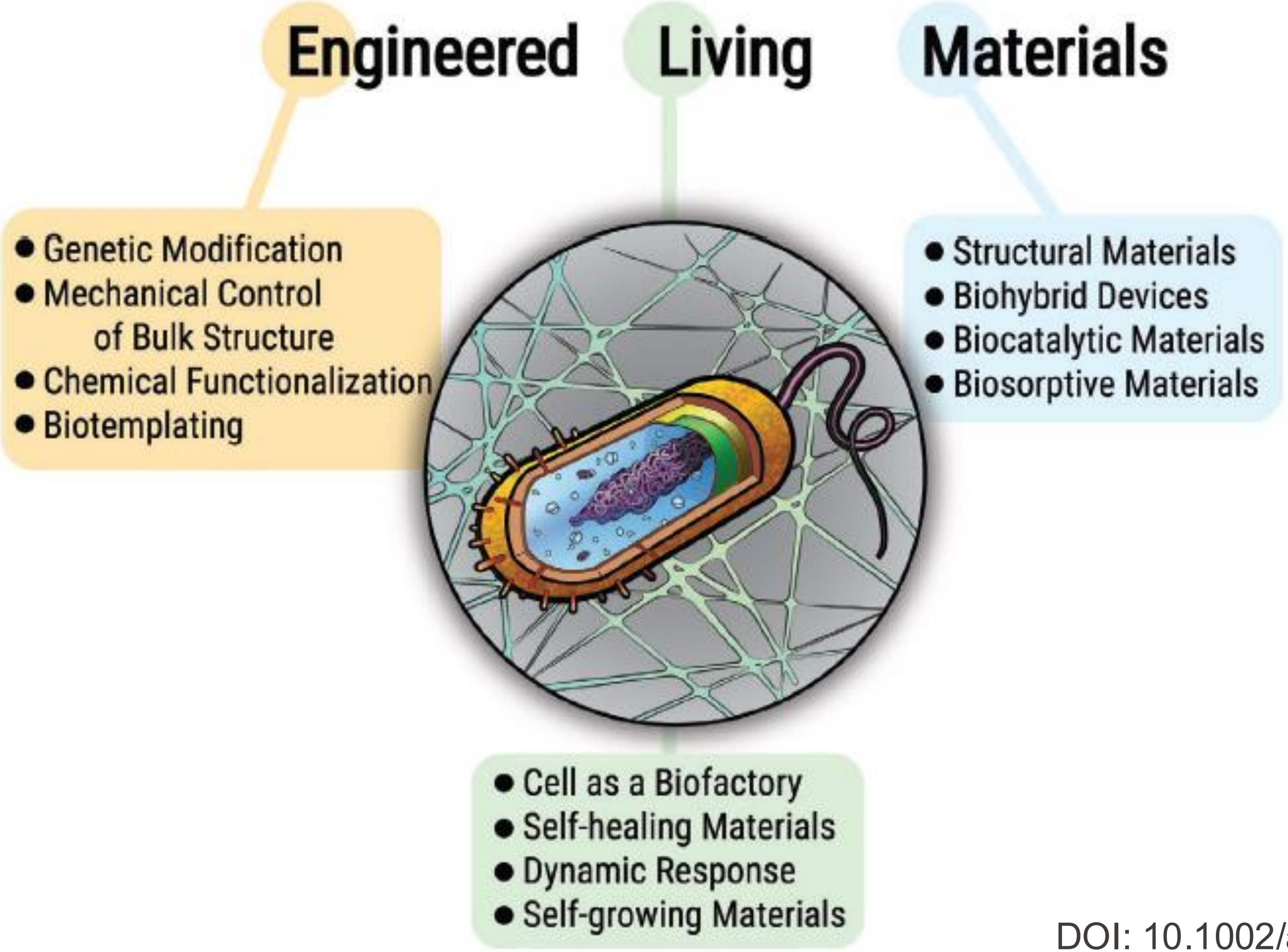
ELM Basics
MSE 493

Prof. Tiffany Abitbol

2025



DOI: 10.1002/adma.201704847

Why study ELMs?





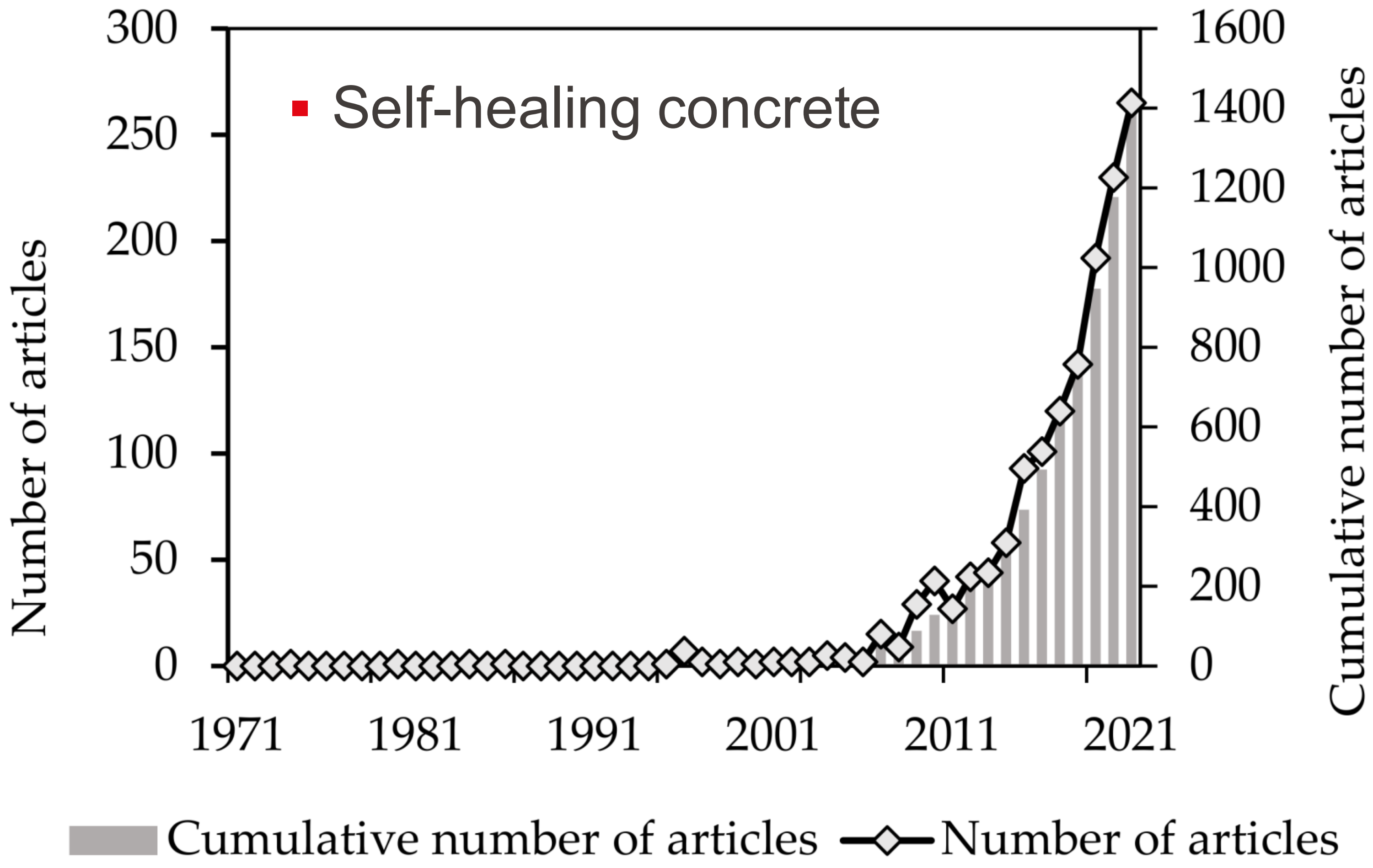



Concrete as an ELM?

- 4-8% of global CO_2 emissions attributed to concrete production related to clinker production
- Concrete lifespan is about 100 years
- Increasing the lifespan of the concrete is one way to reduce environmental impact
- Cracks traditionally repaired by fillers
- Let's start with the 1st paper and then something more recent to see the evolution of the field

$$CaCo_3 + heat \rightarrow CaO + CO_2$$

Before we dive in…



- 1443 documents
- From 2002-2021, 20% annual growth rate

1st paper: Self-healing concrete with bacteria

- Specific bacteria can produce minerals under specific conditions
- This potential to heal concrete cracks was explored by applying bacteria or enzymes to the cracks – still requires monitoring and maintenance
- In this 2010 publication, the researcher's asked: Can we introduce living bacteria into the concrete for a truly self-repairing concrete?
- Their main challenge: identifying a suitable bacteria

https://doi.org/10.1016/j.ecoleng.2008.12.036

Self-healing concrete with bacteria

Ecological Engineering 36 (2010) 230–235

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng

ELSEVIER

Application of bacteria as self-healing agent for the development of sustainable concrete

Henk M. Jonkers[a,*], Arjan Thijssen[a], Gerard Muyzer[b], Oguzhan Copuroglu[a], Erik Schlangen[a]

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Henk Jonkers

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Material science   microbiology

TITLE	CITED BY	YEAR
Application of bacteria as self-healing agent for the development of sustainable concrete HM Jonkers, A Thijssen, G Muyzer, O Copuroglu, E Schlangen Ecological engineering 36 (2), 230-235	1956	2010

https://doi.org/10.1016/j.ecoleng.2008.12.036

Self-healing concrete with bacteria

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https://doi.org/10.1016/j.ecoleng.2008.12.036

Self-healing concrete with bacteria

- High pH (11-13) = alkaliphatic bacteria
- Oxygen tolerant
- *Bacillus* genus =aerobic alkaliphilic spore-forming bacteria
- Gist of experiment: Instead of tap water, suspension of living bacterial cells was added to cement mixture (1–10 × 10^8 spores cm^{-3} cement stone) together with **calcium lactate**

https://doi.org/10.1016/j.ecoleng.2008.12.036

Self-healing concrete with bacteria

- The bacteria produce enzymes to metabolize lactase to CO_2 (lactate dehydrogenases)

- $CO_2 + OH^- \rightarrow HCO_3^- \rightarrow CO_3^{2-}$

- Carbonates will react to form $CaCO_3$ minerals

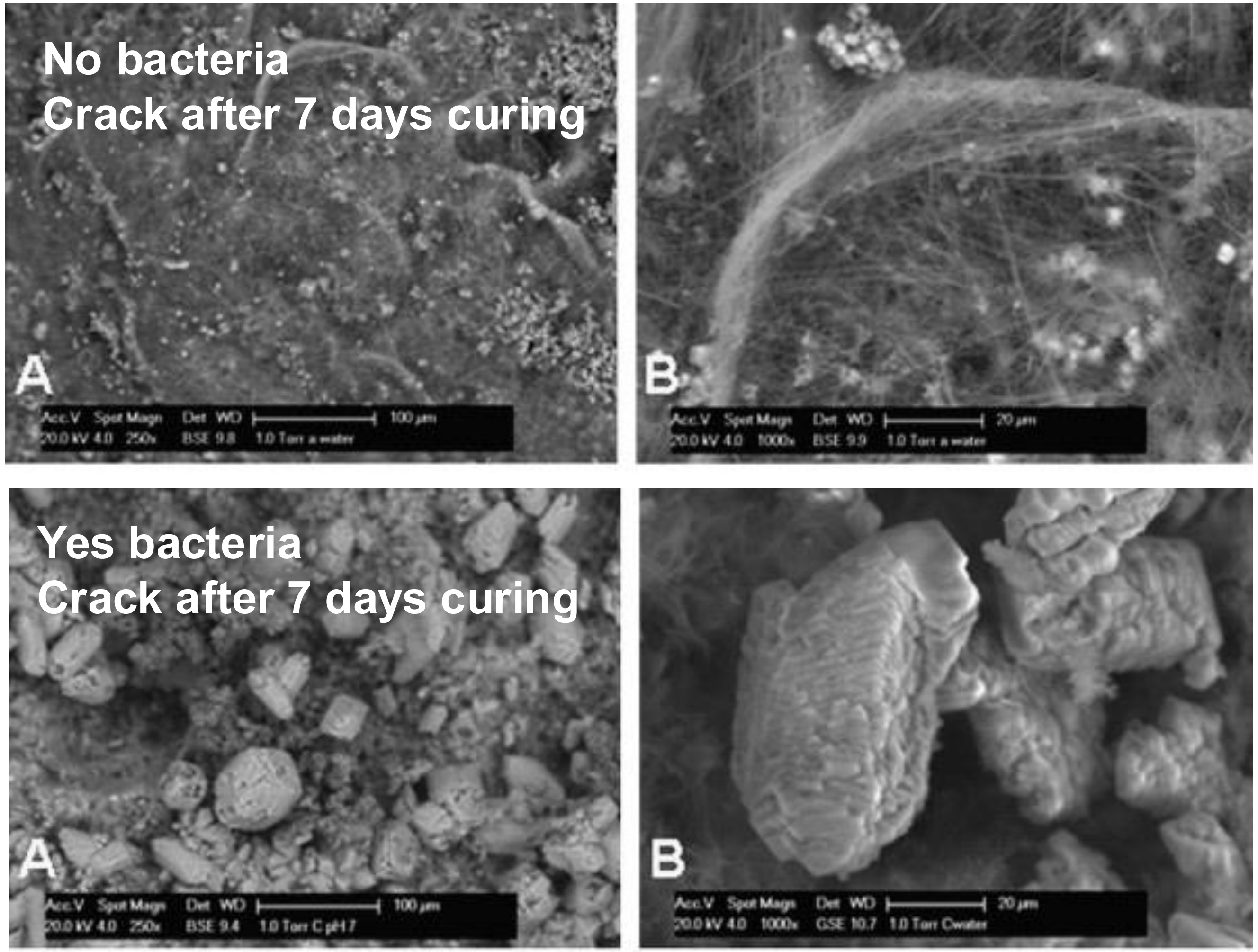
- Amazing! Does it work? Sorta! (Remember this is just the 1st paper in this field…)

https://doi.org/10.1016/j.ecoleng.2008.12.036

EPFL

Self-healing concrete with bacteria

https://doi.org/10.1016/j.ecoleng.2008.12.036



- Spores viable up to 4 months (but only a fraction of the initial content)
- Pore diameter, which decreases with curing time, limits viability (especially below 1 µm – spore size)
- Can only get better, right?

