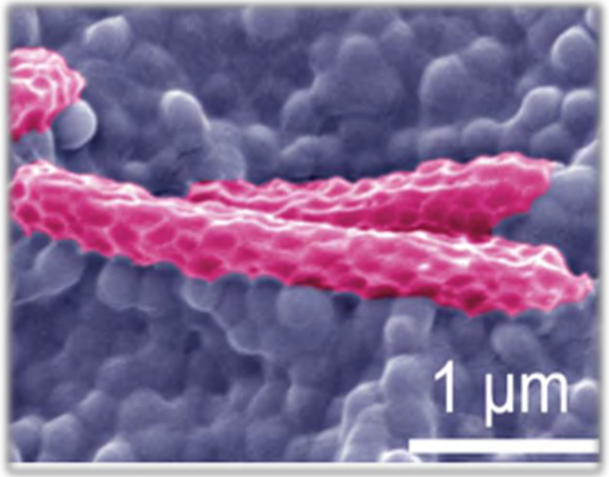
Adding Functionality to Coatings with Non-Growing, Metabolically-Active Bacteria

Joe Keddie[1]

Yuxiu "Phil" Chen[1], Simone Krings[2], Joshua R. Booth[3]
Stefan A. F. Bon[3] and Suzanne Hingley-Wilson[2]

@KeddieLab



@BonLab

[1] Department of Physics, University of Surrey
[2] Department of Microbial Sciences, University of Surrey
[3] Department of Chemistry, University of Warwick

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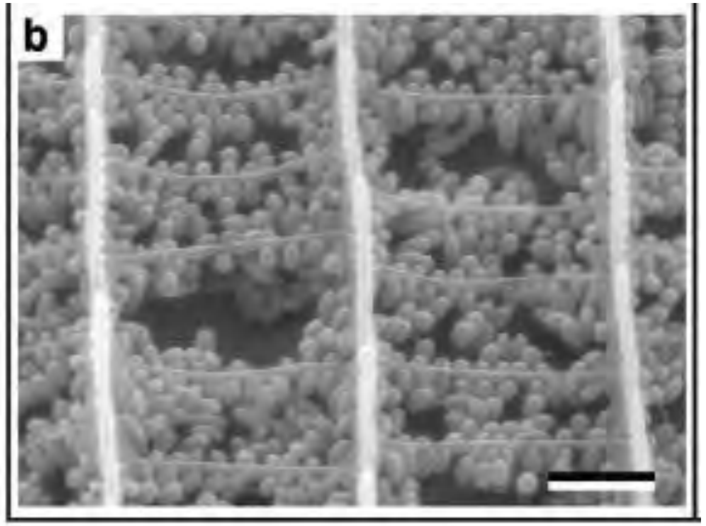
Formulating Functional Films and Coatings III – 19th August, 2020

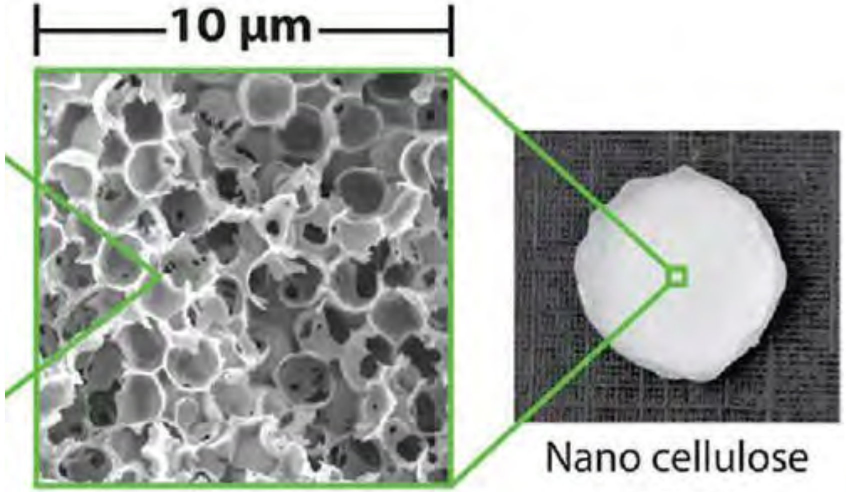
The Biomimetic Strategy

(1)
Observe a useful property in Nature

(2)
Understand the structure and function

(3)
Manufacture from synthetic (or natural) materials





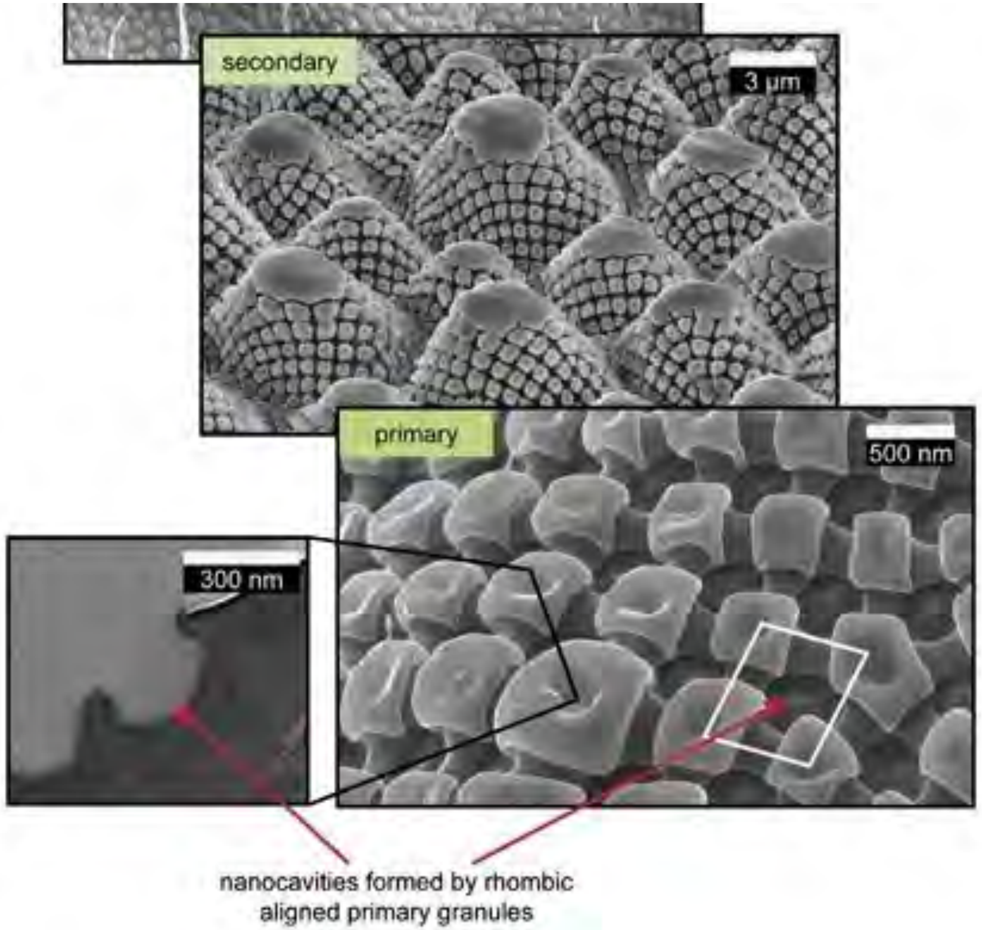
Nano cellulose paper

G. Jacucci *et al.*, (2020) *Adv. Mater*. 2001215

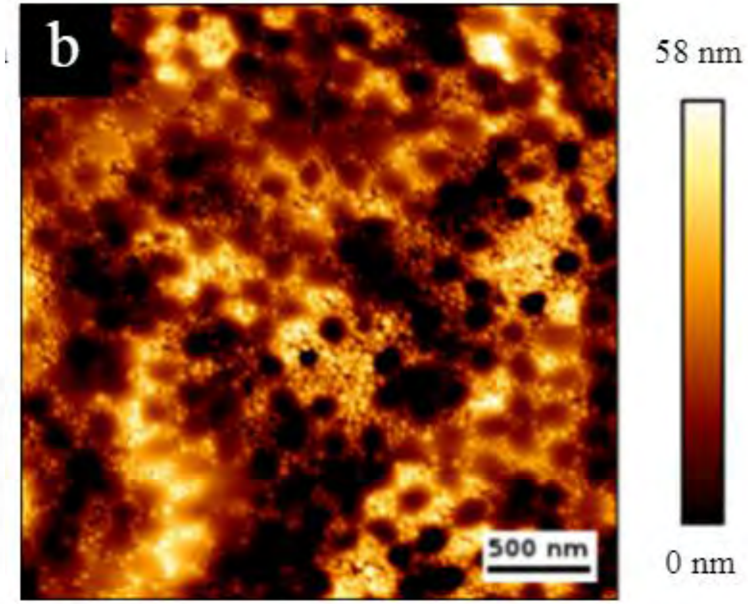
The Biomimetic Strategy

Omniphobic springtail skin

R. Hensel et al., (2013) *NPG Asia Mater*, **5,** e37.
https://doi.org/10.1038/am.2012.66



Hierarchical structure with "overhang"



Coating from a mixture of three sizes of polymer and silica particles

J.D. Tinkler et al. (2021) *J. Coll. Interf. Sci.*, **581**, 729-740.

