# Growing functional multilayers from mycelium

Speaker: Yuki Hayashi

MSE-493 Engineered Living Materials – 17/12/2024

# Where am I from?



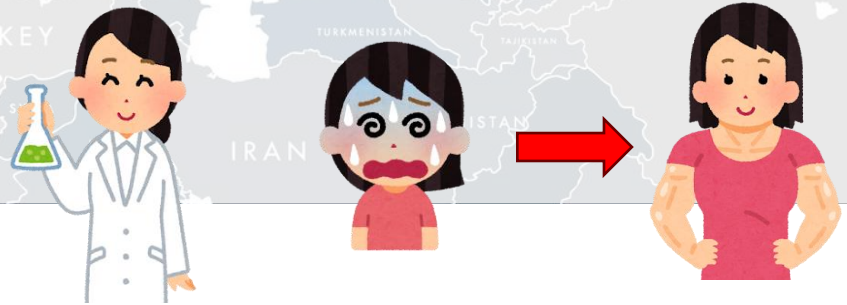
Yuki Hayashi

# When I was 18 years old...

Yuki Hayashi



- University of Sheffield
  - BEng: Materials Science and Engineering



Born & grew up in Fukuoka

**2015-2019**
**Bachelor at University of Sheffield**
Materials Science & Engineering

**2019-2022**
**Master at EPFL**
Materials Science & Engineering
Minor in Chemistry & Chemical Engineering

**2021-2022**
**Sika Technology Body In White part**
Odour improvement of thermal expandable foams

**Industry or Academic career?**

# Reality in Switzerland …

Born & grew up in Fukuoka

Experience and Competencies:
We are looking for experienced candidates with excellent communication skills and a solution focused approach.
What we are looking for:

- At least 7+ years in coating industry preferably with a proven track record in product development, preferred fields include UV curable coatings, acrylic and/or epoxy based, photochemistry, photolithography, stereolithography.
- MS or PhD in chemistry, polymer science or materials science.
- Good knowledge in coating technology and application processes, in photochemistry. Experience in Electronics and PCBT processing is a plus.
- Solid background in polymeric material science.
- Natural leader with an entrepreneurial mindset, self-driven and highly motivated.
- Accountable, reliable and efficient team player with excellent interpersonal, communication and project management skills associated with strong work ethics.
- Excellent oral presentation and written communication skills.
- Fluent oral and written English skills, other language is an asset.

2021-2022
Sika Technology Body In White part
Odour improvement of thermal expandable foams

**Your profile**

- Master's degree in materials science, physics, chemistry or mechanical engineering
- Versatile, passionate, creative and solution-oriented engineer
- More than 10 years' experience in a manufacturing environment, including at least 5 years as a mass production engineer or manager
- Experience in lean manufacturing
- Knowledge of high-tech processes (thin films, lasers, microfabrication, polymers, batteries, clean rooms, etc.)
- Expertise in the economic analysis of industrial processes
- Interest in different technologies, strength of implementation and ability to work in a team
- Languages: German, French, English fluently spoken and written
- Willingness to travel in Switzerland 60-80%

**Your profile :**

- M.Sc., B.Sc. or equivalent FH degree in chemical engineering or similar
- At least 5 years' experience in the design and implementation of complete technical projects
- Very good chemical, heat and hydraulics engineering skills
- Proficiency in complex calculations (using Excel) and process optimization
- Knowledge of electrical and safety standards: ATEX, ISA, IEC, NEMA and instrumentation
- Responsible, rigorous and attentive to detail, able to learn, flexible and solutions-oriented
- Fluent in English (B2/C1) and knowledge of French (A2) with motivation to improve the practice to a B2/C1 level in the medium term
- Swiss nationality, valid work permit or EU citizen eligible for B permit
- Available for 10-30% European and intercontinental travels

- I may need to do a PhD (4 years) to:
  - Gain research experience
  - Be an excellent candidate for employment
  - Acquire French language that I can use at work

Born & grew up in Fukuoka

**2015-2019**
**Bachelor at University of Sheffield**
Materials Science & Engineering

**2019-2022**
**Master at EPFL**
Materials Science & Engineering
Desciplinary in Chemistry & Chemical Engineering

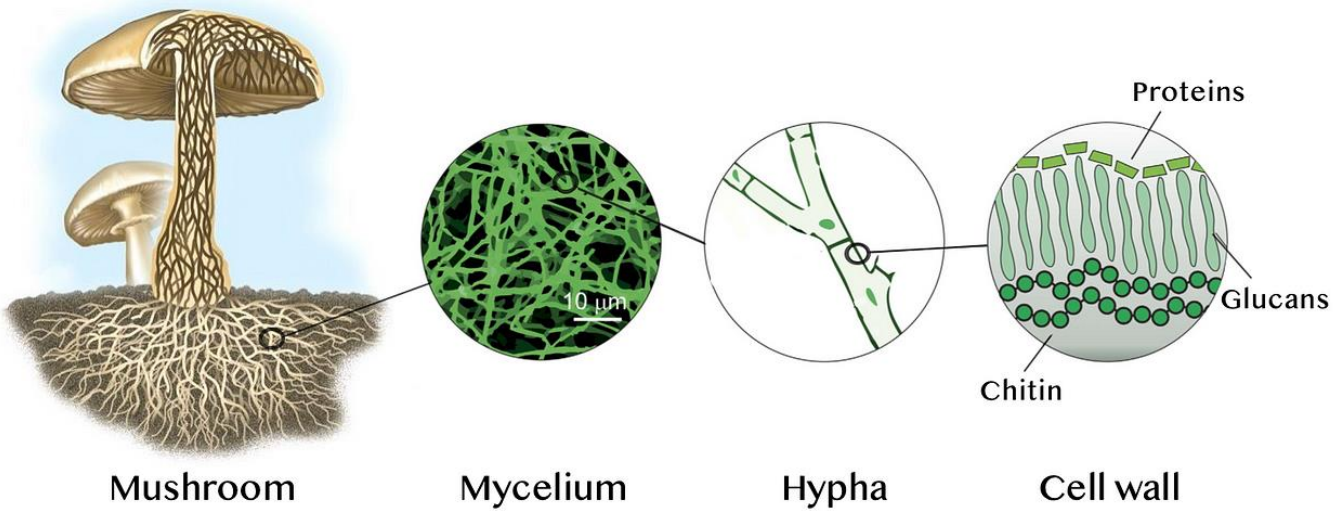
**2021-2022**
**Sika Technology Body In White part**
Odour improvement of thermal expandable foams

**Industry or Academic career?**

**2023-2027 (hopefully)**
**PhD at EPFL**
Mycelium-based multilayer packaging

# Mycelium: What is Mycelium?

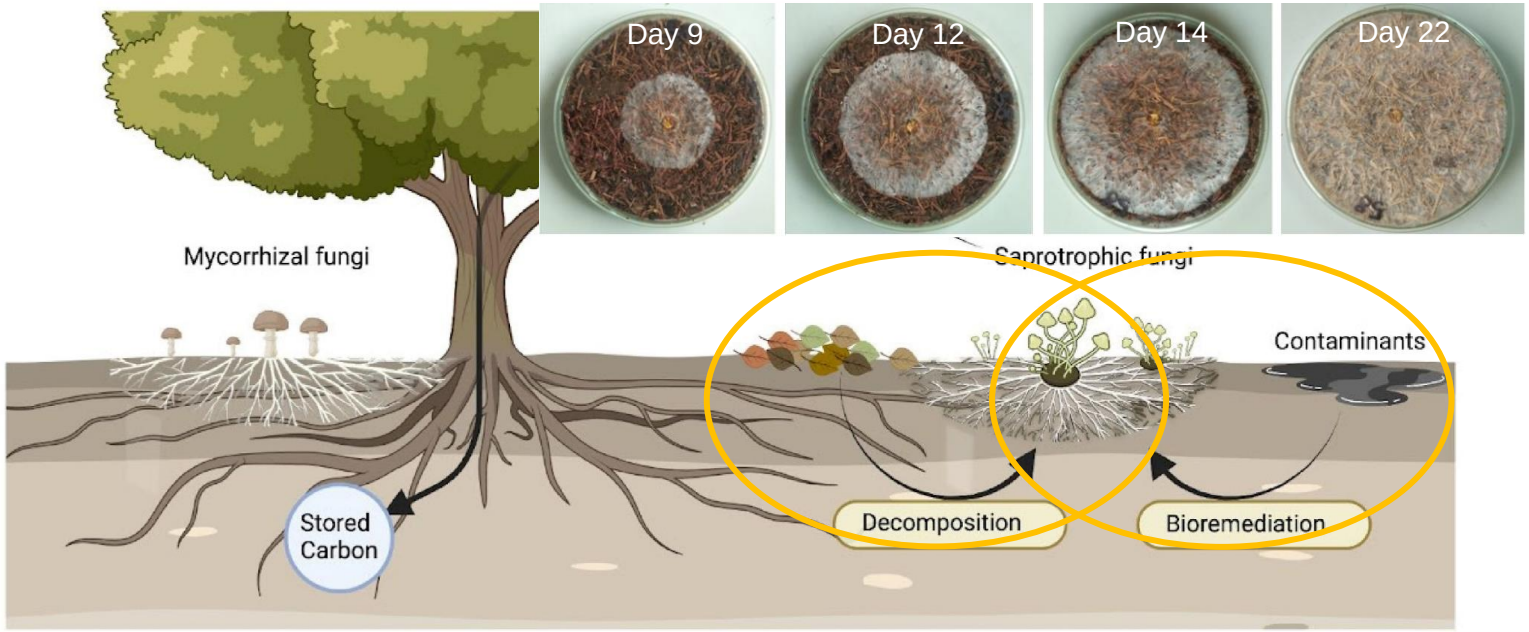
Yuki Hayashi




- 3D network of root-like microfilamentous structures (hyphae) in fungi.
- Biodegradable hybrid material comprising of polysaccharides, proteins, and lipids.

Image adapted from Haneef, M. *et al. Sci Rep.* **2017**, 7, 41292.

# Mycelium: Roles in Nature




- Roles in nature:
  - Decomposing organic compounds into simpler sugars
  - Decontaminating soil and water from heavy metals and toxic compounds

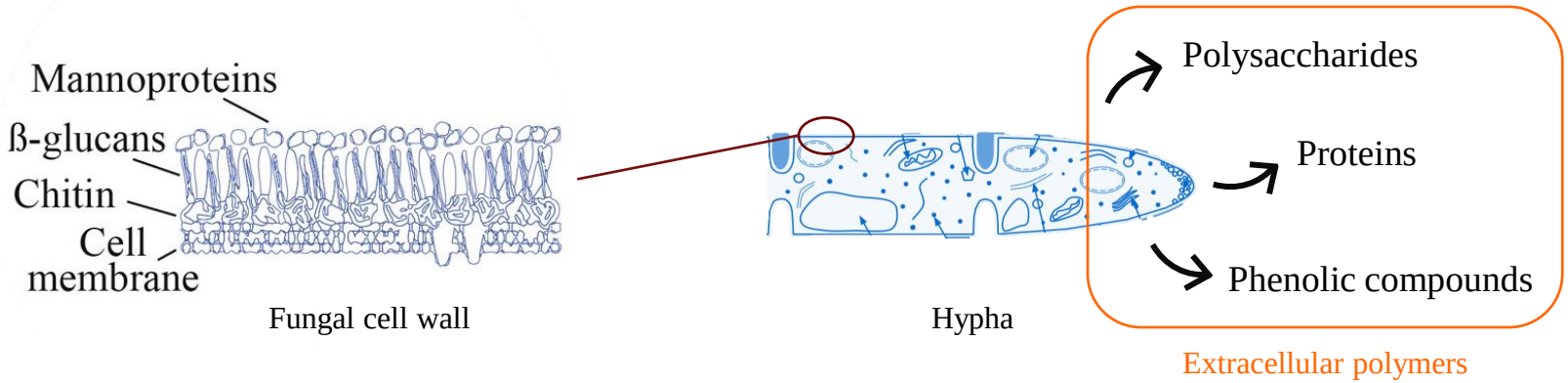
Jo, C. *et al. Materials to Today Bio.* **2023**, 19, 100560.; Attias, N. *et al.* "Developing novel applications of mycelium based bio- composite materials for design and architecture" **2017**.