More recently: Living Building Materials

https://doi.org/10.1016/j.matt.2019.11.016

Matter
CellPress
Volume 2, Issue 2, 5 February 2020, Pages 481-494

Article

Biomineralization and Successive Regeneration of Engineered Living Building Materials

Chelsea M. Heveran [1 2], Sarah L. Williams [6], Jishen Qiu [1], Juliana Artier [3], Mija H. Hubler [1], Sherri M. Cook [1], Jeffrey C. Cameron [3 4 5], Wil V. Srubar III [1 6 7]

Wil V. Srubar III, Ph.D.

Professor, University of Colorado Boulder
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Polymers Cement Biomimetics Carbon Living Materials

Author Contributions

Study design: C.M.H., S.L.W., J.A., J.Q., M.H.H., S.M.C., J.C.C., and W.V.S. Data collection: C.M.H., S.L.W., J.A., and J.Q. Data analysis: C.M.H., S.L.W., and J.Q. Data interpretation: C.M.H., S.L.W., J.Q., and W.V.S. Drafting manuscript: C.M.H., S.L.W., and W.V.S. Approving final version of manuscript: all authors. C.M.H. takes responsibility for the integrity of the data analysis.

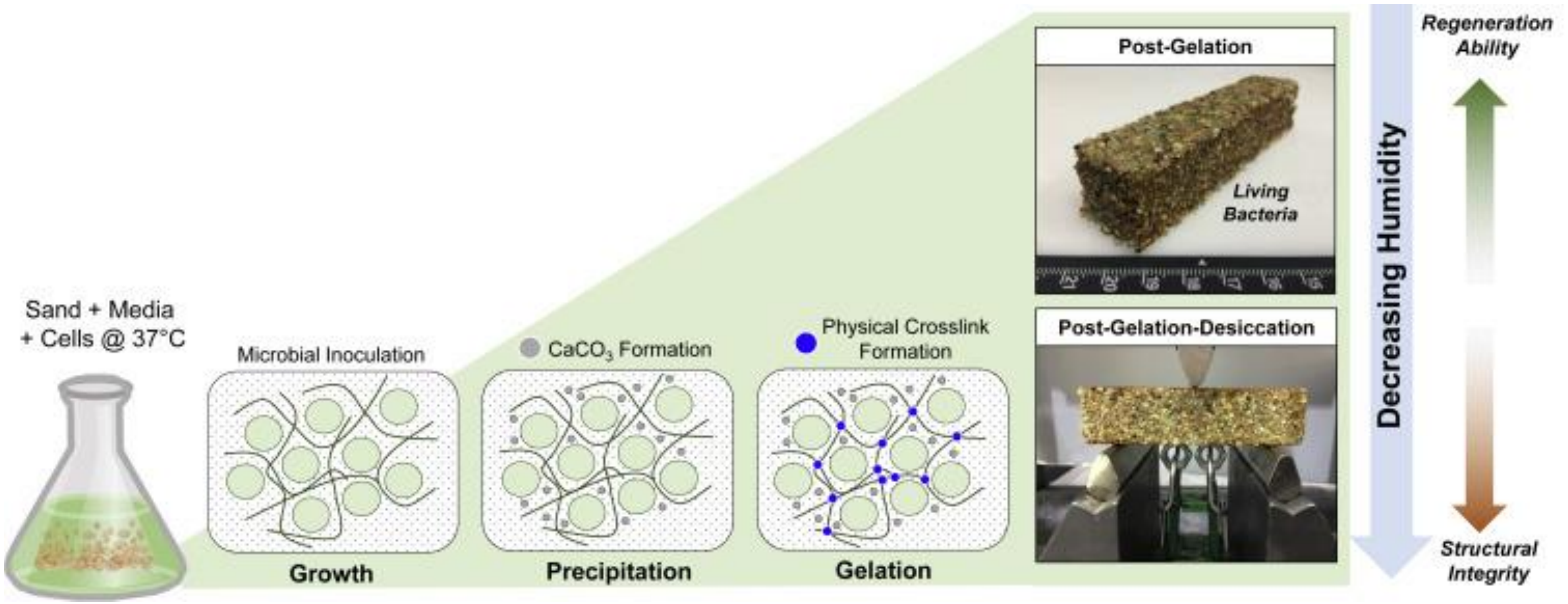
More recently: Living Building Materials

Study objectives:

1. Grow a building material from an inert scaffold and a living component
2. Long-term viability to allow self-repair and regeneration
3. Responsive to switches to move between a dormant and active state
4. Parent inoculum to regenerate