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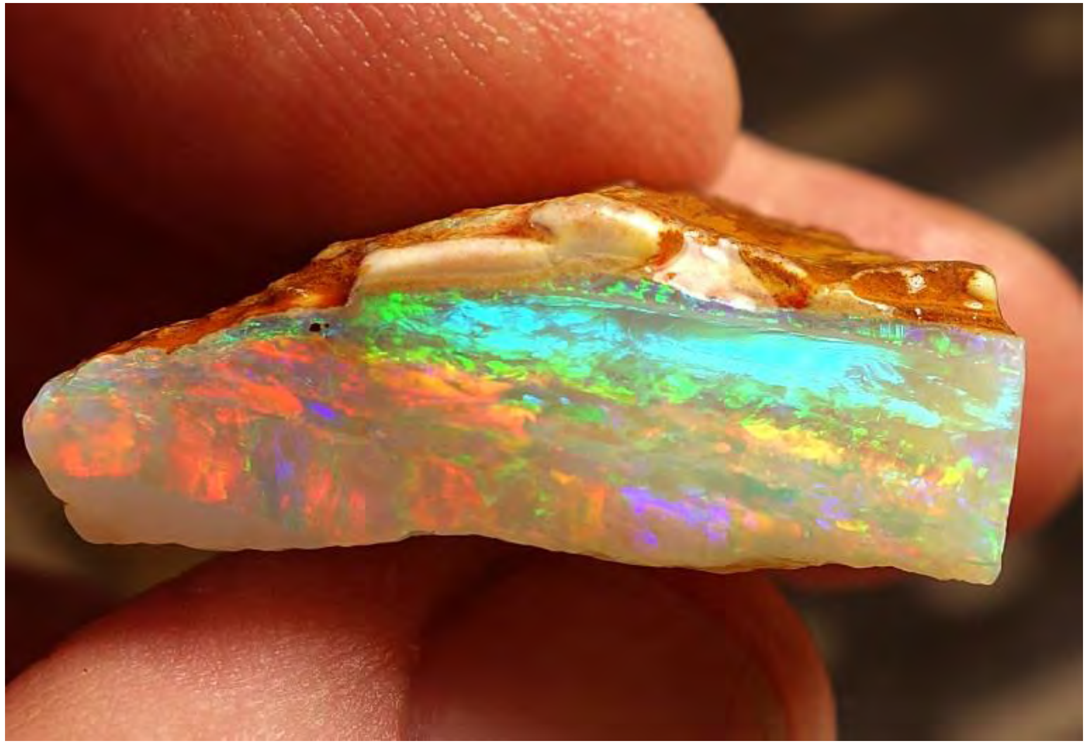
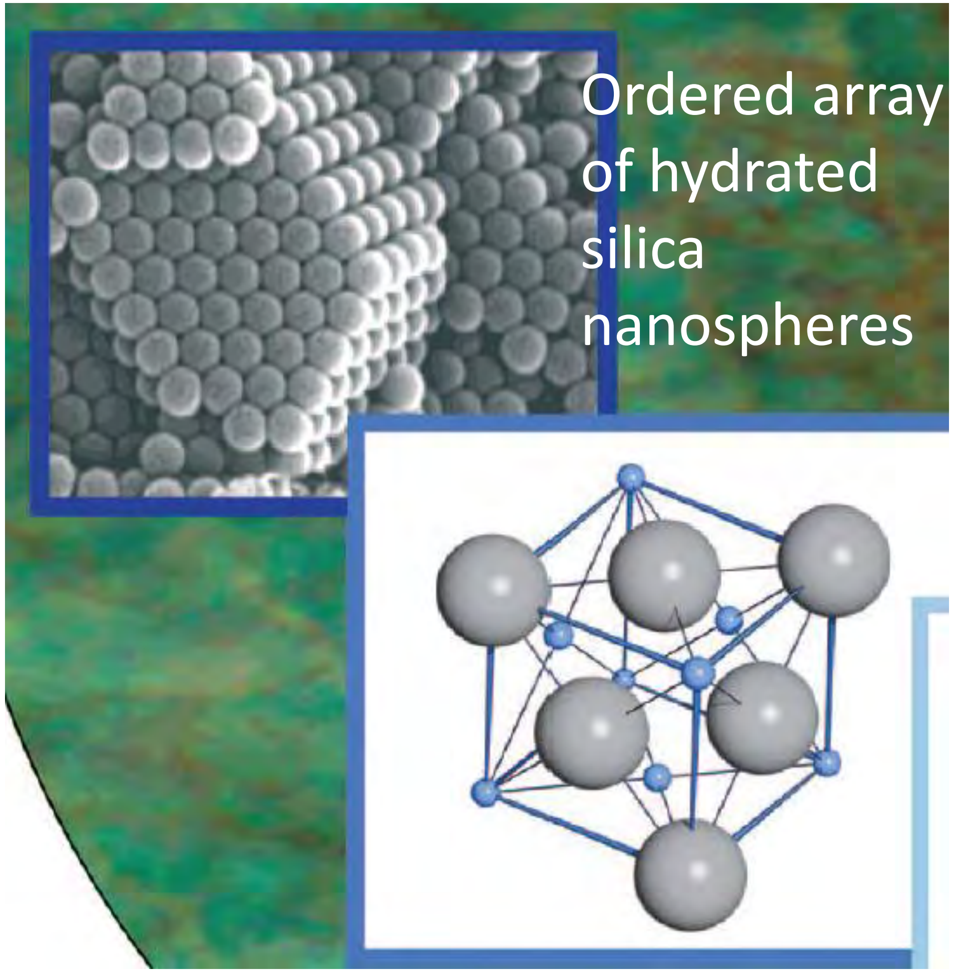
Inspiration from Nature



Opal from Coober Pedy, South Australia

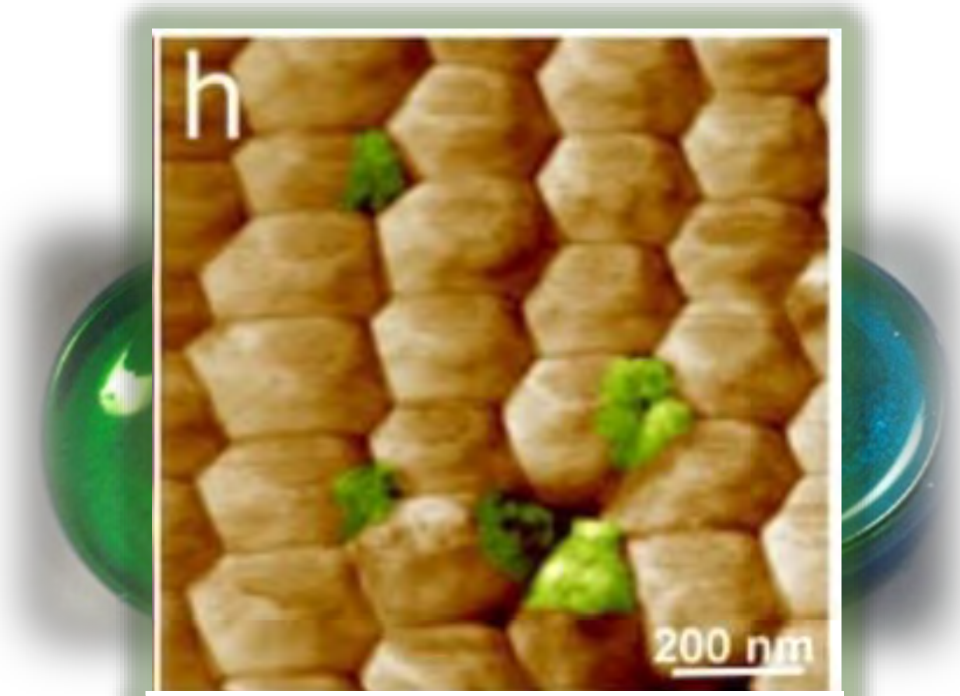
By Dpulitzer - Own work, CC BY-SA 3.0,
https://commons.wikimedia.org/w/index.php?curid=30411934

Nanostructure

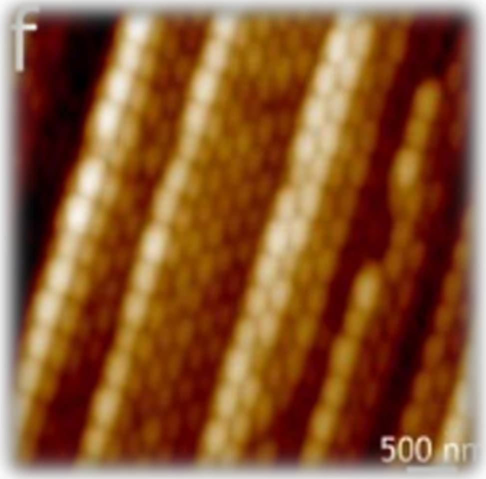


F. Marlow *et al*. (2009) *Agnew. Chem. Int. Ed.*, **48**, 6212-33.

Bio-Inspired Nanomaterials



Graphene sheets between particles



Ordered nanostructure

Izabela Jurewicz

I. Jurewicz *et al*., Mechanochromic and Thermochromic Sensors Based on Graphene Infused Polymer Opals, *Adv. Funct. Mater*. (2020) https://doi.org/10.1002/adfm.202002473

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Mechanochromic Response of Polymer Opals Infused with Graphene

Low concentration of graphene sheets enhances the structural colour because of its

- ❑ wide spectral absorbance
- ❑ high refractive index

Dr. Izabela Jurewicz

I. Jurewicz *et al.*, ***Adv. Funct. Mater.*** (2020) https://doi.org/10.1002/adfm.202002473

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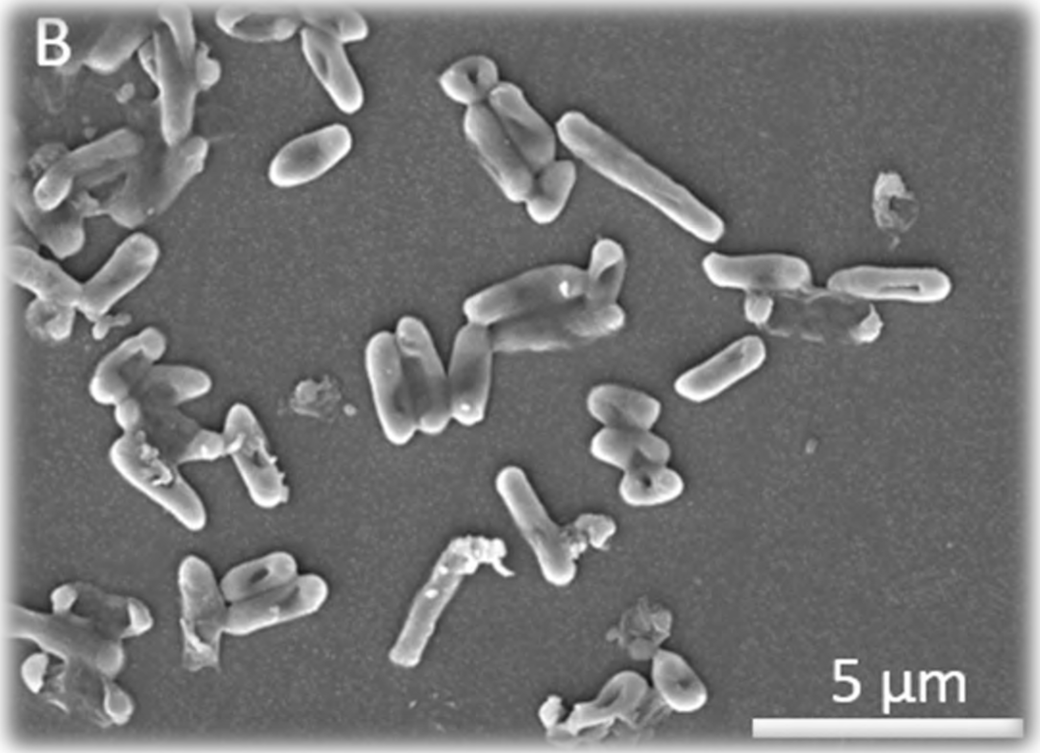
Which Coating Properties Are Desired?

