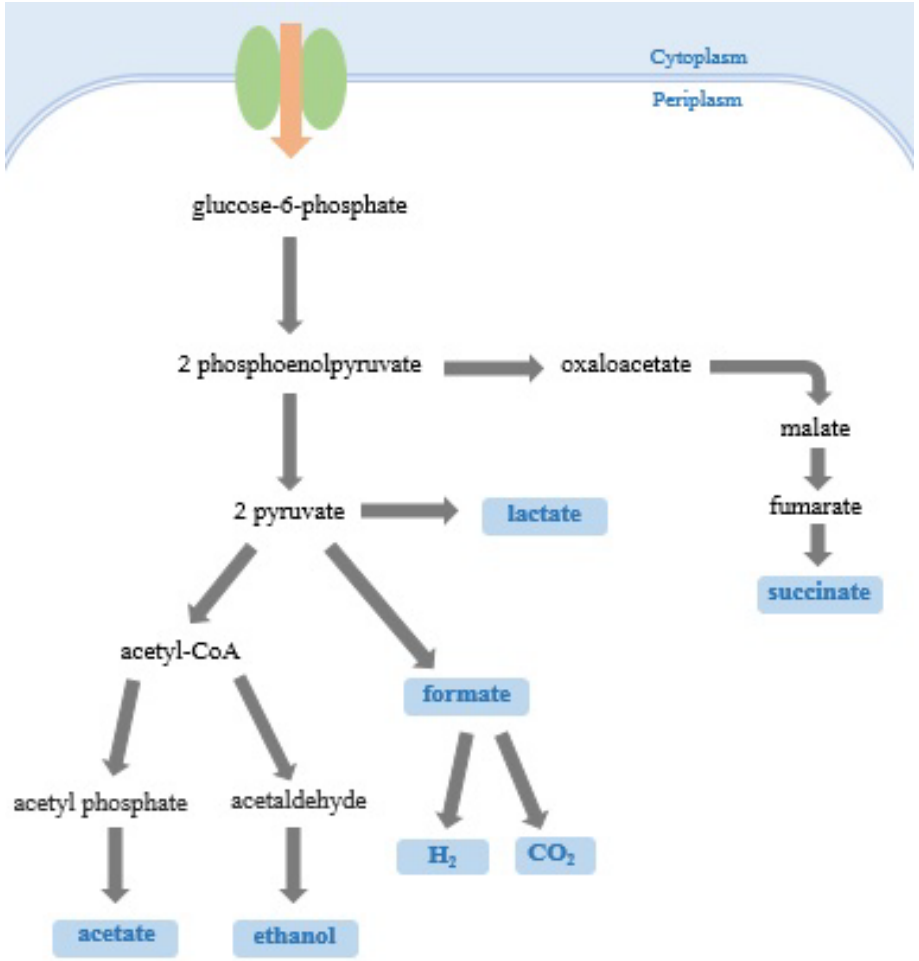
- Used to identify bacteria that produce stable acids
- In digesting glucose, all gut bacteria first produce pyruvic acid
- Some bacteria, use a ‘mixed acid pathway’ to metabolize pyruvic acid to other acids (lactic, acetic, formic)
- These bacteria are methyl red positive – lower the pH and MR is red
- *E. coli* is one of these bacteria (uses mixed acid fermentation in glycolysis)



LHS: positive result
RHS: negative result

Methyl_red

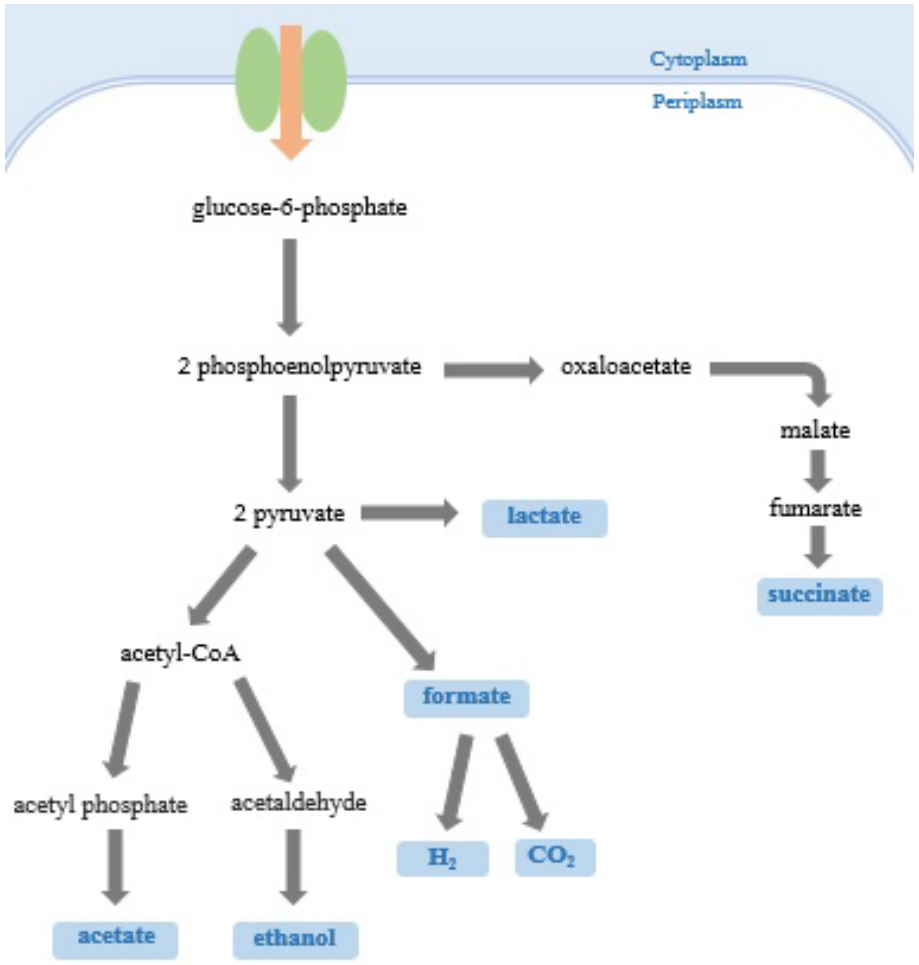
Mixed acid fermentation



- Metabolic process that converts glucose to a complex and variable mixture of acids
- Anaerobic
- Common in bacteria, like *E. coli*

Mixed acid fermentation

Side note – biotech!



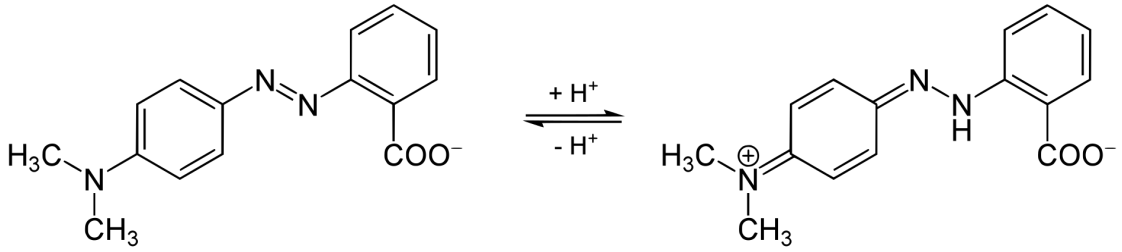
- Bacterial strains metabolically engineered to increase yields of mixed acid fermentation products
- *E. coli* W3110 generates 2 moles of acetate for each mole of glucose
- *E. coli* KO11 to increase ethanol yield
- Overexpression of enzyme PEP carboxylase to increase yield of succinate
- *E. coli* strains to control isomers of lactic acid, important for PLA properties

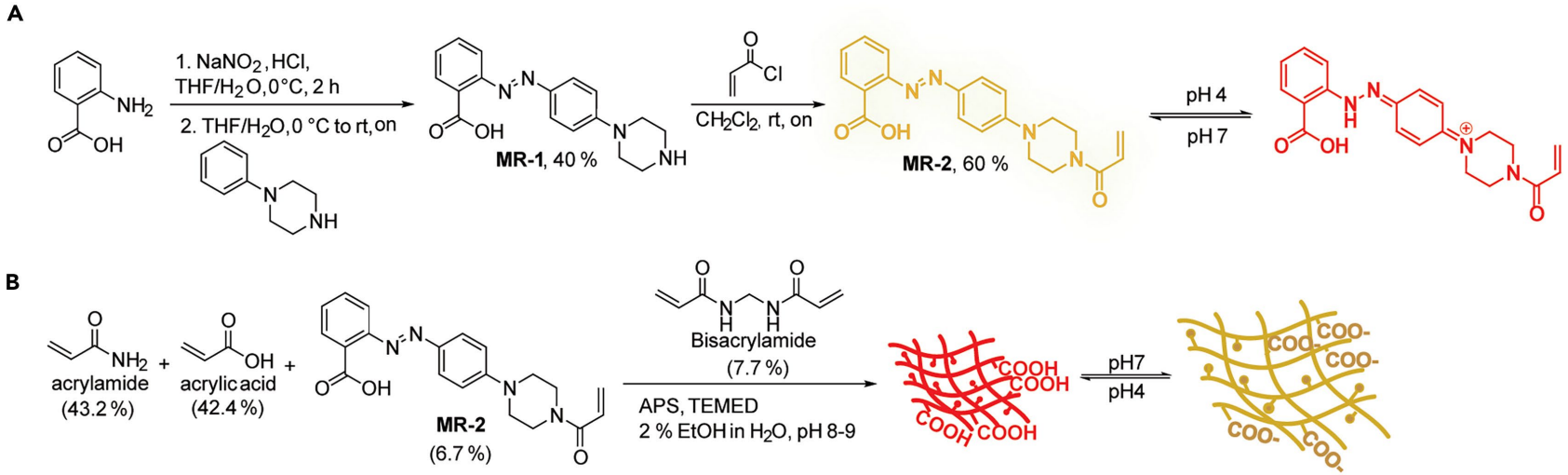
Mixed acid fermentation

Putting it all together?

- Driver of ***pH change*** is metabolism of *E. coli,* which will either generate or consume acids *depending on its conditions of its nutritional environment*
- Nutrients are the ***inducers*** for pH change
- ***Color change*** is based on MR's response to acid (red) or base (yellow)
- ***Shape change*** is based SAP's response to acid (shrink) or base (swell)

A detail ☺

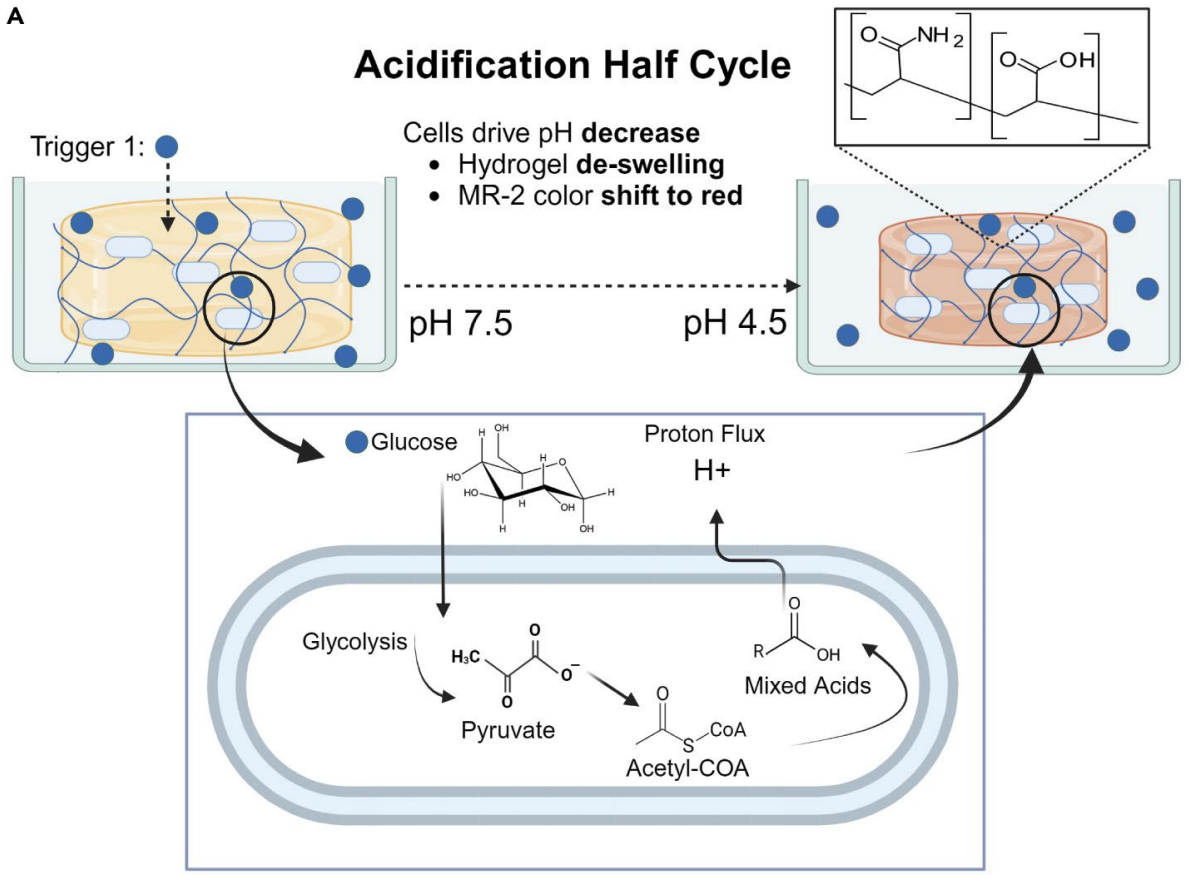




- Just do some chemistry to make a new methyl red analog that has the right chemistry to be crosslinked into the polymer network (no biggie)