https://doi.org/10.1016/j.matt.2019.11.016

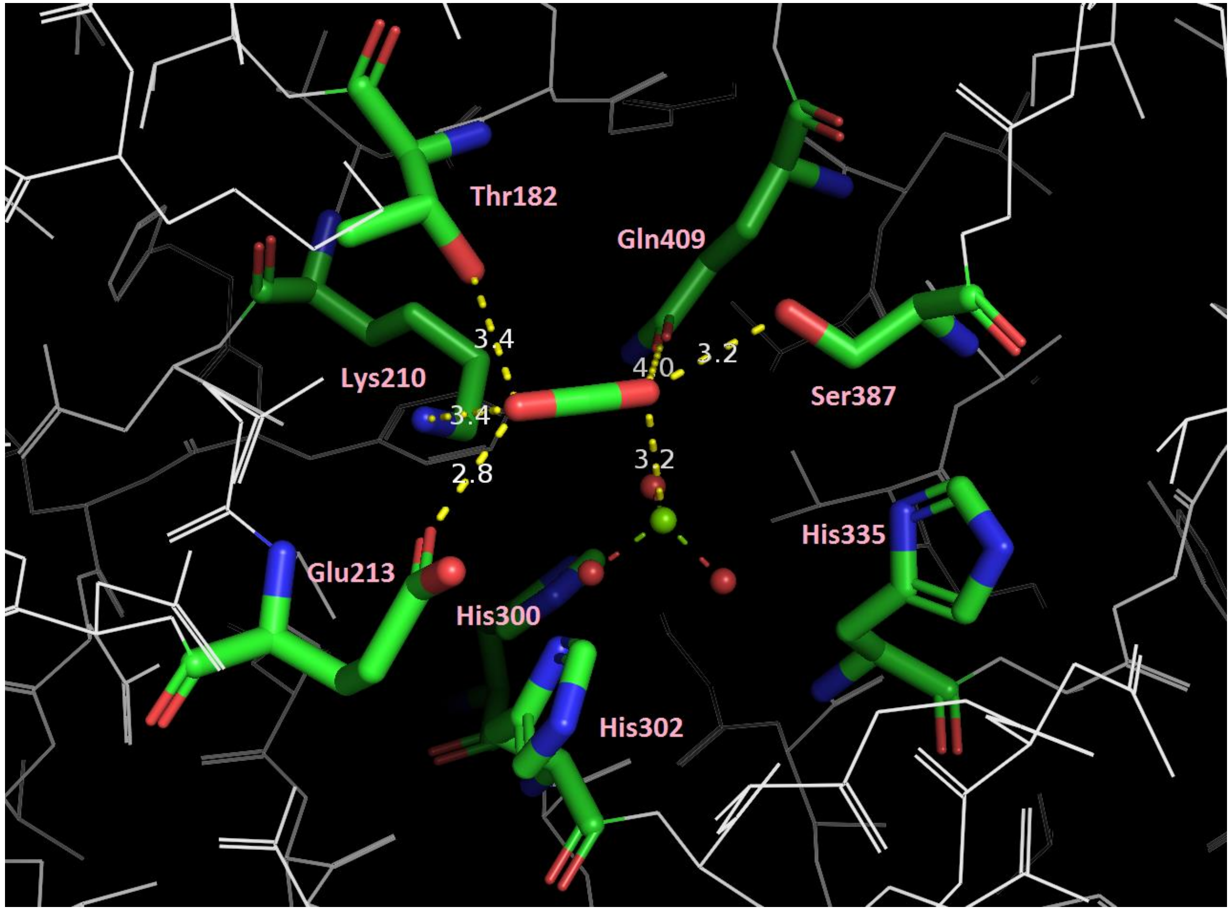
Generalized schematic



- Mineral formation: biology part
- Surrounding media: materials part

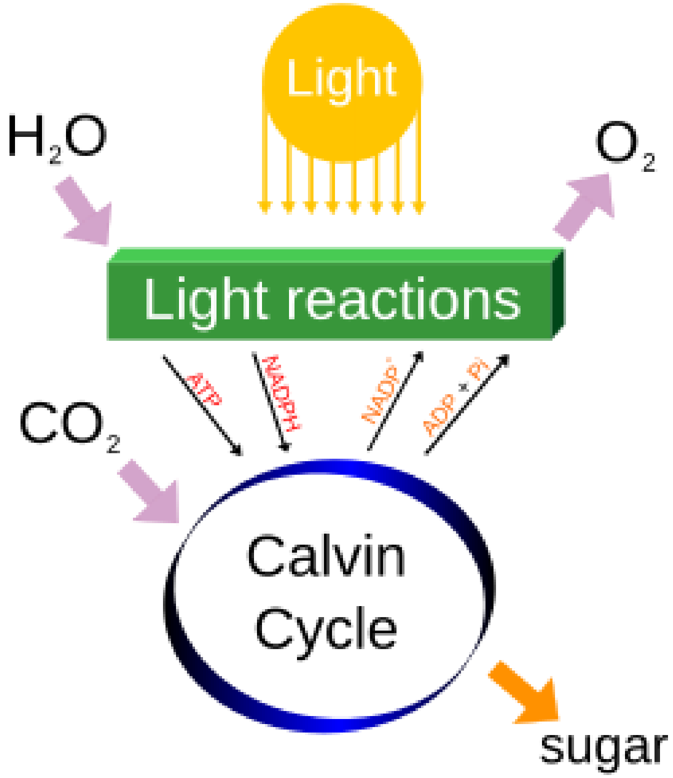
The biology part

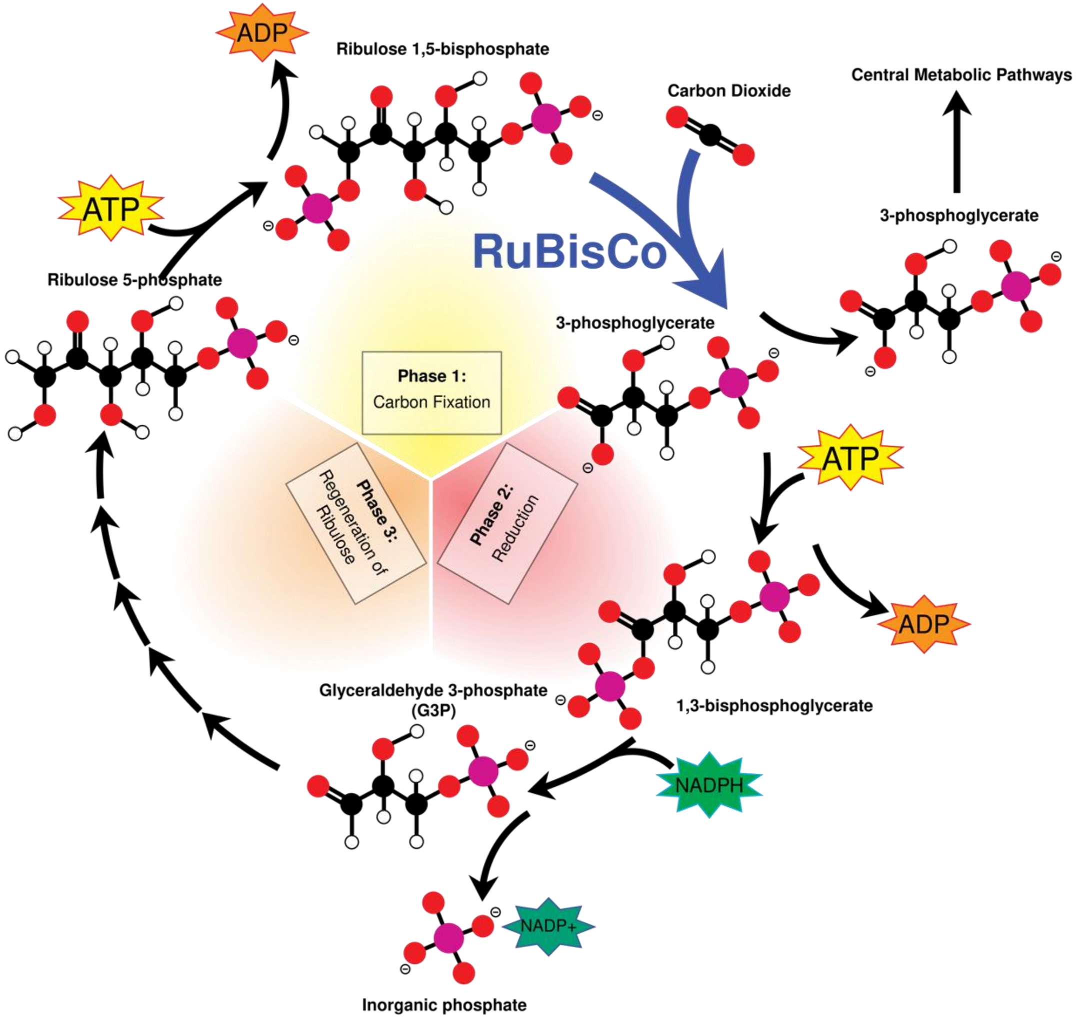
Active site of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco)



- Rubisco binds CO_2 and converts it to sugars in **light-independent** part of photosynthesis
- Allows atmospheric CO_2 to be fixed by plants ands other photosynthetic organisms to produce energy-rich molecules
- In this study, a cyanobacteria that does photosynthesis via Rubisco was used

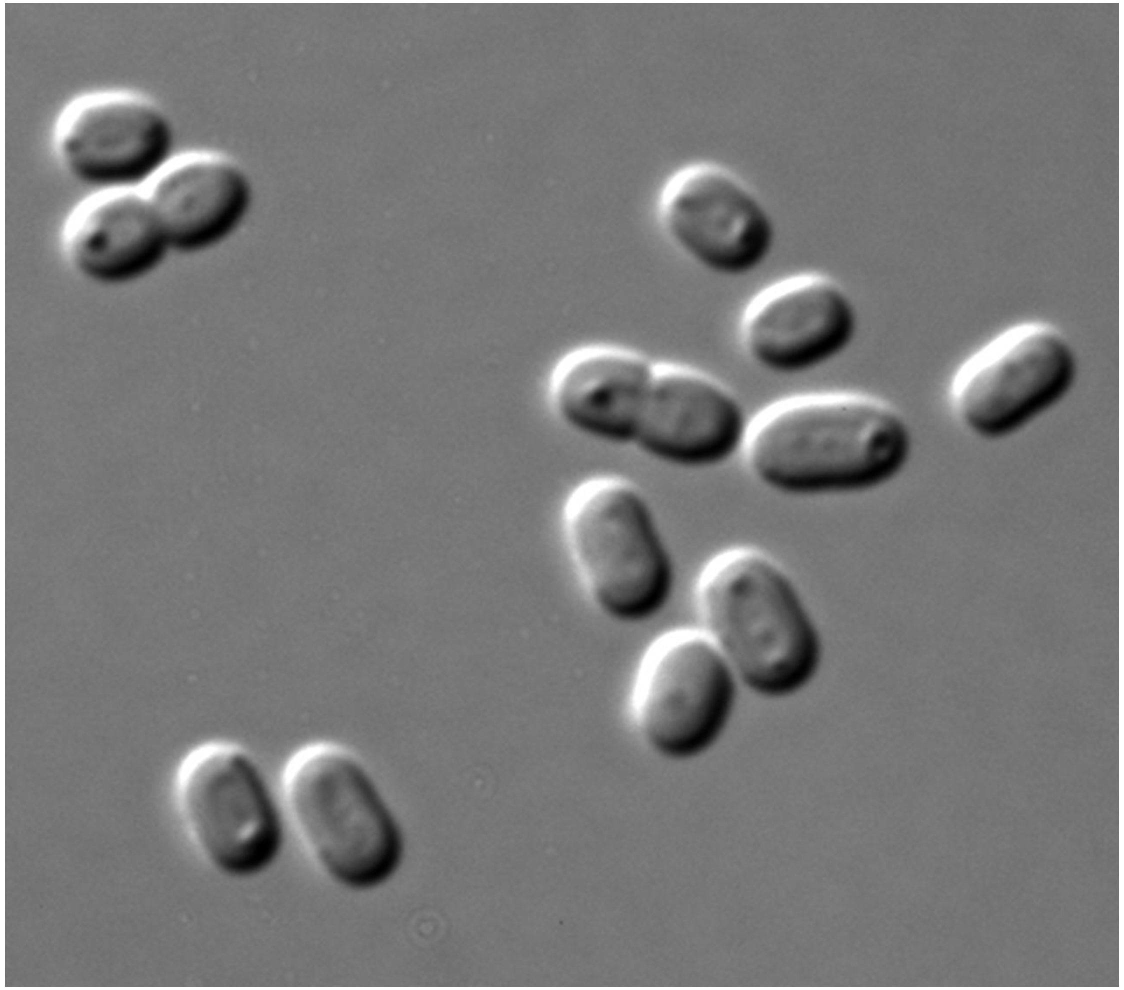
Light-dependent and light-independent photosynthesis reactions





The biology part

Synechococcus PCC 7002 in DIC microscopy.



- Cyanobacteria are photosynthetic prokaryotes
- Cyanobacteria are globally distributed, occurring in freshwater, marine, and terrestrial environments
- Cyanobacteria began oxygenating Earth's oceans and atmosphere about 2.4 billion years ago, during the Great Oxidation Event

The biology part

- In low CO_2 media, O_2 binds to Rubisco instead of CO_2, which wastes energy
- To overcome this, *Synechococcus* boosts the local CO_2 concentration in the cell by pumping in bicarbonate ions (HCO_3^-) that it then converts to CO_2
- The generated OH- is then exported outside the cell, increasing pH
- This creates an alkaline media that promotes carbonate precipitation
- Therefore: Grow in CO_2 depleted media with plenty of bicarbonate and calcium ions

What happens outside the cell?

1. At high pH, equilibrium shifts to carbonate ions:

$$HCO_3^- \rightleftharpoons H^+ + CO_3^{2}$$

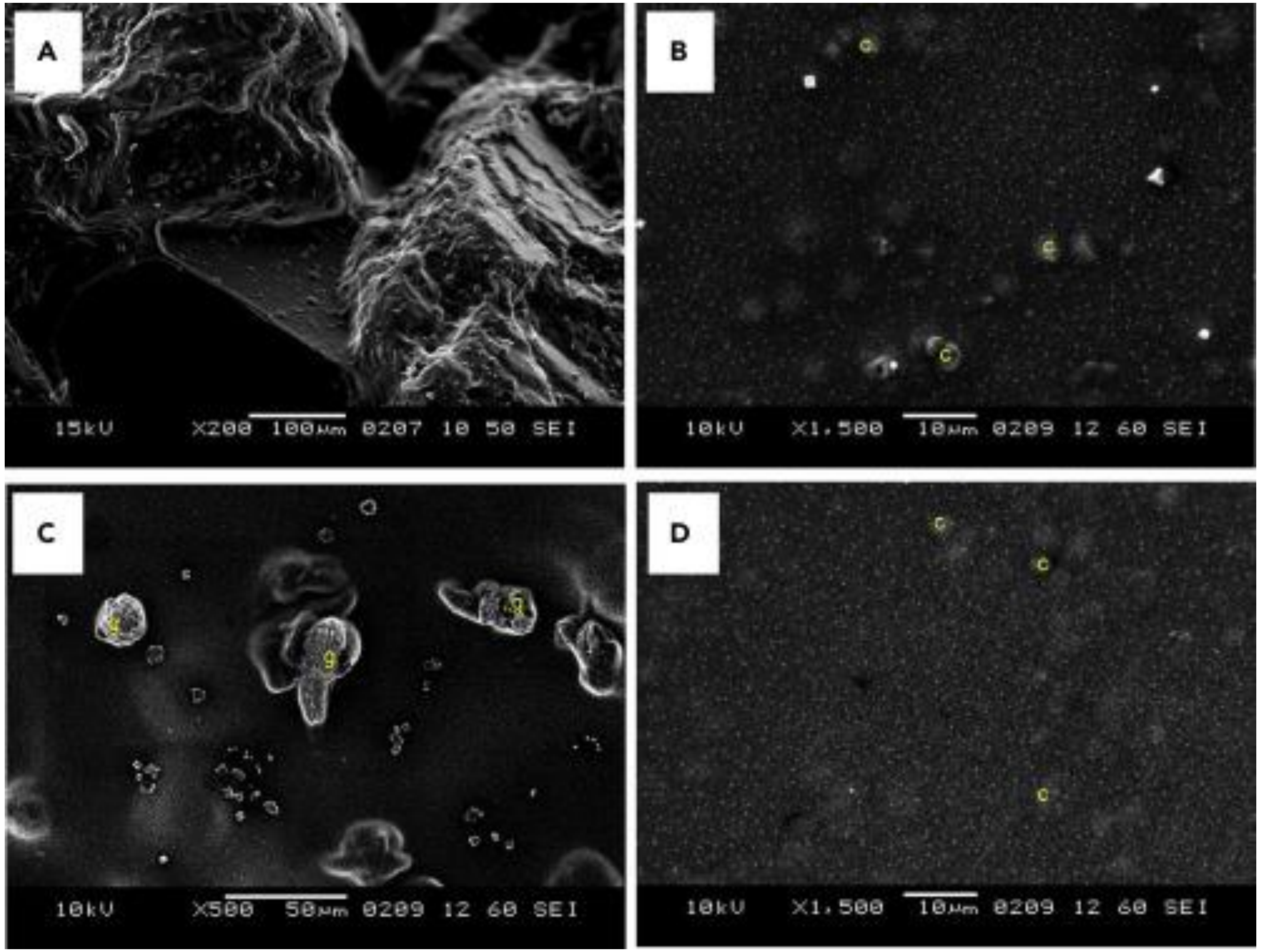
2. Calcium in the media is precipitated:

$$Ca^{2+}(aq) + CO_3^{2-}(aq) \rightarrow CaCO_3(s)$$

The main functional role of the bacteria is to increase the local pH…

https://doi.org/10.1016/j.matt.2019.11.016

Biomineralization



https://doi.org/10.1016/j.matt.2019.11.016

A) For LBMs as well as controls, gelatin bridges sand particles and provides a substrate for mineralization.

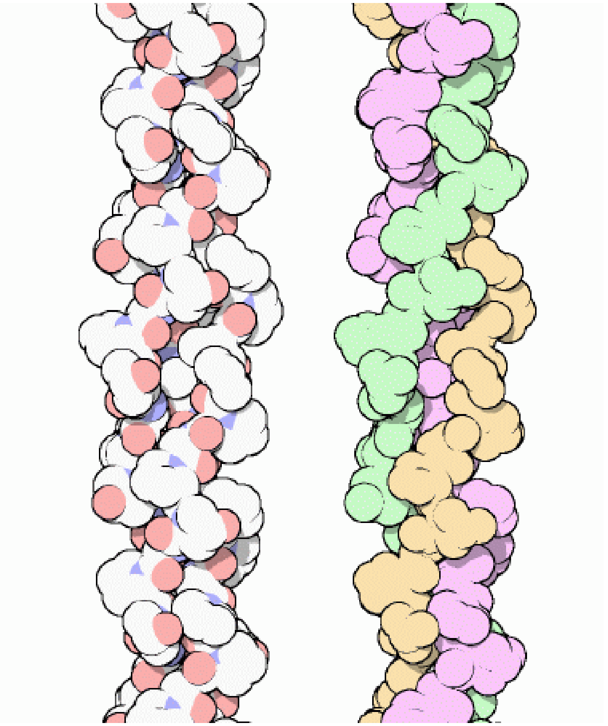
(B) *Synechococcus* sp. PCC 7002 induces precipitation of $CaCO_3$, identified with SEM-EDS, in ALS-gelatin medium.