Would Like to Achieve:

- Colour Control
- Environmental Sensing
- Remediation of Toxins
- Carbon Fixation
- Biofuel Production

The Answer: Bacteria!



E. coli cells viewed in the SEM

Cortez, S., et al., (2017) *Biochemical Engineering Journal*, **121**, 25-37.

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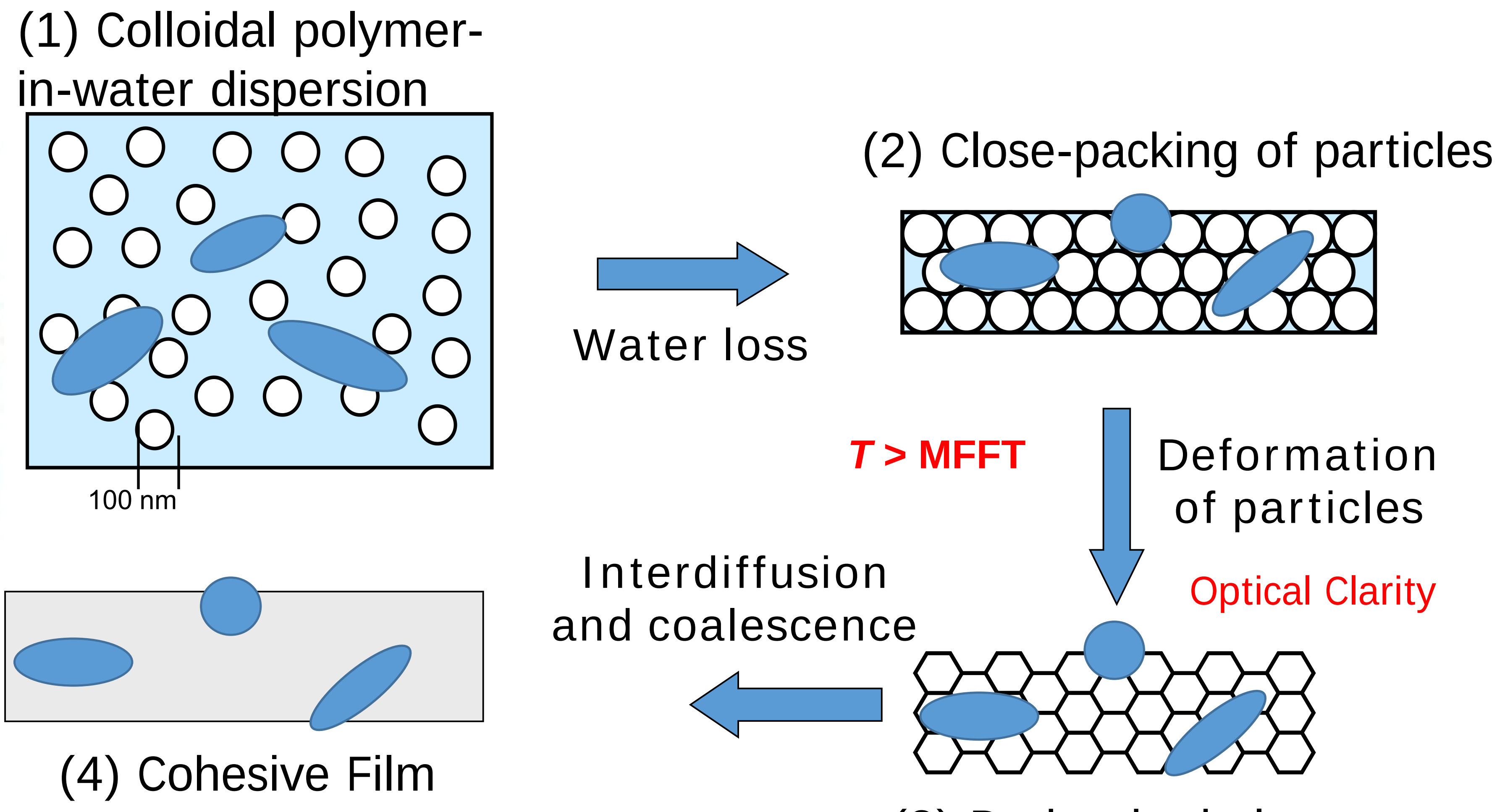
Film Formation of Biocoatings

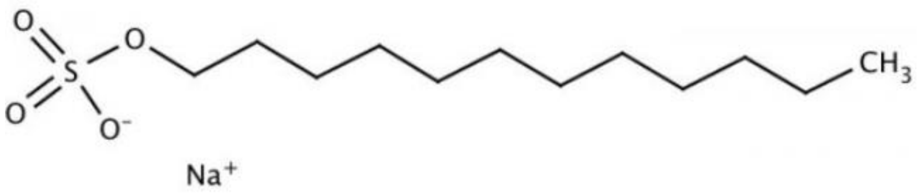
Bacteria encapsulated in a polymer coating



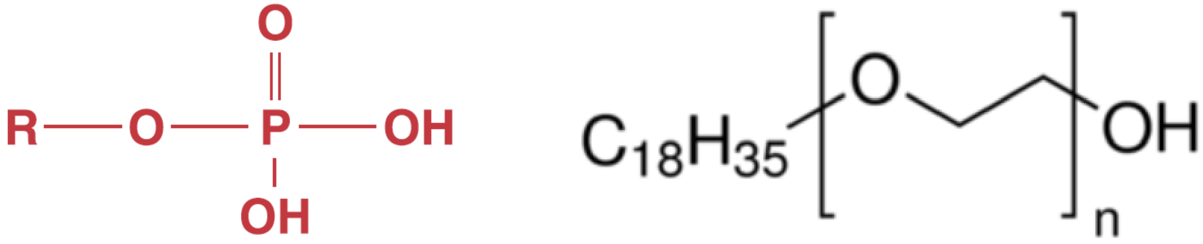
(3) Dodecahedral structure (honey-comb)

Will the bacteria survive dehydration and re-hydration?

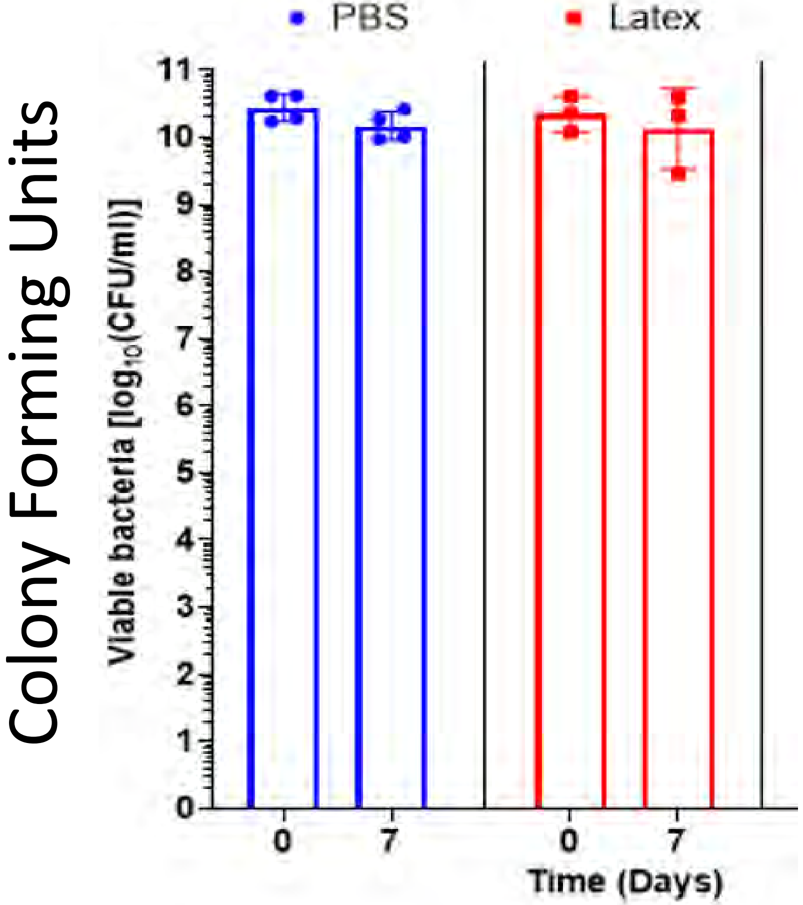
Is Latex Toxic to Bacteria?



Sodium dodecyl sulphate (SDS) is believed to be toxic (damaging to membranes)



Phosphated (anionic) and Brij L23 (non-ionic) surfactants were used instead.



Bacteria were grown inside this latex for 7 days and no toxicity was found

See poster by Josh Booth

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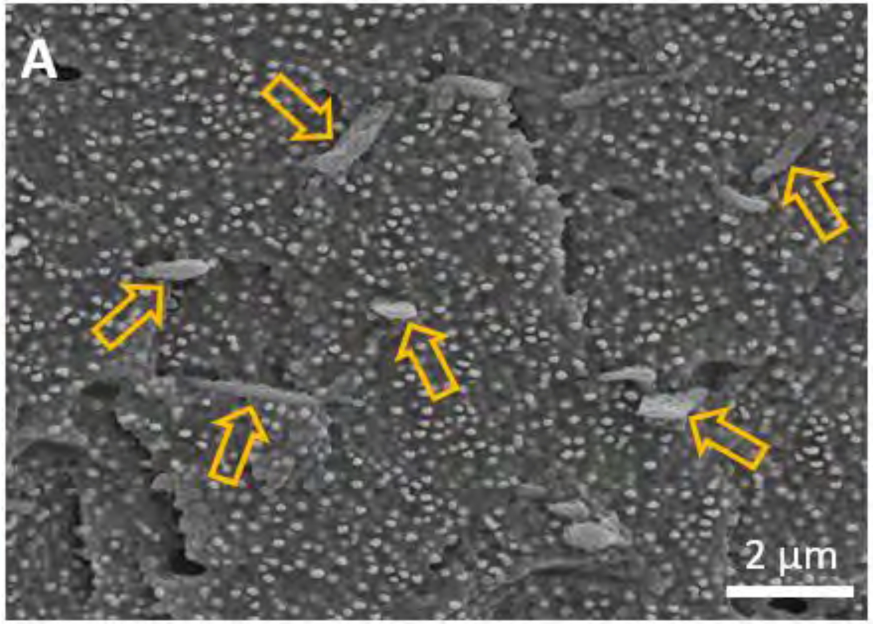
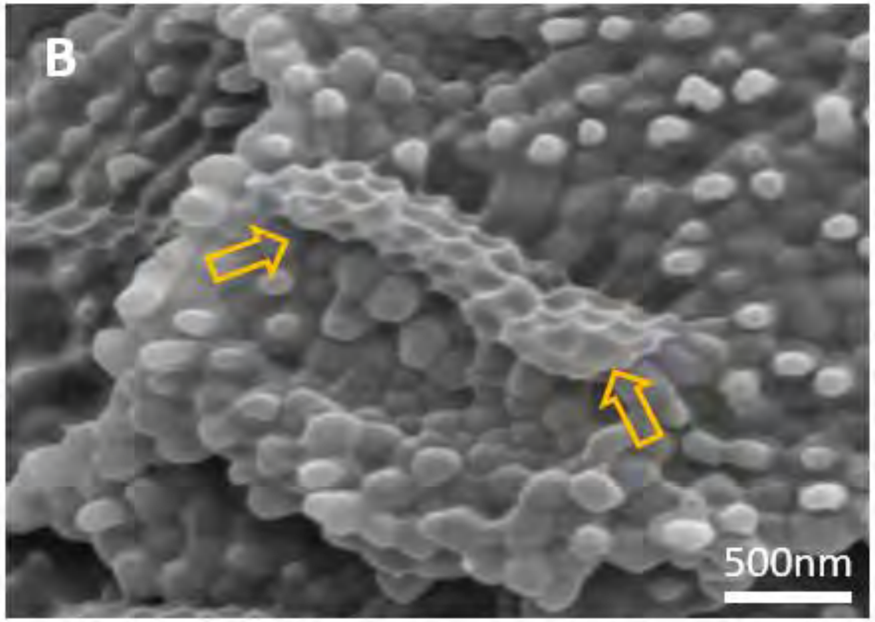
Bacteria in a Polyacrylate Biocoating