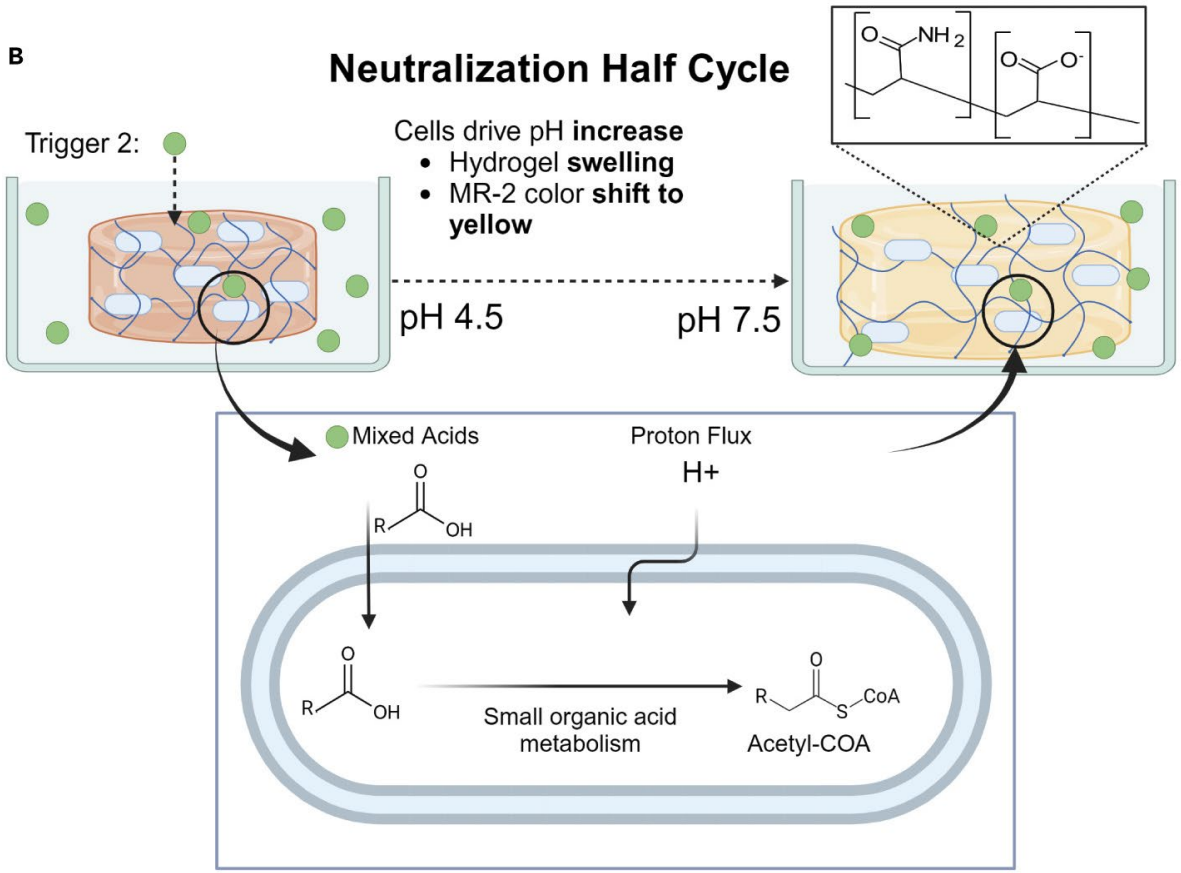
- Why? Otherwise the dye would just leach out of the hydrogel

Putting it all together schematically



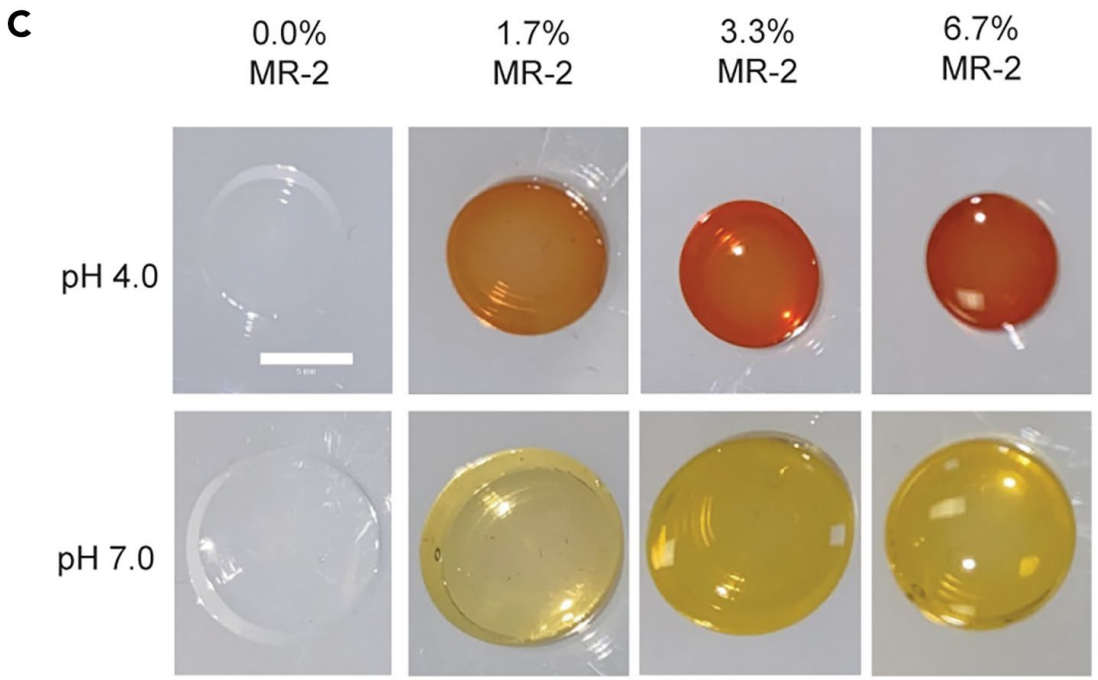
- Trigger 1: excess glucose
- Glucose metabolism at these conditions generate acids, causing the pH to decrease
- This decrease in pH causes the color of the hydrogel to go from yellow to red and the size to shrink

Putting it all together schematically



- Trigger 2: mixed acids
- In the absence of glucose but in the presence of mixed acids, mixed acids are metabolized
- Increase in pH causes color change from red to yellow and the size to swell

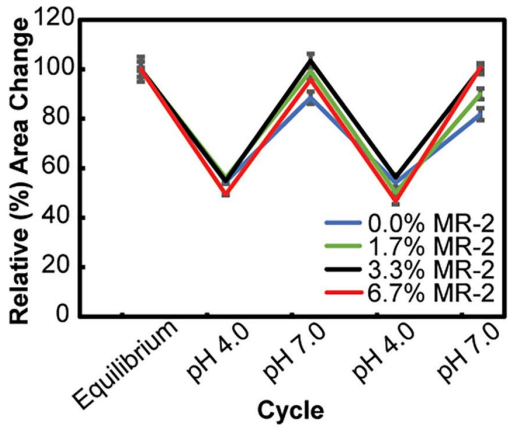
Functional validation of hydrogel (no *E. coli*)



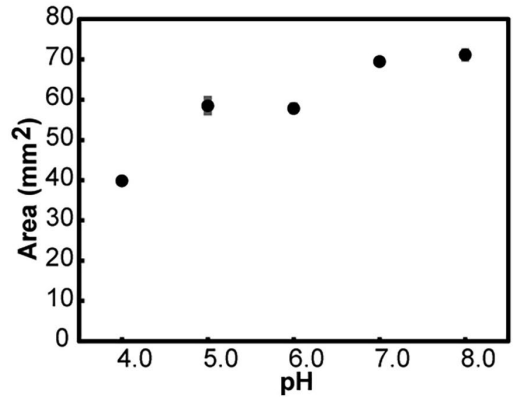
- Co-polymerize acrylic acid, acrylamide, and MR2 in the presence of a crosslinker
- Pucks 6 mm × 2 mm ($d \times h$)
- Different dye loadings
- pH 7: swollen and yellow
- pH 4: deswollen and red

Functional validation of hydrogel (no *E. coli*)
Swelling and Deswelling

D

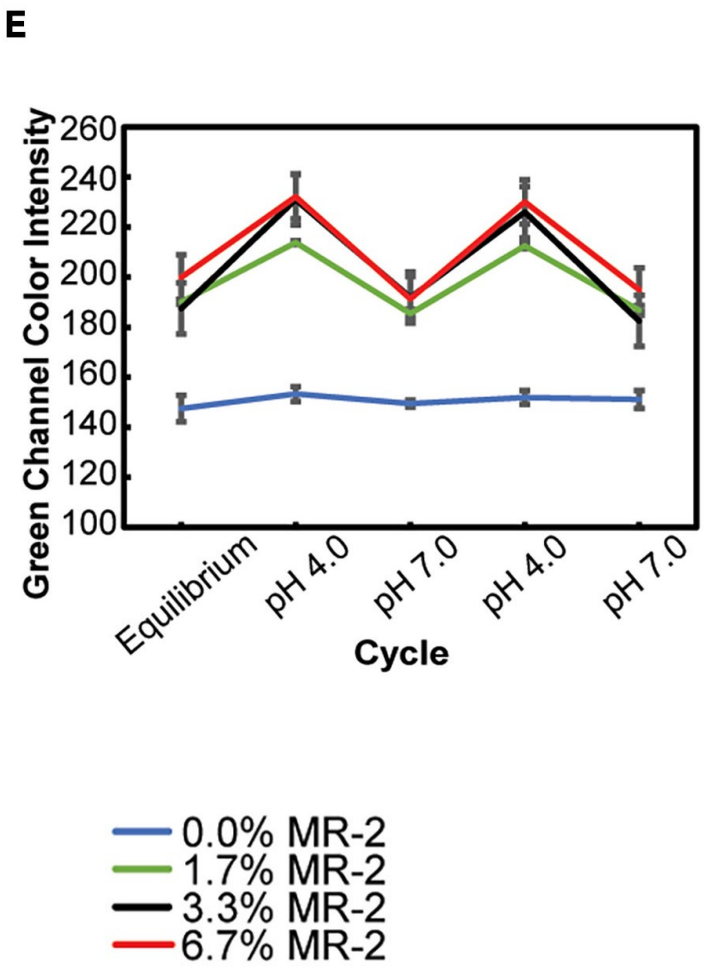


G



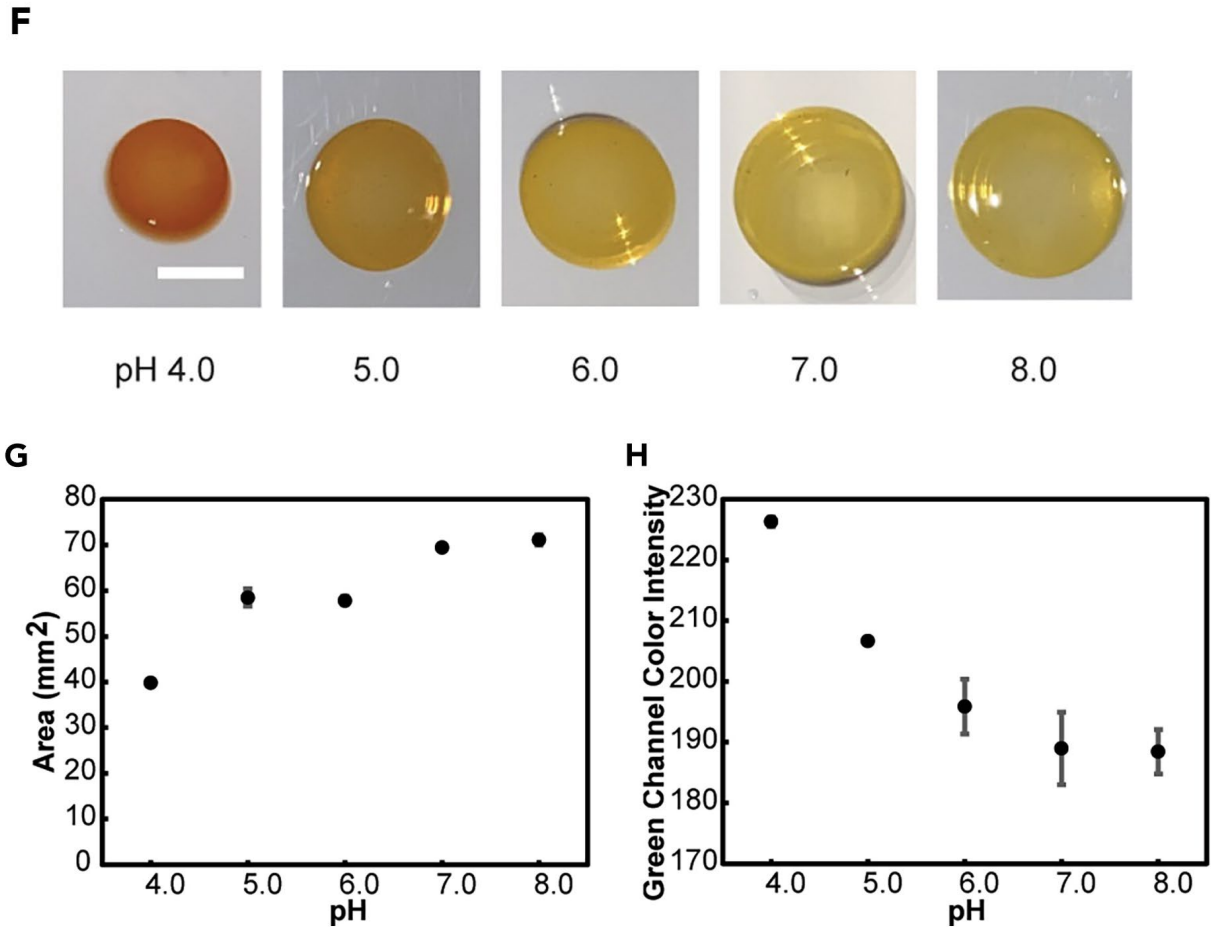
- Pucks 6 mm × 2 mm (*d* × *h*)
- Cross-sectional surface area after polymerization is $\pi \times r^2 = 28$ m^2
- Equilibration @ pH 7: 28 m^2 → **76 m^2** (100% = 76/76 × 100%)
- @ pH 4: 76 m^2 → **40 m^2** (≈ 50% = 40/76 × 100%)
- @ pH 7: 40 m^2 → **74 m^2** (≈ 97% = 74/76 × 100%)

Functional validation of hydrogel (no *E. coli*) Color change



- Images of gels imported into ImageJ (free software) and separated into RBG color channels
- Found that the largest change was in the green color channel (550 nm central wavelength)
- Reported puck color change based on pixel intensity value in the green channel from 0 to 255
- Similar cycles irrespective of MR2 loading
- Selected 6.7% MR2 for all further experiments

Functional validation of hydrogel (no *E. coli*) Color and shape



- 6.7% MR2
- Broader pH range
- Bigger changes in shape and color near pKa's (5.1 for MR2 and 4.2 for AA)

