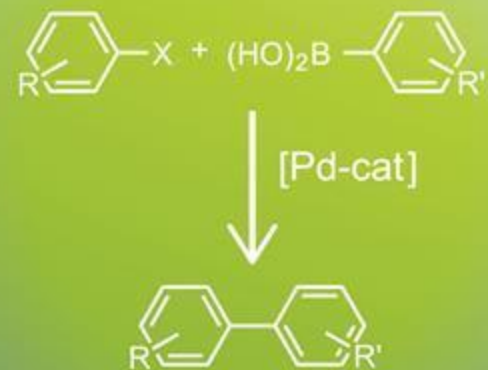
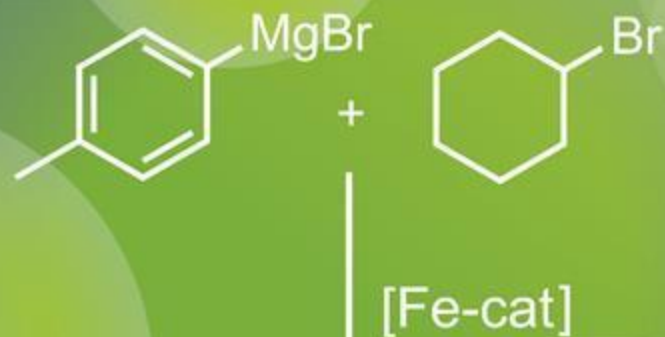
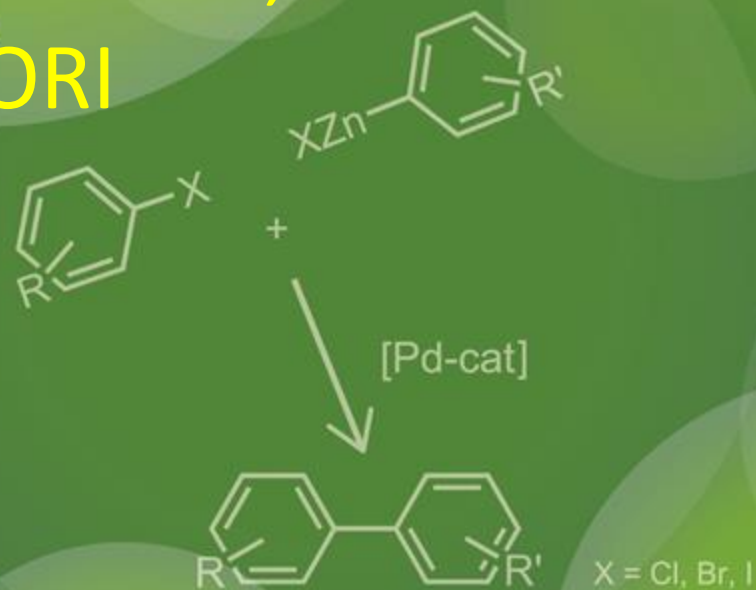




# LA CHIMICA VERDE DELLA LIGNINA NELLA BIORAFFINERIA: COSMECEUTICA, BIOPLASTICHE, BIOINCHIOSTRI E BIOSENSORI





# GREEN CHEMISTRY

## GREEN CHEMISTRY 12 PRINCIPLES

11 analyse in  
real time  
to prevent  
pollution

10 design  
chemicals &  
products to  
degrade after  
use

9 use  
catalysts

8 avoid chemical  
derivatives  
(protein groups)

12 minimize  
potential  
for accidents

7 use  
renewable  
feedstocks

1 prevent  
waste

5 safer solvents  
& reaction -  
conditions

6 increase  
energy  
efficiency

2 maximize  
atom  
economy

4 safer chemicals  
& products

3 less hazardous  
chemical syntheses



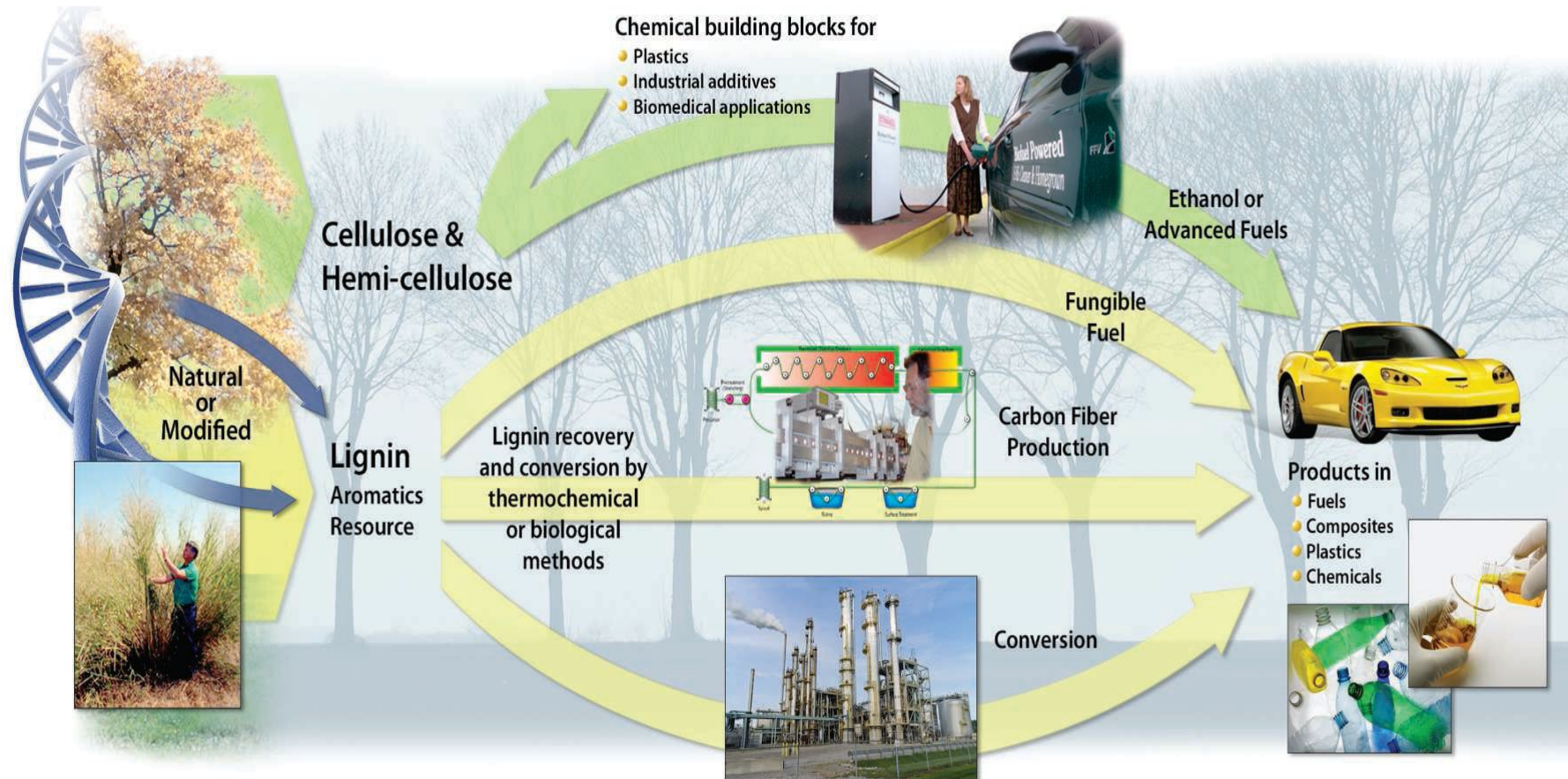
## CIRCULAR ECONOMY Inspired





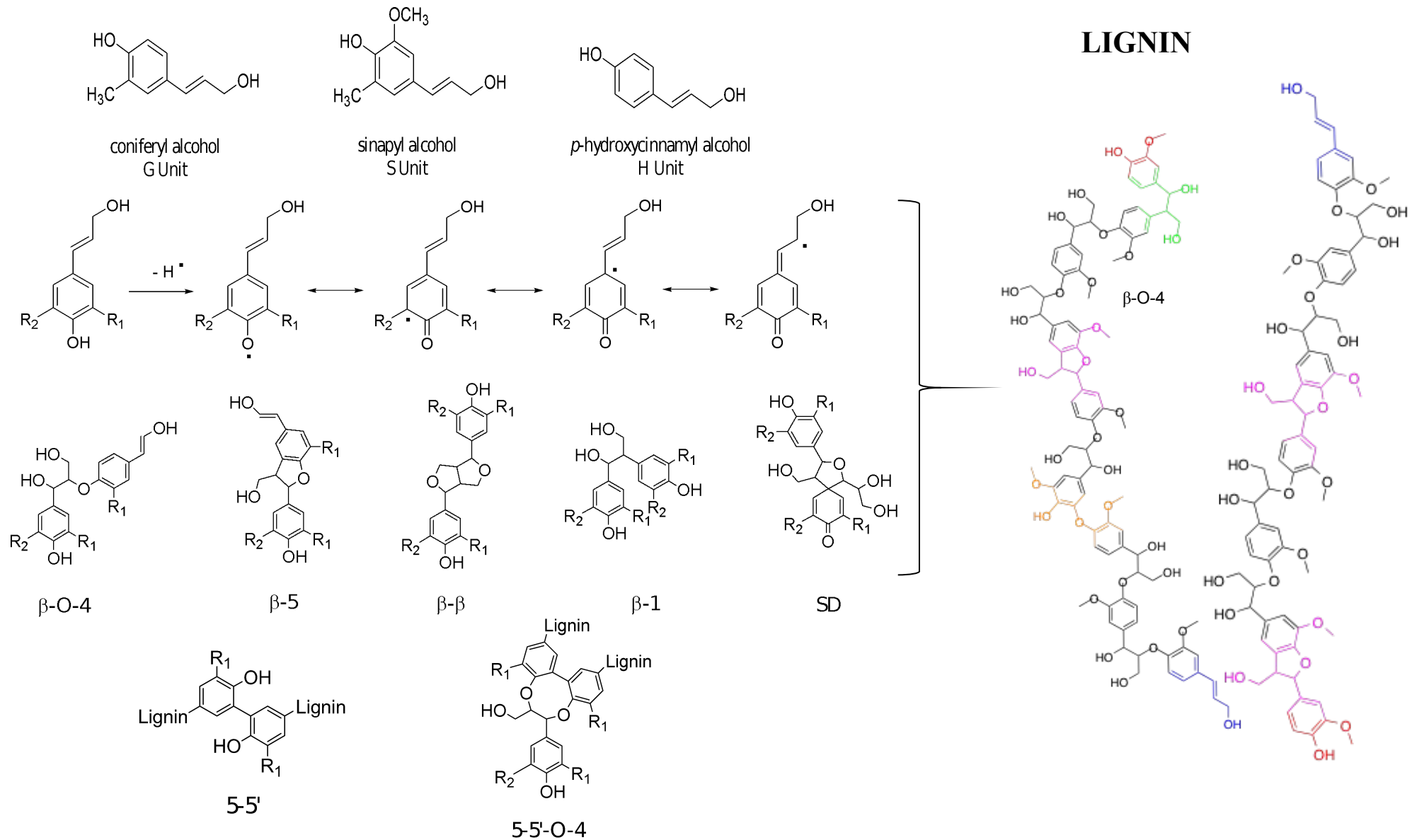
# About lignin...

*«Lignin, nature's dominant aromatic polymer, is found in most terrestrial plants in the approximate range of 15 to 40% dry weight and provides structural integrity»*



*«The U.S. Energy Security and Independence Act of 2007 mandates the development of 79 billion liters of second-generation biofuels annually by 2022. Assuming a yield of 355 liters per dry ton of biomass, 223 million tons of biomass will be used annually producing about 62 million tons of lignin.»*

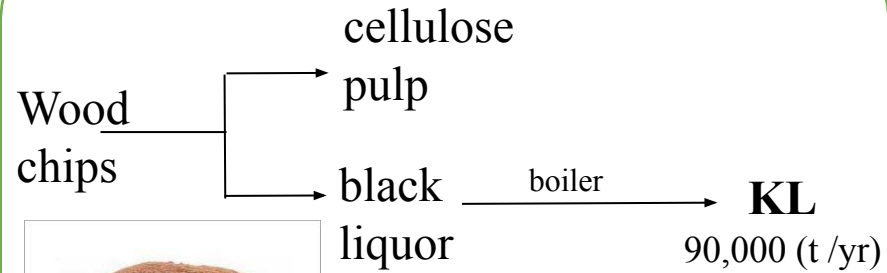
# Chemistry of lignin





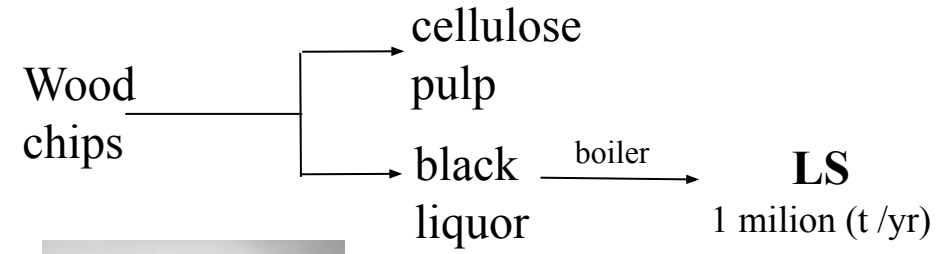
# Lignins from industrial process

## Kraft lignin (KL)

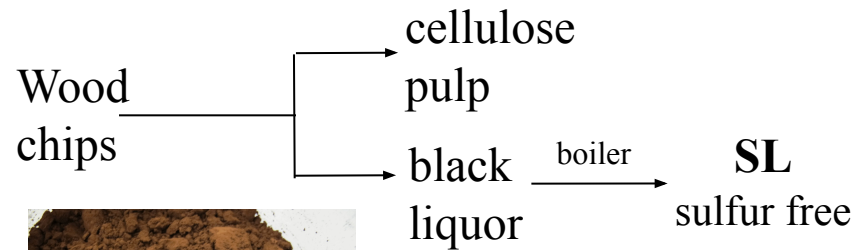


(Z. Strassberger, S. Tanase, G. Rothenberg, RSC Adv. 4 (2014) 25310.)

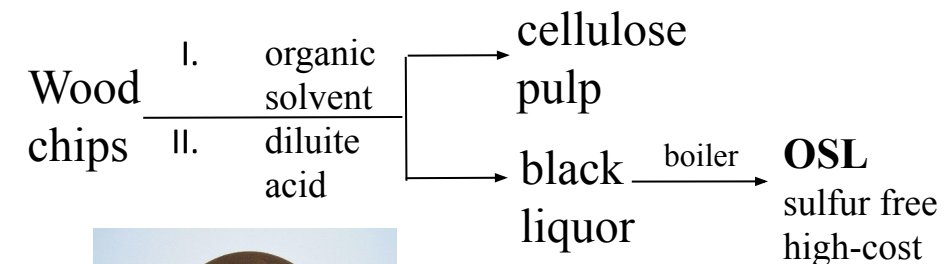
## Lignosulfonated (LS)

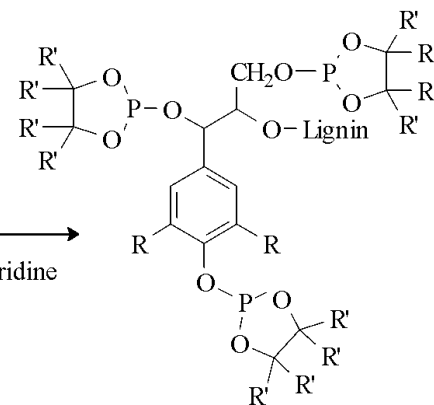
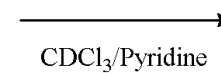
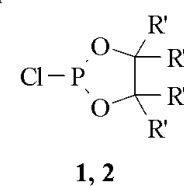
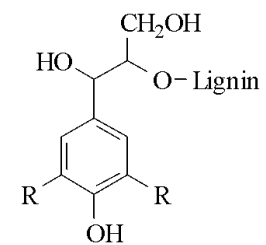
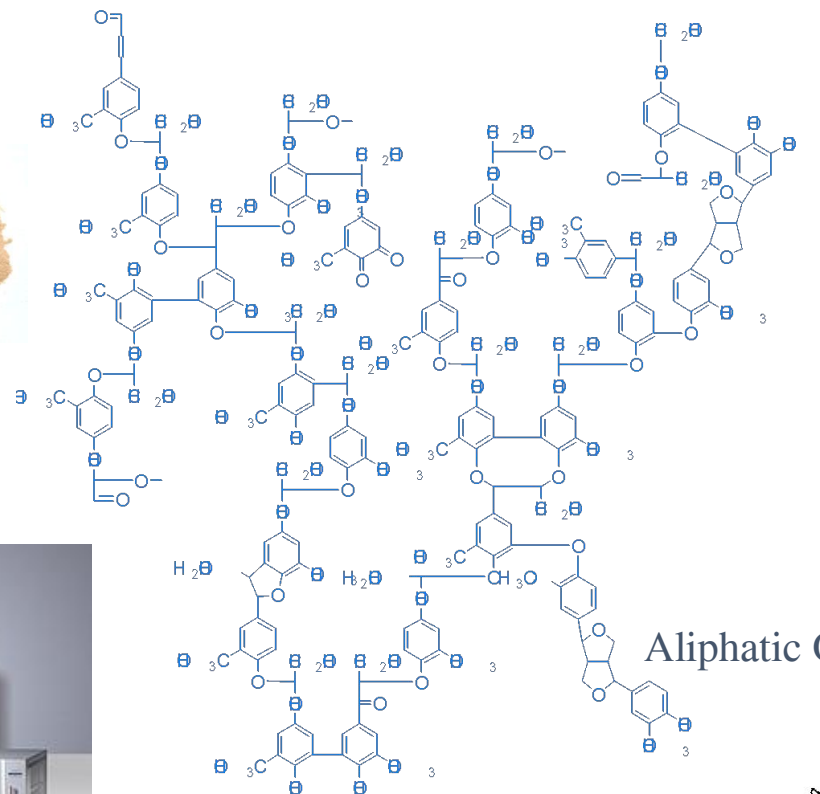
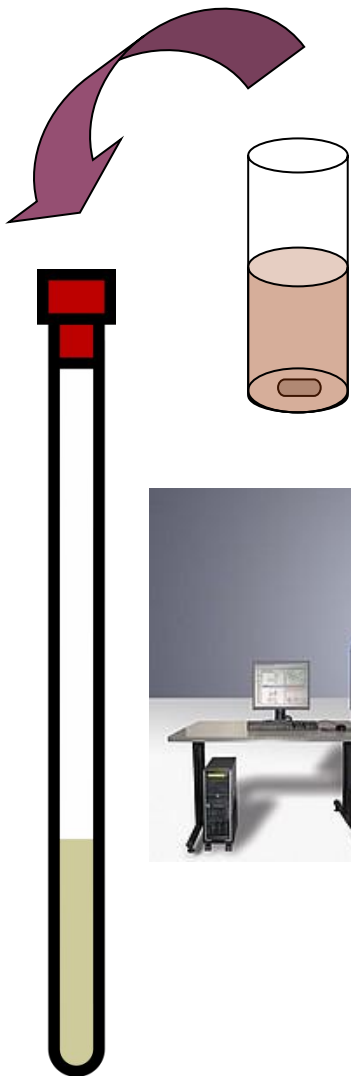