SEM Cross-Sectional Images



E. coli are dimpled!

Glass transition temperature: 34 °C

Film formed at 37 °C

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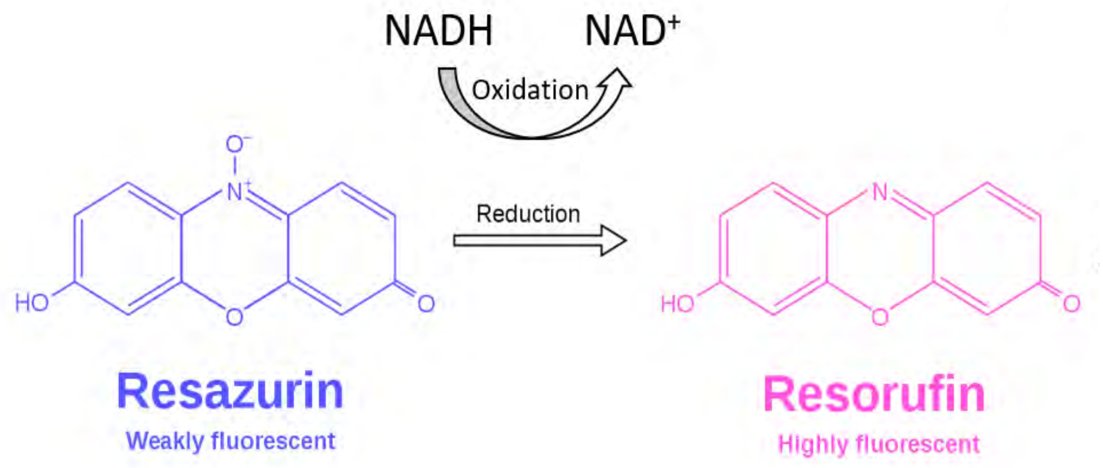
Are the Cells Metabolically Active?



E. coli oxidises NADH to NAD+

Resazurin is reduced to resorufin.

Low metabolic activity

Intermediate metabolic activity

High metabolic activity

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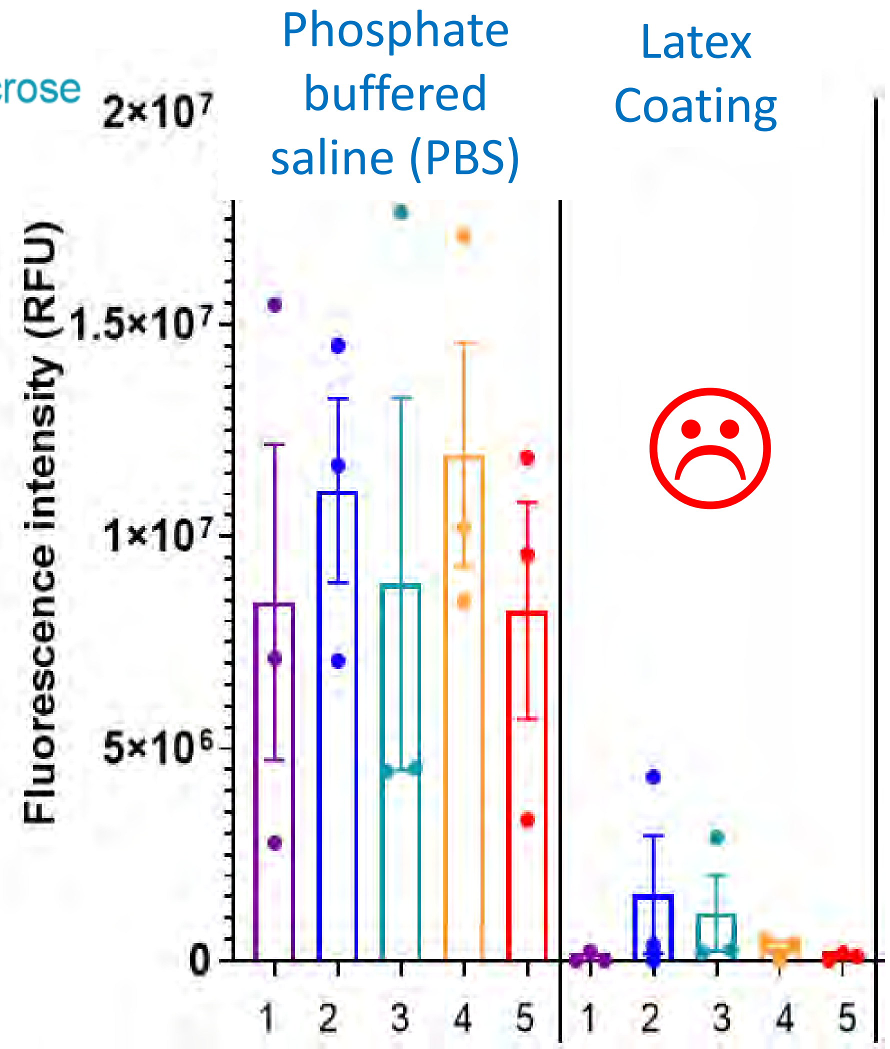
Encapsulated Bacteria Have Low Viability

<u>Osmoprotectants</u>:

1 - Only PBS 2 - Glycerol 3 - Sucrose

4 - Trehalose 5 - Phytoglycogen

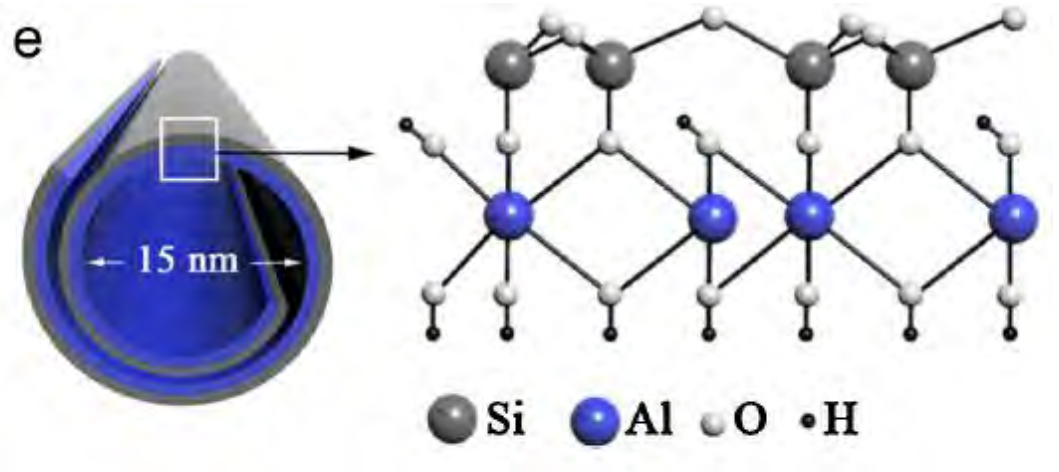


The Problem:

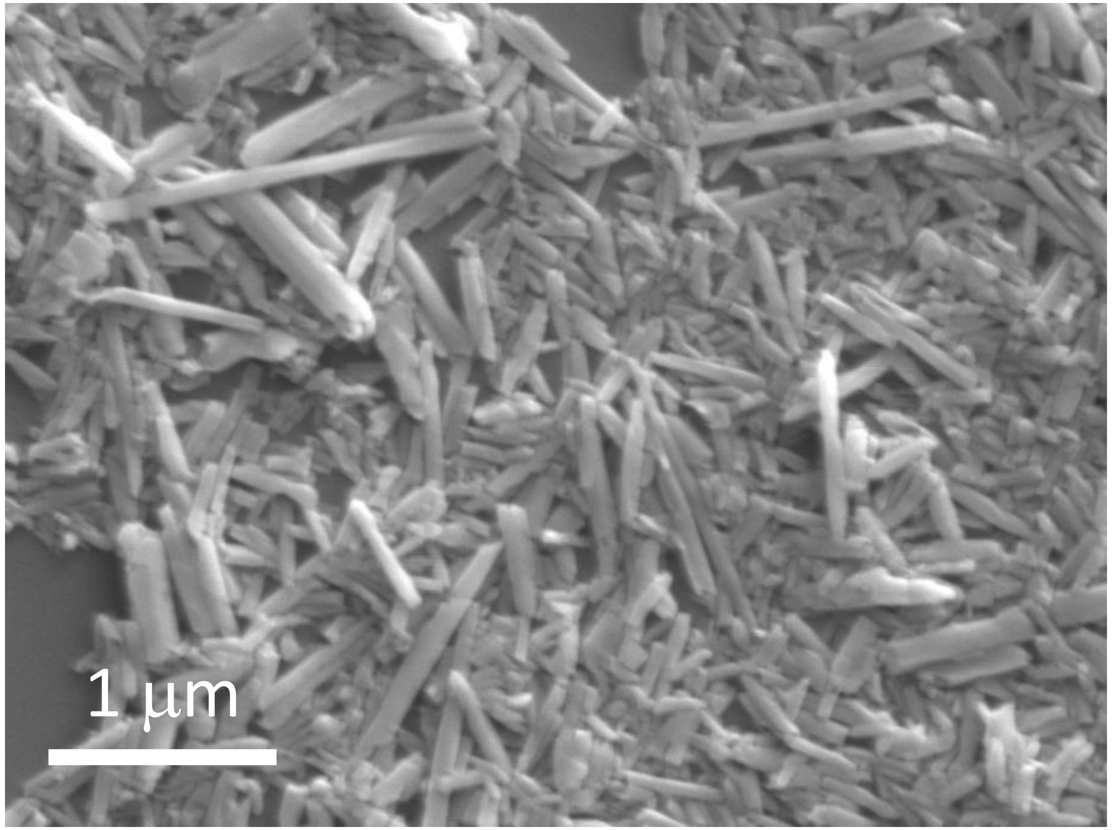
- Latex coatings have **low** permeability.
- Cells are not re-hydrated.
- No metabolic activity.