Yuki Hayashi

Yuki Hayashi

# Mycelium: Chemical Compositions



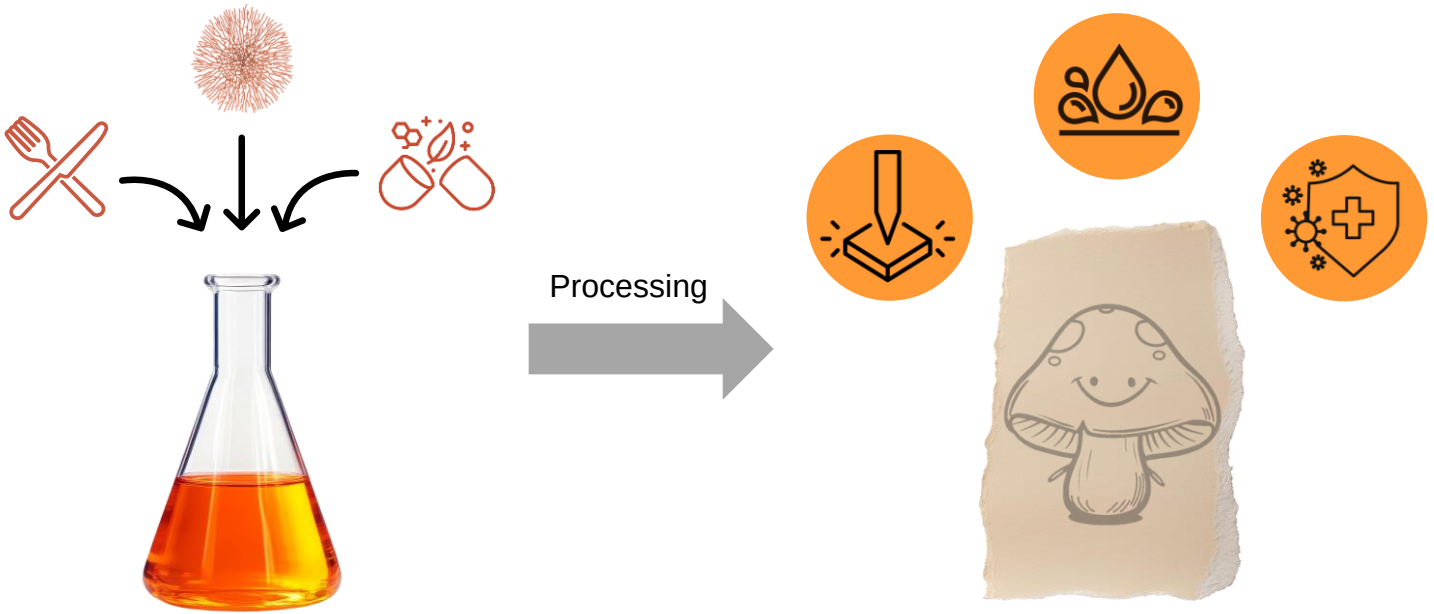

- **Mannoprotein** – ductility and adhesive properties
- **β-glucan** – tensile strength
- **Chitin** – rigidity of fungal cell wall
- Secretion of certain types of compounds during growth
- Different growth environments result in different chemical compositions of the fungal cell wall and extracellular polymers.

Image (left) adopted from Elsacker, E. *et al.*. Sci. Total Environ. **2020**, *725*, 138431.; Image (right) adopted from Wikipedia

Yuki Hayashi

# Objective of My Thesis

## Functionalisation of mycelium-based materials for packaging application




- Can we tailor the chemical composition and the properties of the mycelium-based material by changing the growth environment?

Image modified from Meyer *et al*. *Fungal biol. biotechnol*. **2020**, 7, 5.

# Research Plan

Yuki Hayashi

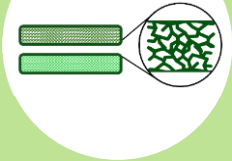
## Single layers and additives

Mycelium production

Mycelium film fabrication

Film characterisation

## Multilayers

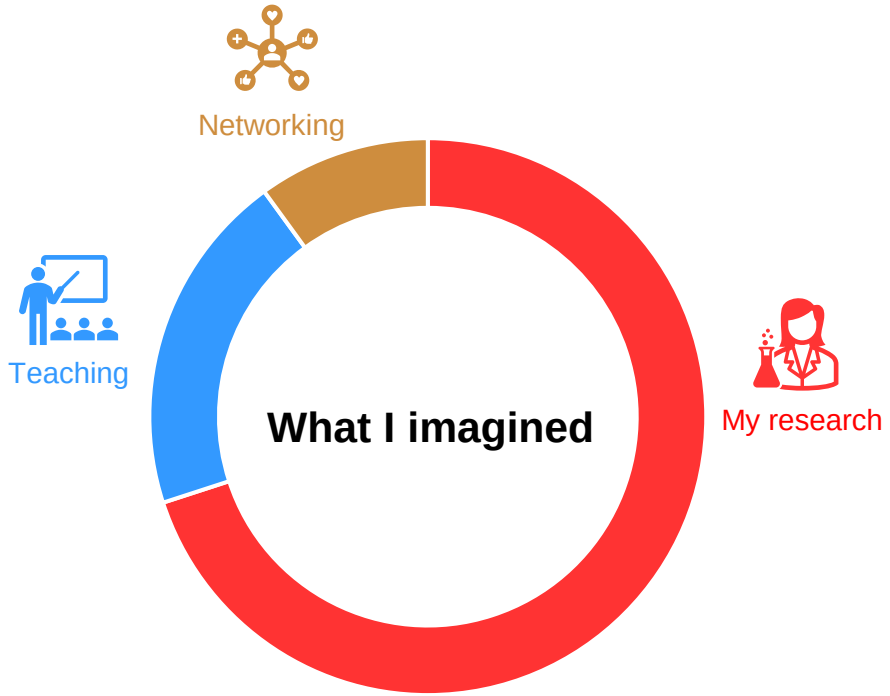


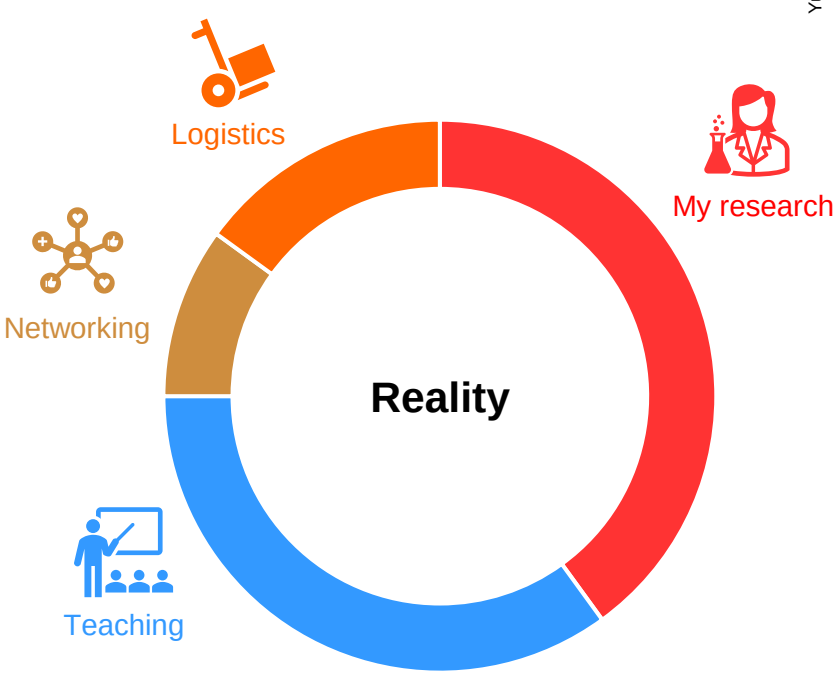
Mycelium multilayer fabrication

Multilayer characterisation

Yuki Hayashi

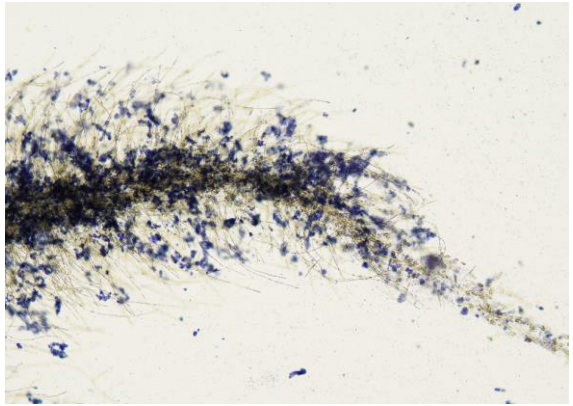
# PhD life (in my case)

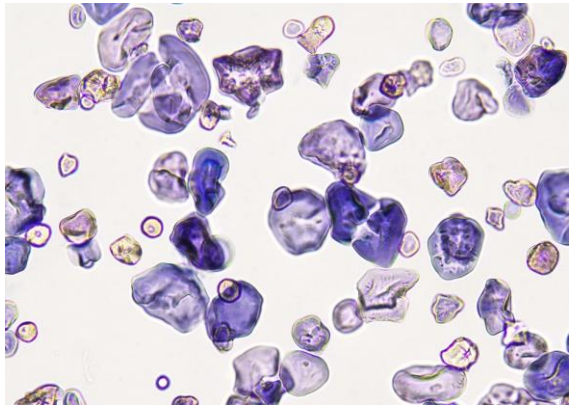



- Doing a PhD isn't just about research, it’s also about other things!

# PhD life (in my case)

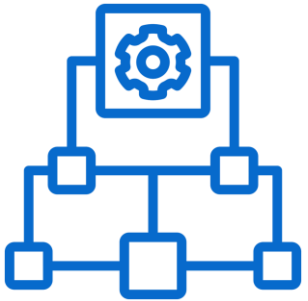




- Doing PhD is hard (default)
- But I like researching and people around me! ☺

# Something I can tell you about PhD

- You are doing research!
  - A framework is given to get some ideas for your project.
  - You are practising to be a researcher!
  - You can (and should) choose your own research direction



- Important things to remember:
  - You are trying to find/do something new
  - There are many possibilities – don't lose your research direction!
  - Have small successes in your personal life for your mentality ☺

# Fungal extracellular matrix for bio-inspired membrane production

15/12/2024

Inyoung Yonah Lee, Sustainable Materials Laboratory

# Who is Inyoung? Why is she here?








# Who is Inyoung? Why is she here?

2020: BSc Chemistry and Chemical Engineering
2022: MSc Chemical Engineering and Biotechnology


# Who is she? Why is she here?

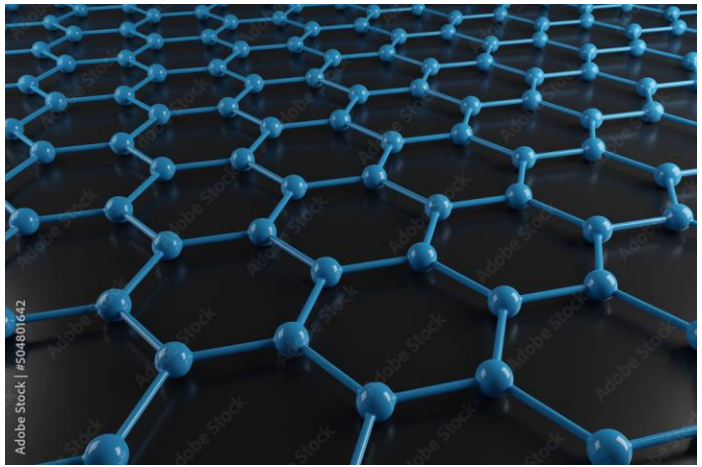


**Why PhD?**

- Tried ~1 year of industrial internship
- Really liked during my master thesis (fabrication of graphene membrane for carbon dioxide separation)
- Felt like I would regret if I didn't try

**How did I find SML?**

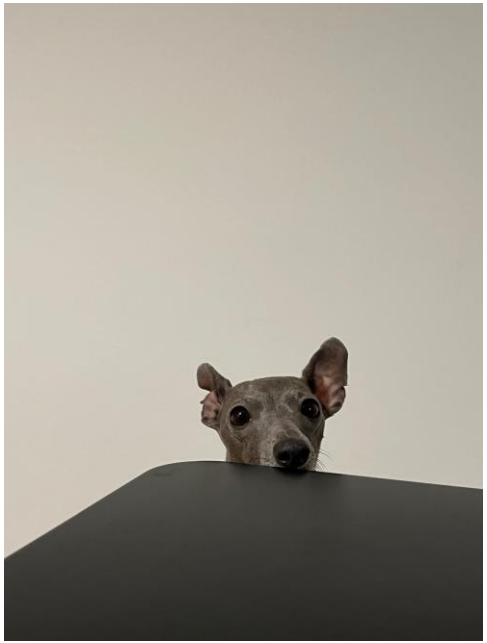
- BNF program
- Ended up having a position