Perfusion chamber for pH cycling - reality

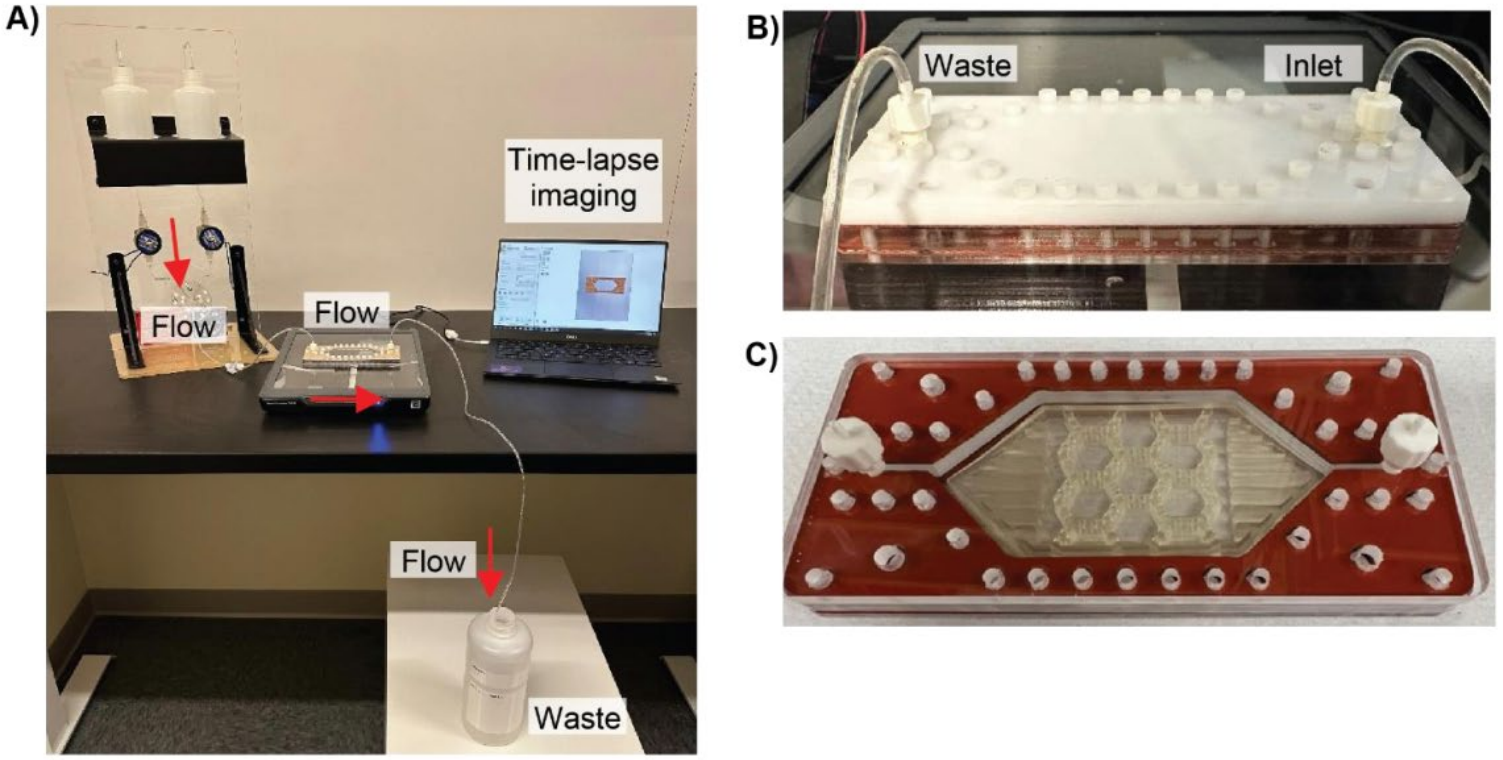
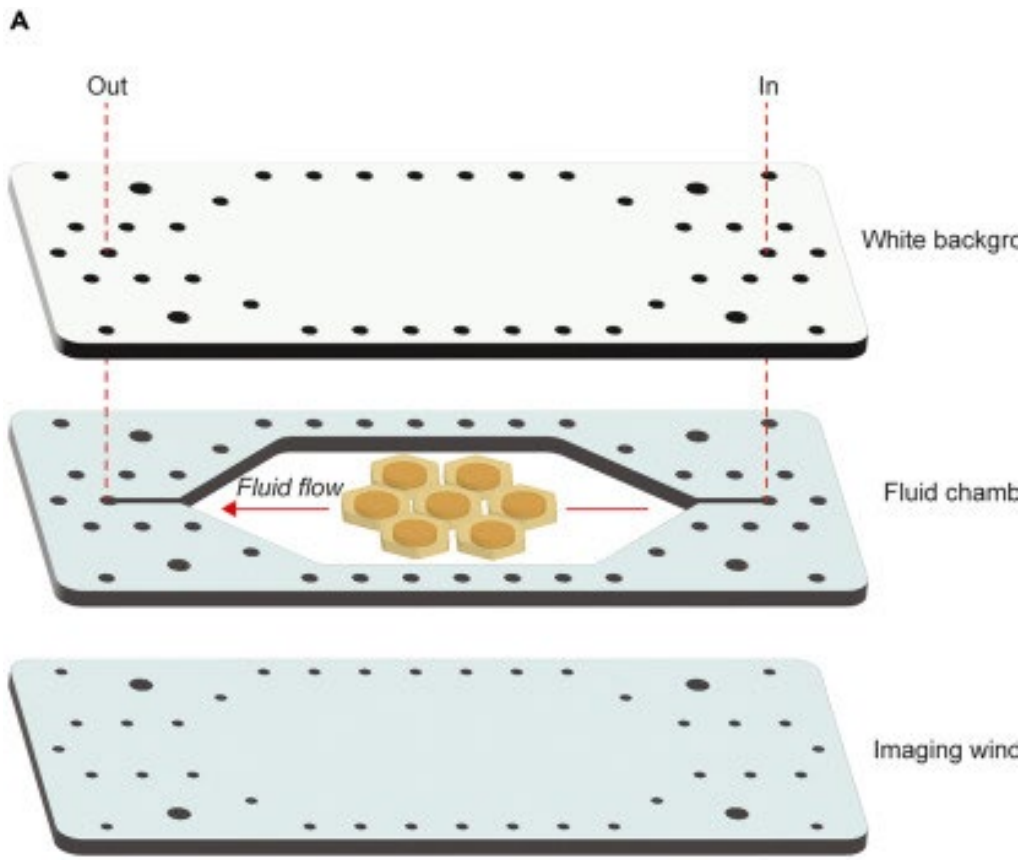


Figure S5. The perfusion chamber set-up. (A) The perfusion chamber consists of two reservoir bottles that flow into a chamber, which sits on a scanner to allow for time-lapse imaging and analysis of hydrogel pucks during cycling experiments. **(B)** Connection of flow to the perfusion chamber fixed with a Luer lock connector to allow for inflow of buffer (*right*) and tubing fixed with a Luer-lock connector for outflow of buffer into a waste container (*left*). **(C)** The fully assembled perfusion chamber (*top-down image*) with a resin puck holder where the hydrogel pucks reside for cycling experiments.

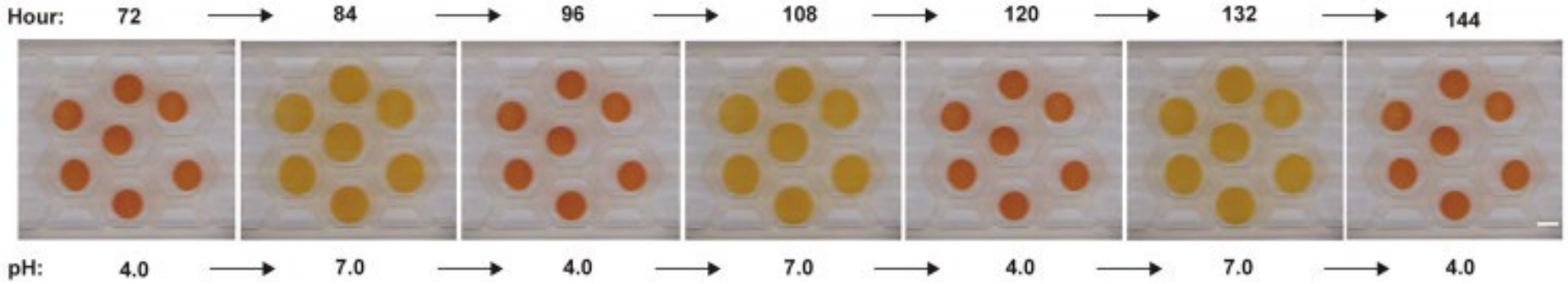
- Custom chamber
- Reservoirs
- Chamber
- Scanner
- Waste collection

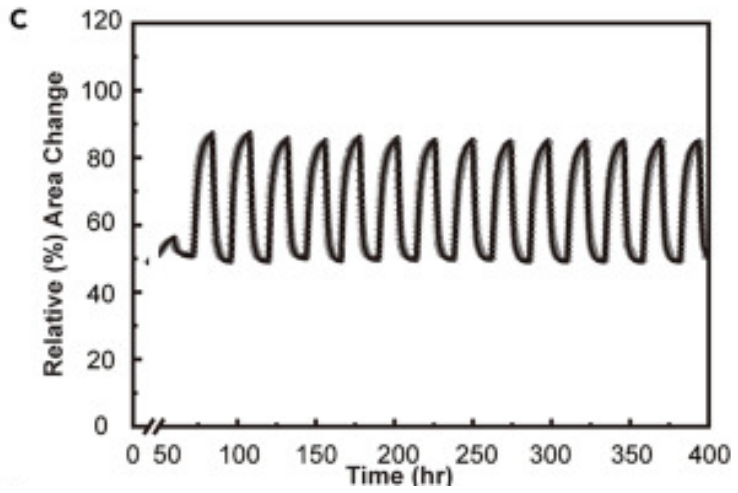
Perfusion chamber for pH cycling - schematic

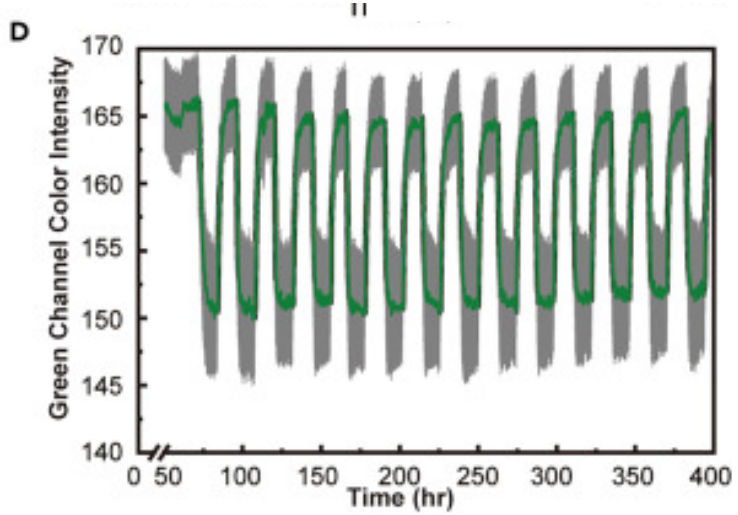


- 7 pucks
- Tracked puck size and color continuously over 2 weeks, 10 min intervals
- Automated fluid exchange/cycling between buffers
- Alternate pH buffers every 12 h
- pH 4 sodium citrate
- pH 7 tris buffer
- 20 mL volume
- Buffer exchanges, approx. 500 mL

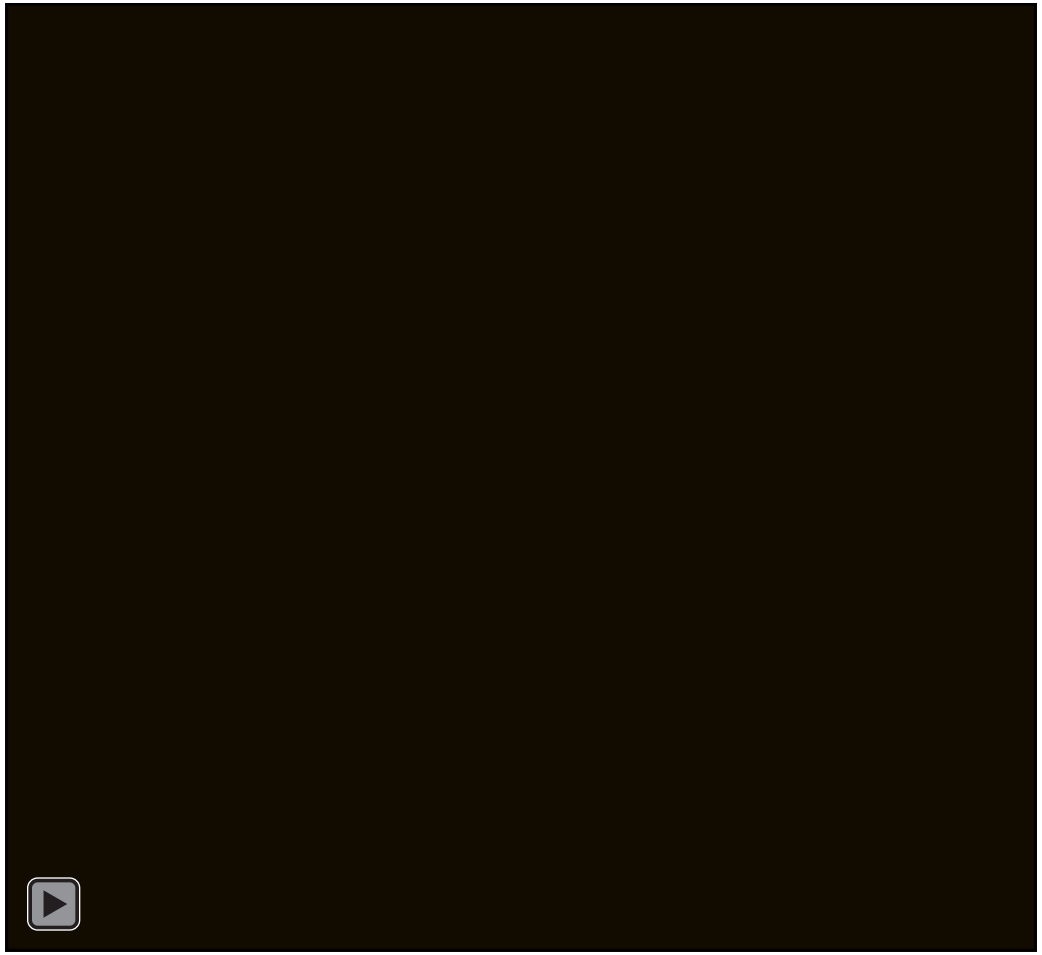
Perfusion chamber for pH cycling - experiment