Solution: Use Clay Nanotubes as Fillers to Introduce Porosity and Increase Permeability

Halloysite

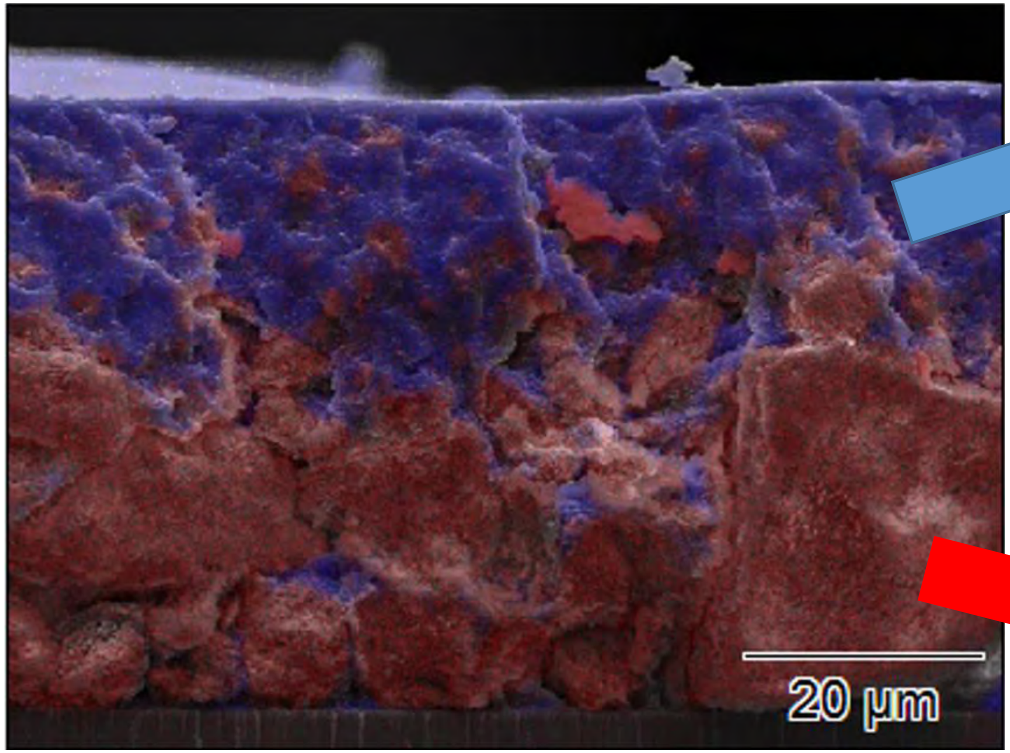


Used previously in latex films to prevent cracking, but also introduced porosity – indicated by film opacity.

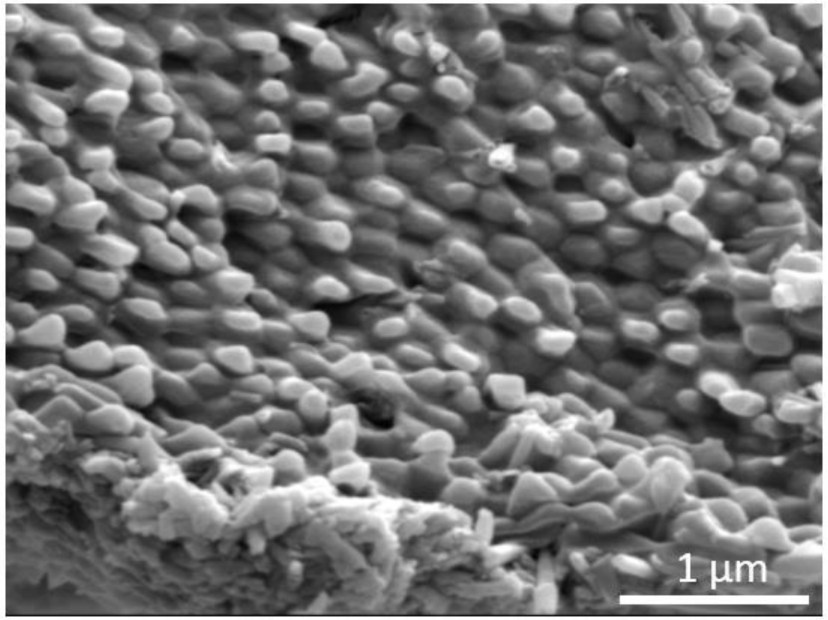


Qiao, J. Q.; Adams, J.; Johannsmann, D. (2012) *Langmuir,* **28** (23), 8674-8680.

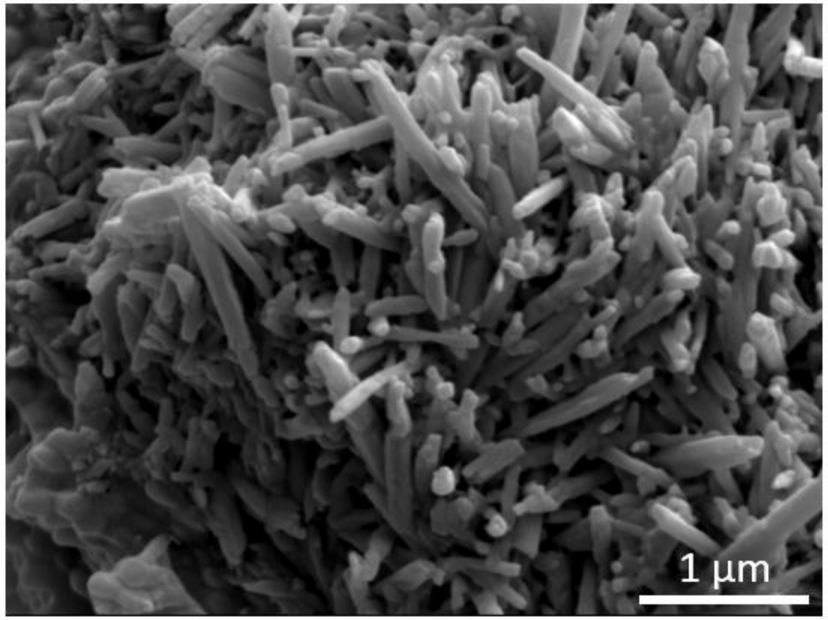
The Problem of Halloysite Sedimentation



EDX image: Al mapped in red.



Polymer-rich phase

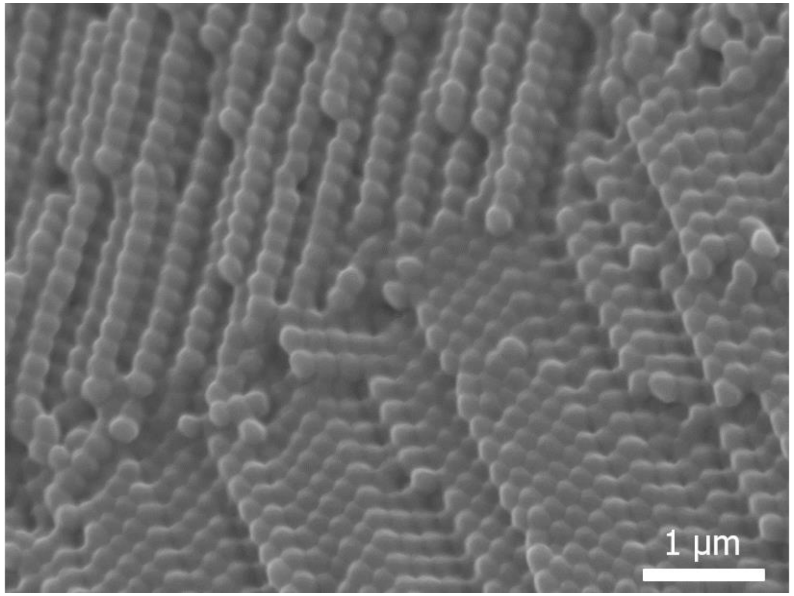


Halloysites-rich phase

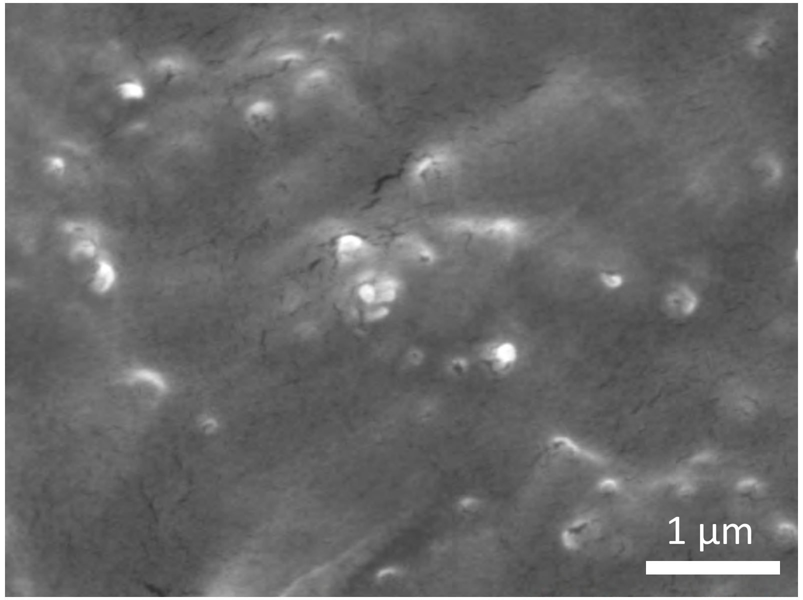
Halloysite Composite Coatings

Solutions:

