Living (bio)materials

BioEng458 - lecture 6

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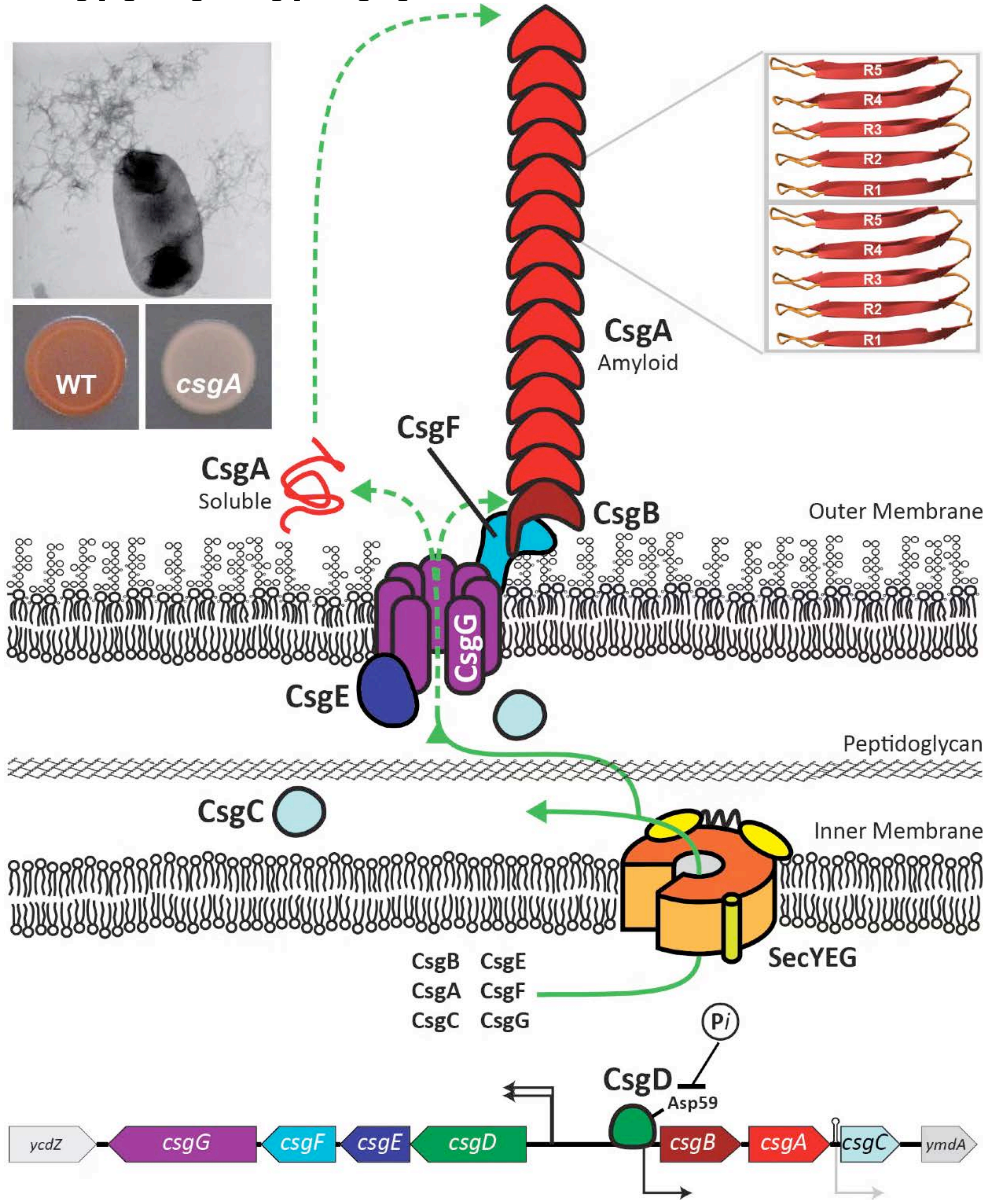
Lecture outline

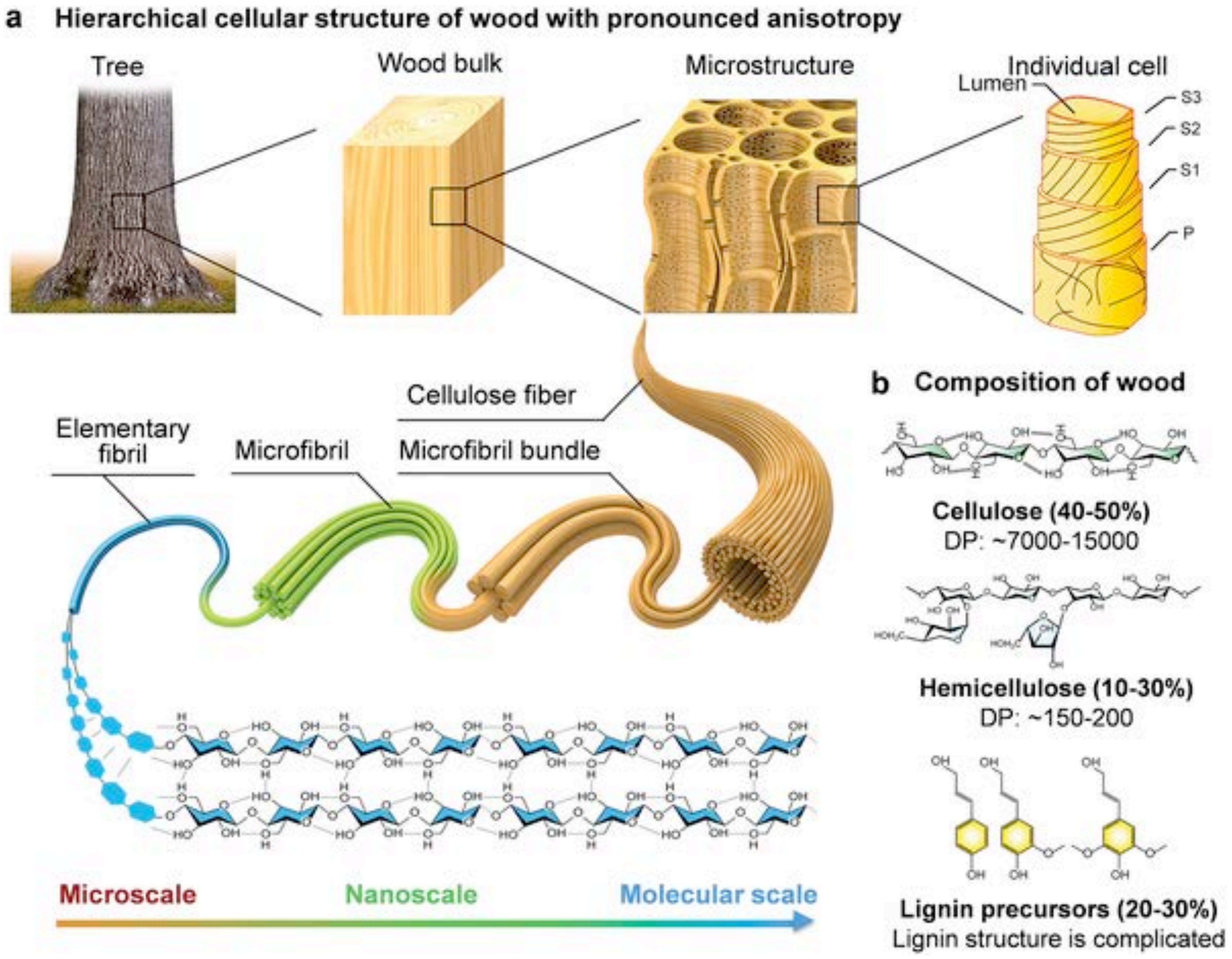
- Living materials: what are they?
- Key properties and capabilities
- Implementing living materials
- Case studies: living materials for sensing and treating

Cells produce biological materials

Bacterial curli

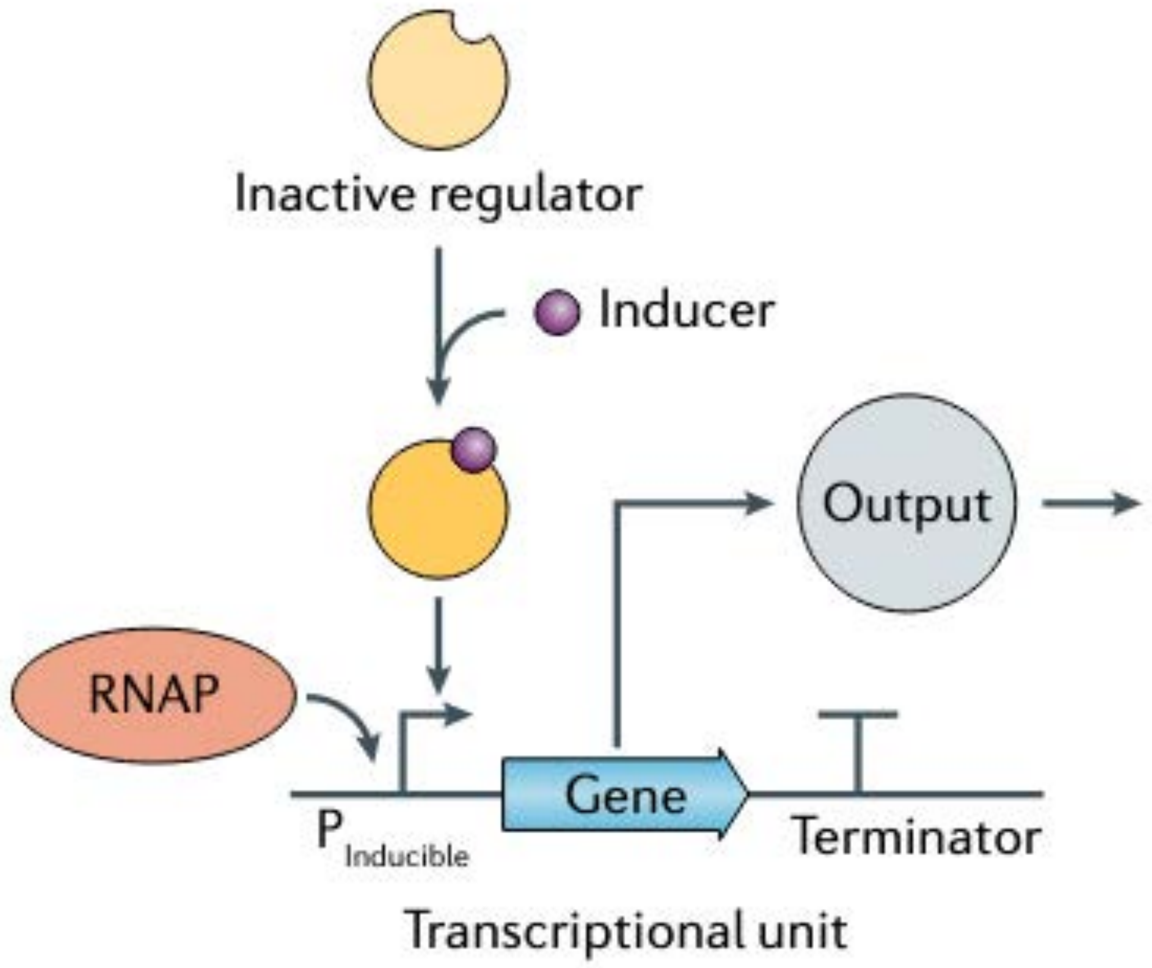


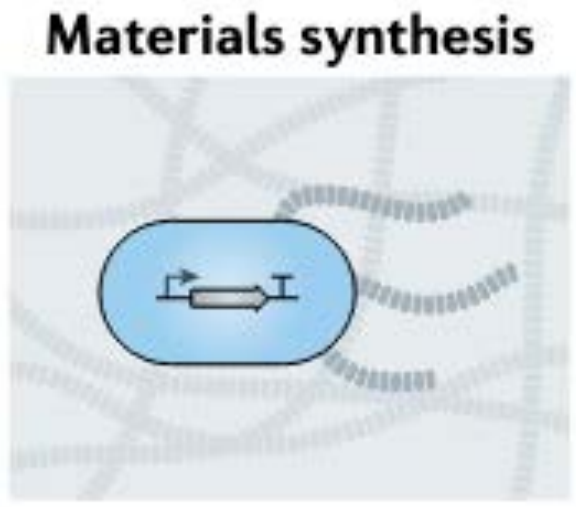
Wood



Recap of lecture 1

Synthetic biology allows control of biomaterial synthesis



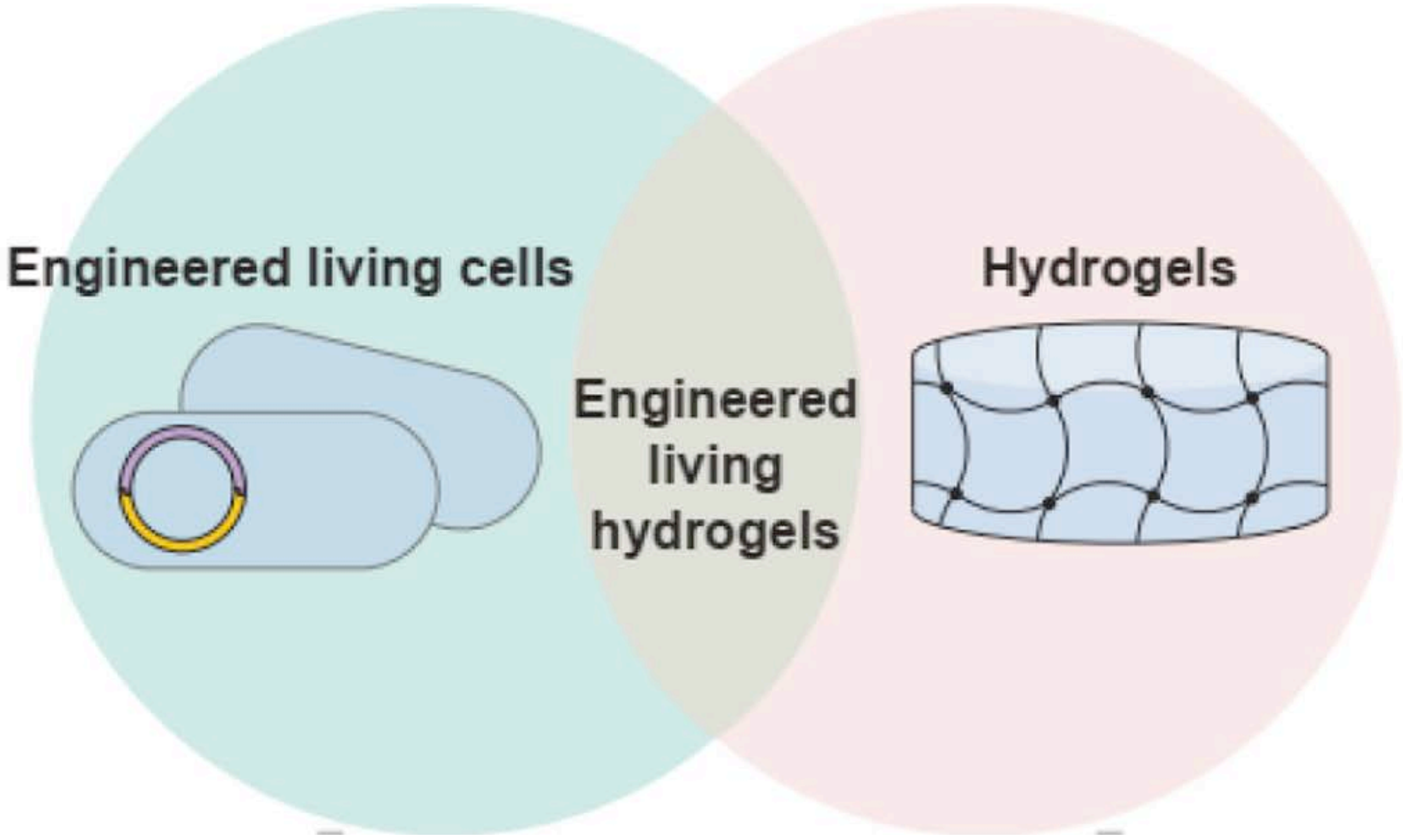


We can program cells as material factories

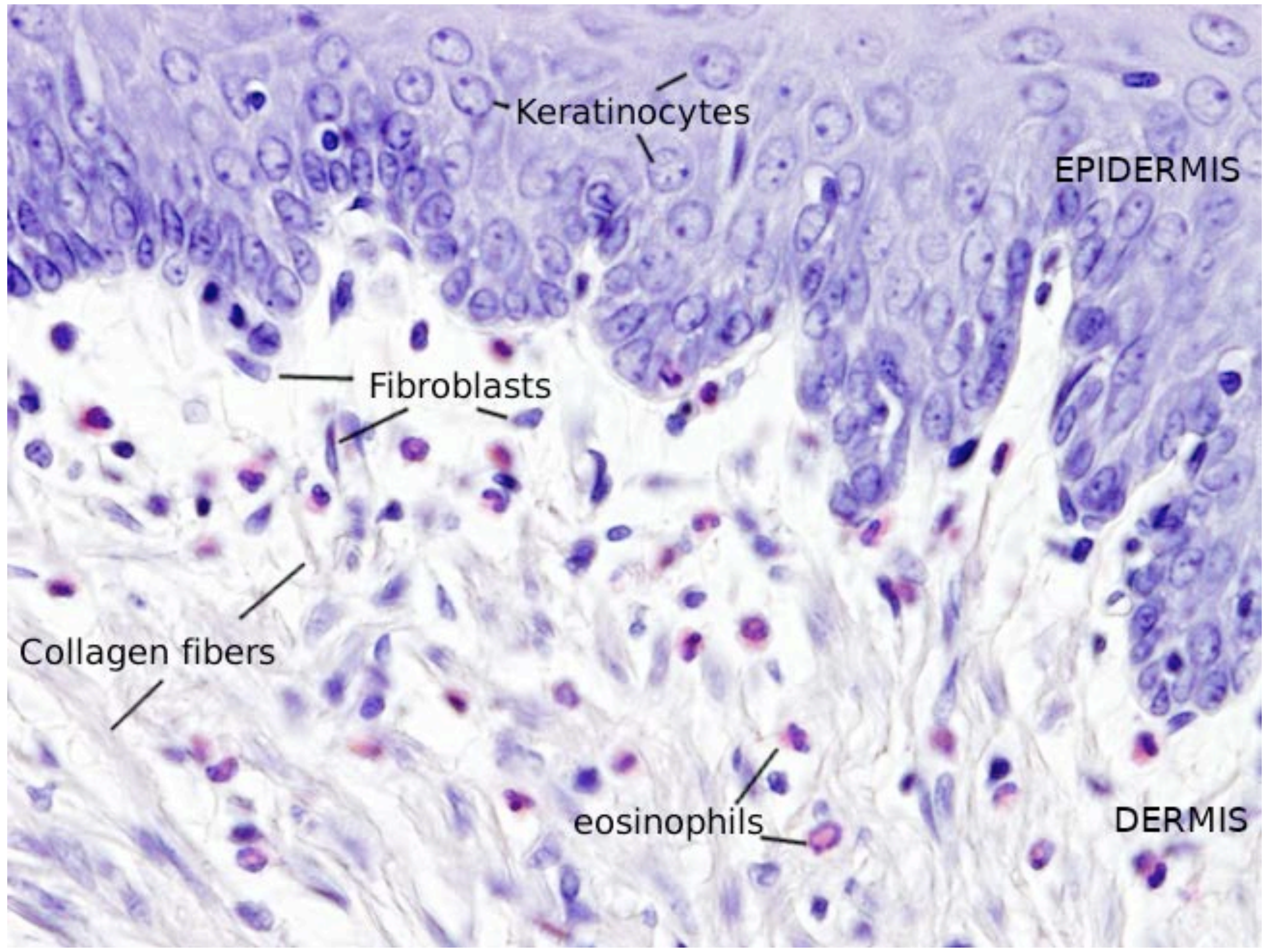
> how about incorporating cells within the biomaterial design?

What are living materials?

Materials that incorporate living cells



Already in nature!



Living biomaterials: engineering

Two approaches

Biohybrid material: embed cells into a synthetic material matrix

> Programmable with synthetic biology, but not at material level

Living material: Cells synthesize biological material

> Material is programmable with synthetic biology

Why living biomaterials?

Self-Healing & Regeneration: Living materials can autonomously repair damage, unlike inert materials that require replacement.

Dynamic Sensing & Response: Living biomaterials can detect changes in their environment (e.g., pH, toxins, temperature) and respond accordingly.

Sustainability & Biodegradability: Living materials can be **biodegradable** and **renewable**, reducing waste compared to synthetic materials.

Integration with Biological Systems: Living biomaterials interact naturally with tissues and cells, reducing rejection risks in medical applications.

Challenges & Considerations

Controllability: Unlike synthetic materials, living biomaterials require precise control over growth and function.

Safety & Ethics: Concerns over containment, regulation, and unintended environmental consequences.

Long-Term Stability: Ensuring that cells remain functional over time in complex environments.

Applications as biomaterials and beyond

Biomedicine & Regenerative Medicine: Living tissue scaffolds for wound healing, drug-releasing implants.

Wearable & Implantable Sensors: Living biosensors that detect infections or changes in body chemistry

Environmental Applications: Living materials for pollutant degradation, carbon capture, and air purification.

Smart Infrastructure: Self-healing concrete, adaptive coatings that respond to weather conditions.

Materials used in living materials

Table 2 | **Materials used in living materials**

Material	System	Advantages	Disadvantages	Refs
Agarose	Drug release; 3D printing	Good for sustaining bacterial populations; gelation by cooling to room temperature	Uncontrolled bacterial growth	28–30
Pluronic F-127	Biosensors; skin patch; 3D printing	Gelation by heating to room temperature; chemically modifiable; physical and chemical crosslinking possible; shear thinning	The physically crosslinked gel dissolves in water	14,25, 31
PVA	Electrospinning; cheap biosensor	Forms porous matrix during drying	Water-soluble	72,127, 129
Alginate	Cheap 3D printing, probiotics	Widely used; forms gel in contact with Ca^{2+}; good printability; chemically modifiable; cheap; accessible	Needs Ca^{2+} to remain a gel; physical crosslinking only	125
PEO	Electrospinning; tissue engineering scaffolds	Water-soluble; substitutive for organic solvents in electrospinning	Can increase water solubility in polymer blends	135
PCL		Thermoplastic; low melting temperature; medical-grade PCL available	Not suitable for live-cell encapsulation during electrospinning	136
Silk fibroin		Genetically modifiable polymer	Expensive	137
Curli nanofibres[a]	Engineered biofilms from *Escherichia coli*	Extensively genetically modifiable	Biodegradable; relatively soft	7,26,85

Biohybrid

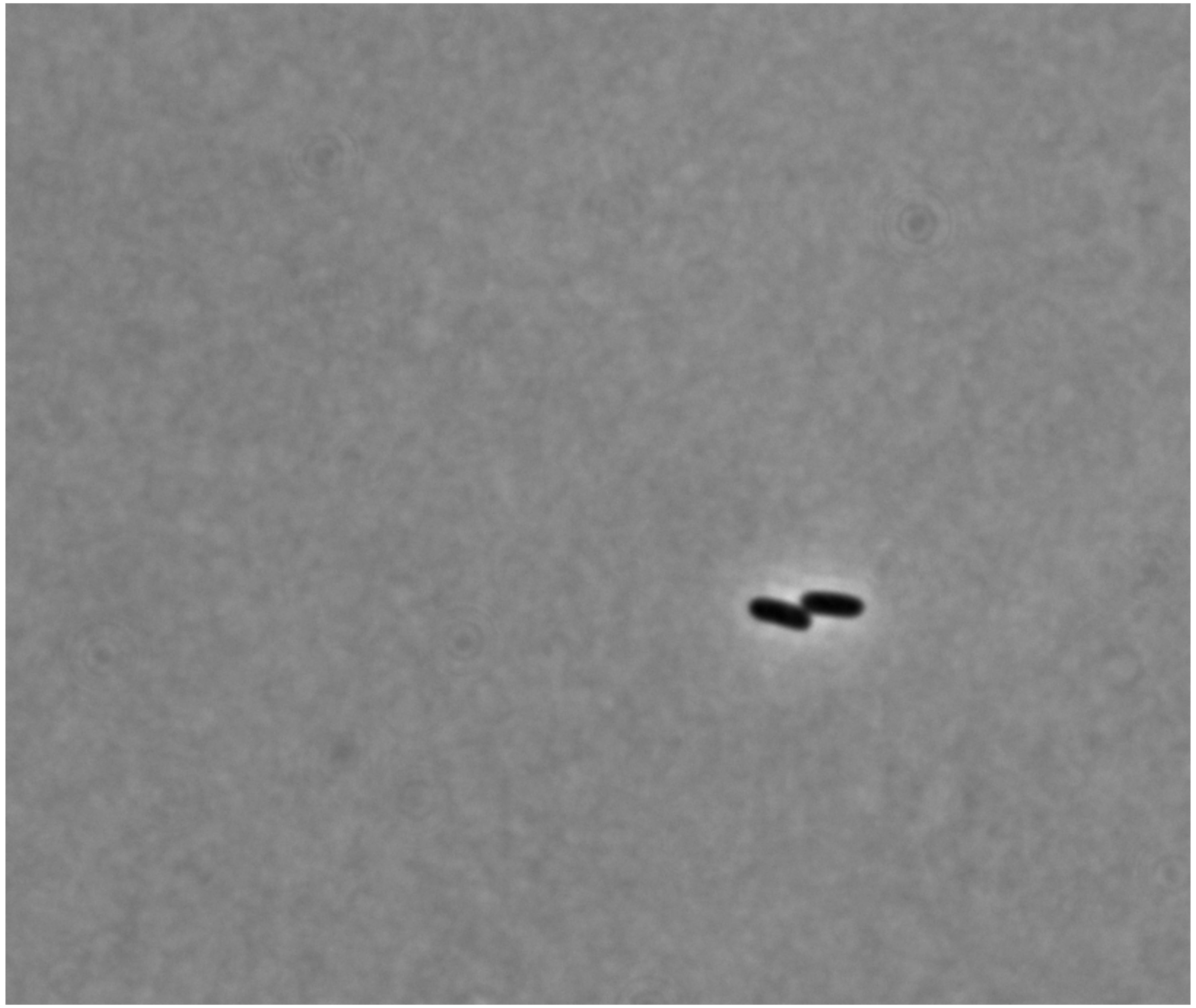
https://www.nature.com/articles/s41578-020-00265-w#article-info

Engineered living materials

- material synthesis
- self organization into higher order structures
- maintenance of structure in response to stimuli

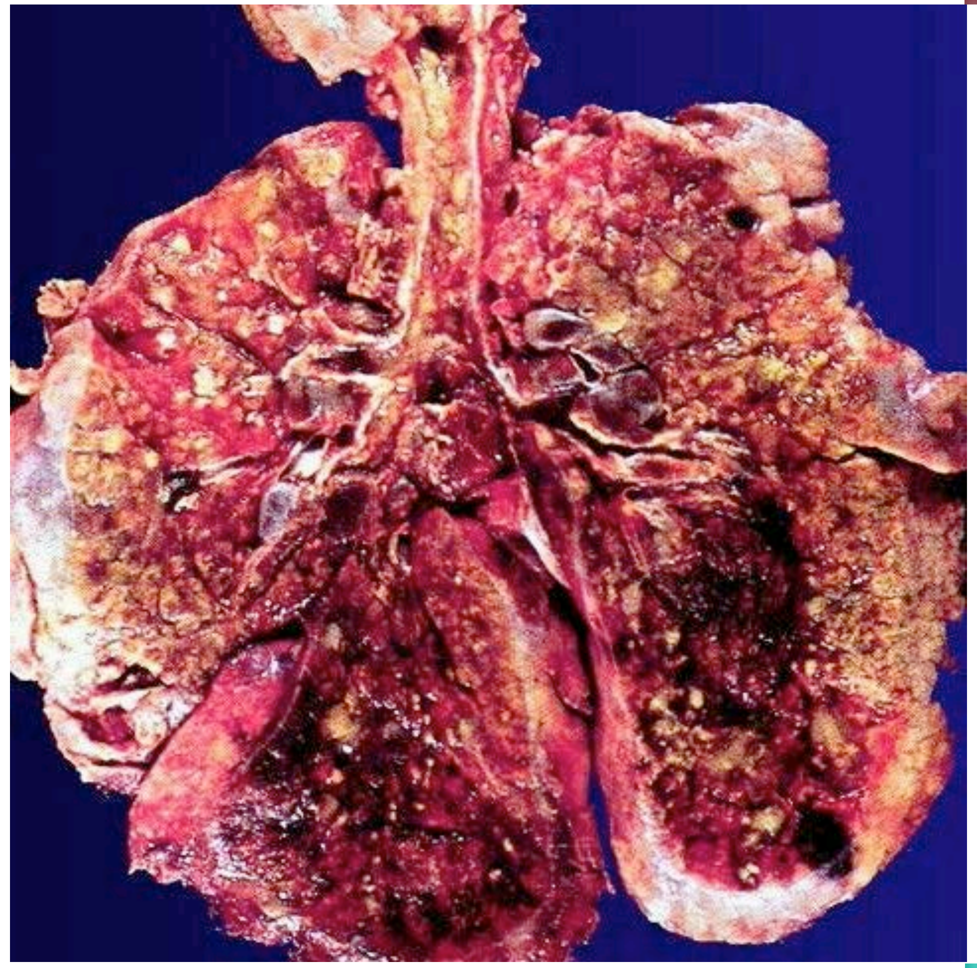
> bacteria can do this - and even more

Bacterial multicellularity



E. coli

Biofilms



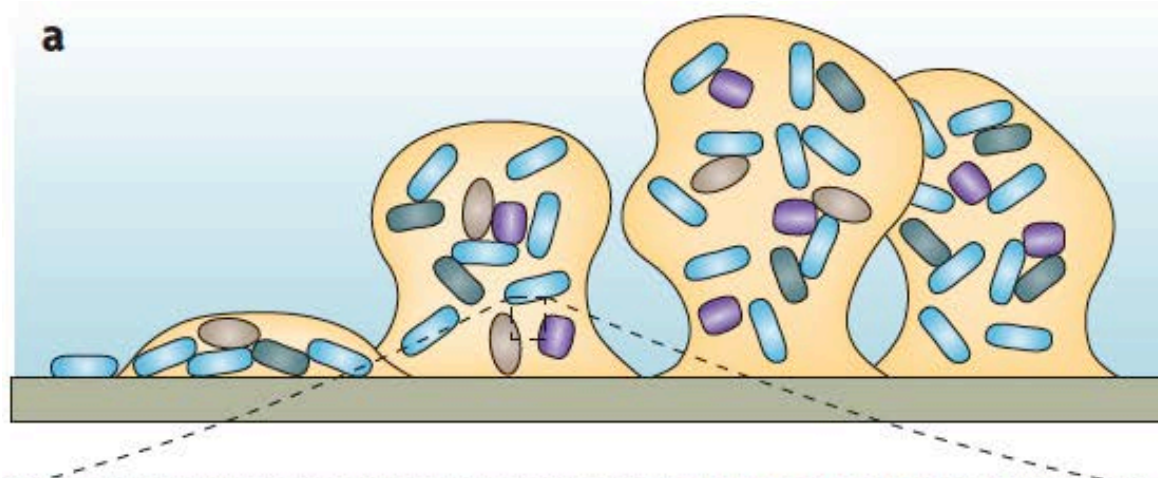
Images:
waterandhealth.org
www.eawag.ch
wikipedia.org
www.cs.montana.edu