(C) The abiotic control (pH 7.6) forms large gypsum particles as well as minor deposits of $CaCO_3$.

(D) The abiotic high-pH (pH 10) control forms $CaCO_3$, although these precipitates are smaller than when bacteria are present

The materials part

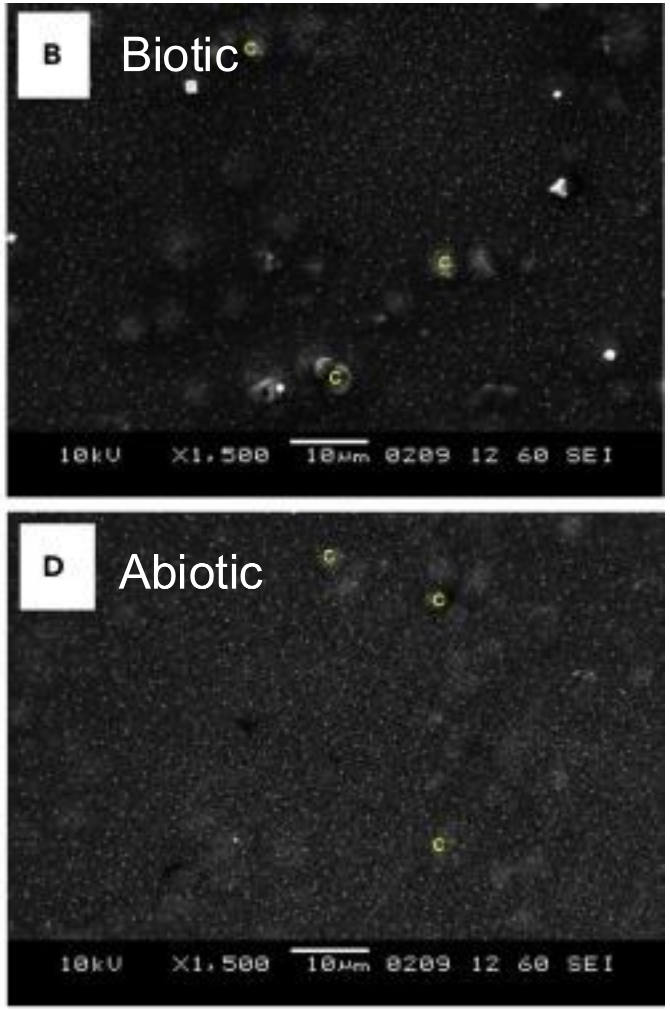
- Gelatin hydrogel
- Gelatin is derived from collagen
- Biocompatible: can host cells
- Liquid when warm (37°C), solidifies when cool
- They also added sand (from a local river)

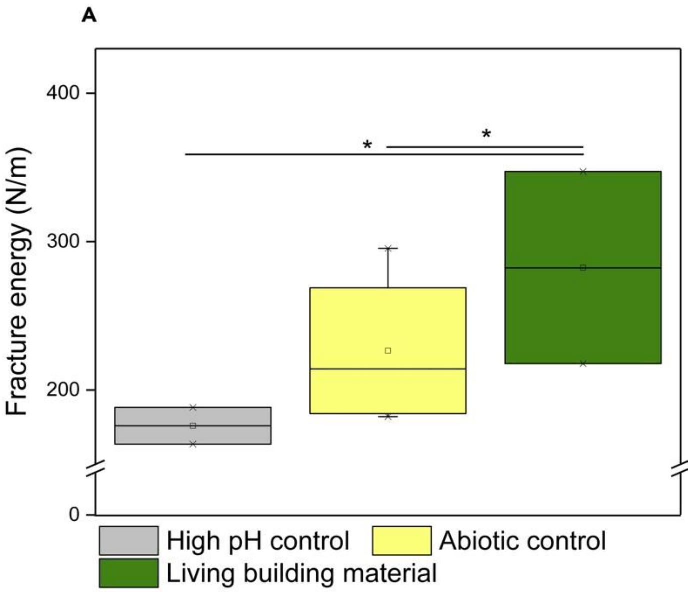


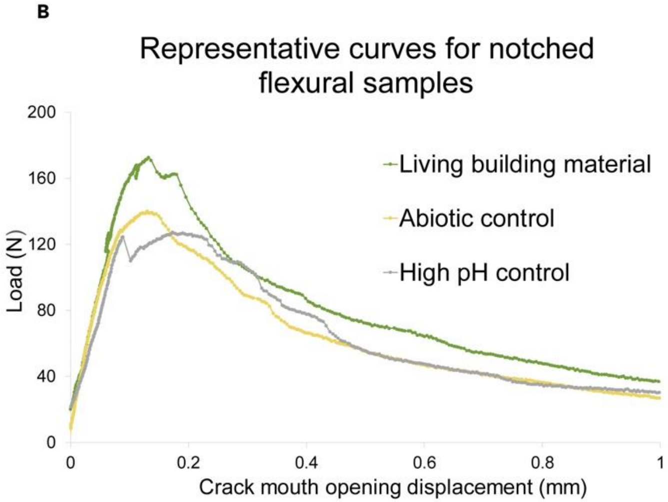
https://doi.org/10.1016/j.matt.2019.11.016

Biomineralization

https://doi.org/10.1016/j.matt.2019.11.016



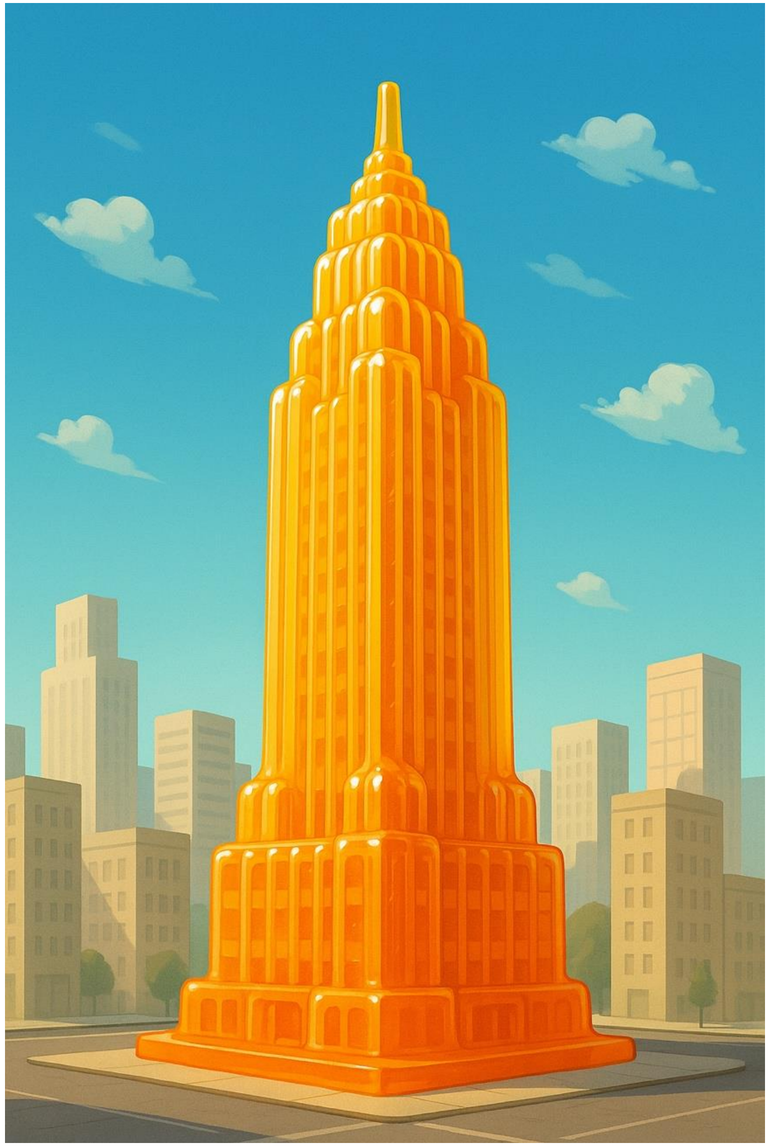




- Neutral pH doesn't work
- High pH without cells, gives smaller/fewer crystals (D)
- Best result is living cells that adjust the pH metabolically, since the pH is key, and perhaps the cells provide a **mineralization nucleation site** (B)
- It's subtle

Are we going to make bricks with gelatin?

- Hmmm, probably not…what happens on a hot day
- Significance is the poof-of-concept
- Cells are viable, their metabolism promotes biomineralization
- The materials are responsive to temperature and humidity in ways we might expect



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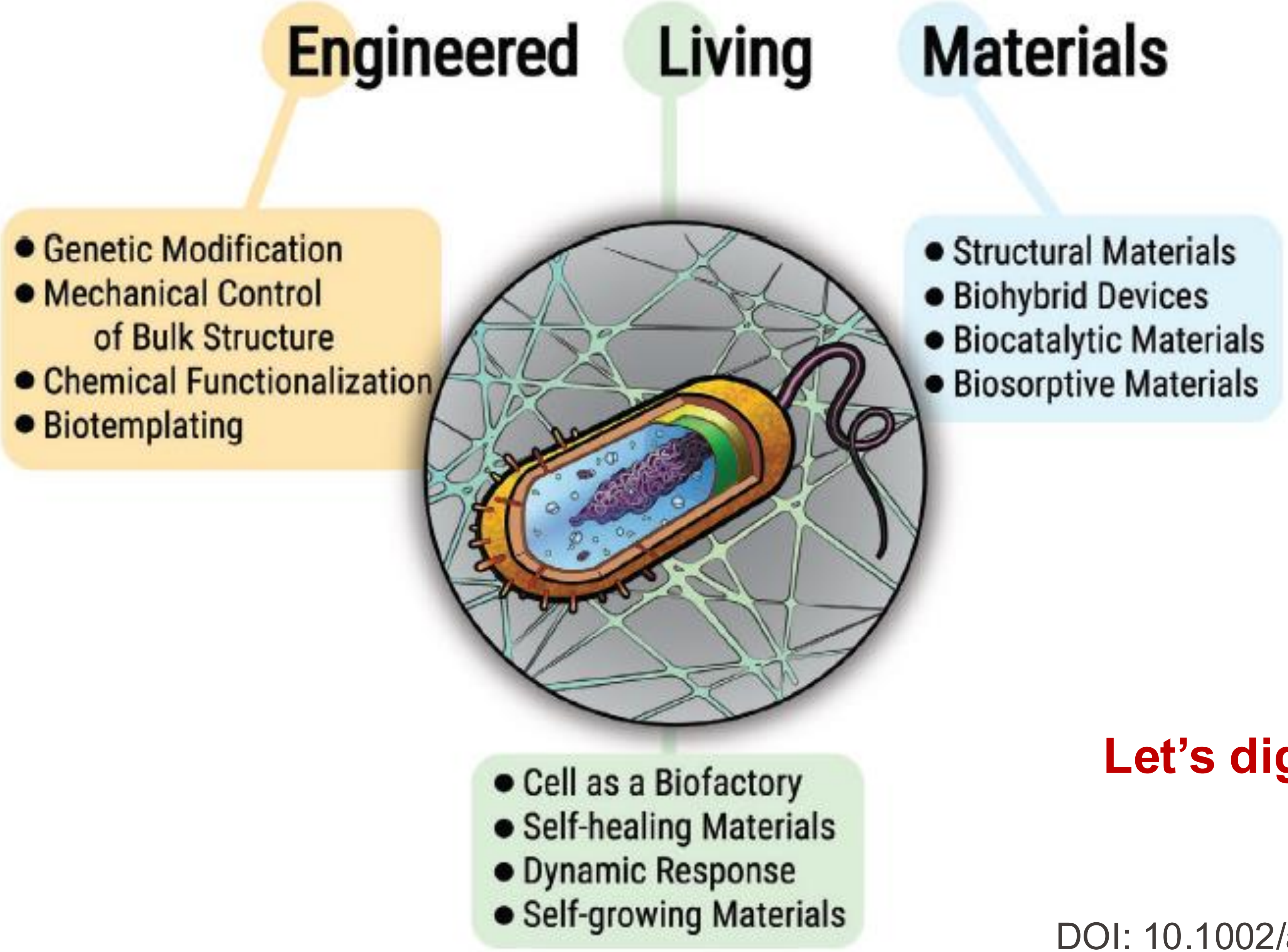
How this class works…