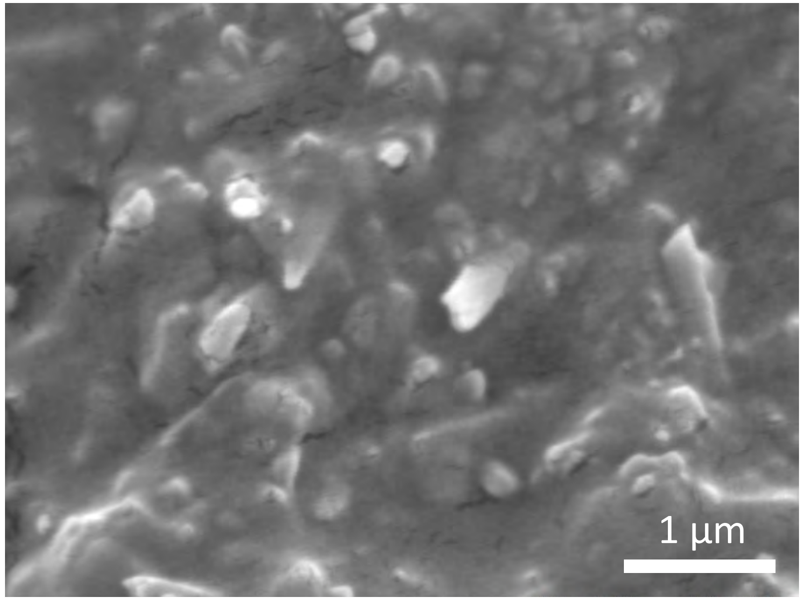
- Used poly(ethylene glycol) for the steric repulsion of halloysite
- Mechanical separation via sonication



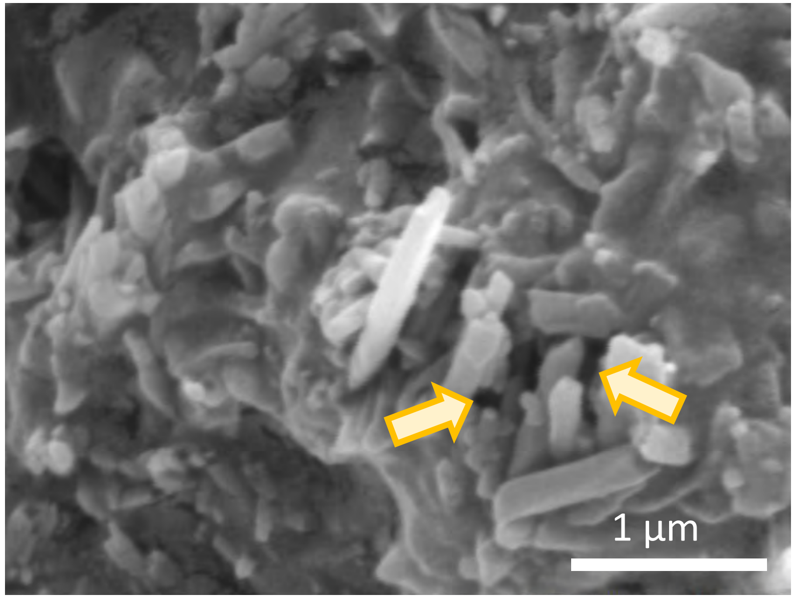
No halloysite



12 vol. % halloysite

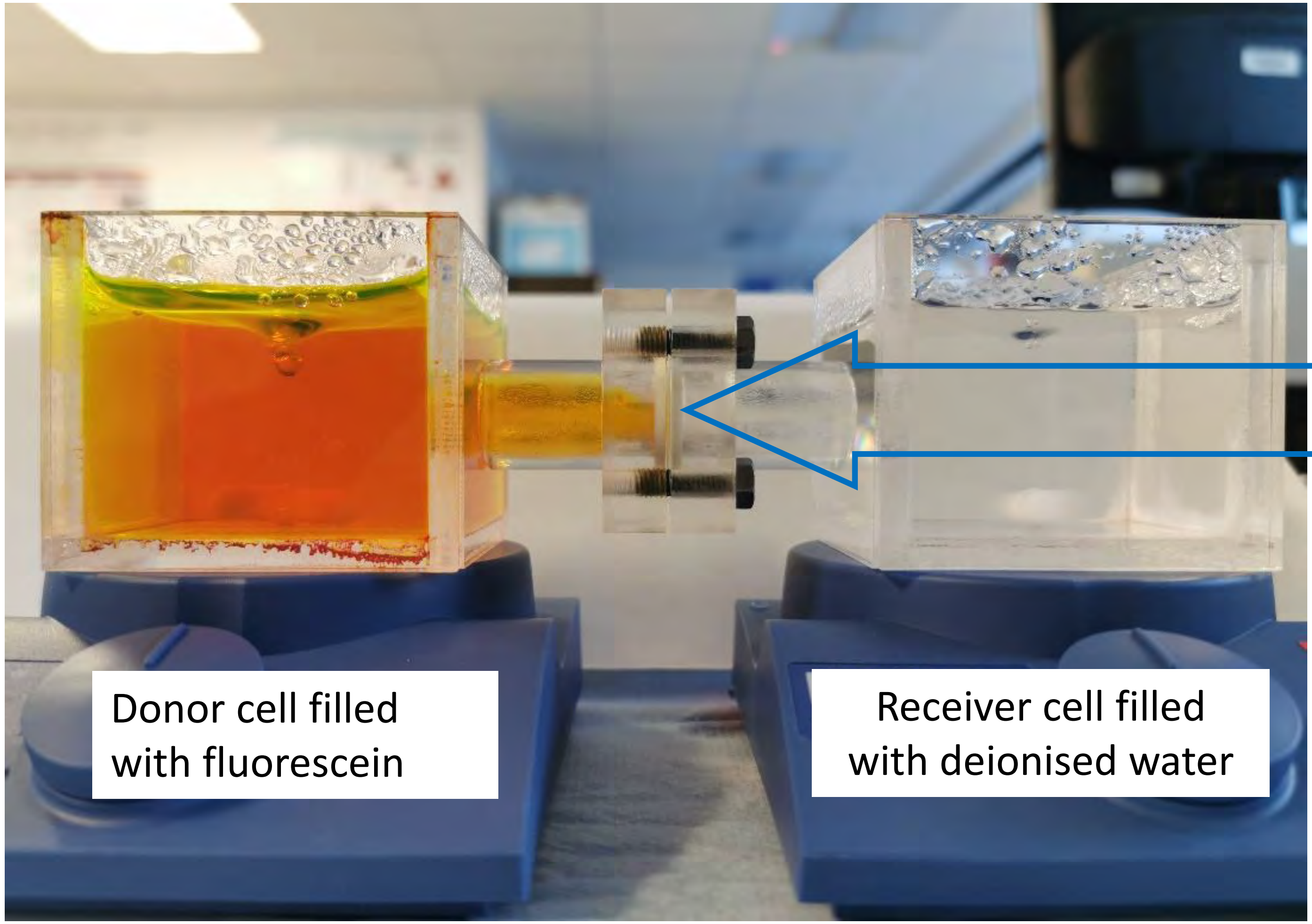


21 vol. % halloysite



29 vol. % halloysite

Does Halloysite Raise the Coating's Permeability?



Permeability Cell

Free-standing biocoating

See poster by Dr. Phil Chen

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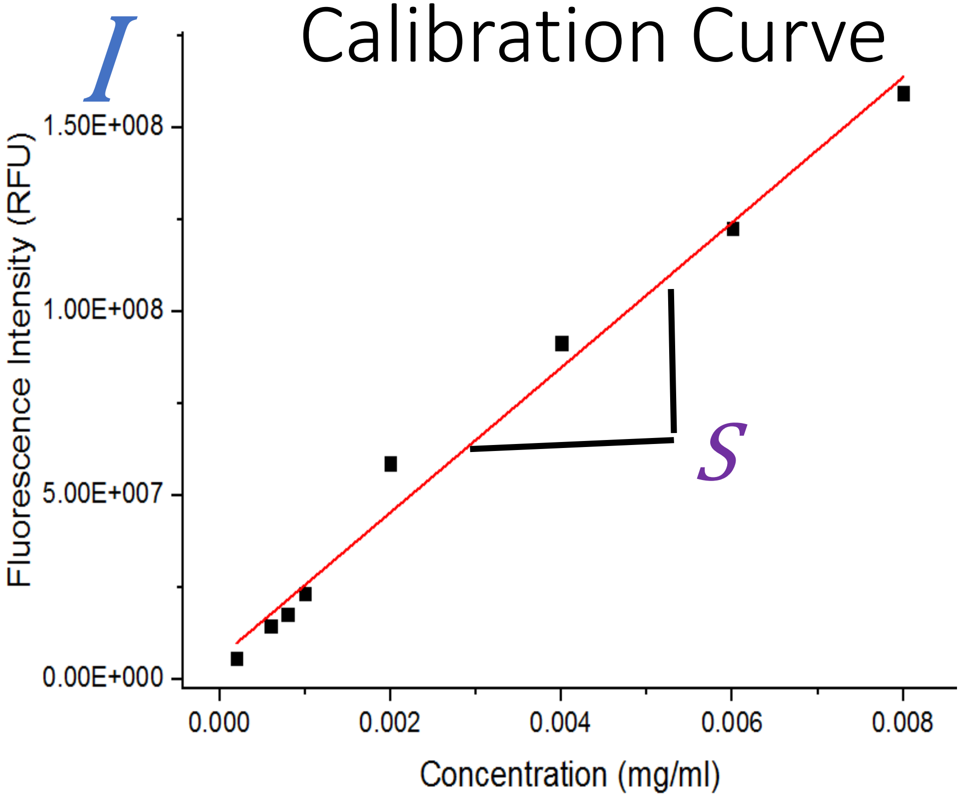
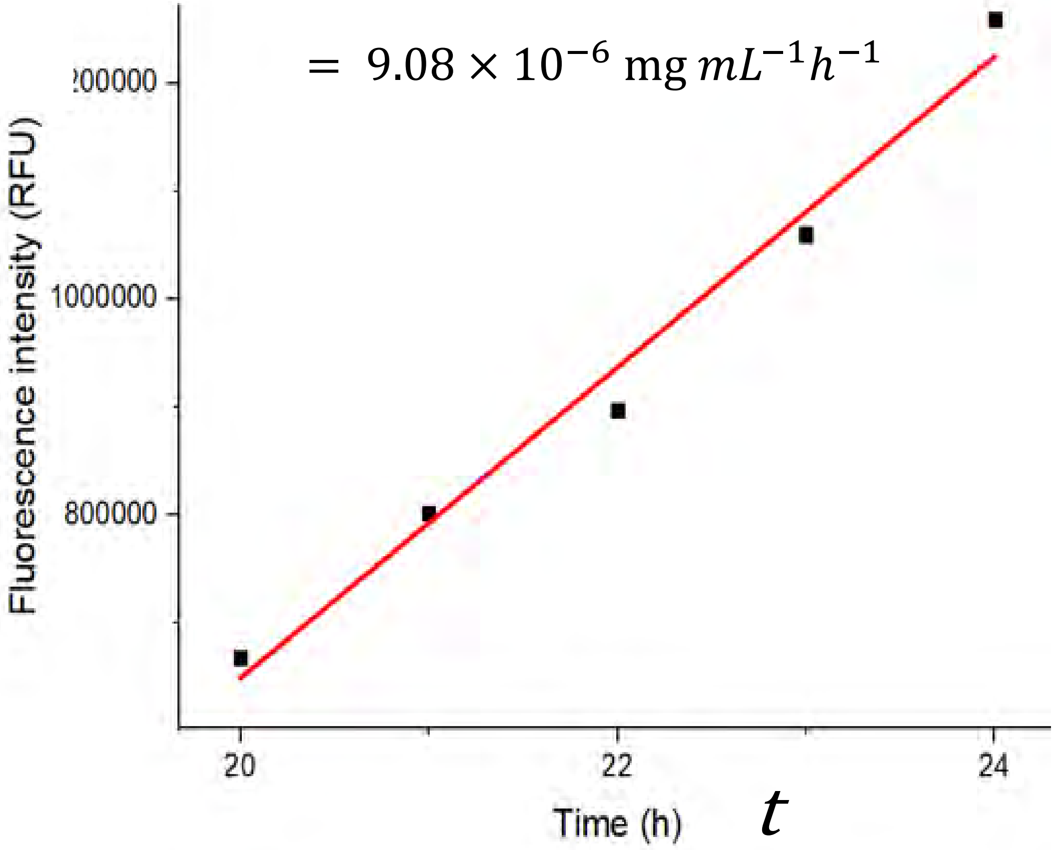
Measurements of Permeability Coefficient, *P*