## Soda lignin (SL)



## Organosolv lignin (OSL)

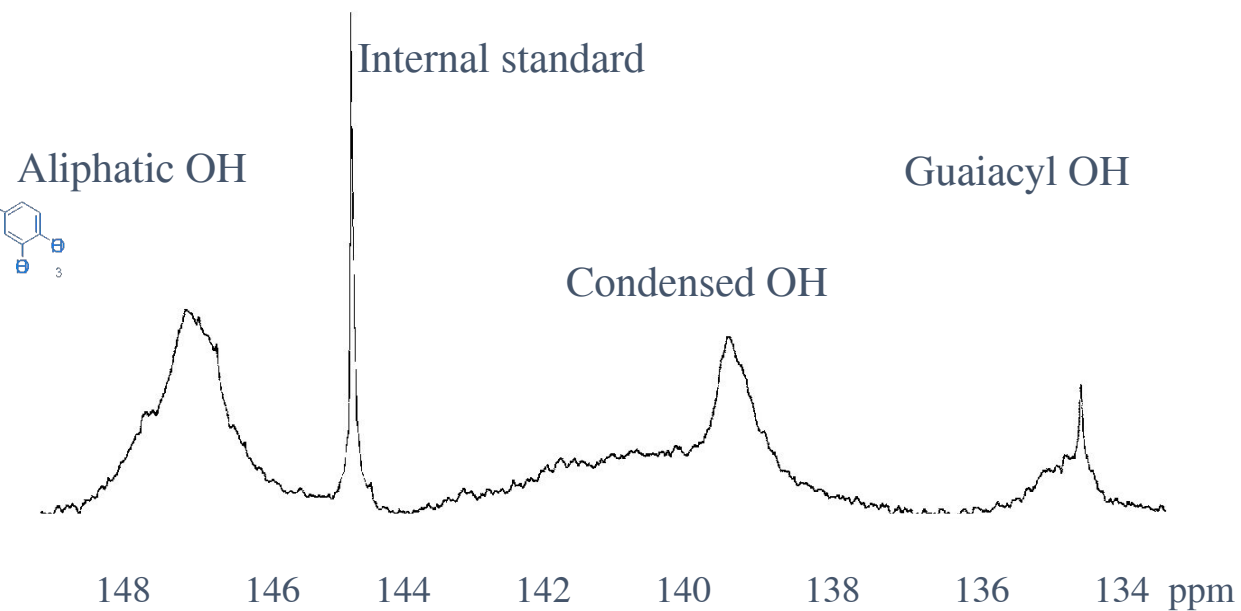




R = H, OCH<sub>3</sub>, Lignin

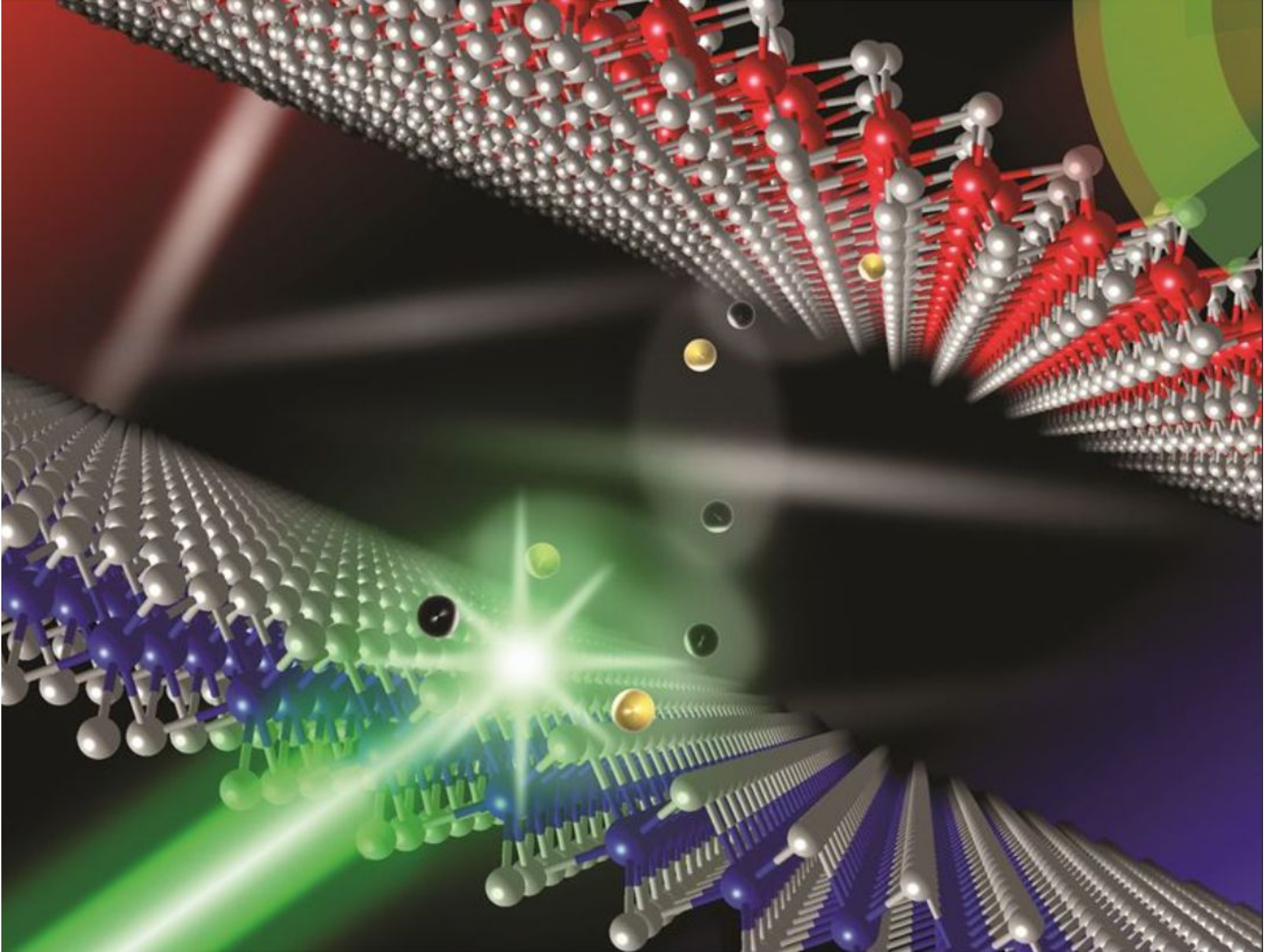
1: R' = H

2: R' = CH<sub>3</sub>





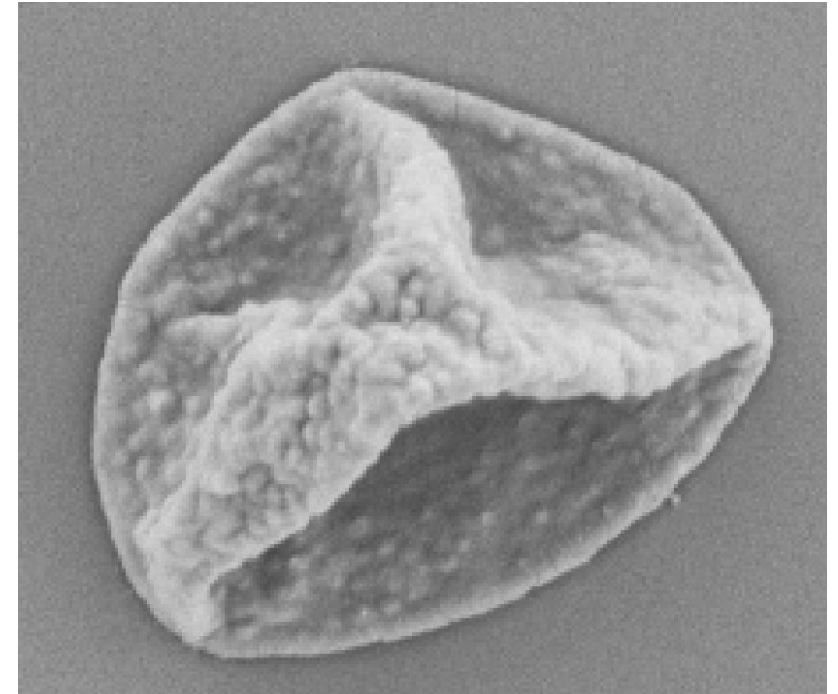
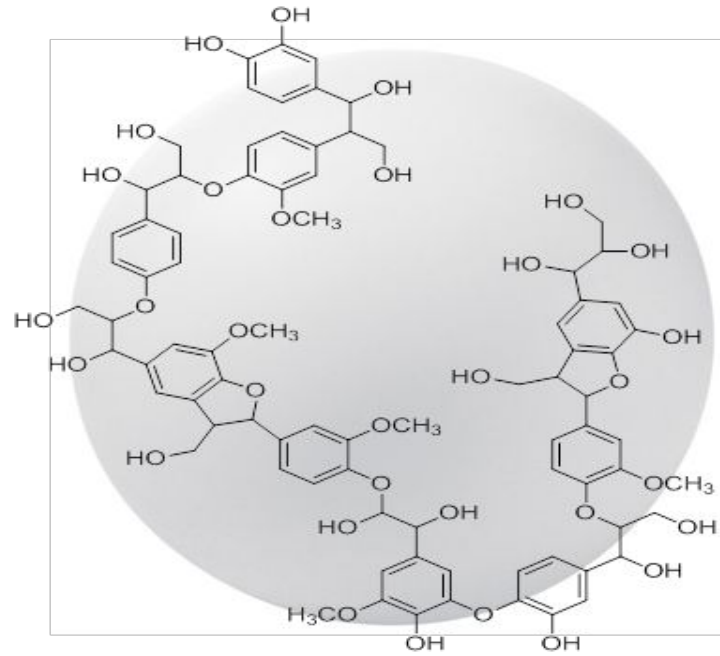
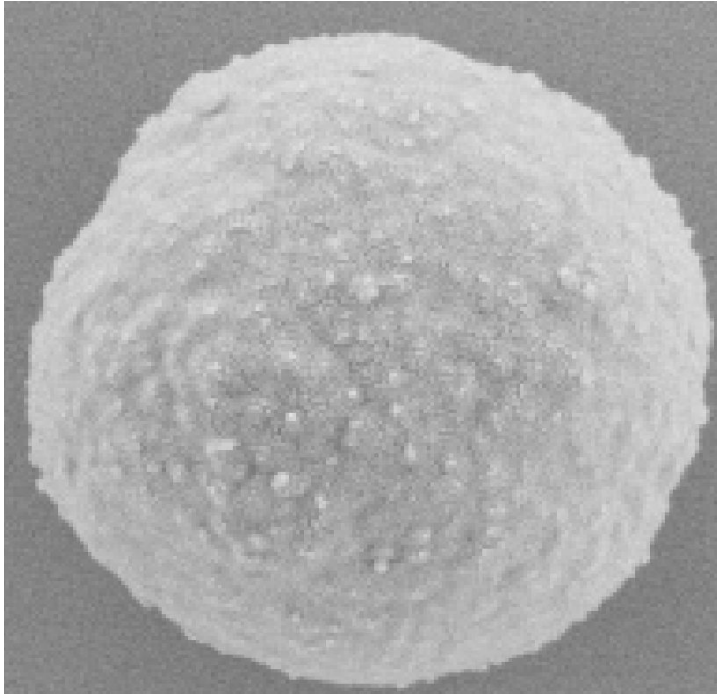
MICRO-SCALE



NANO-SCALE



# Lignin nanocapsule

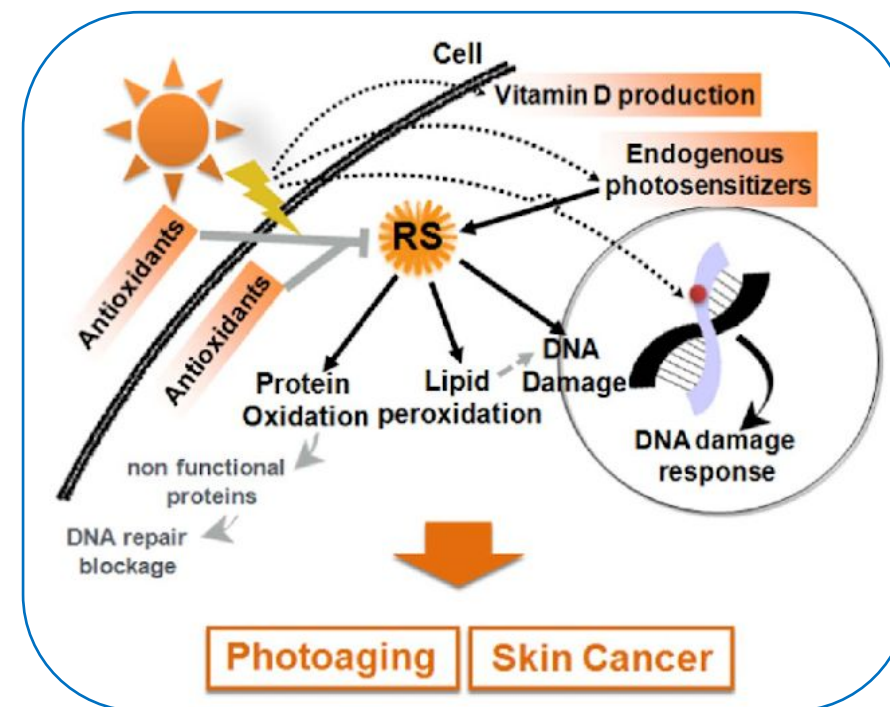
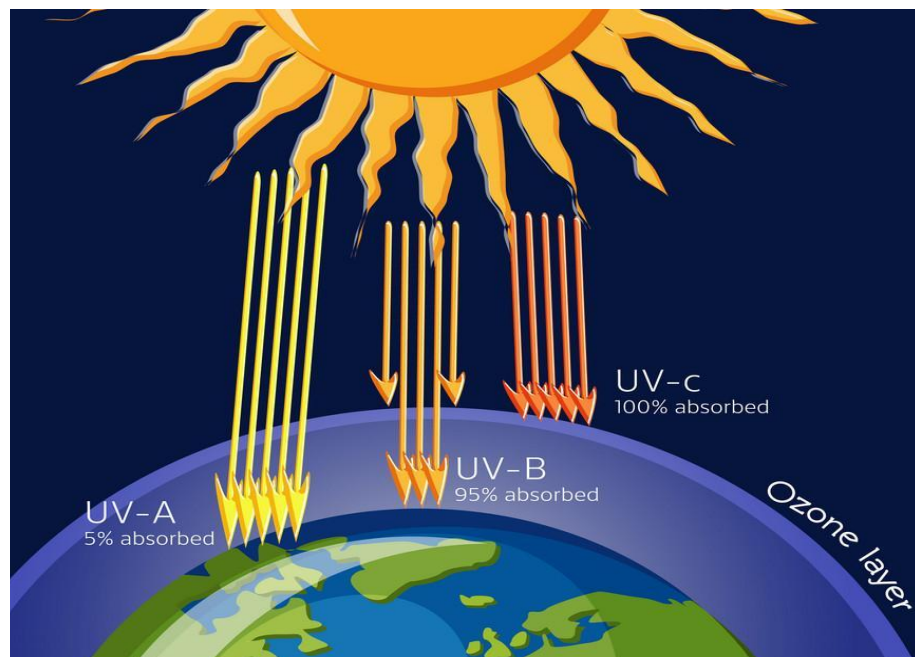
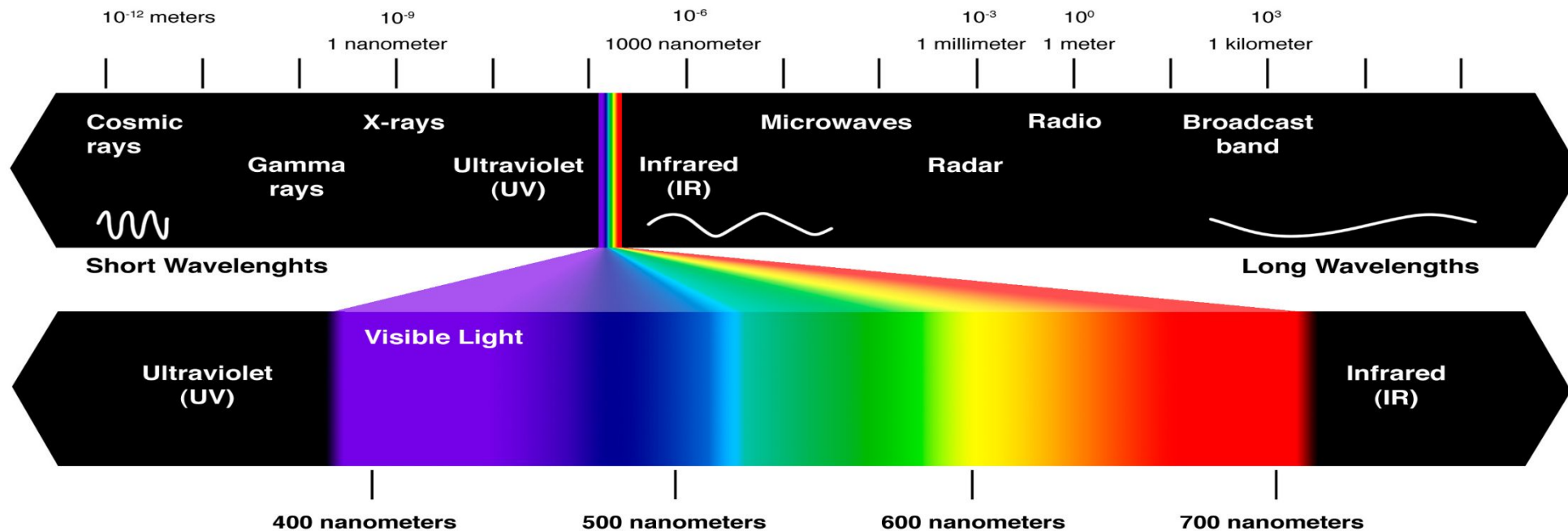




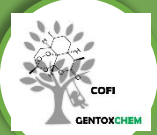
In effettiva collaborazione, nell'ambito del bando POR life 2020 Benessere/Wellbeing, la IDI Farmaceutici e il Dipartimento DEB dell'Università della Tuscia hanno predisposto il progetto “**UNICO**”.

Il progetto integra competenze in ambito biomedico, chimico ed industriale per la realizzazione di un innovativo filtro solare “cosmeceutico” basato su un ingrediente “naturale” a base di **lignina**, un componente del legno non tossico ed ecocompatibile, in grado di assorbire sia UV-A che UV-B.



