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Recent developments in Enzymatic Syntheses and Grafting of Polymers

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PRE-9 Cancún May '15

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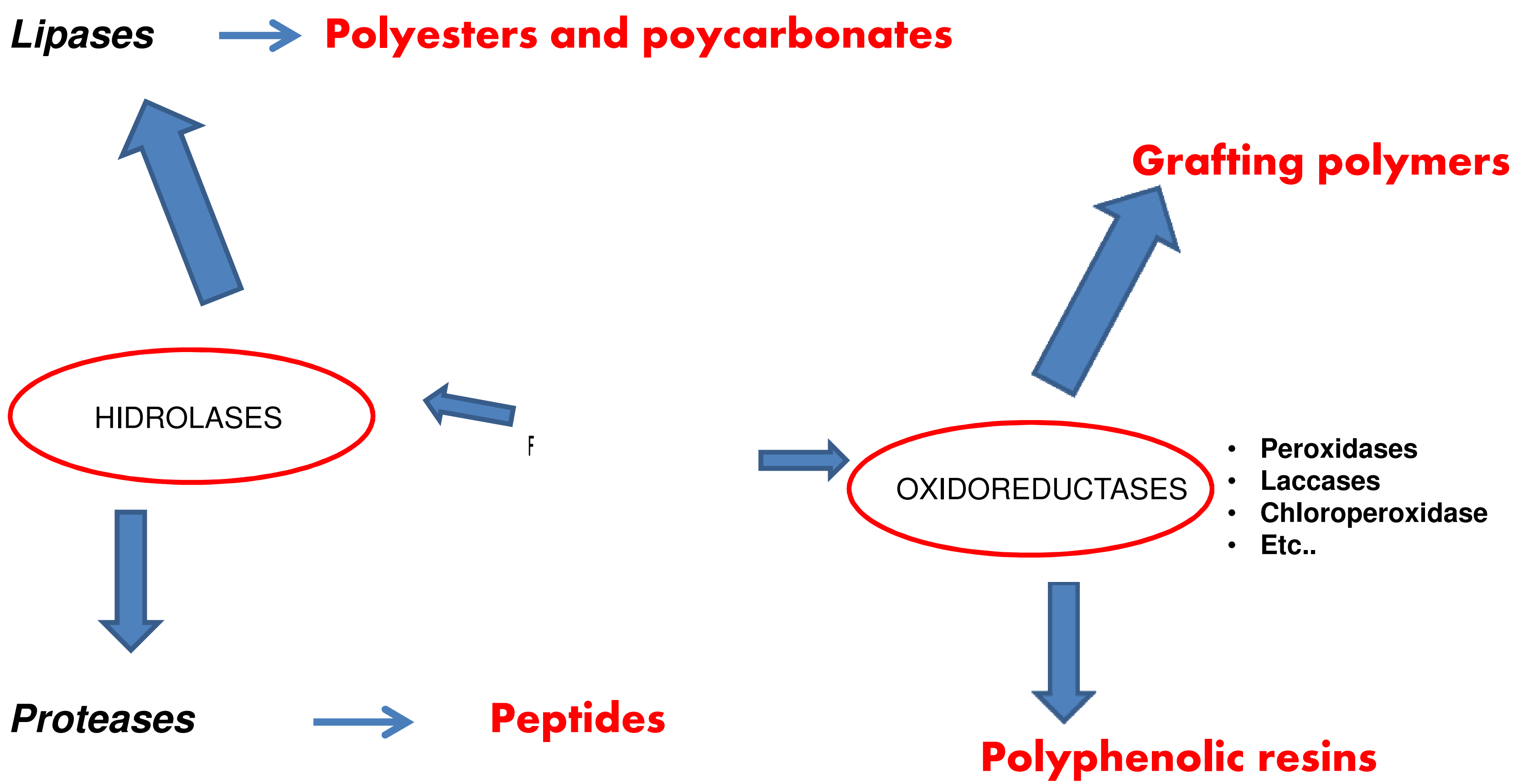
Successful Enzymatic polymerizations



Hydrolases: Biocatalysts in non aqueous media

Naturally existing catalysis in aqueous media

- **Hydrolysis of triglycerides (Ester bonds) (activity at the interface)**
- **Hydrolysis of proteins (amide bonds)**

Hydrophobic and aprotic environment?

Where is the water?

Lipases catalyze

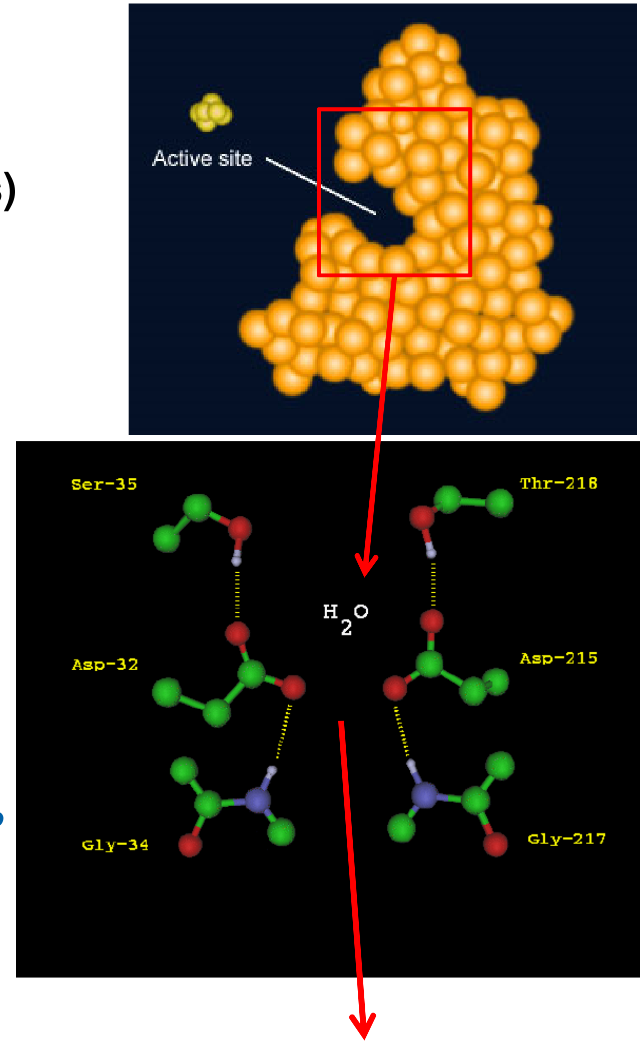
Esterifications / Polyesterifications

and proteases…?

Increase in hydrophilicity

Increase in temperature > 60 ℃

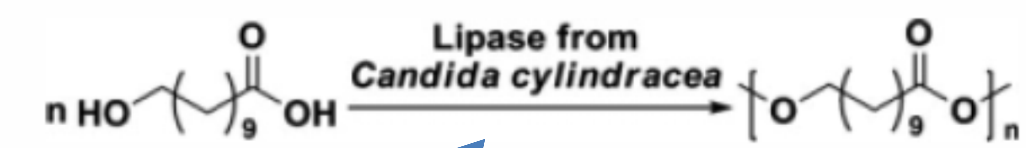
Enzyme deactivation



Adsorbed water molecules (dipole-dipole)

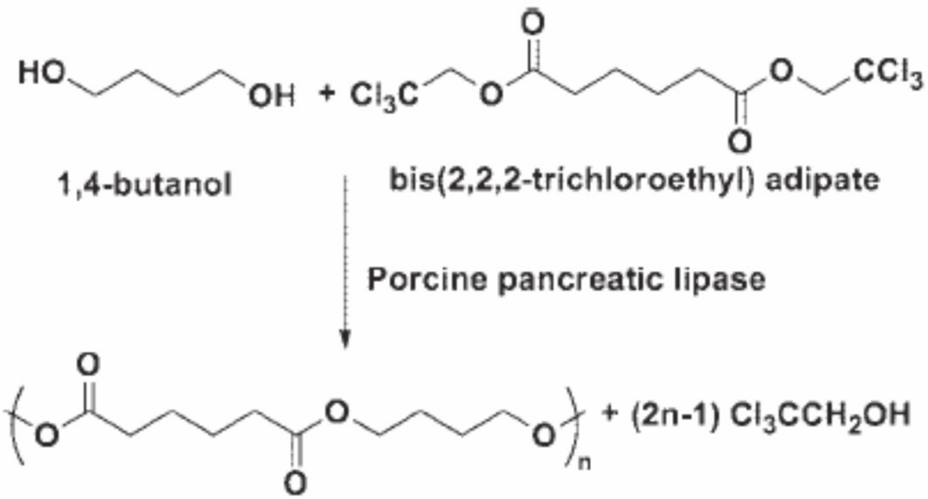
Zaks y Klibanov, Science, 1984, 24,1249-1251

Biocatalysis with lipases in non aqueous media: The synthesis of polyesters and polycarbonates

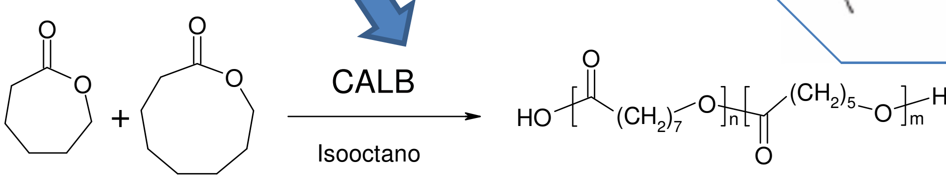


Examples

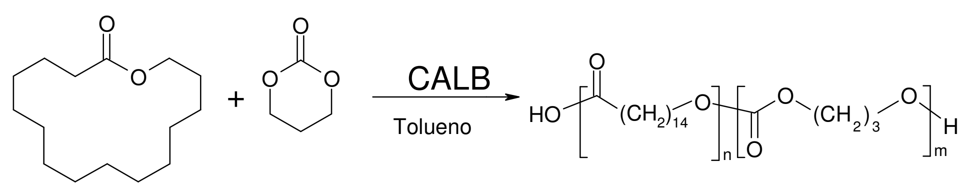
Condensation
(Linear AB structures)



Enzymatic Ring-Opening Polymerizations
eROP
(Lactones)



- **Higher enzyme recognition**
- **Ring opening is driving force of reaction**
- **Entropy favoured**



Kobayashi and Akira. Enzymatic Polymer Synthesis: An Opportunity for Green Polymer Chemistry. *Chemical Reviews* 109, 5288-5353, 2009.

Biocatalysis with lipases in non aqueous media: Other structures

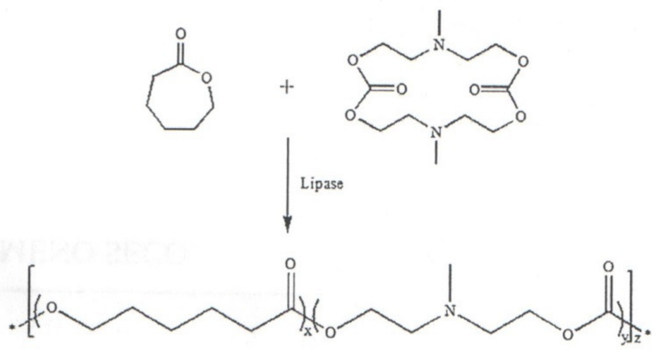


Fig. 3. Enzymatic copolymerization of ε-caprolactone and $(ADMC)_2$.