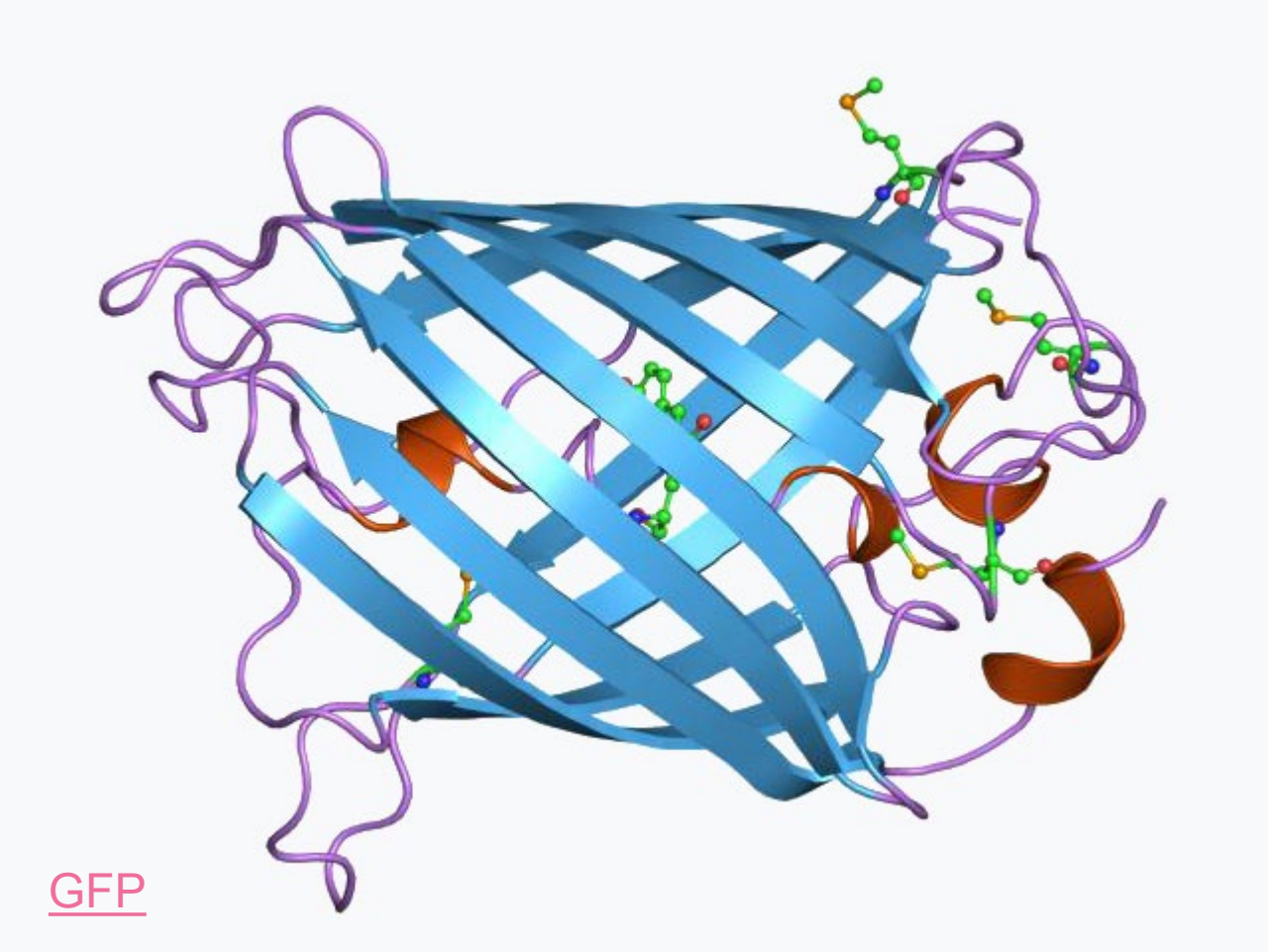


- 10 h equilibration
- Stable response over 2 weeks
- Plots start at 50 h, due to leak at early times



So great! But where is the bacteria?

Encapsulation and viability of *E. coli* in PAAcAAm hydrogels

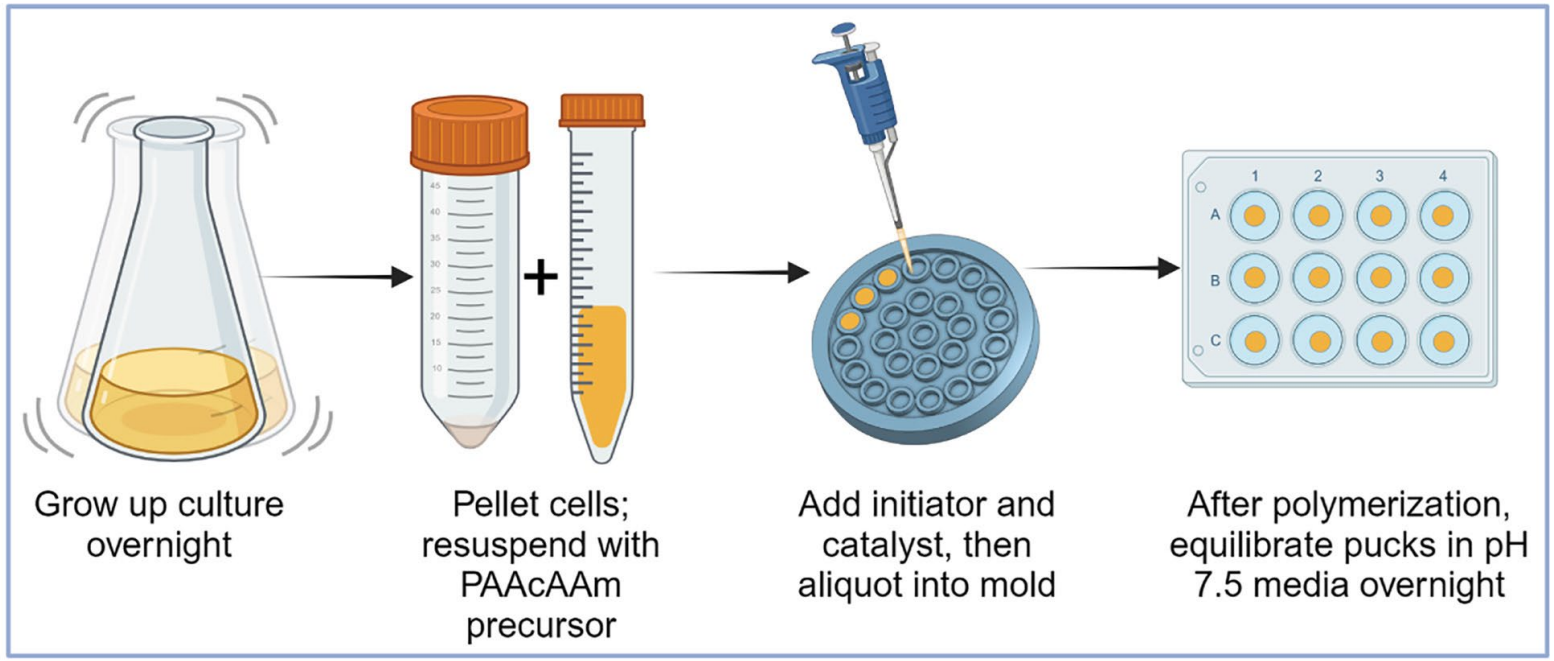


GFP

- 1st transformed E. coli (BL21) with a plasmid encoding green fluorescent protein (GFP) under the control of an inducible promoter (no biggie)
- Recombinant protein technology
- “Inducible promoter” means that the expression of the protein is controlled
- Expression of protein is inactive or low until induced by an external trigger

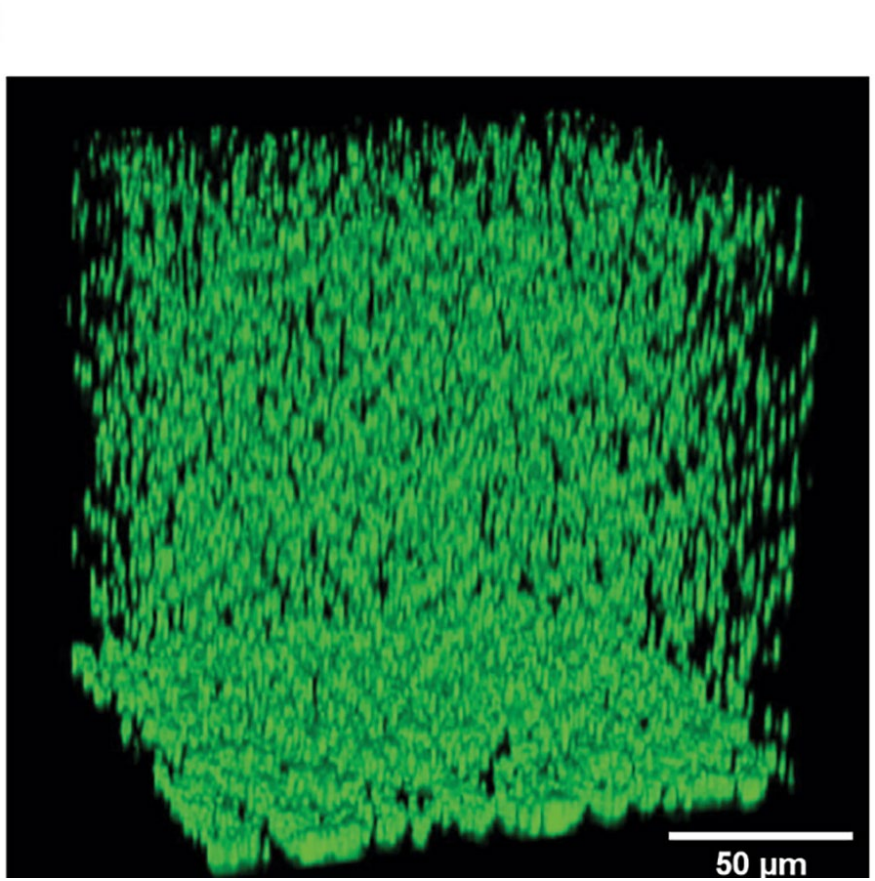
Encapsulation and viability of *E. coli* in PAAcAAm hydrogels

A



- Not obvious that the bacteria will survive the polymerization
- Make pucks with GFP expressing bacteria
- Equilibrate
- If the bacteria is alive, we should see GFP production, right?
- For everything to work, the bacteria should be alive, right?

Encapsulation and viability of *E. coli* in PAAcAAm hydrogels

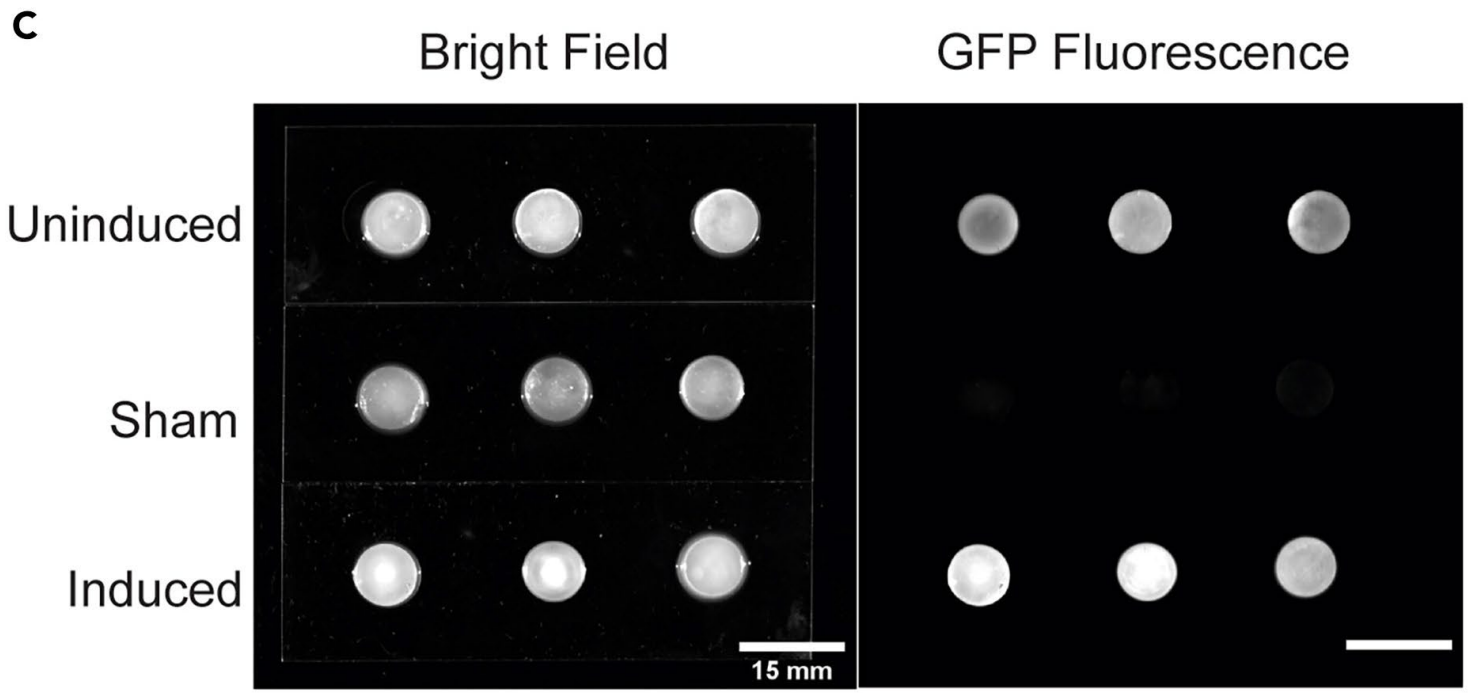


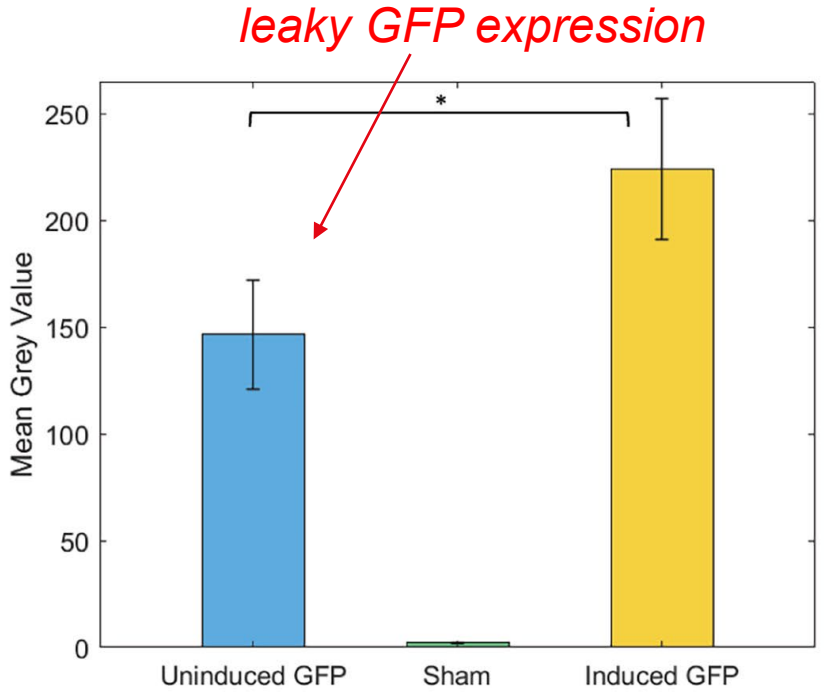
- Confocal fluorescence
- 3D image of gel
- Seems like there is more fluorescence at bottom, attributed to settling of bacteria during the 1 h polymerization
- They thought about recovering the bacteria after encapsulation (alive/dead assay) but not possible due to stability of hydrogel matrix
- Doesn't tell us if alive/dead