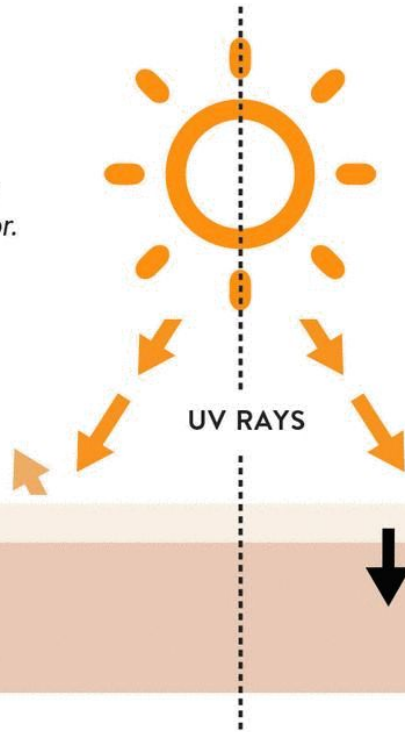






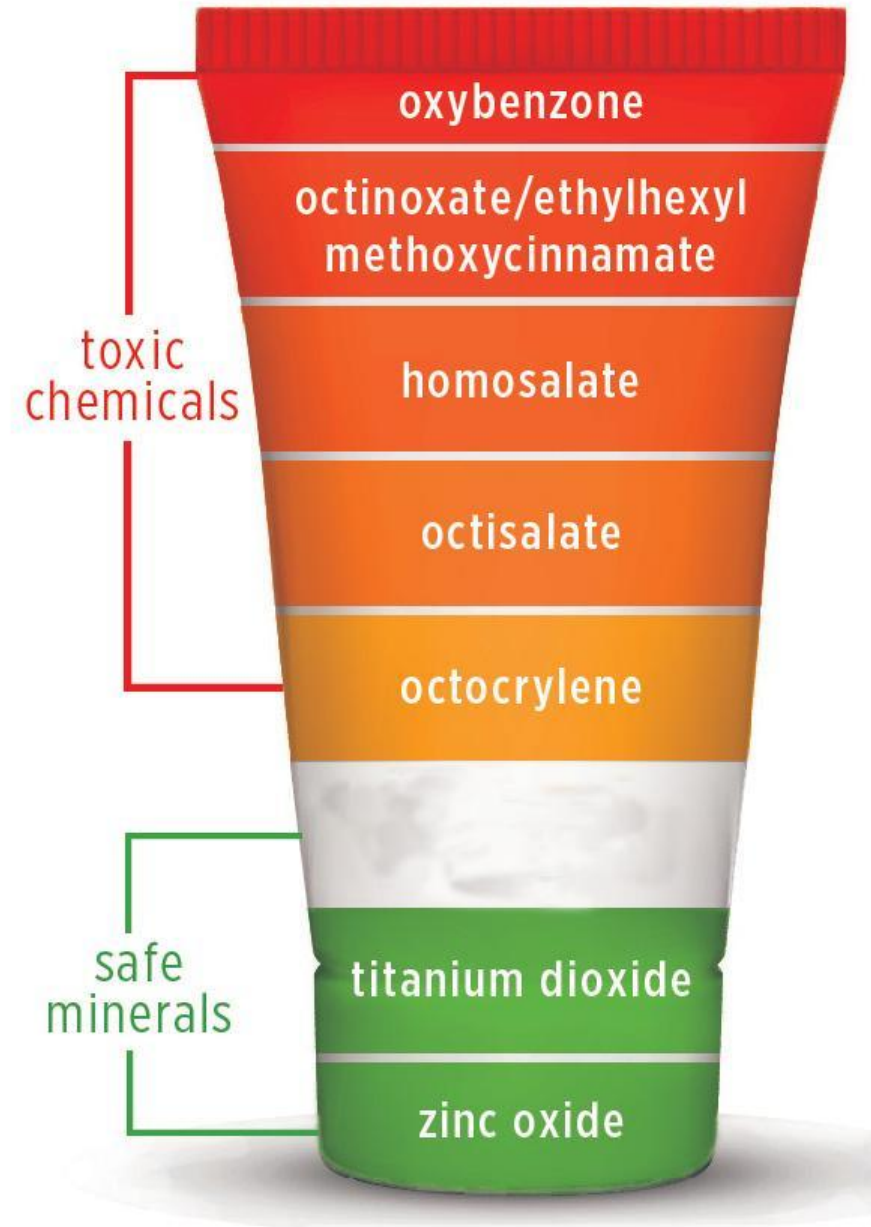
## PHYSICAL SUNSCREENS

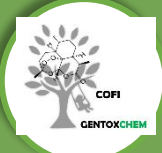
*sit on the top of the skin and reflect the sun rays like a mirror.*



## CHEMICAL SUNSCREENS

*penetrate the skin and absorb sun rays like a sponge.*





# SUNSCREEN CHEMICALS AND MARINE LIFE

How **sunscreen chemicals** enter our environment:



The sunscreen you apply may not stay on your skin.



When we swim or shower, sunscreen may wash off and enter our waterways.



How **sunscreen chemicals** can affect marine life:



**GREEN ALGAE:** Can impair growth and photosynthesis.



**CORAL:** Accumulates in tissues. Can induce bleaching, damage DNA, deform young and even kill.



**MUSSELS:** Can induce defects in young.



**SEA URCHINS:** Can damage immune and reproductive systems, and deform young.



**FISH:** Can decrease fertility and reproduction, and cause female characteristics in male fish.

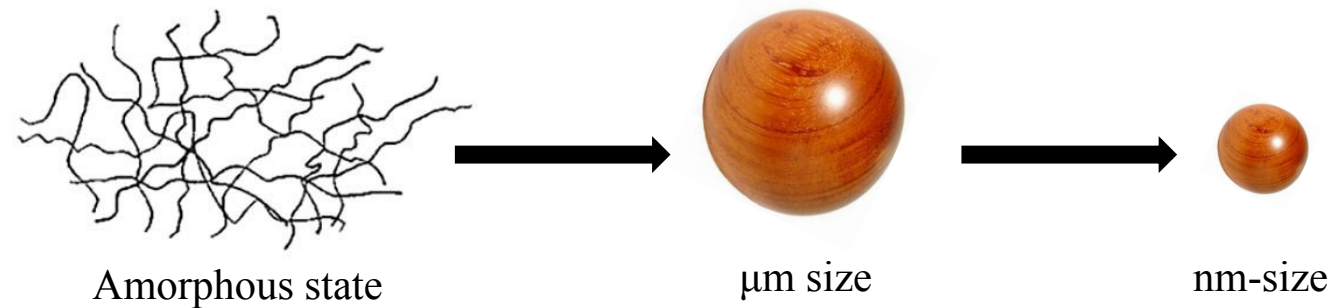
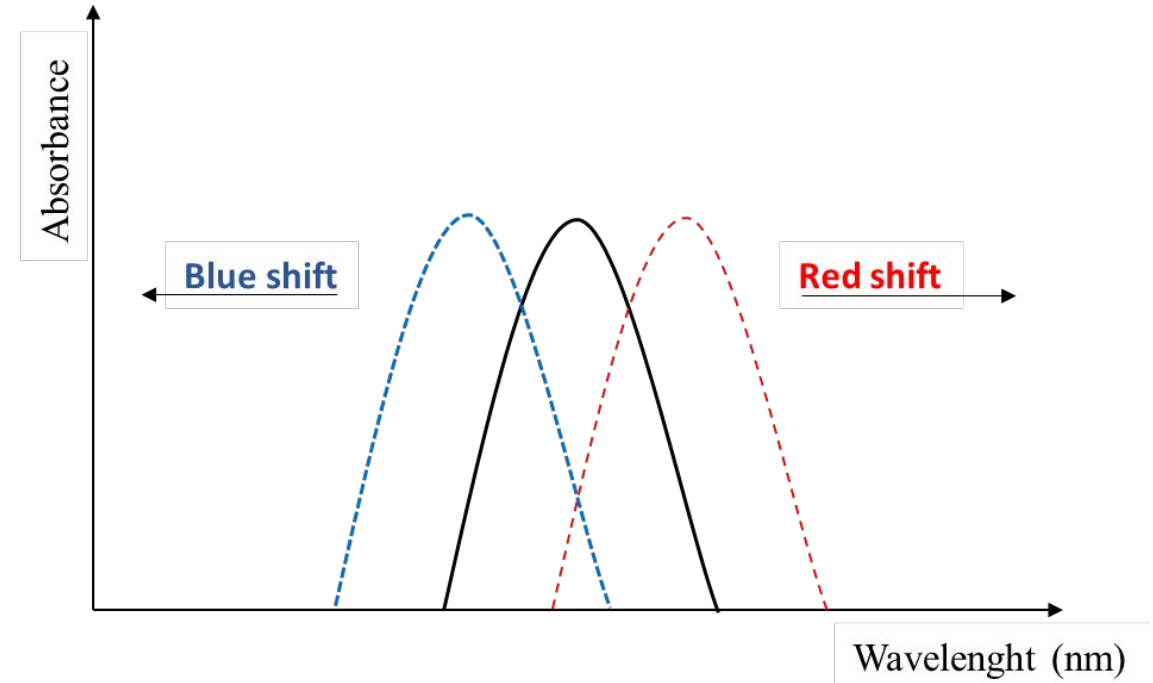
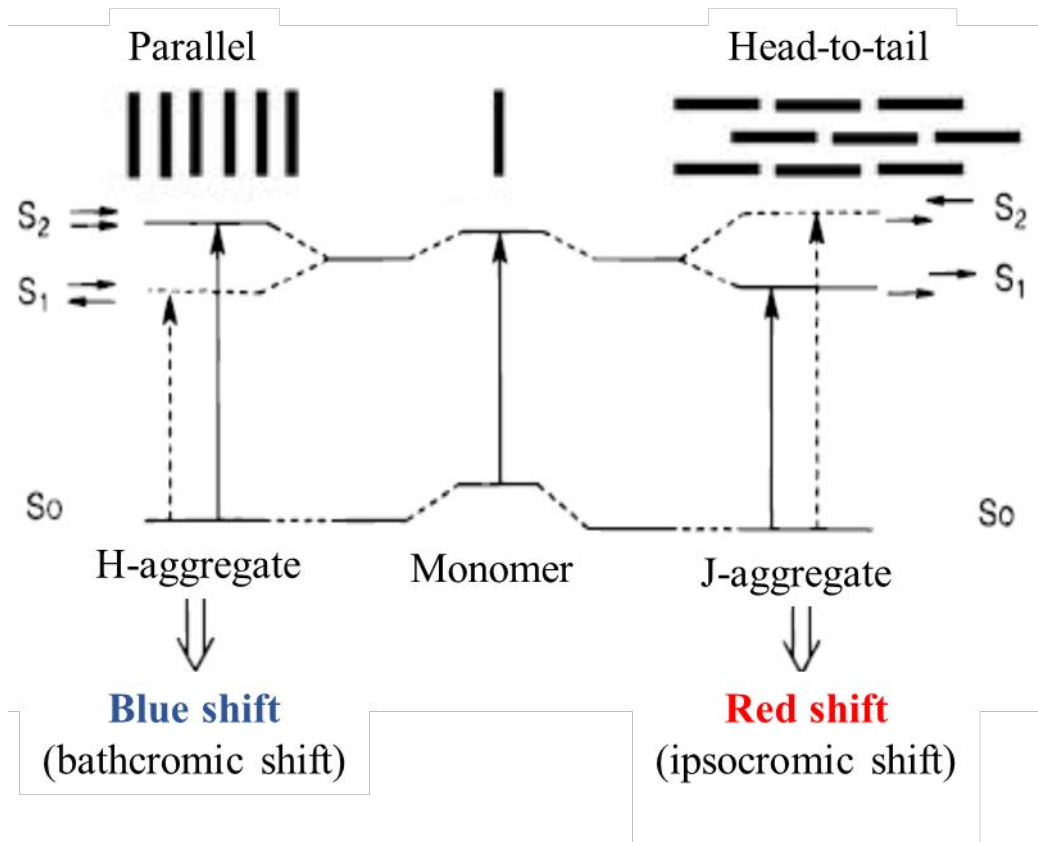


**DOLPHINS:** Can accumulate in tissues and be transferred to young.

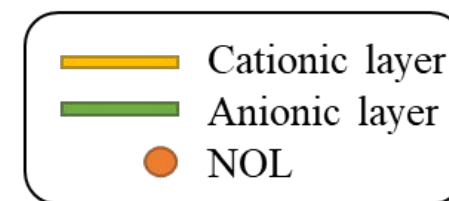
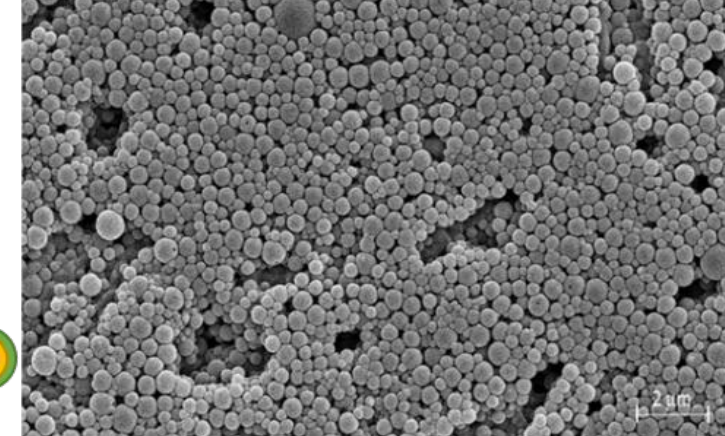
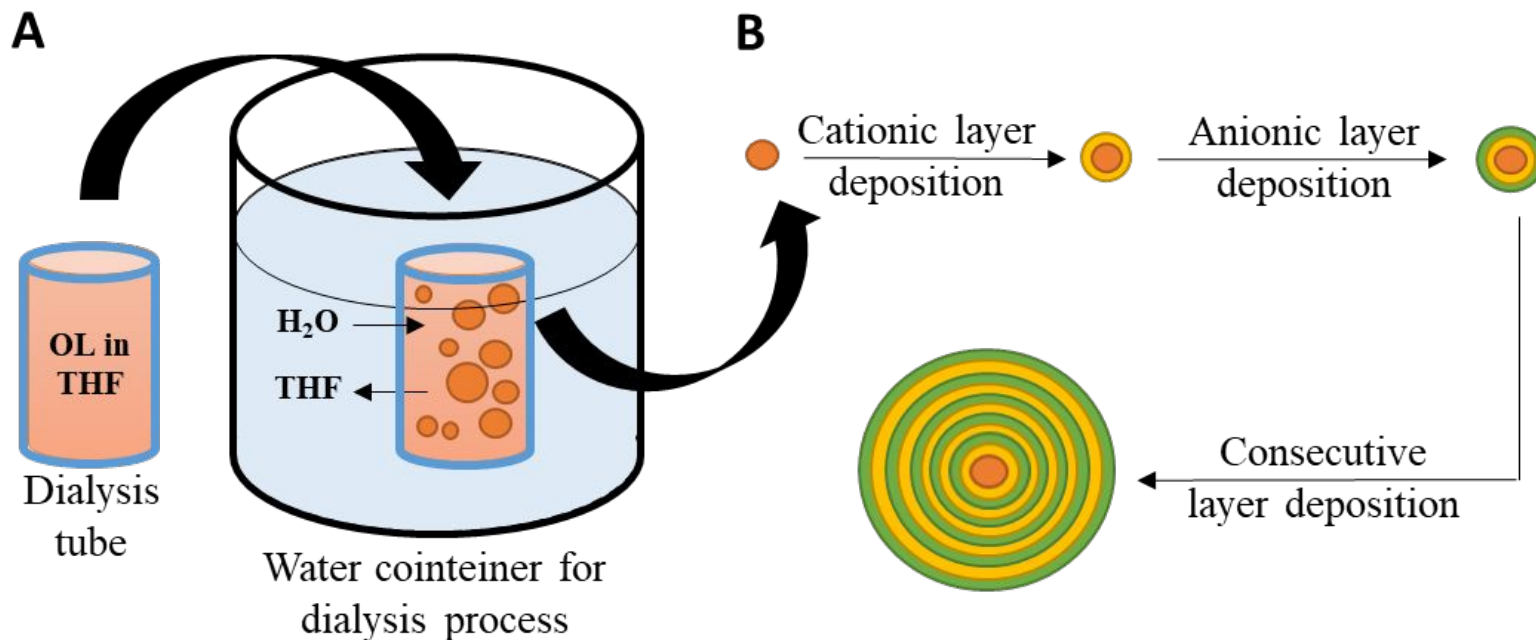




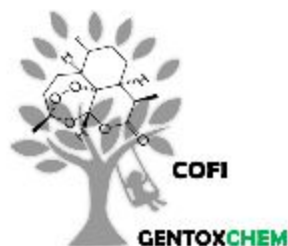
# Structural exciton theory







No. 102018000006058



“Micro- e nano- capsule o particelle a base di polifenoli e loro uso in campo cosmetico e come inchiostro”.