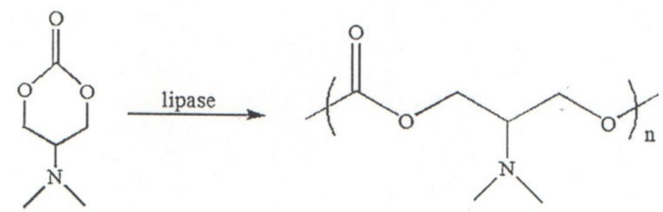


Fig. 2. Enzymatic synthesis of a water-soluble poly(2-dimethylaminotrimethylene

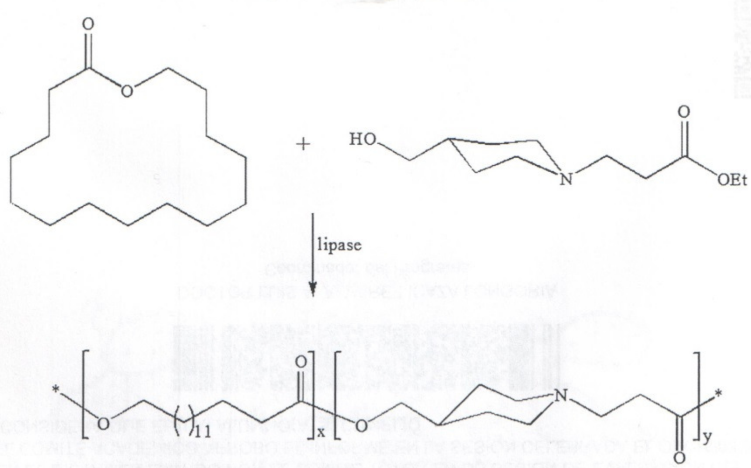


Fig. 9. Lipase-catalyzed copolymerization of ω-pentadecalactone and ethyl 3-(4-(hydroxymethyl)piperidin-1-yl)propanoate.

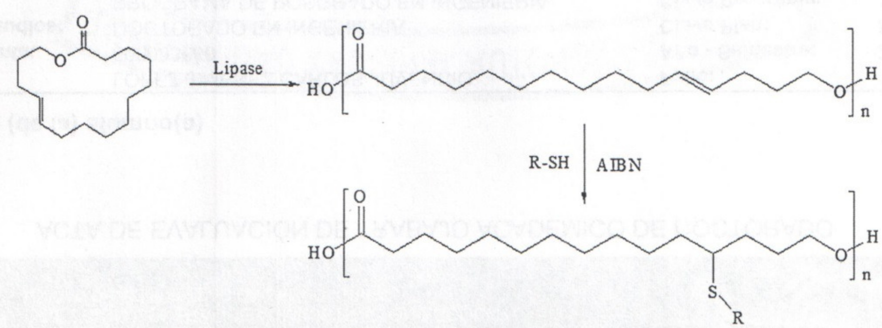


Fig. 10. Enzymatic polymerization of globalide and thiol-ene reaction of poly(globalide) with R-SH

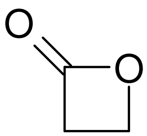
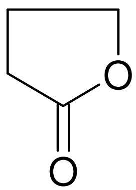
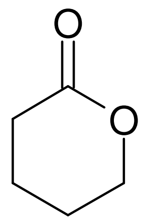
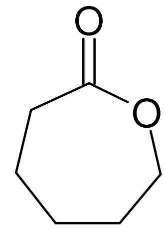
Zhang et al., Process Biochemistry, 49, 797-806, 2014

Enzyme-mediated Polyesterifications using lipases in non aqueous media :

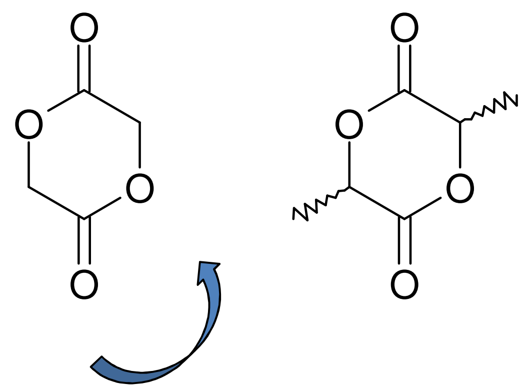
Substrates for eROP

Chemical routes by Stannous octanoate $Sn(Oct)_2$
- **+ Fast**
- **+ Cheap**
- **+ High M_n**

Polyesters

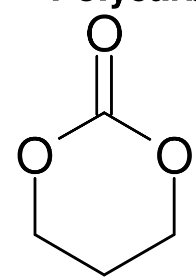





Petroleum based Monomers

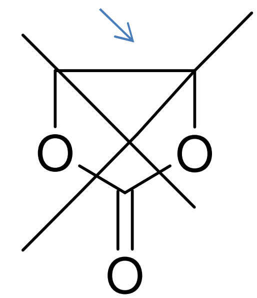


Monomers from renewable sources

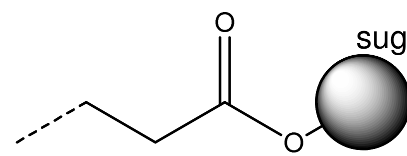
Polycarbonates



Ethylencarbonate not succesfully Polymerized so far



Other contribution…functionalizations



Media: bulk, Toluene, Hexane, cyclohexane, mixtures with dioxane to increase polarity

The eROP mechanism by lipases

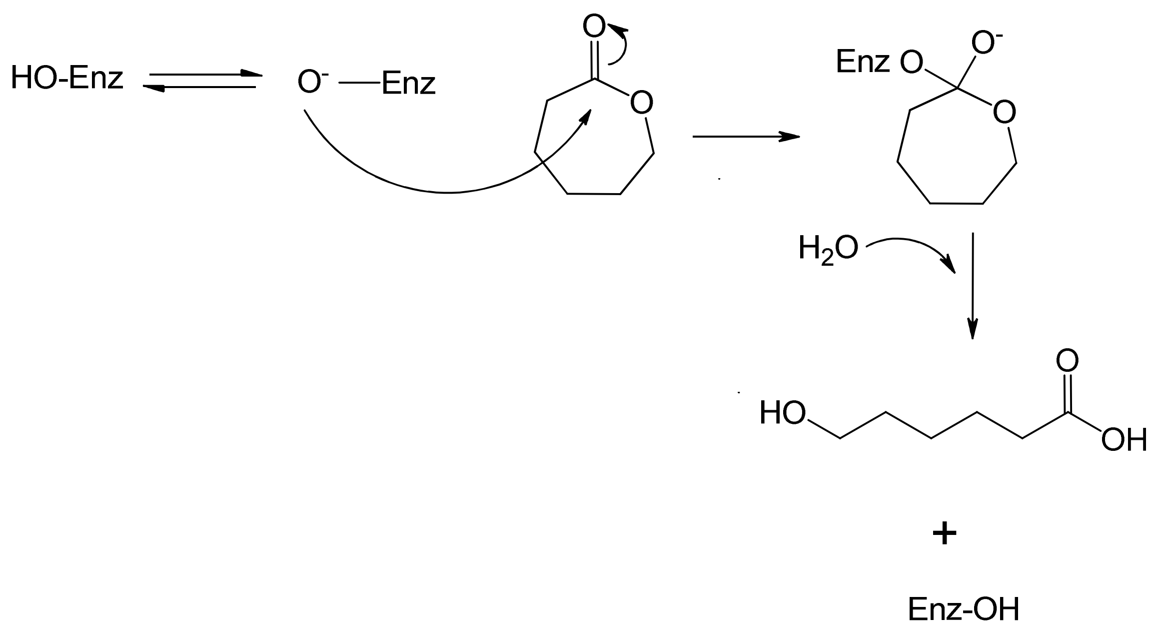
HIDROPHOBIC ENVIRONMENT

To avoid water partitioning from the enzyme active site to the media.

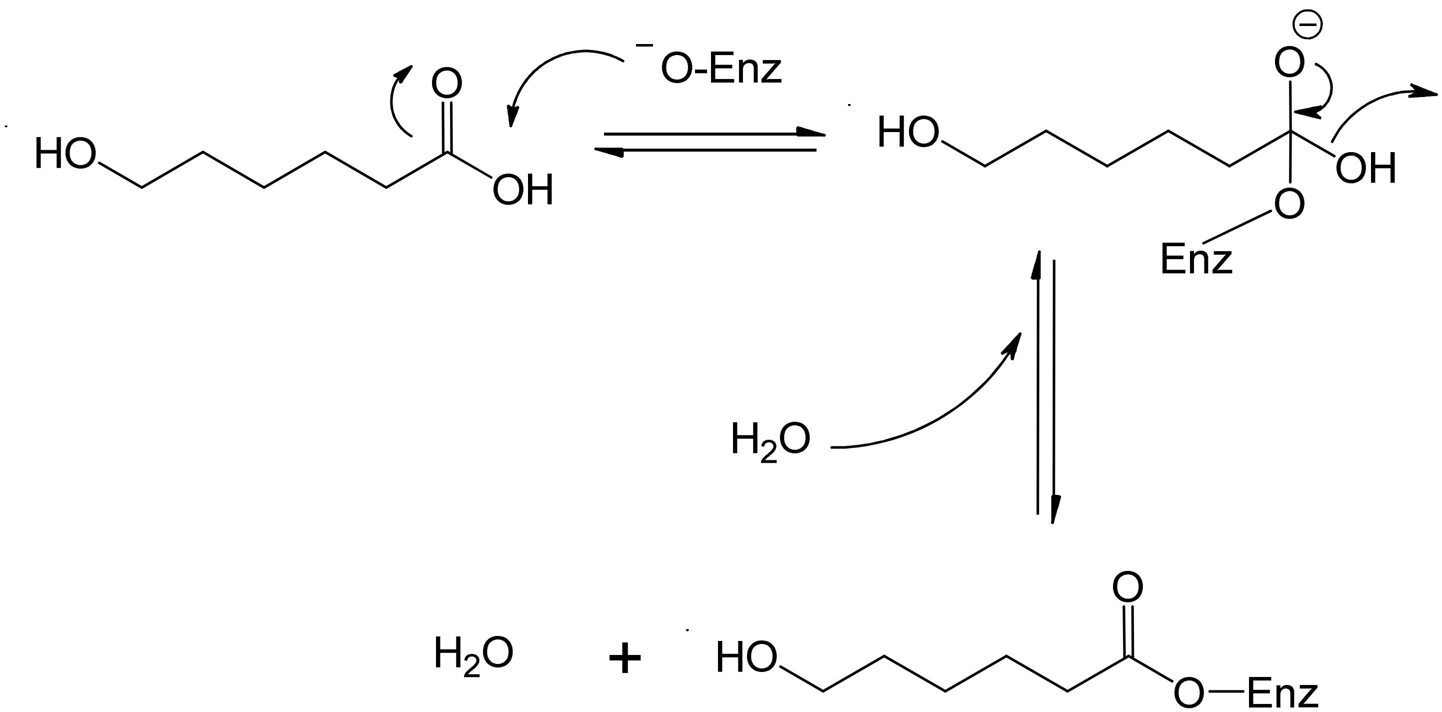
•Dynamic mechanism regarding the water Molecules.