0.00 h
10 µm
cross-sectional view

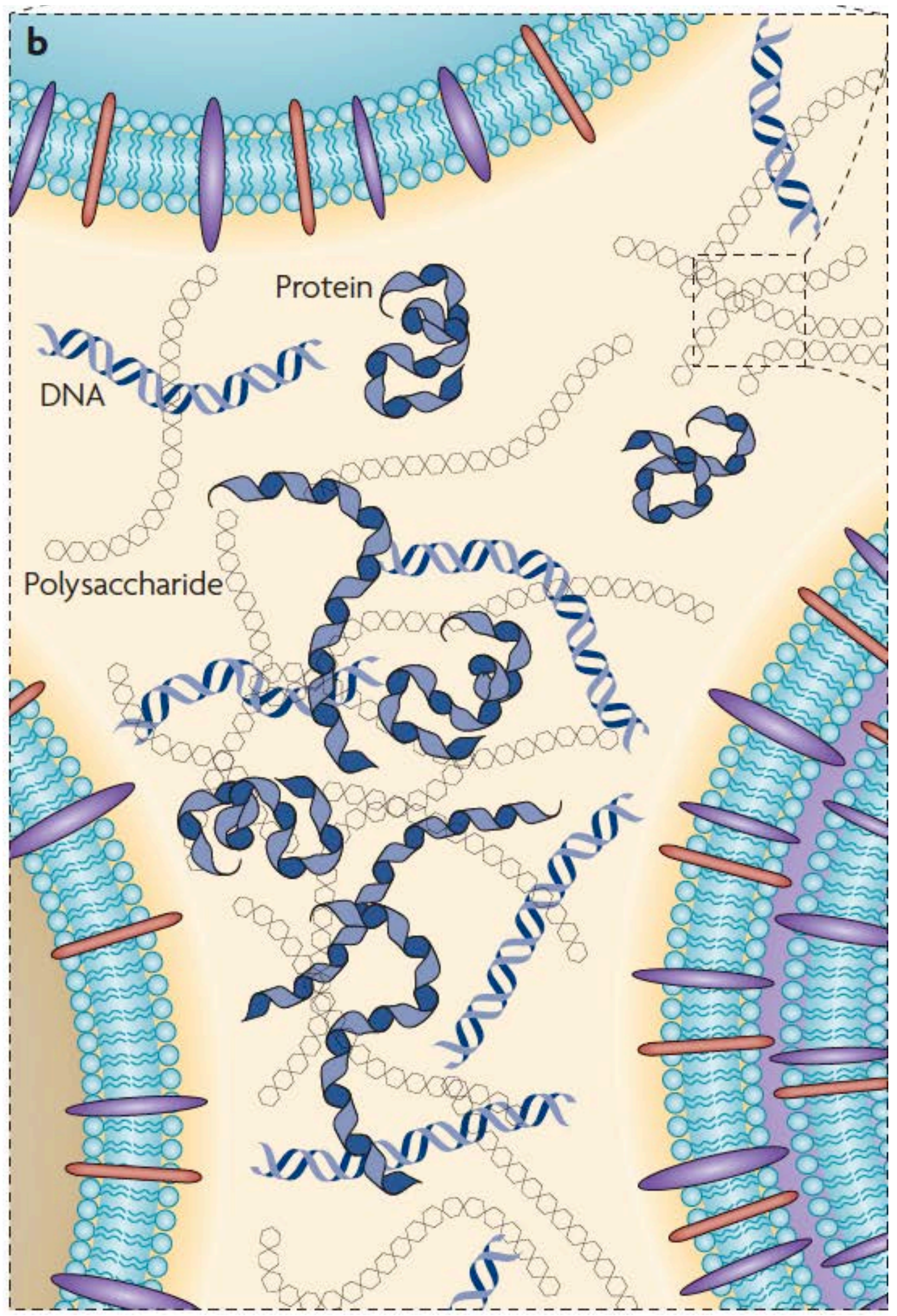
Zooming in on the biofilm matrix

the extracellular polymeric substance (EPS) matrix is a cement for biofilms

Main function: mechanically maintain the cohesion of the community

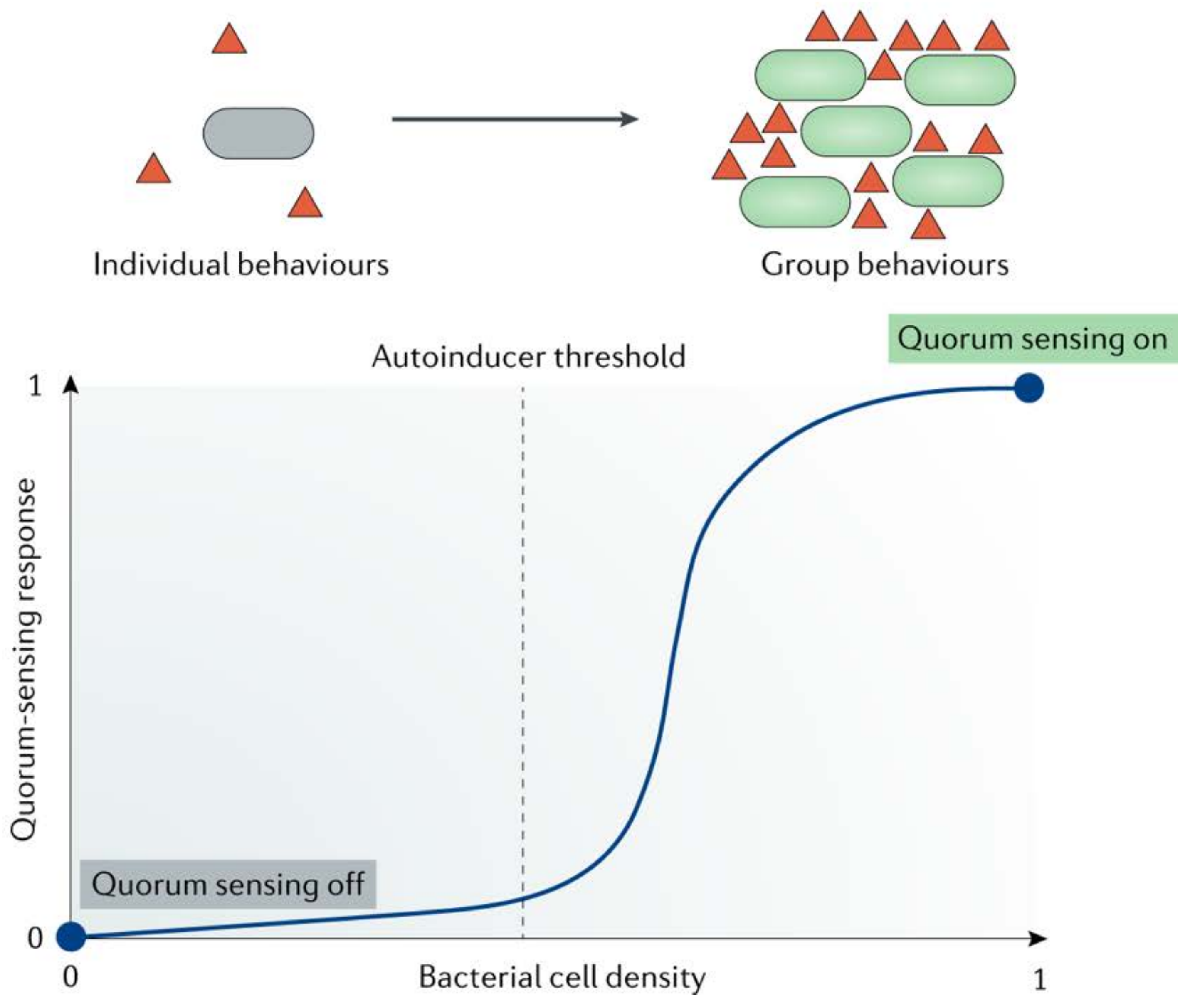


The properties of the matrix sets the architecture of the biofilm and the spatial organization of single cells



Single cells synchronize to build biofilms

- Single cells secrete signaling molecule (autoinducer)
- Autoinducer level depend on bacterial density
- Single cells sense autoinducer levels
- Response is dependent on concentration



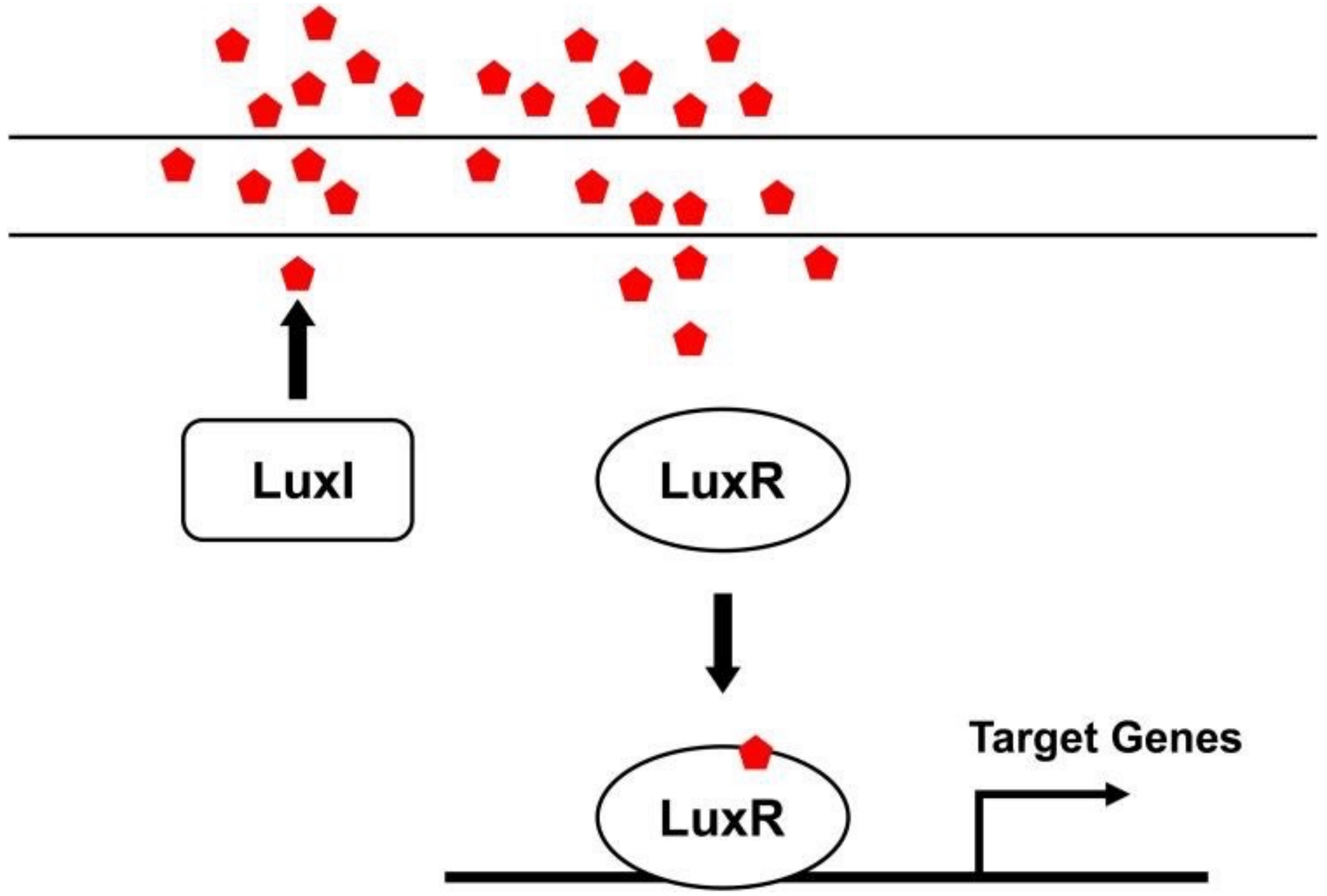
Quorum sensing regulates a breadth of collective phenotypes:

- virulence (patogenicity)
- light production
- biofilm formation

hawaiian bobtail squid
and *Vibrio harveyi* (camouflage)

Engineering cell-cell communication

Quorum sensing system



For example: biofilm matrix

Microbial species for living materials

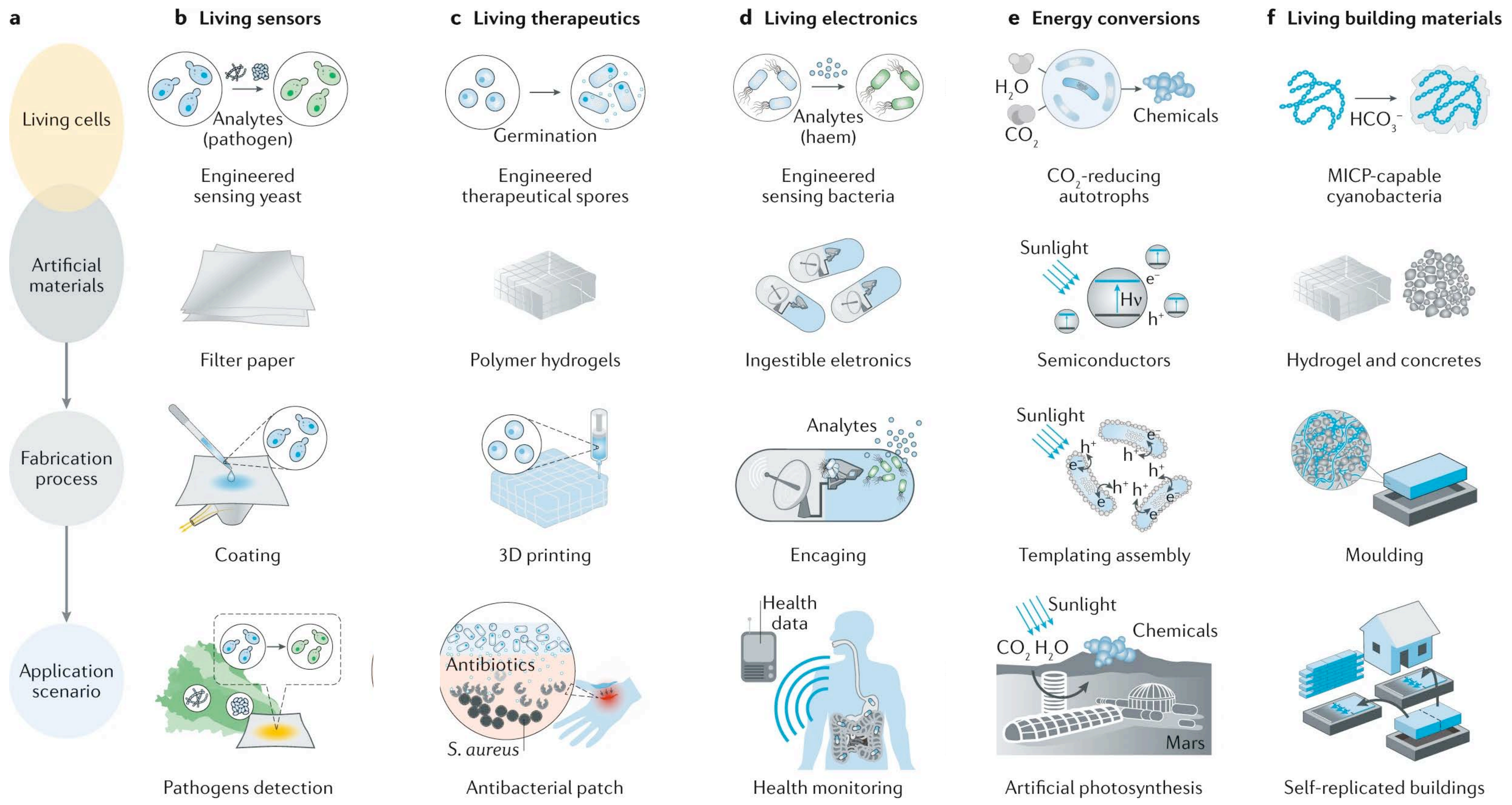
Strain	System	Advantages	Disadvantages	Refs
Escherichia coli Nissle 1917	Biosensor pill for gut bleeding; IBD treatment	Probiotic strain for the GI tract; easy to modify	Endotoxins	26,70
Escherichia coli ClearColi	Light-responsive drug delivery	Endotoxin-free strain; easy to modify	Not a probiotic	29,30
Escherichia coli lab strains	Biosensors; living tattoo; biocatalysis	Fast growth; large genetic toolbox	Endotoxins	14,69,71, 105,186
Lactococcus lactis MG1363/NZ9000	Living biointerfaces	Probiotic strain; adhesive biofilms; good at protein display and secretion; endotoxin-free strain	Limited genetic toolbox	23,90–92, 125
Bacillus subtilis	Low-cost biosensors; skin patches	Forms robust spores; antifungal properties; Gram-positive model organism	Moderate genetic toolbox	24,25,72
Staphylococcus epidermidis	Skin patches	Skin commensal; antifungal properties	Opportunistic pathogen inside tissues	79
Lactobacillus; *Bifidobacterium*; *Streptococcus*; *Enterococcus*	Probiotic microcapsules	Probiotic strains for the GI tract	Limited genetic toolbox	41–46
Acetobacter xylinum	Living skin graft	Bacterial cellulose producer	Not a probiotic; not genetically tractable	138
Spirulina	Stem cell, neuroregenerative and anti-thrombogenic scaffold	Anti-inflammatory; antibacterial; antifungal; antimetastatic; antioxidant; regenerative	Only lysed extracts used for these applications	135–137
Penicillium chrysogenum	Drug release	Naturally produces penicillin	Minimal availability of genetic tools	28
Saccharomyces cerevisiae	Biosensor; biocatalysis; bioremediation	Food-grade; cheap; large genetic toolbox	Ethanol and CO_2 production undesirable for some biomedical applications	73,132, 150,151

GI, gastrointestinal.

Genetic parts for living material

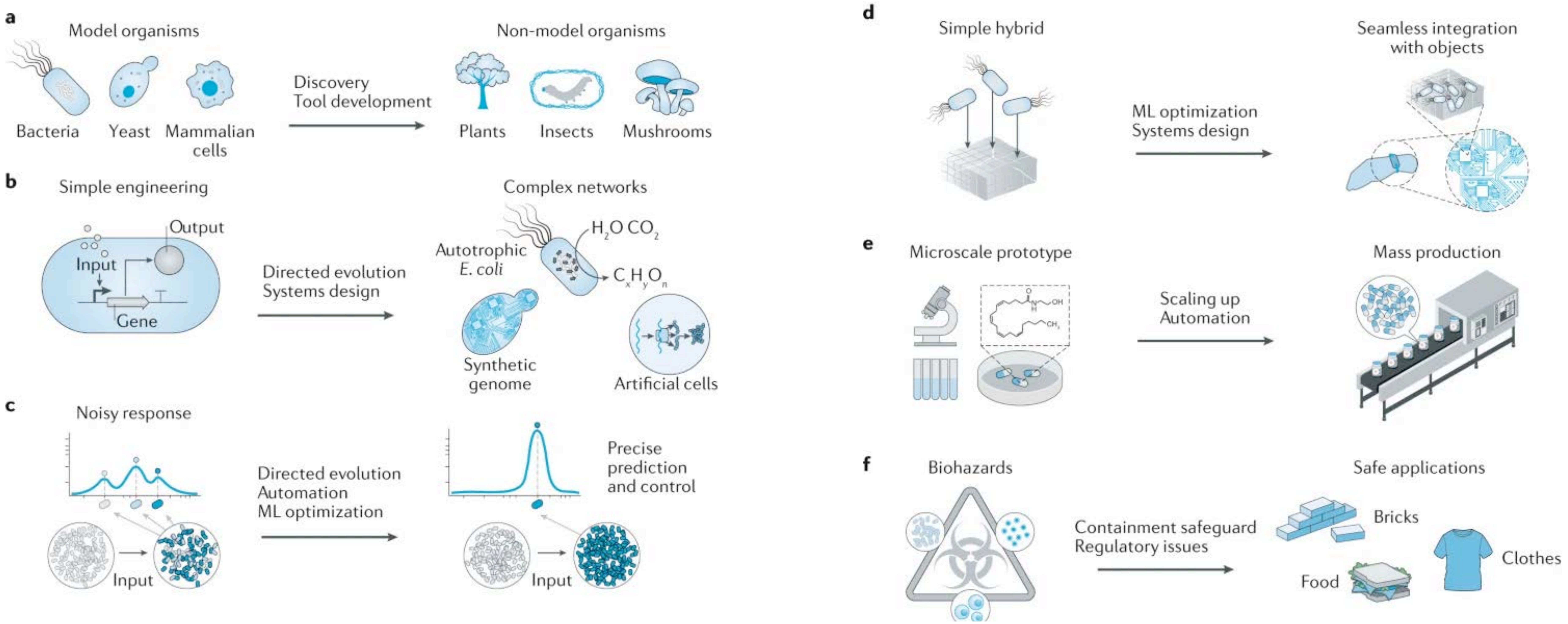
Genetic part	Type	Examples	Refs
Input			
Chemical	Small molecules	IPTG (pLac-LacI); arabinose (pBad-AraC)	229
	Heavy-metal ions	Arsenic (pArs-ArsR); mercury (pMer-MerR)	90,154
	Biomolecules	Steroid (pLexA-XVE); haem (pHrt-HrtR)	175,230
Electrical	Redox potential change	pSox-SoxR	180
Optical	Red light	Cph8/OmpR; phyB/PIF	140
	Green light	CcaS/R	140
	Blue light	YF1/fixJ; Cry2/CIB1; EL222	140,231
Thermal	Heat	Heat-shock-response mechanism	39
	Cold	Cold-shock-response mechanism	232
Mechanical	Pressure	Mechanosensitive channels	233
Computation			
Boolean logic	AND gate	Riboregulators; recombinases; split regulators; regulator cascades; CRISPR–Cas	64,234
	NAND gate		235
	NOR gate		43,48,236
Memory	Recording	Retrons; self-targeting CRISPR–Cas	37,237
	Timer	Feedforward loop	40
	Counter	Recombinase cascade	238
State change	Toggle switch	Repressor feedback loops	8
	Oscillator	Repressor cascade	9,146
	State change	Recombinase-based state machine	66
Communication			
Diffusion (chemicals)	Quorum sensing	AHL (lux, rhl, las, cin, tra, rpa)	24,67
Diffusion (peptides)	GPCR-based sensing	Yeast mating factor	68
Contact	Surface receptor	synNotch	26
Output			
Fluorescence	Fluorescent proteins	GFP; RFP; BFP	58
Bioluminescence	Luciferases	Firefly luciferase; NanoLuc	59
Colour change	Chromoproteins	aeBlue; amilCP; tsPurple	60
	Pigments	Carotenoid; melanin	61,239
	Opacity change	Cephalopod reflectin	112
Bioplastics	Monomers for bioplastic	Polyhydroxyalkanoates	240
Electricity	Current production	Extracellular electron transfer	241
	Radical polymerization		131
Protein materials	Amyloid fibres	Curli (CsgA); TasA	25,87,88
	Adhesives	Mussel foot proteins	86,101
	Adhesins	Substrate-binding peptides; nanobodies	87,149,242
	Silk	Silkworm silk; spider silk	205,243
	Protein ligase	SpyTag-SpyCatcher	244
Polysaccharide materials	Cellulose	Bacterial cellulose	73,130
	Chitin and chitosan	GlcN and GlcNAc	245
Mineralization	Magnets	Ferritin; magnetosome organelle	246,247
	Calcium carbonate	Microbially induced $CaCO_3$ precipitation	248
	Silica	Diatom silaffin	102
	Quantum dots	CdSe; CdS	19,249
Acoustic property	Gas vesicle	Gas-vesicle-forming proteins	89,113

Hybrid living materials



https://www.nature.com/articles/s41578-020-00265-w#article-info

Challenges in living materials



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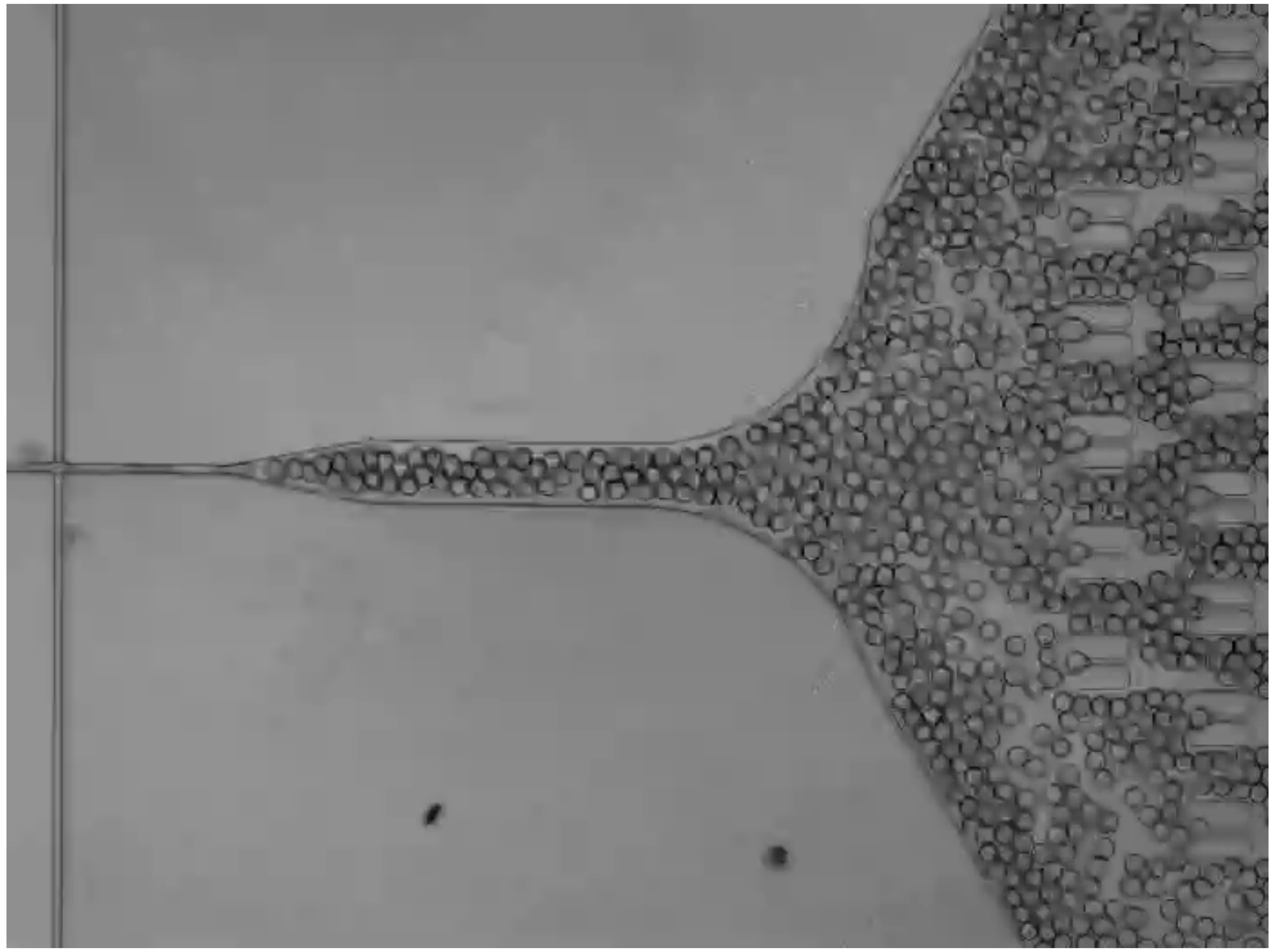
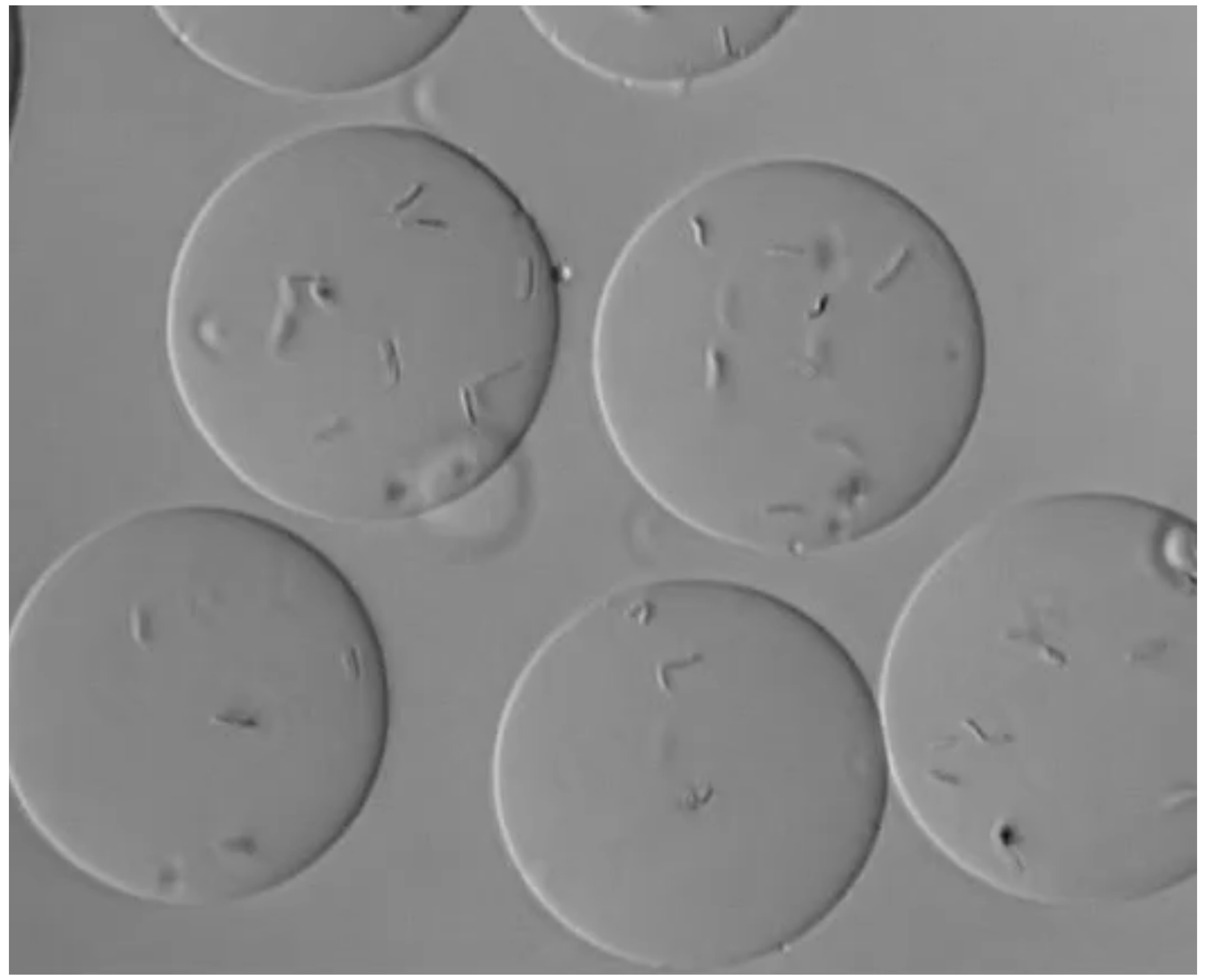
Incorporate (synthetic) living organisms in materials > sensors