*Bio*MACROMOLECULES

Subscriber access provided by UNIV OF DURHAM

Article

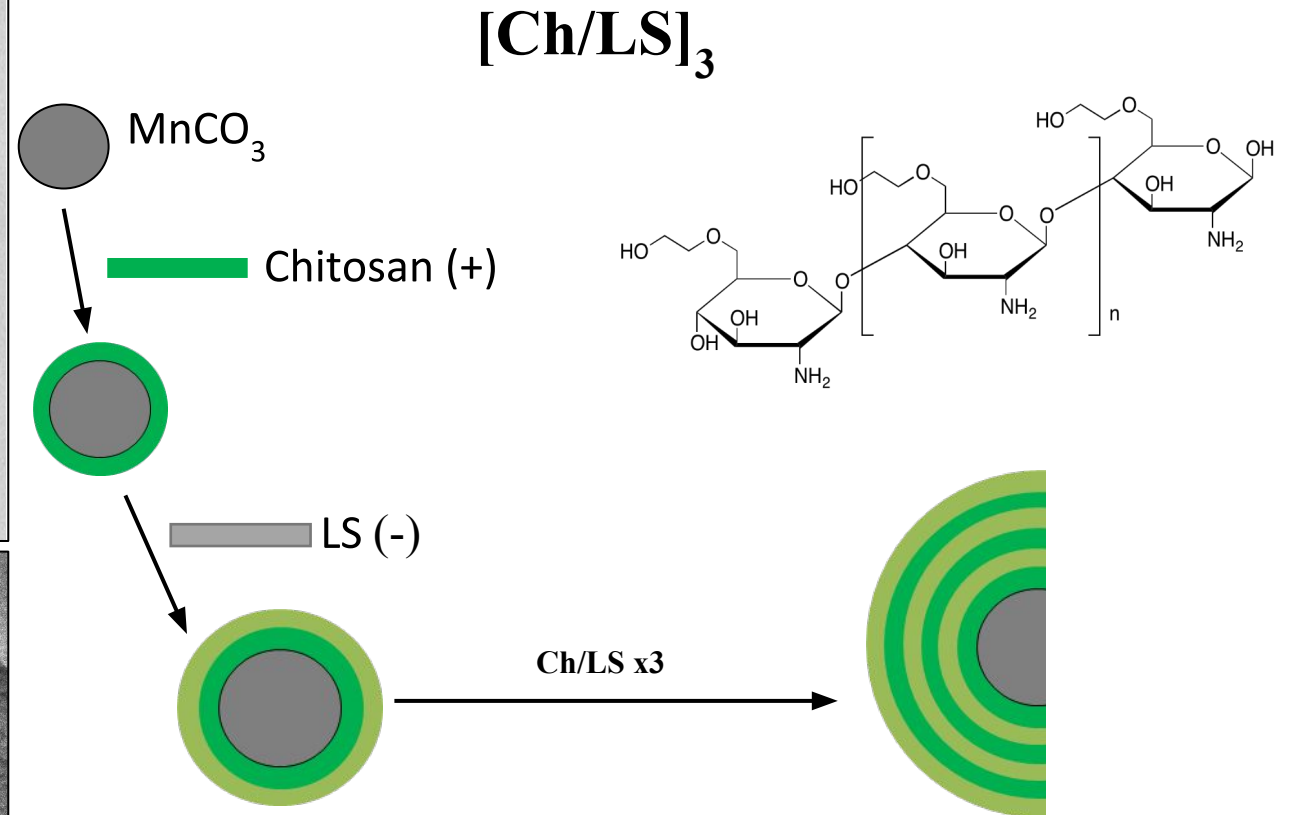
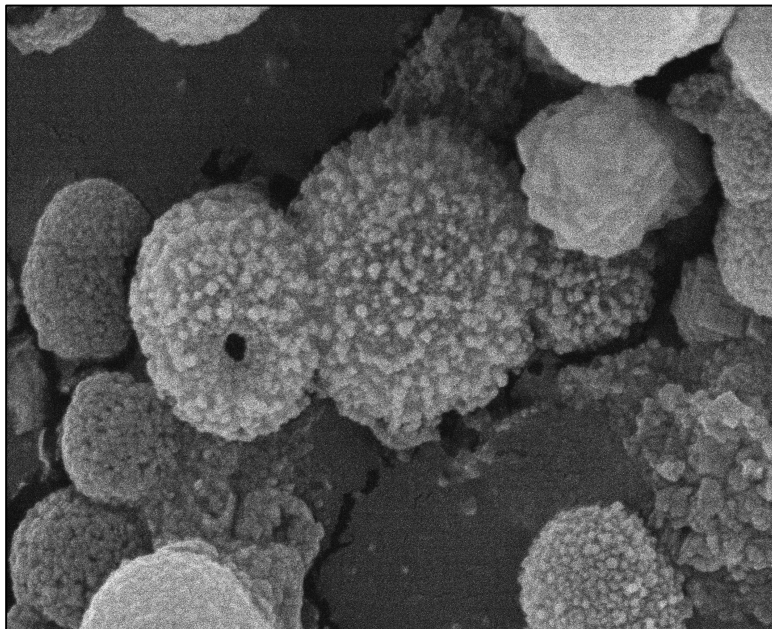
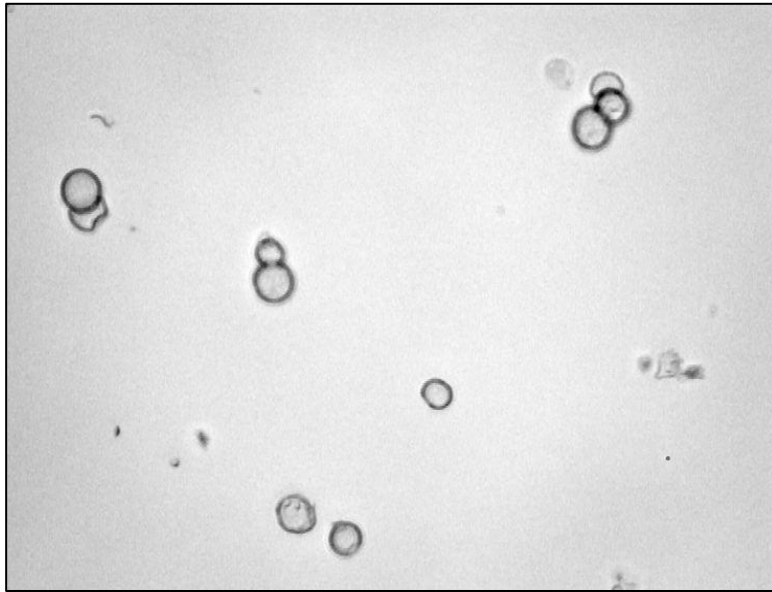
**Layer-by-layer preparation of microcapsules and nanocapsules of mixed polyphenols with high antioxidant and UV-shielding properties.**

Davide Piccinino, Eliana Capecchi, Lorenzo Botta, Bruno Mattia Bizzarri, Paolo Bollella, Riccarda Antiochia, and Raffaele Saladino

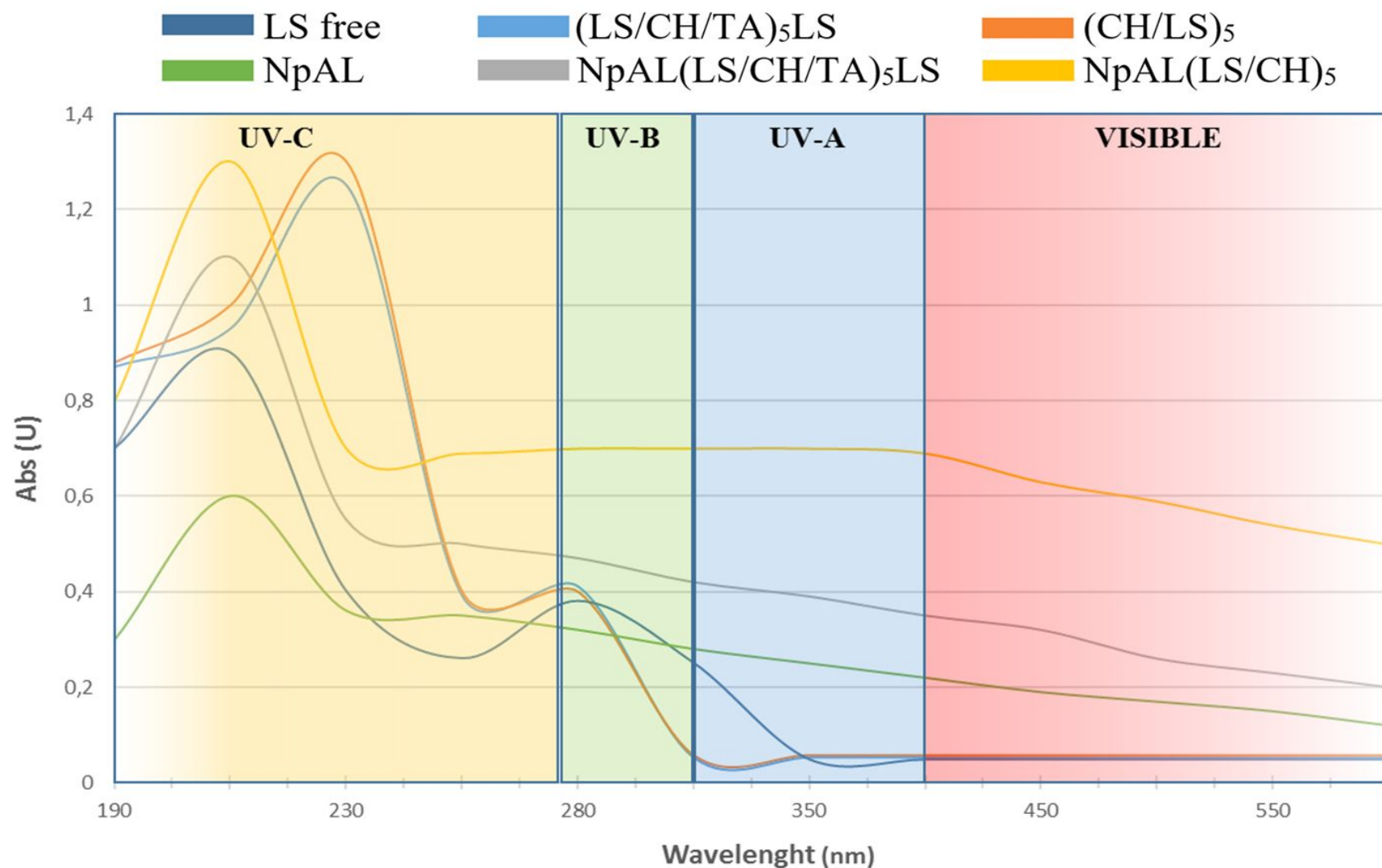
*Biomacromolecules*, Just Accepted Manuscript • DOI: 10.1021/acs.biomac.8b01006 • Publication Date (Web): 08 Aug 2018

Downloaded from <http://pubs.acs.org> on August 9, 2018

# Lignin nanocapsule obtained by LbL method

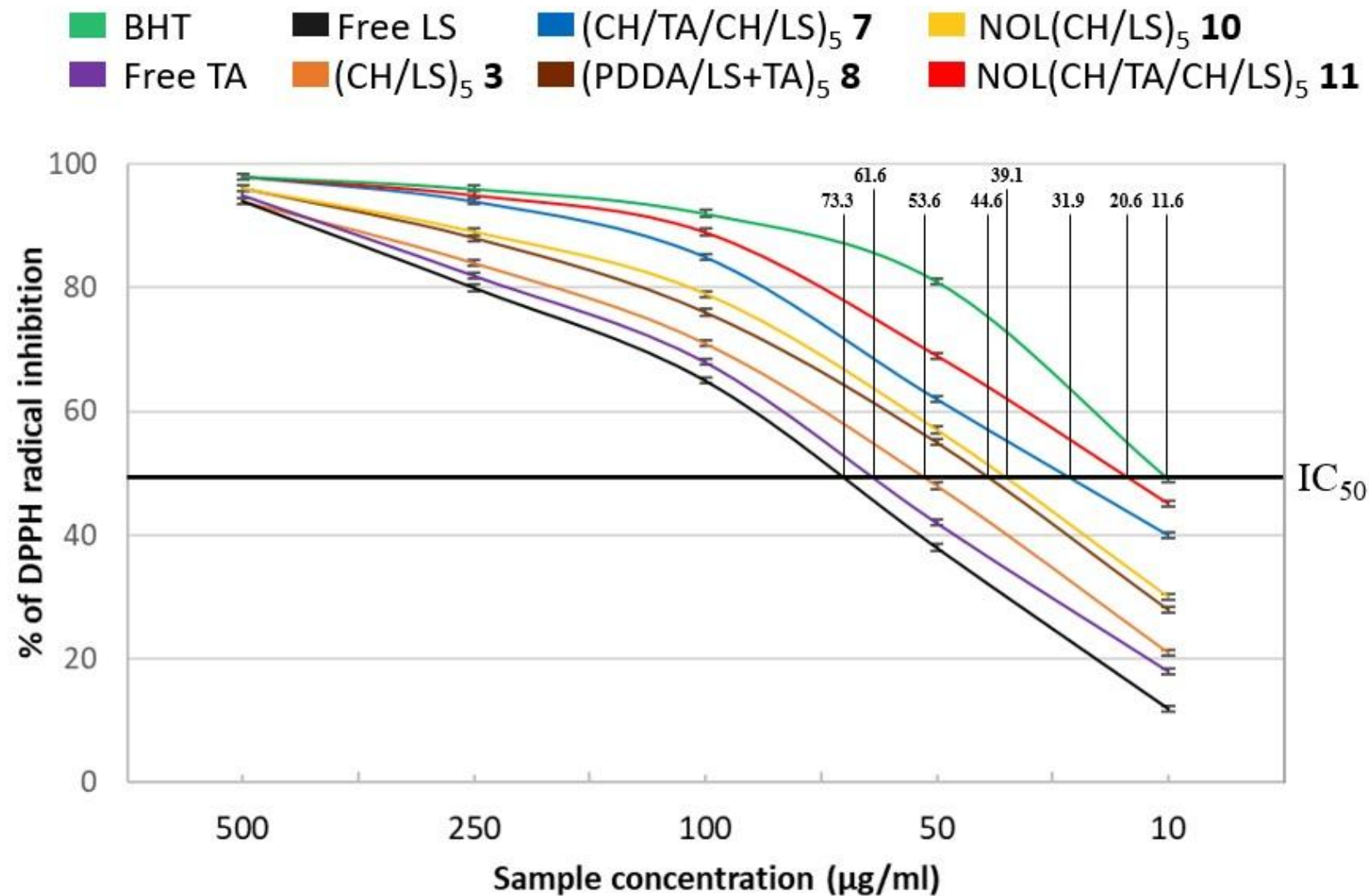


# POTERE ASSORBENTE UV-VISIBILE





# POTERE ANTI-RADICALE TRAMITE SAGGIO DPPH





UNIVERSITÀ  
PERUGIA



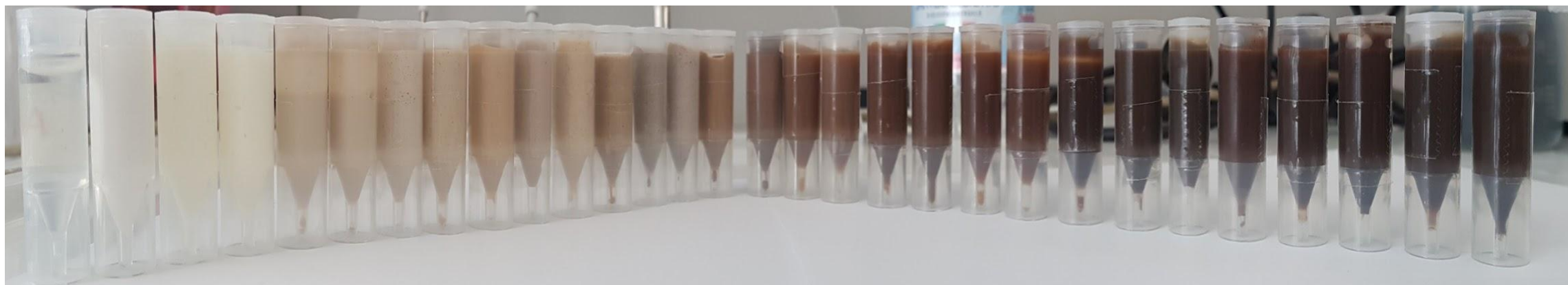
UNICO

TESTIMONIAL DAY

|    |              |             |       |                |             |                   |     |
|----|--------------|-------------|-------|----------------|-------------|-------------------|-----|
| 1  | campione A   | GEL BASE A  | g 100 | glicerina 5 g  | etanolo g 5 |                   | 110 |
| 2  | campione B   | IDI-OLS     | g 1   | glicerina 5 g  | etanolo g 5 | GEL BASE A g 100  | 116 |
| 3  | campione C   | IDI-OLSC    | g 1   | glicerina 5 g  | etanolo g 5 | GEL BASE A g 100  | 116 |
| 4  | campione D   | IDI-OLT     | g 1   | glicerina 5 g  | etanolo g 5 | GEL BASE A g 100  | 116 |
| 5  | campione E   | IDI-OLTC    | g 1   | glicerina 5 g  | etanolo g 5 | GEL BASE A g 100  | 116 |
|    |              |             |       |                |             |                   |     |
| 6  | campione 1   | IDI-OLS     | g 5   | glicerina 10 g | etanolo g 5 | GEL BASE A g 100  | 130 |
| 7  | campione 2   | IDI-OLSC    | g 5   | glicerina 10 g | etanolo g 5 | GEL BASE A g 100  | 130 |
| 8  | campione 3   | IDI-OLT     | g 5   | glicerina 10 g | etanolo g 5 | GEL BASE A g 100  | 130 |
| 9  | campione 4   | IDI-OLTC    | g 5   | glicerina 10 g | etanolo g 5 | GEL BASE A g 100  | 130 |
|    |              |             |       |                |             |                   |     |
| 10 | campione 1A  | GEL BASE 1A | g 10  | glicerina 5 g  | etanolo g 5 |                   | 110 |
| 11 | campione 1B  | IDI-OLS     | g 1   | glicerina 5 g  | etanolo g 5 | GEL BASE 1A g 100 | 116 |
| 12 | campione 1C  | IDI-OLSC    | g 1   | glicerina 5 g  | etanolo g 5 | GEL BASE 1A g 100 | 116 |
| 13 | campione 1D  | IDI-OLT     | g 1   | glicerina 5 g  | etanolo g 5 | GEL BASE 1A g 100 | 116 |
| 14 | campione 1E  | IDI-OLTC    | g 1   | glicerina 5 g  | etanolo g 5 | GEL BASE 1A g 100 | 116 |
|    |              |             |       |                |             |                   |     |
| 15 | campione 1-1 | IDI-OLS     | g 5   | glicerina 10 g | etanolo g 5 | GEL BASE 1A g 100 | 130 |
| 16 | campione 1-2 | IDI-OLSC    | g 5   | glicerina 10 g | etanolo g 5 | GEL BASE 1A g 100 | 130 |
| 17 | campione 1-3 | IDI-OLT     | g 5   | glicerina 10 g | etanolo g 5 | GEL BASE 1A g 100 | 130 |
| 18 | campione 1-4 | IDI-OLTC    | g 5   | glicerina 10 g | etanolo g 5 | GEL BASE 1A g 100 | 130 |



Calculation of sun protection factor (SPF)





TESTIMONIAL DAY



+



4%



SPF  
12

UVA/UVB unique filter !



+



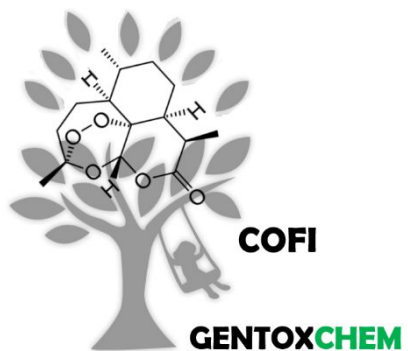
2%



SPF  
52

Booster effect at low concentration!