(Water needed for enzymatic activity, for ring opening and enzyme regeneration but plays against propagation due to reverse hydrolysis reaction

First step ring opening / lactone hydrolisis



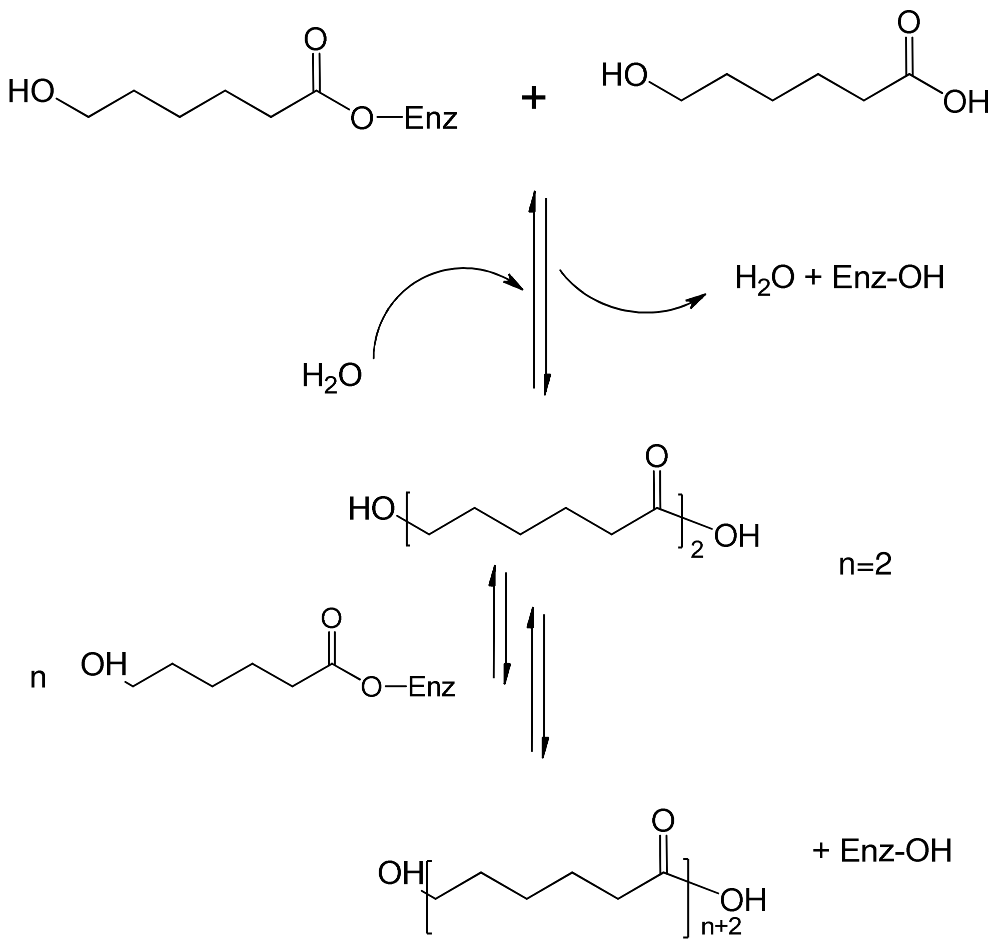
Second step: Acyl enzyme formation



Acyl enzyme intermediate

Water removal would favour the equilibrium toward the intermediate formation

Third step: propagation



Propagation takes place mainly by addition of acylenzyme units to the growing polyester chains

Active site recognizes lactone or small open forms

Best common solvent reported: Toluene

Common organic solvents

- Volatile organic compounds(VOCs)
- Toxic - Non biocompatibles
- Environmentally unfriendly

Green solvents

Compressed Fluids

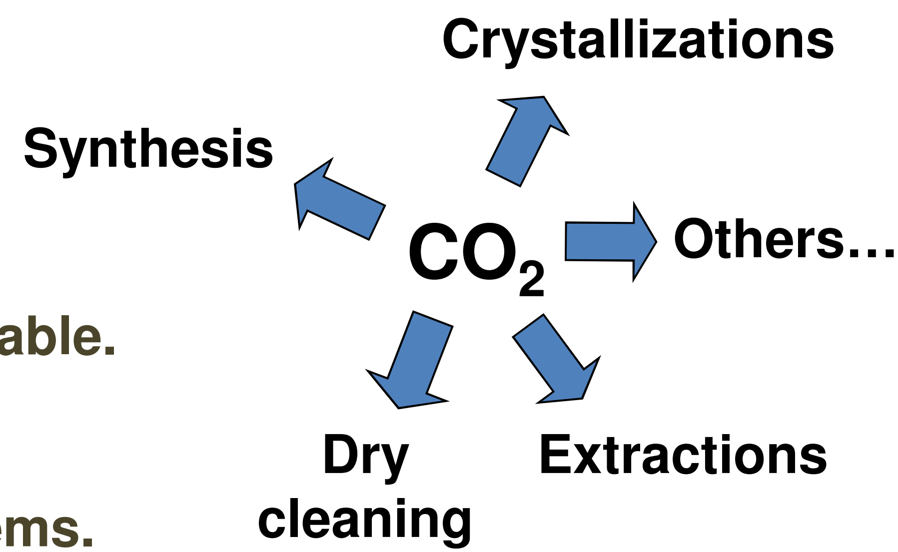
Supercritical and subcritical

Ionic Liquids, are they green?

Compressed fluids as green solvents

CO_2

- Non ozone depleting potential.
- Industrial subproduct.
- Low cost, low toxicity, non flammable.
- Easily recycle.
- Designable to use in closed systems.
- Supercritical state easily reachable (T=35℃, P=80bar).
- No traces of solvent in products.



Compressed fluids

ALTERNATIVE TO VOCs

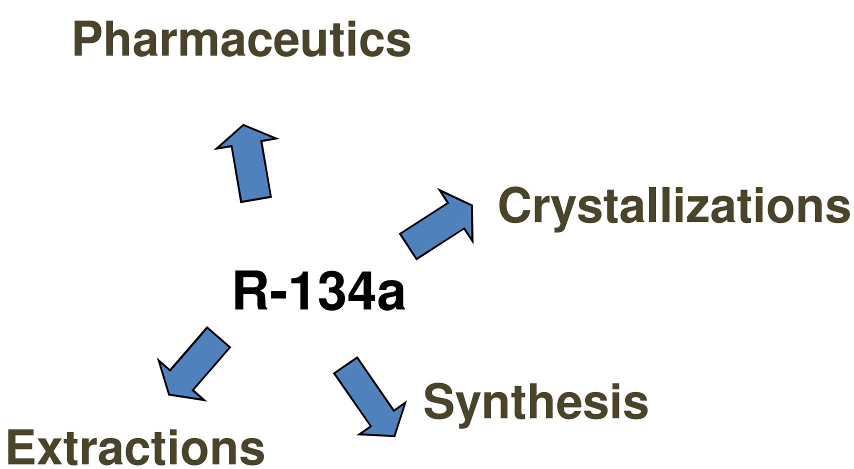
CO_2 disadvantages

NON POLAR-Low solubility of polar compounds

1,1,1,2-Tetrafluoroethane

$(CF_3 - CFH_2)$

- Non toxic (GRAS), no-flammable, recyclable
- Non ozone depleting potential
- Polar (ε = 5.0, DM = 2.1D)
- Similar properties to $scCO_2$ in liquid state



¿What are ionic liquids? Are they green?

- Ionic liquids are organic salts with negligible vapor pressure.

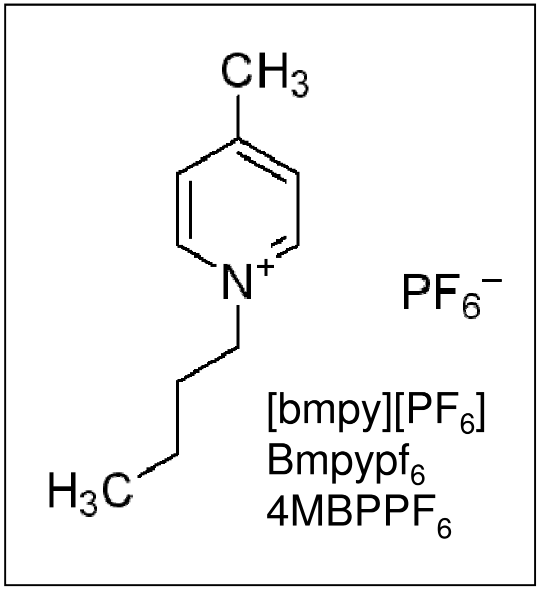
They are toxic → the "***green***" moniker is doubtful…

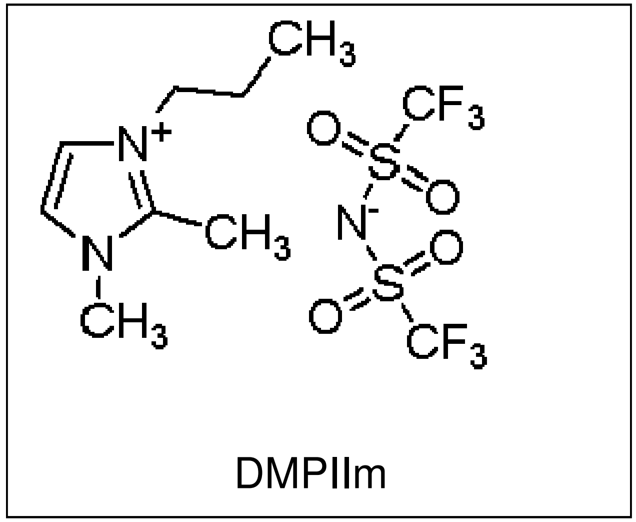
2 Advantages of IL in enzymology:

- Some hydrophobic IL allows for high lipase activity in reactions up to 100 ℃
  (but some others are highly deactivating for hydrolases)

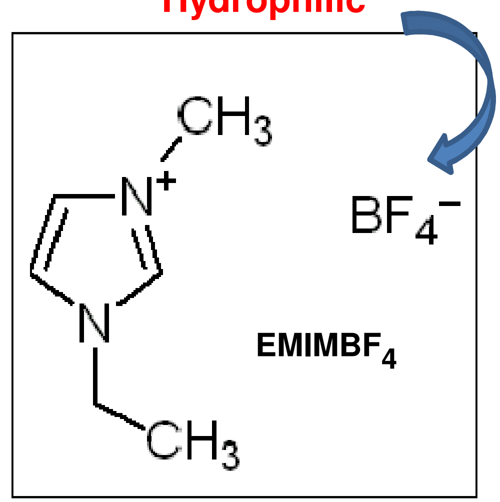
- High solubility of polar and non polar substances in an hydrophobic environment.