Encapsulation and viability of *E. coli* in PAAcAAm hydrogels

- Relied on a qualitative assessment of viability
- Top and bottom contain bacteria with inducible genes that encode for GFP
- Middle contains bacteria with a sham plasmid
- Higher fluorescence in induced gels is taken as an indicator of viability

GFP expressing bacteria is only to assess viability – moving on just BL21





Microbial modulation of environmental pH

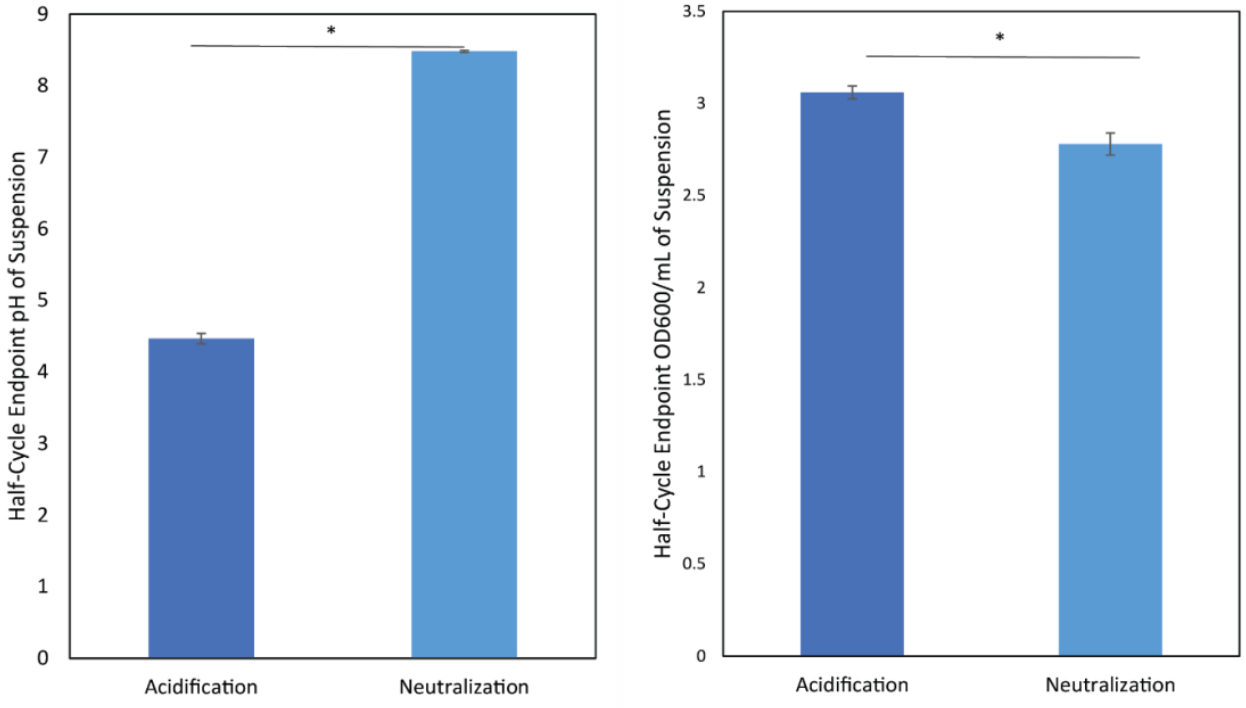


Figure S7. Endpoint pH (*left*) and OD_{600}/mL (*right*) of the suspension media (n=3) from hydrogel-free suspension culture experiment. This data shows that cells in suspension supplemented with a carbon source (glucose for acidification and acetate for neutralization) can decrease and increase the pH, respectively. The starting pH for the acidification cycle is 7.5 and the starting pH of the neutralization cycle is 4.5. Paired t-tests were used to compare half-cycle endpoints within a given group (n=4, (ns) $p > 0.05$, (*) $p < 0.05$).

- OK, but we actually need to show that BL21 can modulate pH
- Acidification: If grown with glucose, pH drops from 7.5 to 4.5 (doubling of population)
- Neutralization: If grown with acetate with pH adjusted to 4.5, pH increases to 8.5 (doubling of population)
- Seems promising!

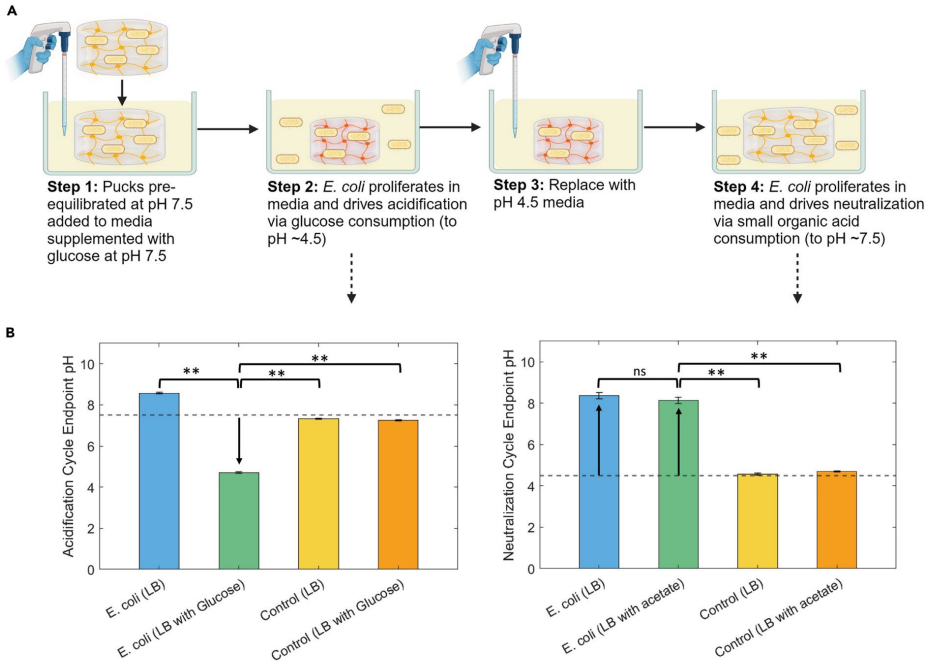
Microbial modulation of environmental pH

Two inducers (carbon sources):

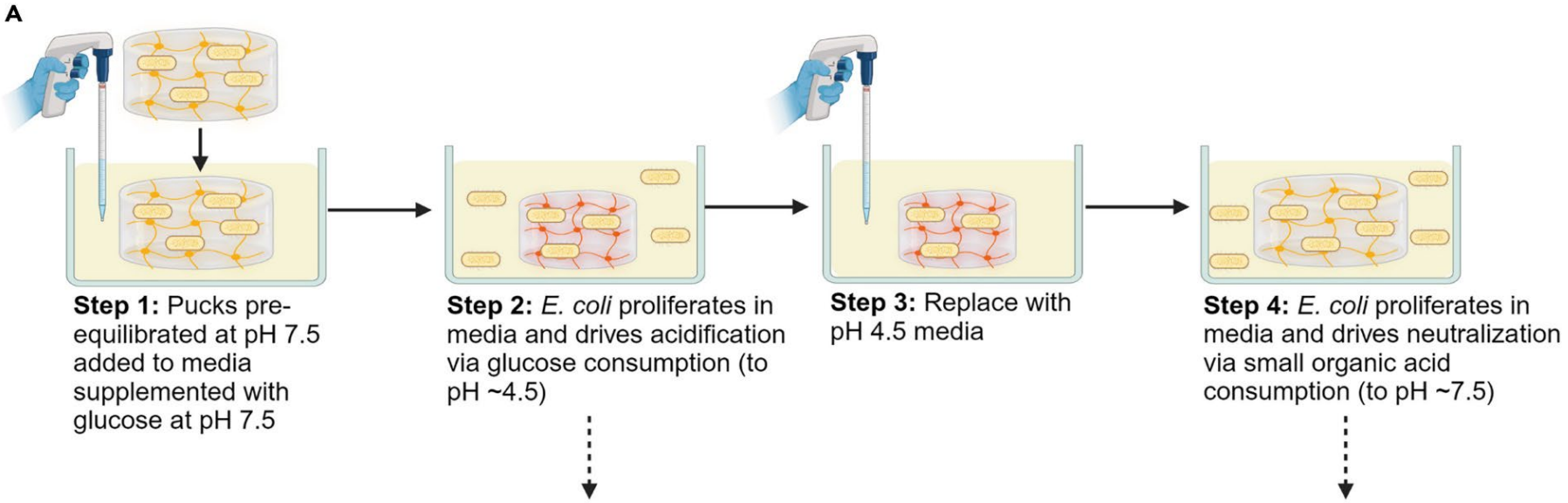
1. Glucose induces acidification
2. Acetic acid induces neutralization

Four samples:

1. Hydrogels containing *E. coli* in LB media (no inducers) - blue
2. Hydrogels containing *E. coli* in LB media with inducers (either glucose or acetic acid) - green
3. Cell-free control hydrogels in LB media (no inducers) - yellow
4. Cell-free control hydrogels in LB media with inducers (either glucose or acetic acid) - orange

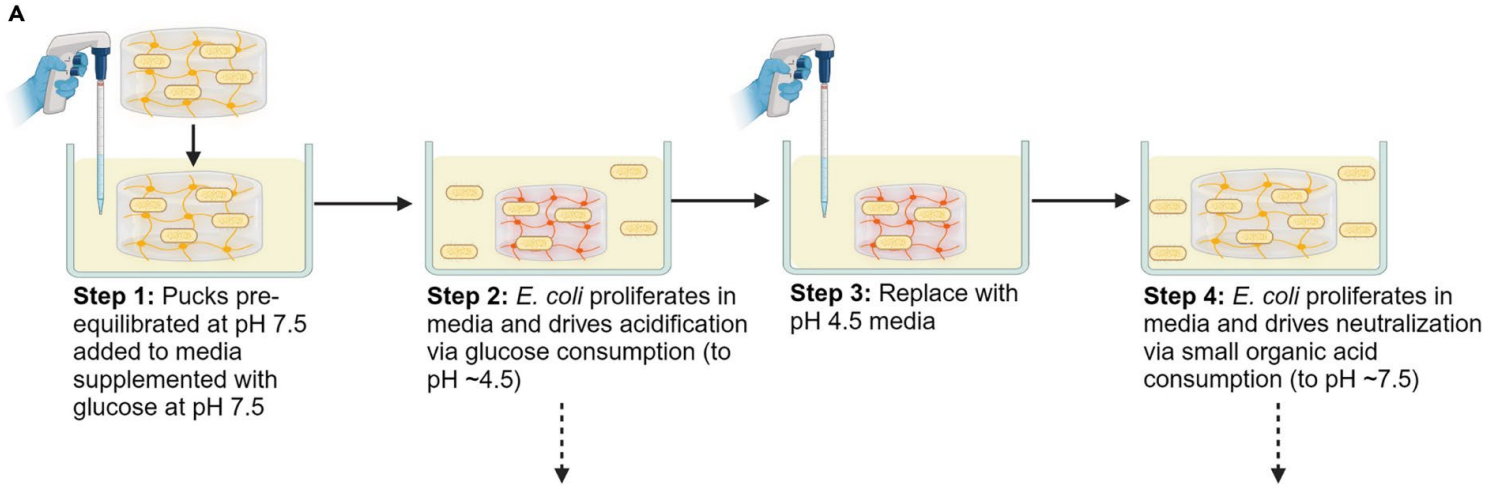


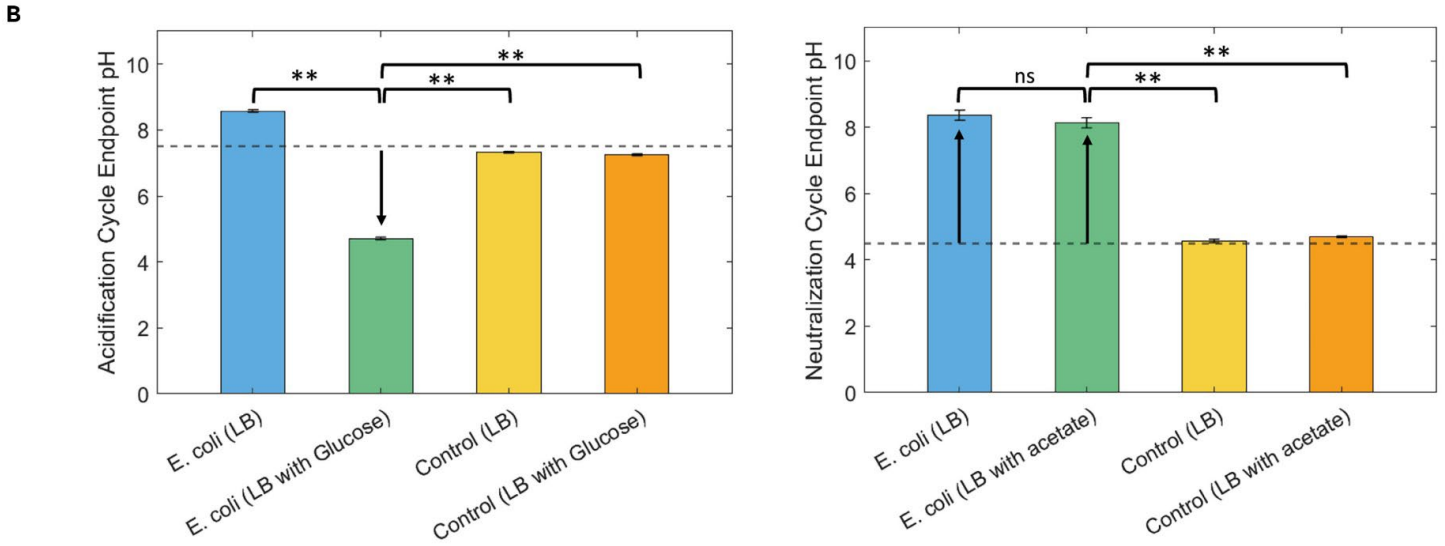
Microbial modulation of environmental pH



- Step 1: Supplement media with glucose
- Step 2: *E. coli* grows and drives acidification
- Step 3: Replace media with acidic media
- Step 4: *E. coli* grows and consumes acid, driving neutralization
- Control experiments with no cells (negative control) and with cells but no inducers (positive control)