$$\frac{dC_t}{dt} = \frac{dI}{dt}\Big/ S = \frac{1.79\times10^{5}\ h^{-1}}{1.97\times10^{10}\ \text{mL}\ mg^{-1}} = 9.08\times10^{-6}\ \text{mg}\ mL^{-1}h^{-1}$$

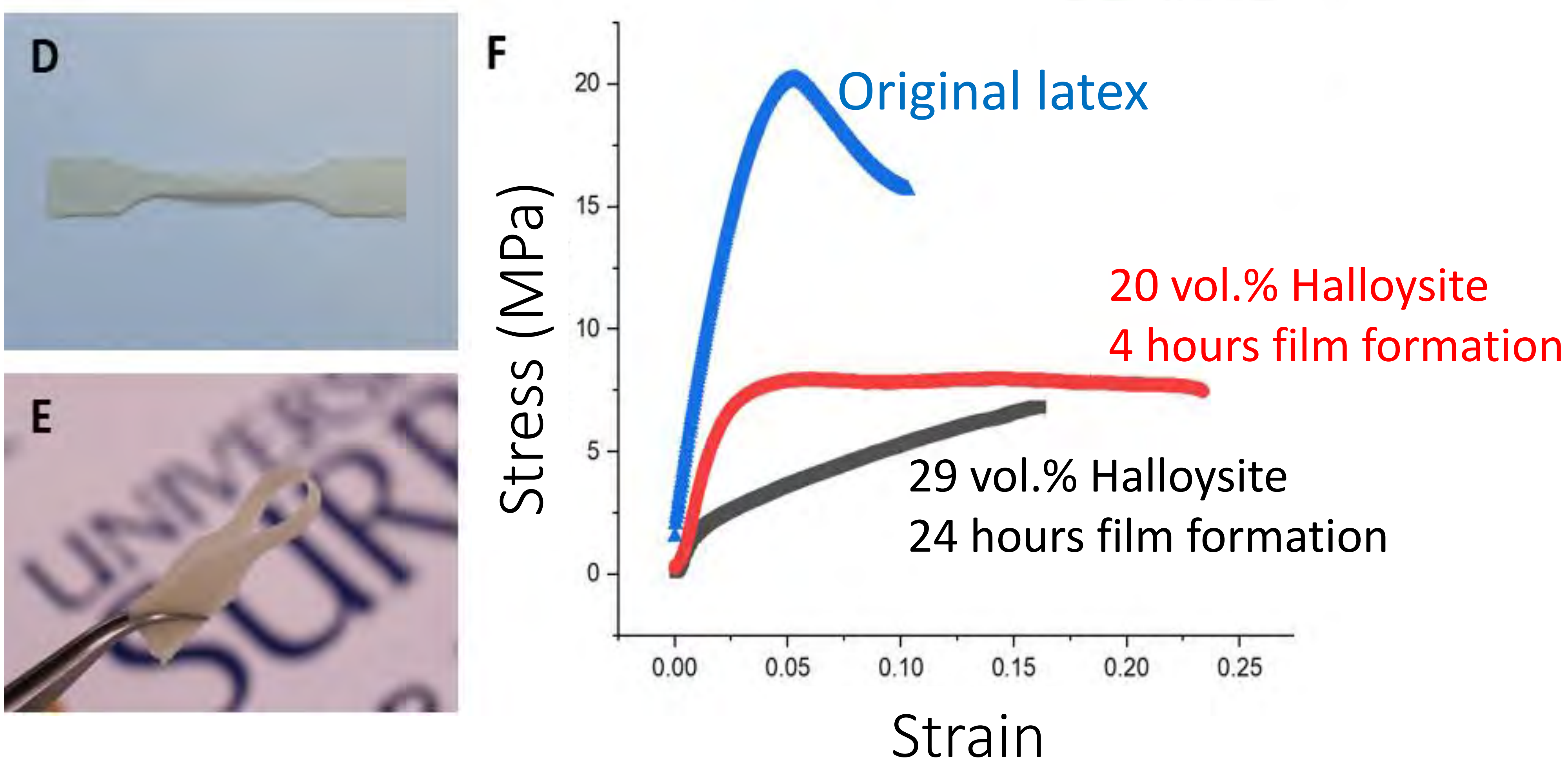


Fluorescein concentration, *C*



$$P = \frac{V}{C_0 A}\left(\frac{dC_t}{dt}\right) = \frac{1\times10^{-4}\ m^{3}}{1\times10^{3}\ \text{g}\ m^{-3}\times 7.85\times10^{-5}\ m^{2}}\times 9.08\ \times\frac{10^{-3}\text{g}}{m^{3}\cdot h} = 1.16\times10^{-5} m\ h^{-1}$$

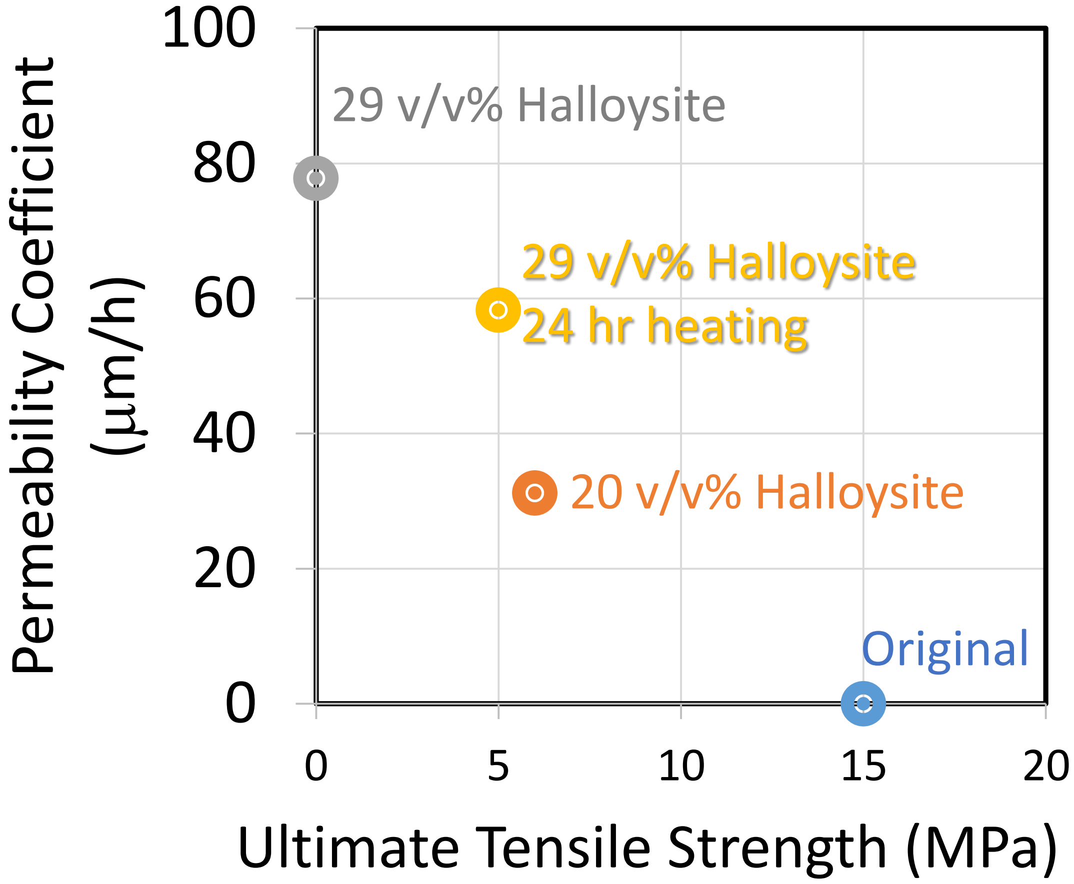
Halloysite Increases the Extensibility and Decreases the Modulus