Producing bacterial capsules

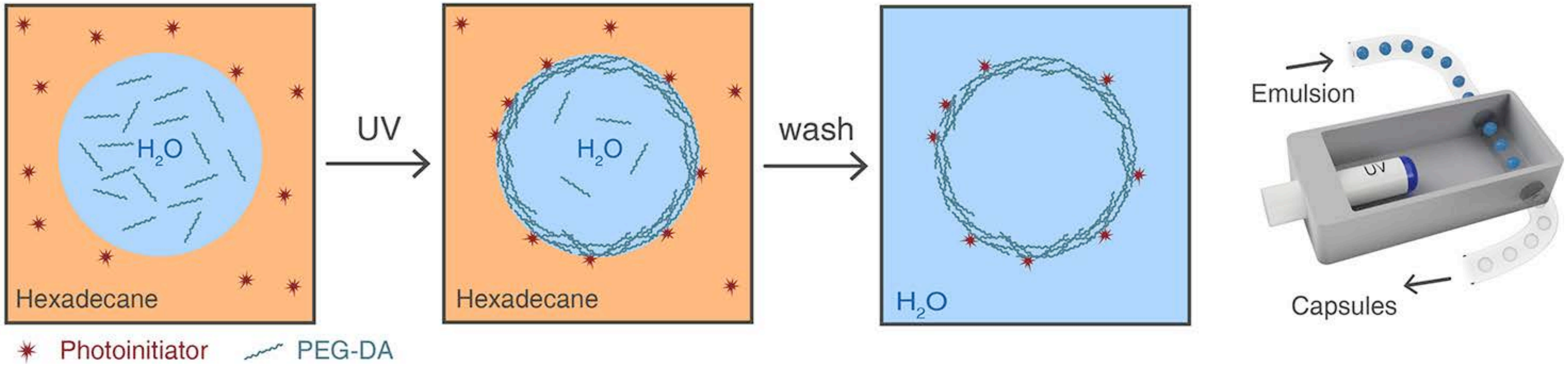


Microfluidic droplet generator



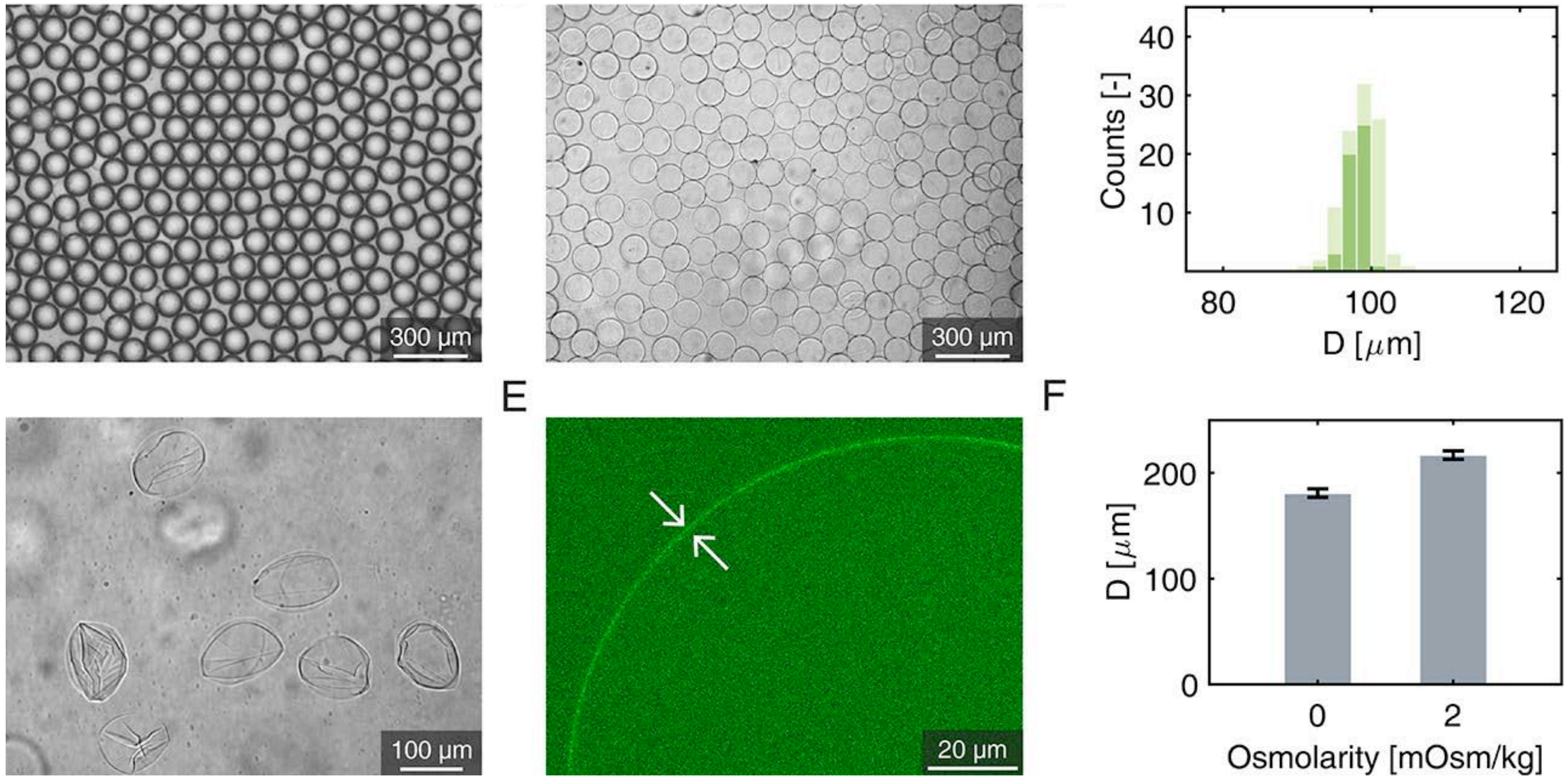
Bacterial growth (20h)

Bacterial capsules: core-shell



https://doi.org/10.1021/acsami.1c00230

Core-shell particles at high throughput



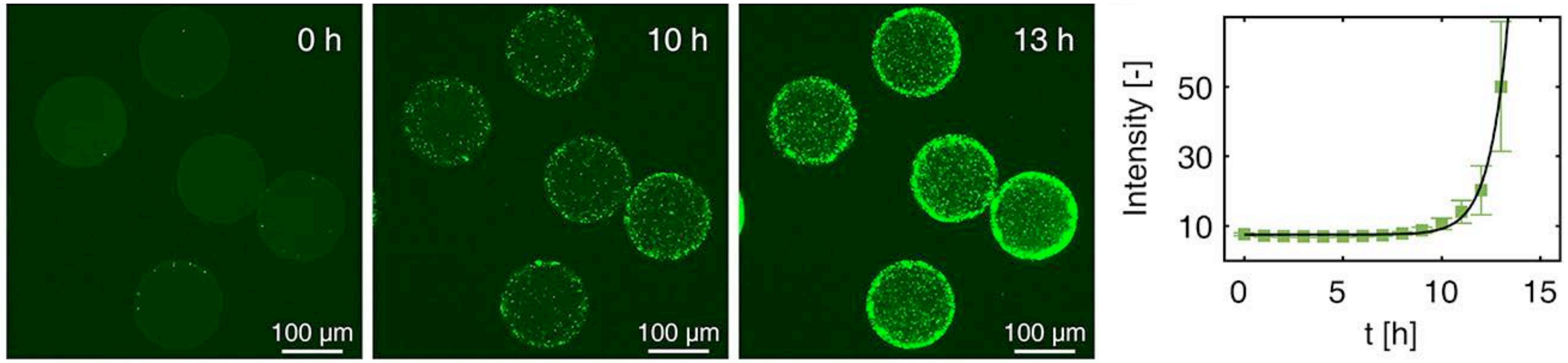
Emulsion in oil

Particles in water

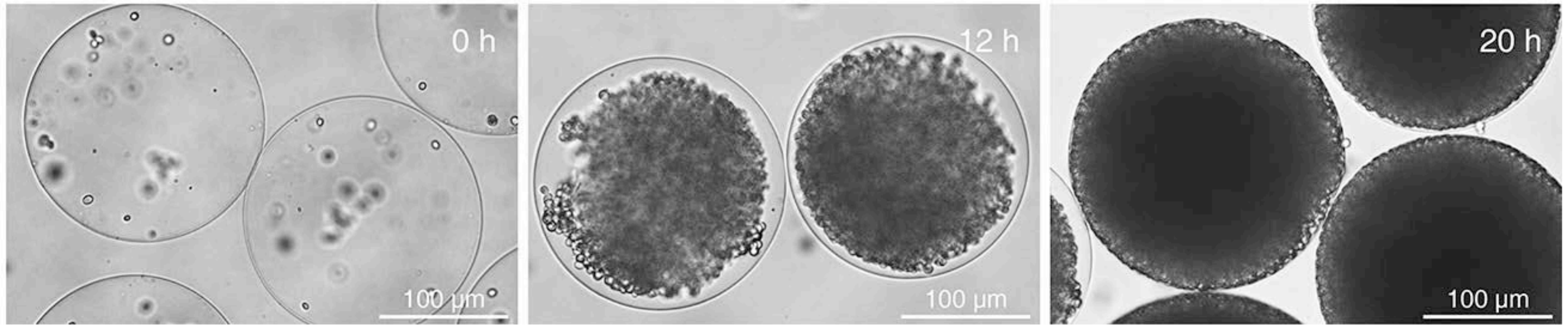
Osmotically-shocked particles

Fluorescent visualization

Growth in core-shell capsules



Fluorescent bacteria growth

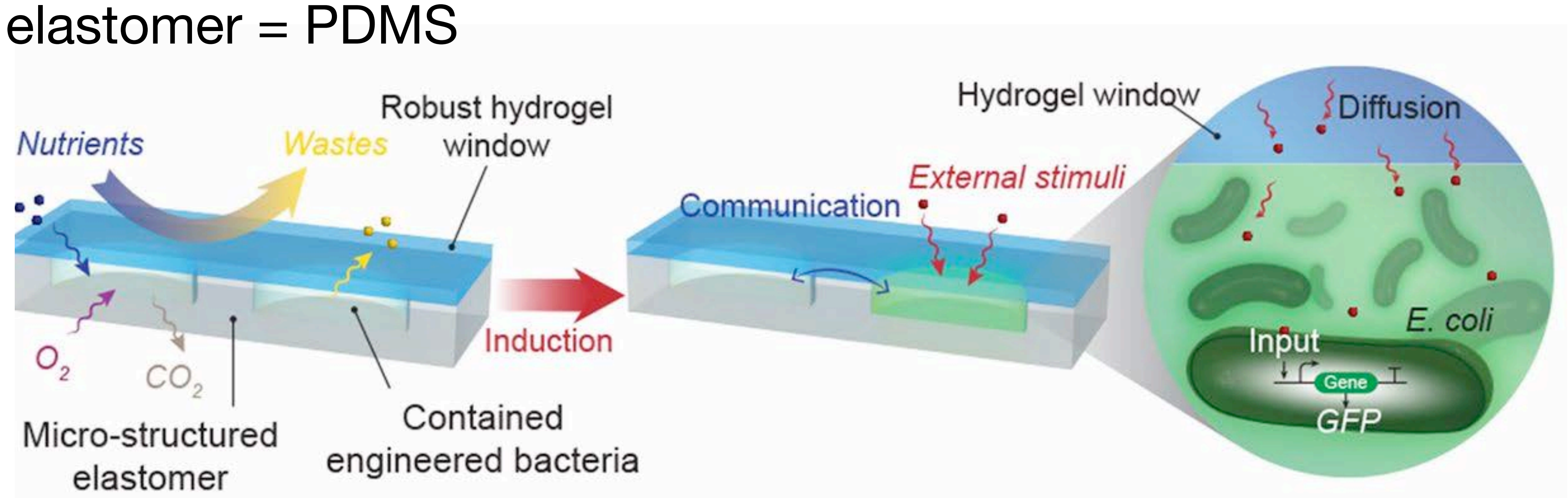


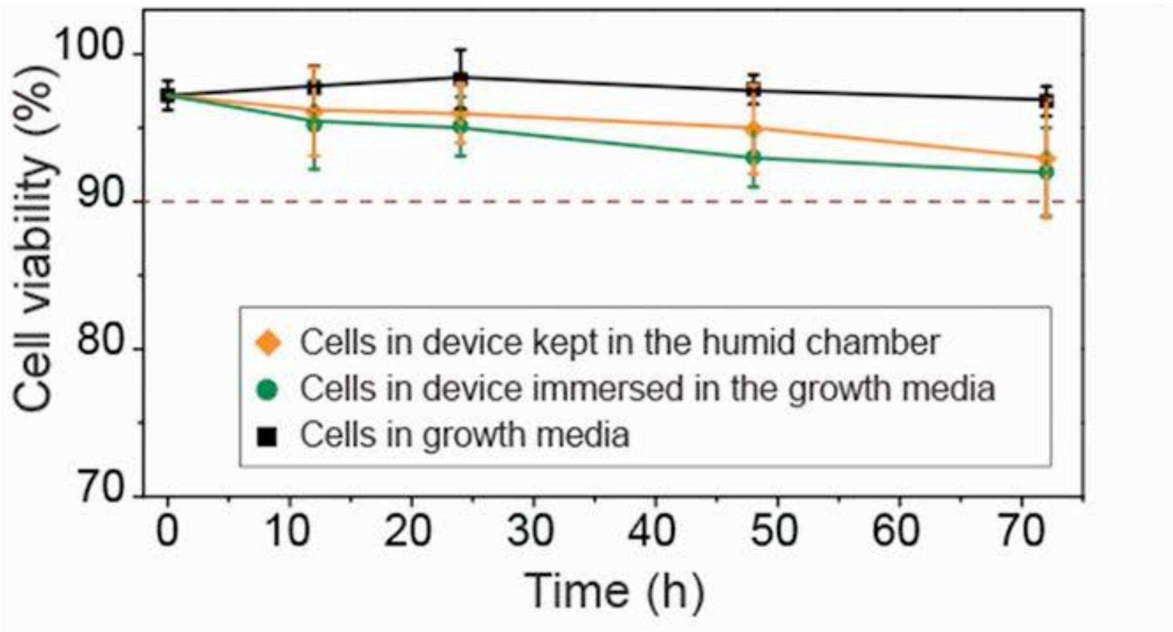
Yeast growth

Stretchable living materials with programmed cells

Concept of fabrication

hydrogel = polyacrylamide+alginate
elastomer = PDMS

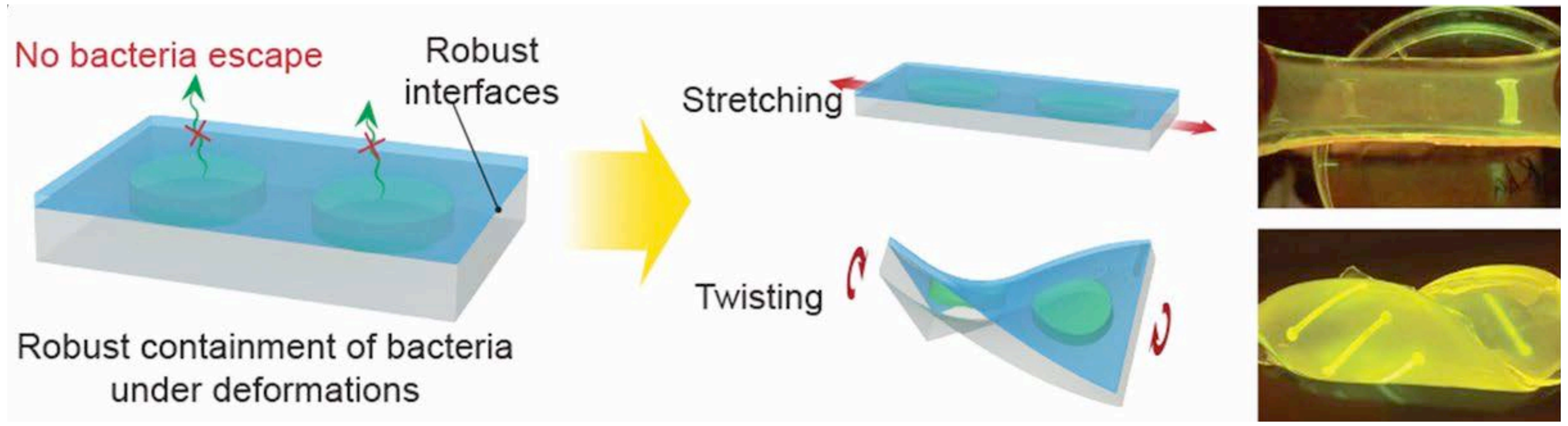




Cell viability

Key capabilities:

- containment
- deformability



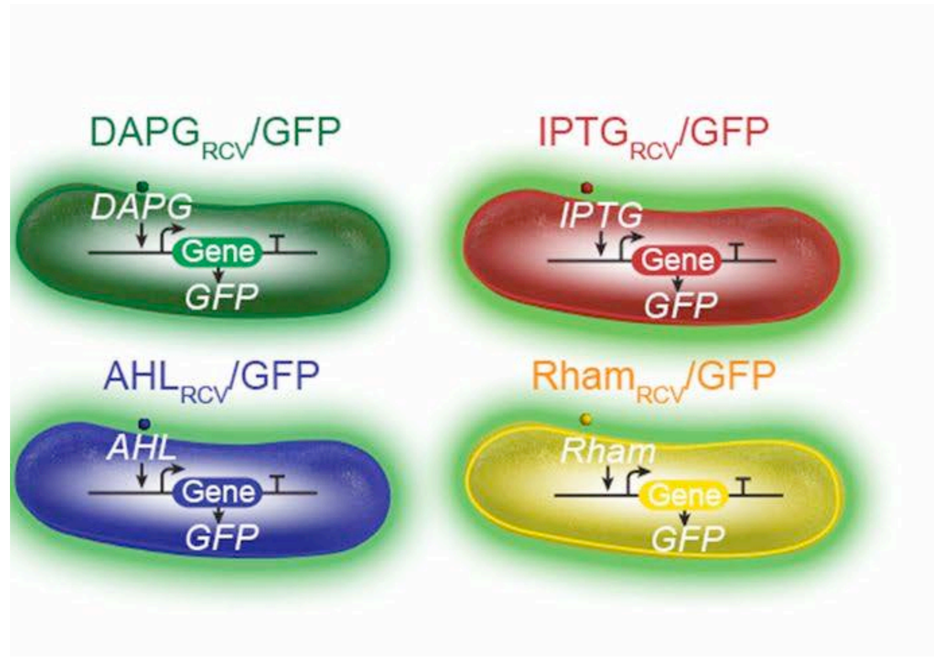
https://www.pnas.org/doi/full/10.1073/pnas.1618307114