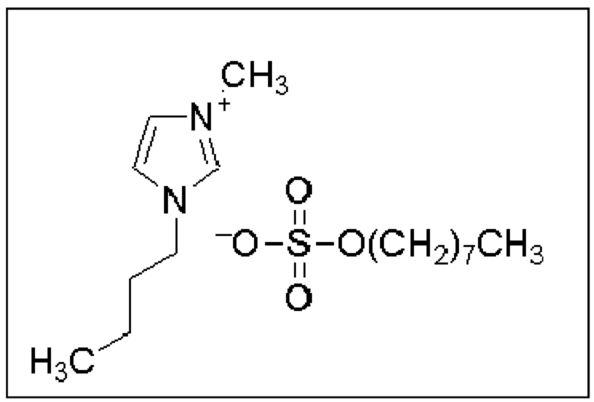
Examples of ILs

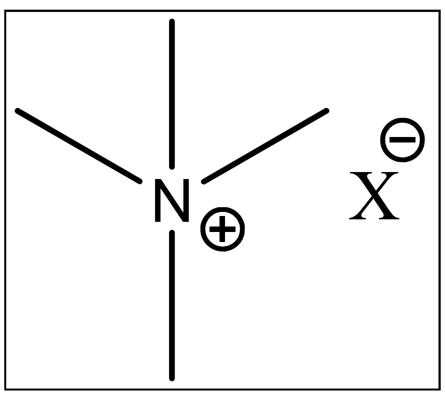




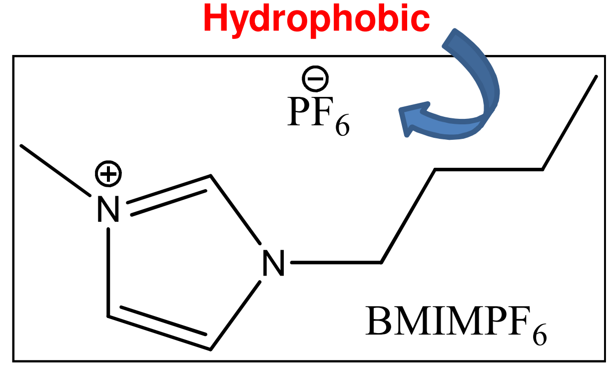
Hydrophilic



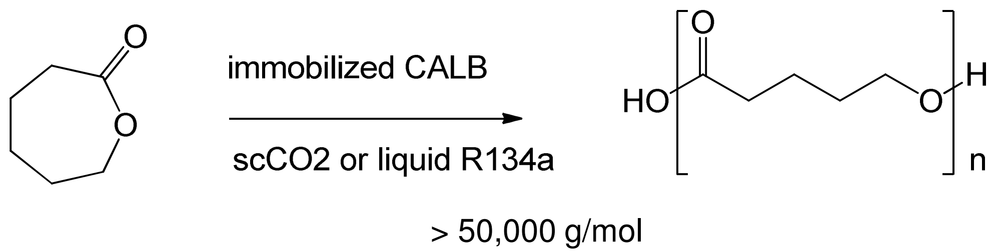




Hydrophobic

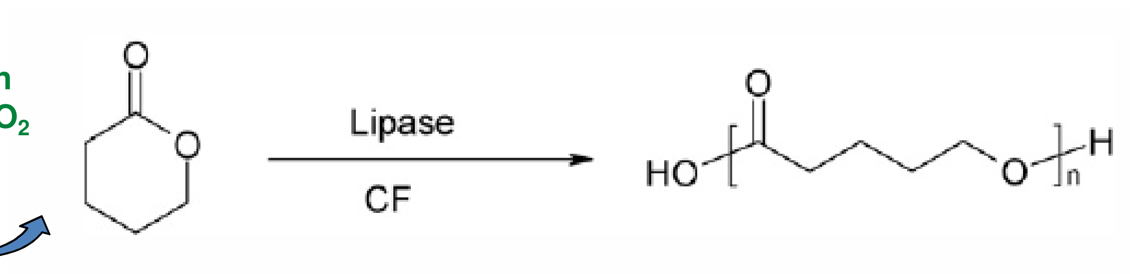


Syntheses in CFs & ILs

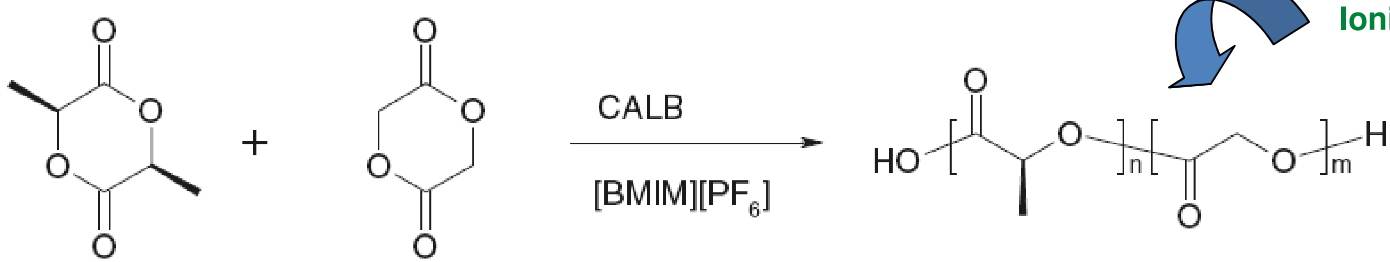


Polymerization in solution
Liquid CF_3-CFH_2 (65 °C and 25 bar) or
scCO2 (65 °C and 80 bar) similar products

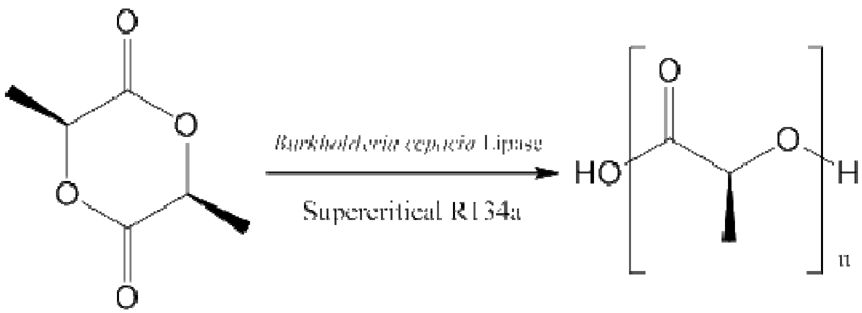
Polymerization in solution
in liquid R-134a and $scCO_2$

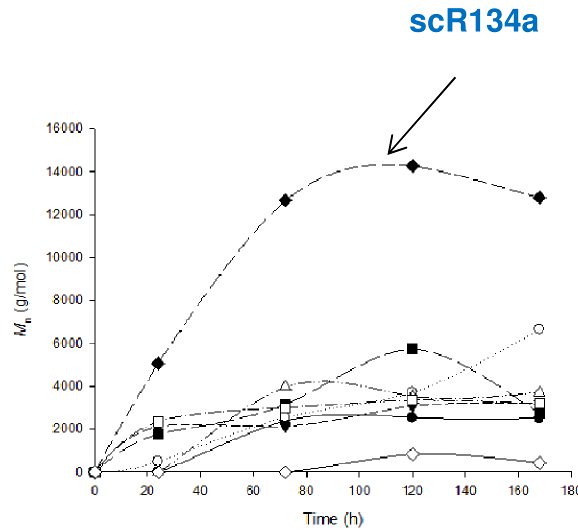


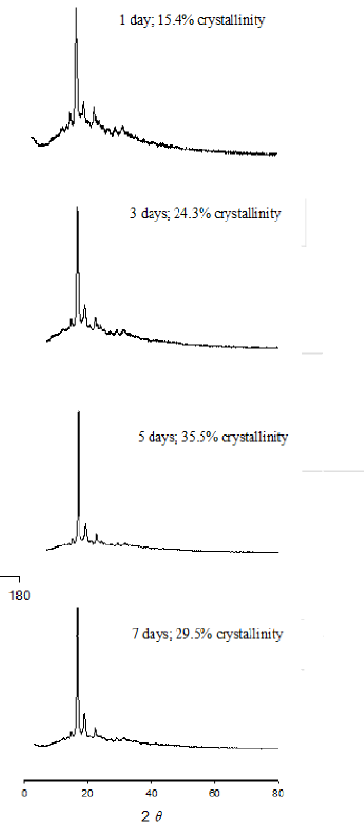
Ionic liquids



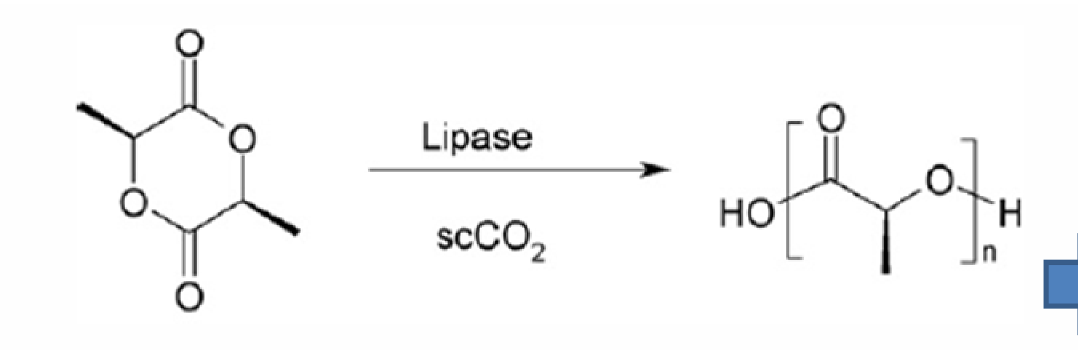
Loecker, *et al.* Macromolecules, 37, 2450-2453, 2004.
García-Arrazola, *et al. Macromolecules,* 40, 4119-4120, 2007.
Chanfreau, *et al. Bioprocess and Biosystems Engineering*, 33, 629-638, 2010.