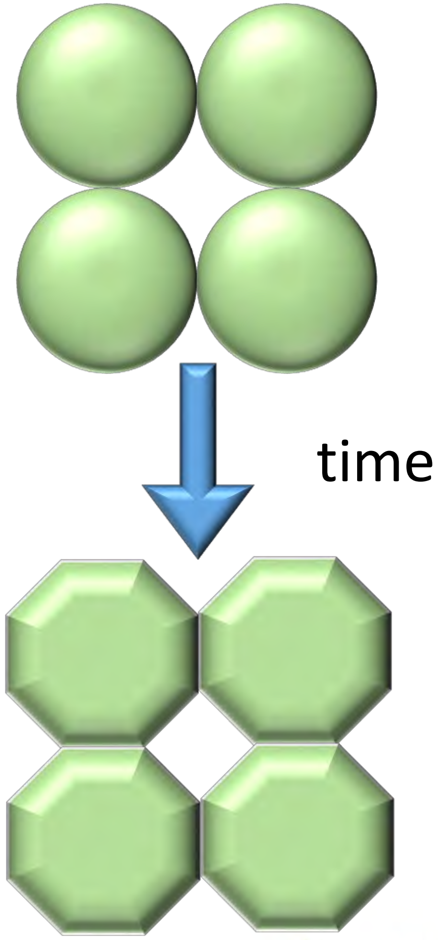


Y. Chen et al. (2020)
Biomacromolecules, ASAP
DOI: 10.1021/acs.biomac.0c00649

See poster by Phil Chen

Trade-Off of Properties: Permeability and Tensile Strength

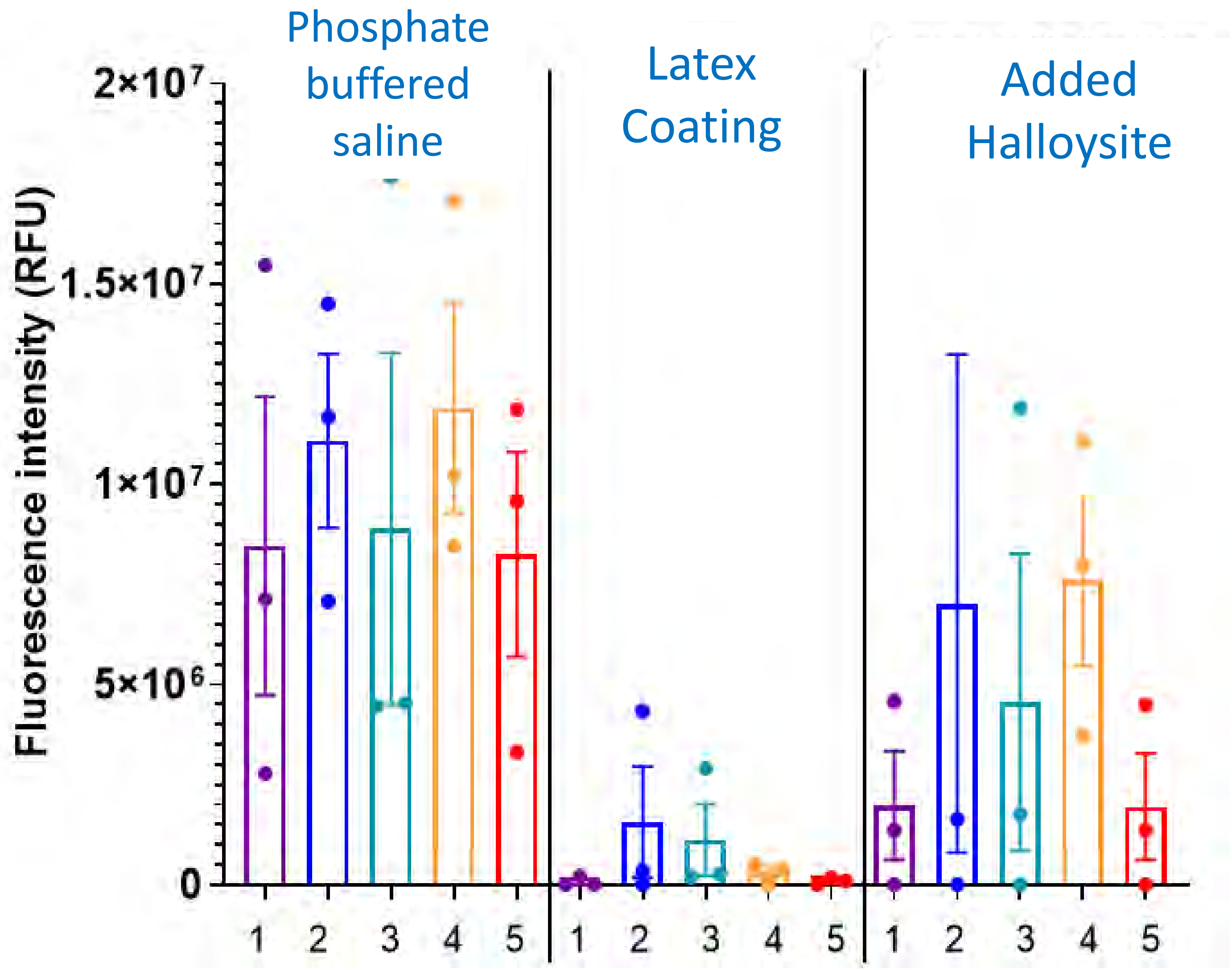




Y. Chen *et al.* (2020)
Biomacromolecules, ASAP

DOI: 10.1021/acs.biomac.0c00649

Adding Halloysite Increases the Metabolic Activity



Osmoprotectants

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4 - Trehalose 5 - Phytoglycogen

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Biomacromolecules, ASAP

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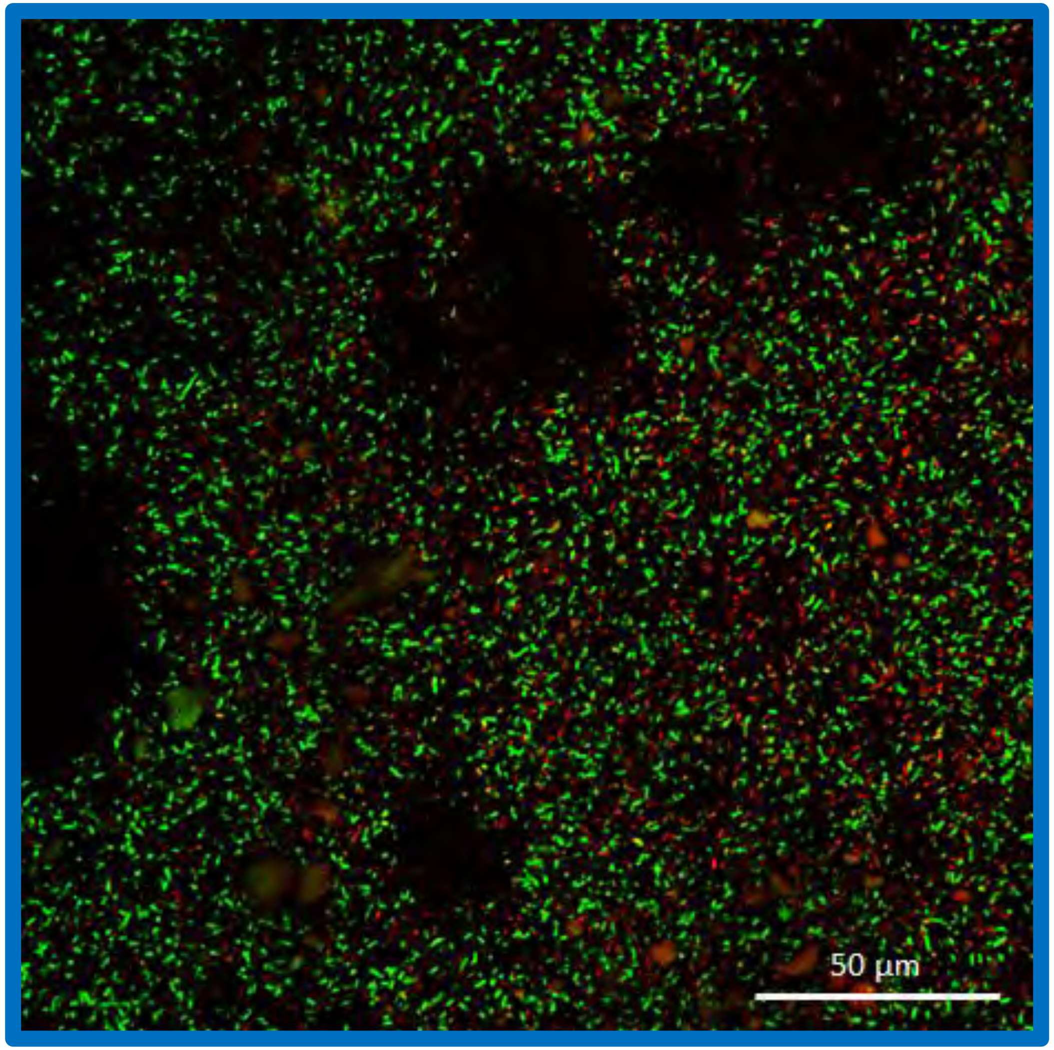
Imaging Viable Cells with Confocal Microscopy



***E. coli* in polyacrylate coating**

Green dots: live bacteria
(yellow fluorescent protein)

Red dots: dead bacteria
(propidium iodide)

Summary

- Nature can inspire the materials design for use in coatings.

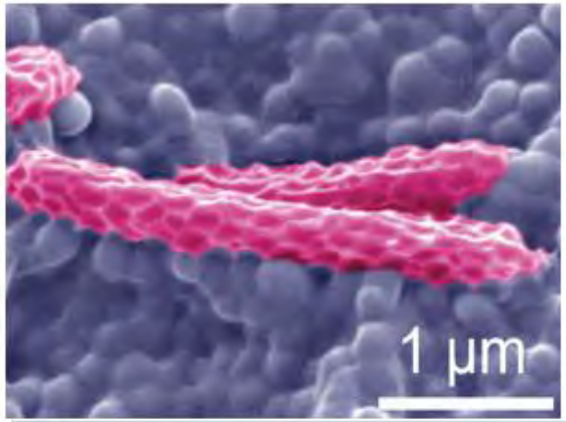
- Rather than try to re-create or mimic Nature, we can use living things *directly* in hybrid materials, such as our *E. coli* in waterborne polyacrylate coatings.

- The permeability needed to rehydrate the cells in the biocoatings was achieved by adding clay nanotube (halloysite) fillers.

- Increased water permeability allows faster rehydration of *E. coli* after desiccation.

- The bacteria encapsulated in the biocoatings were metabolically-active (according to rezasurin assays) and viable (expressed yellow fluorescent protein).

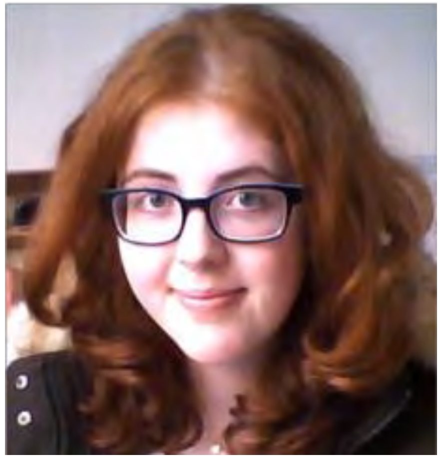
- Biocoatings can be used for waste-water treatment, environmental remediation, biosensing, carbon capture, and fuel/biomass creation.



M. Flickinger, *et al.*, (2017) *J. Coat. Technol. Res.* **14** (4), 791-808.
Y. Chen *et al.* (2020) *Biomacromolecules*, ASAP

Acknowledgements

Dr. Yuxiu (Phil) Chen



Simone Krings

Josh Booth

Prof. Stefan Bon

Dr. Suzie Hingley-Wilson

@KeddieLab

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