Bio-based packaging  
products that are  
biodegradable/compostable  
and/or recyclable



Proposal number: 863697

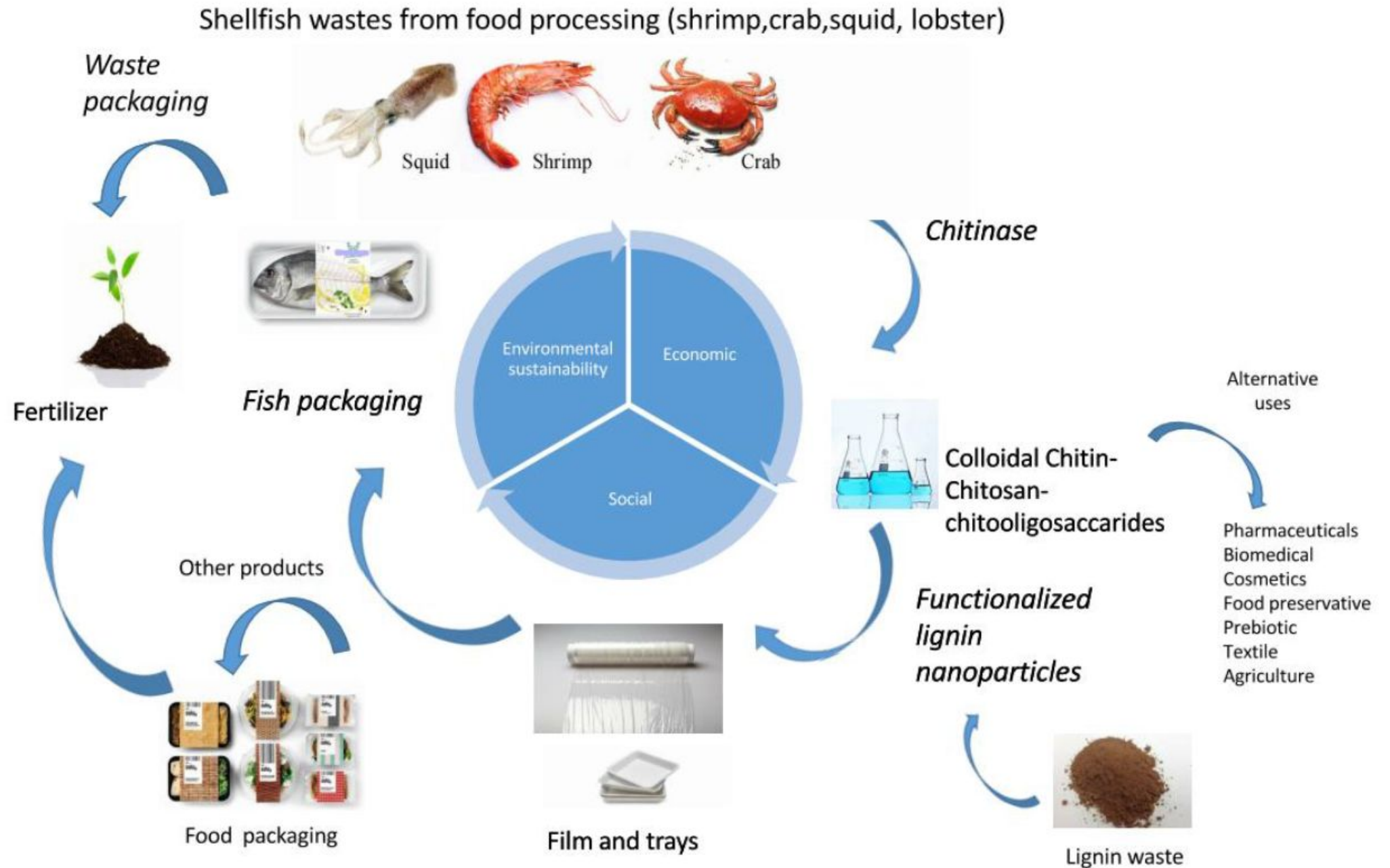
Proposal acronym: FISH4FISH



Il progetto europeo “**FISH4FISH**” integra competenze in ambito chimico ed industriale per la realizzazione di un innovativo blend realizzato con bioplastiche e **lignina**, un componente del legno non tossico a basso costo ed ecocompatibile, in grado di aumentare le proprietà reologiche e chimico fisiche delle bioplastiche attualmente in commercio.

L'obiettivo del progetto è quello di ottenere un materiale attivo a basso costo da sfruttare per le applicazioni industriali di Imballaggi alimentari.

The FISH4FISH concept is reported in the following scheme:







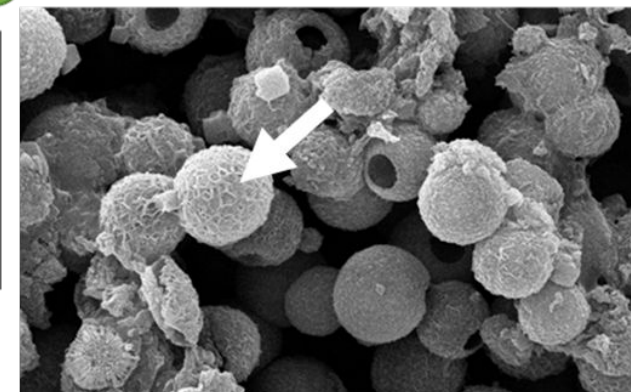
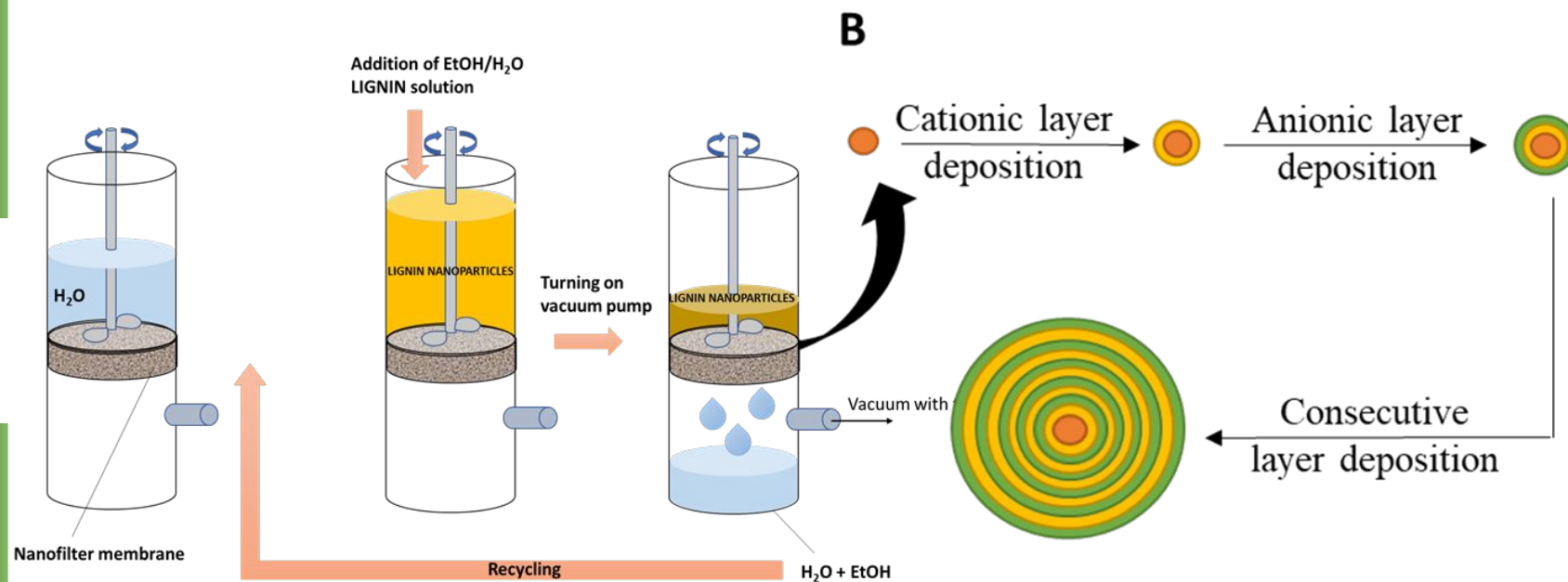
UNIVERSITÀ  
di Siena



FISH  
4  
FISH



NEXT  
TECHNOLOGY



No. 102018000008001  
submitted

Preparazione di nanoparticelle e sistemi colloidali per la  
produzione di bioplastiche

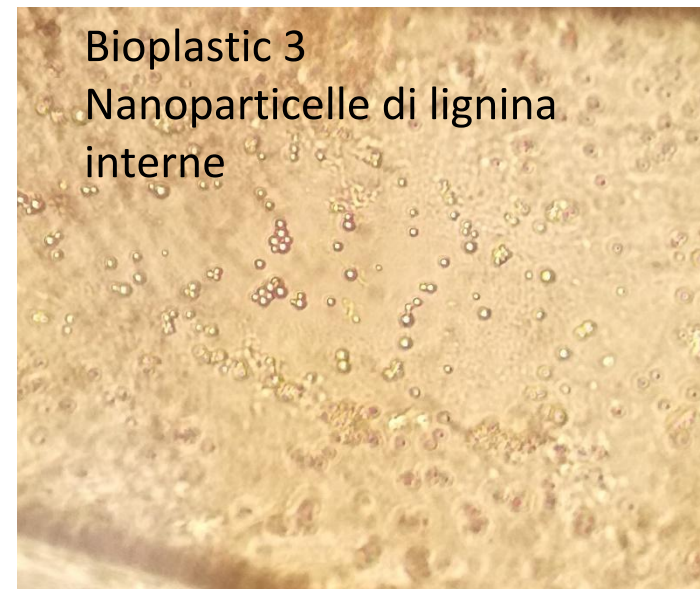
Bioplastic 3 Livello  
Macroscopico



Bioplastic 3  
Livello  
Microscopico



Bioplastic 3  
Nanoparticelle di lignina  
interne





HEALTH & FOOD / PROJECT

# EU-IBISBA

**European Industrial Biotechnology Innovation and Synthetic Biology Accelerator**

An accelerator for research and innovation in Industrial Biotechnology and Synthetic Biology



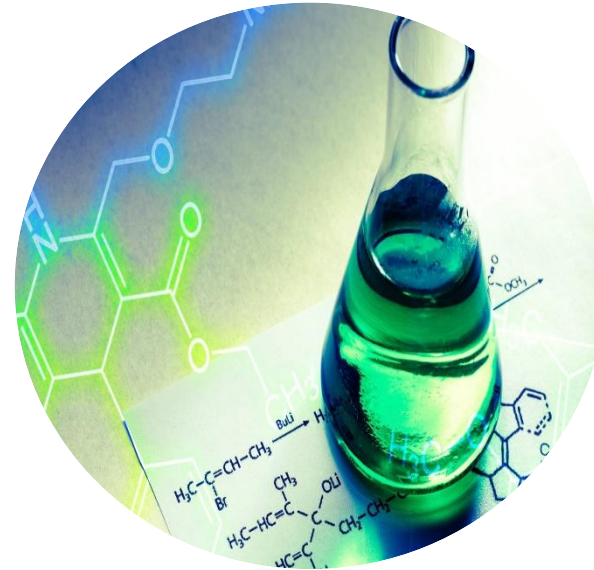
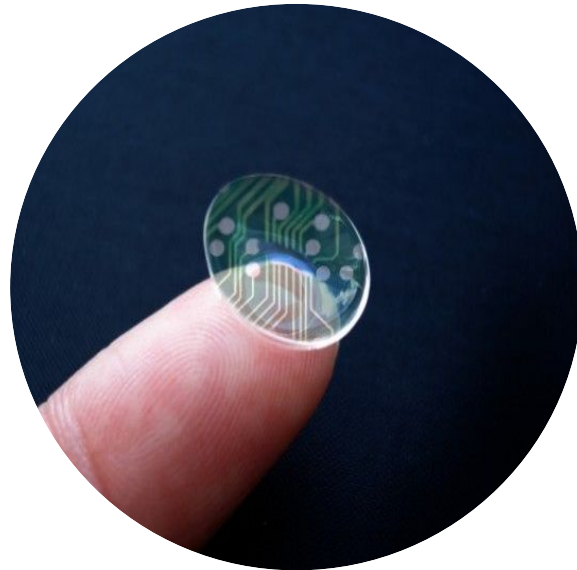
**IBISBA<sup>1.0</sup>**





UNIVERSITÀ  
DEGLI STUDI DELLA  
**Tuscia**

# Design of bioinks



**PhD Student Eliana Capecchi**

*Mail: [e.capecchi@unitus.it](mailto:e.capecchi@unitus.it)*

# INKs GLOBAL ISSUES

## Health, Environmental and Regulamentation Concerns



ABSTRACT

ORIGINAL RESEARCH

### TATTOOS: What Do People Really Know About the Medical Risks of Body Ink?

by ILIANA A. RAHIMI, BSc; IGOR EBERHARD, PhD; and ERICH KASTEN, PhD  
Mrs. Rahimi and Professor Kasten are with the Medical School Hamburg in Hamburg, Germany. Dr. Eberhard is with the Department of Social and Cultural Anthropology at the University of Vienna in Vienna, Austria.

J Clin Aesthet Dermatol. 2018;11(3):30–35

21%

Malignant melanoma

HEALTH  
RISK

- Use of toxic phenol-formaldehyde resins
- Can contain PAH (*polycyclic aromatic hydrocarbons*)
- Photosensitivity
- Enhanced psoriasis and autoimmune problems

## INK'S ENVIRONMENTAL FOOTPRINT



Decomposition time  
450 to 1000 Years

- VOC (Volatile Organic Compounds)
- Energy demanding

## Regulamentation



# ABOUT INKS

## How ink is made

Physical and stability Properties

Specific guidelines for the inks

Complex Composition

Chemistry Formulation

Viscosity  
Surface Tension  
Light Fastness



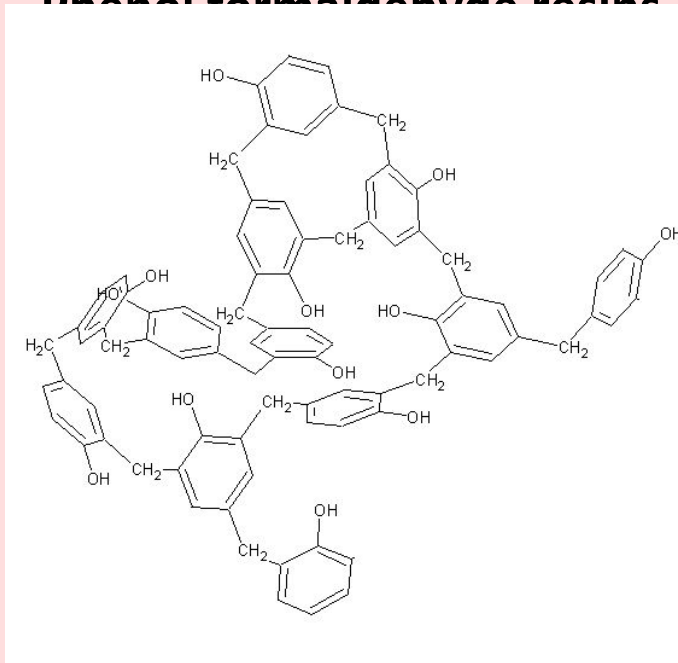
Additive 0.1-10%  
Vehicle or Binders 6-7%  
Pigments 33-34%  
Solvent 60%



# ECO FRIENDLY BINDER

- Synthetic polymers obtained by the reaction of phenol or substituted phenol with formaldehyde.
- Carcinogenic formaldehyde (Toxicity) High Environmental impact for air pollutants emitted

## Phenol formaldehyde resins

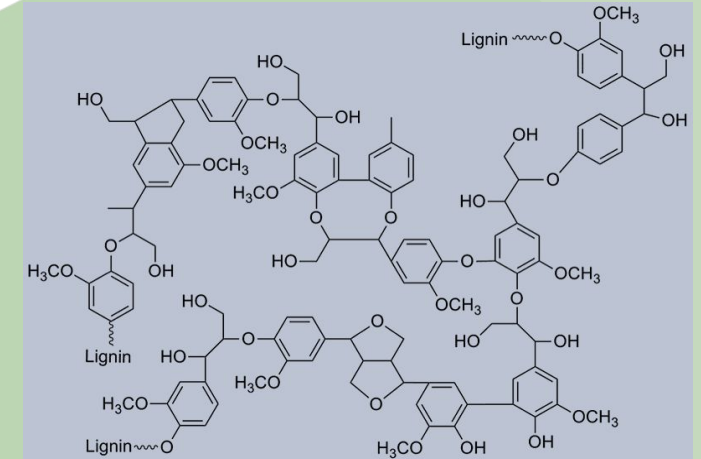


V

S



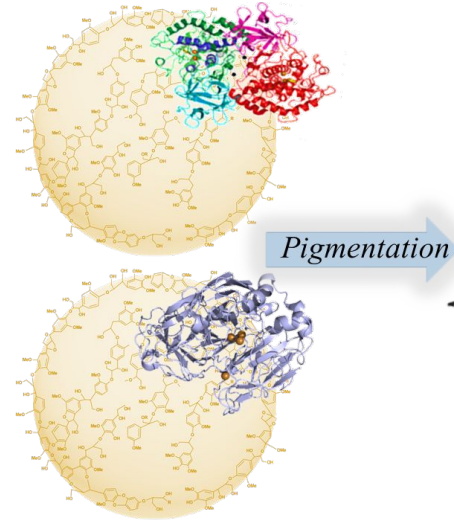
## Lignin Based Binders



- The actual structure of the lignin molecule it is similar of a fully set formaldehyde polymer.
- Lignin based binders allow for raw materials cost savings
- Lignin as a raw material is non toxic and easy to handle.
- improving the viscosity of the medium

# Our technology for an innovative Bioink

## Lignin Nanoparticles with Biocatalytic Functionalization



Cite This: *Biomacromolecules* XXXX, XXX, XXX–XXX

Article

[pubs.acs.org/Biomac](https://pubs.acs.org/Biomac)

## Enzyme-Lignin Nanocapsules Are Sustainable Catalysts and Vehicles for the Preparation of Unique Polyvalent Bioinks

Eliana Capecchi,<sup>†</sup> Davide Piccinino,<sup>†</sup> Bruno Mattia Bizzarri,<sup>‡</sup> Daniele Avitabile,<sup>‡</sup> Claudia Pelosi,<sup>§</sup> Claudia Colantonio,<sup>§</sup> Giuseppe Calabrò,<sup>§</sup> and Raffaele Saladino<sup>\*,†</sup>

<sup>†</sup>Department of Biological and Ecological Sciences (DEB), Tuscia University, Via S. Camillo de Lellis snc, 01100 Viterbo, Italy

<sup>‡</sup>IDI Farmaceutici srl, Via dei Castelli Romani 83/85, Pomezia, 00040 Rome, Italy

<sup>§</sup>Department of Economics, Engineering, Society and Business Organization (DEIM), Tuscia University, 01100 Viterbo, Italy



No. 102018000006058

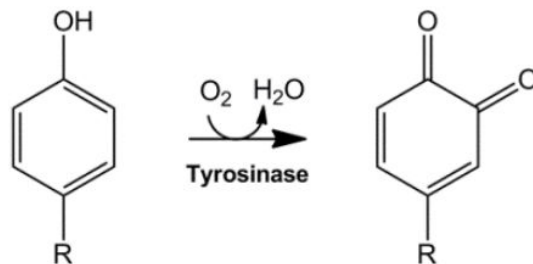
“Micro- e nano- capsule o particelle a base di polifenoli e loro uso in campo cosmetico e come inchiostro”.