# Who is she? Why is she here?

But also…

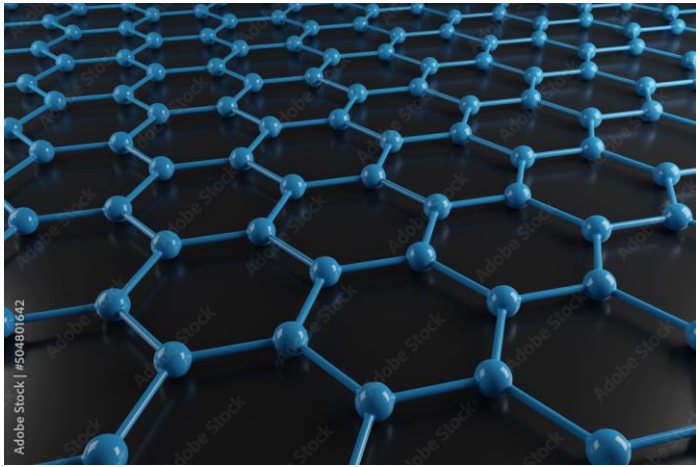
- Got married with a PhD student at EPFL, so wanted to stay in Switzerland for a while
- AND we have a lovely dog



# Research background



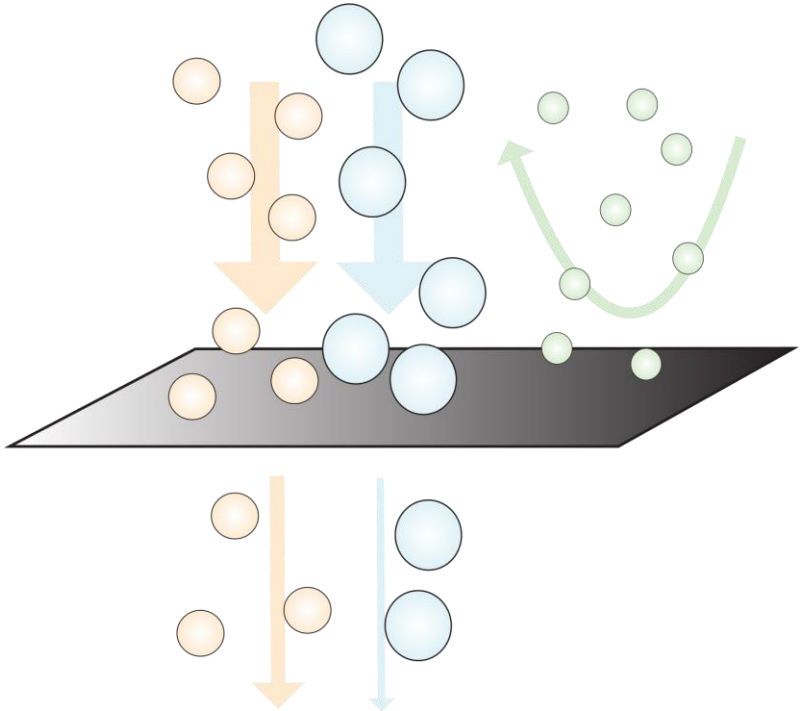
## Filtration, membranes, purification

# Why membranes?

**Membrane**

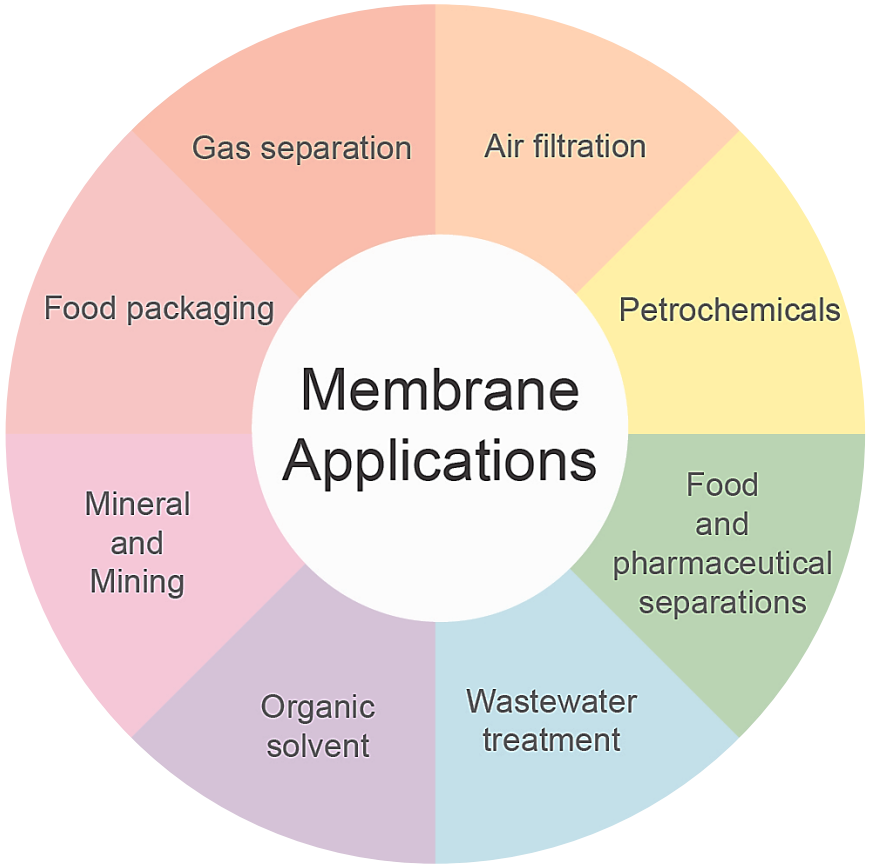
interfaces between two adjacent phases,
regulating the transport of substances
between the two compartments





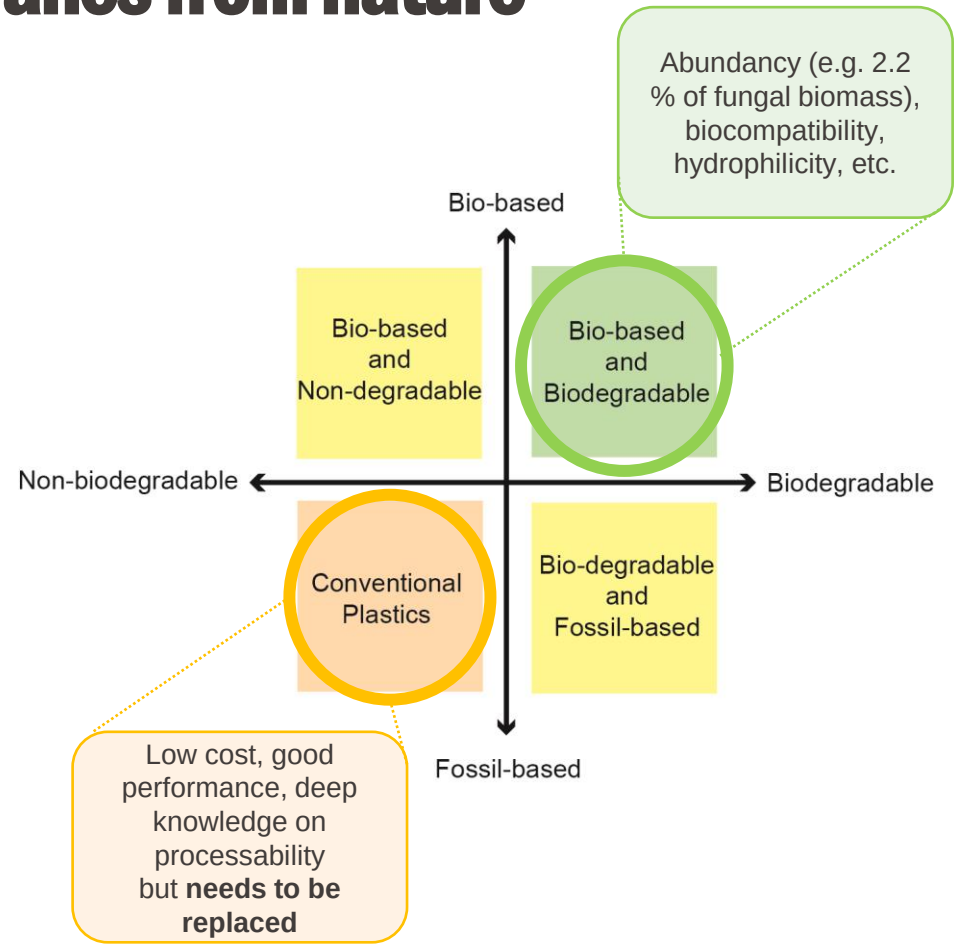
Inyoung Yonah LEE

# Why membranes?

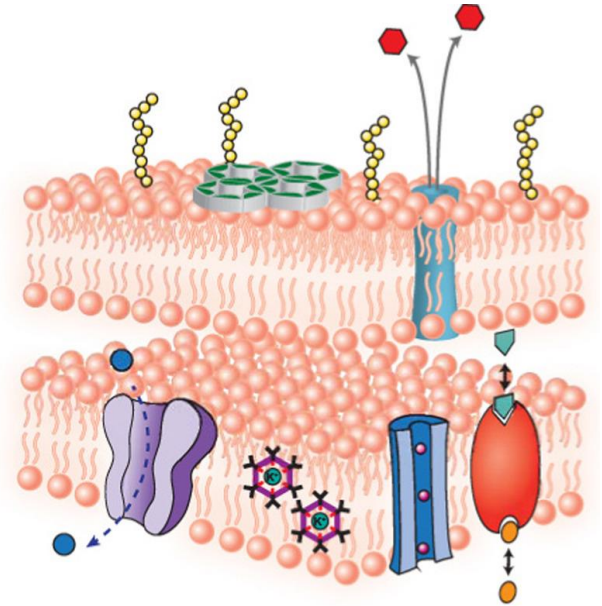



Readapted from Karki, S., et al. (2024)

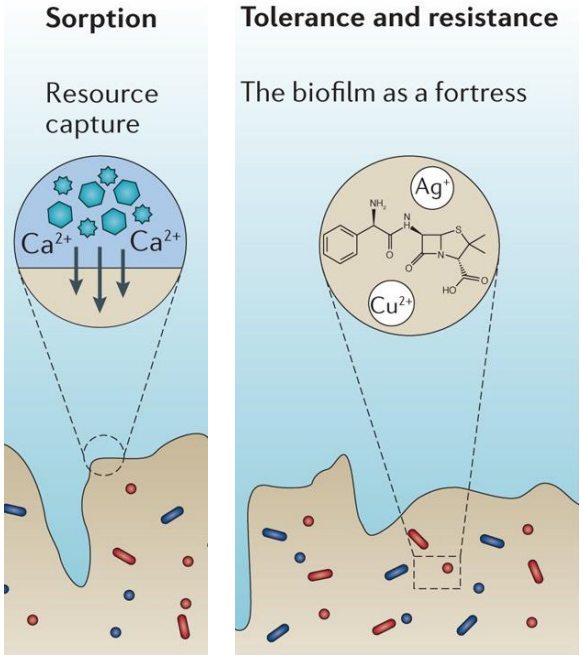
# Membranes from nature



# Membranes from nature

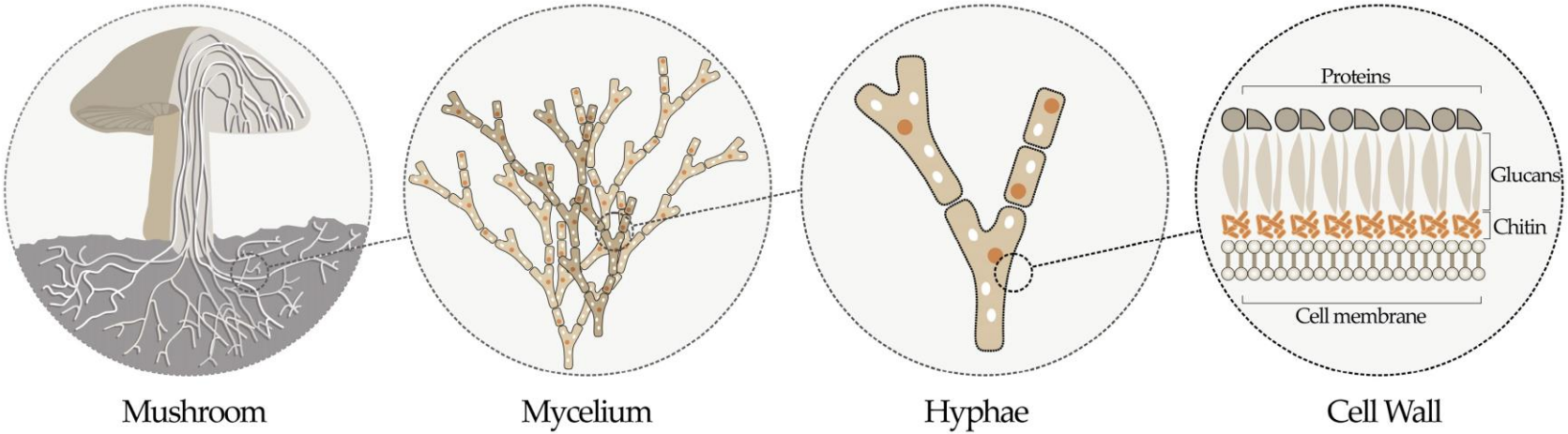


Shen, Y.-x., et al. (2014)