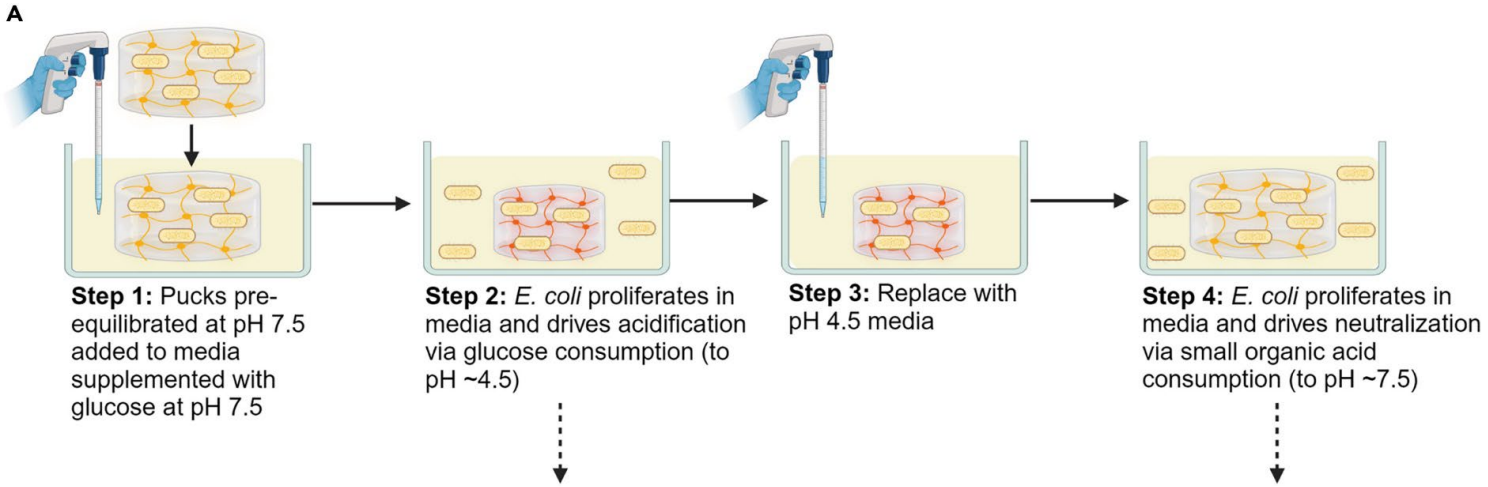
Microbial modulation of environmental pH

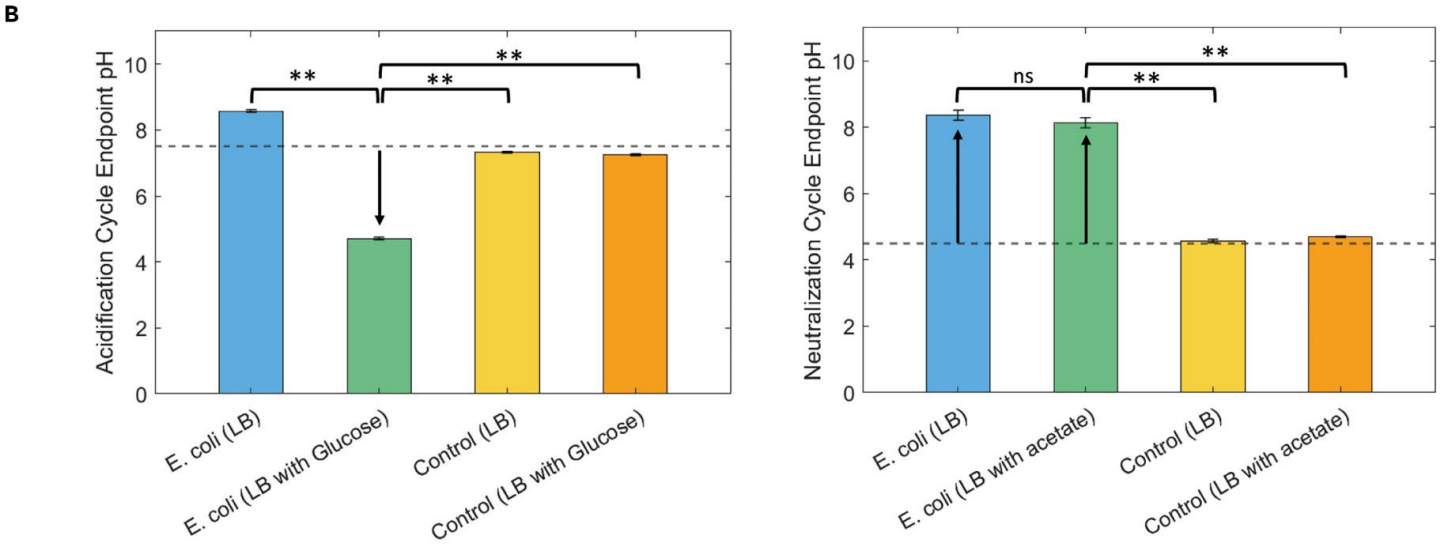




- Dotted line is initial pH
- *E. coli* with LB only is unchanged without glucose supplementation, but results in a neutralization without an acetate supplementation but with the starting pH at 4.5
- "likely due to *E. coli*'s ability to consume mixed organic acids that already exist in LB medium"
- So even without inducers, *E. coli* will result in pH change from acid to neutral

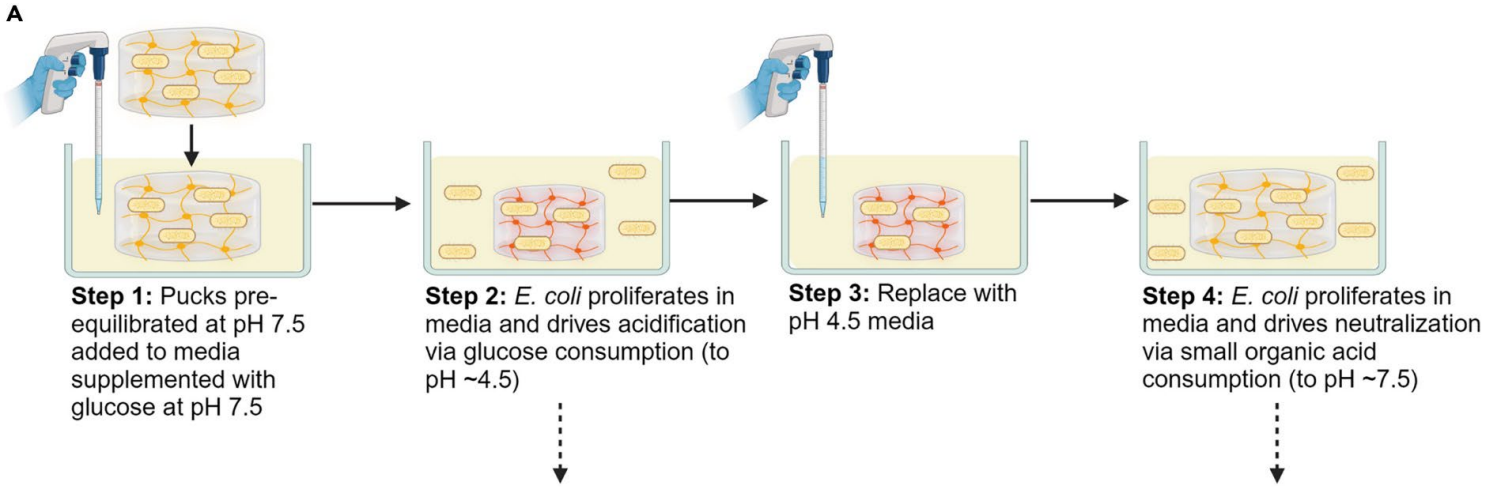
Microbial modulation of environmental pH

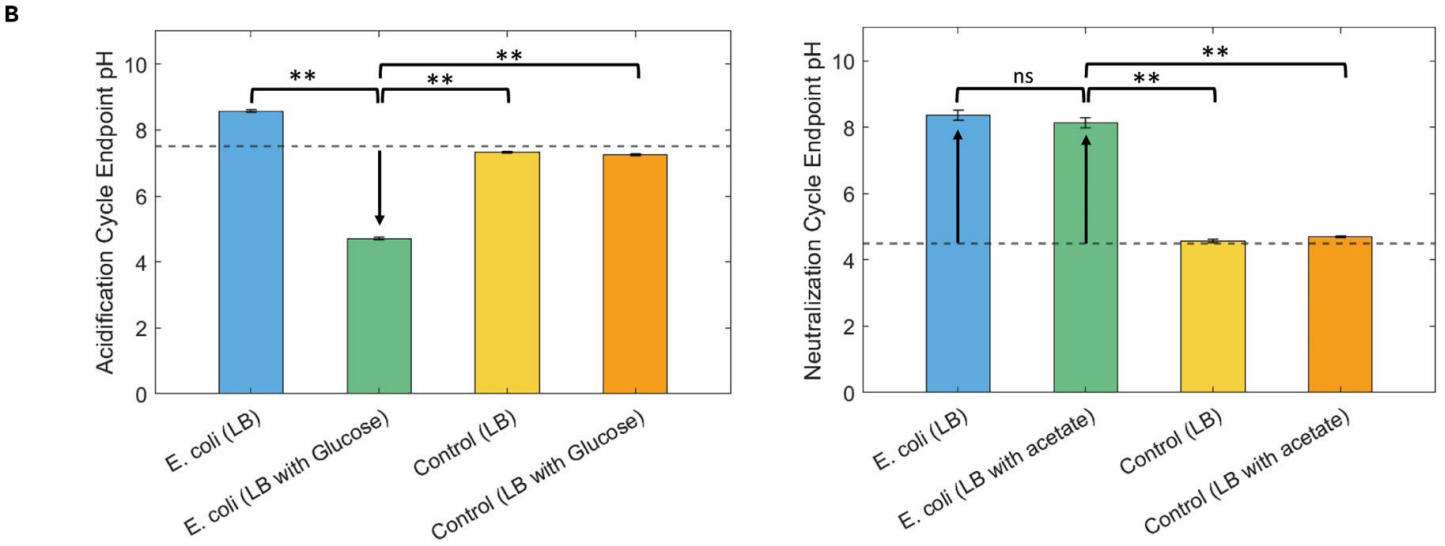




- *E. coli* with inducers behaves as expected
- With glucose supplementation, acidification is observed
- With acetate supplementation, neutralization is observed

Microbial modulation of environmental pH

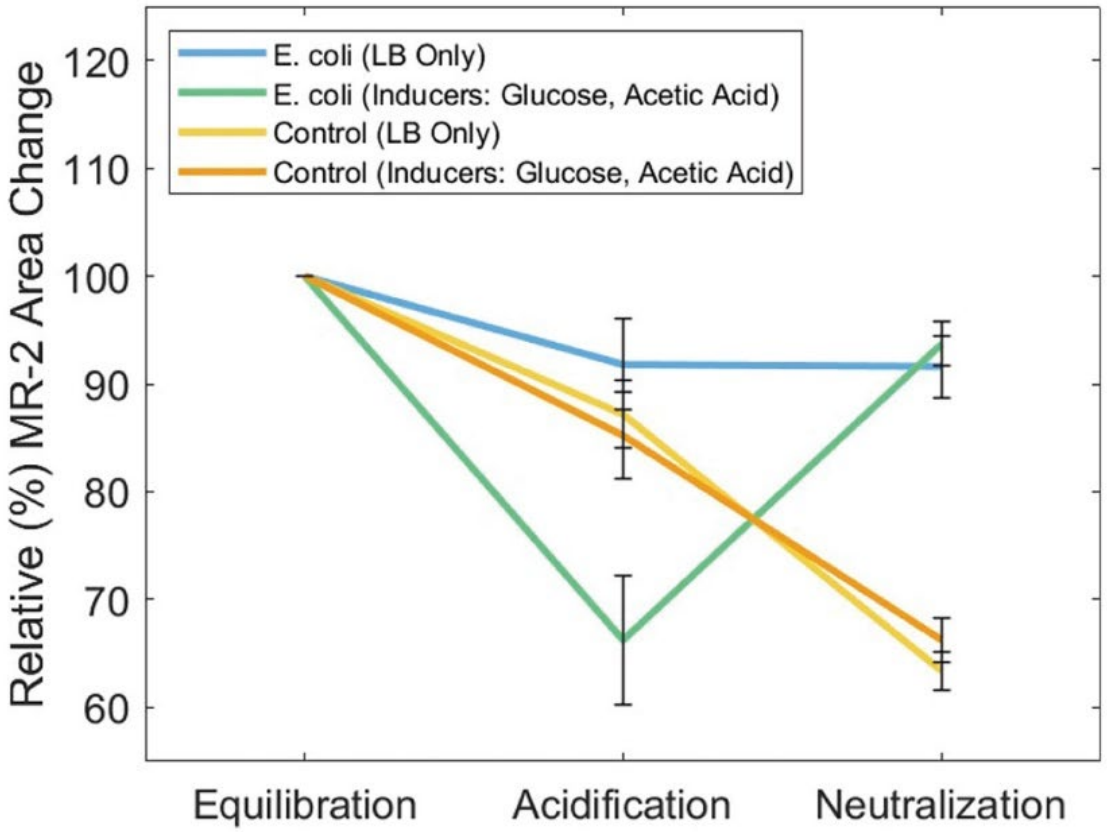




- Cell free controls have no mechanism to switch pH, so they keep the pH they started with, regardless of whether inducers are present or not

Microbial modulation of environmental pH, hydrogel swelling, and color

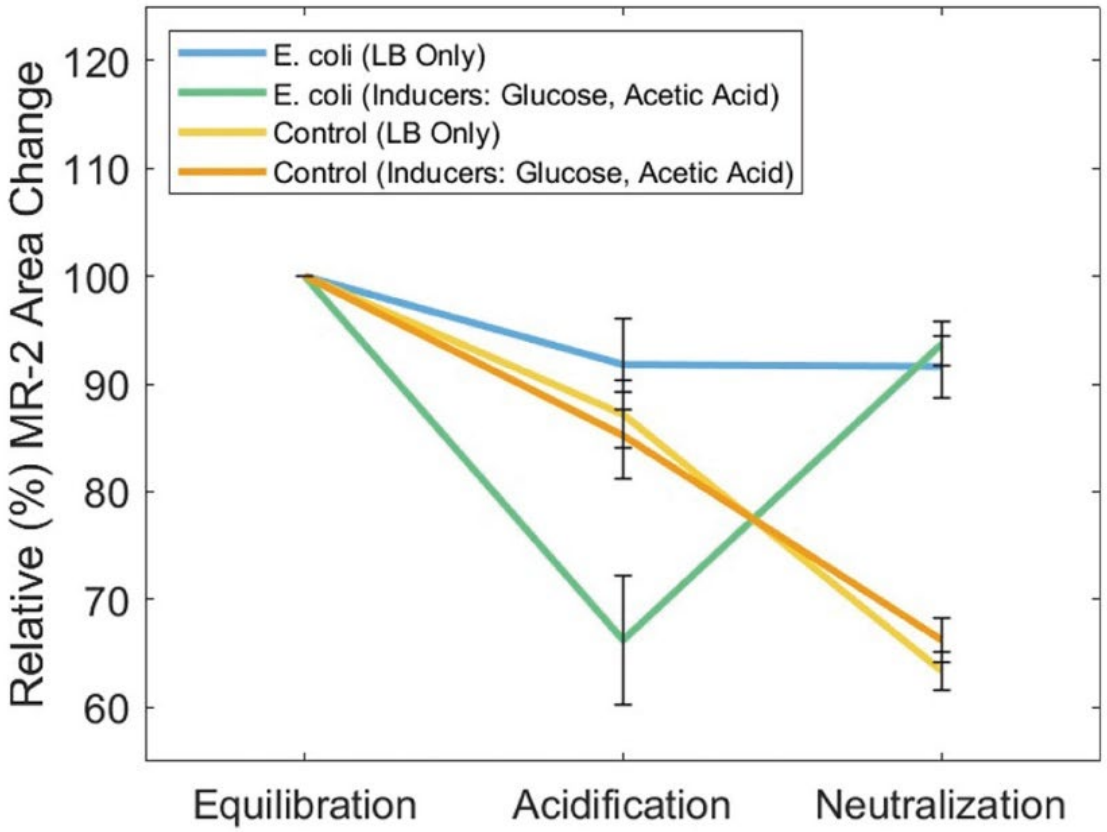
C



- *E. coli* with no inducers (LB only) does not cycle in size with acidification and neutralization
- Keep in mind that this is relative to initial swollen state equilibrated at pH 7.5