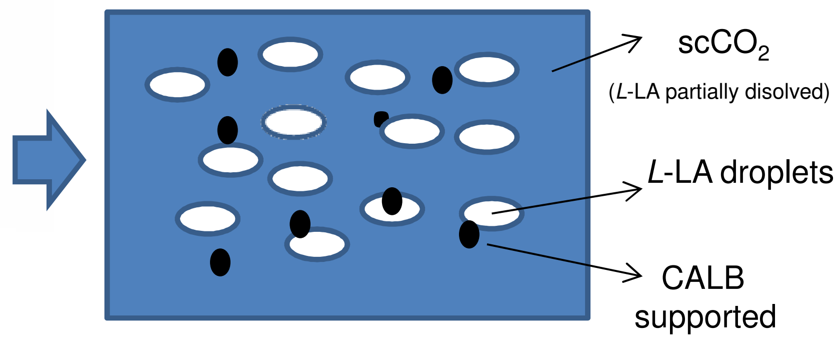






Guzmán-Lagunes *et al. The Journal of Supercritical Fluids* 72, 186-190, 2012

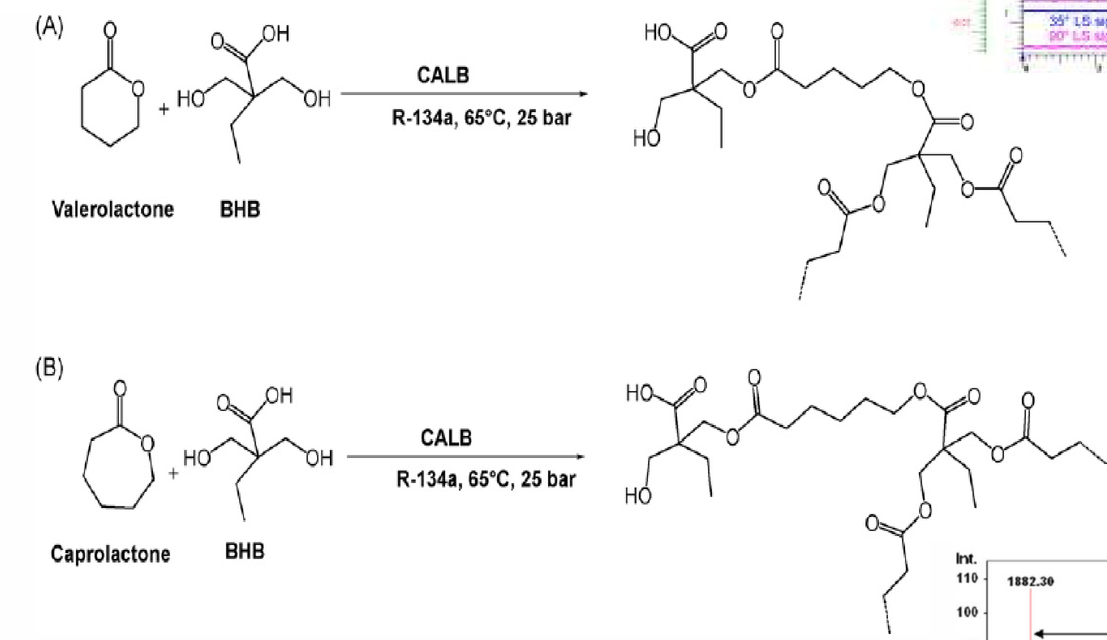


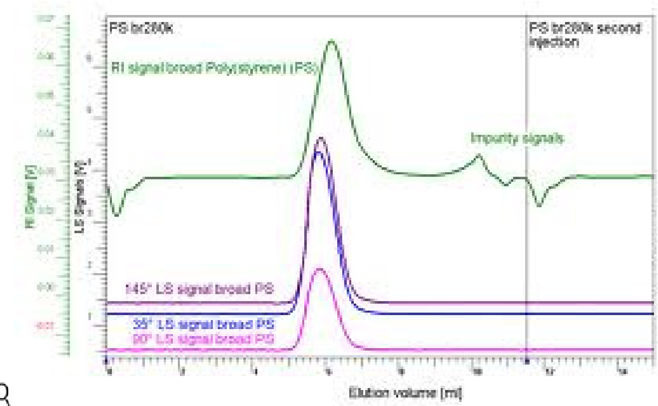


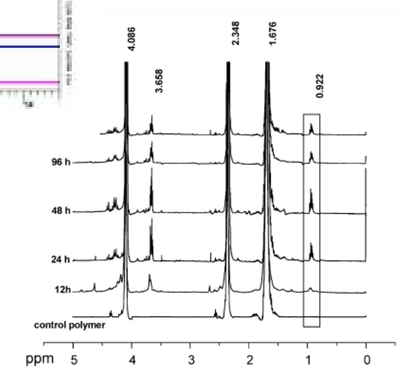
Emulsion type polymerization at 300 bar and 65 ℃

García-Arrazola, *et al. The Journal of Supercritical Fluids*, 51, 197-201, 2009.

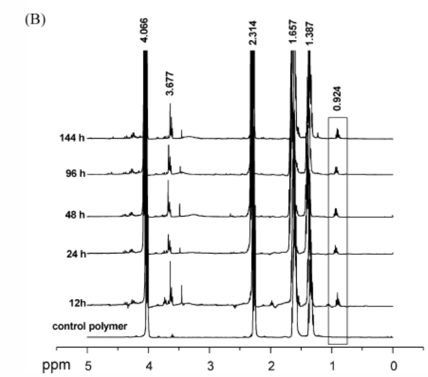
Hyperbranching

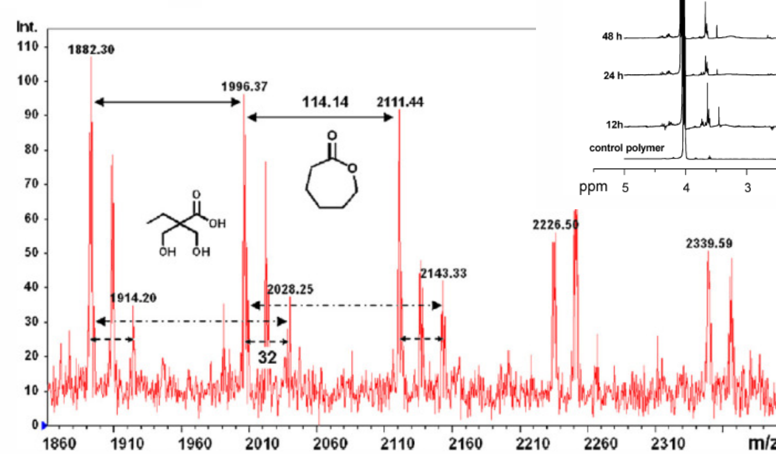




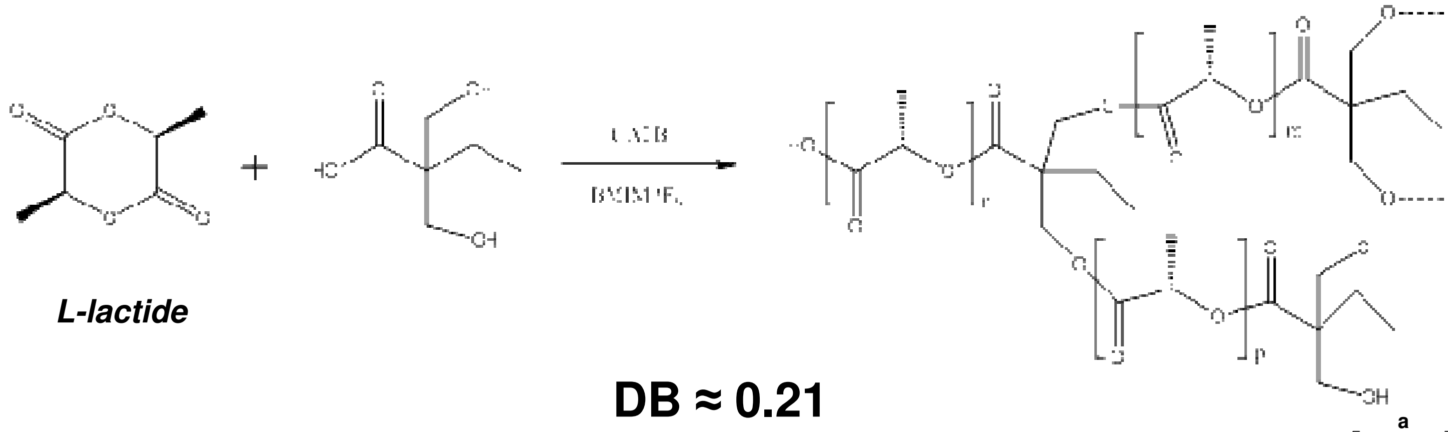


DB ≈ 0.1

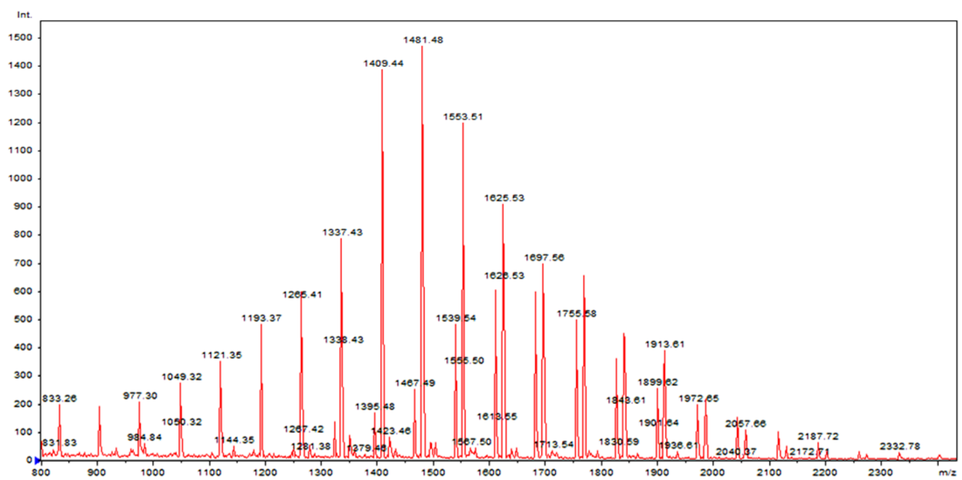


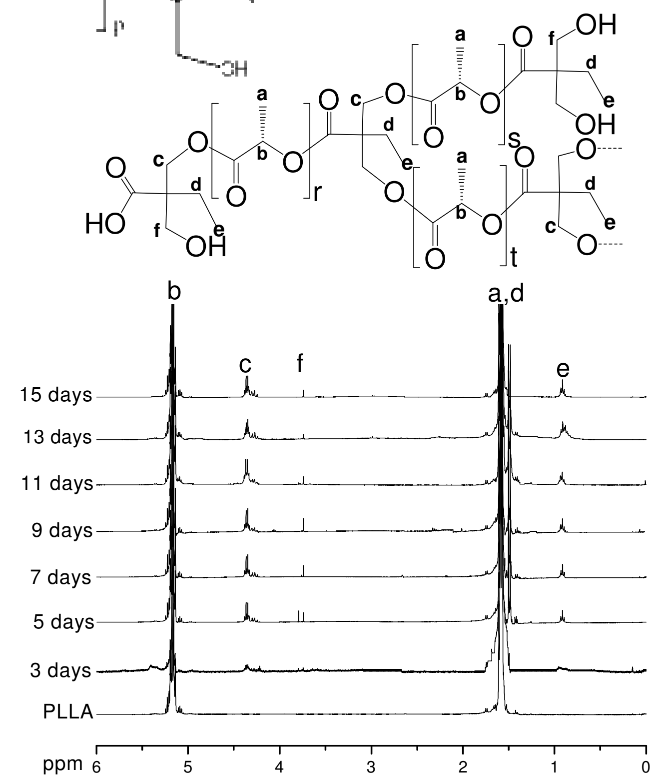


López-Luna *et al.*, *Journal of Molecular Catalysis B: Enzymatic*, 67, 143-149, 2010.