Readapted from Flemming, H. C. et al. (2016)

# Fungal mycelium



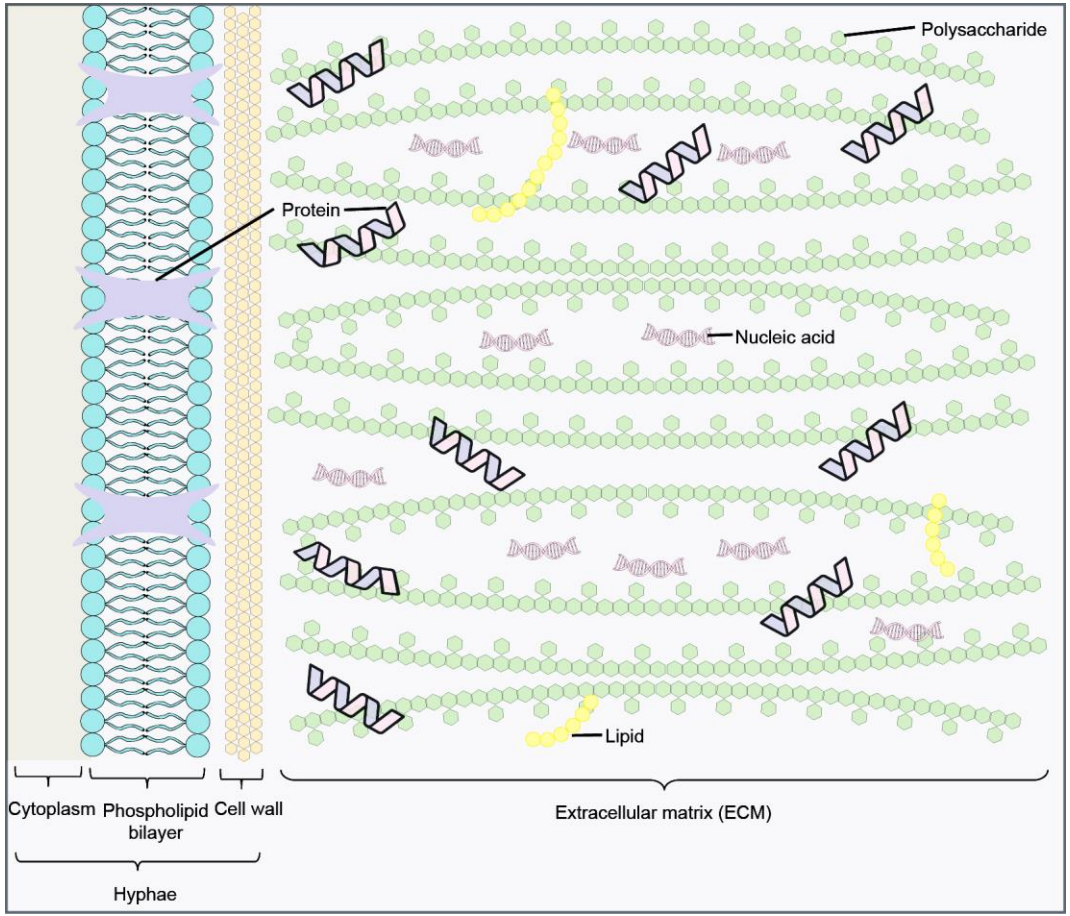

Mohseni, A., et al. (2023)

# Fungal extracellular matrix

**Extracellular matrix (ECM)**
composed of

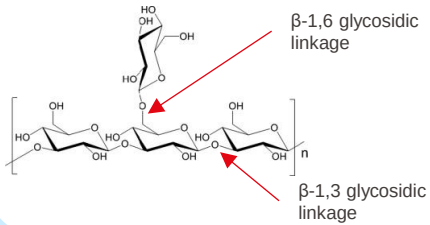
**Extracellular polymers**

= Polysaccharide
+
Protein
+
Lipid
+
Nucleic acid



# Schizophyllan (SPG)

Schizophyllan (SPG)

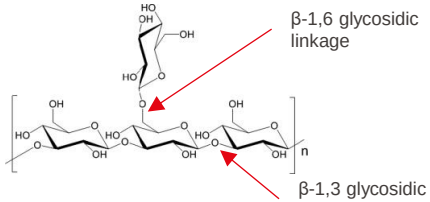


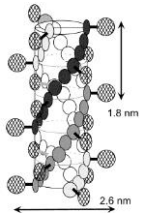

Applications

- Environmental
- Cosmetics
- Biomedical

# Schizophyllan (SPG)

Schizophyllan (SPG)




Sakurai, K., et al. (2003)

**Supramolecular Structures**
1) Triple-helix
2) Liquid crystalline

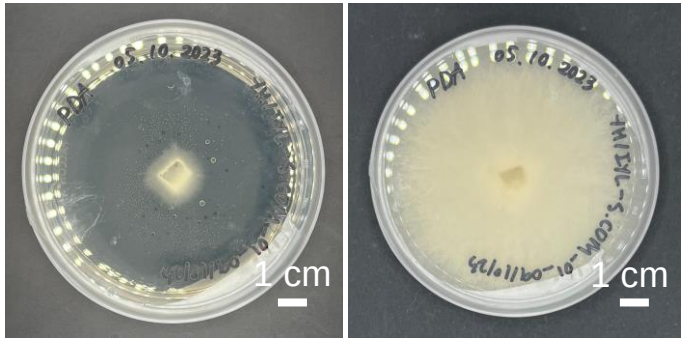
**SPG membrane**

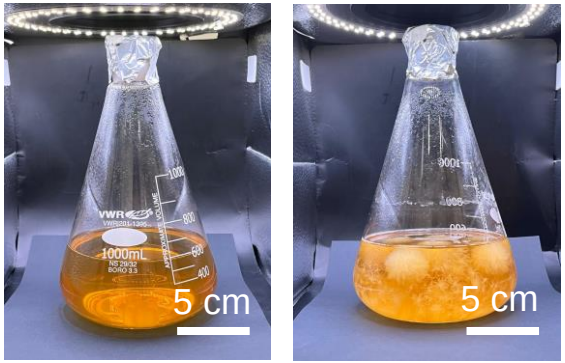
**Rheology**

**Other properties**
Anti-tumor, anti-oxidant, thickening, emulsifying, water-uptake, $O_2$ barrier

# Production of extracellular polymers

**Production**



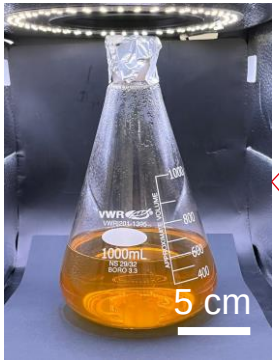



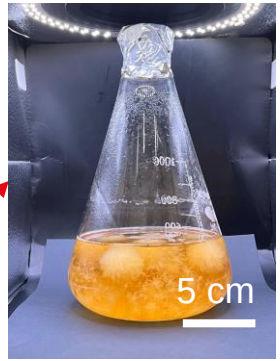

**Isolation**

**Diversity in isolation**
e.g. dialysis and precipitation

# My project in ELM perspective

"Composed of living cells that **form or assemble the material or modulate the functional performance** of the material"

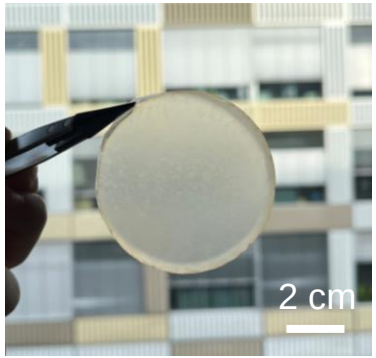




**Variables**:
Time, nutrients, etc.

**Properties**:
Supramolecular structures
Composition
Film properties



EPFL

SML
SUSTAINABLE MATERIALS LAB

Blanca de Miguel Martinez

# BIONANOMATERIALS FROM FUNGAL FERMENTATION

# A BIT ABOUT ME

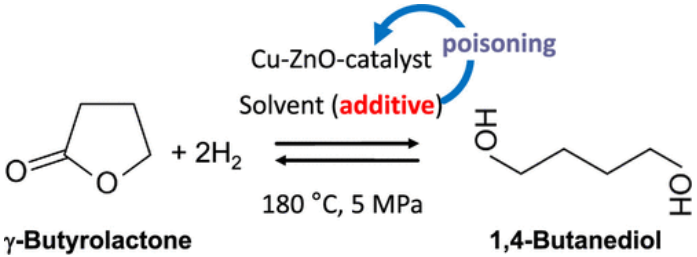
| | |
|---|---|
| 2017 – 2021 | BSc in **Chemical Engineering**<br>Universidad Complutense of Madrid (UCM) |
| 2021 – 2022 | MSc in **Sustainable Energy – BioEnergy**<br>Danmarks Tekniske Universitet (DTU) |
| 2022 – present | PhD in **Materials Science**<br>École Polytechnique Fédérale de Lausanne (EPFL)<br>Sustainable Materials Laboratory (SML) |

# WHAT I WORKED ON

BSc in **Chemical Engineering** (UCM)

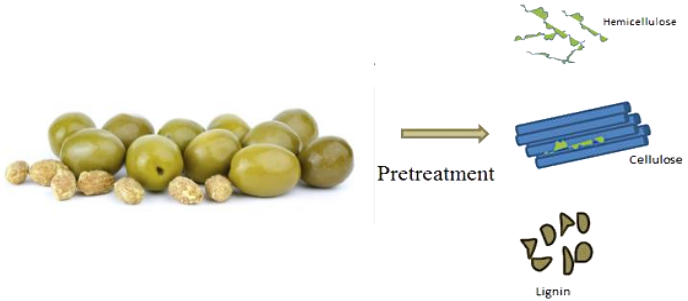
- CSIC – Institute of Catalysis and Petrochemistry (ICP)
  - Solvent-induce deactivation
  - Biomass fractionation pretreatment for lignocellulosic feedstocks (olive seeds)

- BSc Thesis – Physico-Chemistry of Industrial and Environmental Processes (FQPIMA)
  - Enzymatic microbioreactor for gluconic acid production




Additive = 2,6-di-tert-butyl-4-methylphenol, BHT

*Ind. Eng. Chem. Res.* 2021, 60, 44, 15999-16010

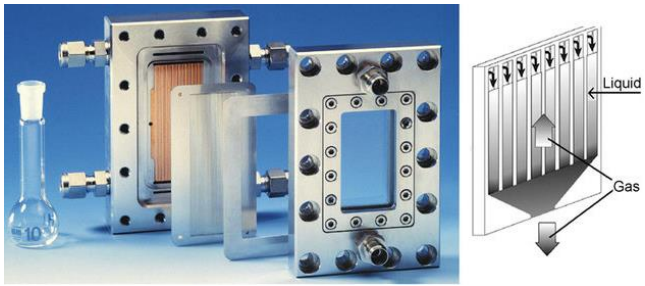


# WHAT I WORKED ON

BSc in **Chemical Engineering** (UCM)

- CSIC – Institute of Catalysis and Petrochemistry (ICP)
  - Solvent-induce deactivation
  - Biomass fractionation pretreatment for lignocellulosic feedstocks (olive seeds)

- BSc Thesis – Physico-Chemistry of Industrial and Environmental Processes (FQPIMA)
  - Enzymatic microbioreactor for gluconic acid production




*Chem. Eng. Sc.* 2008, 63 (21): 5149-5159

S-L-G three-phase reaction

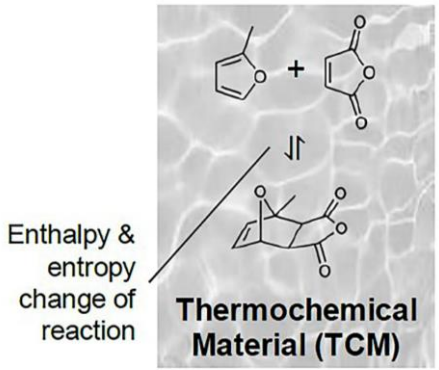
Enzyme immobilization

# WHAT I WORKED ON

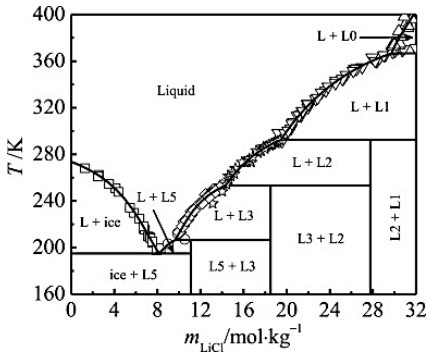
MSc in **Sustainable Energy – BioEnergy** (DTU)