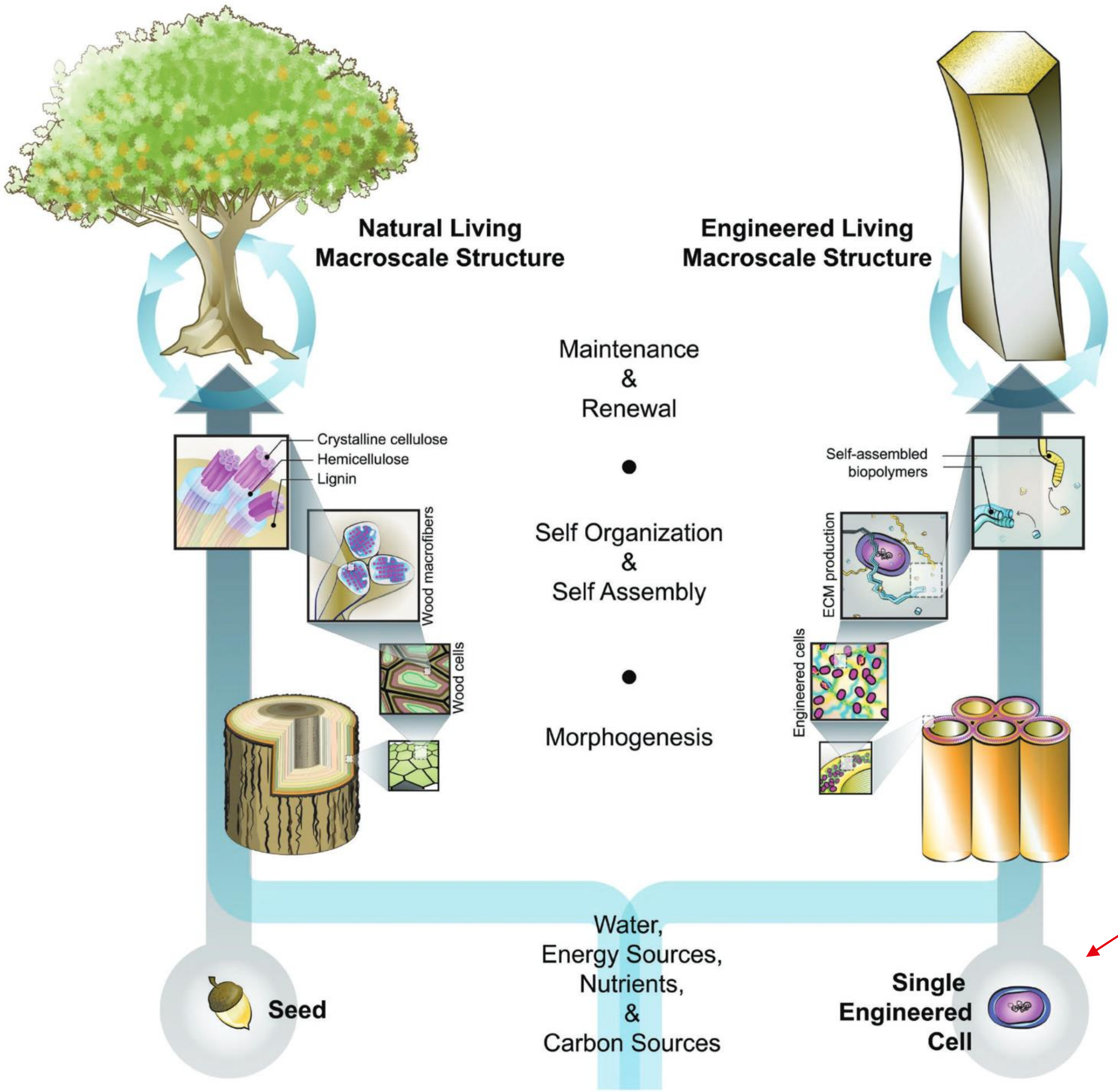
- Mostly, we look at emerging literature
- I try to give context as to why this is a topic of study
- I try to give background on the materials part (more familiar) and the biology part (less familiar)
- We sometimes look at who did the research, where, journal metric, etc.,
- It's more interesting if you read before class (sometimes prompted by a short assignment)

https://doi.org/10.1016/j.matt.2019.11.016



Let's dig in a bit more

DOI: 10.1002/adma.201704847



ELMs as bioinspired materials engineering

Uses tools of synthetic biology

DOI: 10.1002/adma.201704847

OK, so what is an ELM?

- Living cells **form or assemble the material**
- These living cells are materials factories, drawing energy from their environment, to create biopolymer building blocks and to guide material formation and maintenance
- Allows for stimuli-responsiveness over the course of the lifetime of the cells (adaptive gene expression)
- Composed of **cells** and/or of extracellular secretions (**biofilm**)
- If desired, can be processed or programmed to kill the living cells at a given time, keeping the material intact (no concern for maintenance or potential biohazard threats)

DOI: 10.1002/adma.201704847

The cells

The Microbes

In this section, we survey the representatives of microbes and the main methods to view them. Microbial diversity is the range of different kinds of unicellular organisms, including bacteria, archaea, protists, and fungi. Various microbes thrive throughout distinct strata of the biosphere. The resident microbes in any given biosphere often define the limits of life and create conditions conducive to the survival and evolution of other living beings. Entries include algae, eukaryotes and prokaryotes, and bacteria.

Microbes

Microbial Methods

- Human genome project
  1990-2003
- Human microbiome project
  2007-2016

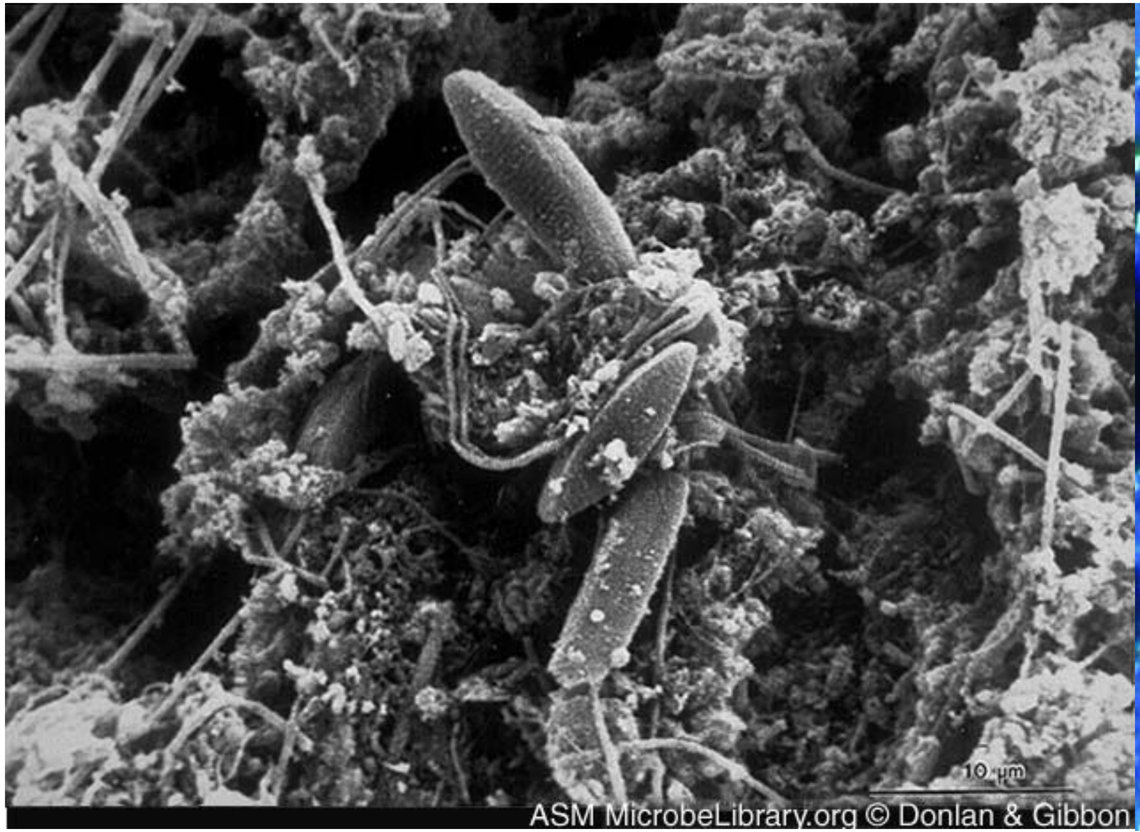
Genome project
Human microbiome project

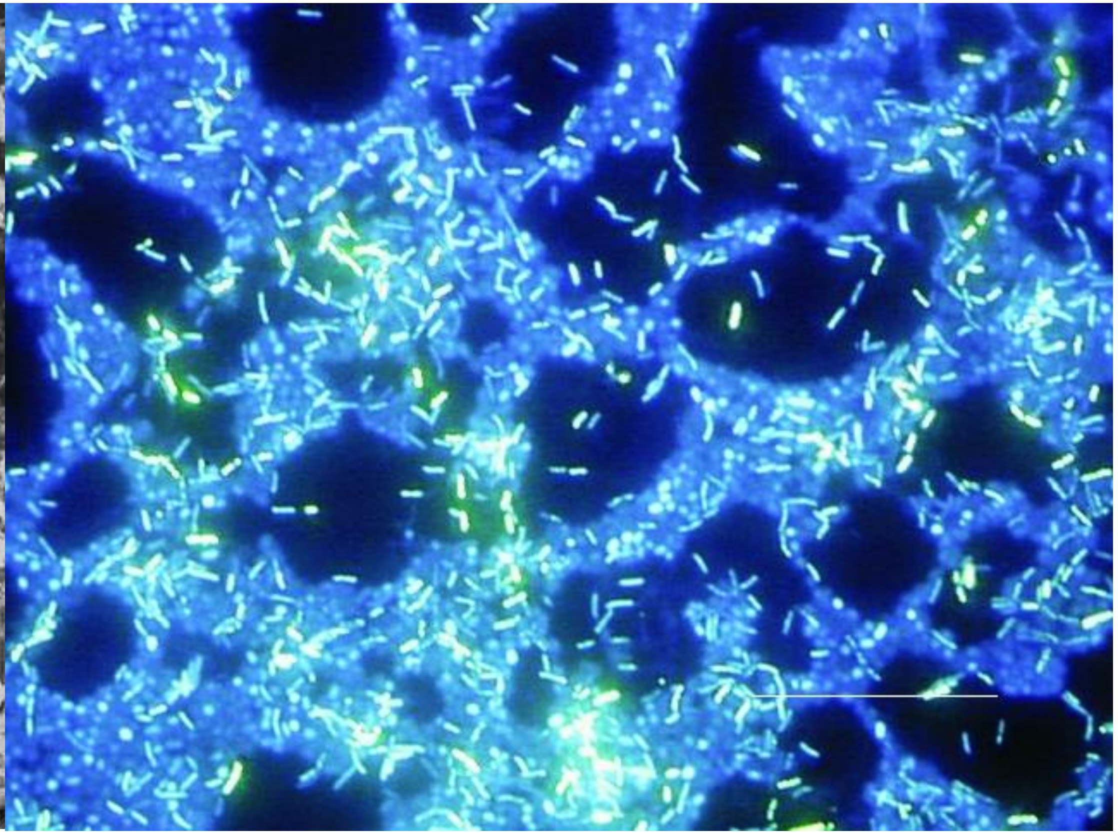
Principles of Microbiology

Microbes

Microbe examples	Description	Strategies for obtaining nutrients
Bacteria	Single-celled organisms that lack a nucleus	Mostly heterotrophs
Fungi	Includes yeasts and molds, which can be single-celled or multicellular	Mostly heterotrophs
Algae	Simple, typically aquatic organisms that can conduct photosynthesis, including both single-celled and multicellular forms	Mostly autotrophs - photosynthesis