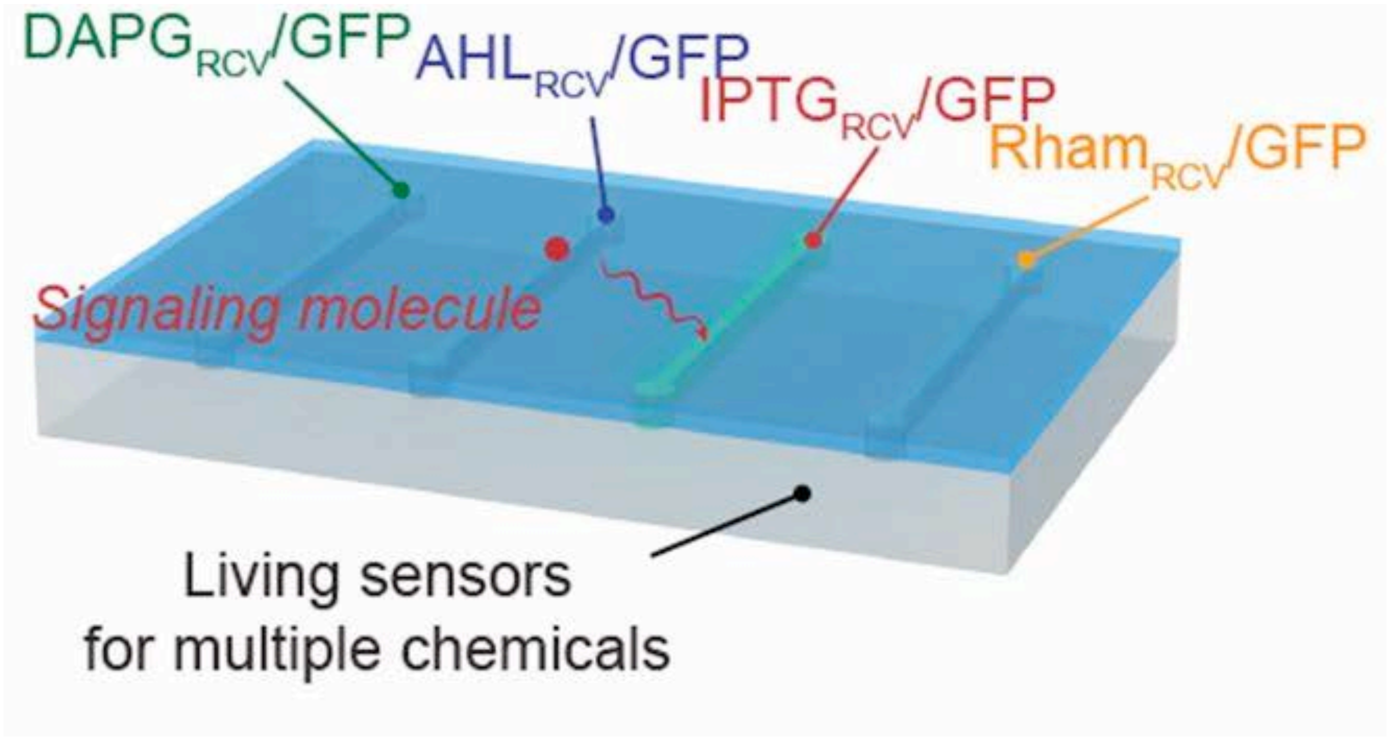
Living sensor: detection of chemicals

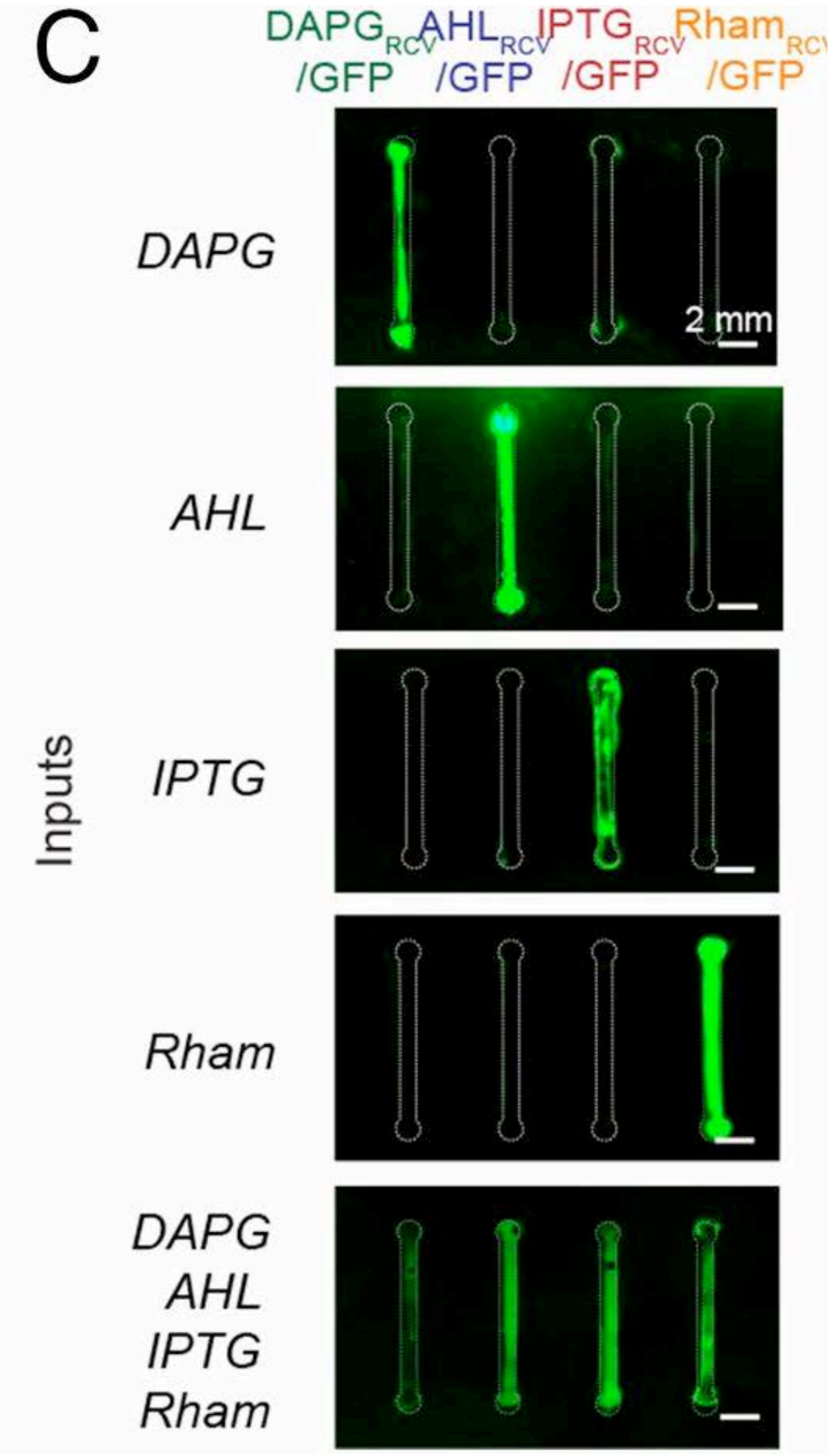
4 engineered bacterial strains

tune gene expresssion based on input

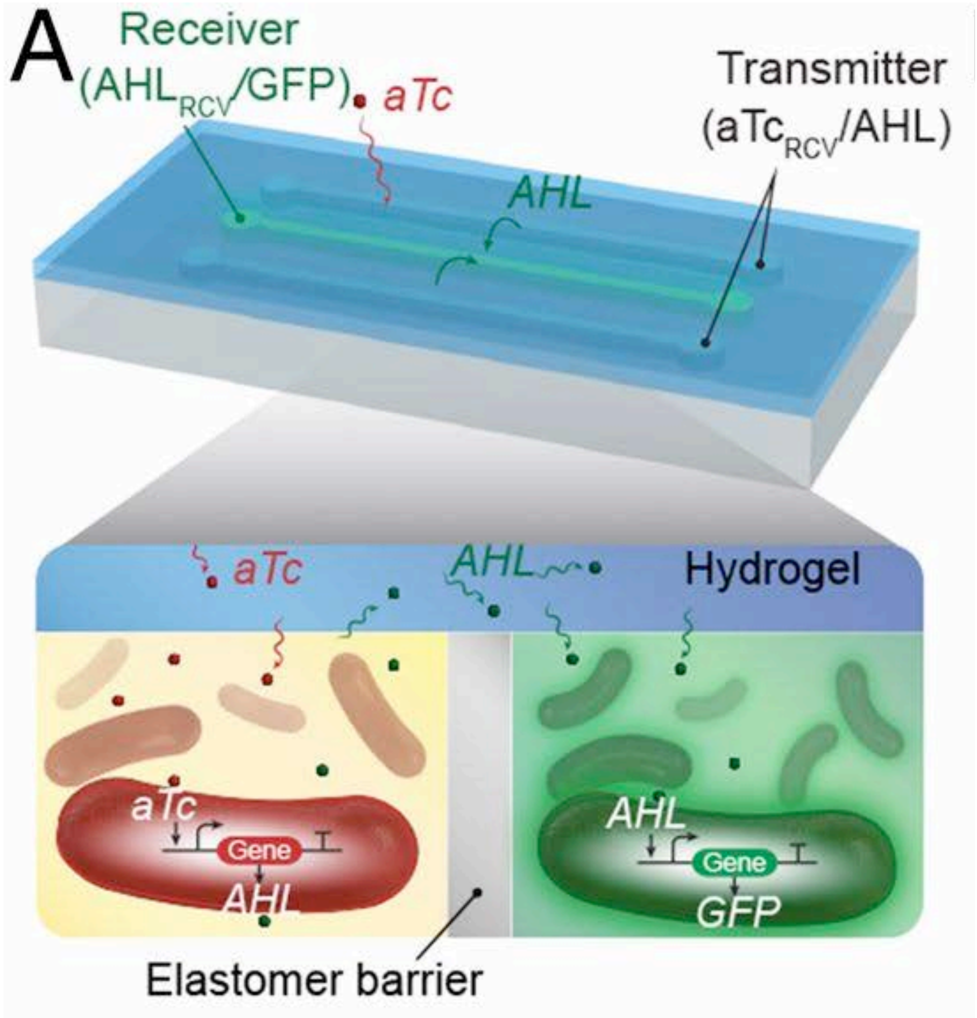
responsive to distinct chemicals

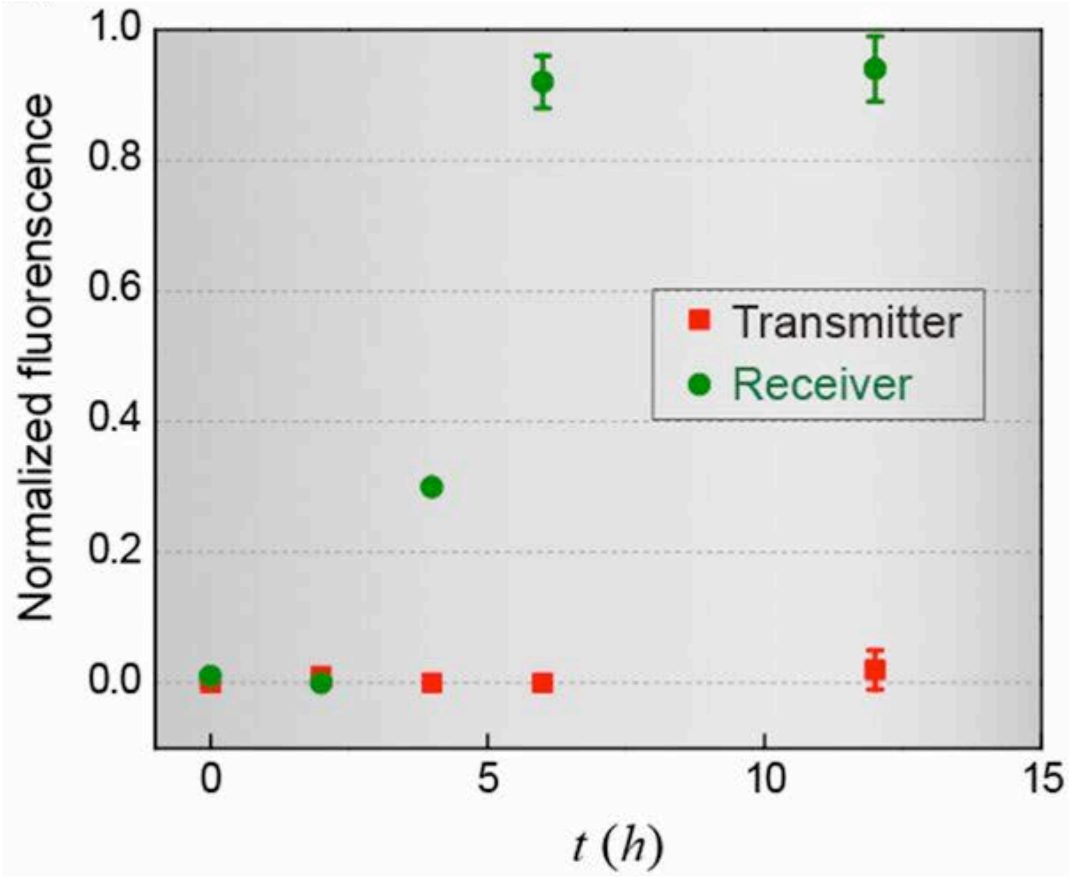


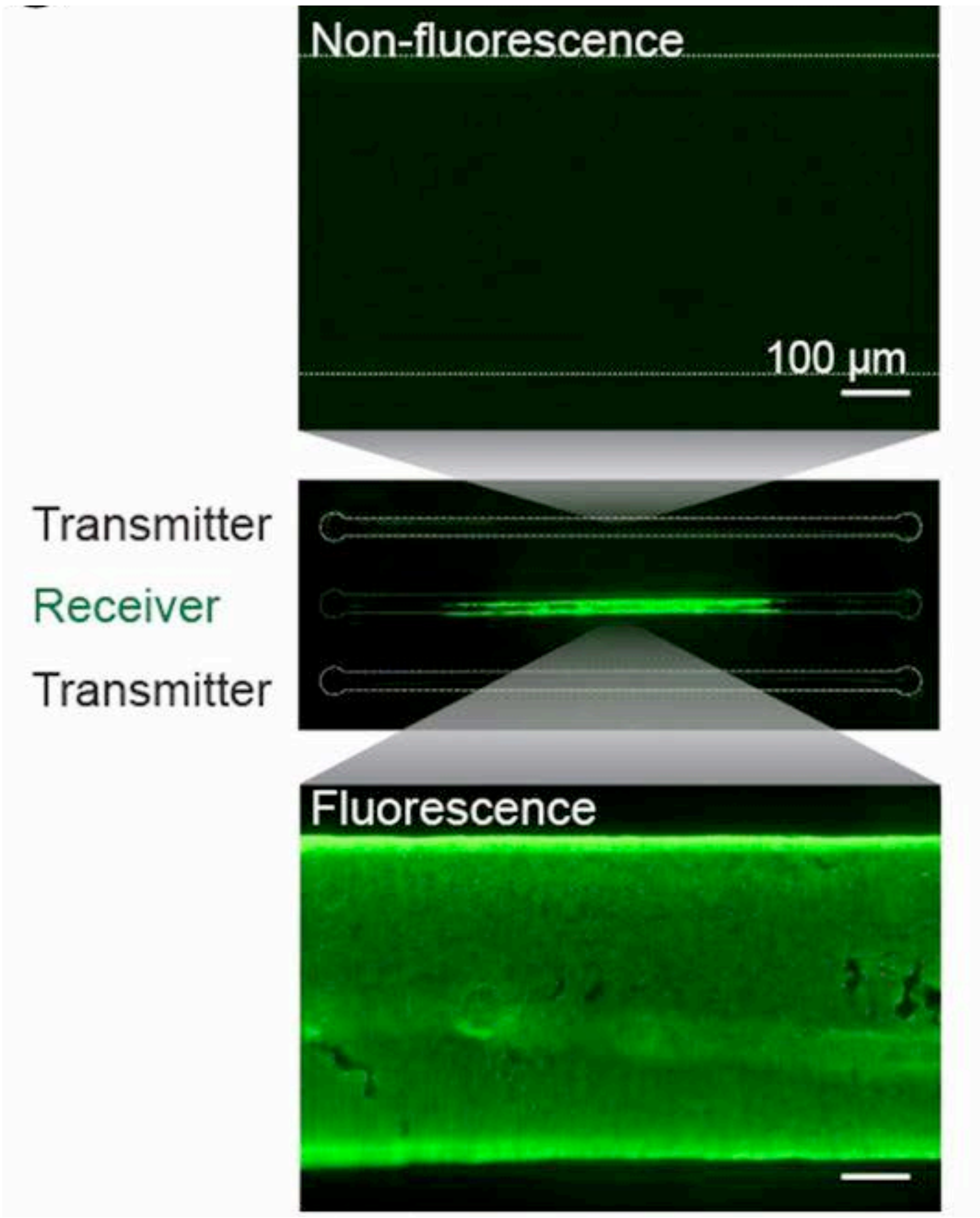




Interactive gene circuit

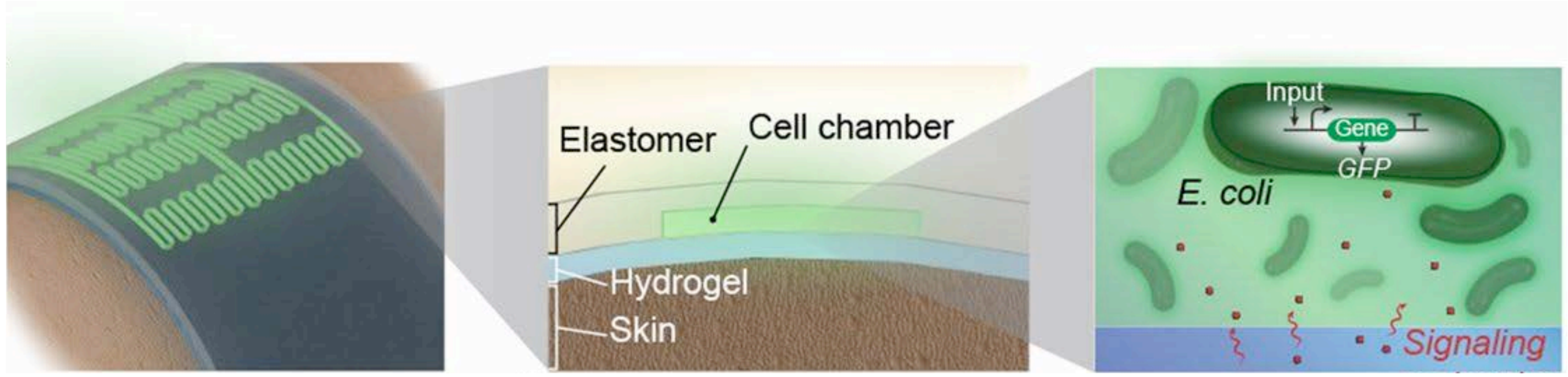


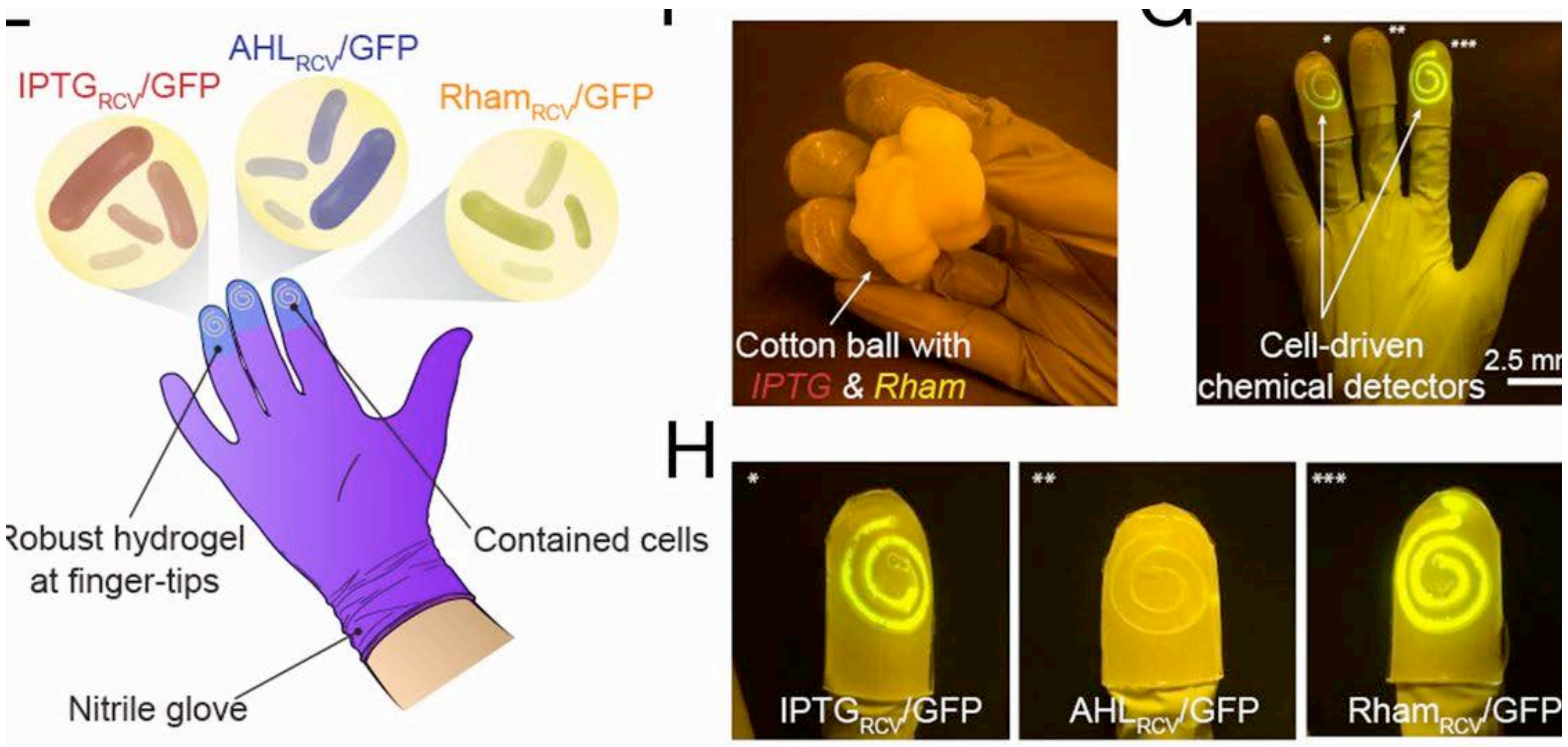




cells within hydrogels can interact via diffusion of chemical signals

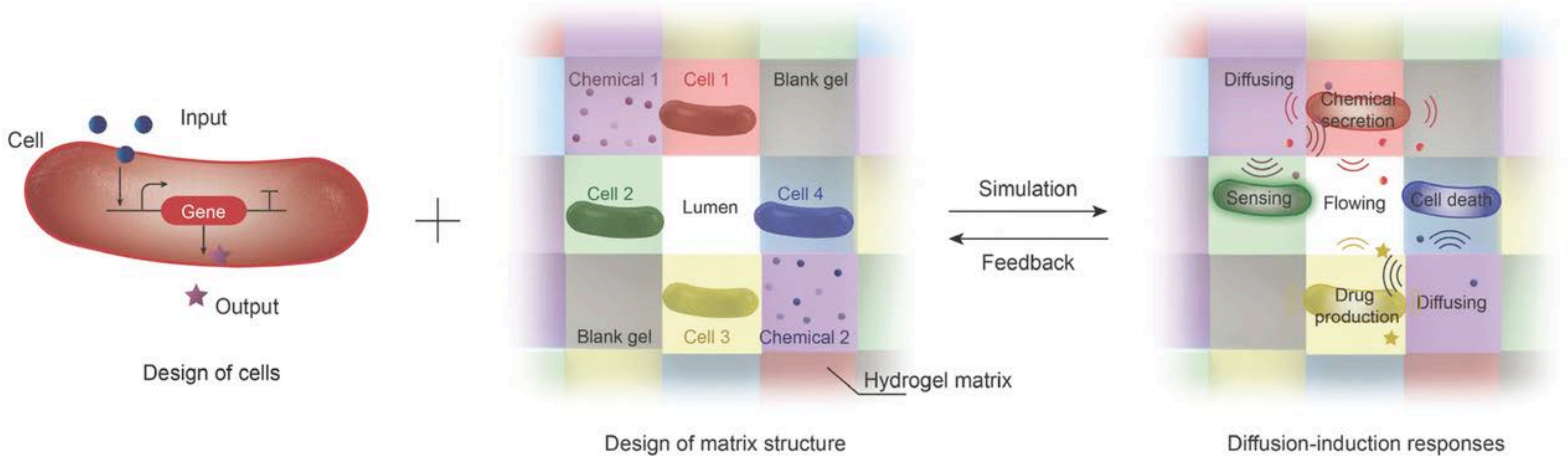
Living wearable device



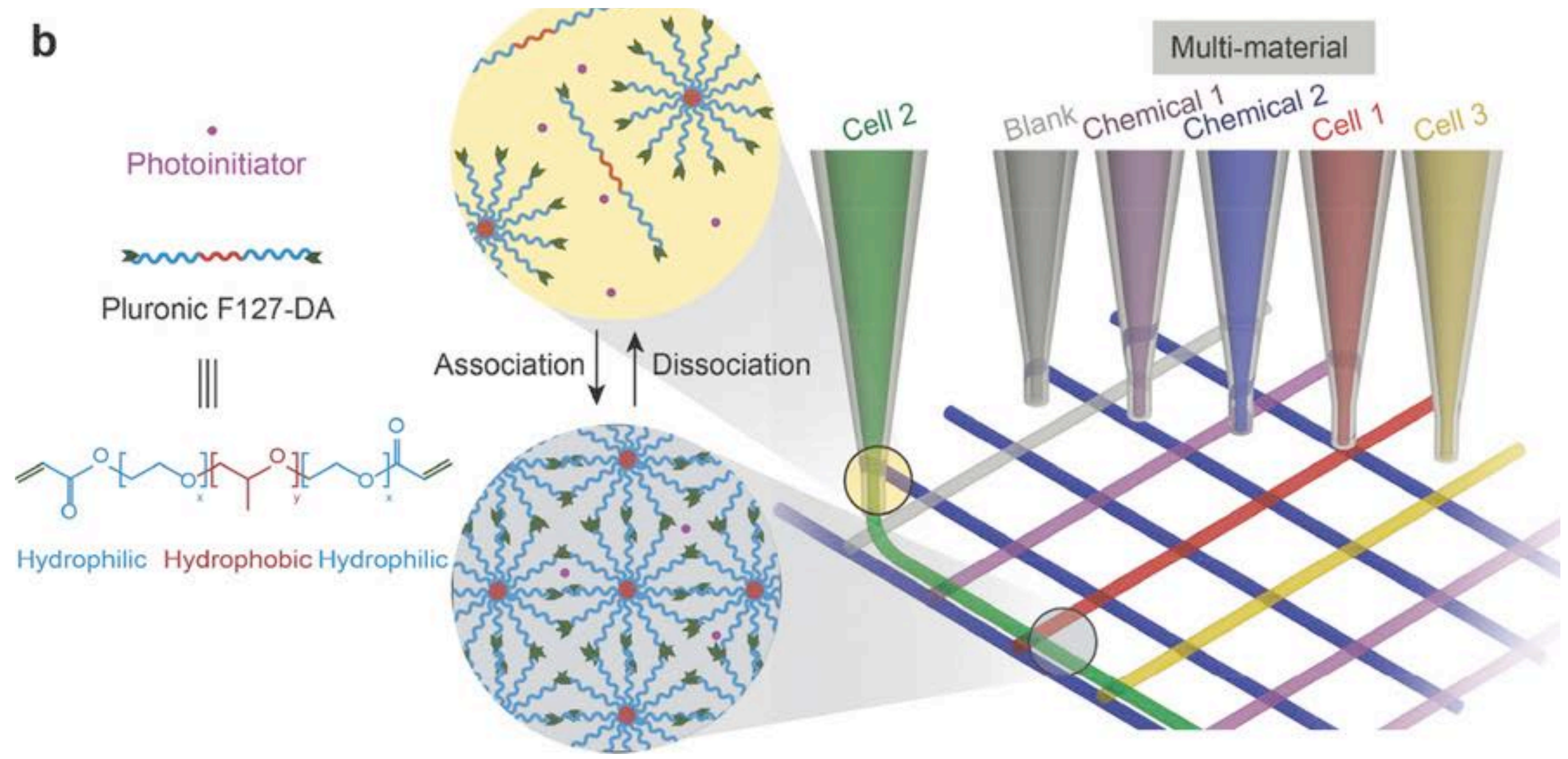


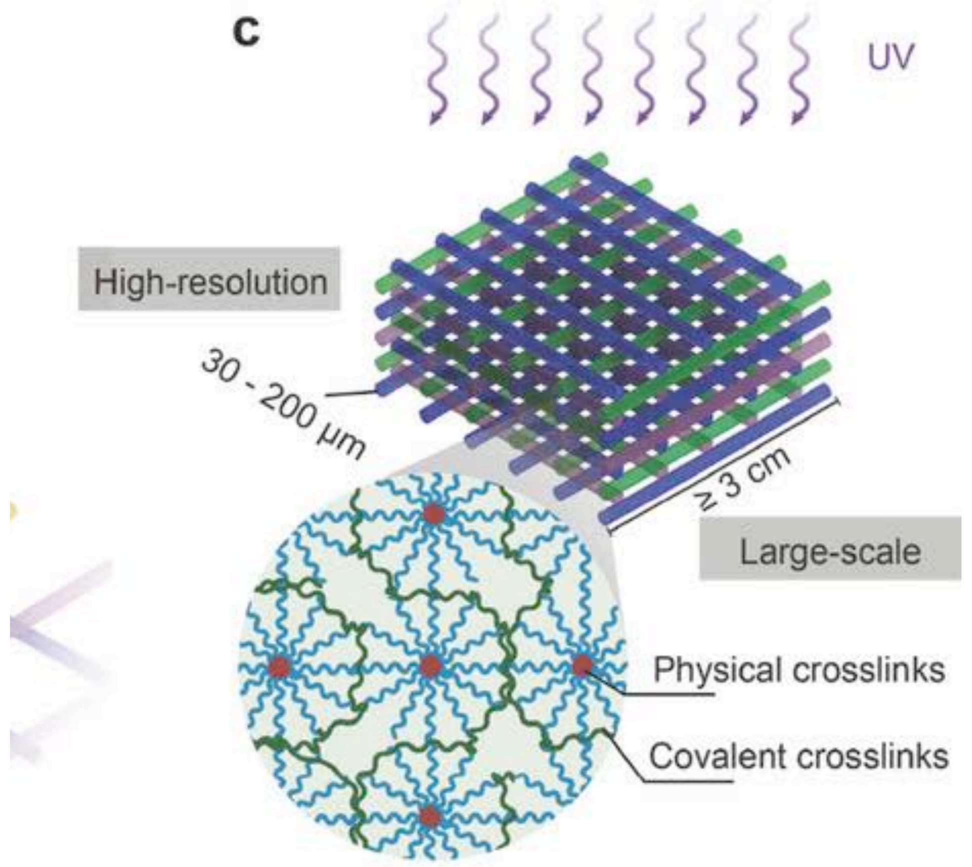
3D-printing of live biomaterials

Concept



Implementation

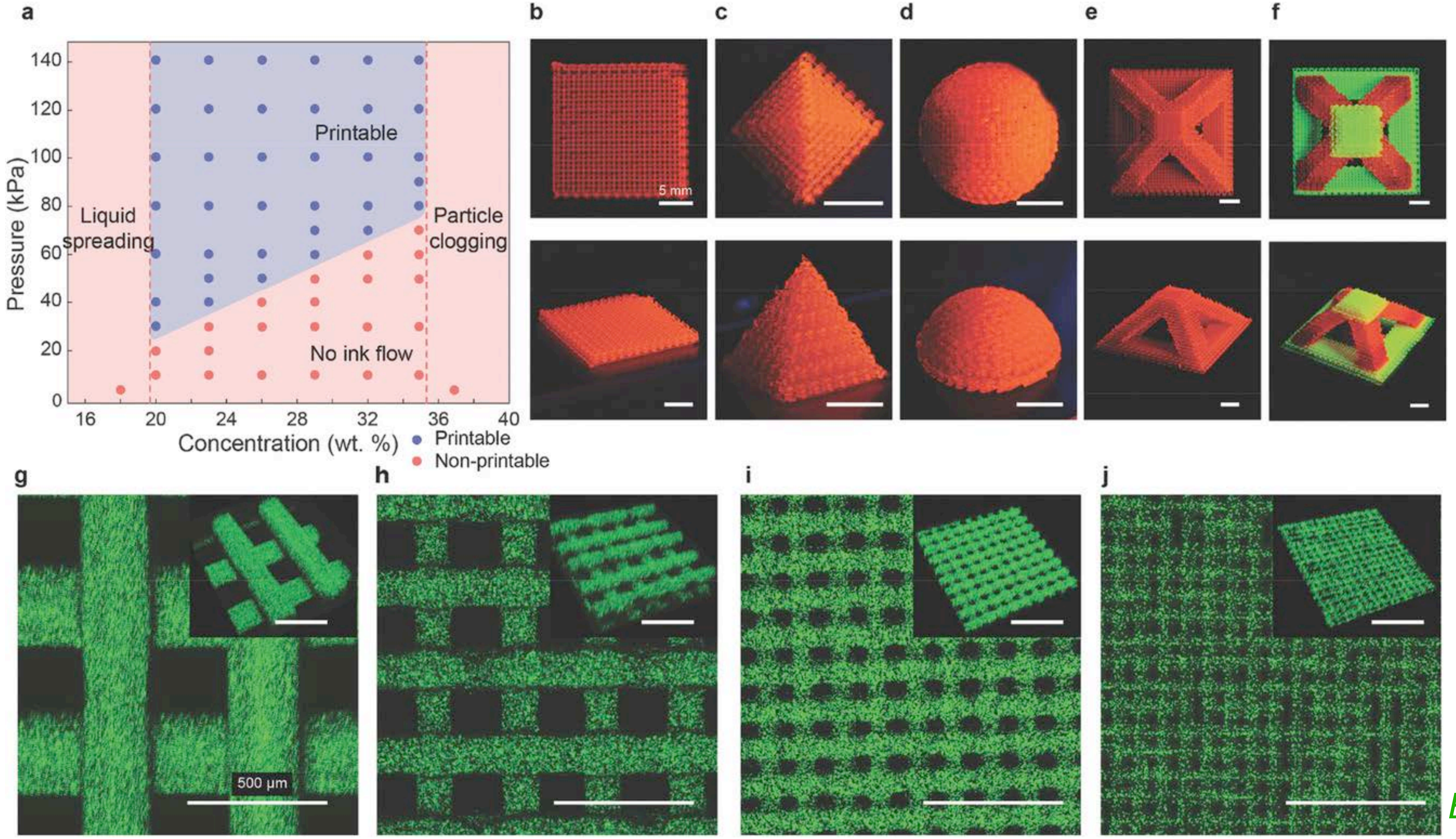




https://doi.org/10.1002/adma.201704821

3d-printing of bacteria-laden ink

Pluronic F127 diacrylate as ink

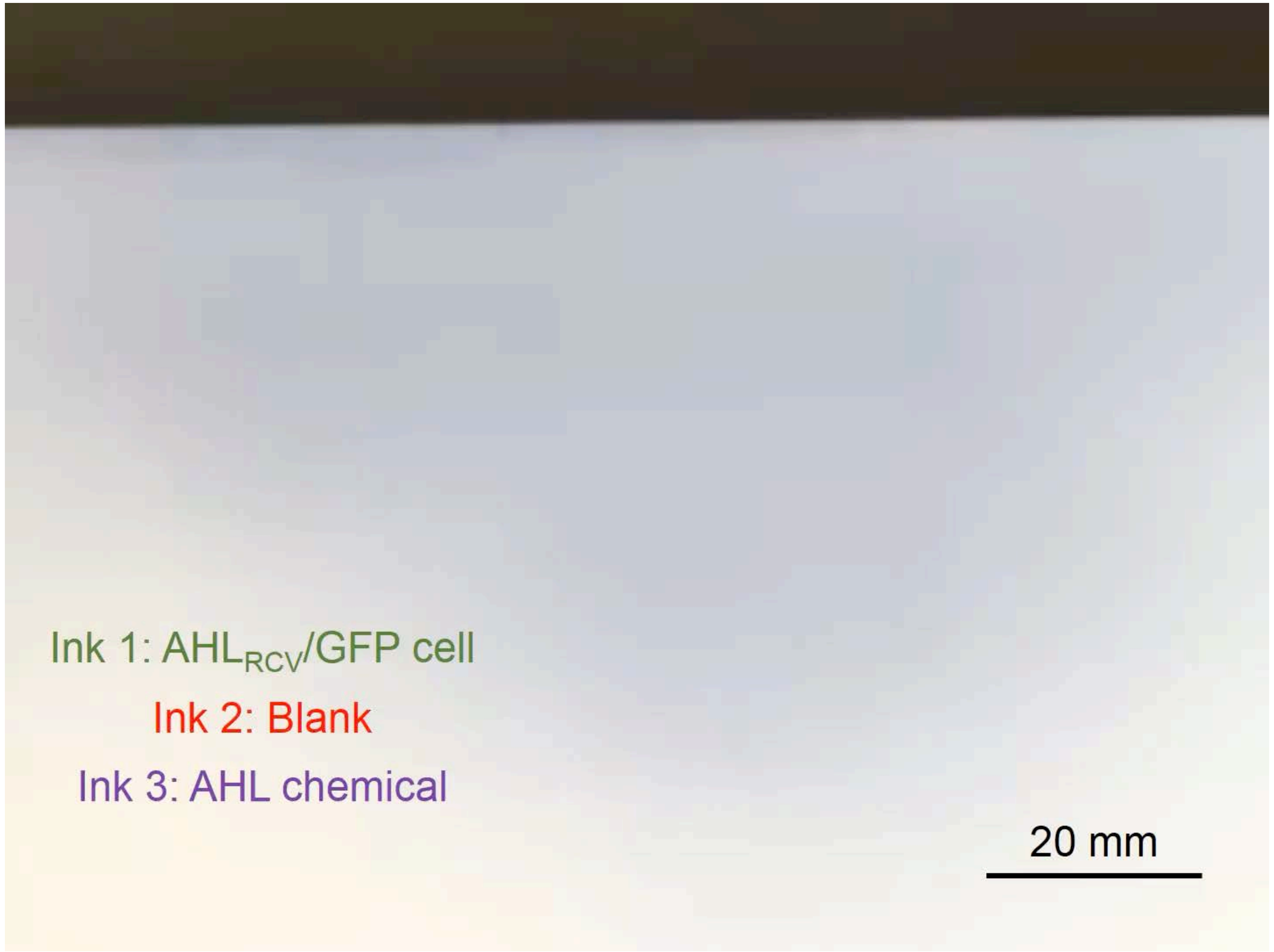


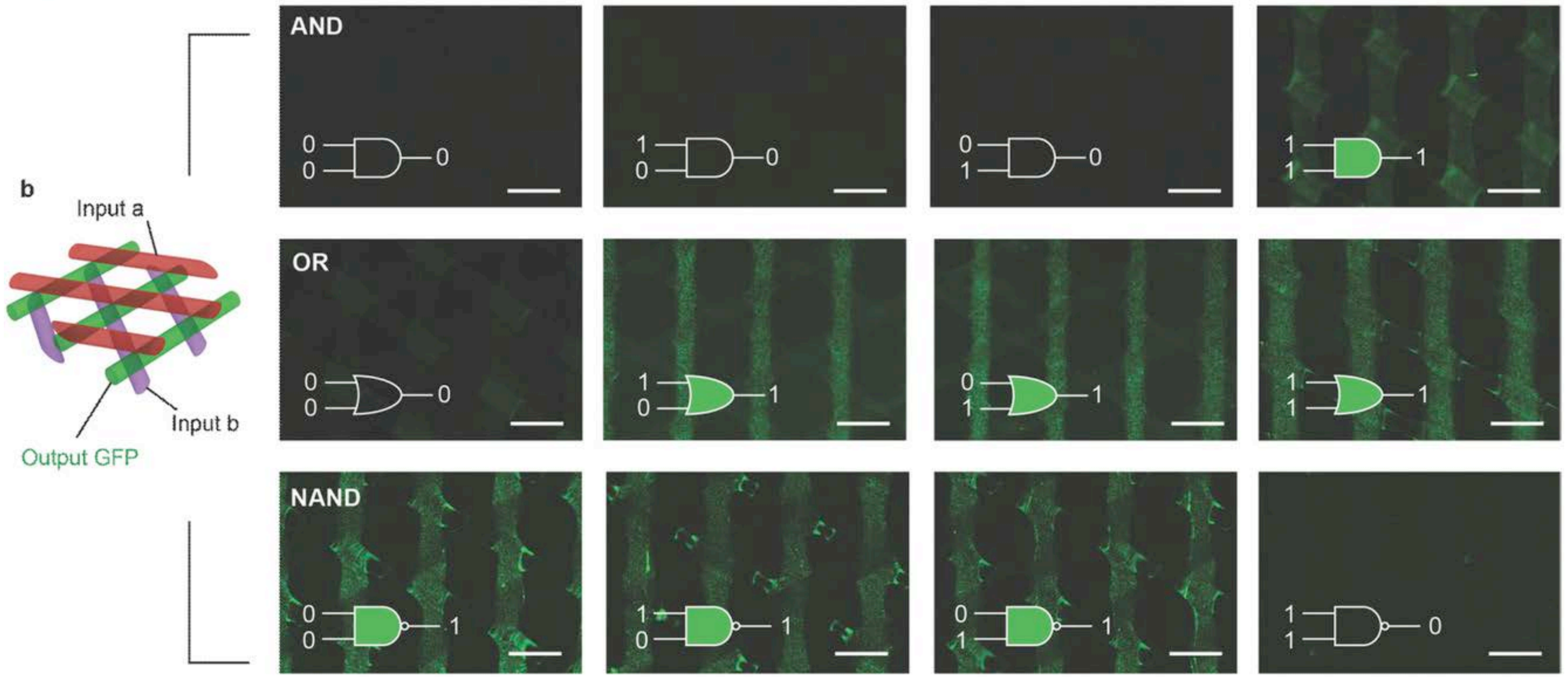
rhodamine