- BRTA – CIC energyGUNE
  - Development of Phase Change Materials for Power-to-heat (P2H): TES integrated heating

- MSc Thesis – Bloom + SML
  - Advanced cellulose products using a novel type of cellulose




Salts



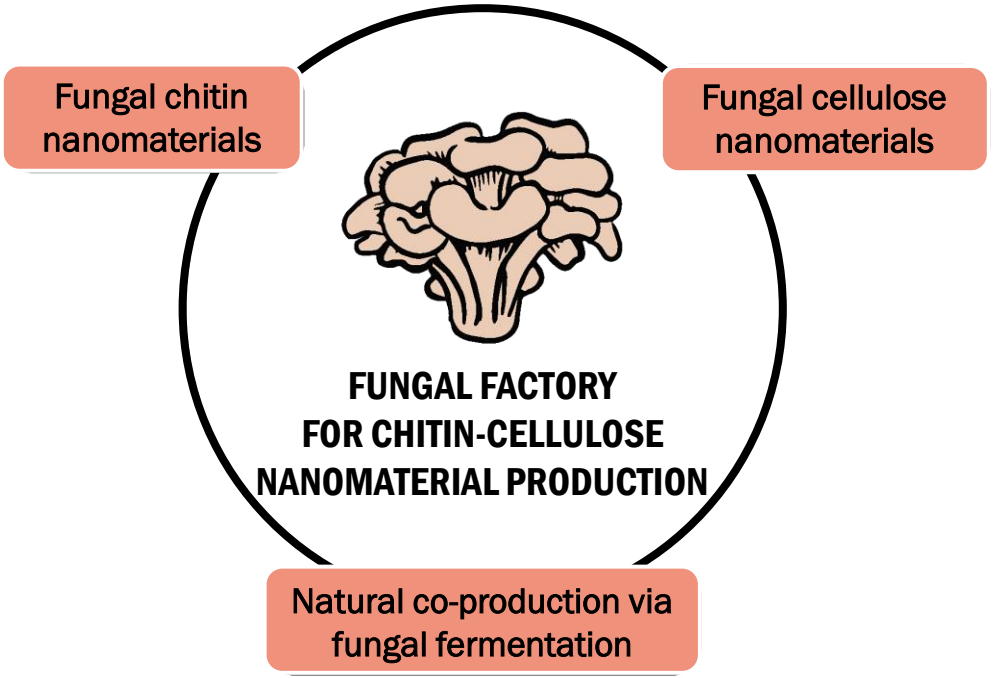

*J. Appl. Phys*. 2021, 130, 220903

*Calphad*. 2015, 51, 1-12

Blanca de Miguel Martínez

# BIONANOMATERIALS FROM FUNGAL FERMENTATION

Objective of my PhD

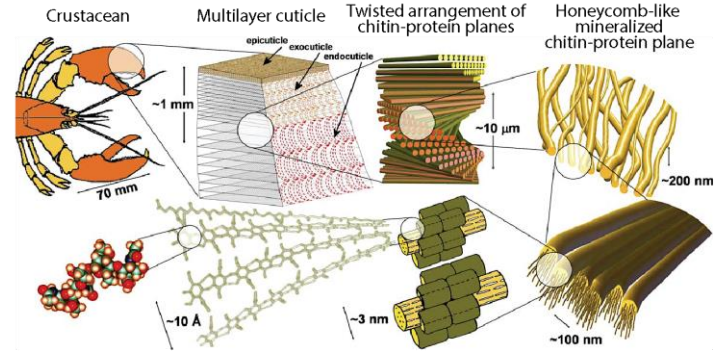


Blanca de Miguel Martínez

# BIONANOMATERIALS FROM FUNGAL FERMENTATION

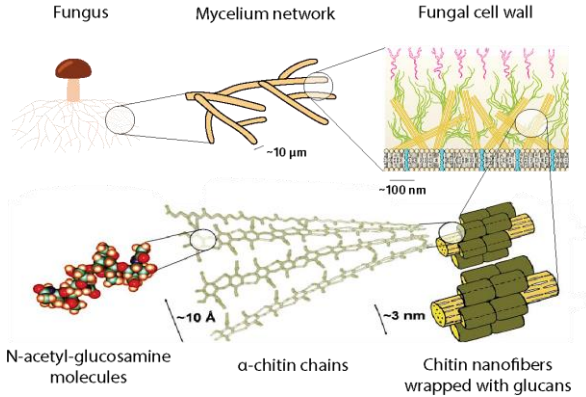
## Polysaccharide sources: Chitin

### Crustaceans




Adapted from Raabe et al., 2005. *Acta Mater*, Vol. 53, Issue 15, p 4281-4292.

### Fungi




Adapted from Liao et al., 2023. *Carbohydrate Polymers*, Vol. 305, 120553.

**Current Problems**

Heterogeneous chitin

Difficult isolation

Allergic reactions

**Advantages**

Homogeneous chitin

Easier isolation

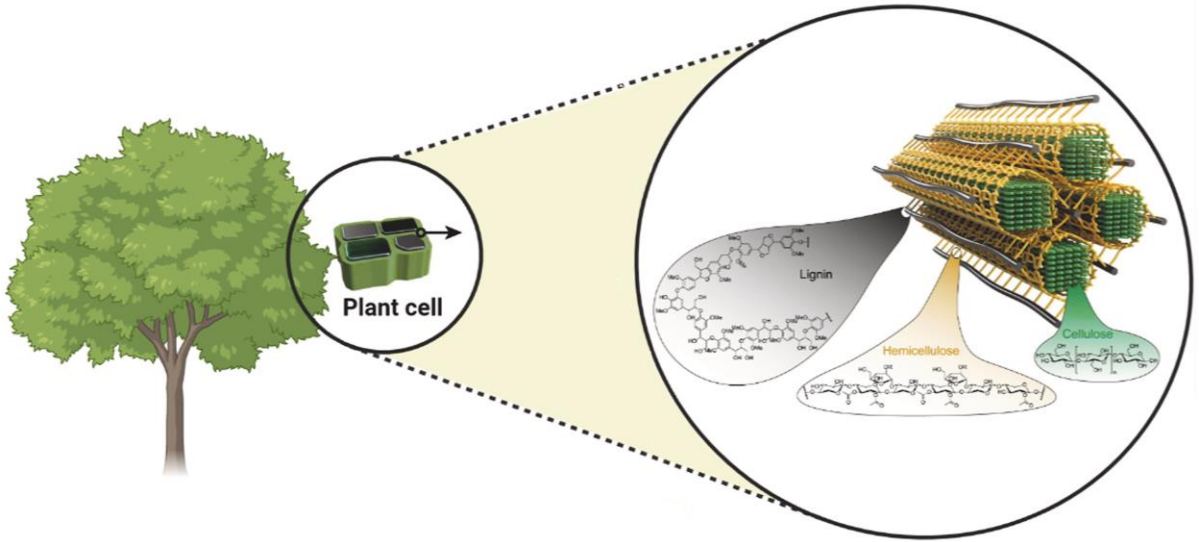
Modify by growth conditions

Blanca de Miguel Martínez

# BIONANOMATERIALS FROM FUNGAL FERMENTATION

## Polysaccharide sources: Cellulose

Lignocellulosic biomass



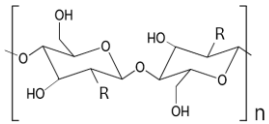

*J. Cleaner Production*. 2023, 402, 136815.

Blanca de Miguel Martínez

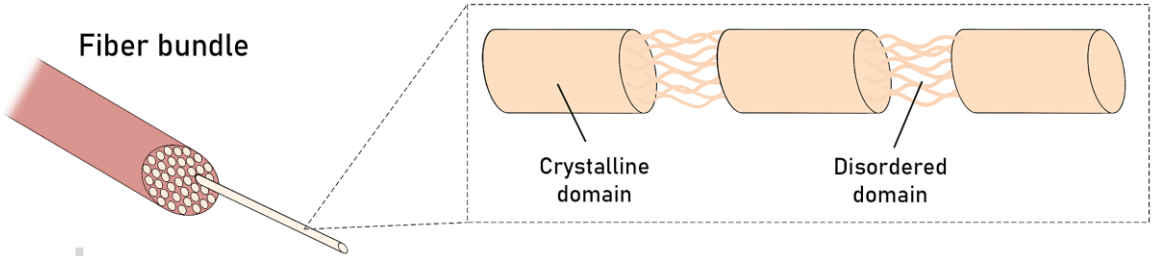
# BIONANOMATERIALS FROM FUNGAL FERMENTATION

## Polysaccharide nanomaterials

**Chitin or Cellulose**




Cellulose: R = OH
Chitin: R = $NCOCH_3$
Chitosan: R = $NH_2$




TOP-DOWN APPROACHES

**Nanocrystals Isolation**

- Chemical treatment
- Biological treatment

→ CHITIN OR CELLULOSE NANOCRYSTALS

**Nanofibers Isolation**

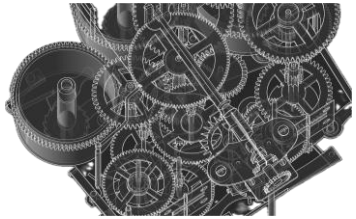
- Mechanical treatment
- Biological treatment

→ CHITIN OR CELLULOSE NANOFIBERS

Blanca de Miguel Martínez

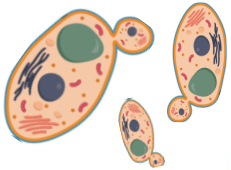
# BIONANOMATERIALS FROM FUNGAL FERMENTATION

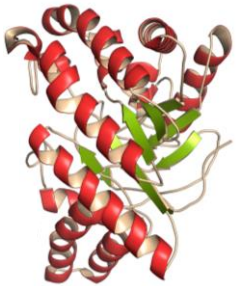
## Polysaccharide nanomaterials



**Chemical and Mechanical treatments**

**Biological treatments**





**Current Problems**

Chemically intensive

Energy intensive

**Advantages**

Greener treatments

Efficient technology

Blanca de Miguel Martínez

# BIONANOMATERIALS FROM FUNGAL FERMENTATION

## Cellulose Nanomaterials from fungi

### Treatments based on fungal enzymatic mechanisms

- Enzymatic cocktails with fungal hydrolytic and oxidative enzymes
- Successfully used to isolate CNFs and CNCs from different cellulosic feedstocks

### Fungal treatments

- Aid the breakdown (higher yields)
- Natural control growth conditions
- Substrate-tailored mechanism
- Technically and economically attractive

**Never performed**

Alonso-Lerma et al., 2020. *Communications Materials*, Vol. 1:57.
Zhou et al., 2019. *Cellulose*, Vol. 26, p 543-555.
Koskela et al., 2019. *Green Chemistry*, Vol. 21, 5924.