# THE BASES OF OUR TECHNOLOGY

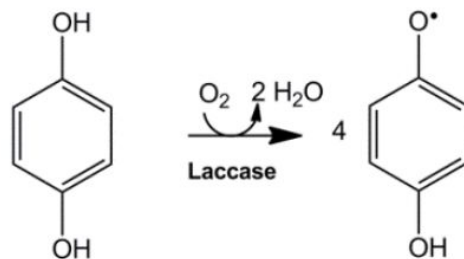
## Biocatalytic Component

(From Mushroom)

### TYROSINASE (EC 1.14.18.1)



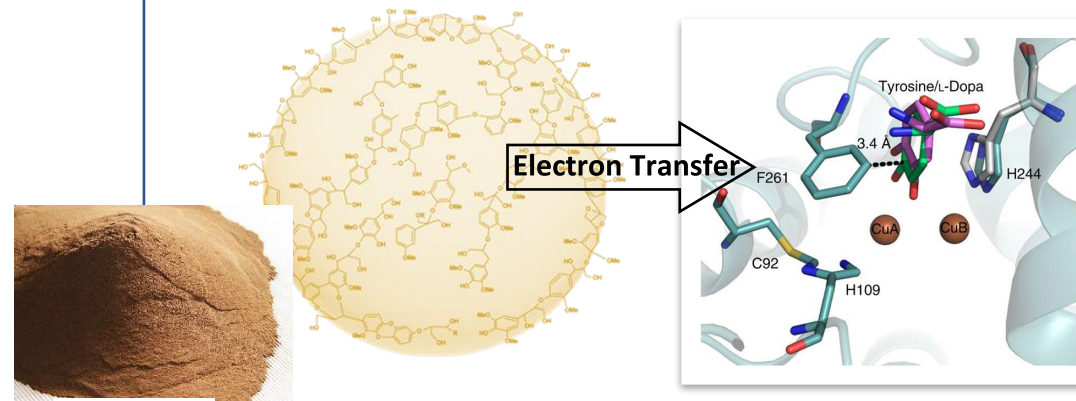
### LACCASE (EC 1.10.3.2)



FUNGI  
EXTRACTION

## Electro Active support For Enzymatic Immobilization

### LIGNIN NANOPARTICLES



LIGNIN



Article

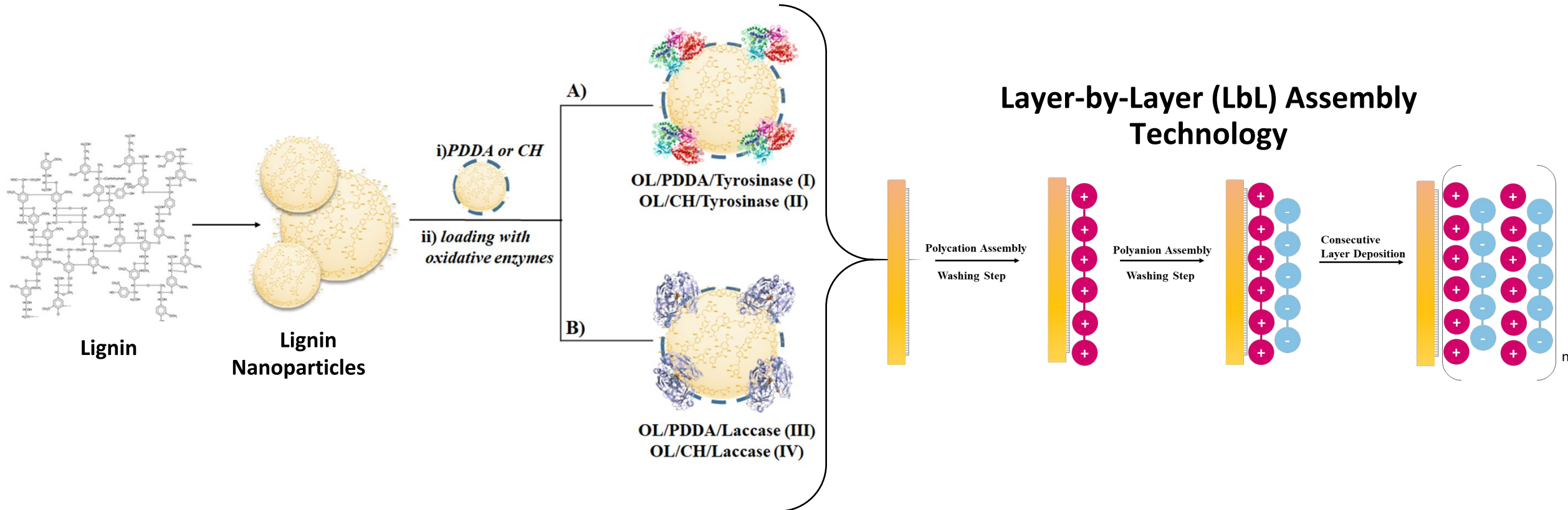
## Functionalized Tyrosinase-Lignin Nanoparticles as Sustainable Catalysts for the Oxidation of Phenols

Eliana Capecchi <sup>1</sup>, Davide Piccinino <sup>1</sup>, Ines Delfino <sup>1</sup>, Paolo Bollella <sup>2</sup>, Riccarda Antiochia <sup>2</sup>   
and Raffaele Saladino <sup>1,\*</sup>



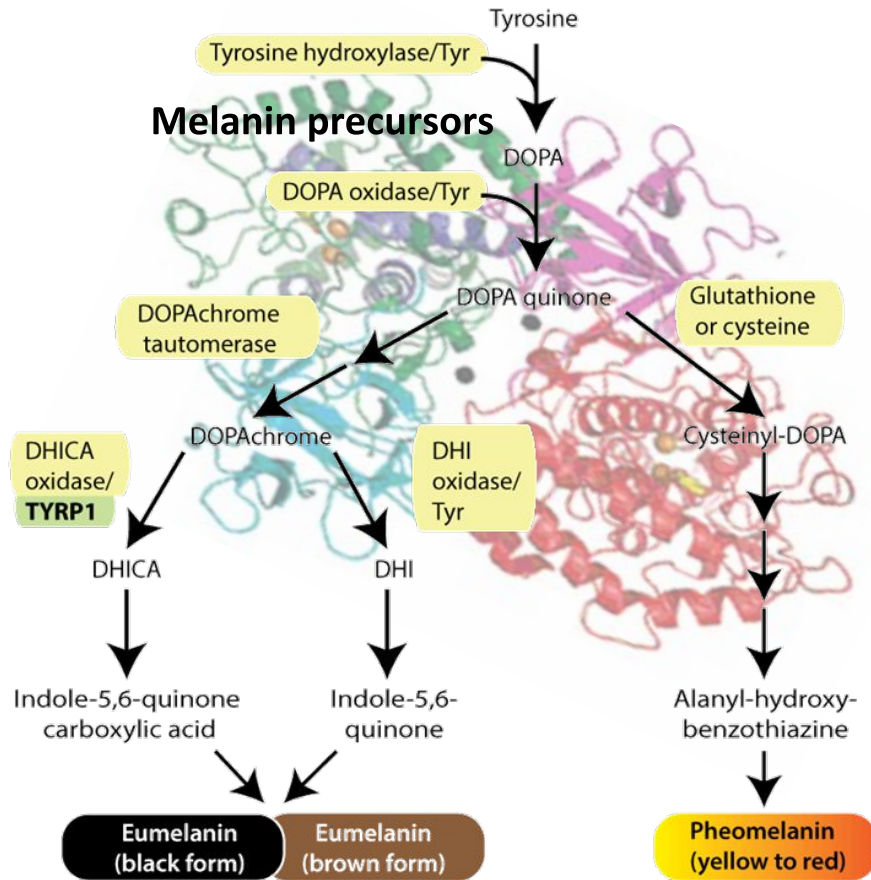
# BIOTECHNOLOGY APPROACH

## Layer by Layer Assembly Technology for the enzymatic functionalization of Lignin nanoparticles

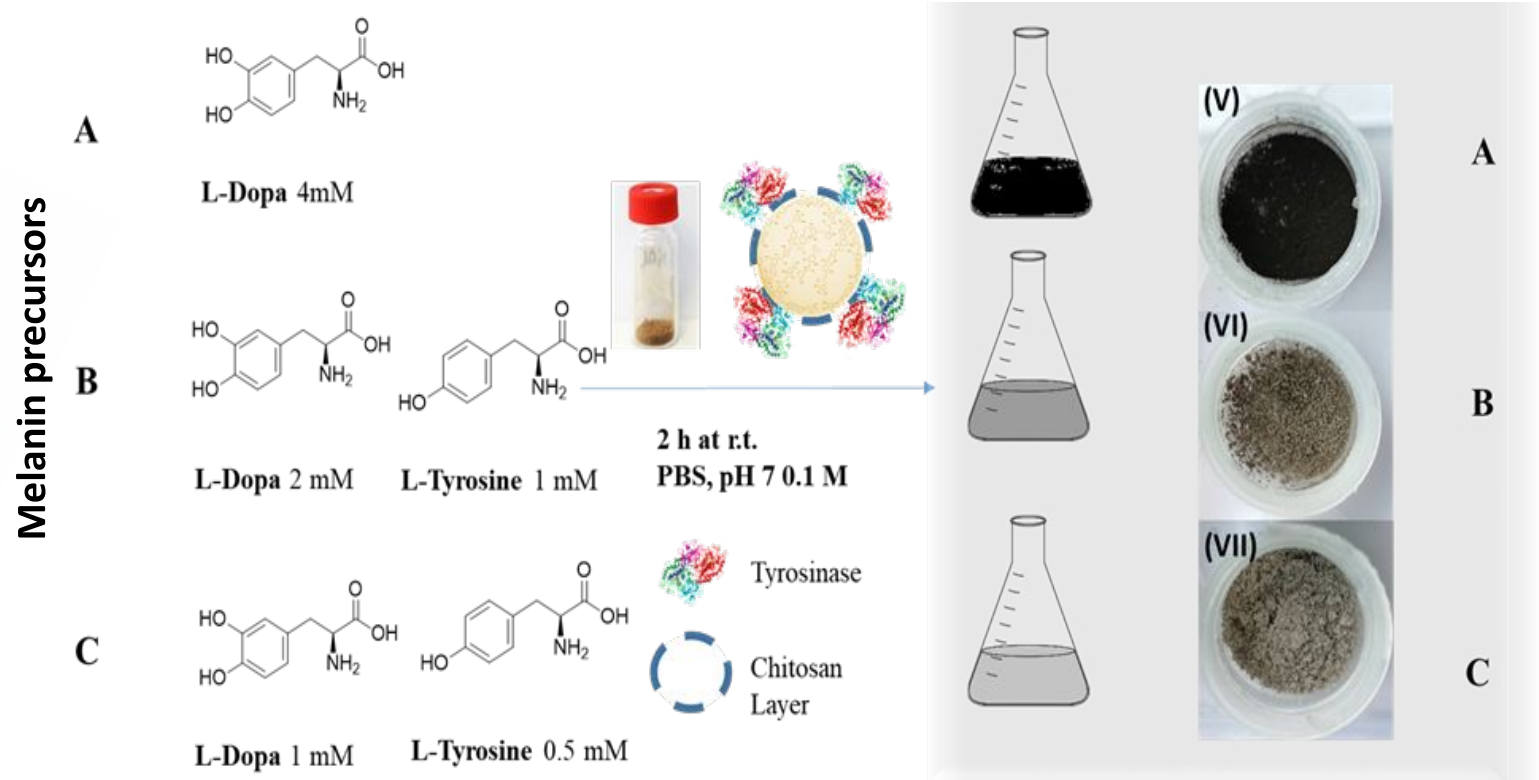


# BIOINKS PRODUCTION

## Tyrosinase in melanin pathway

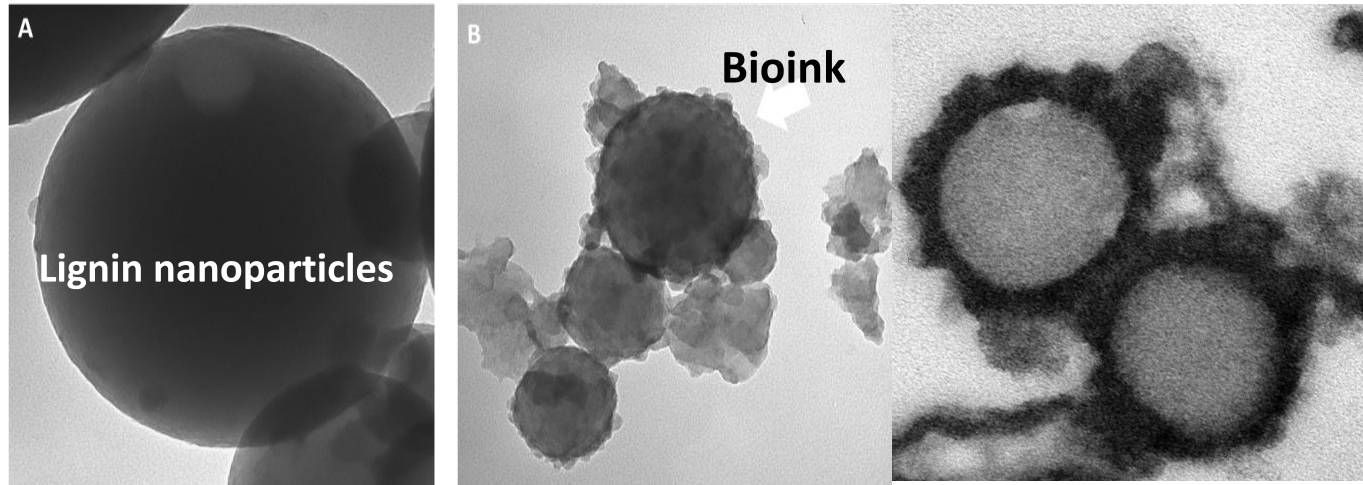


## Immobilized Tyrosinase in Bioink Technology

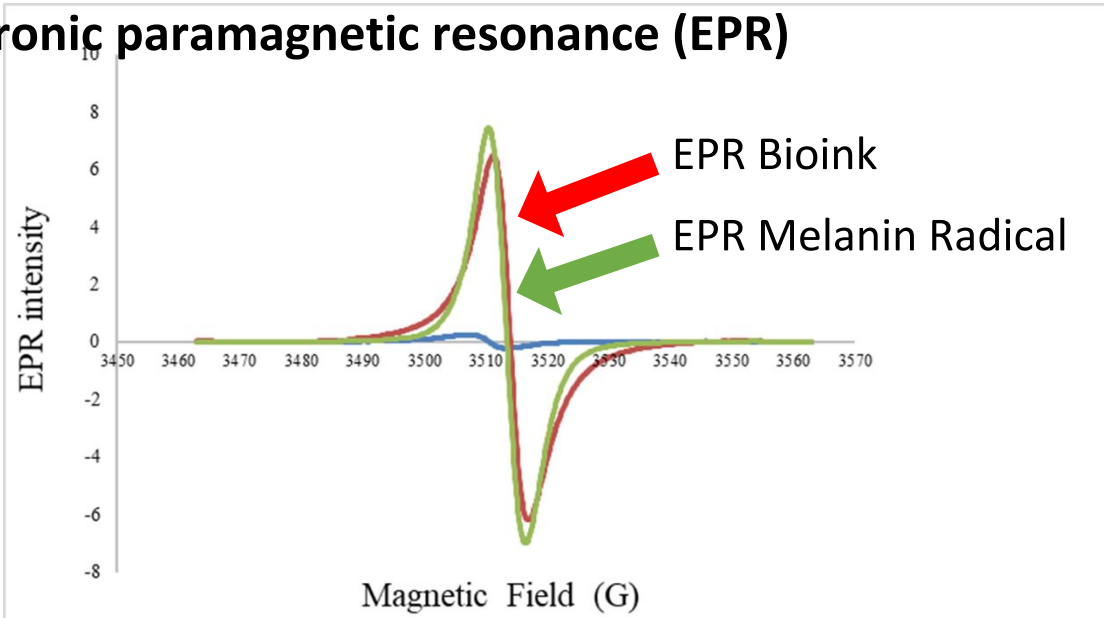


# BIOINKS CHARACTERIZATION

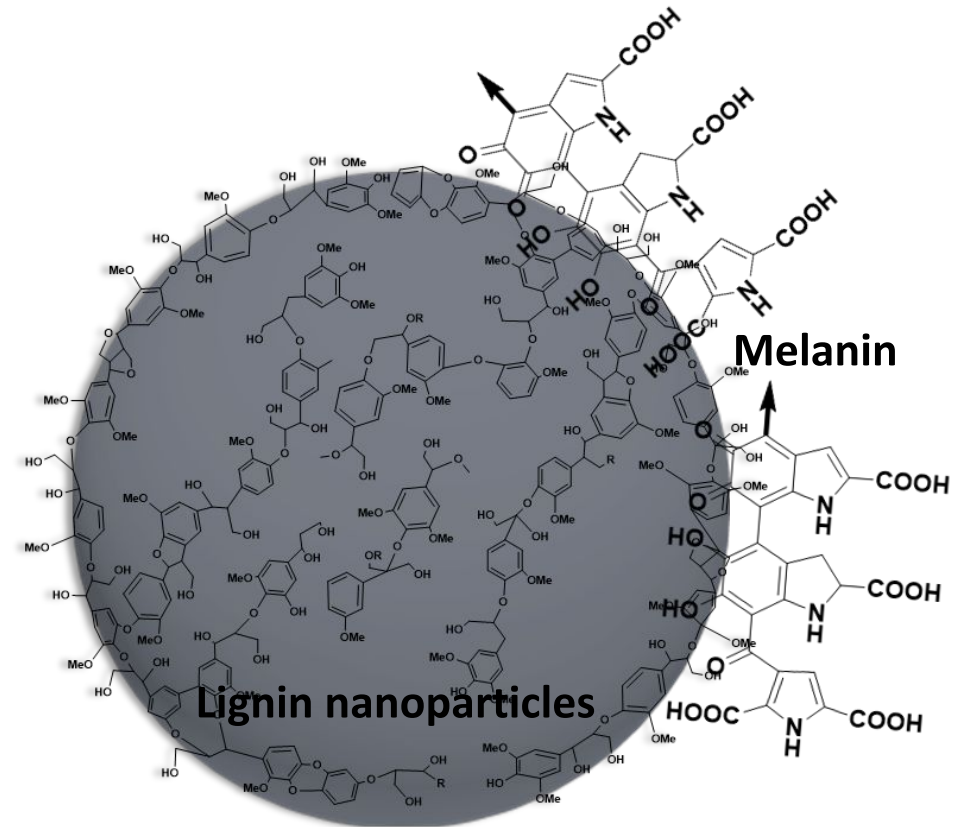
## Transmission Electron Microscopy (TEM)