Biofilms



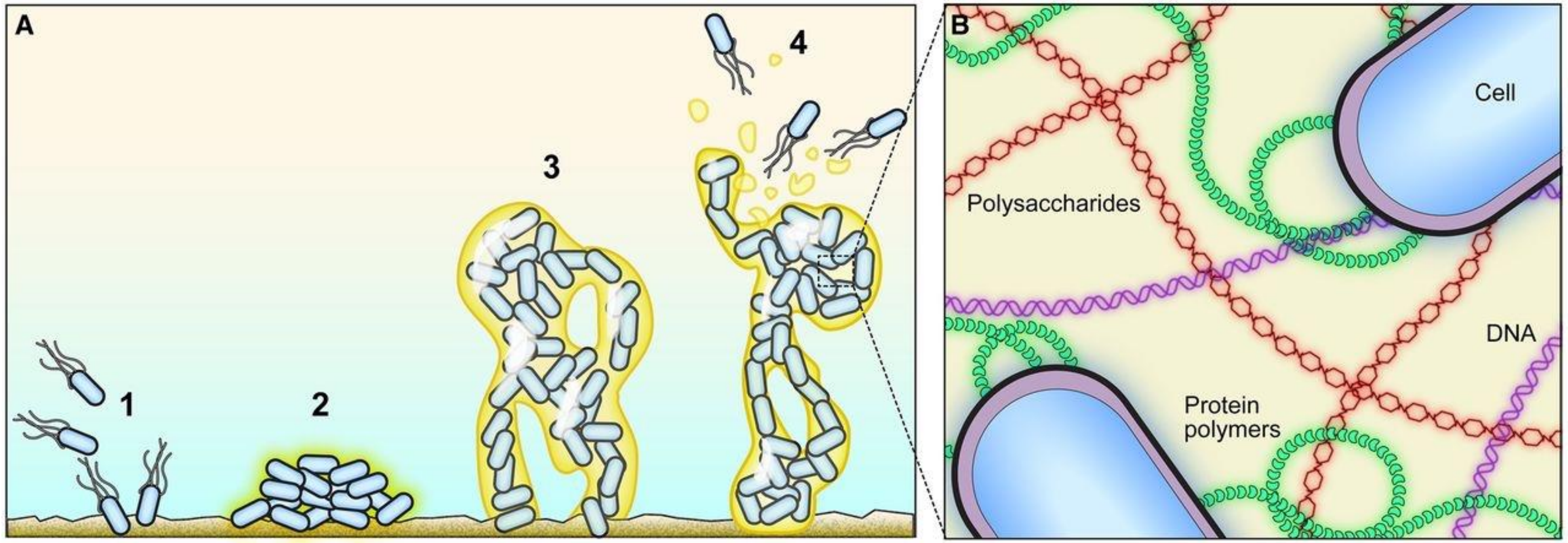


Biofilms on steel surfaces

doi: 10.3201/eid0809.020063

Biofilms

https://doi.org/10.1042/BST20160348



- A community of surface-associated microbial cells
- Protect microorganisms by encapsulation in extracellular matrix (ECM)
- Attach (1), ECM generation (2), mature biofilm (3), and biofilm dispersal for colonization (4)
- Three main components: collectively called extracellular polymeric substances (EPS) = polysaccharides, DNA, **self-assembled protein polymers/fibers**

OK, so what is not an ELM? (No strict definition actually…)

- There are many examples of “biohybrids” in the literature where the biological component is “just one element” of the final material **and does not actively create or modulate the bulk material structure**

REVIEW
Engineered Living Materials

ADVANCED MATERIALS
www.advmat.de

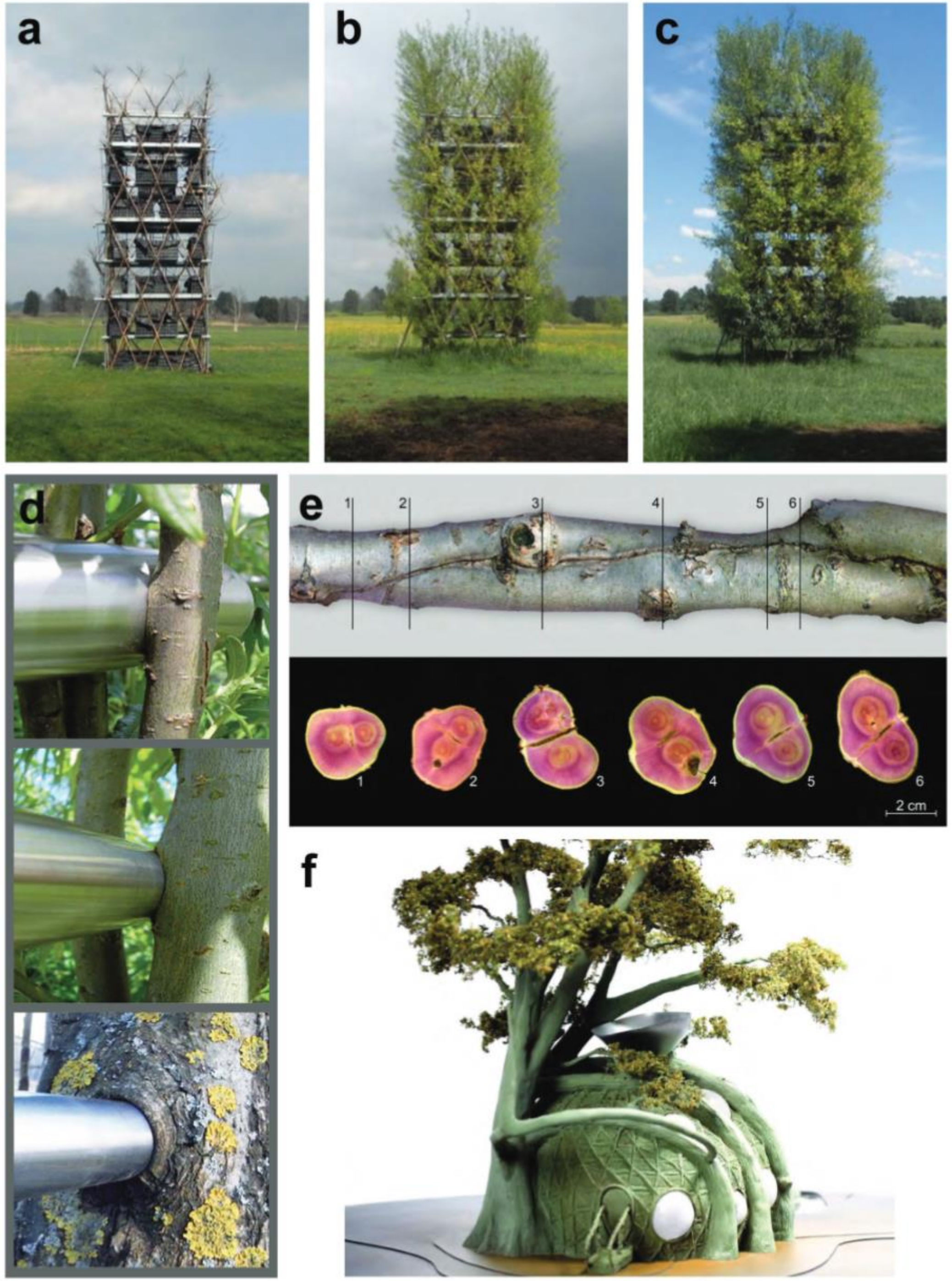
Engineered Living Materials: Prospects and Challenges for Using Biological Systems to Direct the Assembly of Smart Materials

*Peter Q. Nguyen, Noémie-Manuelle Dorval Courchesne, Anna Duraj-Thatte, Pichet Praveschotinunt, and Neel S. Joshi**

DOI: 10.1002/adma.201704847

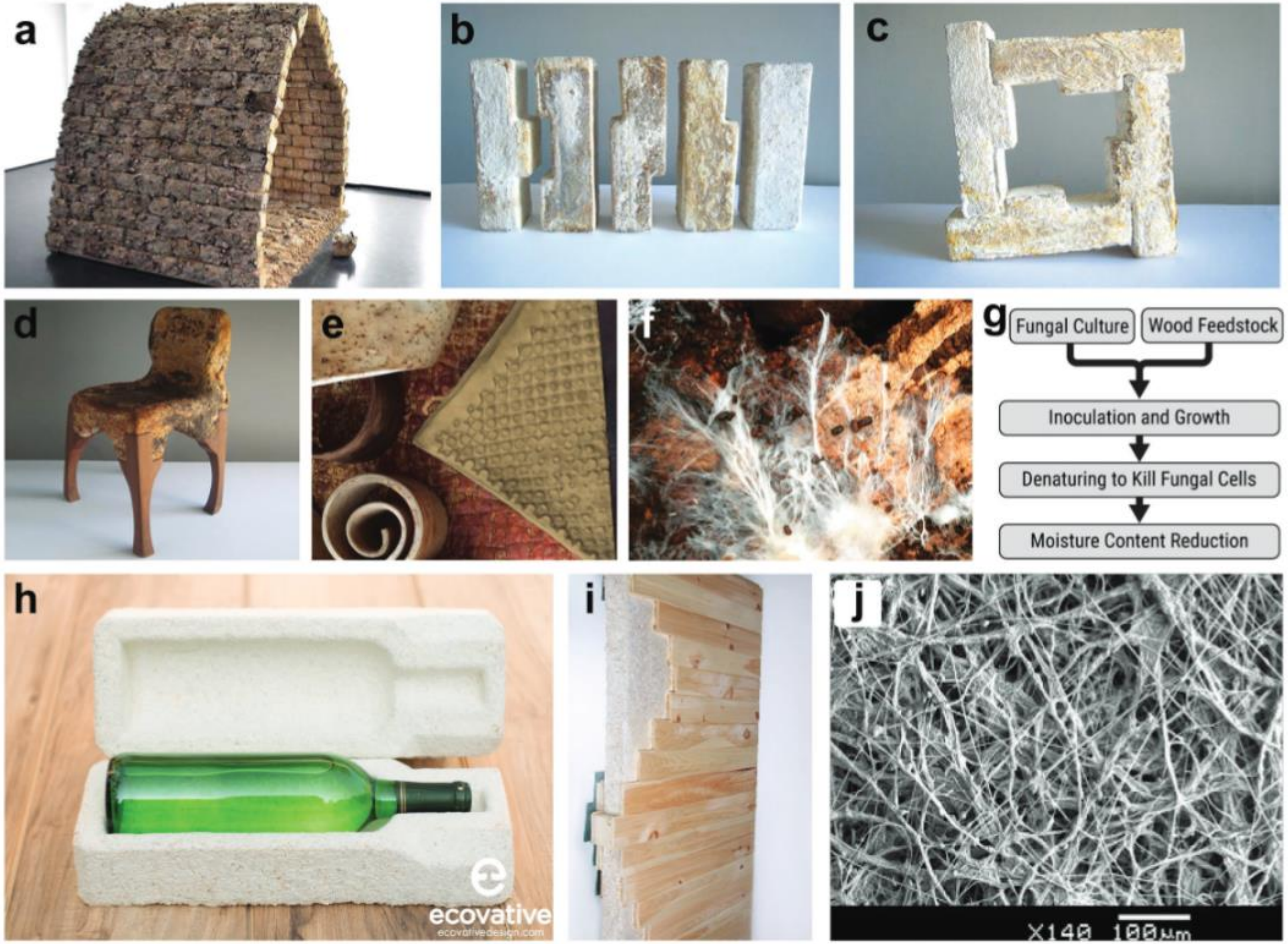
Be aware!

- In this course, we sometimes cover topics that are not ELM’s under the strict definition as put forth in this review
- Indeed, not all papers agree



Lignocellulosic living organisms as ELMs

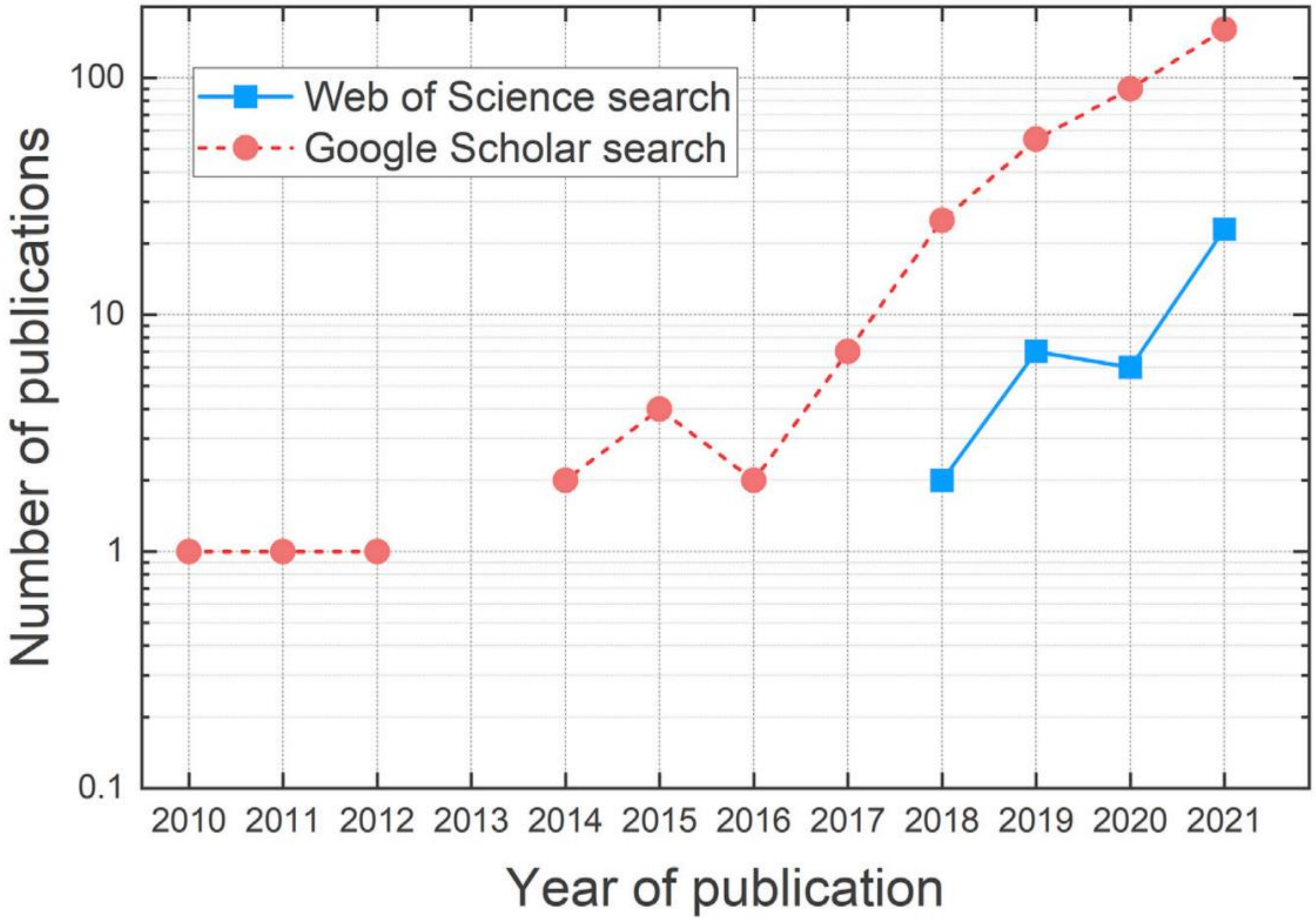
DOI: 10.1002/adma.201704847



Mycelium-based ELM materials

DOI: 10.1002/adma.201704847

ELMs - an emergent field of materials science



Annual number of publications obtained from a Web of Science and Google Scholar search using "engineered living materials" as keywords.