Blanca de Miguel Martínez

# BIONANOMATERIALS FROM FUNGAL FERMENTATION

## Cellulose & Chitin Nanomaterials Combination

### Individual production of NMs and later combination

- Enhanced barrier and mechanical properties
- Successfully used to produce functional nanocomposites with chitin and cellulose nanomaterials

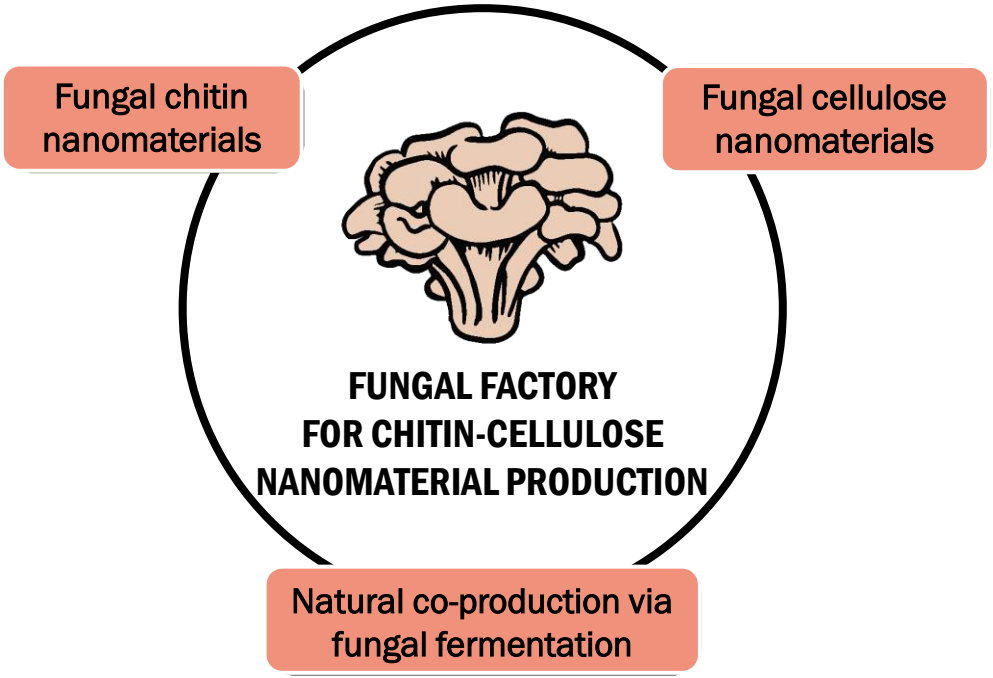
### Combined production of Nanocomposites

- One-step process for the simultaneous assembly of chitin and disassembly of cellulose
- More environmentally friendly process
- Natural combination of cellulose and chitin

**Never performed**

# BIONANOMATERIALS FROM FUNGAL FERMENTATION

Objective of my PhD



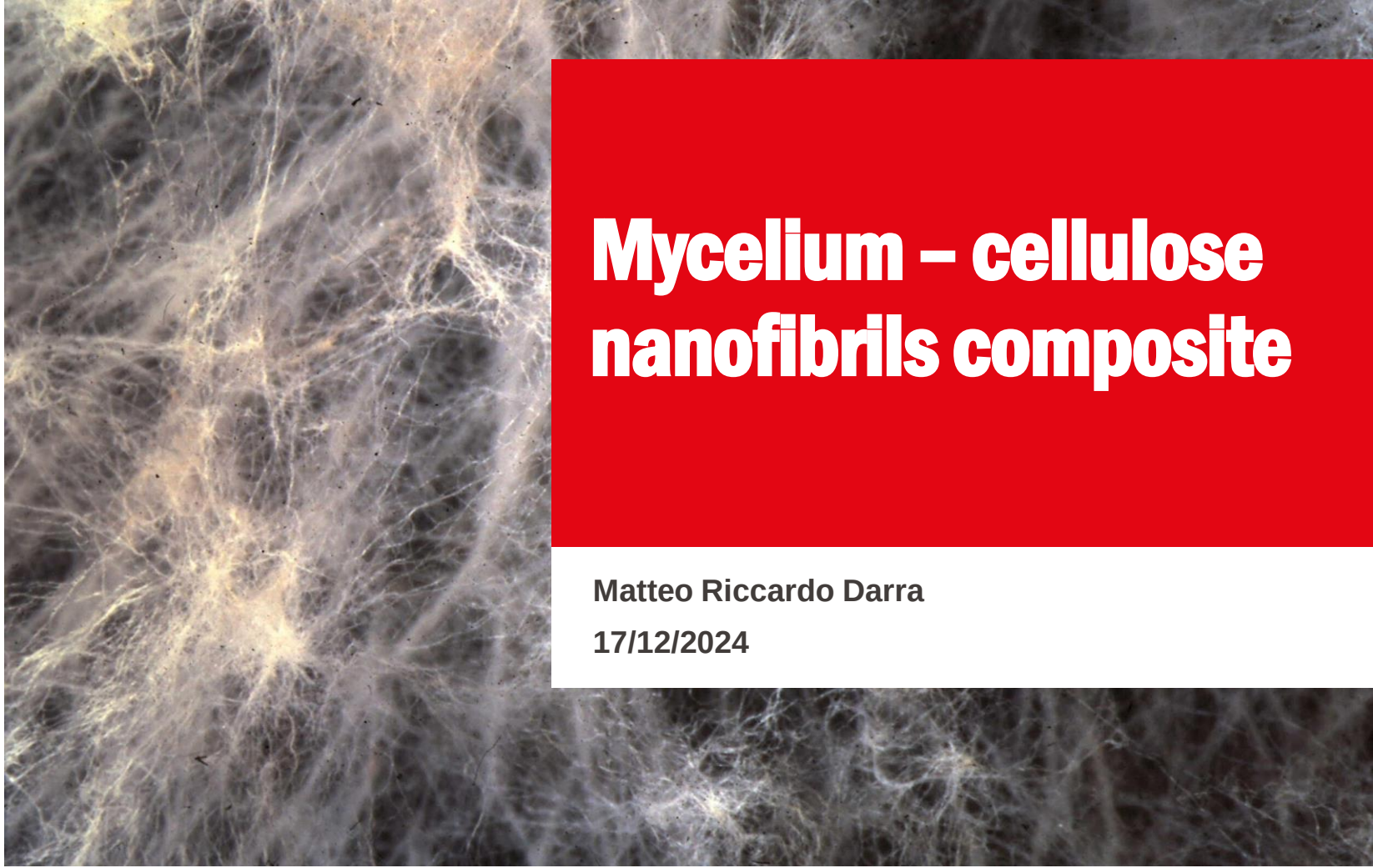
EPFL

SML
SUSTAINABLE MATERIALS LAB

# ANY QUESTIONS?

# THANK YOU!

Blanca de Miguel Martínez



# Mycelium – cellulose nanofibrils composite

Matteo Riccardo Darra

17/12/2024

# My life so far...


**Bachelor:** Chemistry and Materials Chemistry.

**Master:** Photochemistry and Molecular Materials.

# More recently

~ 1 year

Who knows..?

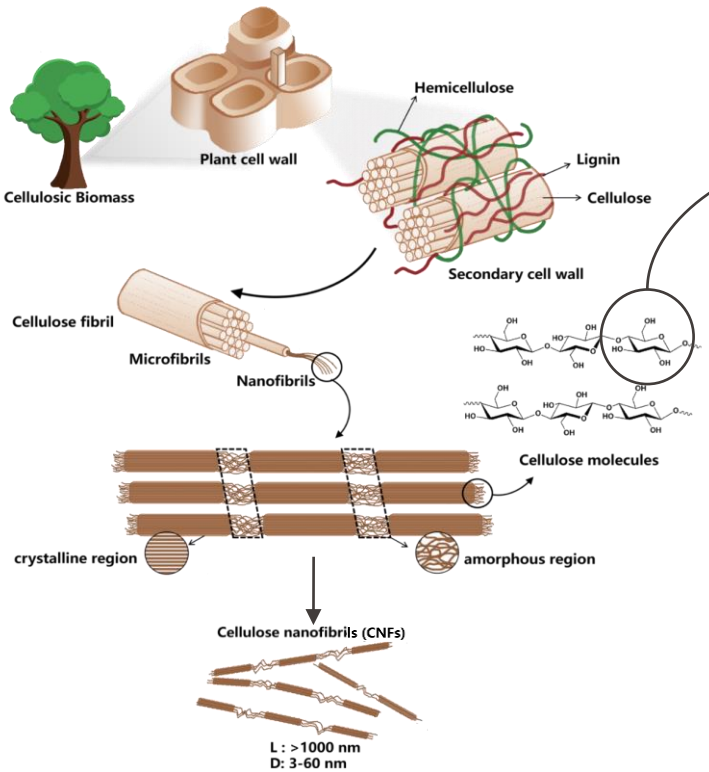
# Currently

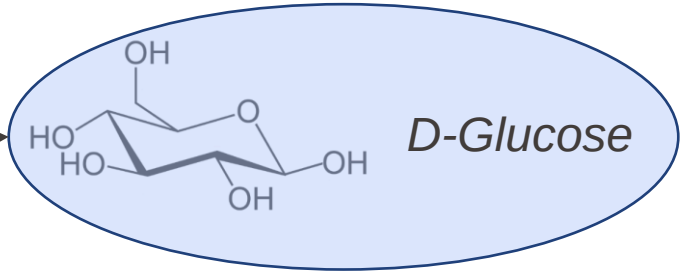
- PhD Student at Sustainable Materials Laboratory

## *Mycelium-Cellulose nanofibrils hybrid materials*

Matteo Riccardo Darra

# What are cellulose nanofibrils (CNFs)?




- **Mechanical properties**:

Elastic modulus of 100-130 GPa

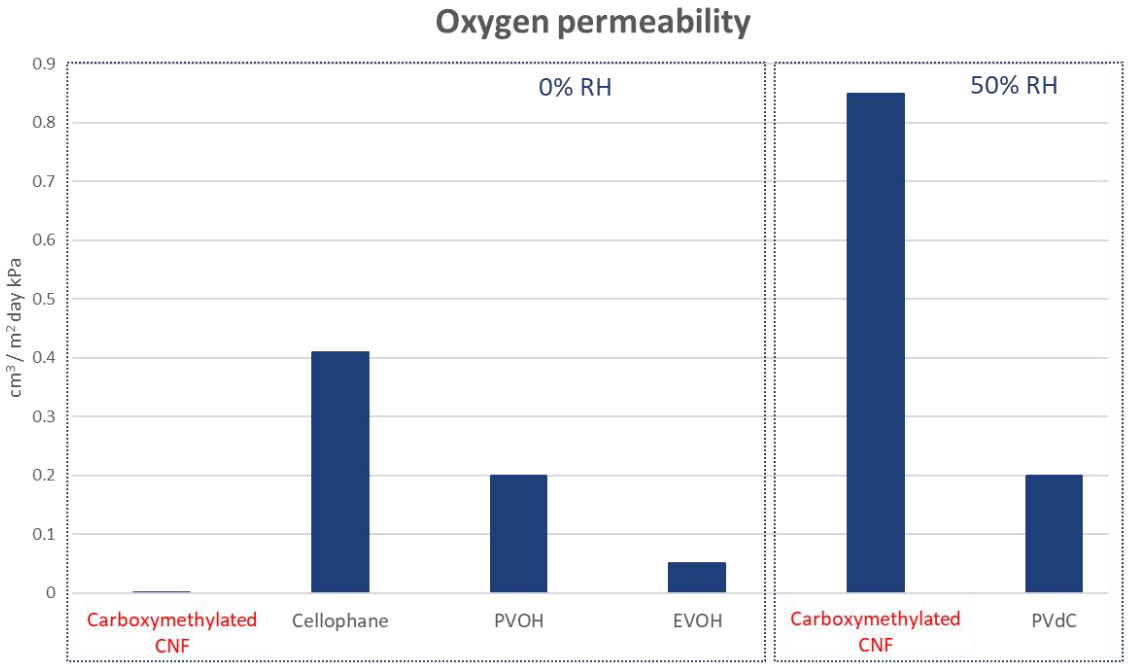
- **Oxygen barrier properties**:

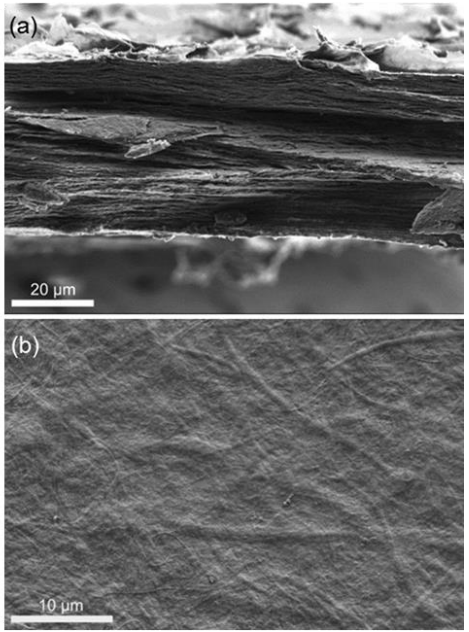
$\sim 0.01 \frac{cm^3}{m^2\ day\ kPa}$



Kong et al., Chapter 1 - Preparation and properties of nanocellulose,-Surface Modifications of Nanocellulose, Elsevier, 2024.
Zhai et al., 2018, Proc. SPIE 10597, Nano-, Bio-, Info-Tech Sensors, and 3D Systems II.