fluorescein

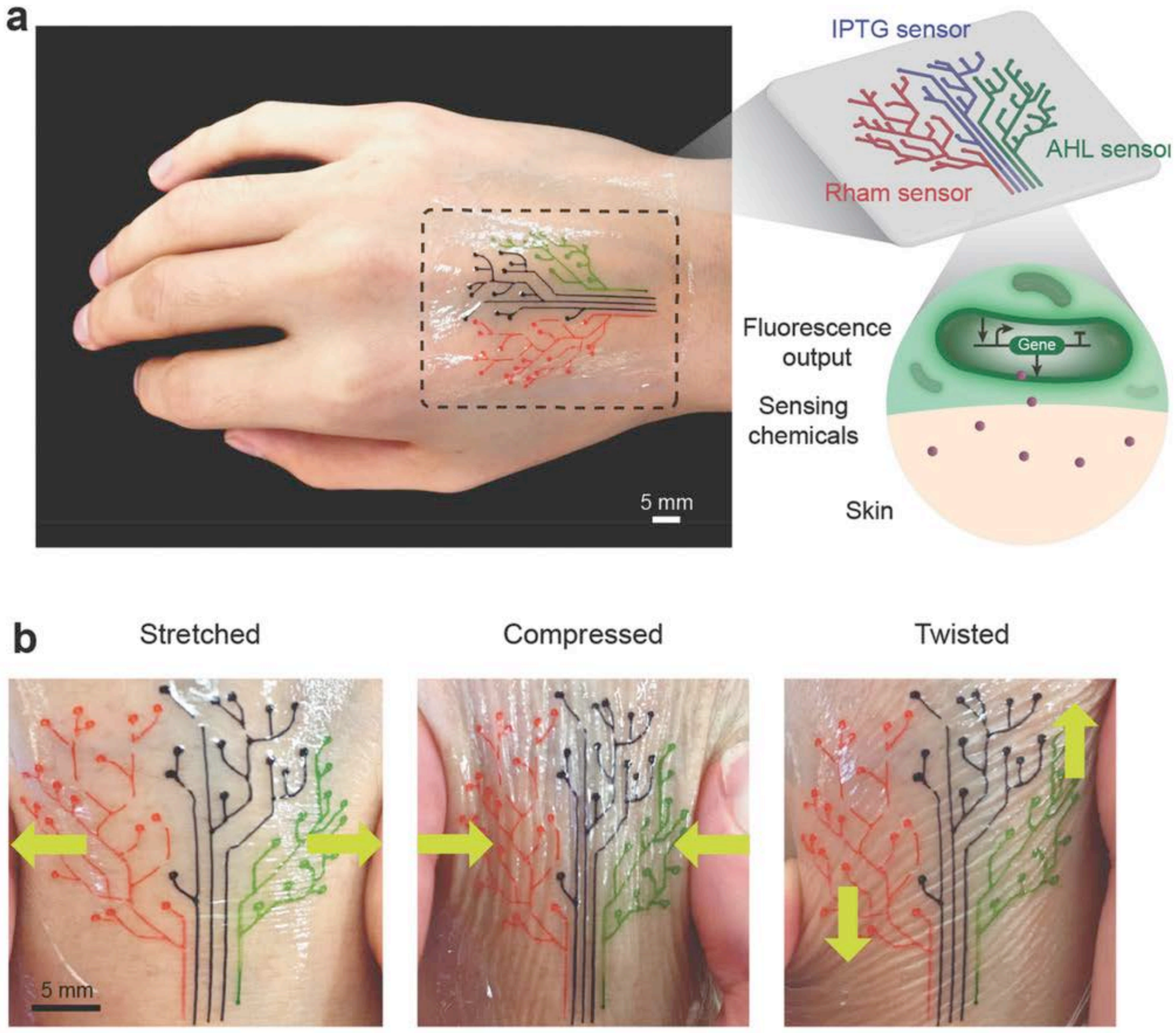
E. coli GFP

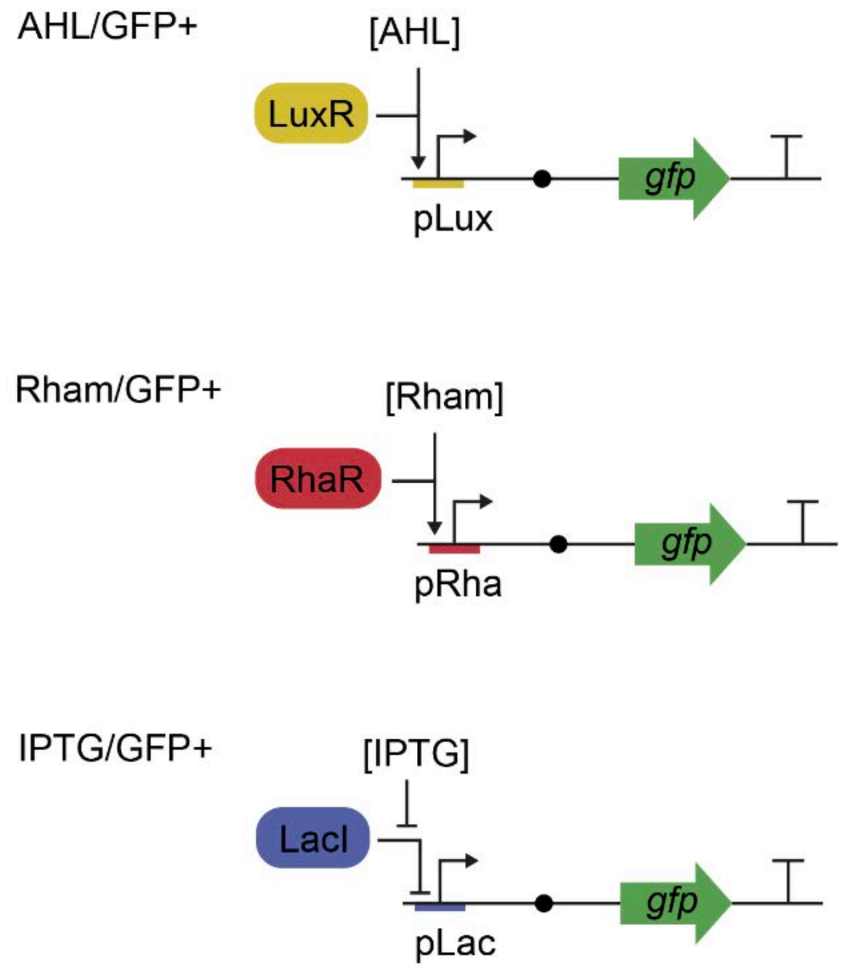
Implementation of logic gates 3D-printed biomaterial



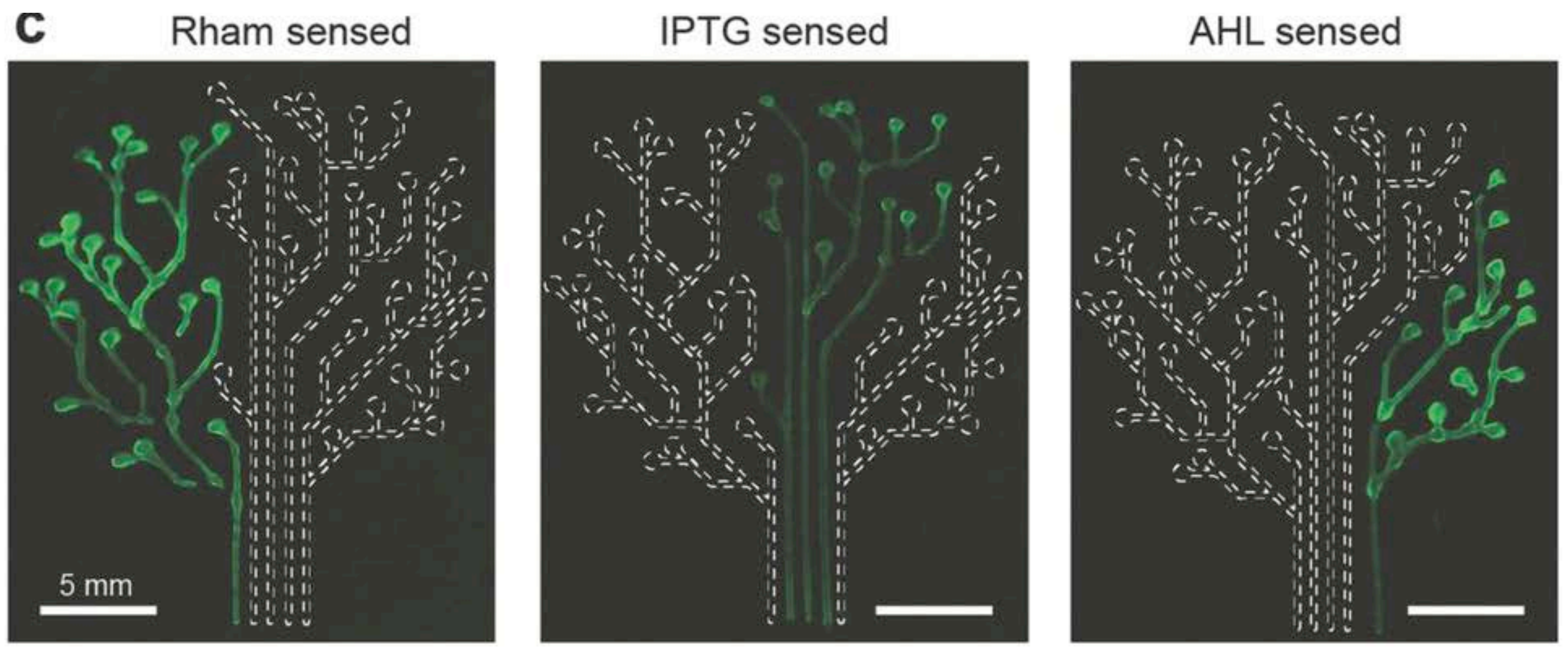


3D-printed living tattoo

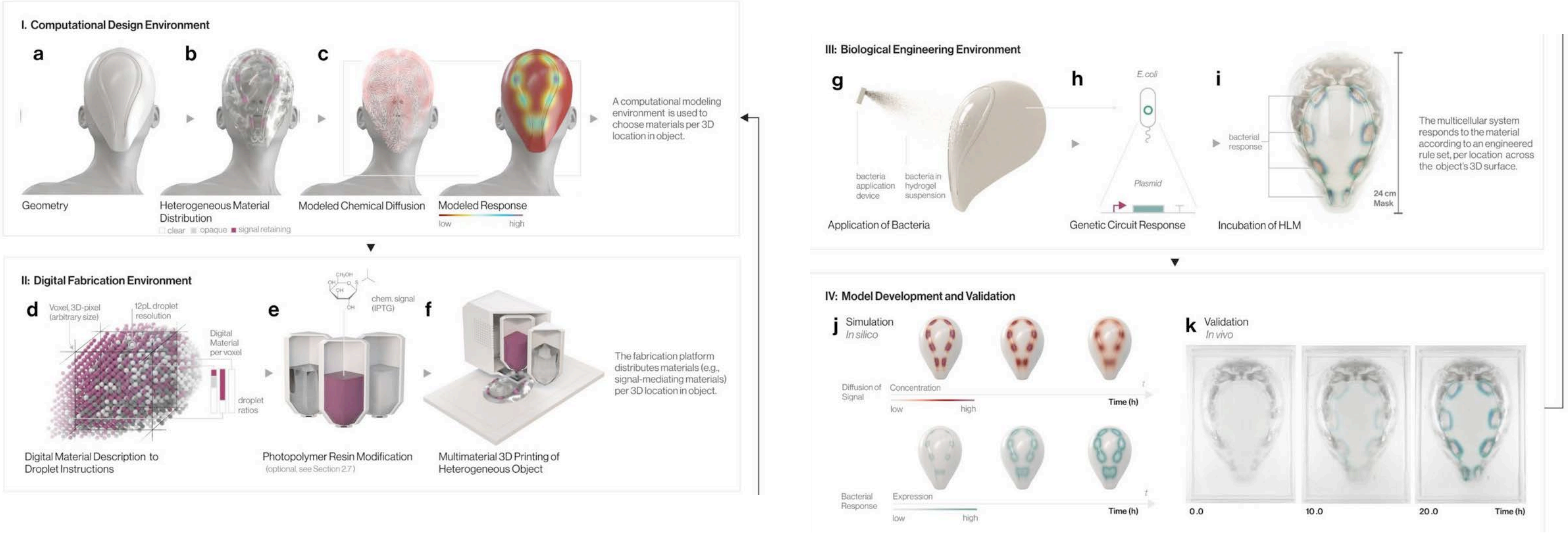




Signal introduced on skin



Scaling up: living biohybrid material

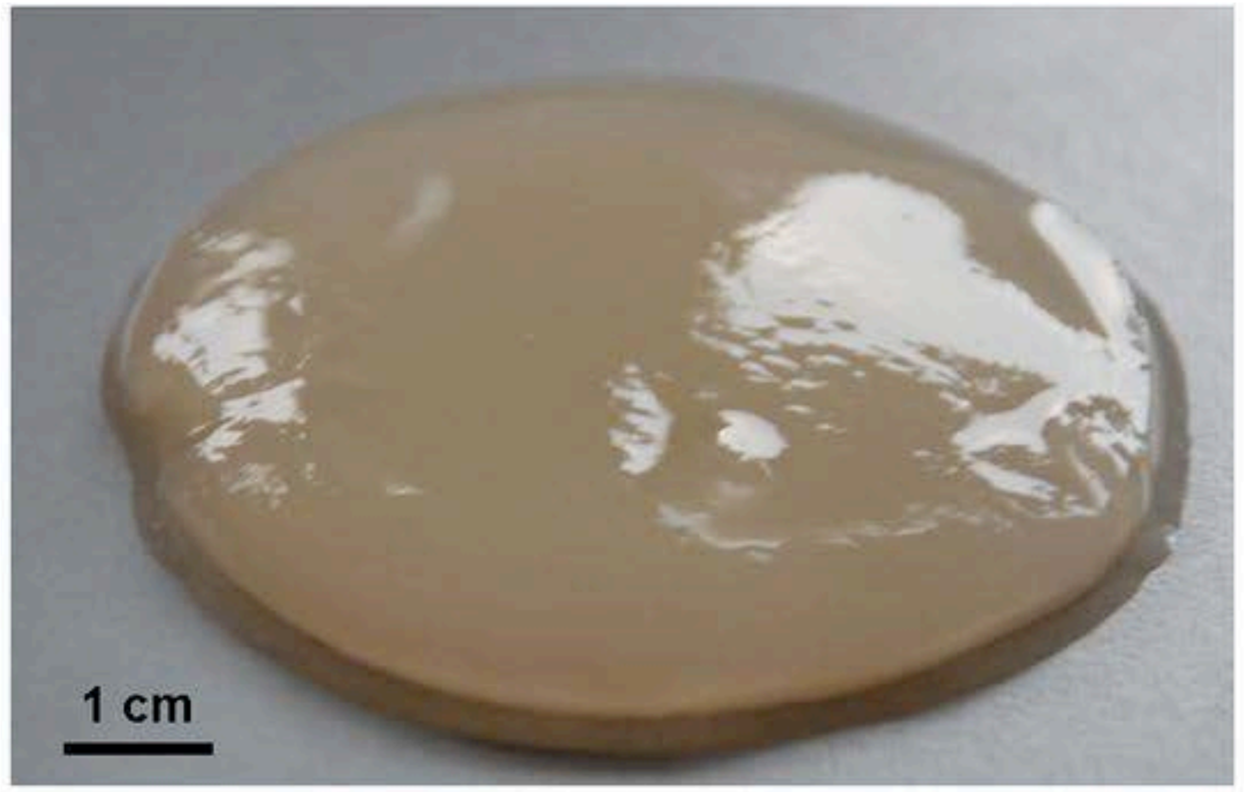


https://doi.org/10.1002/adfm.201907401

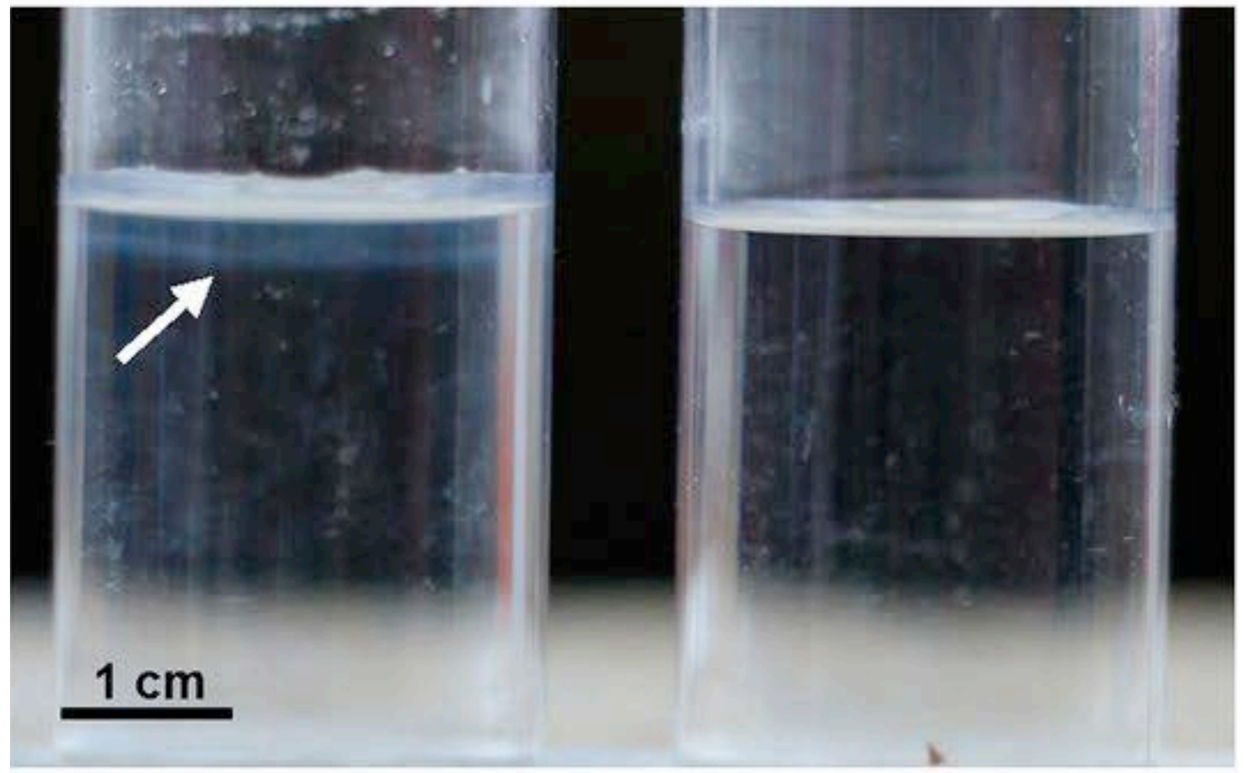
https://www.media.mit.edu/projects/hybrid-living-materials/overview/

Living materials with self regeneration: cellulose

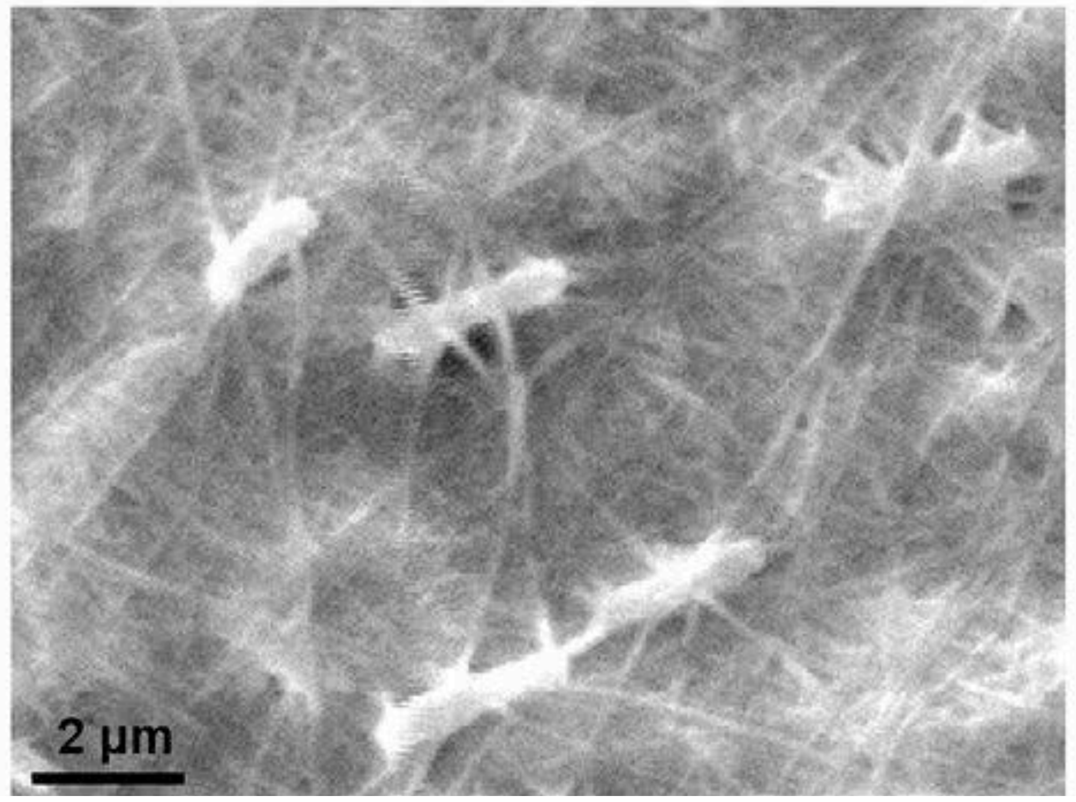
Bacterial cellulose production



Komagataeibacter rhaeticus forms a pellicle of cellulose



K. rhaeticus *E. coli*

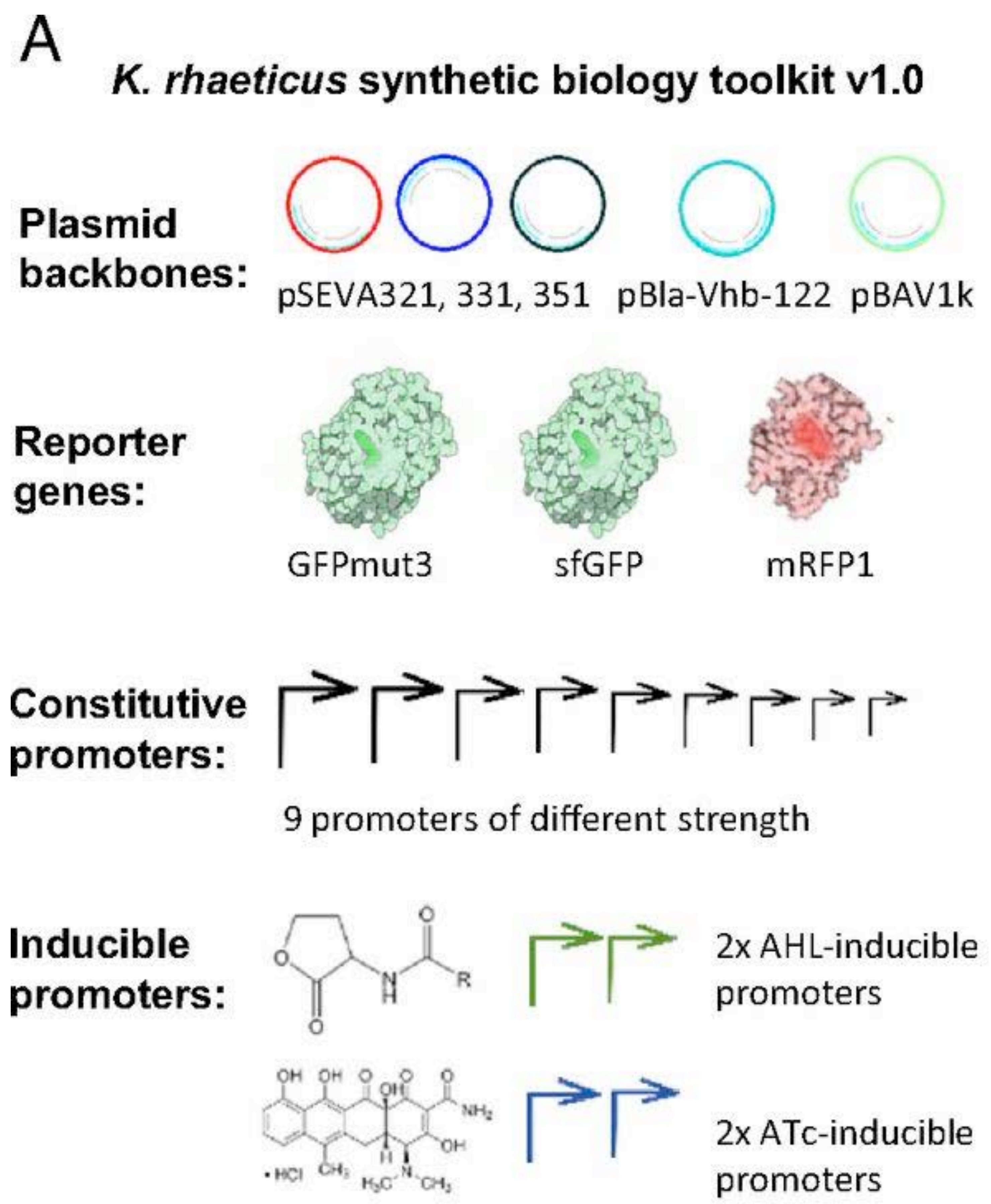


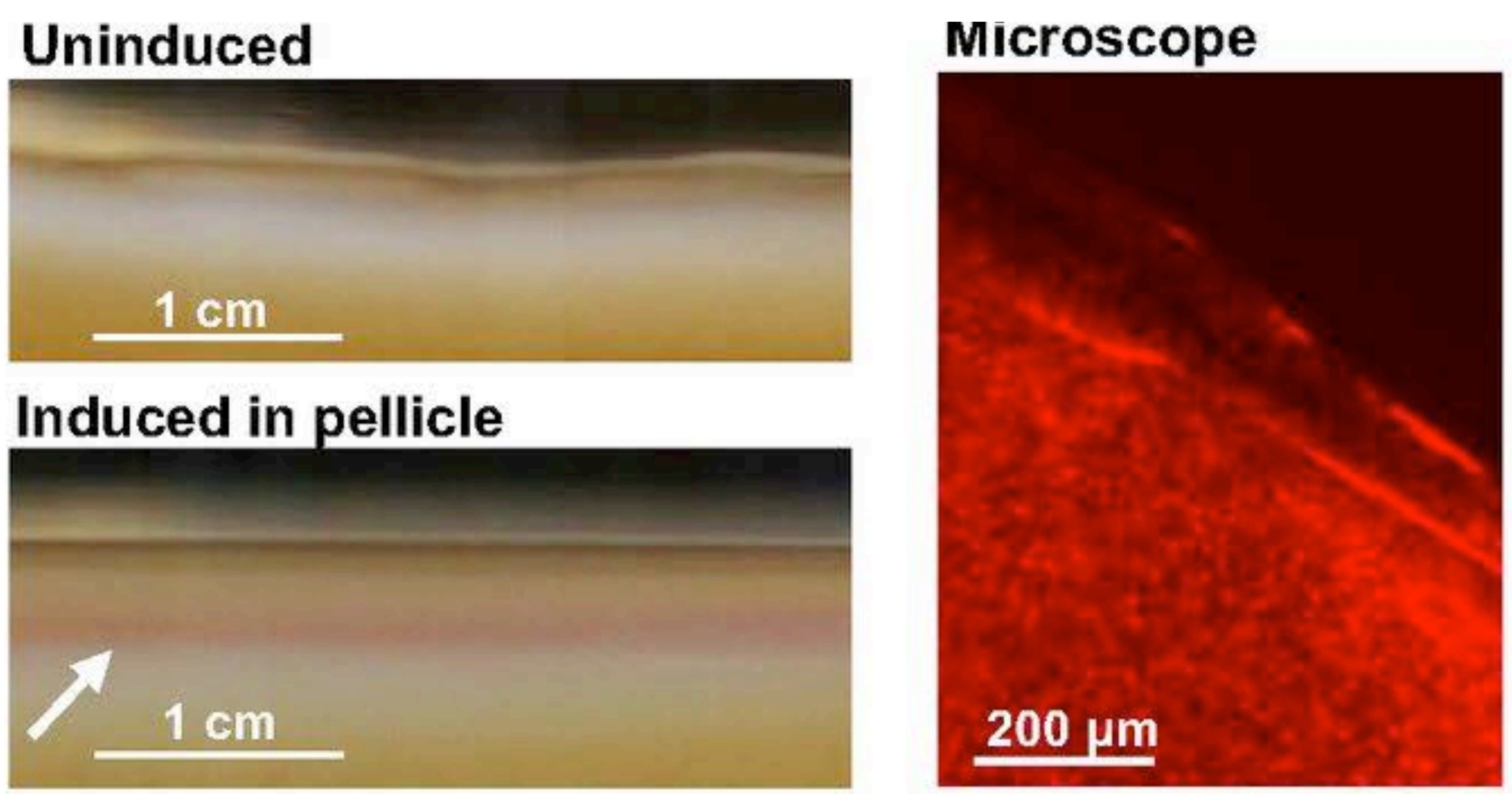
SEM of cellulose pellicle

https://www.pnas.org/doi/full/10.1073/pnas.1522985113

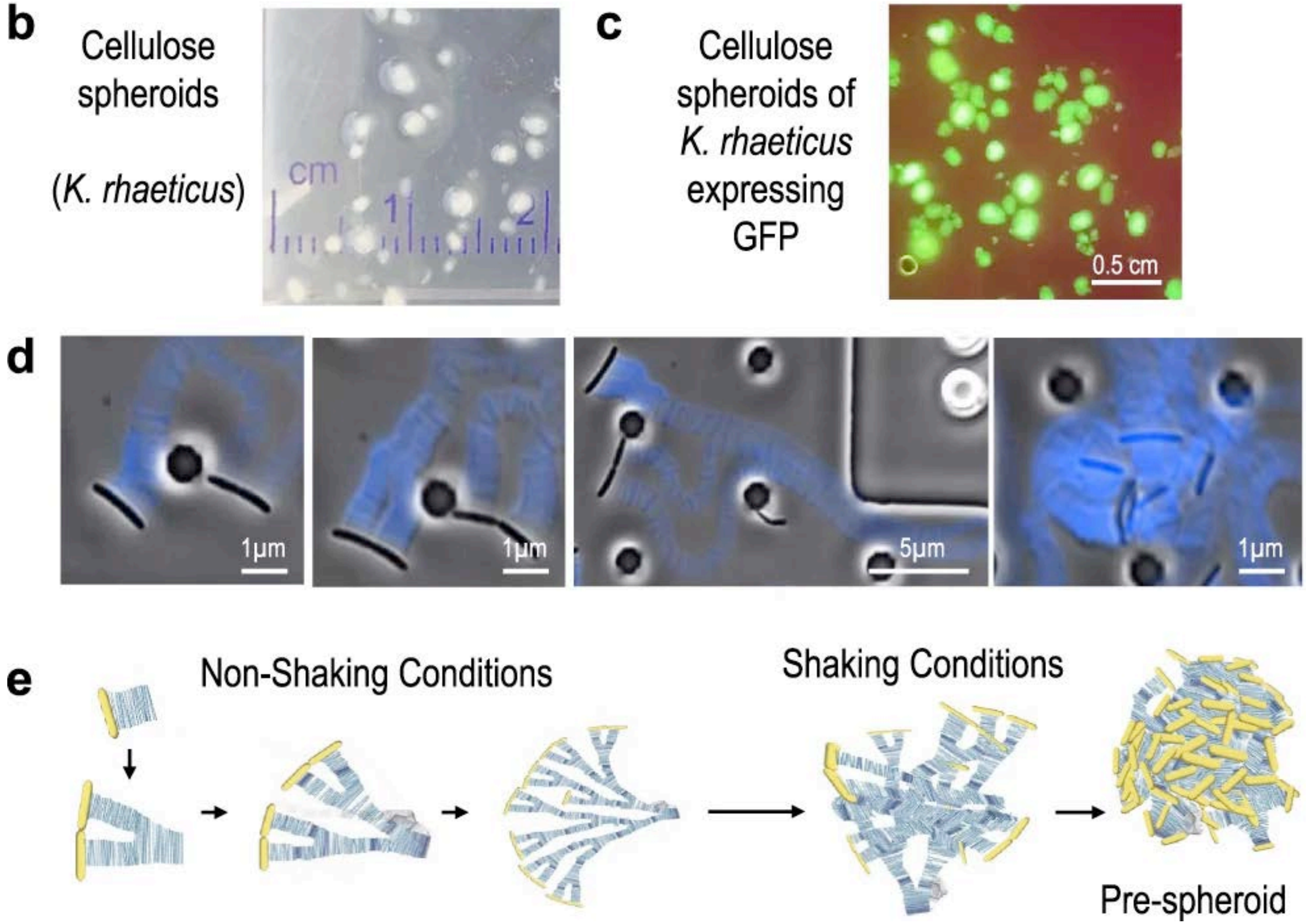
A synthetic biology toolkit for *K. rhaeticus*