## Electronic paramagnetic resonance (EPR)



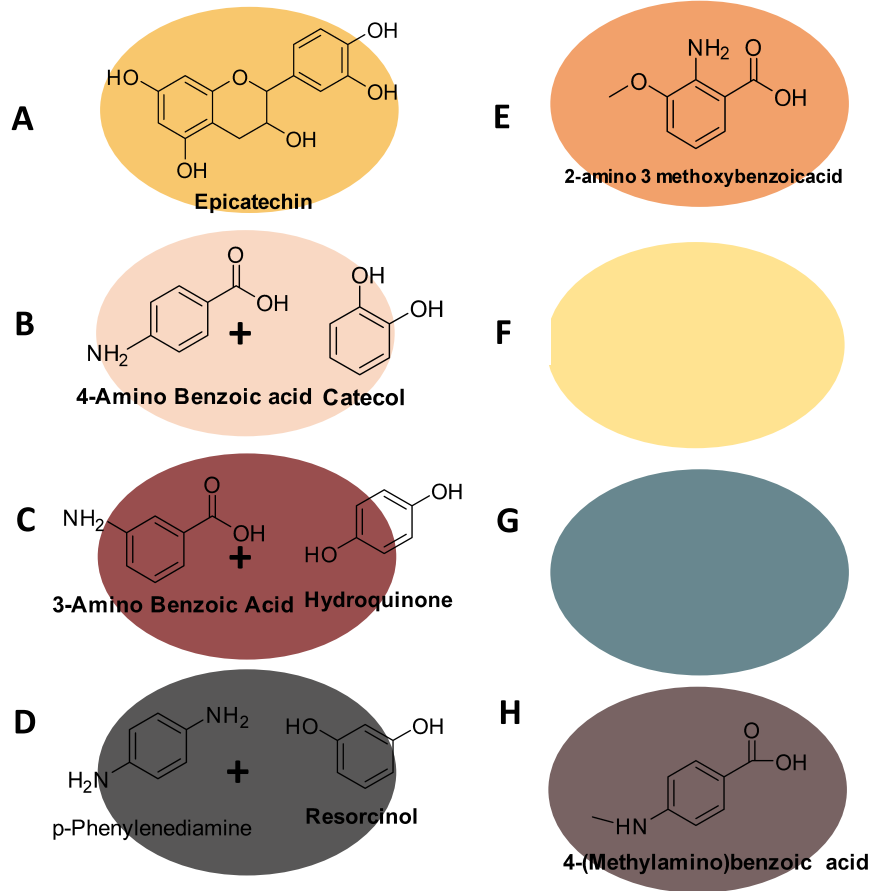
## BioInk Chemical Structure





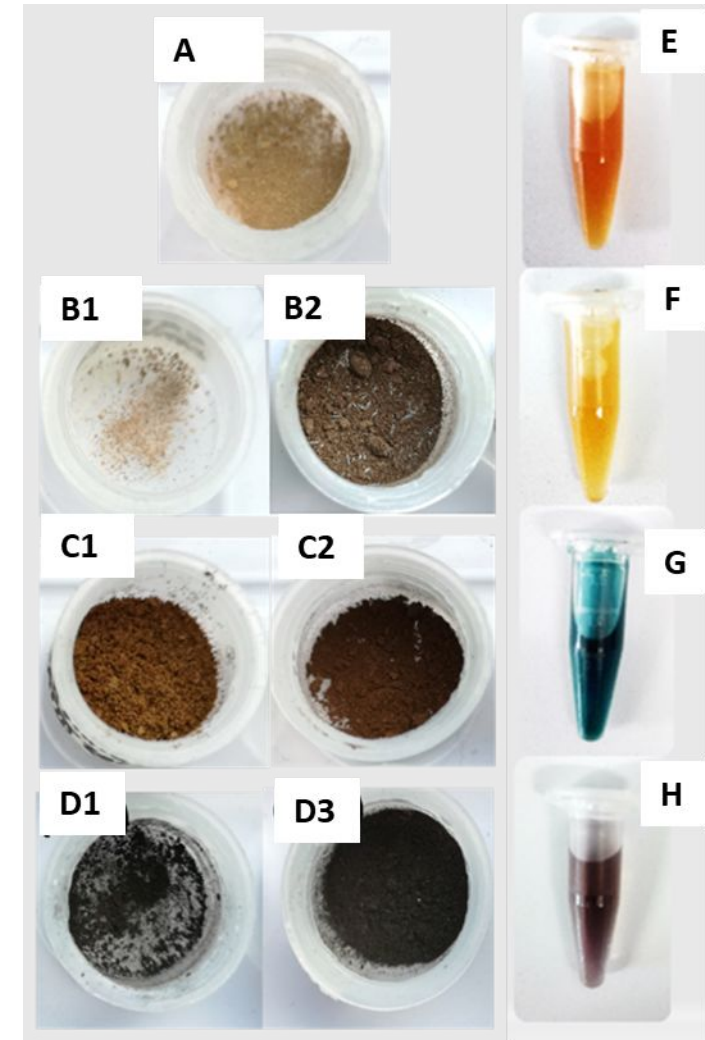
# BIOINKS PRODUCTION

## Laccase Chromogenic Substrates



rt.  
NaAcetate, pH 5 0.1 M, EtOH 2%

## Immobilized Laccase in Bioink Technology



# PHYSICAL PROPERTIES OF BIOINKS

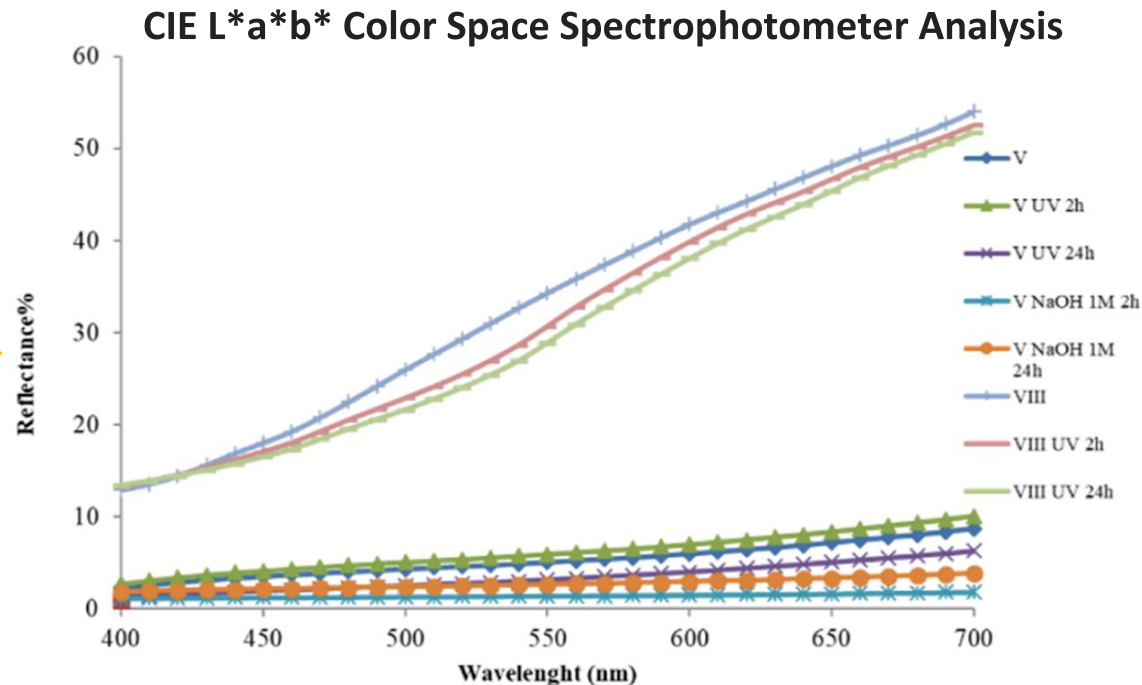
## LIGHT FASTNESS STABILITY TEST

Color strength and light fastness are important physical properties for the quality, weather resistance, and applications of the inks. Alkaline medium and artificial and natural light may cause most pigments to degrade resulting in color change.



Bio-Ink

-Alkaline medium  
-Artificial light



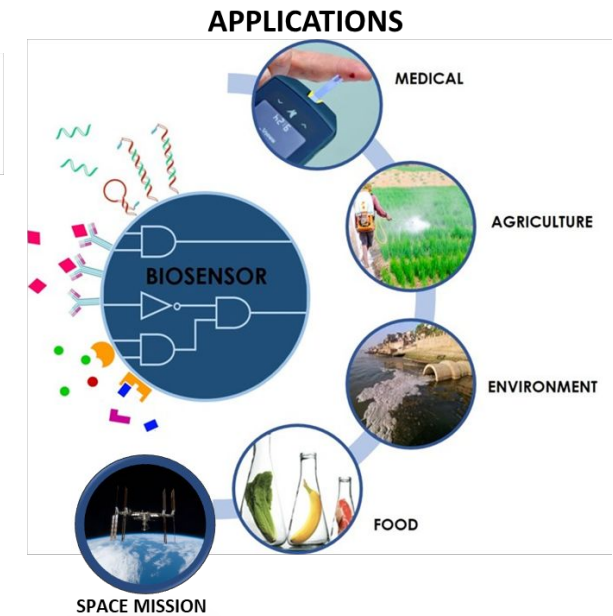
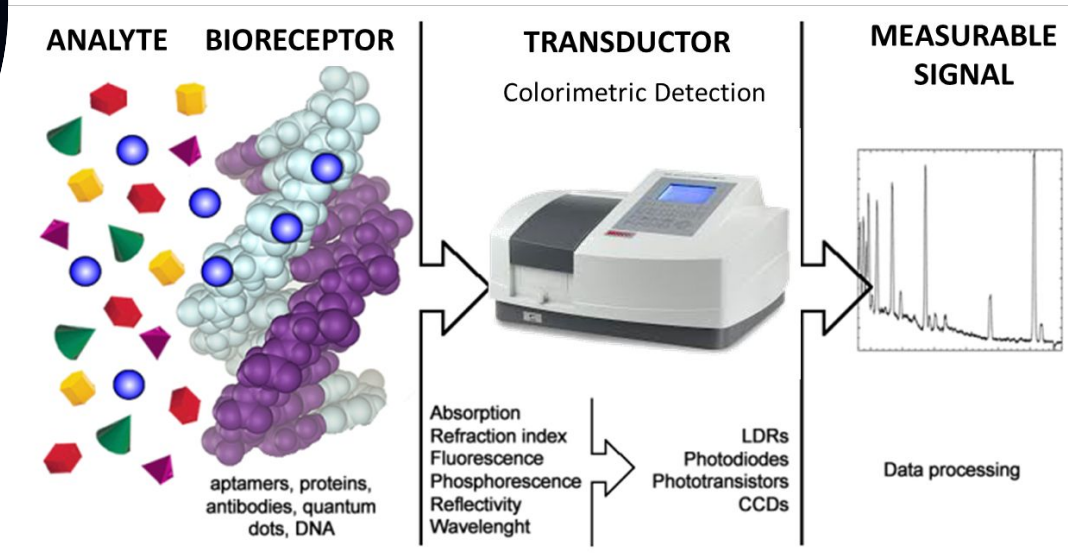
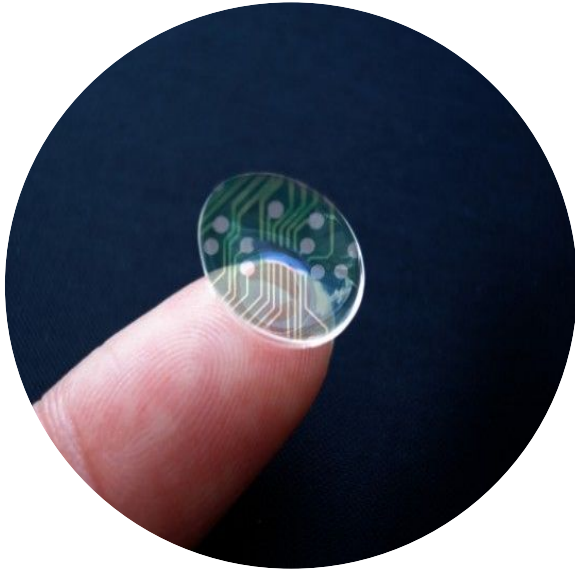
### Results

- 1.No Colour Changes
- 2.Pigments not degraded
- 3.Bio-Ink stable



UNIVERSITÀ  
DEGLI STUDI DELLA  
**Tuscia**

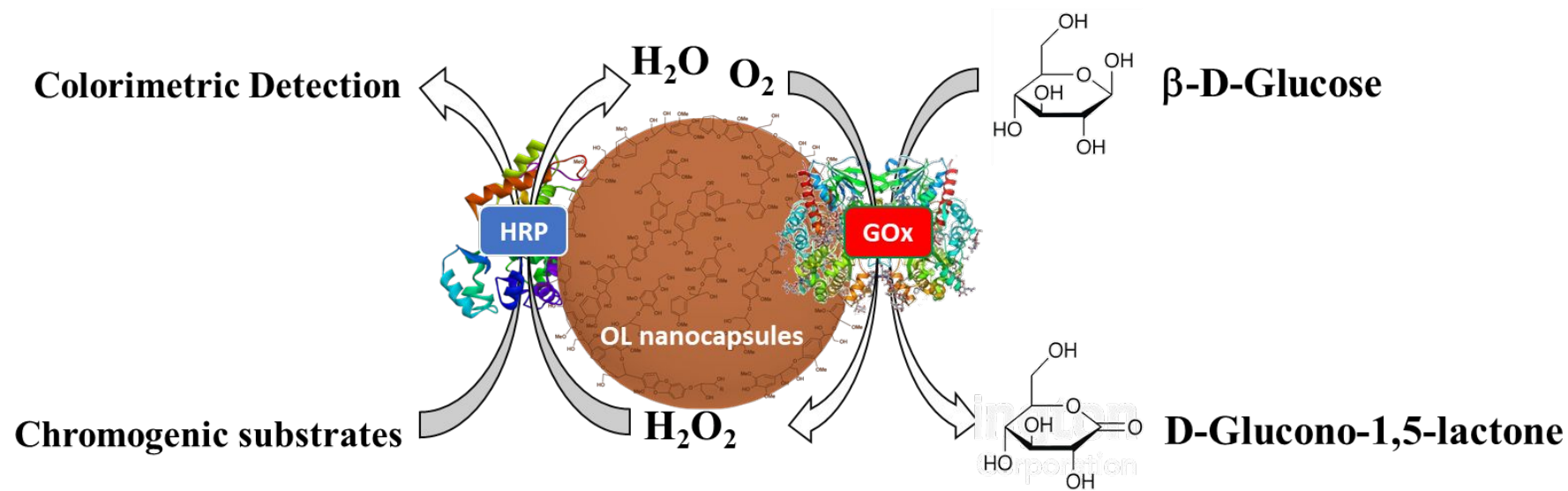
# Design of colorimetric biosensors



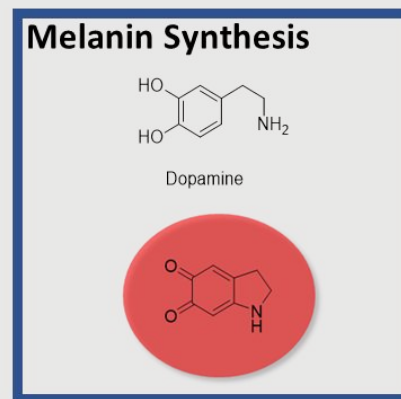
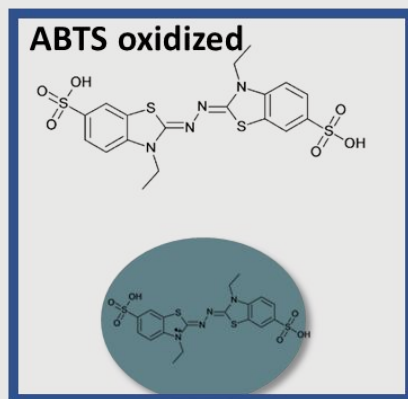


# Design of colorimetric biosensors

A novel Biosensors that combining colorimetric and Electrochemical detection

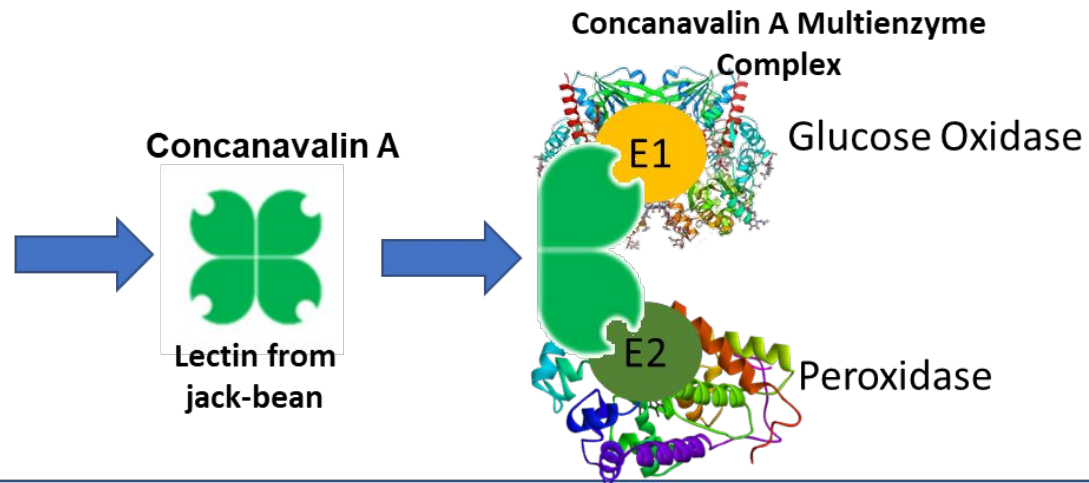


## Chromogenic Substrates

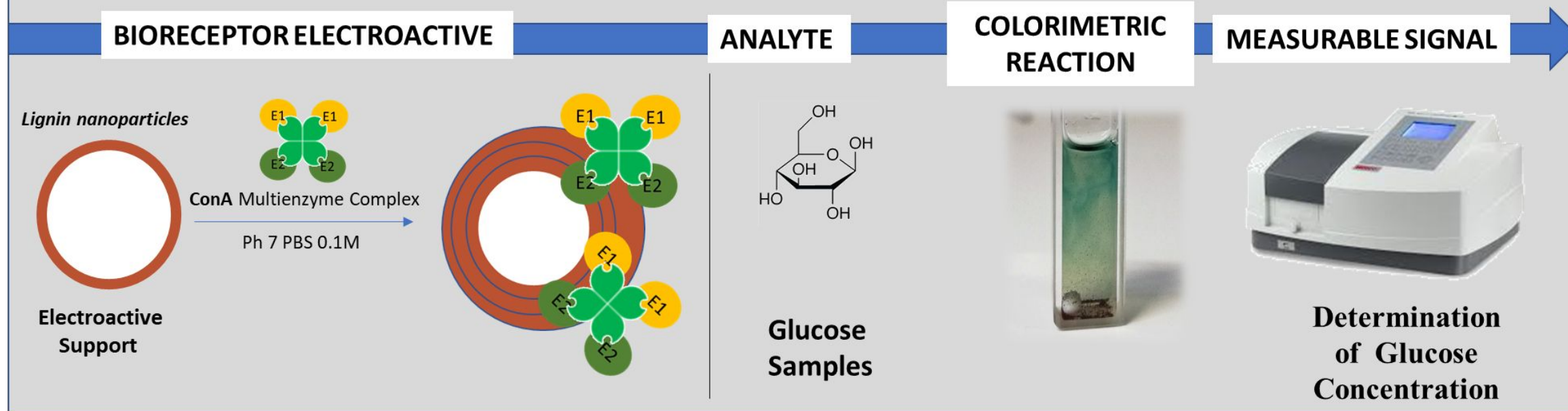


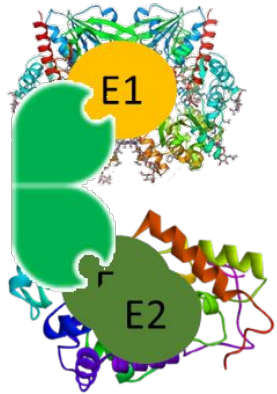
# Design of colorimetric biosensors

## BIOTECHNOLOGY STRATEGY

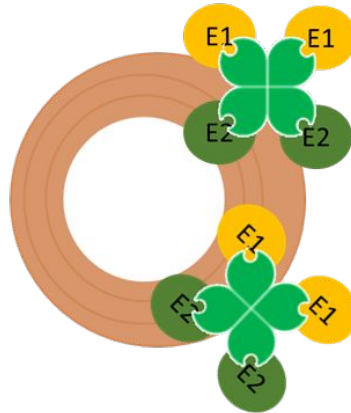


## DESIGN OF GLUCOSE BIOSENSORS BASED ON RAW MATERIAL SUPPORT

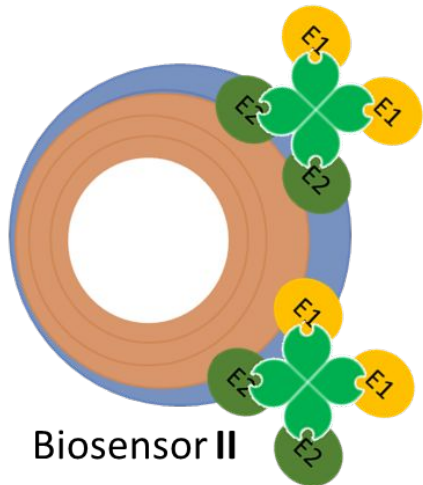




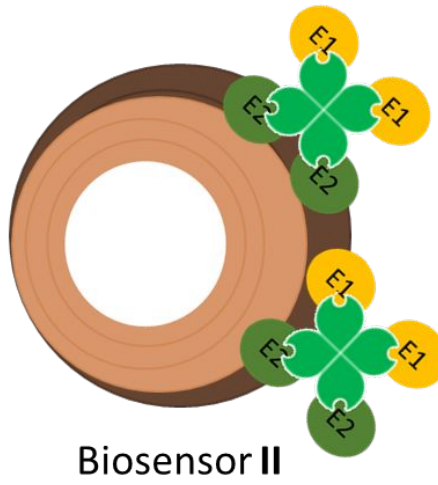
GOx-ConA-HRP Aggregate



Biosensor I



Biosensor II

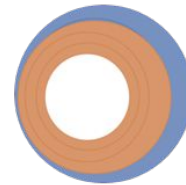


Biosensor II

## Biodegradable Support



Lignin Nanoparticles



Lignin Nanoparticles/Chitosan



Lignin Nanoparticles/CATLIG



Concanavalin A (ConA)