# Oxygen barrier properties

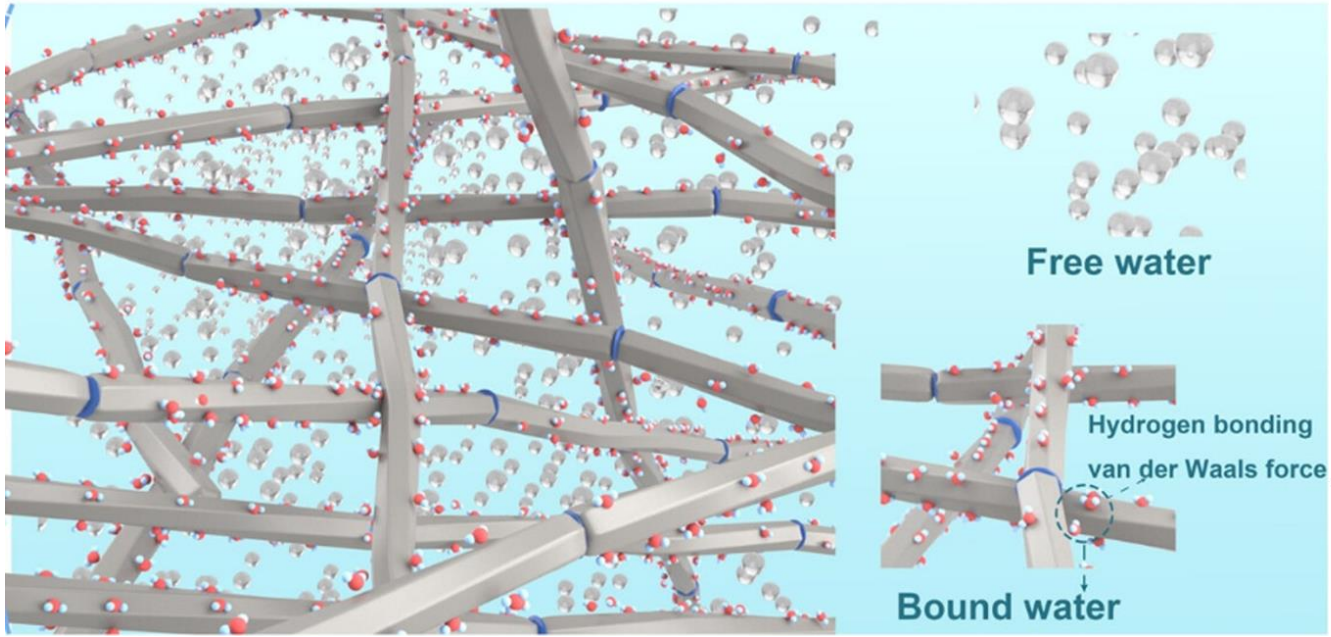


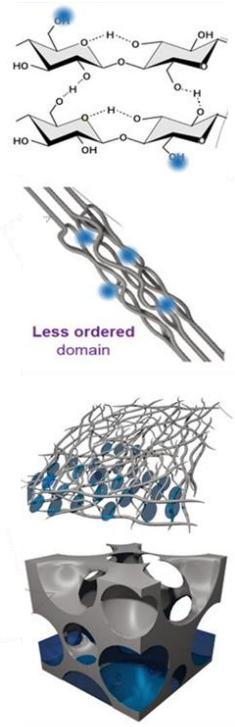




Nair et al., Sustain Chem Process 2, 23 (2014).
ACS Appl. Mater. Interfaces 2016, 8, 24, 15607–15614

Matteo Riccardo Darra

# Water influence on CNFs




Solhi et al., Chem. Rev. 2023, 123, 5, 1925–2015, 2023

# What is Mycelium?



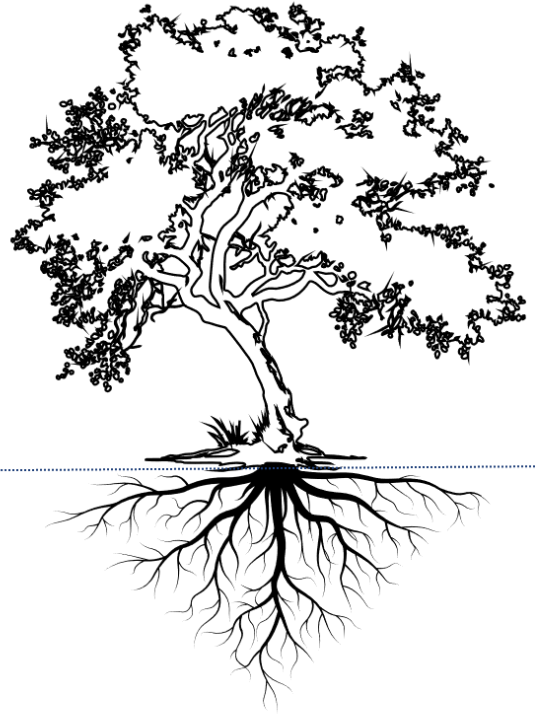

Matteo Riccardo Darra

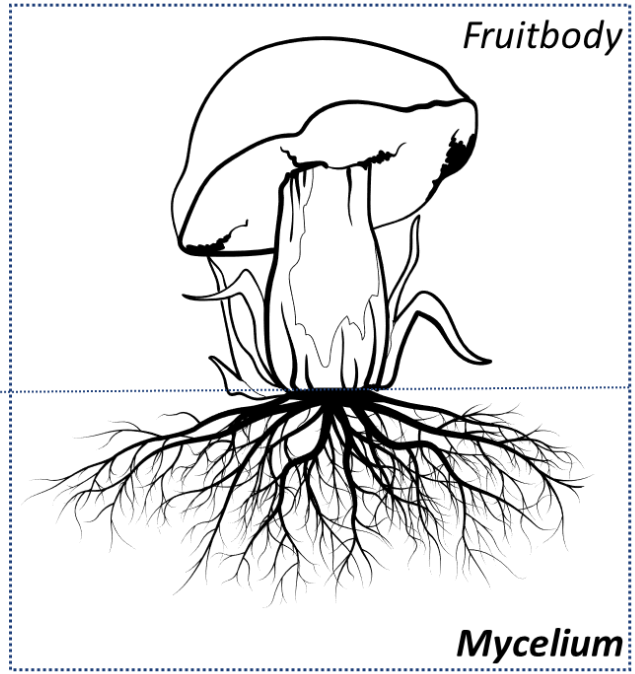
# What is Mycelium?