https://doi.org/10.1016/j.xcrp.2022.100807

1st use of term in 2017

Biochemical Society Transactions (2017) **45** 585–597
DOI: 10.1042/BST20160348

Review Article

Synthetic biology engineering of biofilms as nanomaterials factories

Peter Q. Nguyen[1,2]

[1]Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA 02115, U.S.A. and [2]School of Engineering and Applied Sciences, Harvard University, Cambridge, MA 02138, U.S.A.

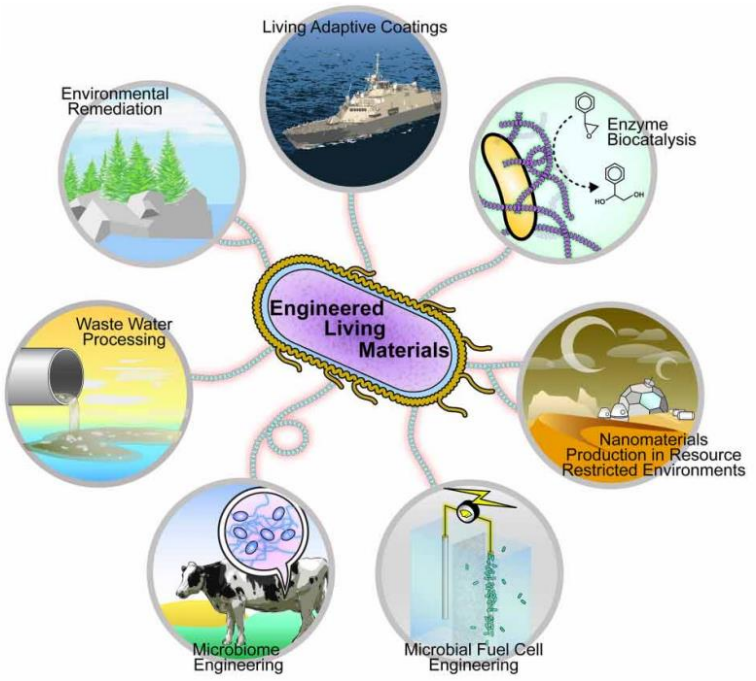
Correspondence: Peter Q. Nguyen (peter.nguyen@wyss.harvard.edu)

https://doi.org/10.1042/BST20160348

https://wyss.harvard.edu/

1st use of term in 2017

"An integration of *nanomaterials science* and *synthetic biology approaches* could allow the development of *nanomaterial-producing systems* that are able to *adapt dynamically to stimuli, modulate their properties over time, and heal themselves*, much like tissues are capable of doing. Such engineered systems can be thought of as '**engineered living materials**', materials that have *integrated living and non-living components*, respectively, the cells and the materials they generate."
– *Peter Q. Nguyen*



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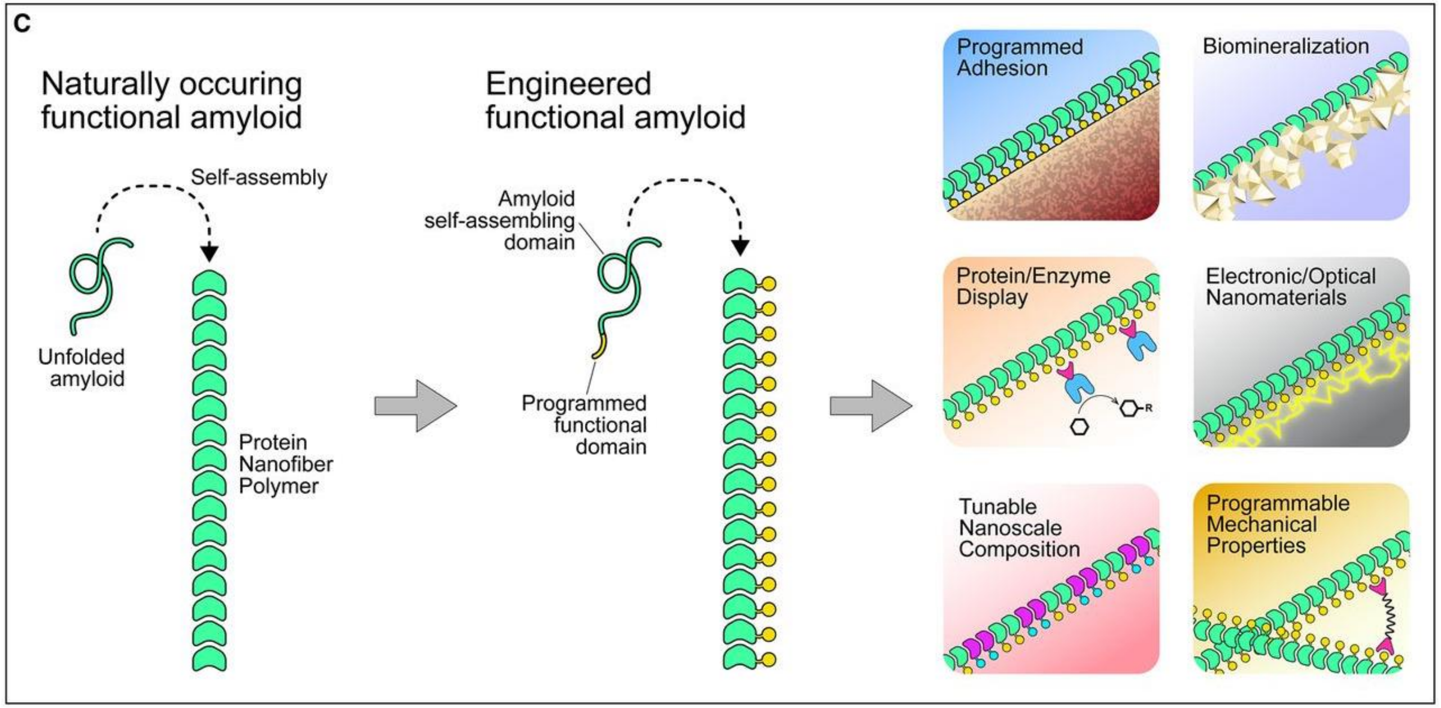
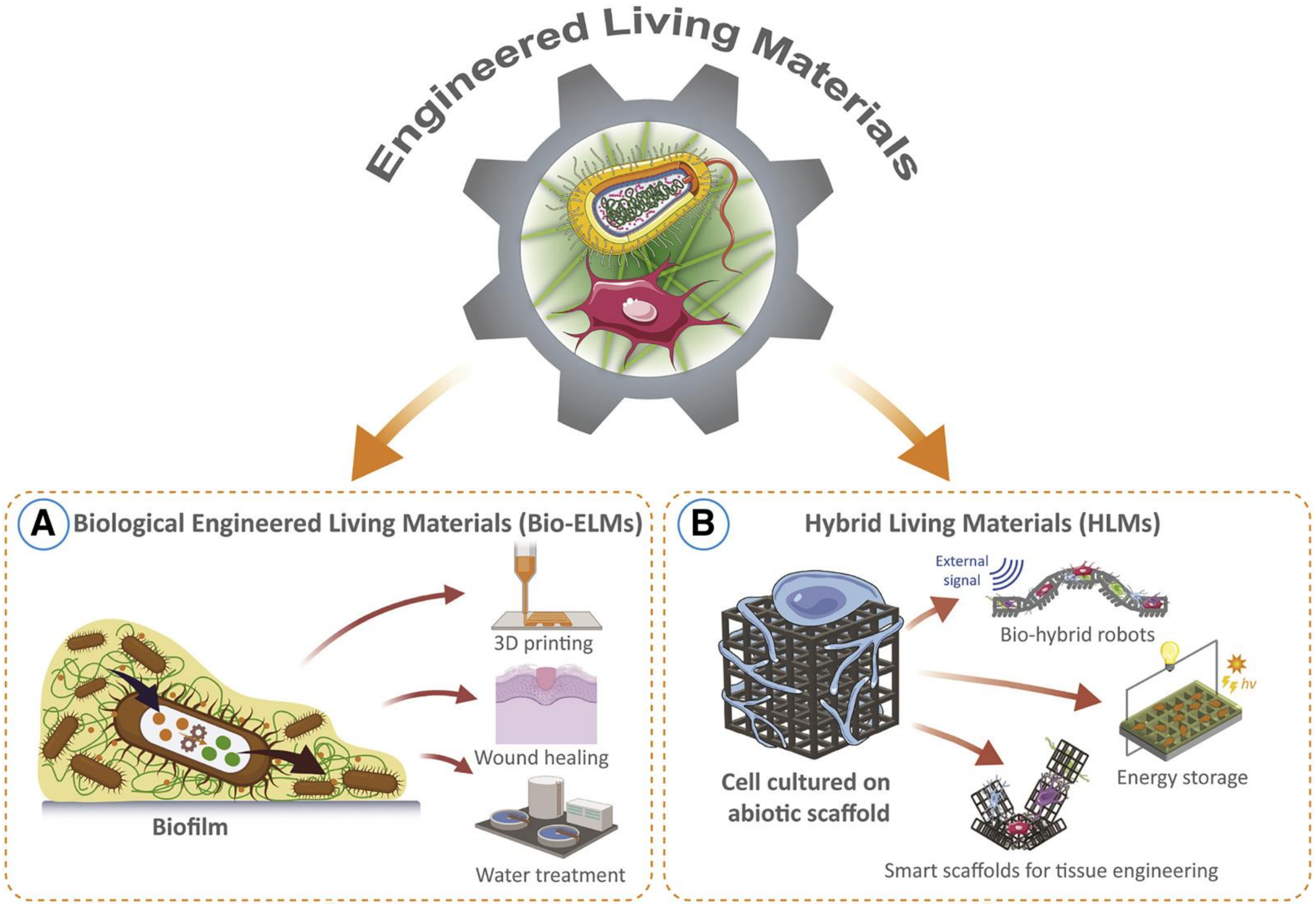
1st use of term in 2017