Glucose Oxidase (GoX)

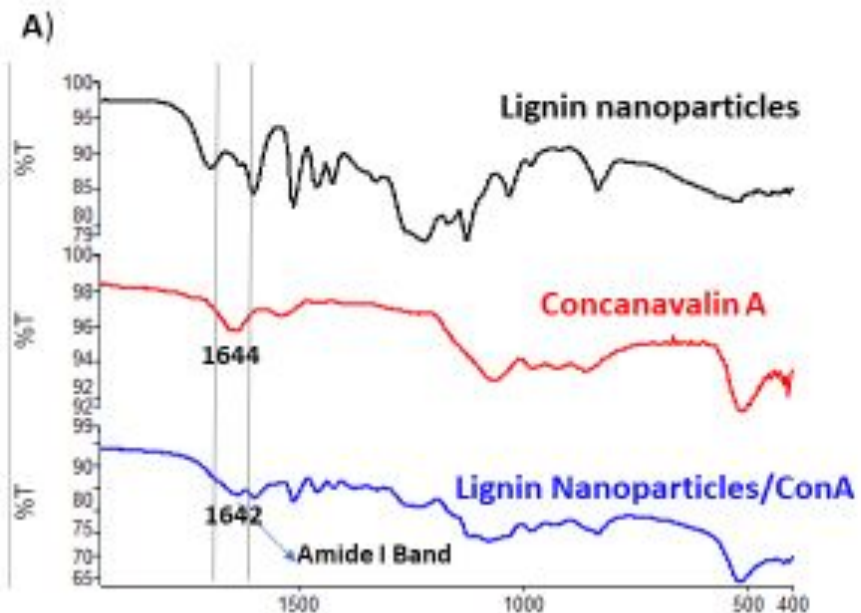


Horseradish Peroxidase (HRP)

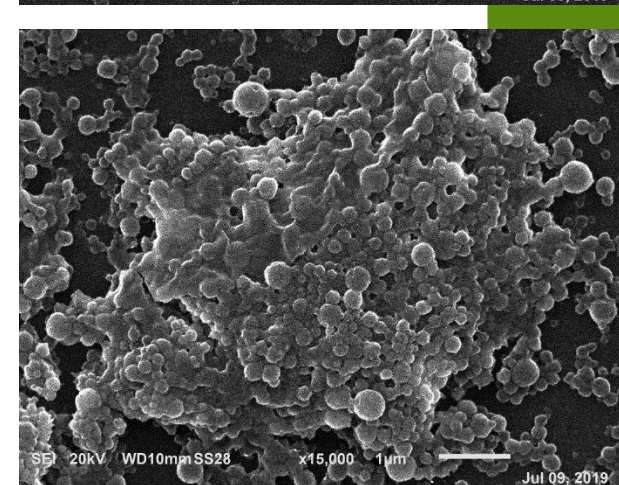
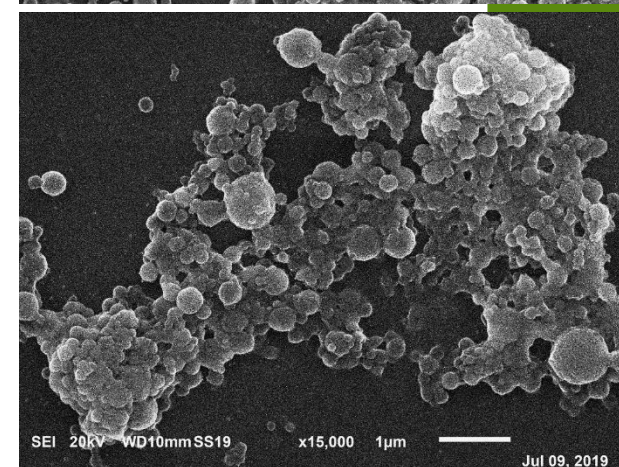
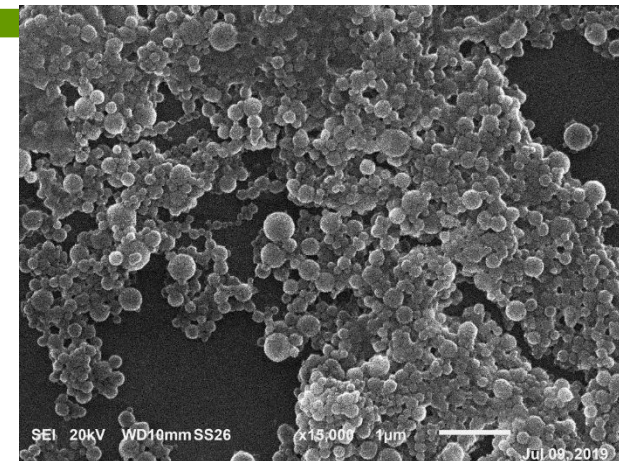
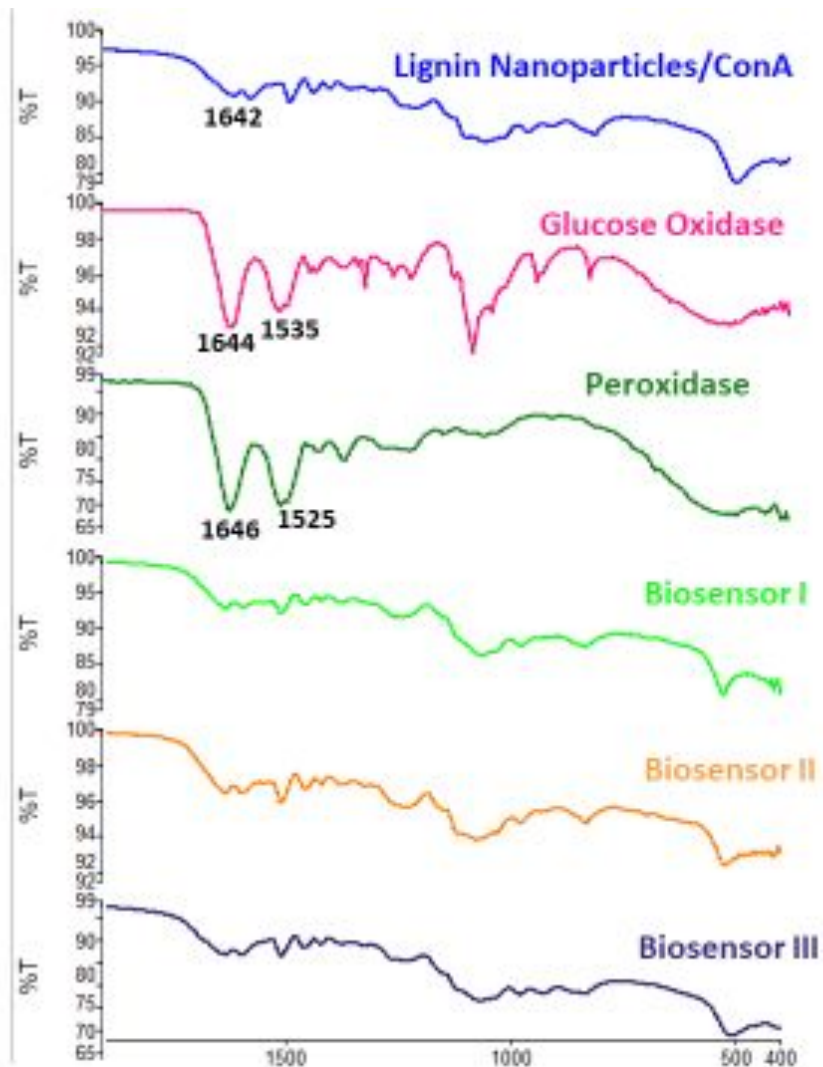


# BIORECEPTOR ELECTROACTIVE

## Fourier transform infrared spectra (FTIR) in ATR mode



Fourier transform infrared spectra (FTIR) of Lignin Nanoparticles, ConA and ConA-Lignin Nanoparticles, showing a typical C=O peak at 1600 cm<sup>-1</sup> for Lignin Nanoparticles with ConA, a similar trend is reported in previous work. [ref]

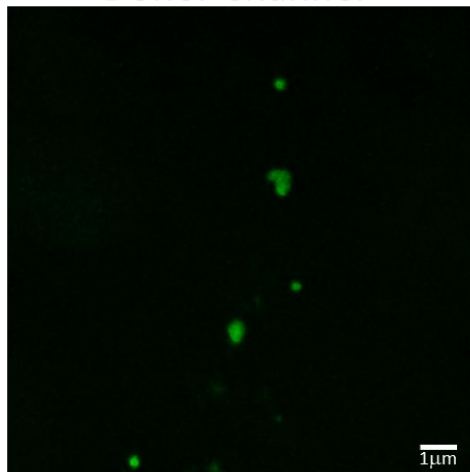


# Design of colorimetric biosensors

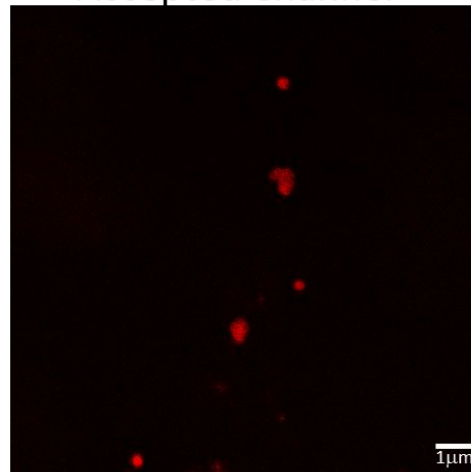
BIORECEPTOR ELECTROACTIVE

FITC donatore  
GOX

Donor Channel

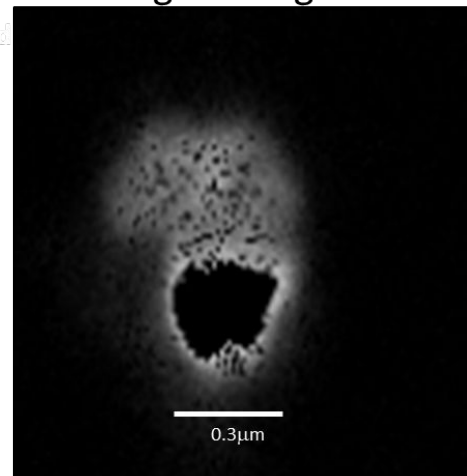


Accepted Channel



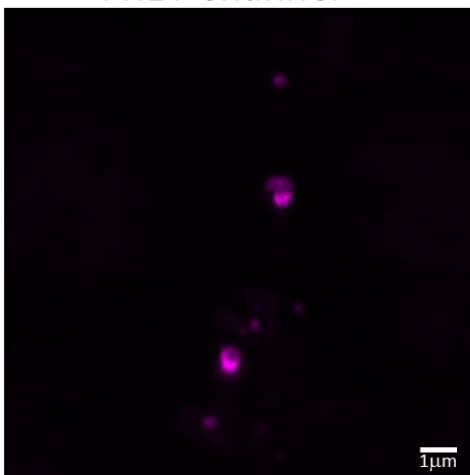
FRET signal Magnification

Merged

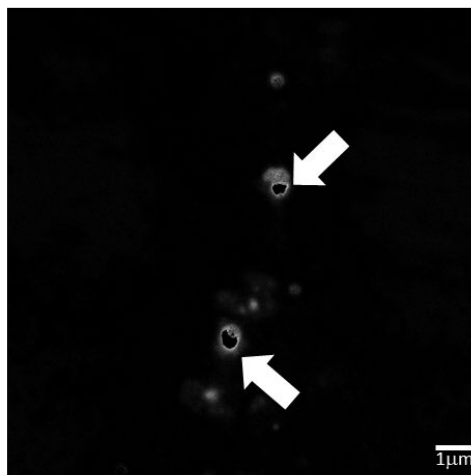


**RESULTS**  
**R=7nm**

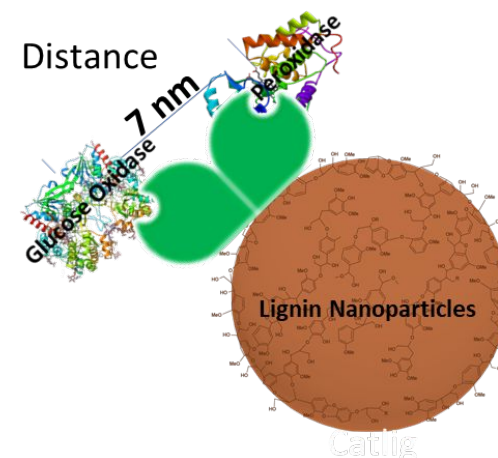
FRET Channel



Corrected FRET



La distanza ottimale tra GOX-HRP è circa 6-10nm





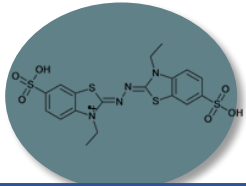
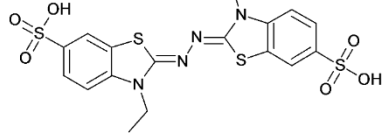
ANALYTE

COLORIMETRIC  
REACTION

MEASURABLE SIGNAL

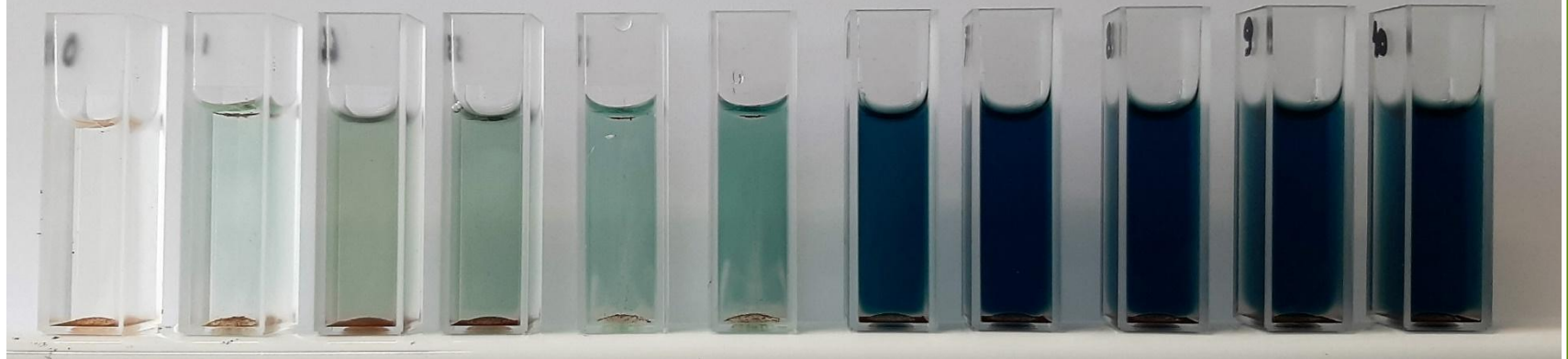
BIOSENSOR  
SENSITIVITY  
(LOD)

ABTS oxidized

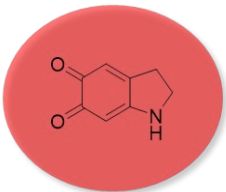


GLUCOSE CONCENTRATION (0-20 mM)

0 mM 0.04mM 0.1mM 0.2mM 0.4mM 0.5mM 2 mM 5 mM 10 mM 15 mM 20 mM

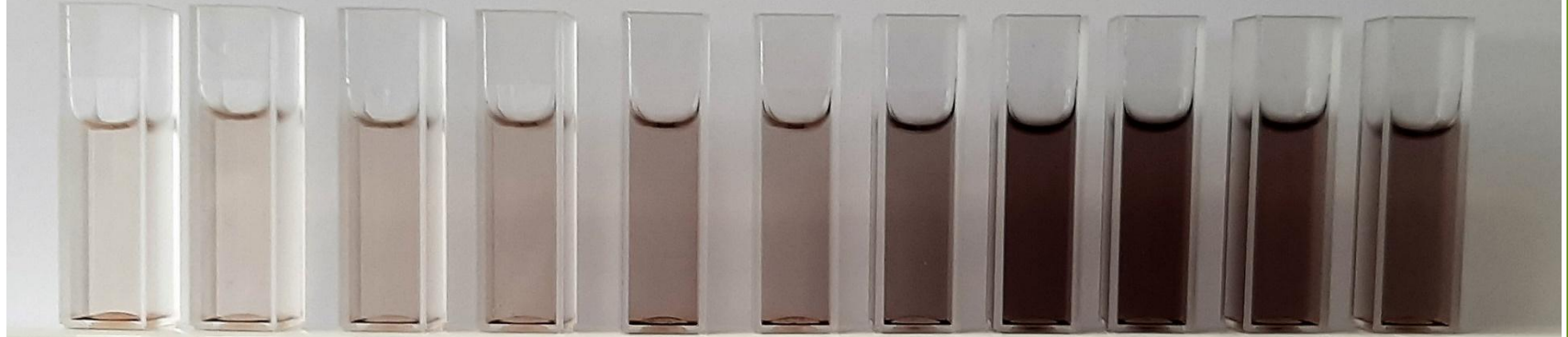


Melanin Synthesis



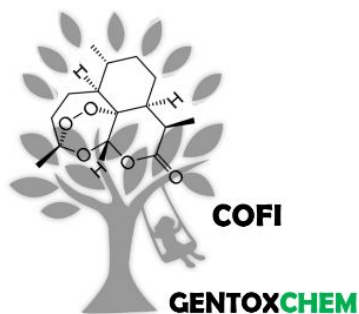
MELANIN PRODUCTION IN COLORIMETRIC ASSAY

0 mM 0.04mM 0.1mM 0.2mM 0.4mM 0.5mM 2 mM 5 mM 10 mM 15 mM 20 mM





## COFI



Raffaele Saladino

## GentoxChem



Stefano Poponi  
Raffaele Saladino

**GENTOX**chem



Bruno M Bizzarri



Lorenzo Botta



Davide Piccinino



Eliana Capecchi

Dott. Claudio Zippilli

Dott.ssa Silvia Cesarini

Dott. Maria Pelosi

Dott. Carmela Gallo

Dott.ssa Angelica Fanelli

Dott.ssa Elisabetta Tomaino



**THANK YOU  
FOR YOUR ATTENTION**