Exposed to light

In darkness






Matteo Riccardo Darra

# Mycelium-based materials



Matteo Riccardo Darra

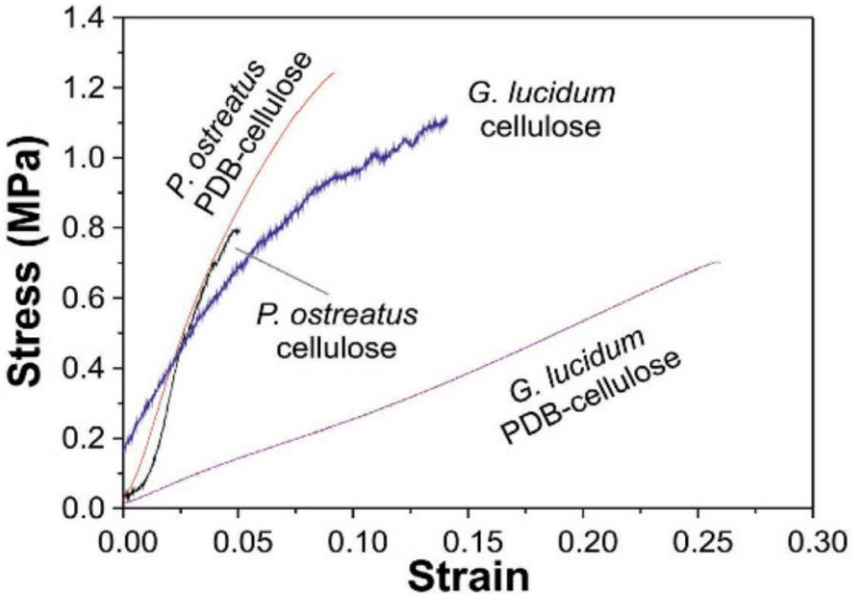
©Ecovative mycelium packaging

Mycelium-based leather alternative from Reishi™

Mycelium-based shoes from Adidas™

Mycelium thermal insulator brick from Greensulate™

# Mycelium-based materials

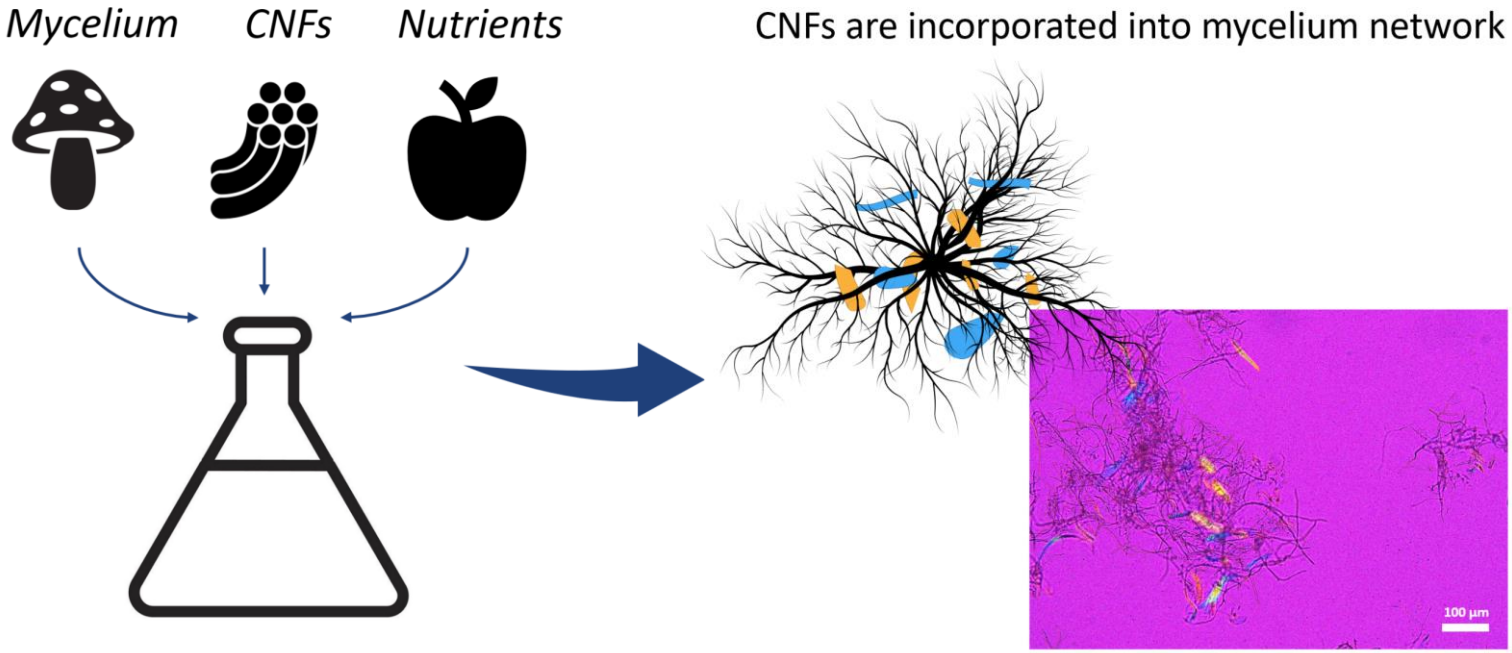



Haneef et al., Scientific Reports | *7:41292*



Matteo Riccardo Darra

# What I am doing

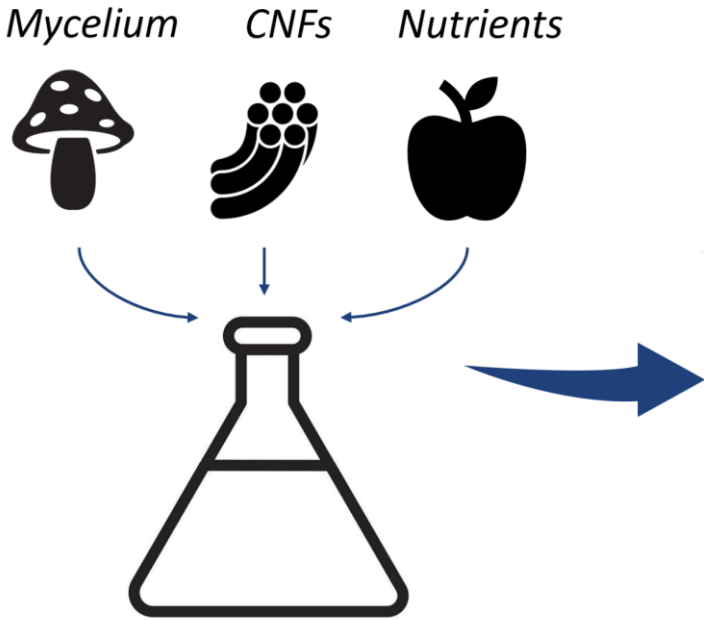



Matteo Riccardo Darra

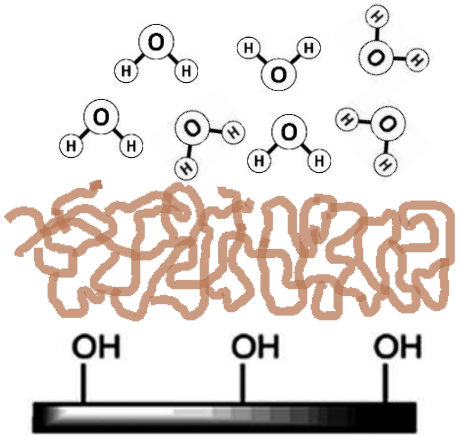
# Mycelium effect on CNFs



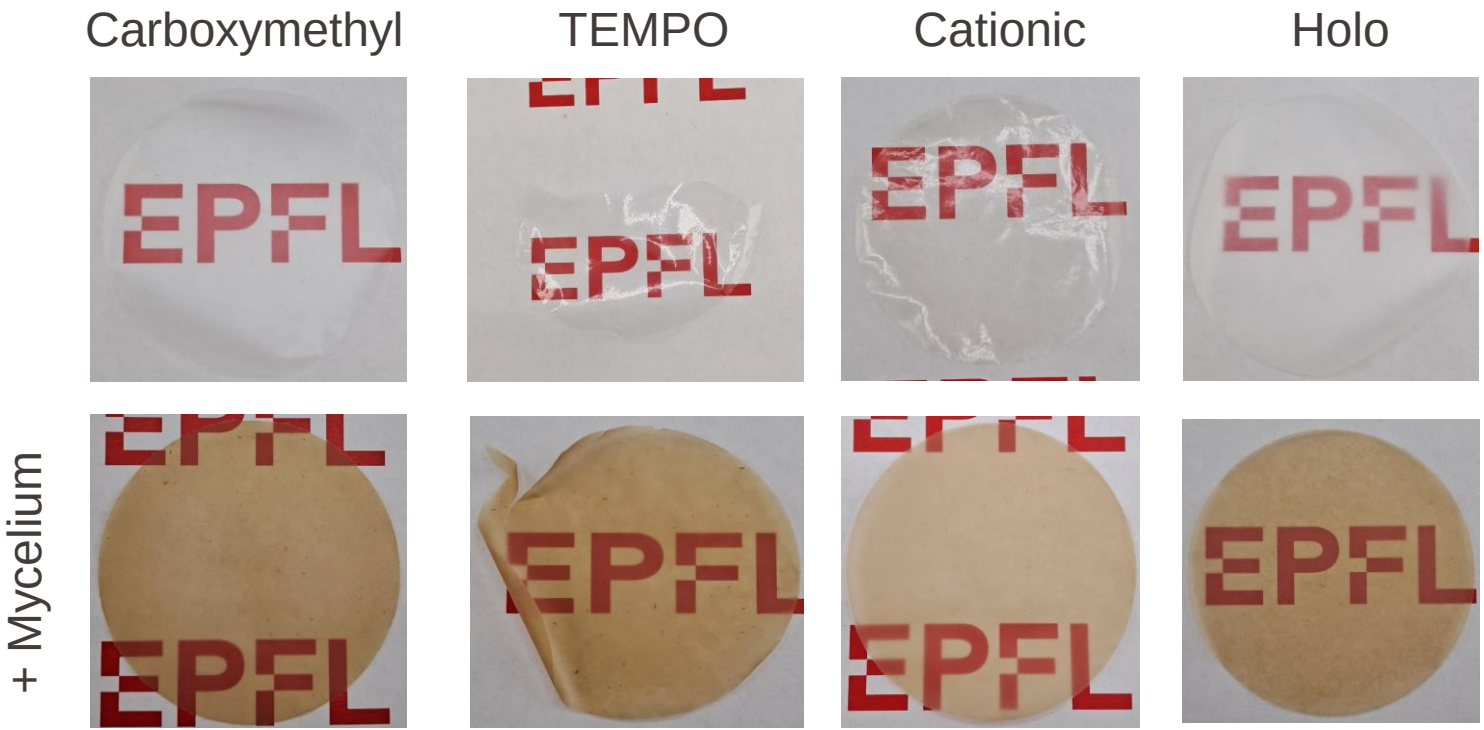
Matteo Riccardo Darra




*Surface passivation due to mycelium presence*



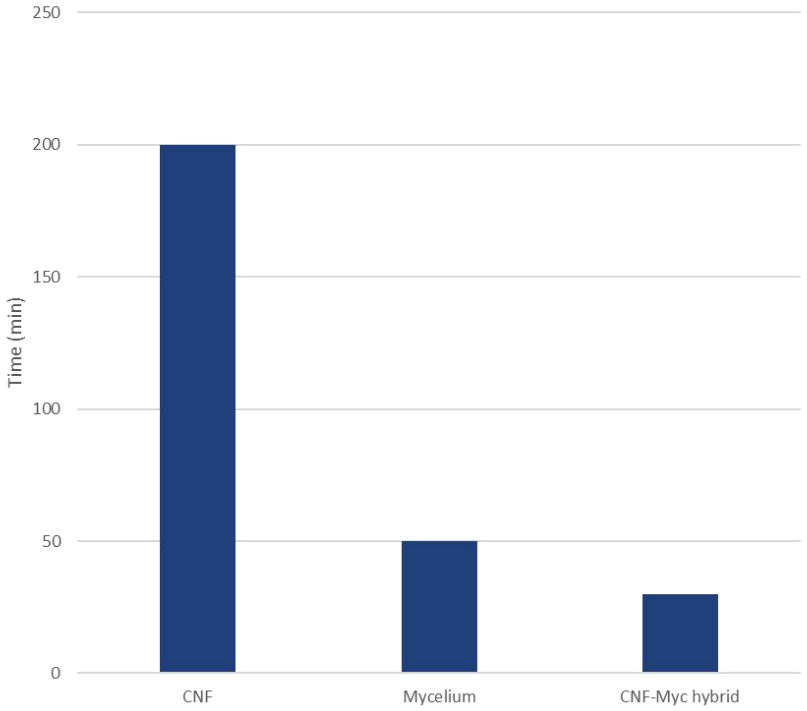
# Different type of CNF can be used






Matteo Riccardo Darra

# Time needed for film production





*Pure CNF*


*Myc-CNF composite*



Matteo Riccardo Darra

# Applications



Matteo Riccardo Darra


Films

Paper additive

Coatings


# Thank you, questions are welcome!

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

Federica Daus
Visiting PhD student
Department of chemistry «G.Ciamician», University of Bologna

17-12-2024

# A little bit about my background

Master degree at the
University of Pisa, 2021

Supervisor: prof. F. Pineider

What you don't see:

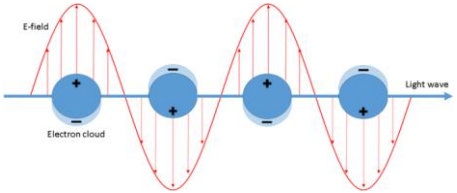
ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

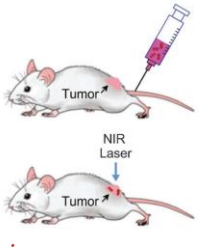
# A little bit about my background

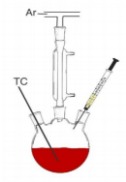
Master degree at the University of Pisa, 2021

Supervisor: prof. F. Pineider

During my master project, I worked on plasmonic NPs for optical hyperthermia

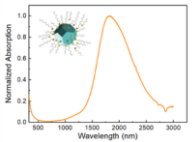


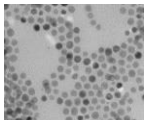



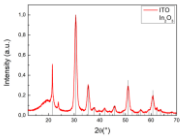



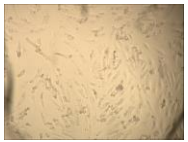

Synthesis

Characterization





Test *in vitro*



ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

# A little bit about my background

What do I do now?

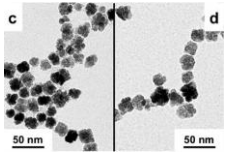
SCIENCES SORBONNE UNIVERSITÉ

Internship at the Sorbonne University, Paris, 6 months, 2022

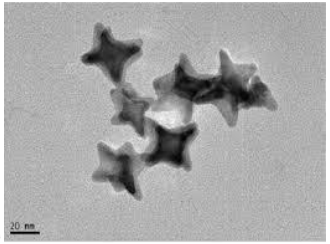
prof. A-A. Hassan

PHENIX

$Fe_2O_3$ and $Fe_3O_4$ nanoflowers for optical hyperthermia




Au and CuS@Au nanostars for biomedical application

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

# A little bit about my background

What do I do now?

PhD in Nanoscience for Medicine and the Environment, University of Bologna, 2022-present

prof. M-L. Focarete

The oldest university of the world (founded in 1088)

UNESCO world Heritage Site since 2021

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

# A little bit about my background

What do I do now?

PhD in Nanoscience for Medicine and the Environment, University of Bologna, 2022-present

prof. M-L. Focarete

# My PhD


+

Bn

Bioniks s.r.l., Verona, Italy.

Kombucha Tea

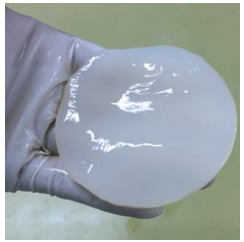
SCOBY

**First reaction: shock becäüṣḛ..**
Never worked with biomaterials before, nevever done a TGA, DSC, stress-strain..analysis before.



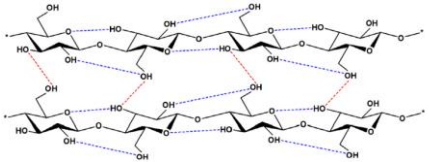
ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

# Bacterial Cellulose



Characterized by <u>many interesting properties</u>:

- Hydrophilicity
- High degree of cristallinity
- High surface area
- Great water-holding capacity



3D network of hydrogen bonds between the microfibrils

*Suitable <u>alternative green material</u>, which can be employed in a wide range of applications:*

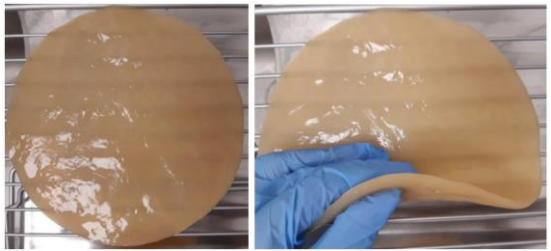
- **Kombucha tea** is a slightly alcoholic sugared beverage produced by the fermentation of tea leaves and sugar.
- During fermentation, bacterial cellulose is produced in form of a membrane, better known as SCOBY (Symbiotic Culture Of Bacteria and Yeasts).

# Aim of my PhD project

- Develop a **sustainable purification procedure**, suitable for **industrial-scale applications**;
- Improving the mechanical and physical-chemical properties of the resulting cellulose, in order to develop **biomedical devices** from it.

# Aim of my PhD project

- Develop a **sustainable purification procedure**, suitable for **industrial-scale applications**;
- Improving the mechanical and physical-chemical properties of the resulting cellulose, in order to develop **biomedical devices** from it.



Scoby

**Purification**

Bacterial cellulose

# Aim of my PhD project

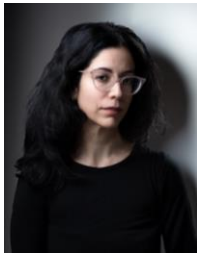
- Develop a **sustainable purification procedure**, suitable for **industrial-scale applications**;
- Improving the mechanical and physical-chemical properties of the resulting cellulose, in order to develop **biomedical devices** from it.

Bacterial cellulose

Plastified bacterial cellulose with glycerol

# My period abroad at EPFL

6 months at the EPFL,
September 2024-February 2025



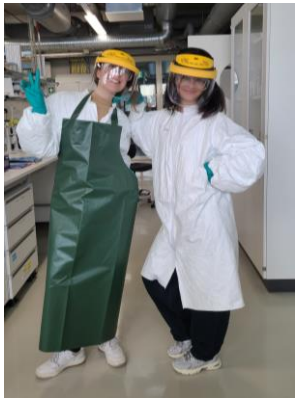
prof. T. Abitbol

Aim of this period abroad:

Bacterial cellulose nanocrystals extraction

Hydrolysis with sulfuric acid

Enzimatic hydrolysis

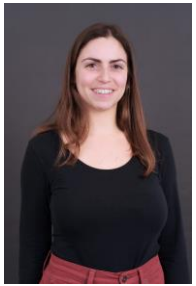


Me and Buse before making CNCs..

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

# My period abroad at EPFL

- Food product mainly consumed in Asia;
- During the fermentation process Acetobacter Xylinum ferments coconut water and convert glucose into cellulose;
- Acetic acid is produced, which helps in preserving the food product.



Dr. Chiara Moretti

Au NPs expert

Back to the origins…

Develop a material by combining bacterial cellulose from *nata de coco* and **metallic nanoparticles** (e.g., Au NPs) to create a patch or biomedical device for photothermal cancer therapy.

Good luck to us!

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

# My University/Life Survival Kit

Stay positive!

- Do not worry too much about what is coming next, trust the process
- Something will come your way anyway
- Even if you are scared of a new adventure, do not let fear hold you back—just go for it!

Wish you all the best for your future!