It is not a model organism > hard to engineer

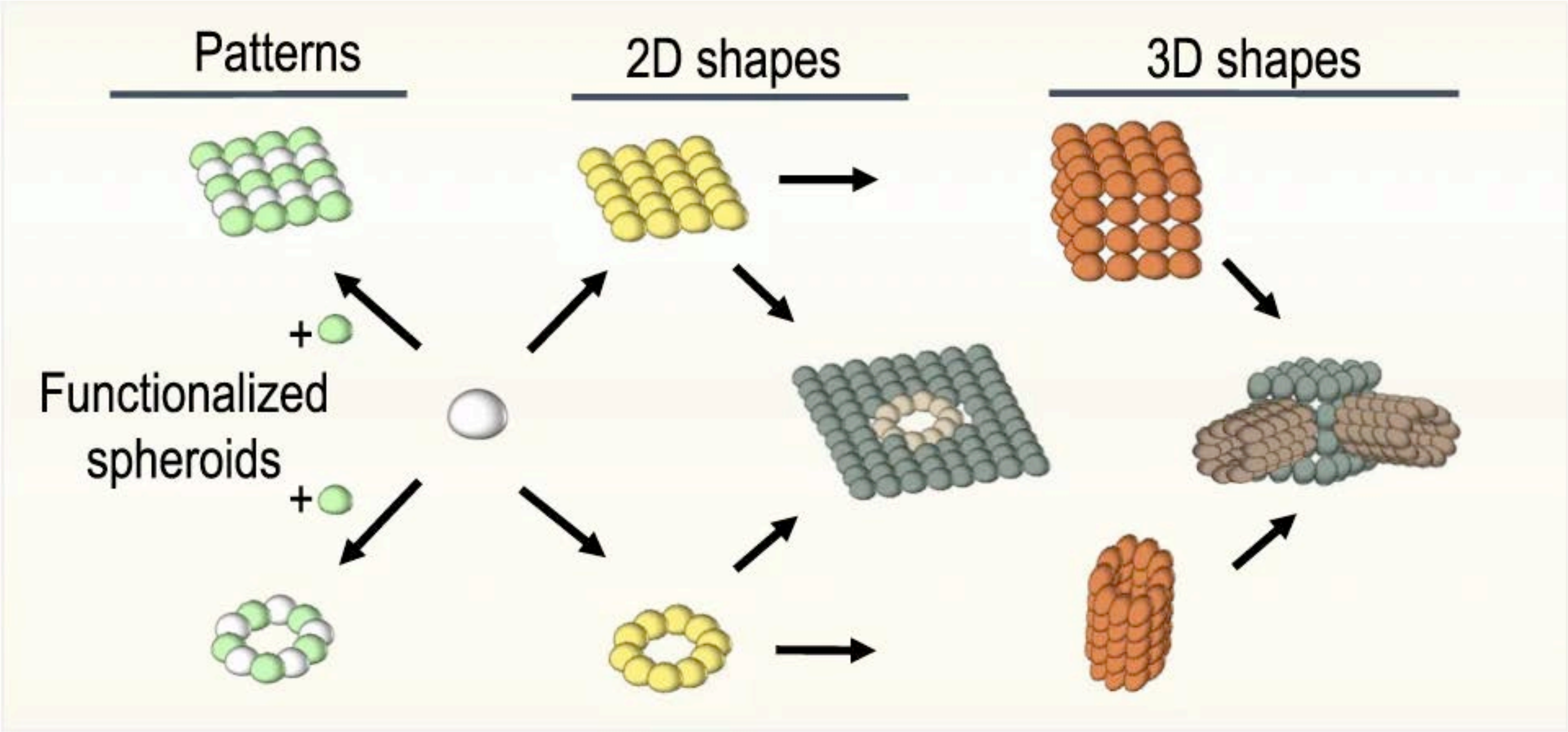




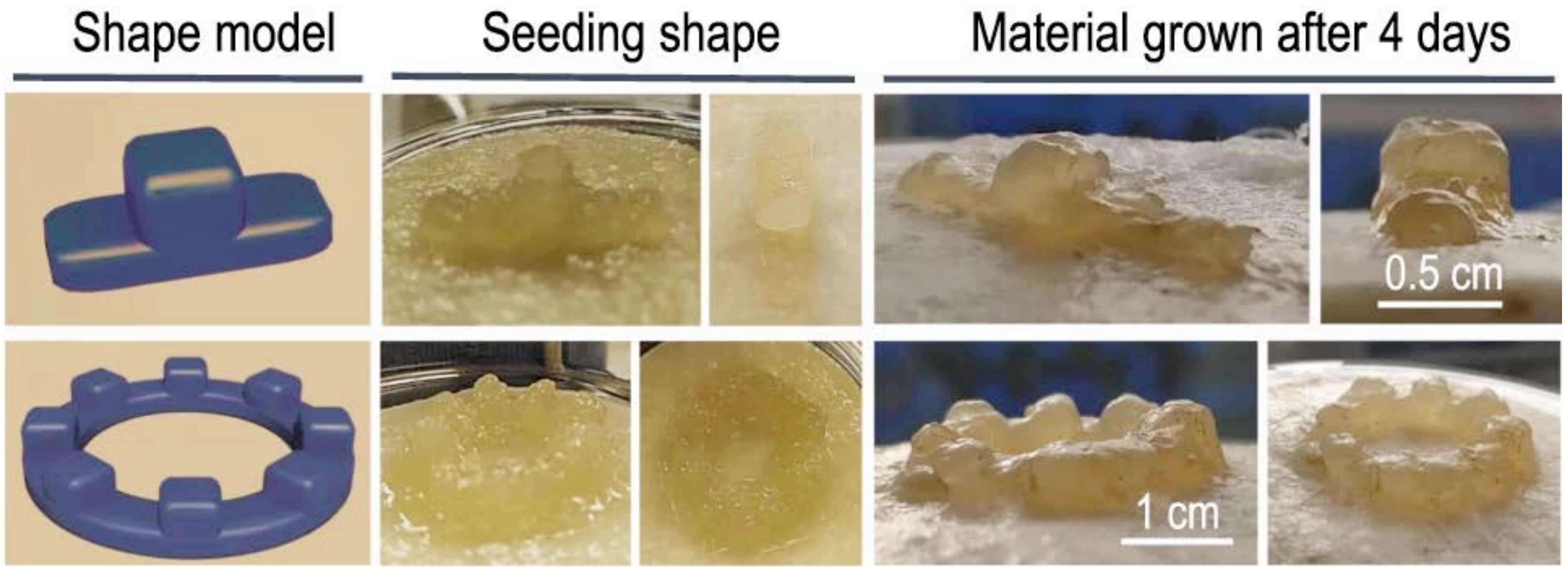
Production of living cellulose spheroids



Spheroids as building blocks

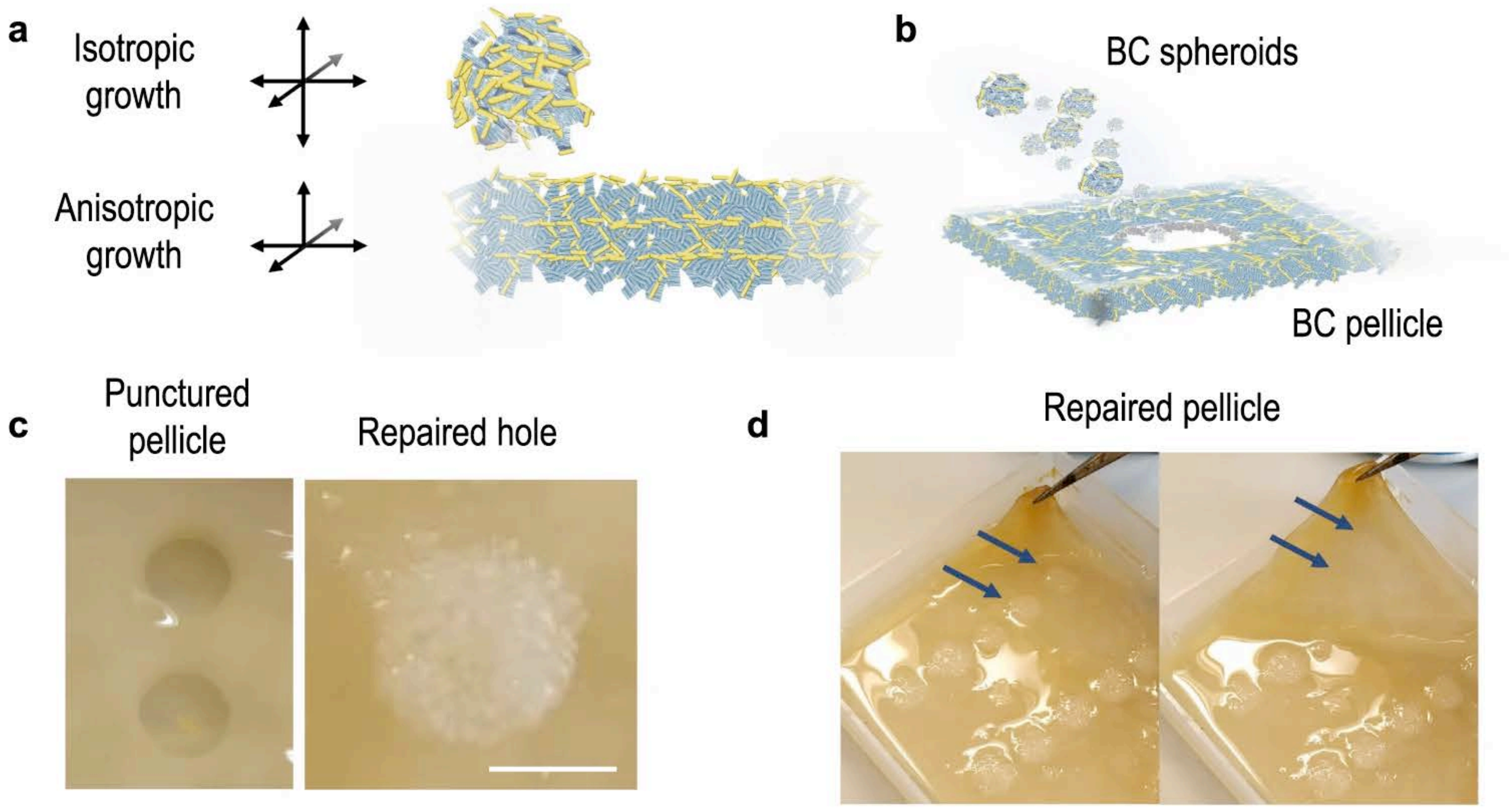


(manual assembly)

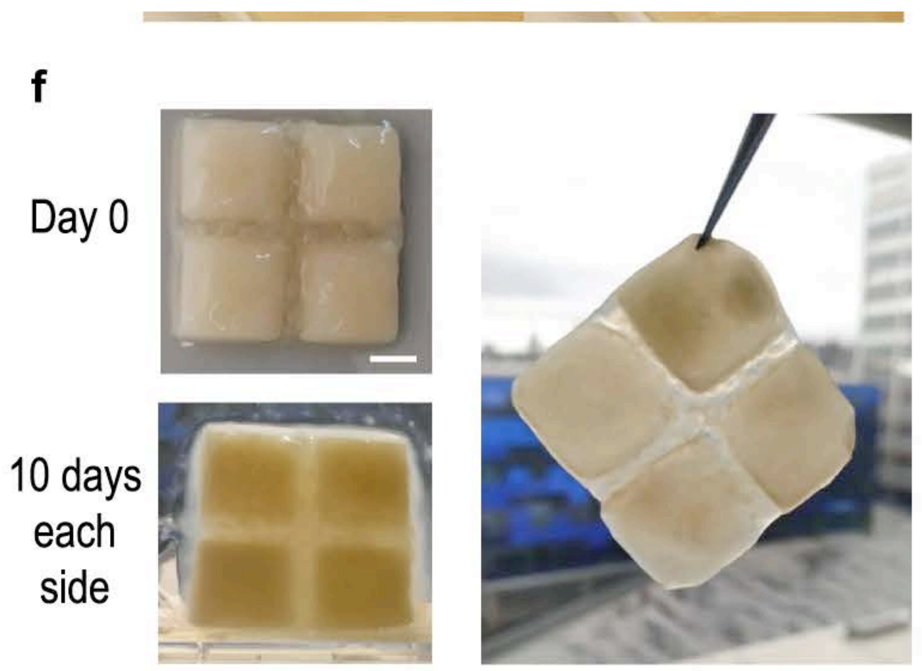


Material self-healing

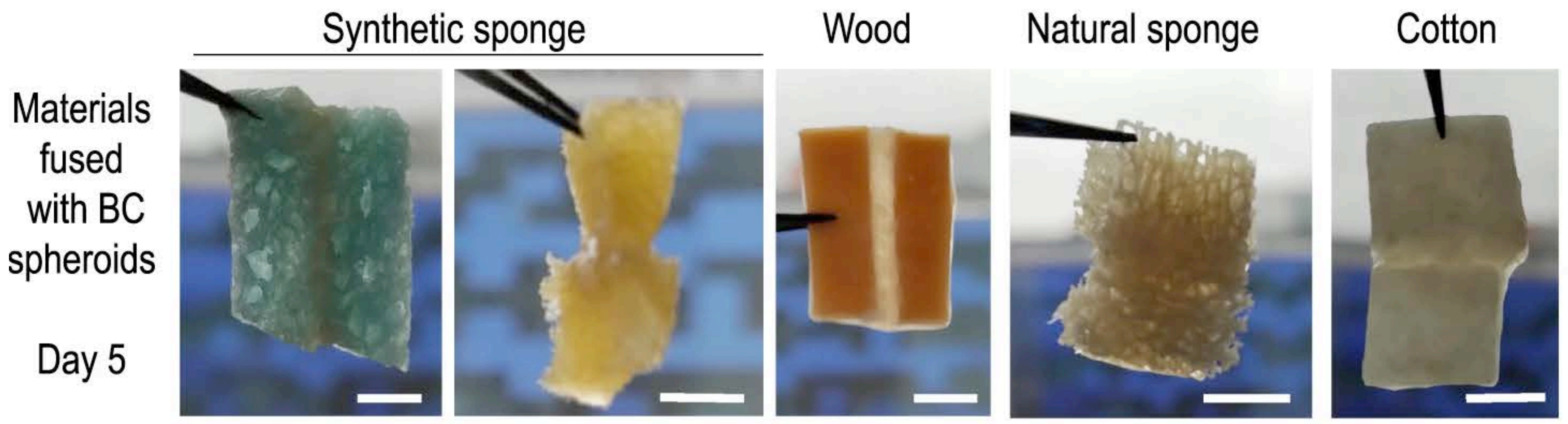
repair



fusion: cellulose patches



fusion: hybrid materials



A great living material, but spatial, temporal and biological control remains complex

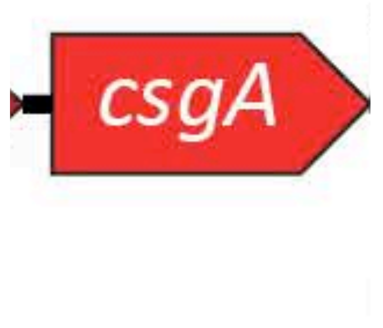
Scaling up bacterial cellulose production

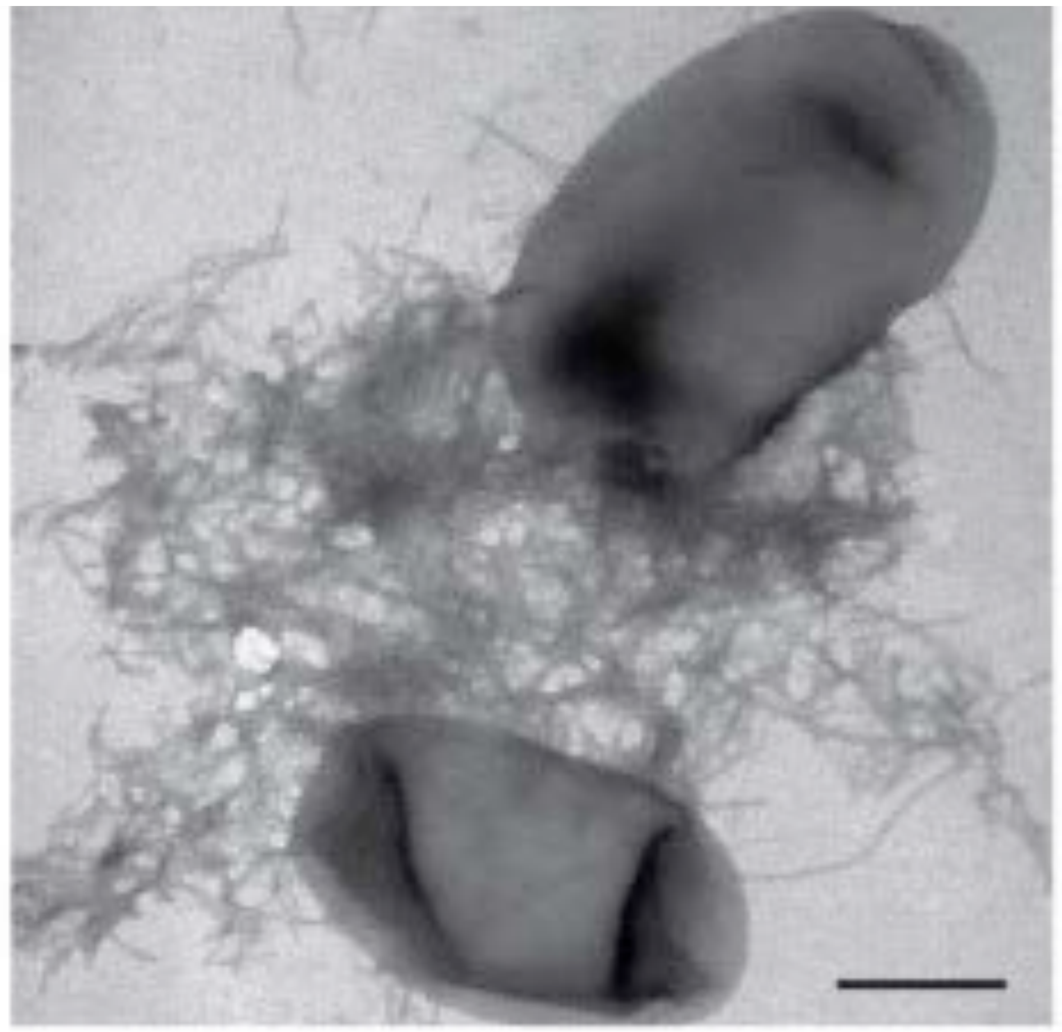
Improving living material precision: turn to protein-based materials

Remember the amyloid filaments?

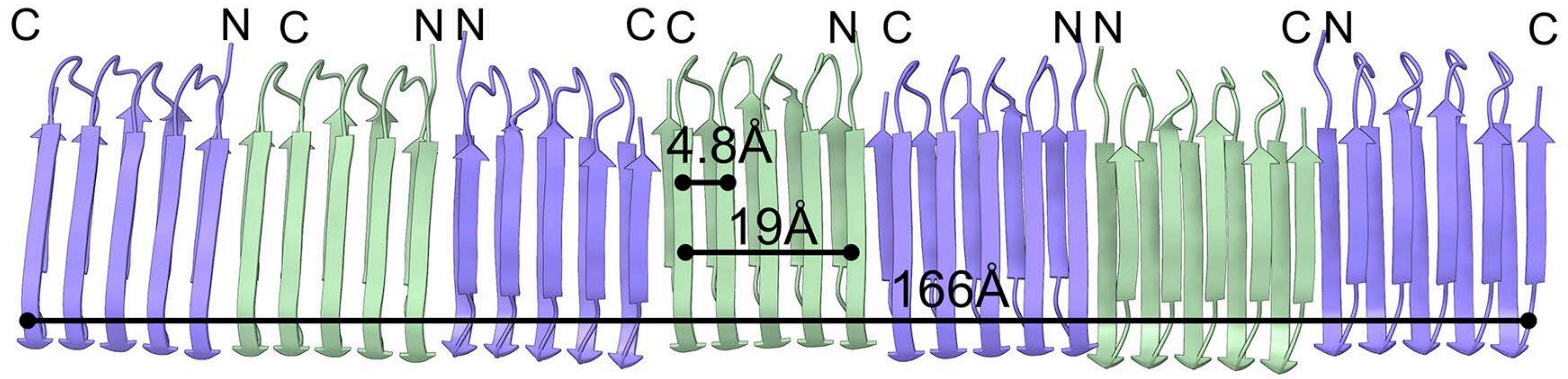
Protein material: easier to control production

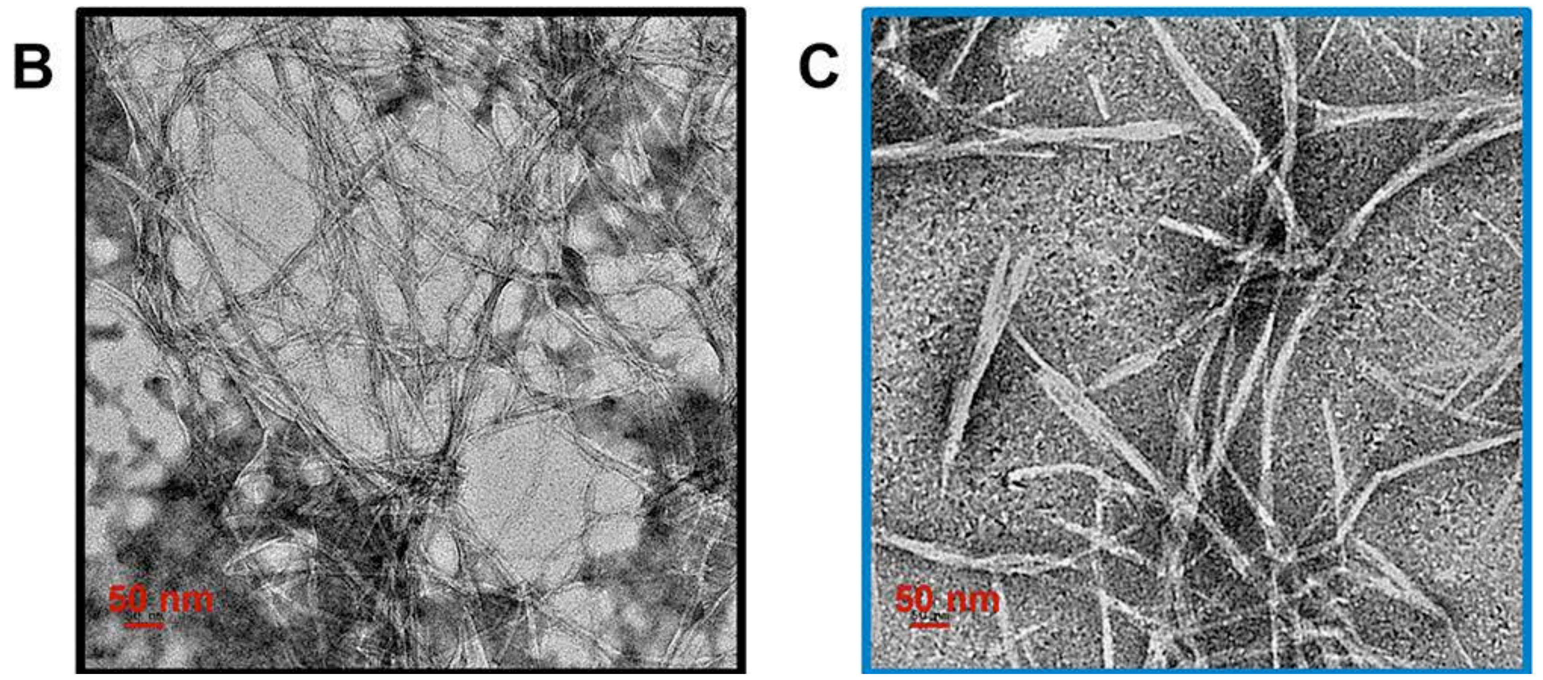
CsgA gene





CsgA polymer



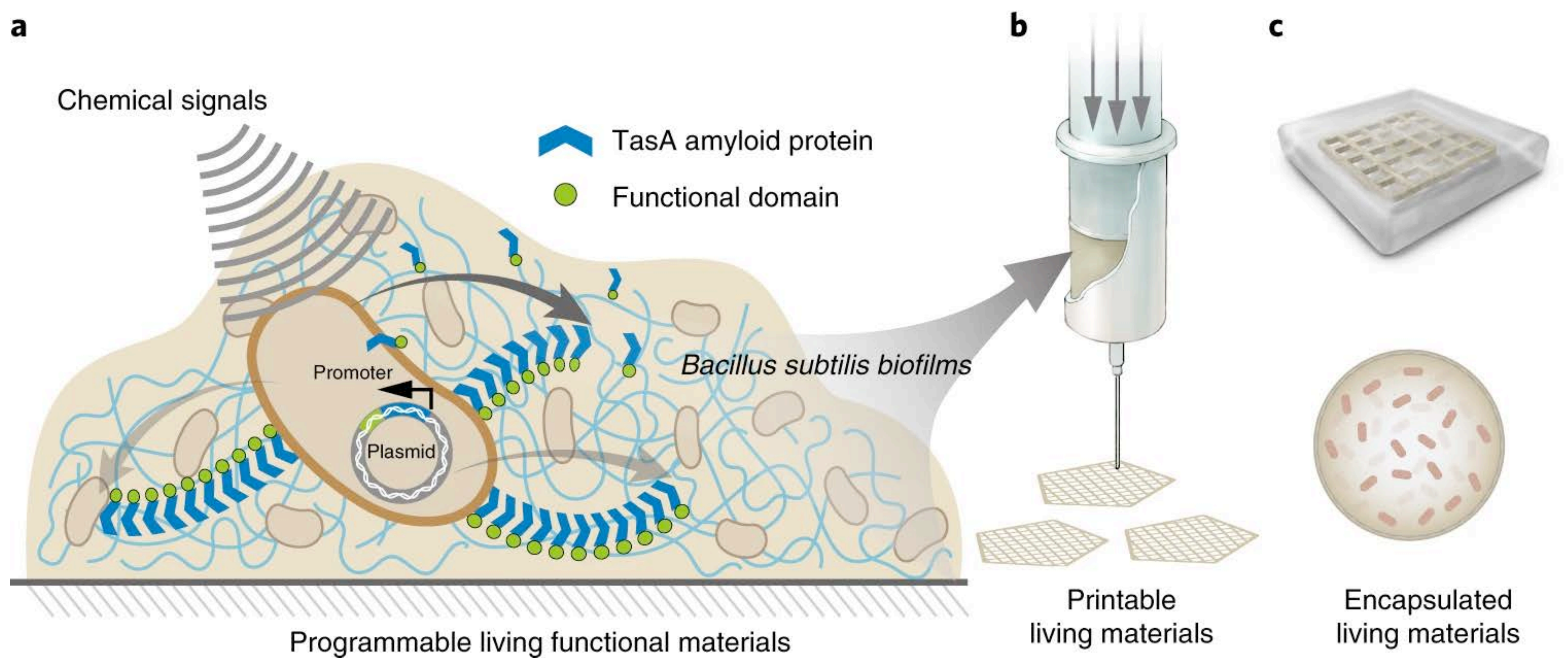


Curli filaments (amyloid)