Programmable nanomaterials – expanding the **synthetic biology toolkit** for biofilm materials

https://doi.org/10.1042/BST20160348

Other definitions…



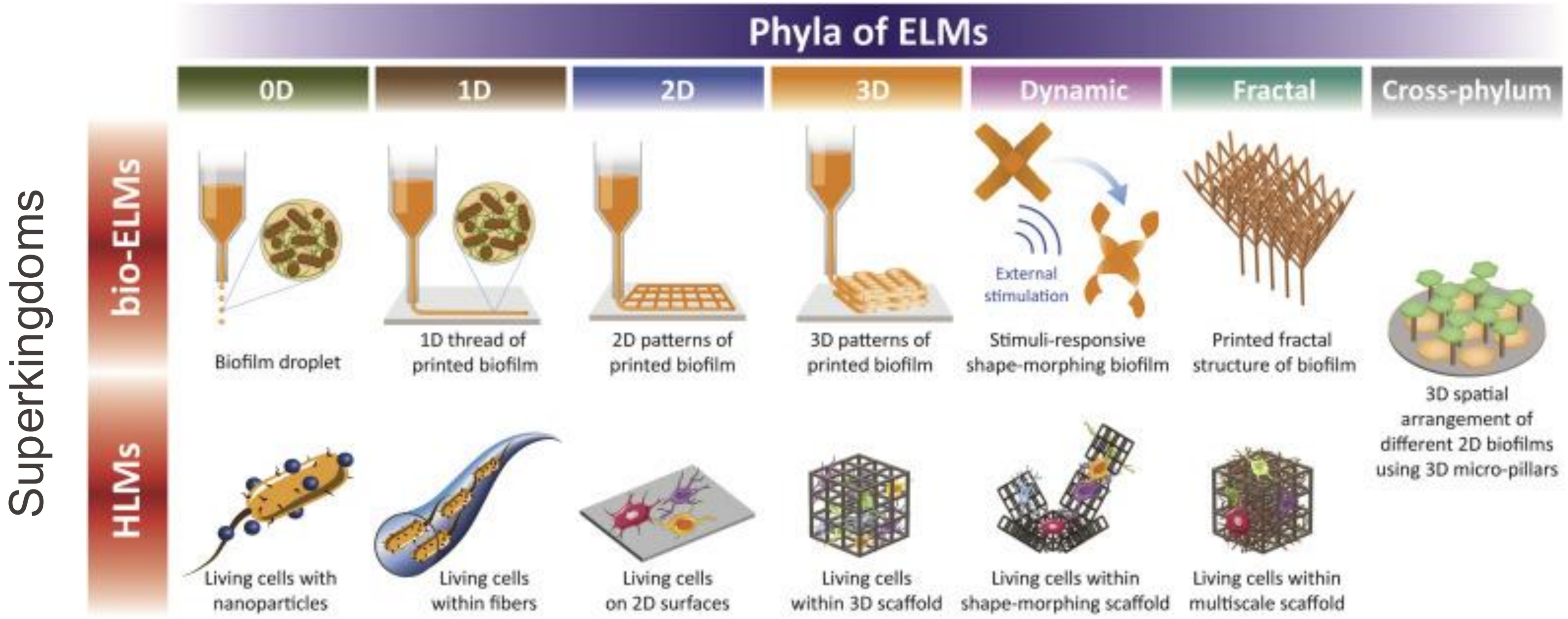
Defines ELMs as:

- Bio-ELMs – self-assembly of living cells
- Hybrid ELMs – incorporating living cells in a scaffolding structure (not a true ELM according to previous definition – but who's keeping track?)

https://doi.org/10.1016/j.xcrp.2022.100807

Proposed taxonomy of ELMs

Domain – *superkingdom* – kingdom – *phylum* – class – family



https://doi.org/10.1016/j.xcrp.2022.100807

Proposed taxonomy of ELMs

Domain – superkingdom – kingdom – phylum – *class* – *family*

https://doi.org/10.1016/j.xcrp.2022.100807

Proposed taxonomy of ELMs

Table 1. *Continued*

Pioneering examples of engineered living materials (ELMs)		Classification according to proposed taxonomy					
Described ELMs	Purpose/application field	Domain	Superkingdom	Kingdom	Phylum	Class	Family
Sand-hydrogel scaffolds with photosynthetic cyanobacteria[42]	smart buildings	bacterial	hybrid living materials	eubacterial ELMs	3D ELMs	living composites	living polymer matrix composite
Bacteria cultured on polymeric scaffolds growing ceramics[43]	materials production	bacterial	hybrid living materials	eubacterial ELMs	3D ELMs	living composites	living ceramic matrix composite
Self-healing, bacterial-loaded concrete[44]	smart buildings	bacterial	hybrid living materials	eubacterial ELMs	3D ELMs	living ceramics	living concrete

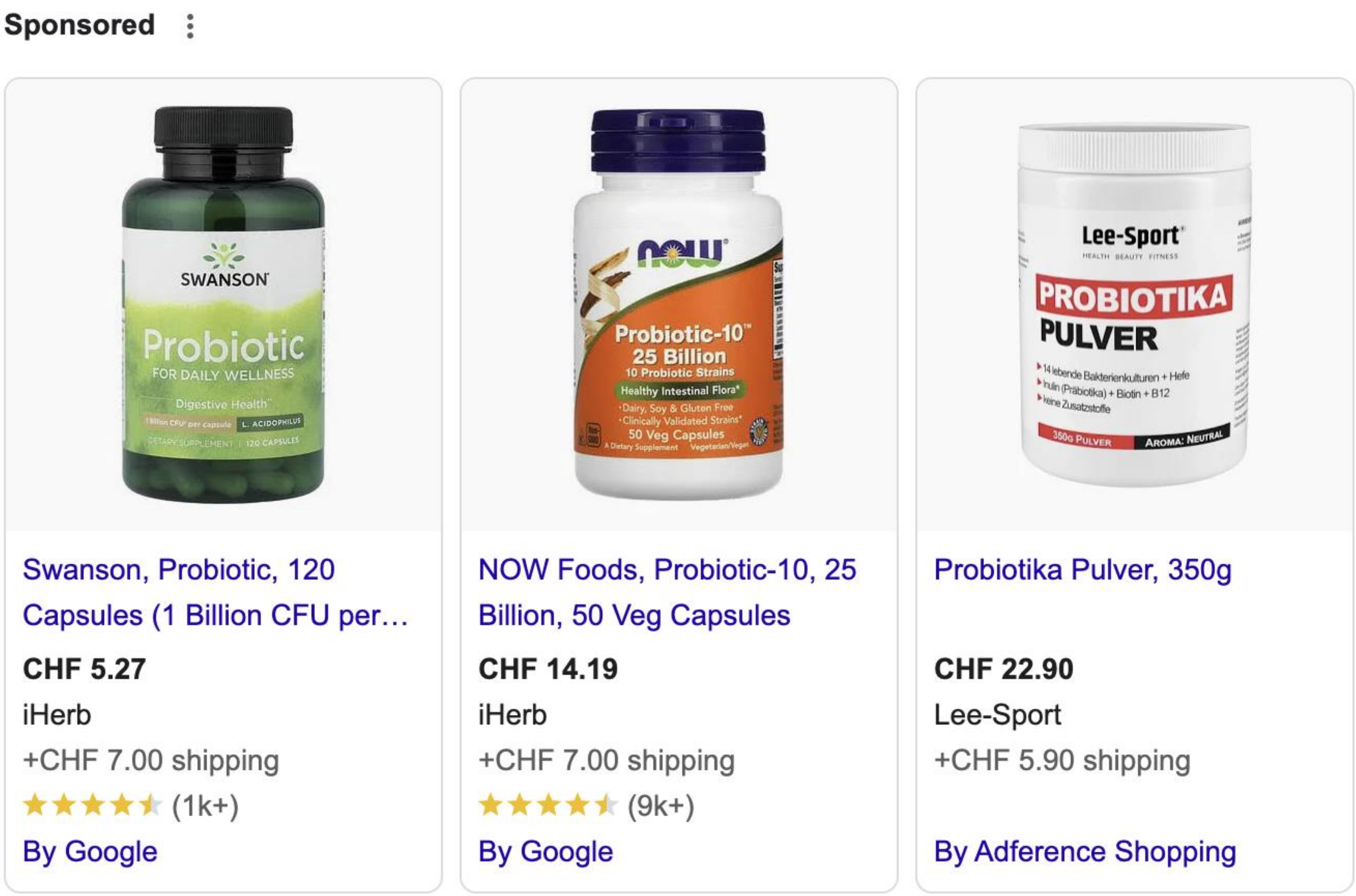
Table 1. *Continued*

Pioneering examples of engineered living materials (ELMs)		Classification according to proposed taxonomy					
Described ELMs	Purpose/application field	Domain	Superkingdom	Kingdom	Phylum	Class	Family
Musculoskeletal cells attached to hair[83]	robotics	eukaryotic	hybrid living materials	animal ELMs	1D ELMs	living polymers	living elastomers
3D printed bio-ink containing cardiac tissue[84]	smart surfaces and structures	eukaryotic	hybrid living materials	animal ELMs	dynamic ELMs	living polymers	living hydrogels
Cardiomyocytes with CNTs[18]	robotics	eukaryotic	hybrid living materials	animal ELMs	dynamic ELMs	living carbon	living CNTs
Mycelium-based composites (fungi into organic substrate)[85]	materials production	eukaryotic	hybrid living materials	fungal ELMs	3D ELMs	living polymers	other living polymers (fibers)
Bioprinted bioinks with baker's yeast[86]	biotechnology and bioprocessing	eukaryotic	hybrid living materials	fungal ELMs	3D ELMs	living polymers	living thermoplastics

https://doi.org/10.1016/j.xcrp.2022.100807

ELM?

The Next Frontier:
"*L. lactis* has been engineered to secrete anti-inflammatory drugs to combat inflammatory bowel disease[49,50] and *Lactobacillus jensenii* has been engineered to produce antivirals to inhibit vaginal HIV entry[51]."



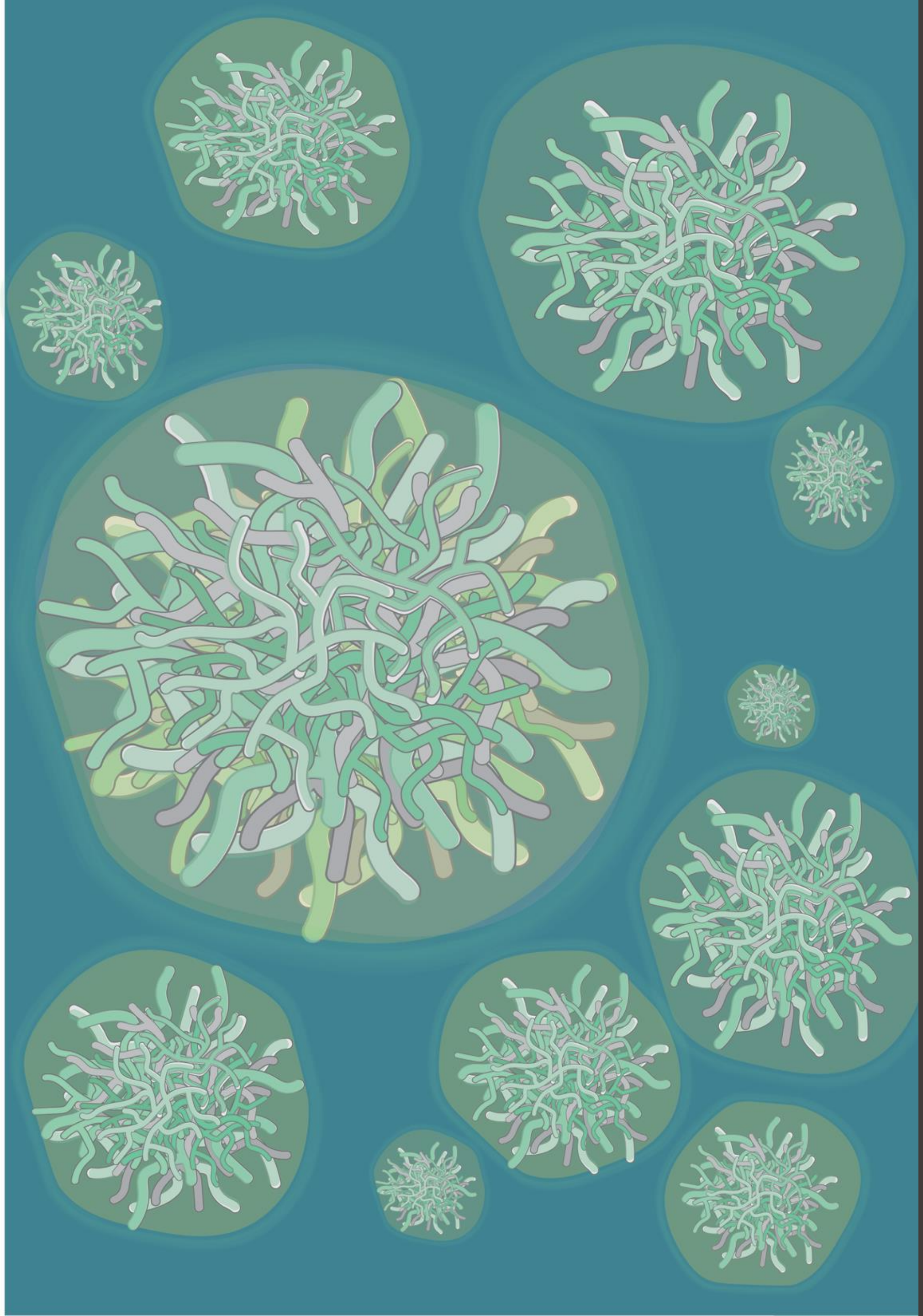
https://doi.org/10.1038/s41578-021-00350-8

Take aways

- You are in this class because:
  - You are interested in the interface between biology and materials
  - You want to learn more about how high-level research is done, all the nitty gritty, not just the popular science headline

- After today:
  - You have an idea how this class is structured and what is being asked of you
  - You are more familiar with ELMs
  - You have a basic understanding of where ELMs might be used

- Next week, it’s going to be a crash course on biology concepts that come up *over and over and over again* in this field



See you next week!