VISCOSITY ? → **critical mixture IL/R134a?***





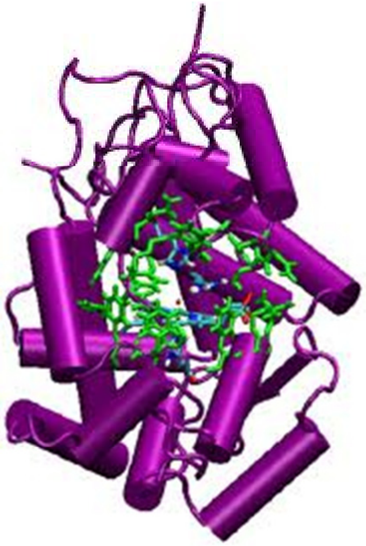
Mena *et al. Bioprocess and Biosystems Engineering* 36, 383-387, 2013
***Mena *et al. The journal of Supercritical Fluids.* in Press**

Applicability?

Free of toxic catalysts ➡ **BIOMEDICAL?**

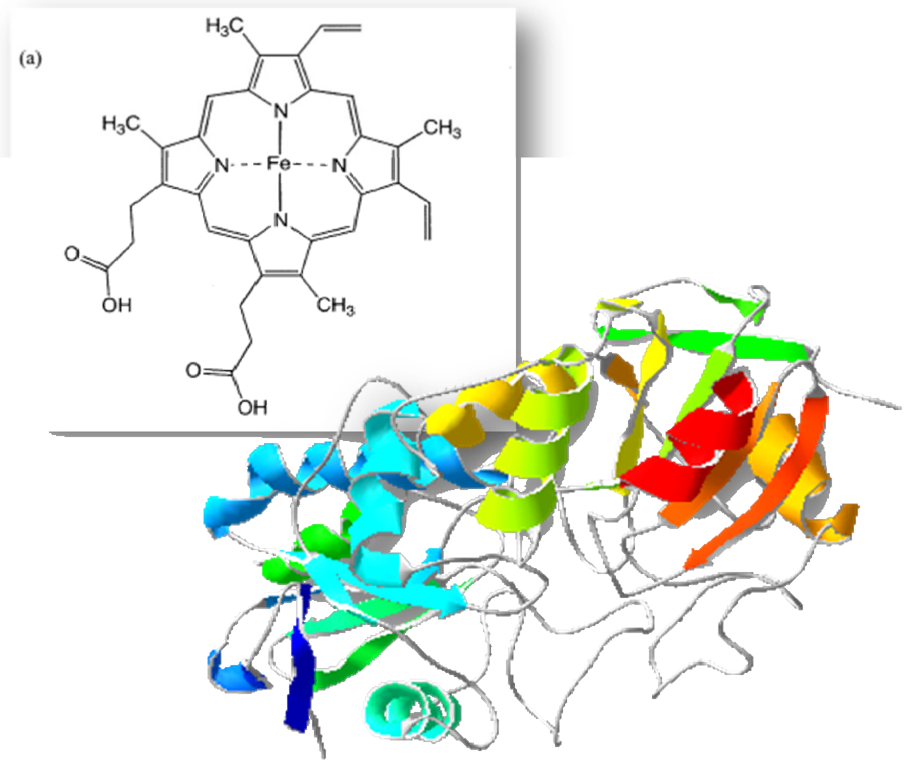
- **Research Challenges**:

- Reduction in polymerization times
- Reduction in the costs of commercial biocatalysts
- Increase polymer yields and molecular weights



Biocatalysis by

OXIDOREDUCTASES



OXIDOREDUCTASES IN POLYMER SYNTHESES

Oxidation toward polyphenolic resins

Polymer grafting

Enzymatic syntheses of polyphenolic resins

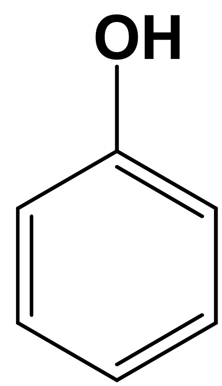
Oxidative polymerization mediated by oxidoreductases
(peroxidases, laccases, manganese peroxidases, phenoloxidases, chloroperoxidases)

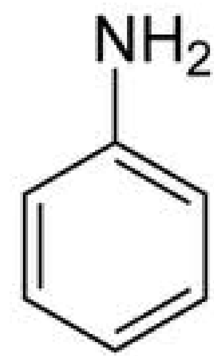
Chemical Routes

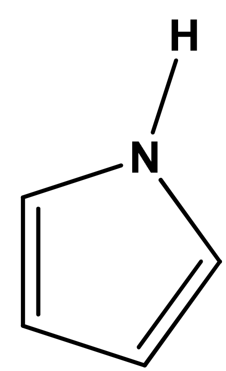
Toxic Copper catalysts
Pyridine solvent
Extreme pH conditions
Formaldehyde presence (class I carcinogenic)

Enzymatic advantages & challenges

- Fast reactions
- Mild reaction conditions (RT)
- High molecular weights and yields
- Relatively low costs of biocatalysts
- Only recognize phenolics and anilines (pirrole in less extent) substrates.
- Propagation by free-radical mechanism.
- Oxidoreductases require aqueous media.
- Solubility of substrates and polymers vs enzymatic activity.



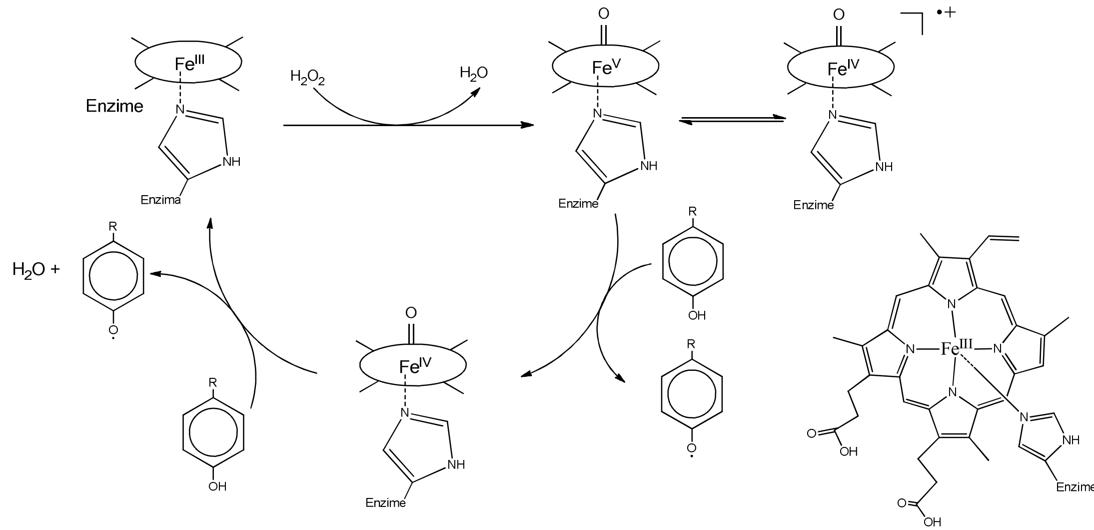




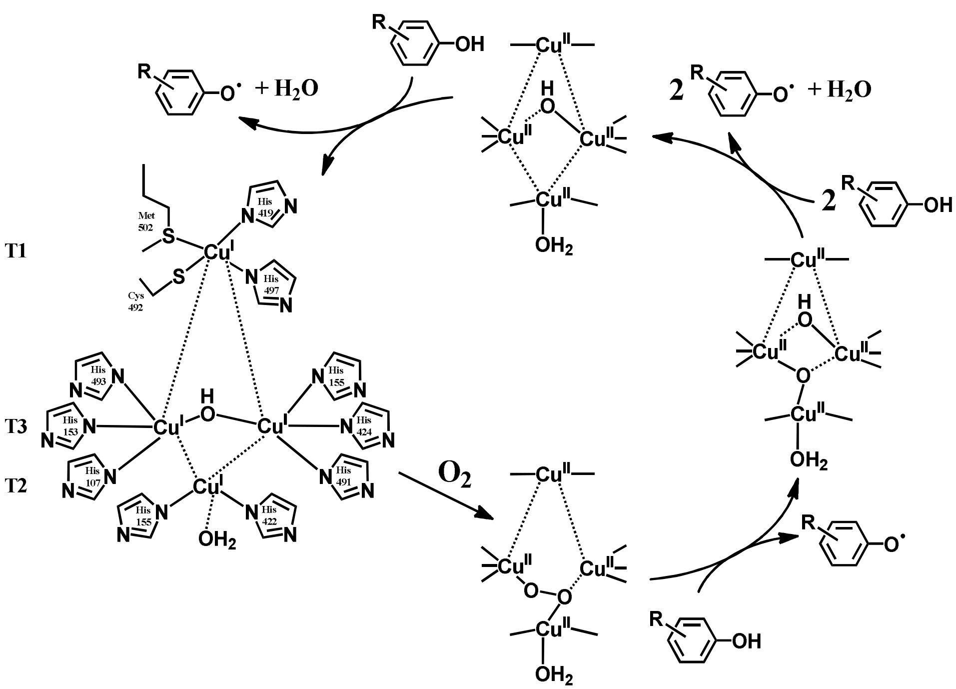
Mixed aqueous media with miscible solvents (MeOH, Acetone, DMSO, DMF)
Decrease in activity but increase in solubility

The oxidoreductases at the active site

PEROXIDASE

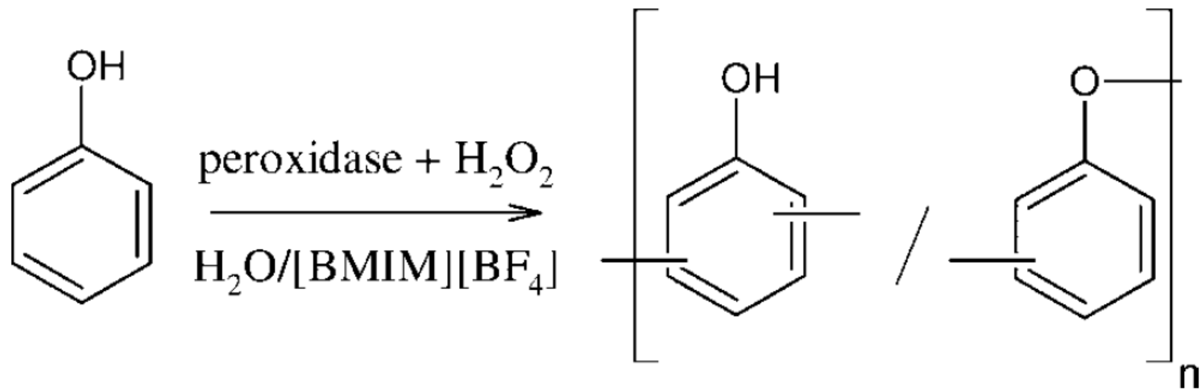


HRP Active site
Iron-Protoporphyrin



LACCASE

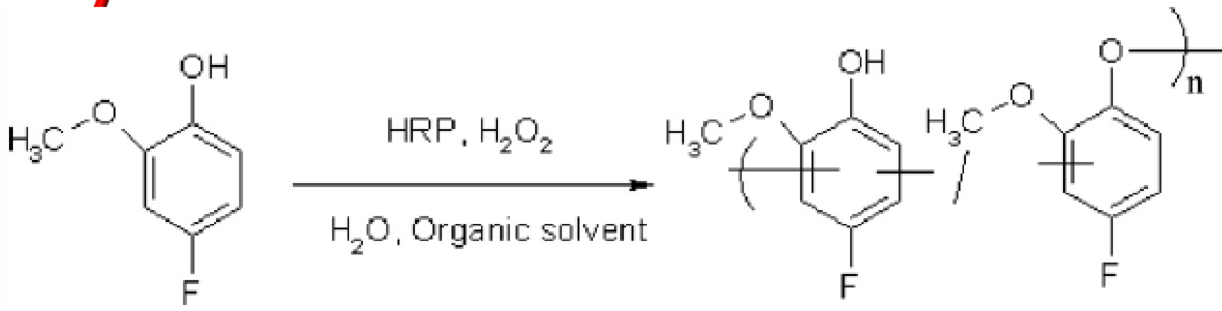
Synthesis:



Hydrophilic/water miscible Ionic liquids allow for enzymatic activity. ILs can be easily recovered and recycled owing to zero vapor pressure

Dordick, *et al.* , *Biotechnology and Bioengeneering*, 30, 31-35, 1987
Ecker, *et al.*, *Journal of Molecular Catalysis B Enzymatic*, 59, 177-184, 2009
Zaragoza-Gasca, *et al. Polymers for Advanced Technologies*, 21, 454-456, 2010.

Synthesis:



Photoconductive

Fig. 1. Reaction scheme for de HRP-catalyzed polymerization of 4-fluoro-2-methoxyphenol.

Soluble in organic solvents/water non-soluble. Thermostable > 350℃

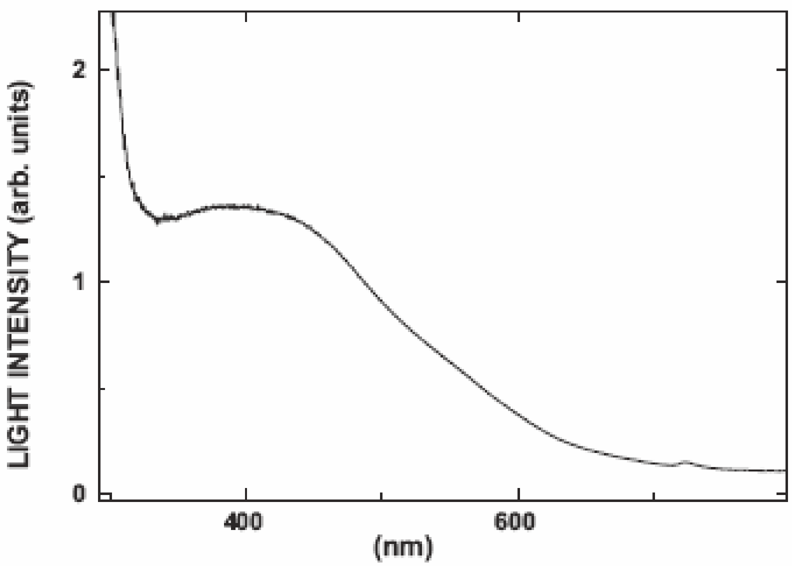


Fig. 2. Optical absorptions in the visible for PFMP.

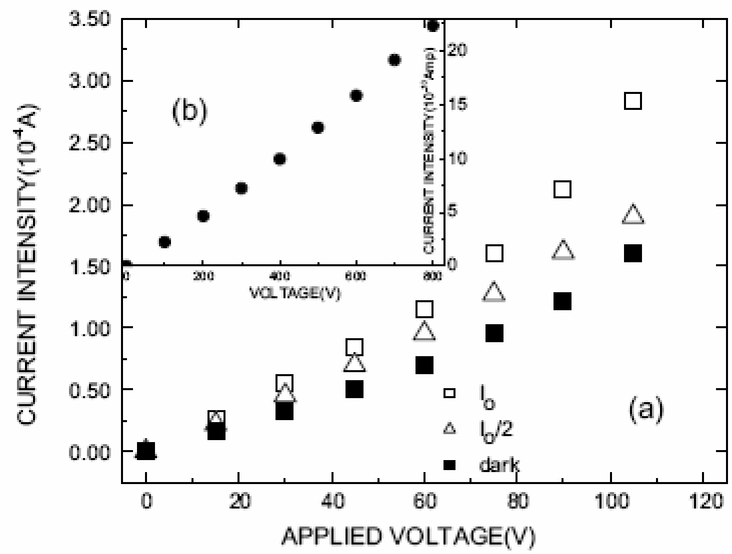
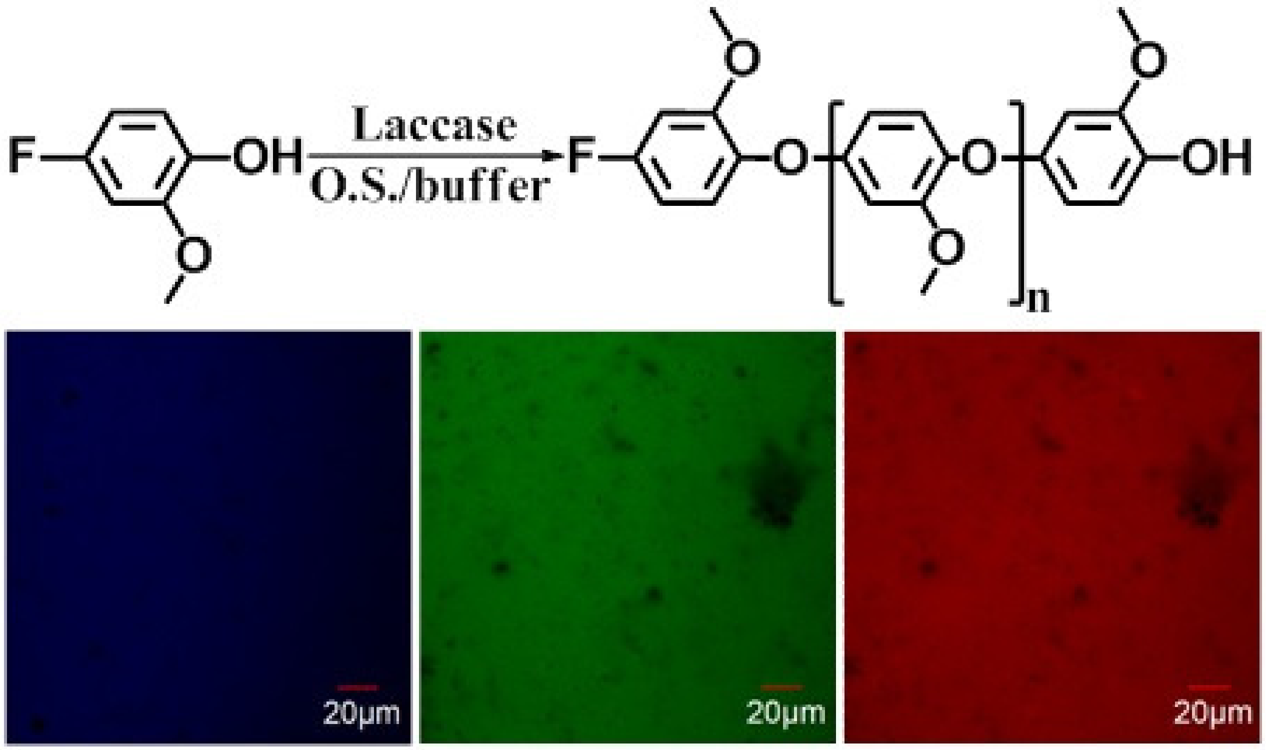


Fig. 3. (a) Photocurrent intensity of PFMP sample and inset above in the left (b) corresponds to blank sample, I_0 value was 0.82 W/cm².

Zaragoza-Gasca, *et al. Journal of Molecular Catalysis B: Enzymatic* 72, 25-27, 2011.

Synthesis:



Photoluminiscent !

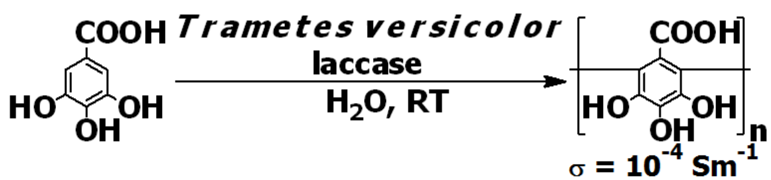
Soluble in organic solvents/water non-soluble. Thermostable

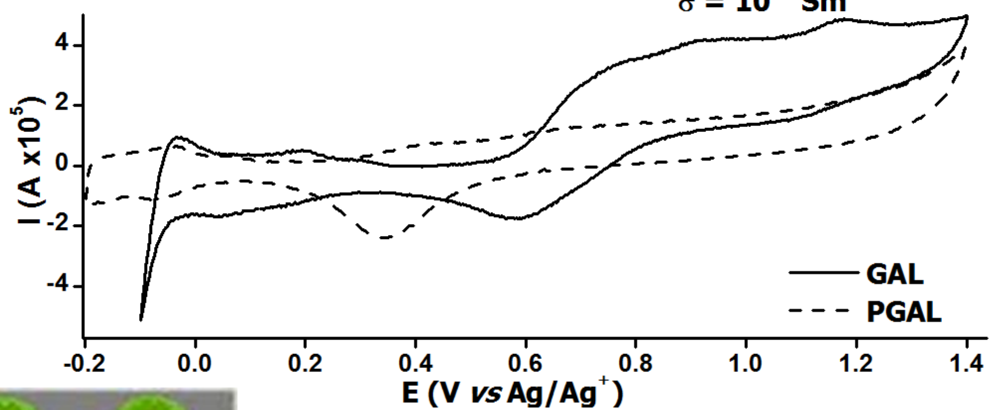
López *et al. Journal of Molecular Catalysis B: Enzymatic* 109, 70-75, 2014.