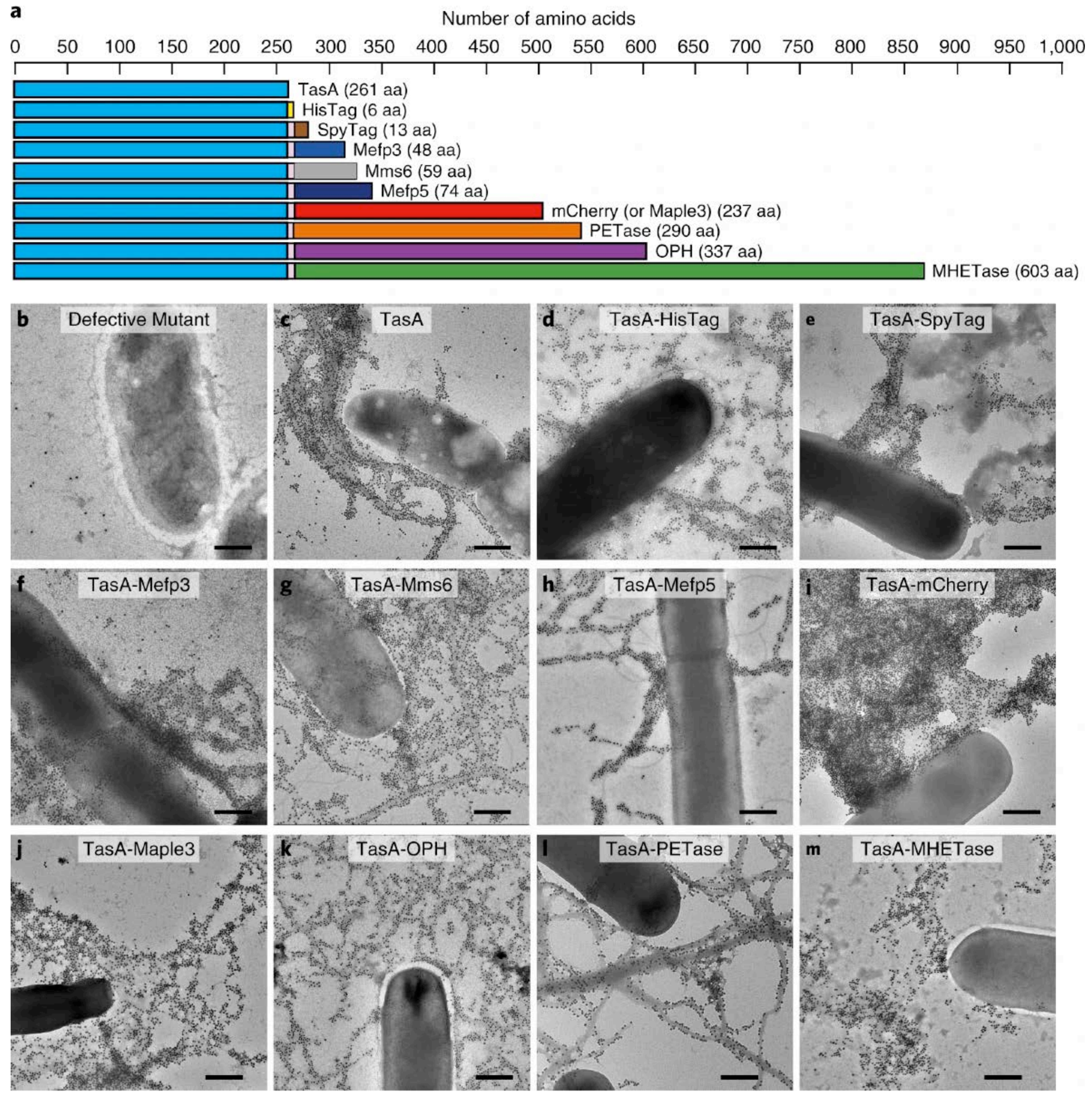
3D printing engineered living materials

Leverage *B. subtilis* biofilm amyloids and fusions

A biofilm printer - with functionalized amyloids



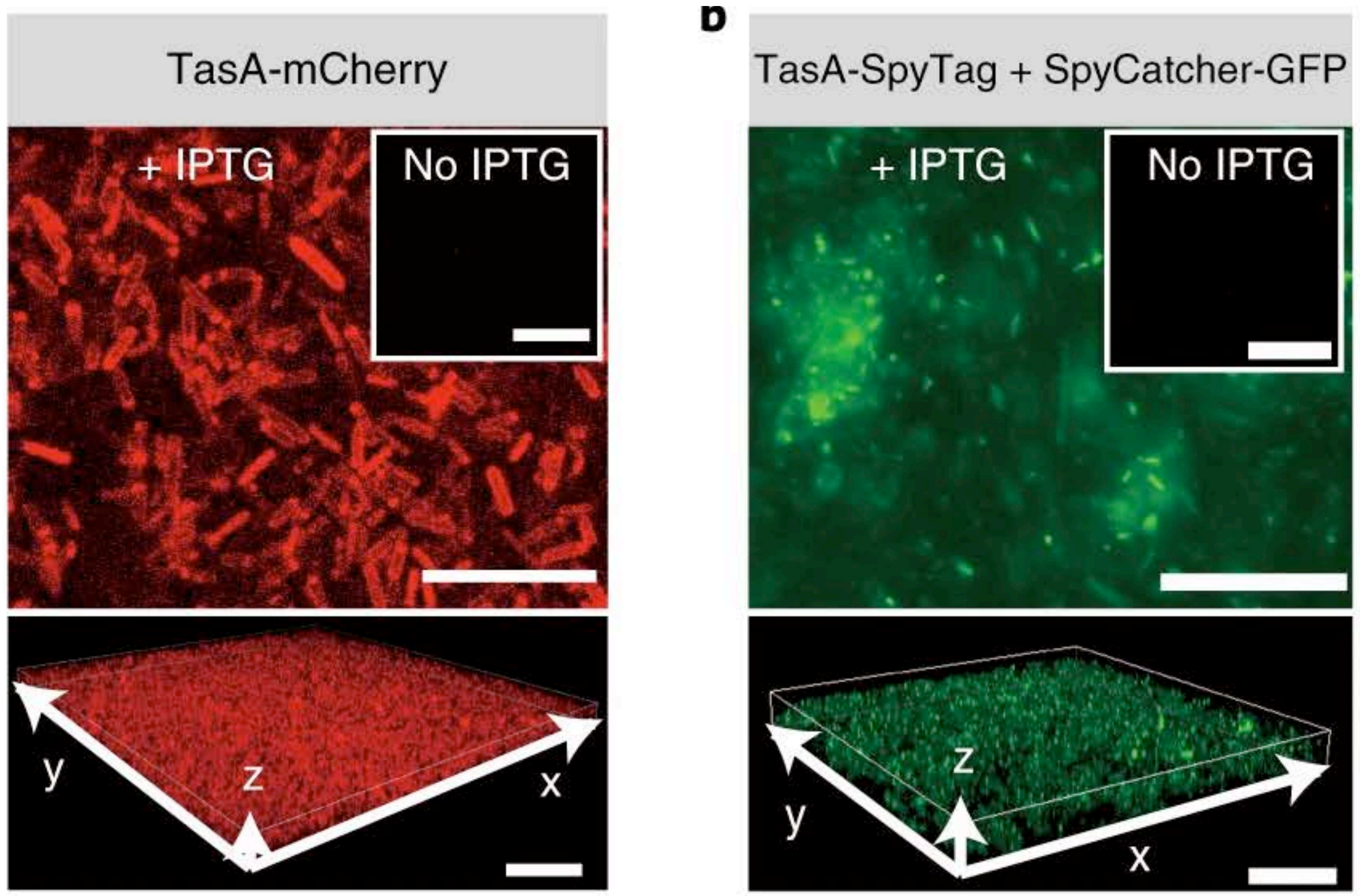
Construction of functionalized amyloids



https://doi.org/10.1038/s41589-018-0169-2

Amyloid functionalization

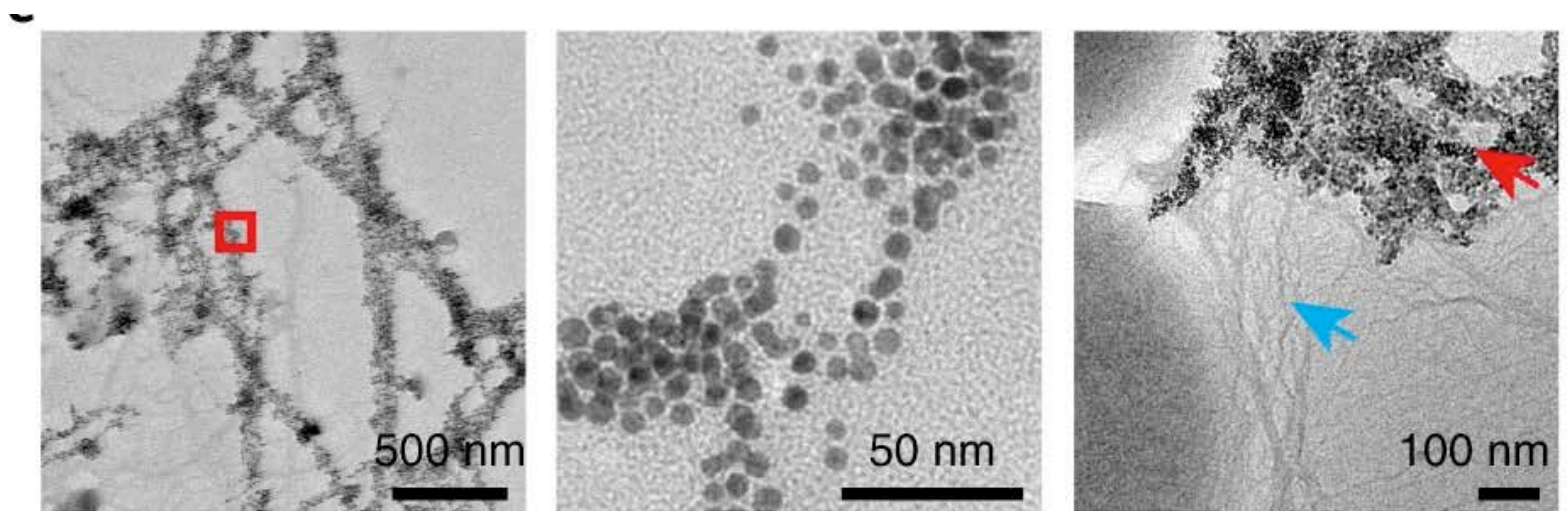
Fluorescent labeling



Gold nanoparticles

TasA-HisTag

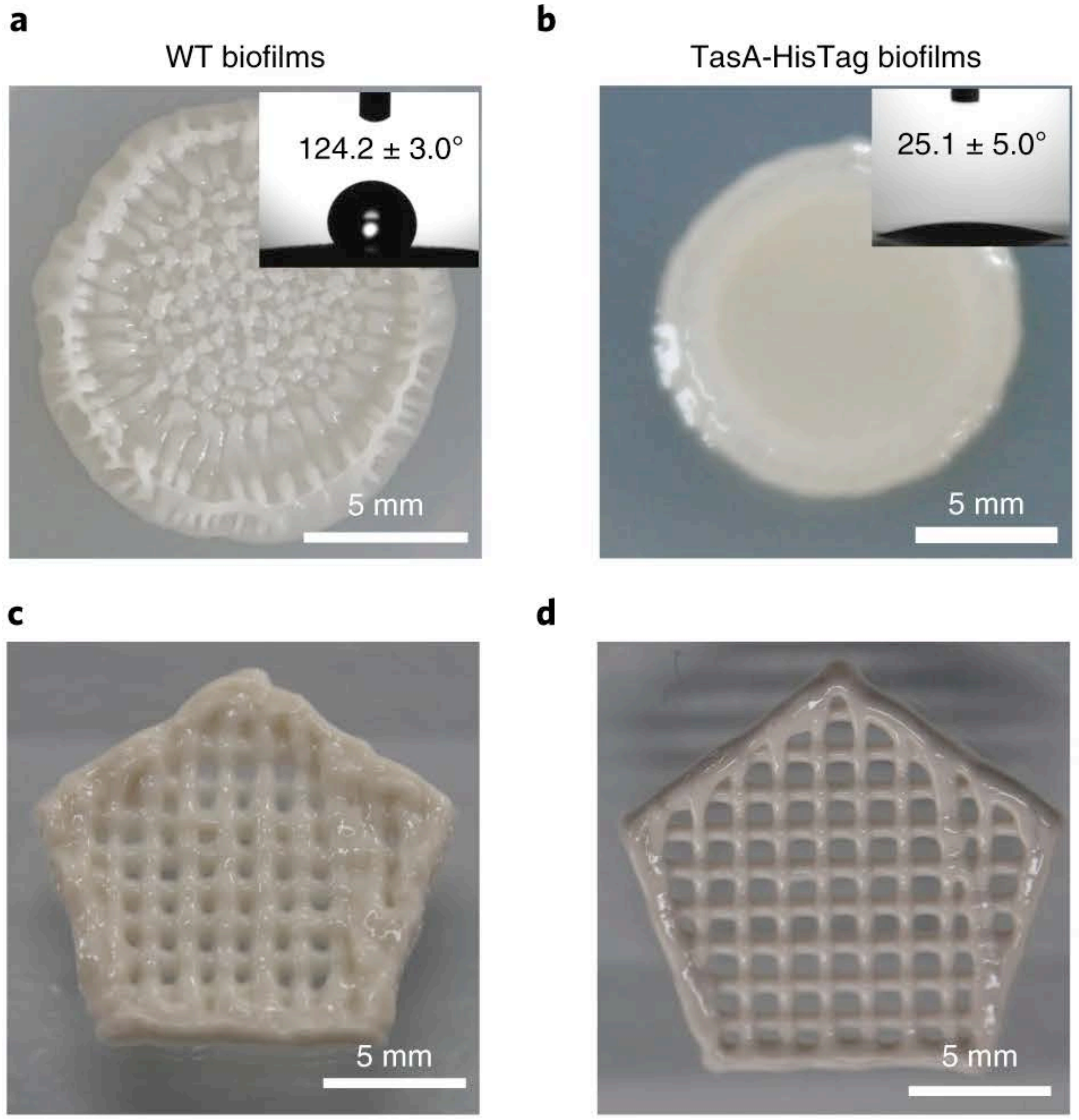
Ni-NTA-decorated AuNPs



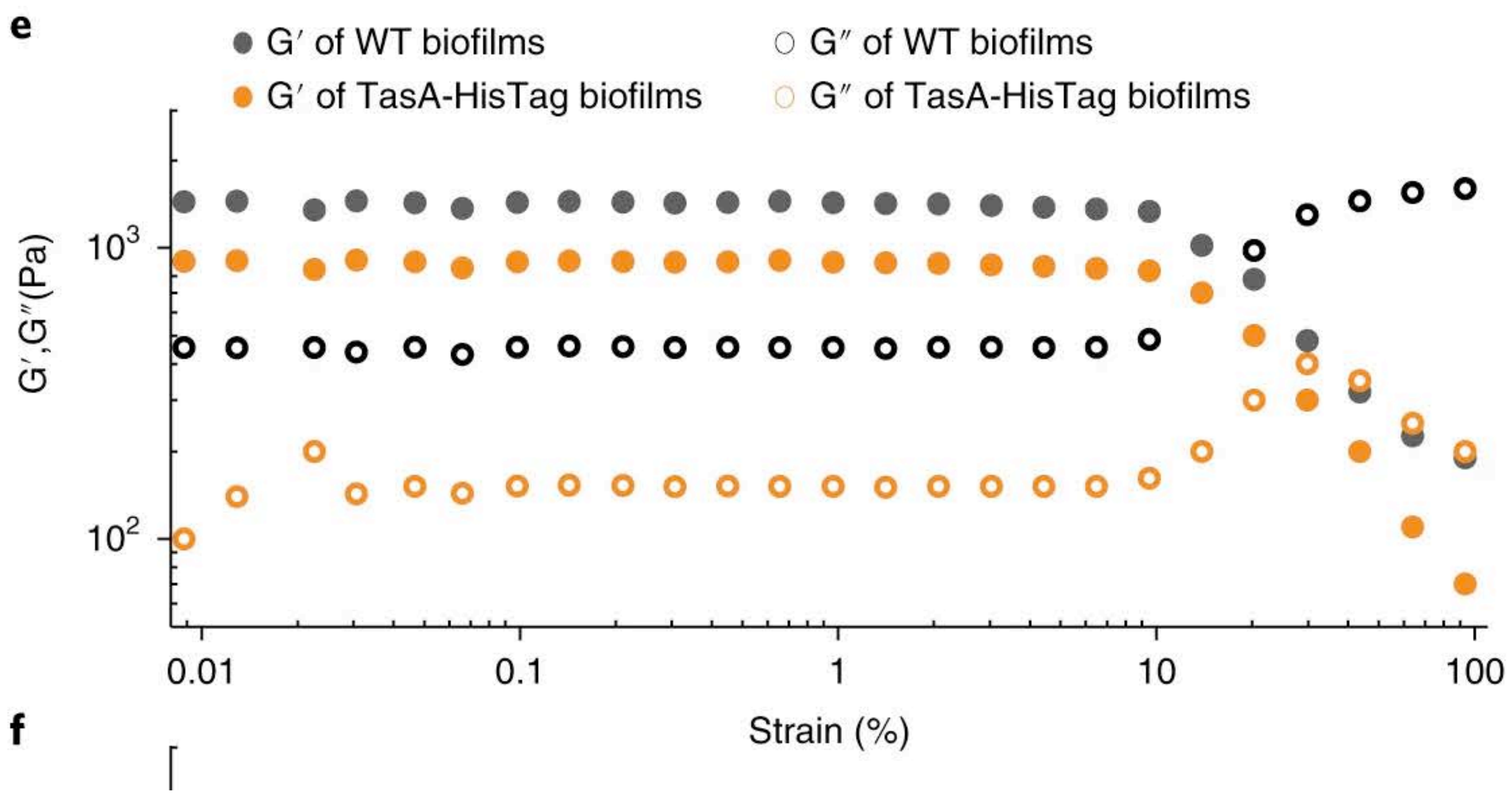
Fibers

Biofilm

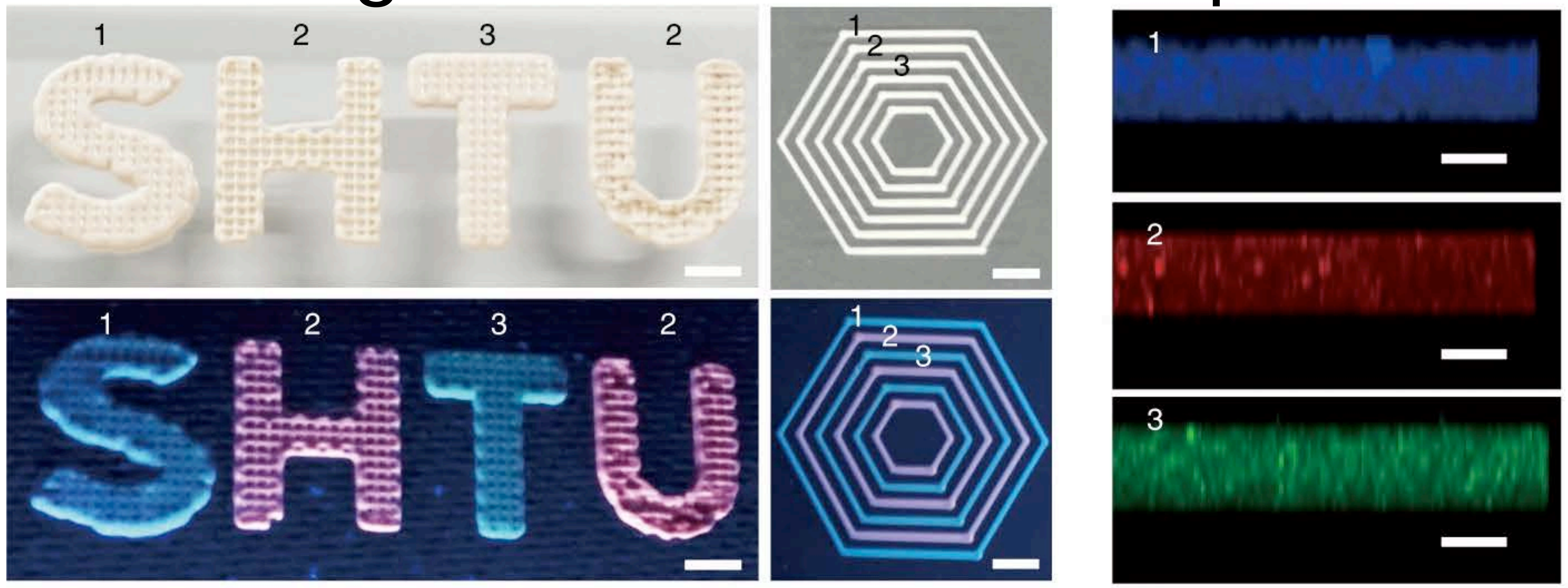
Printing biofilms



Mechanical properties

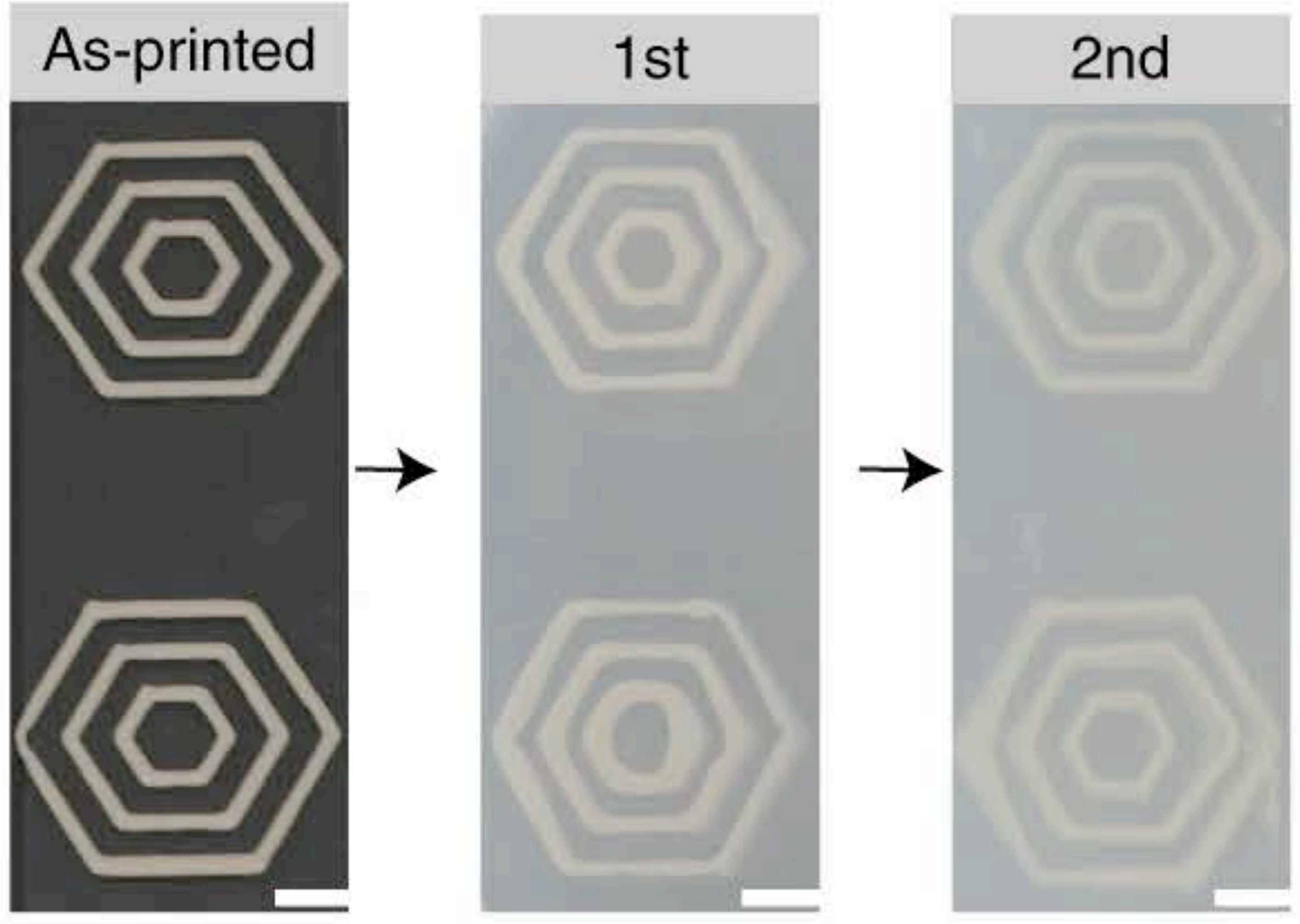


Printing+incubation with nanoparticles



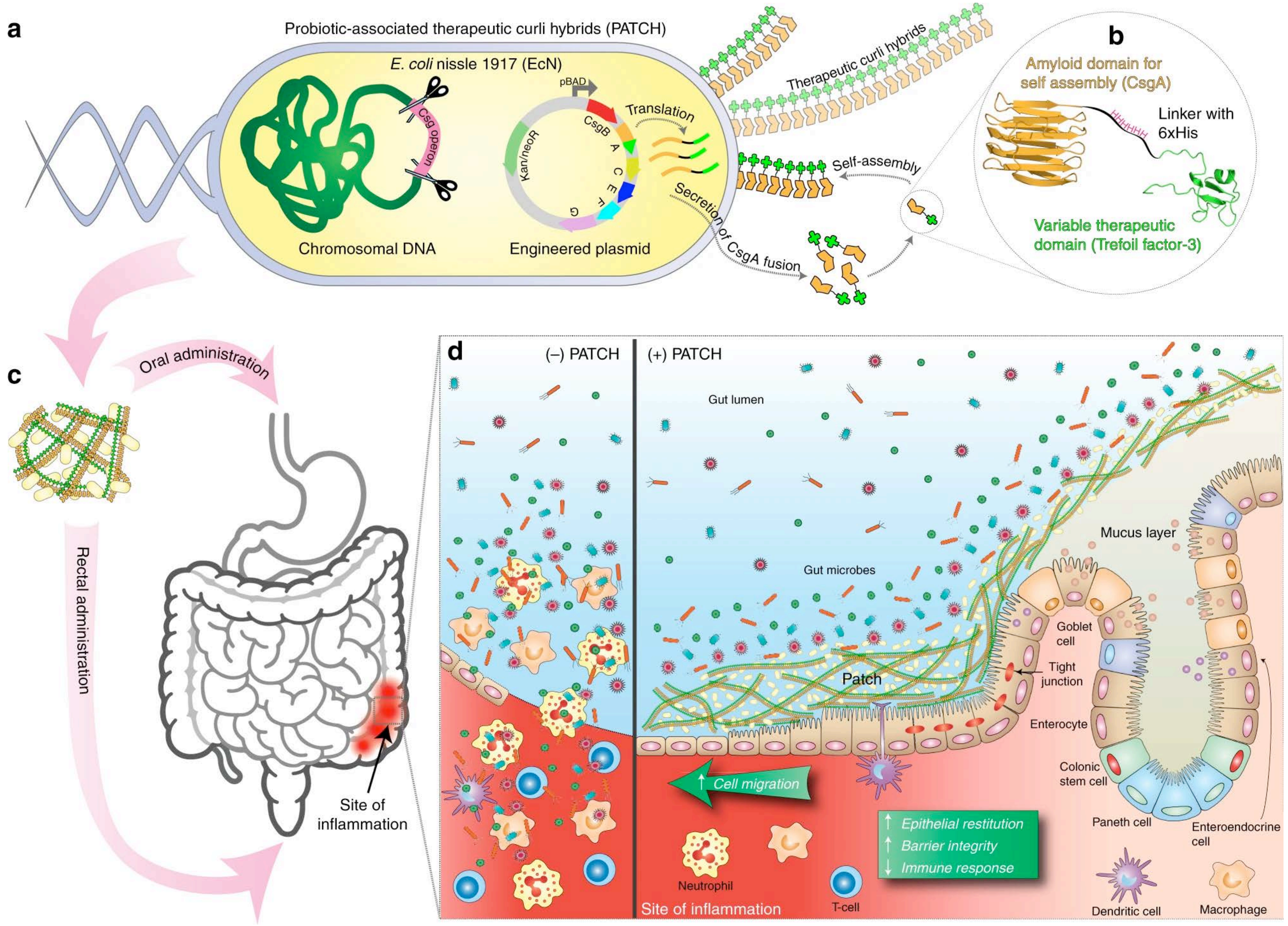
Replica of printed biofilms

use bacteria's ability to stick and grow



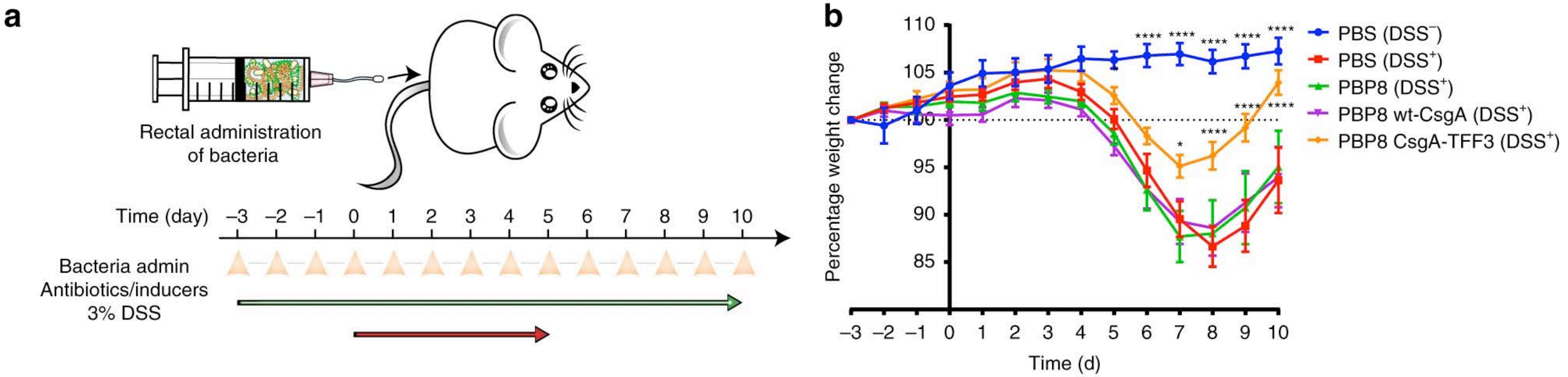
Therapeutic living biomaterial

CsgA probiotic to treat intestinal permeability



Efficacy during colitis

DSS induces colitis

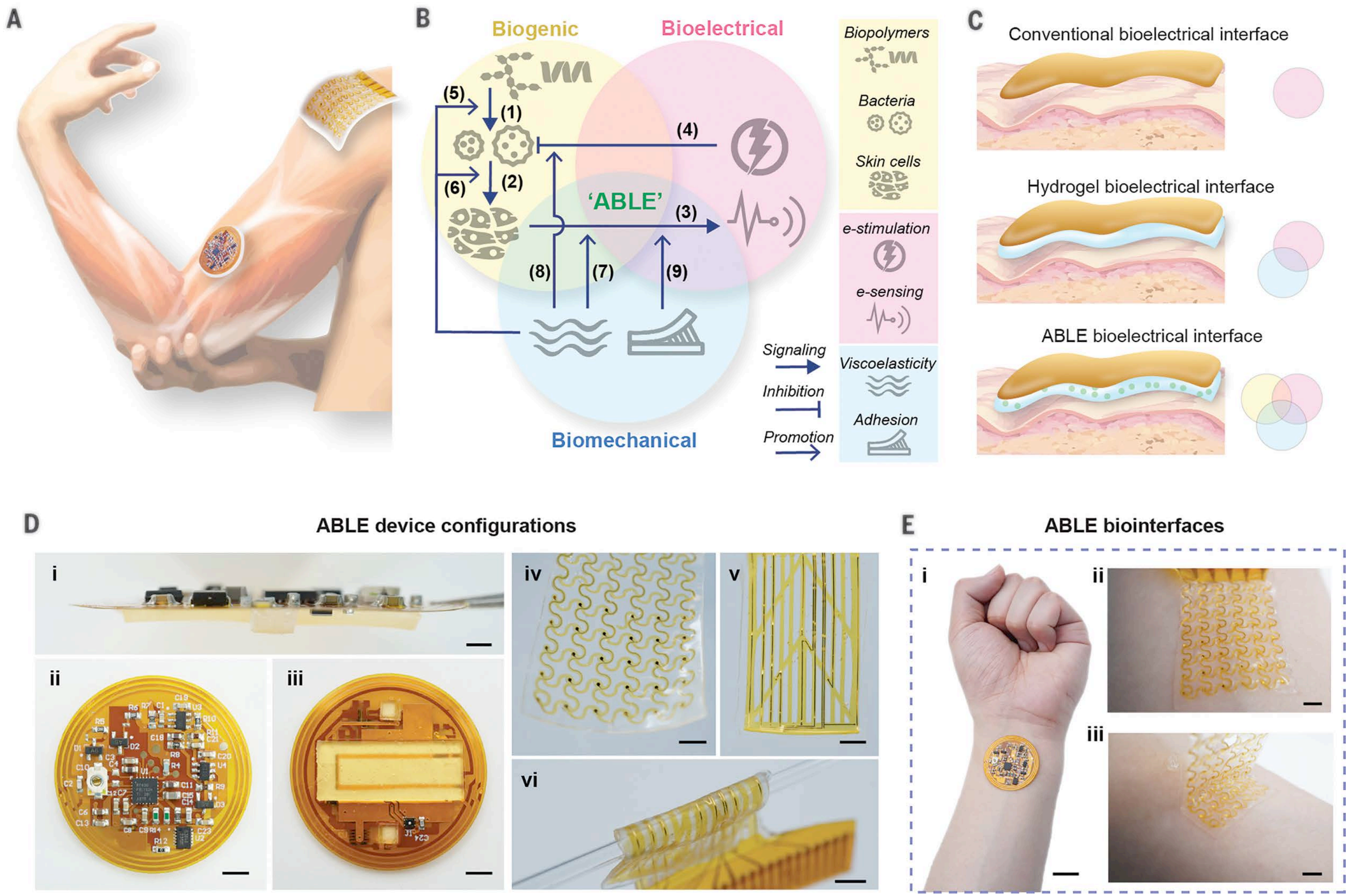