Microbial modulation of environmental pH, hydrogel swelling, and color

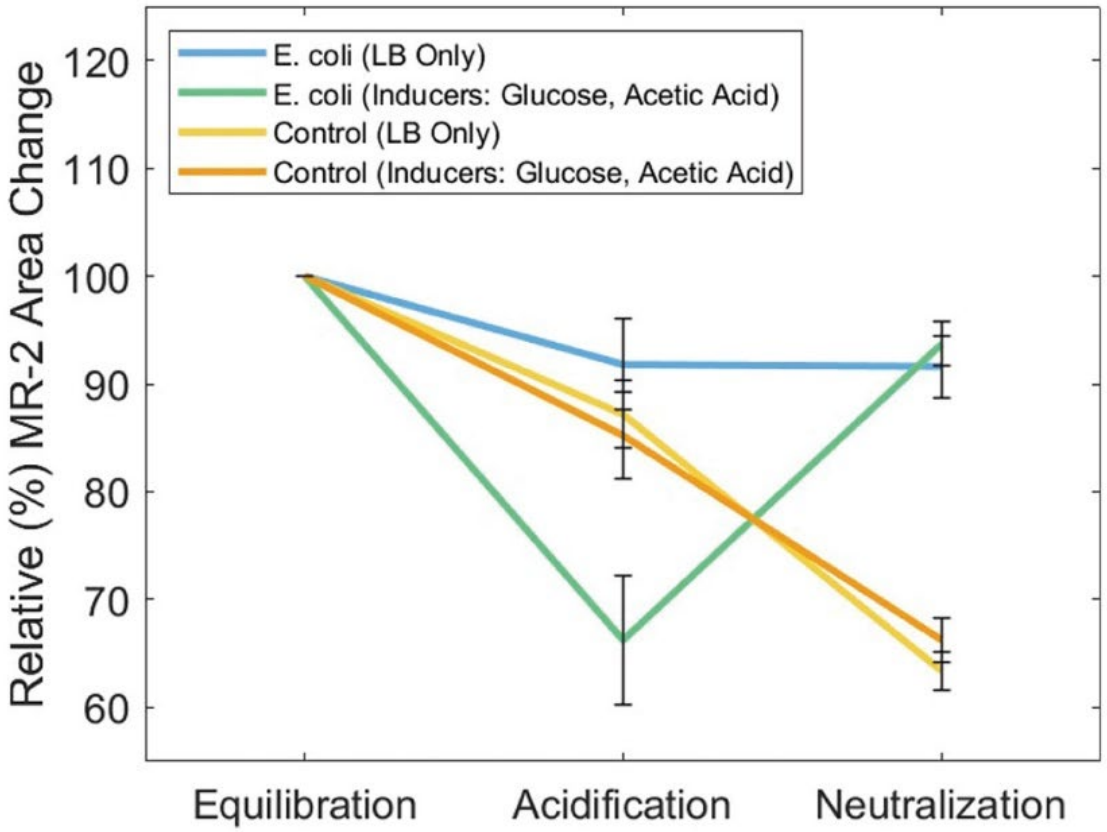
c



- *E. coli* with inducers shrinks with acidification and swells back up with neutralization, as expected
- 30% decrease in cross-sectional surface area with acidification (induced by glucose), size recovered with neutralization (induced by acetic acid)

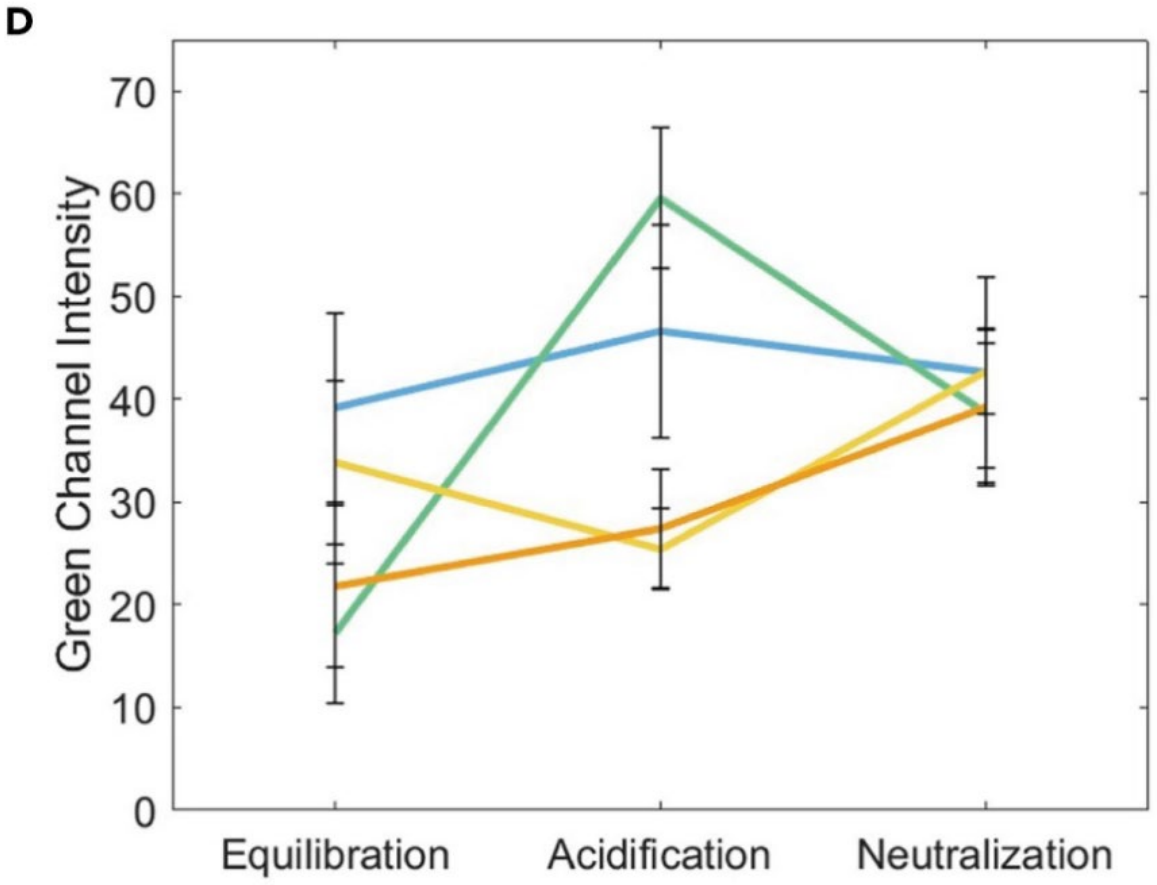
Microbial modulation of environmental pH, hydrogel swelling, and color

c



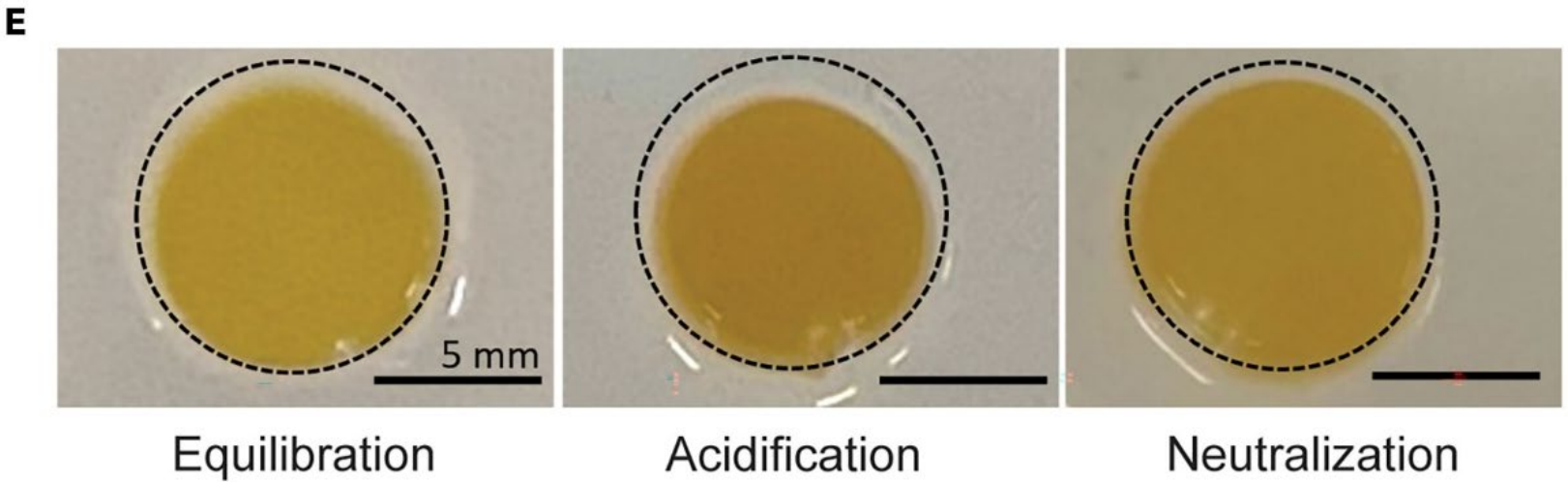
- Cell-free controls (with or without inducers) decrease in size with glucose (not significant) and then further with acetic acid (significant)
- Why do you think so?

Microbial modulation of environmental pH, hydrogel swelling, and color



- More of a clear color switching behavior with induced cells (green)
- Overlapping error bars…

Microbial modulation of environmental pH, hydrogel swelling, and color



@ endpoint of half cycles

- Dotted line shows 100% size
- Shrinkage with acidification
- Reswelling with neutralization
- Regardless of medium conditions, surrounding media became turbid due to growth of cells
- Color change is not super dramatic or vibrant – scattering from bacteria

Testing turbidity

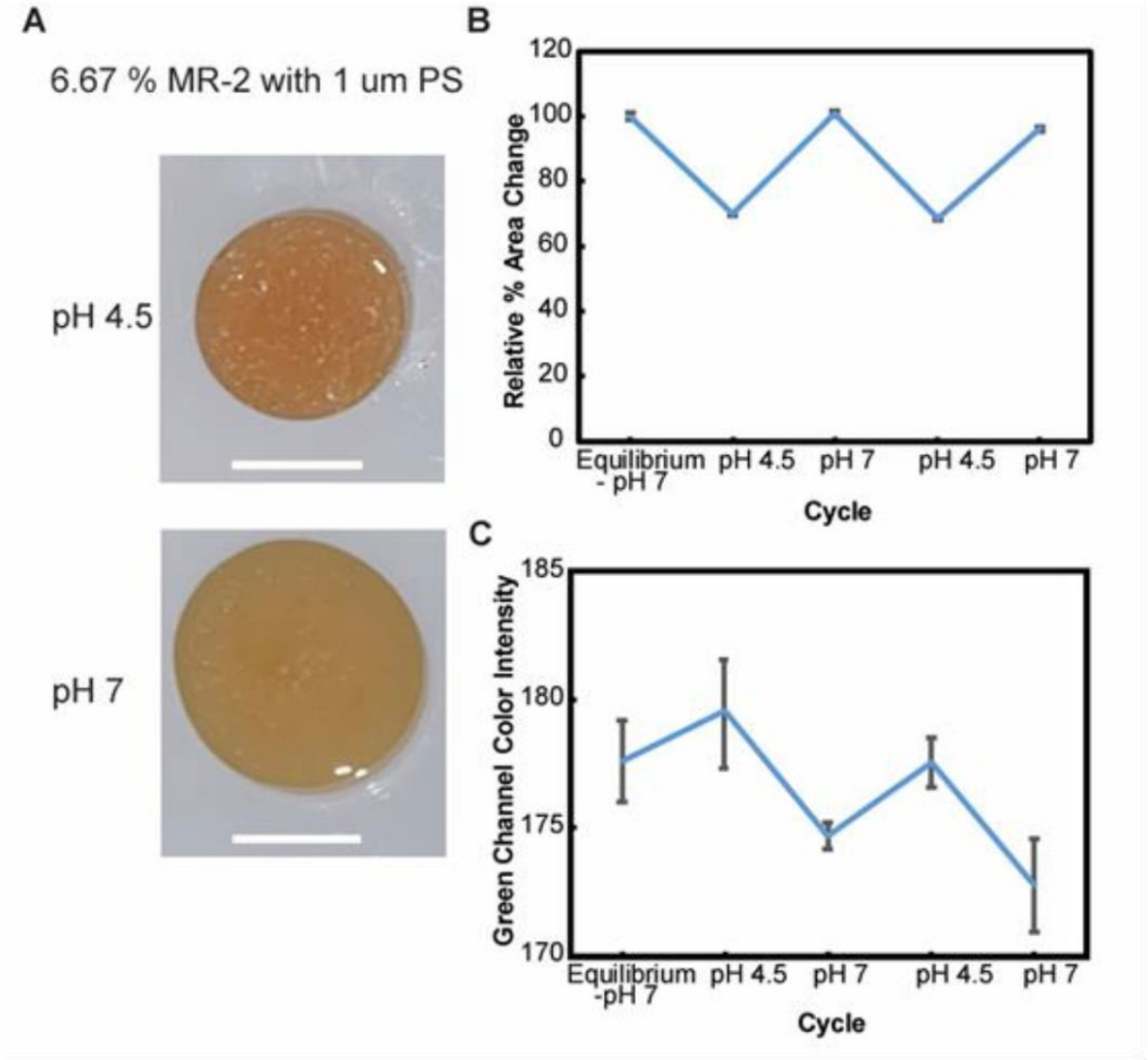
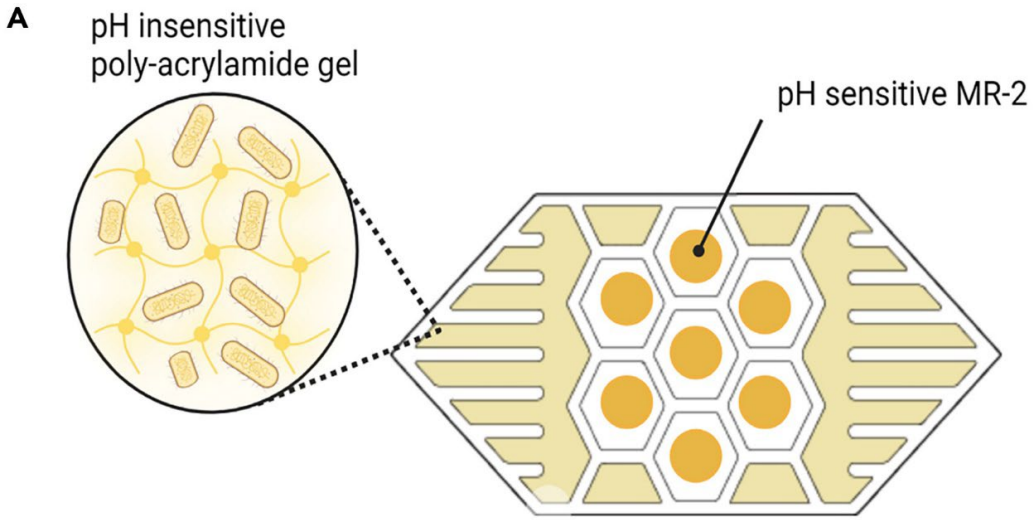


Figure S10. 1 µm polystyrene nanoparticles encapsulated into MR-2 loaded hydrogels to mimic the presence of *E. Coli* cells. After overnight equilibration, hydrogel pucks (n=3) were cycles between pH 4.5 and pH 7.0 sodium citrate and tris buffers, respectively. **(A)** Representative images of the color and size of MR-2 loaded pucks with polystyrene beads and pH 4.5 (top) and pH 7.0 (bottom). **(B)** Relative area change of pucks. **(C)** Relative color intensity change of pucks. Data in B and C are represented as mean ± standard deviation.