Curli production improves disease symptoms

Fully integrated living material and bioelectronic

A biointegrated wearable living electronic

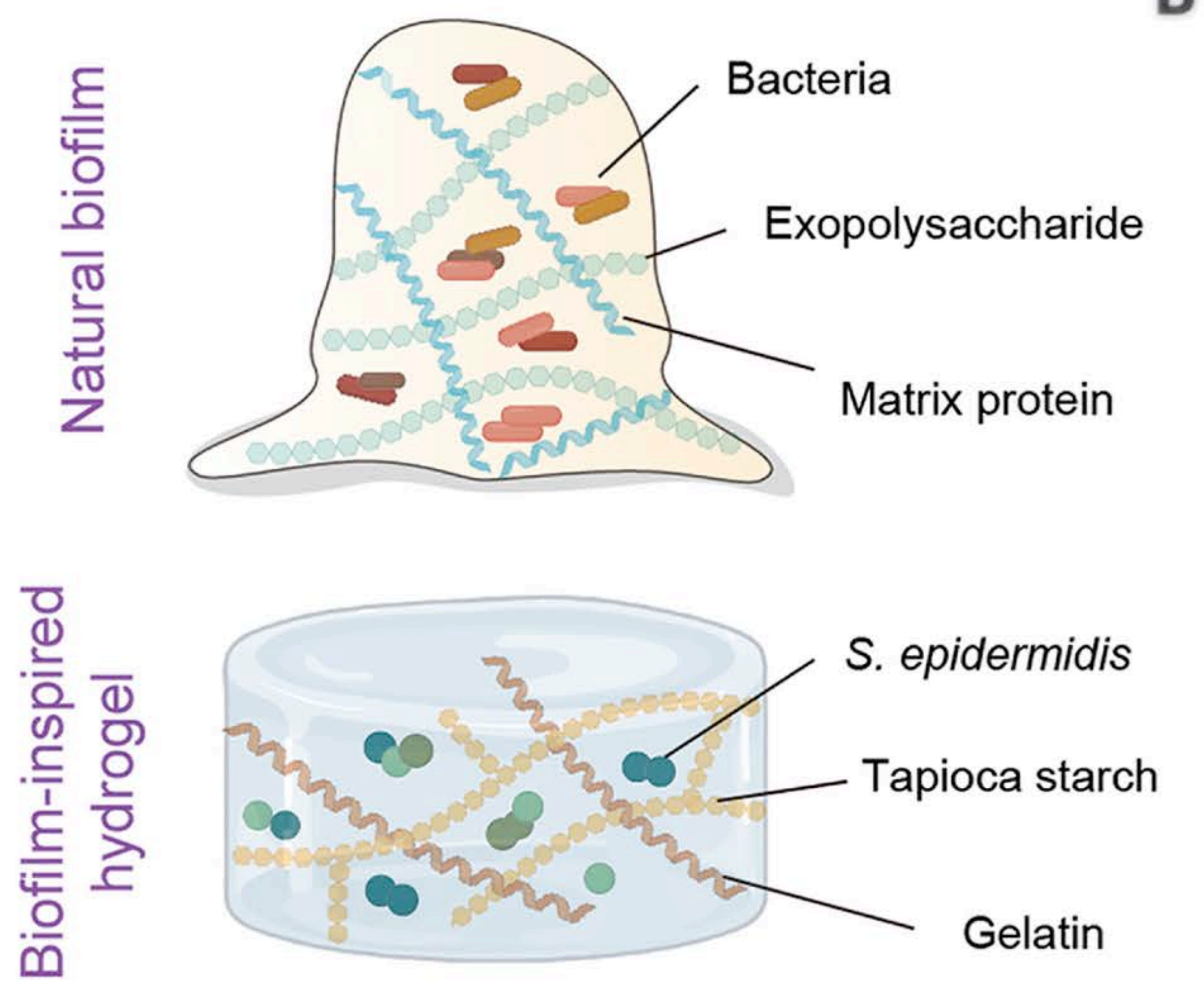
Device focused on skin diagnosis and therapy

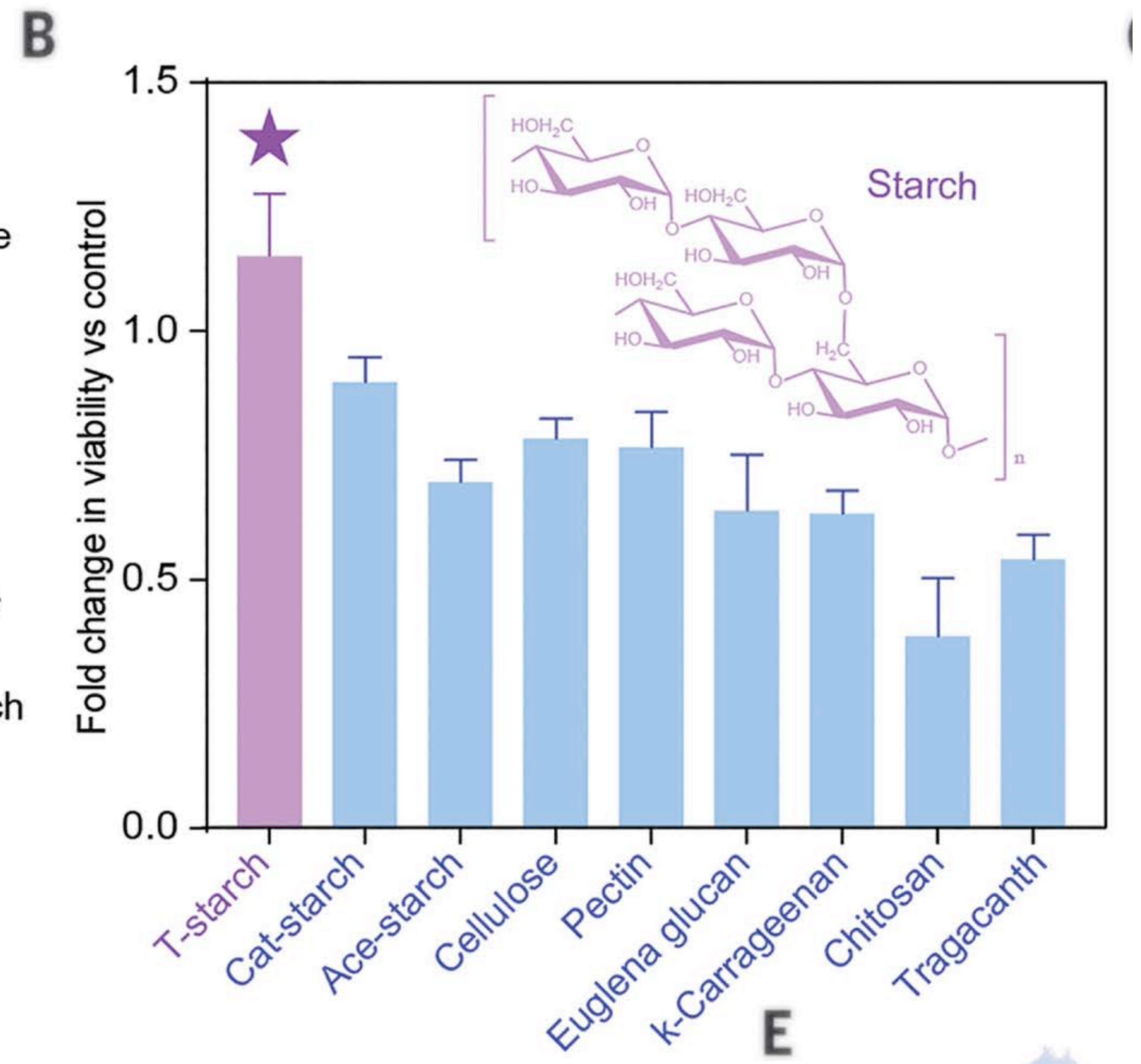


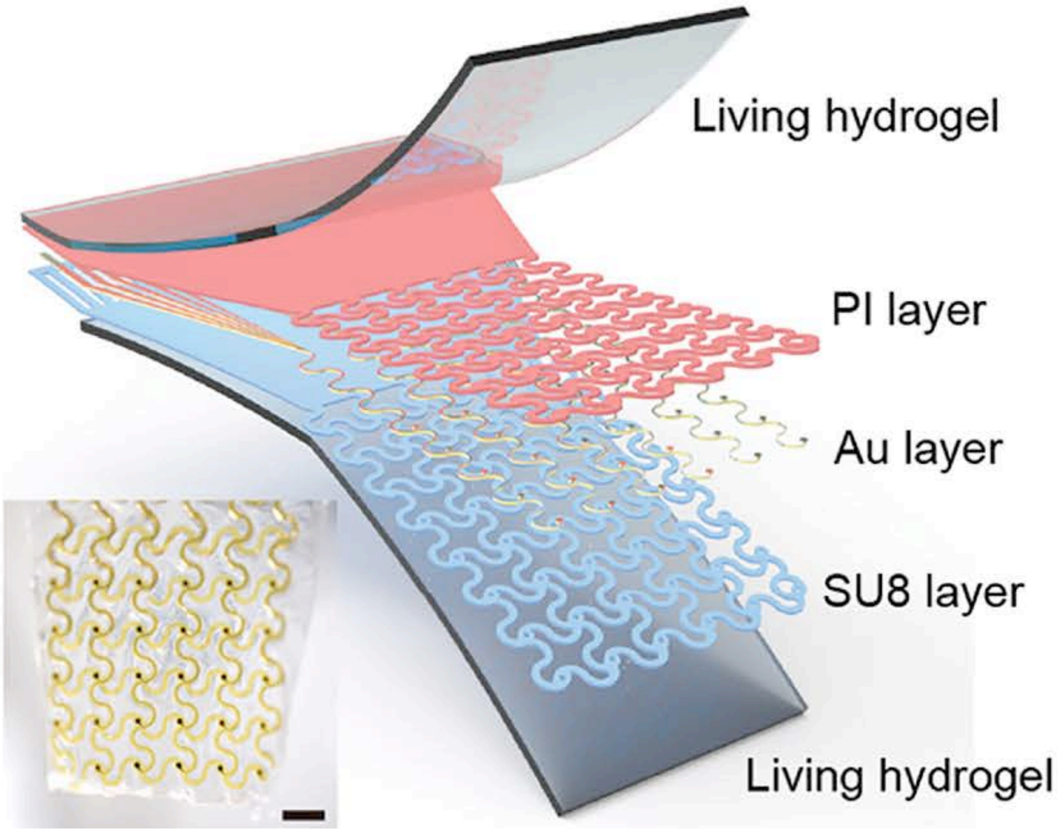
https://doi.org/10.1126/science.adl1102

Bacterial encapsulation

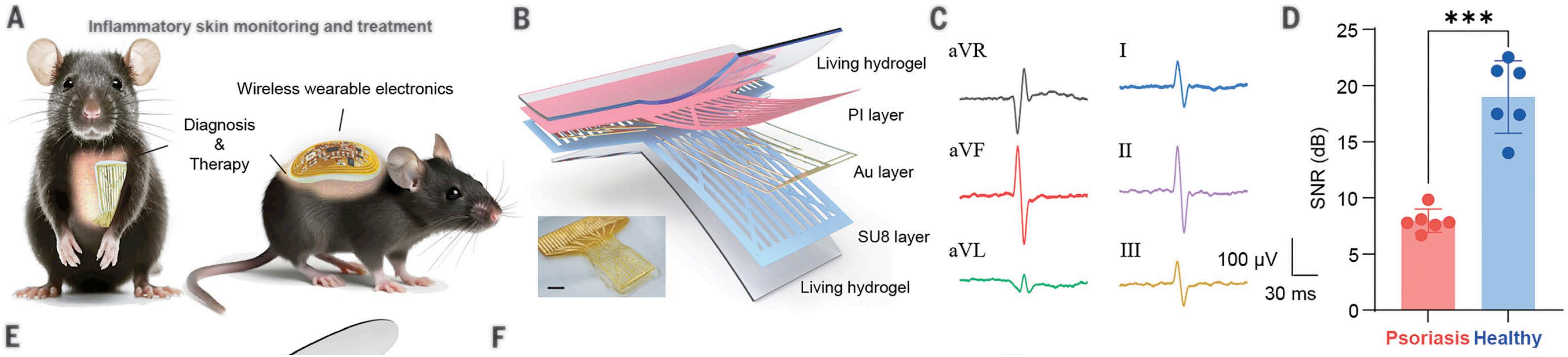
Tapioca starch





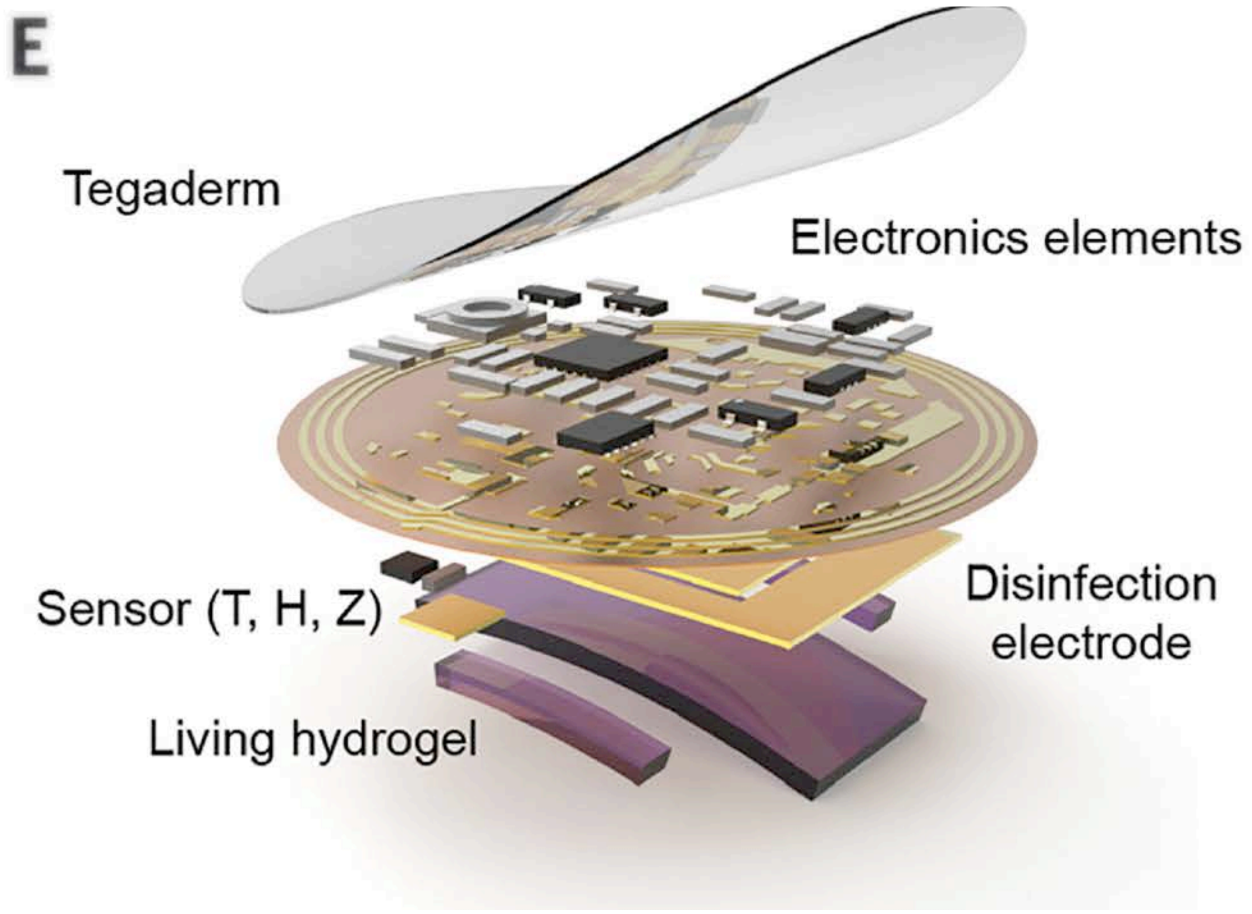


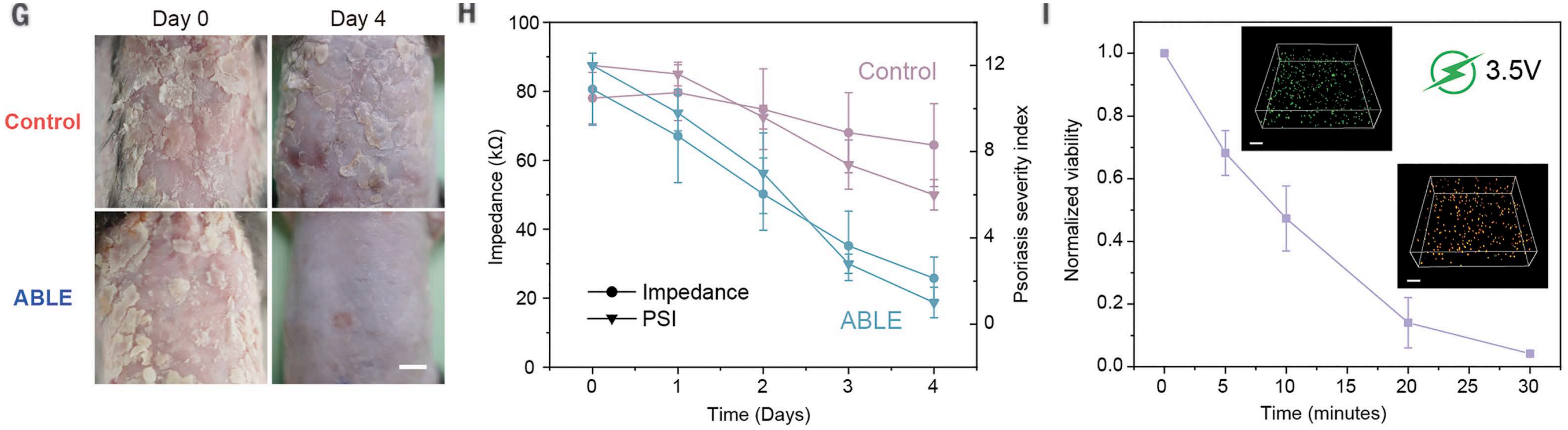
Psoriasis diagnosis



Lower SNR due to skin thickening

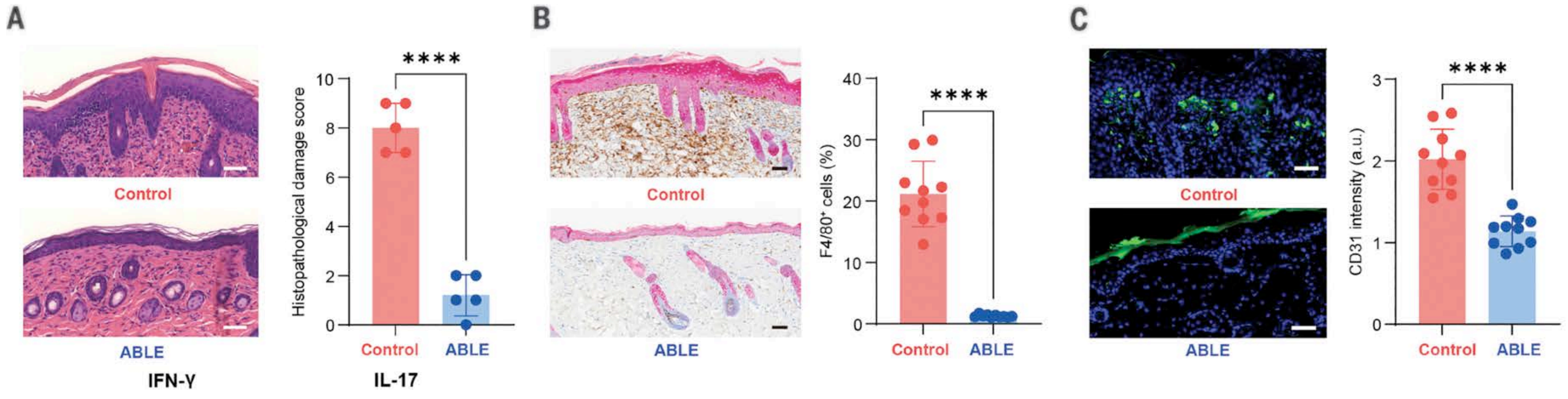
Psoriasis diagnosis and treatment

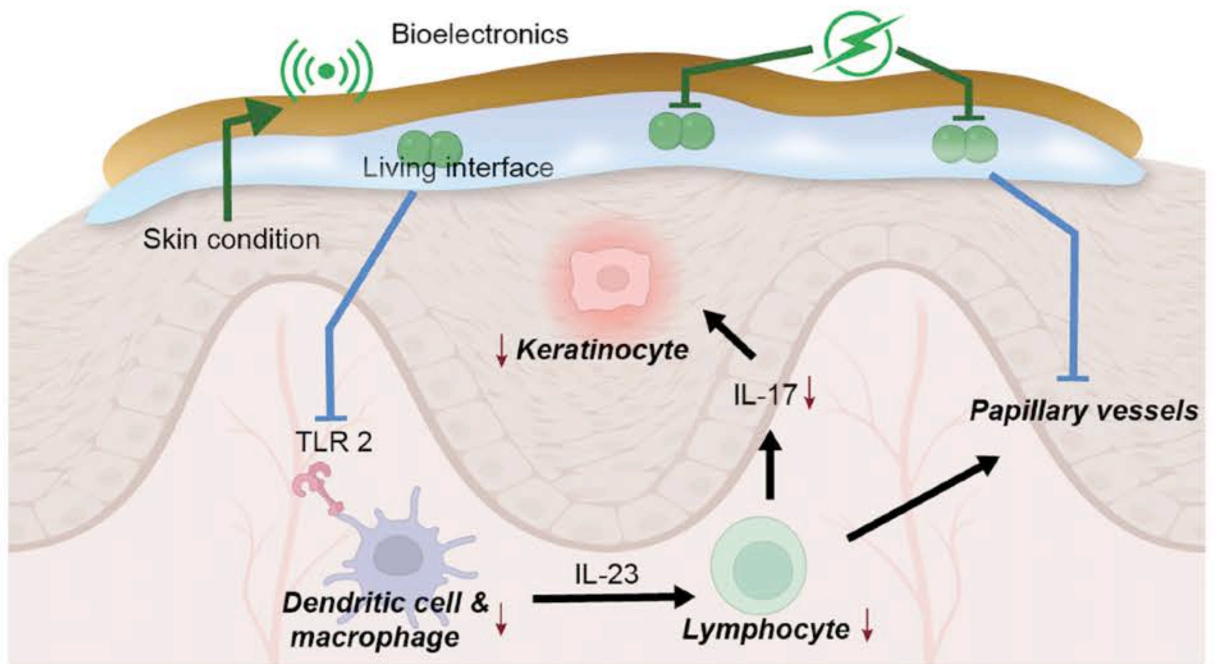




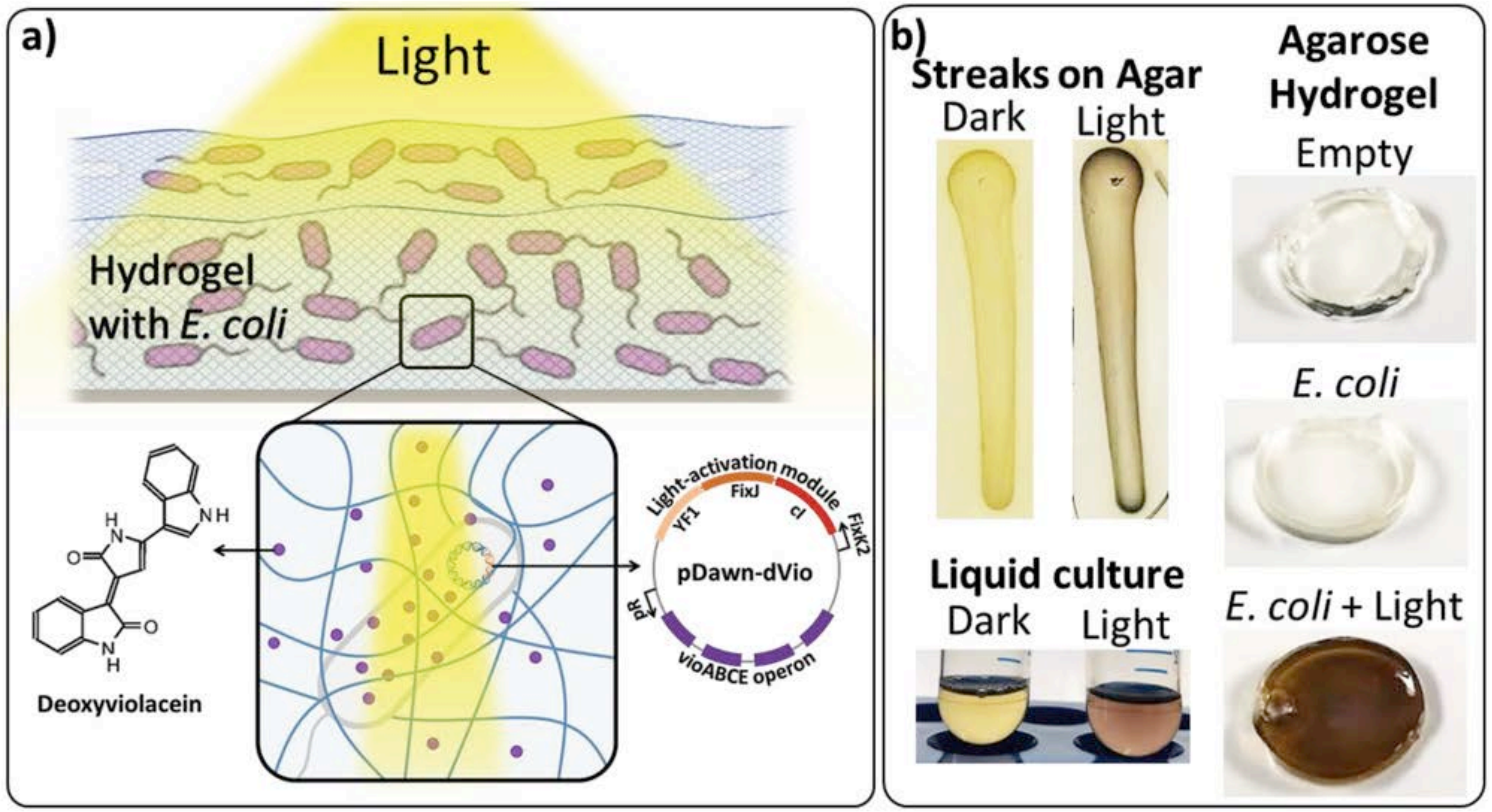
How does it work?

microbes tame inflammatory response





Living materials that respond to light



pDawn is photoactivable

dVio is an antifungal, expressed upon light stimulation

https://doi.org/10.1002/smll.201804717

Untapped biology: bacteria follow magnetic fields

https://doi.org/10.1002/smll.201804717