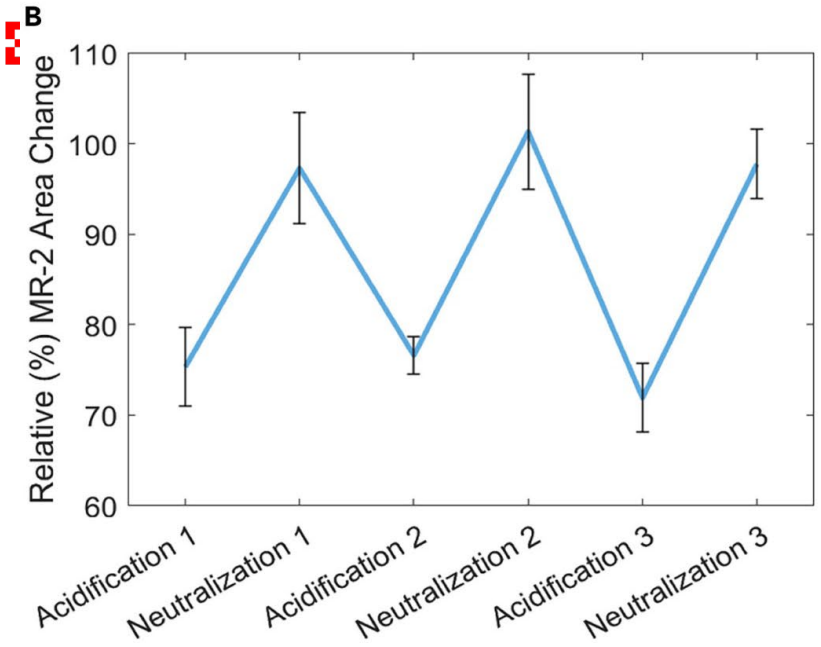
- Encapsulated 1 µm polystyrene beads
- Similar swelling/deswelling behavior and decrease in color intensity
- Therefore, conclude scattering is indeed the culprit with the cell-containing hydrogels
- Decide they need to redesign their system to address this… have to compartmentalize the cells apart from the dye

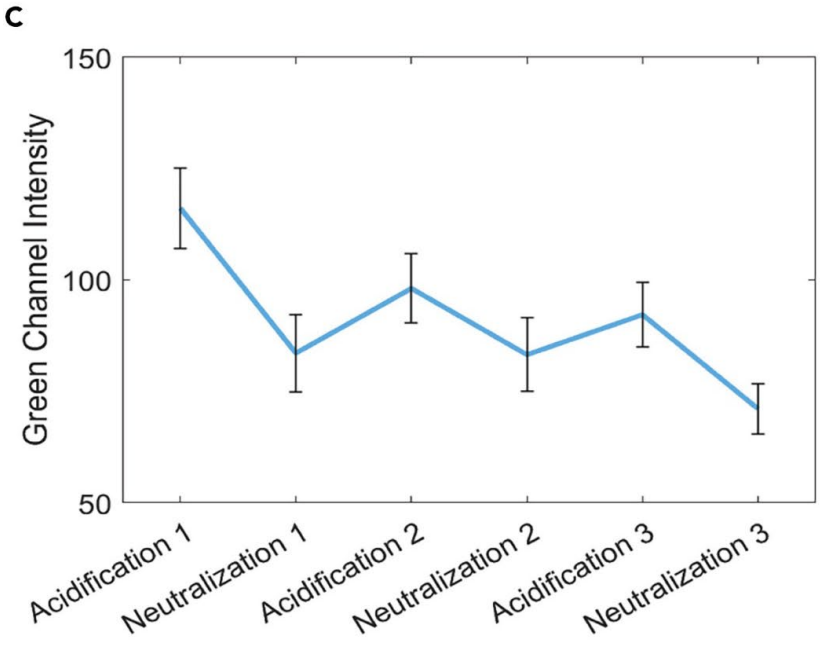
pH-responsive display configuration

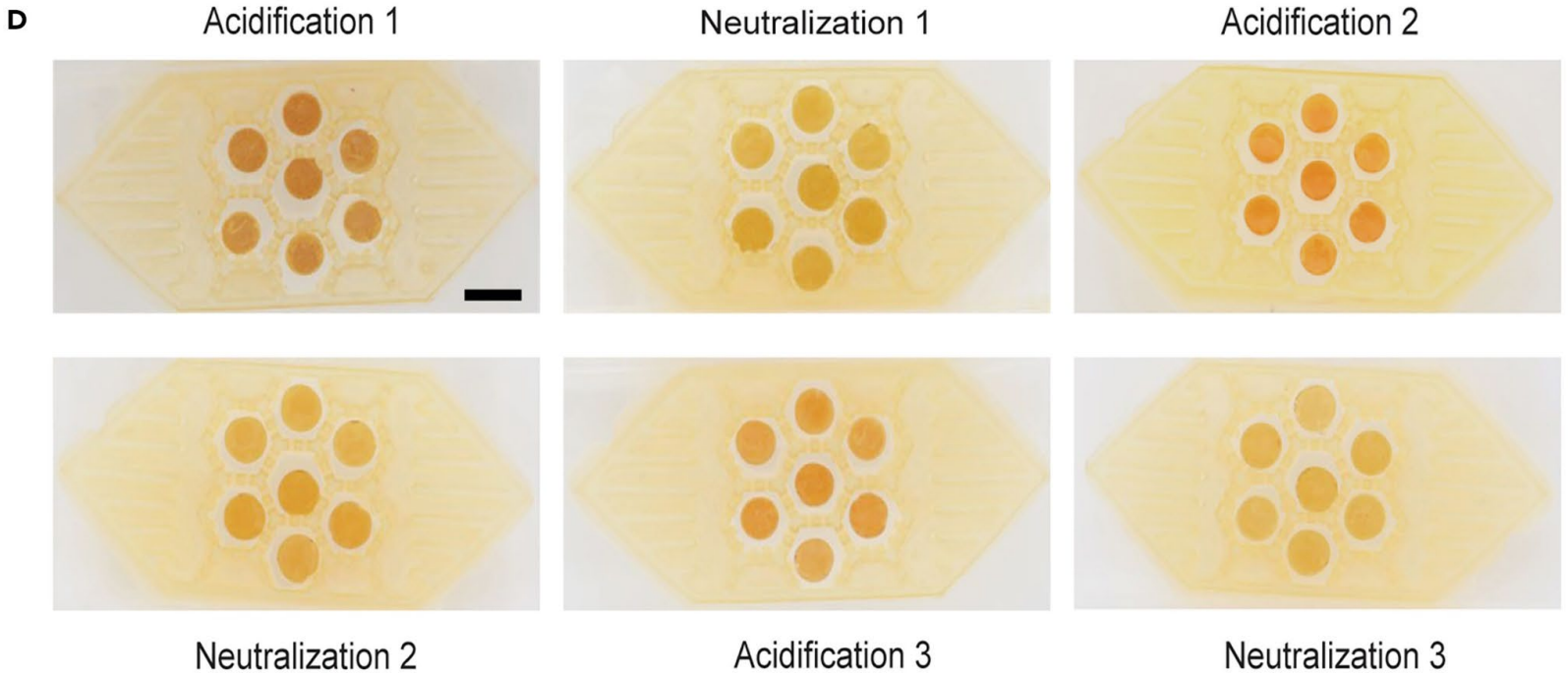


New design!

- 7 cell-free MR-2 loaded pH sensitive hydrogels – the same hydrogels as before
- Cell-containing comb structure made up of pH insensitive polyacrylamide hydrogels







3 full cycles of acidification and neutralization!

- Same shape behavior as for individual pucks
- Much more vibrant color behavior (significance?)

Conclusions

- Successful demonstration of the concept of reversible size and color change in a material driven by an active metabolic process

- “While our initial goal was to demonstrate that cells could alter their pH surroundings while remaining fully encapsulated in the hydrogel matrix, we found it difficult to prevent cellular escape into the surrounding medium, where their growth and metabolic activity would outpace the encapsulated cells.”

- “Considering this design concept, practical deployment would have to address containment of the living cells either through physical or genetic methods.”

- “While these changes driven by *E. coli* rely on the native metabolism, future iterations of this ELM could implement genetic control over acidification and neutralization. Introducing a living driving force could allow for more complex input-output functions than currently exist for such materials.”

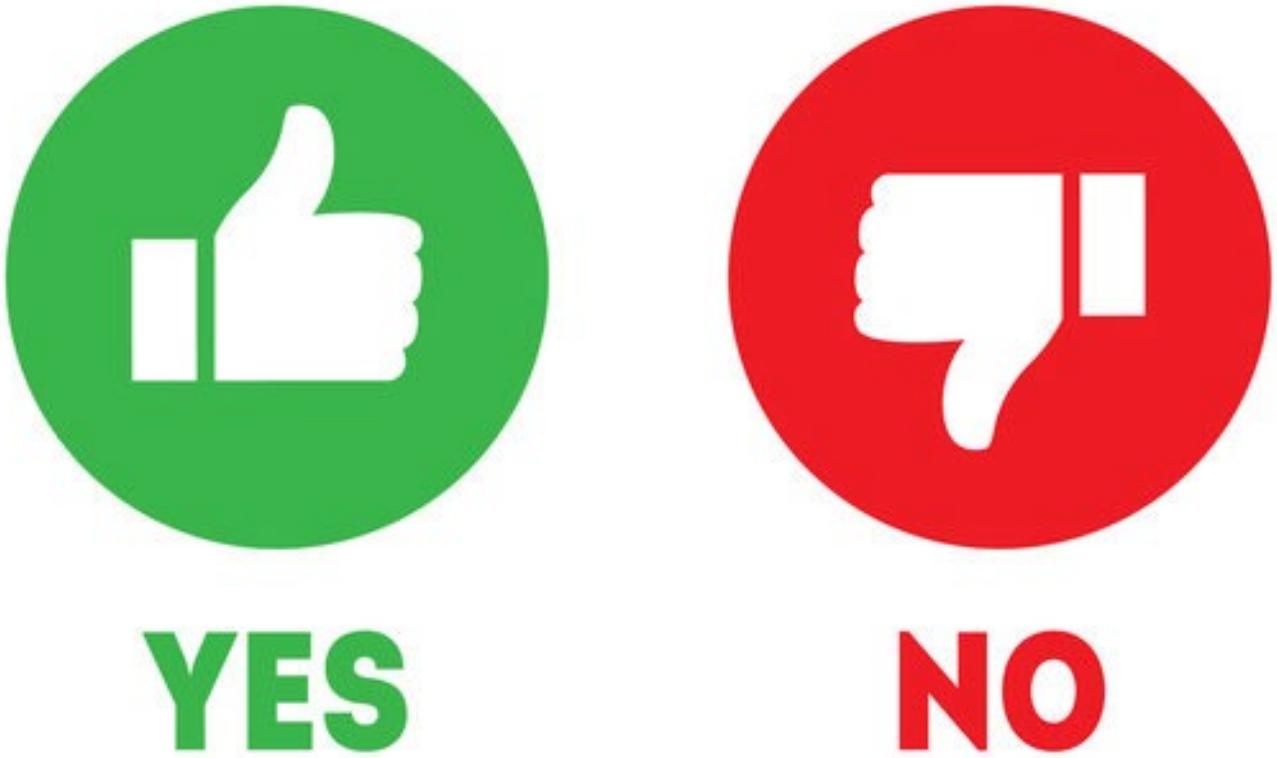
Applications

- Previous studies have demonstrated a range of applications of pH-responsive hydrogels: soft robotics, actuation systems, sensors, tissue engineering, and drug delivery.

- Introducing microbes, known for their diverse sensing capabilities, expands the potential input-output functions of these materials.

- Furthermore, these hybrid living systems enable the development of sensing systems and devices that may *reduce or eliminate the dependency to external power supplies*, since microbes only require biochemical fuel to function.

Did you like this paper? Why or why not?



A note on style and telling a story

Popular replies (1)

Dean Whitehead
Federation University Australia

Geoff - it depends on the context. If it's a 'hard, factual' academic article then it's best written in the third person. If, however, it's more of a 'softer science' i.e. humanities/social sciences and/or might be conceptual, opinion-piece, editorial, review, experiential etc - then writing in the first person is fine. I always tell students etc the same thing.

- These authors told us their research story in a way that felt personal
- This is accomplished by an intentional use of the 1st person: we, our…
- Chronology may be true or not, but the manuscript was written in a way that felt like the events occurred in chronological order
- This is interesting and valuable as it helps us to see the story through the eyes of the authors, to understand why they made certain decisions in time, etc.,
- Both of these aspects make the reader invested in the story (research)

A note on collaboration

Microbially driven reversible size- and color-changing materials

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9 Lead contact

Lesson takeaways

- You should know what an ELM is
- You have just learned about a concrete example of a living material, where the living thing is critical to defining the dynamic state of the material (color/shape)
- You have just now seen how ELM research is truly cross-disciplinary – experts in organic synthesis, polymer chemistry, molecular engineering, and computer science were needed to make this happen
- Each little piece could have been a complete story, but the pieces together stitched up something powerful