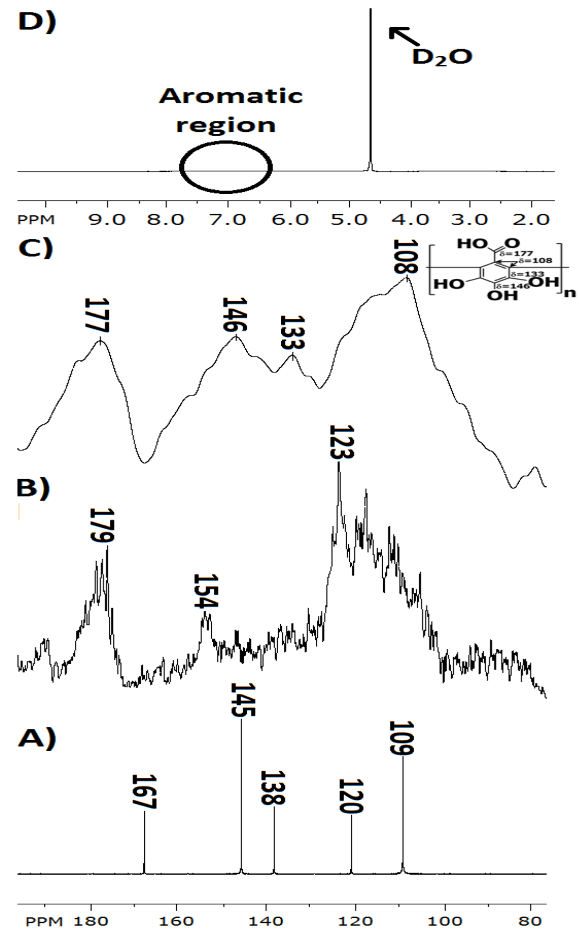
Synthesis:

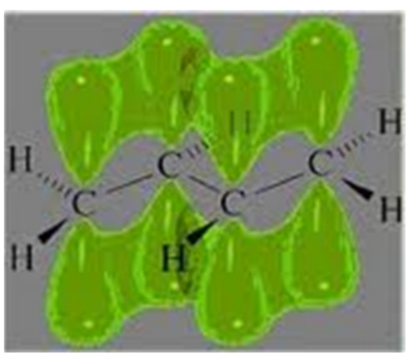
Polyoxidation of gallic acid in water media using Laccase from *Trametes versicolor*

Semiconducting properties / Water soluble Natural origin. Other applications?



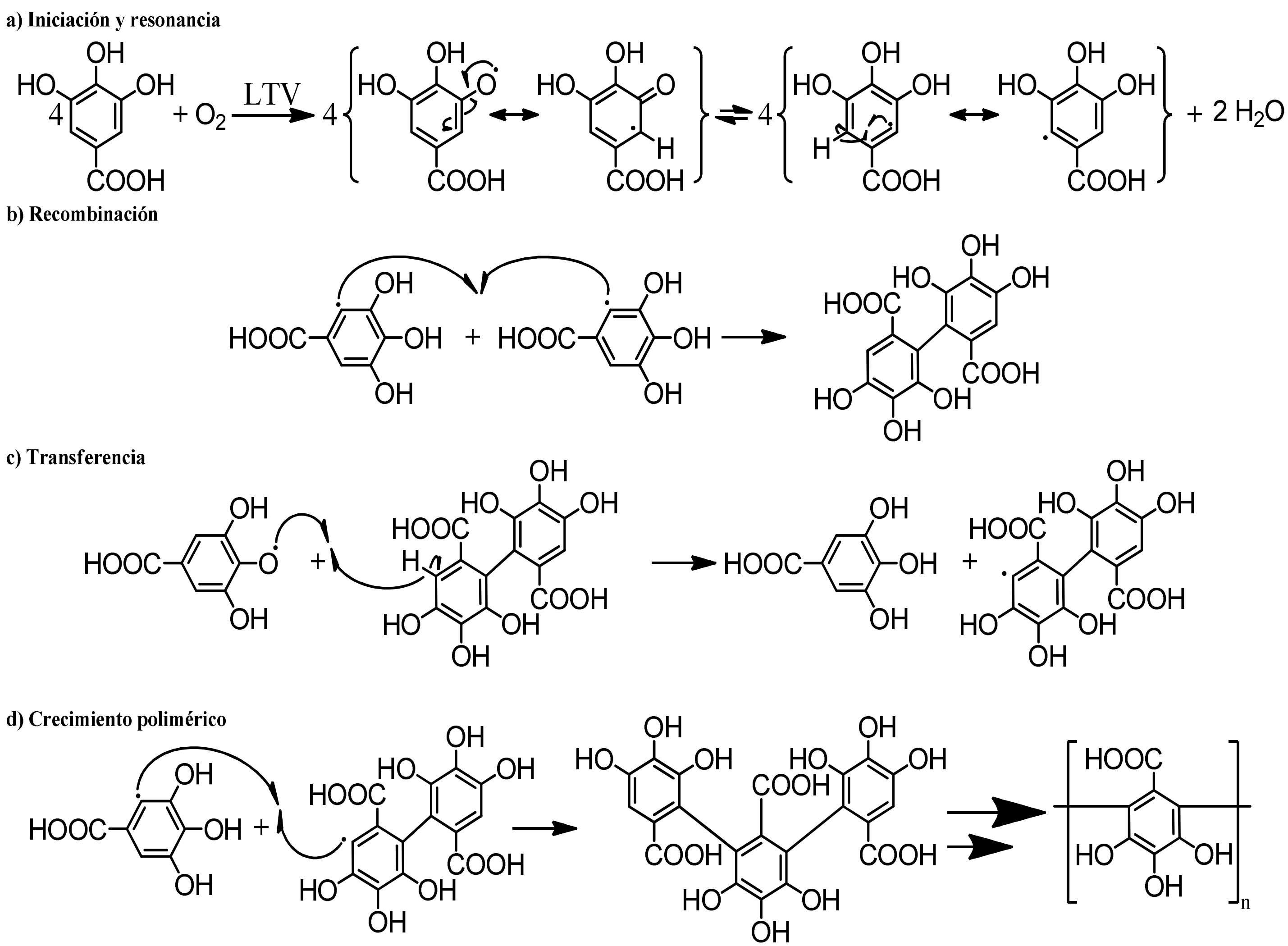






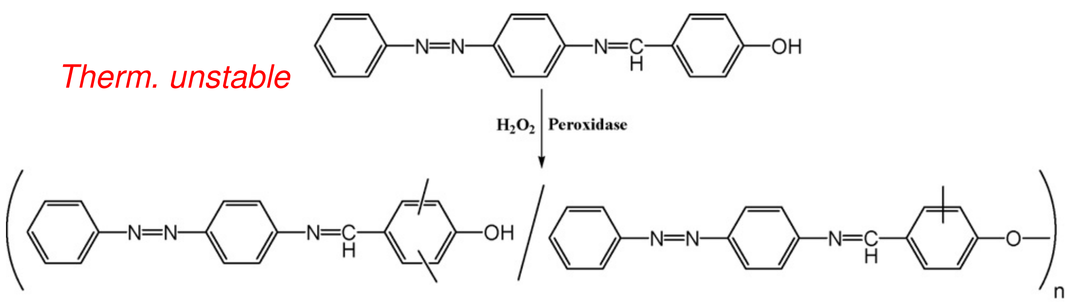
López *et al. Journal of Molecular Catalysis B Enzymatic*, 97,100-105, 2013

Proposed mechanism

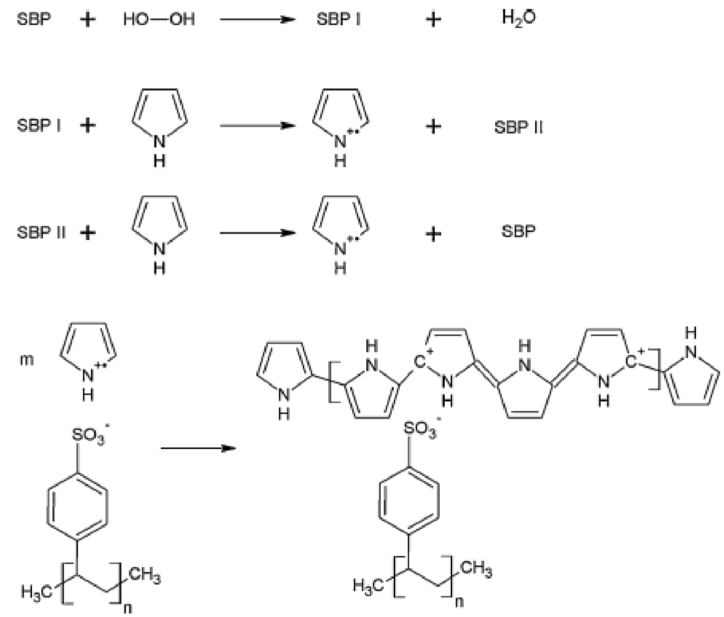


Other reports

Therm. unstable

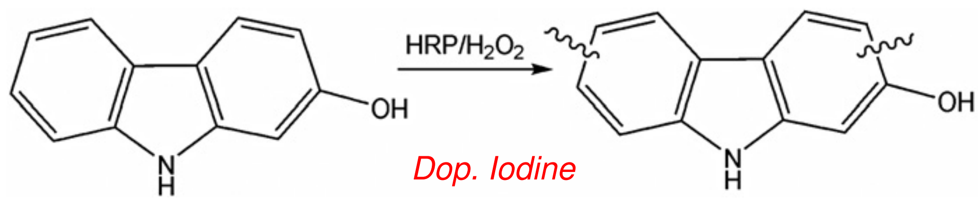


Turac *et al.*, Synthetic Metals *160 ,169–172, 2010*



Bouldin *et al.*, Synthetic Metals 161, 1611-1617, 2011

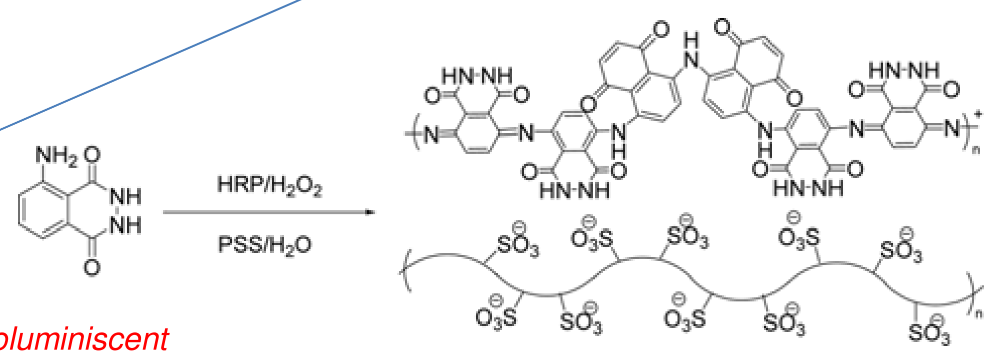
Semiconducting (pirrol recognition)



Dop. Iodine

Bilici *et al.* Journal of Molecular Catalysis B: Enzymatic *64 ,89–95, 2010*

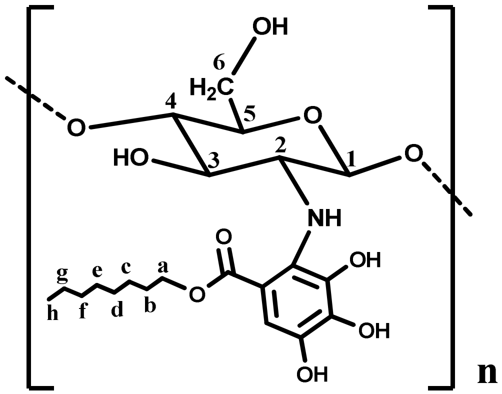
Semiconducting σ 10^{-8} - σ 10^{-4}



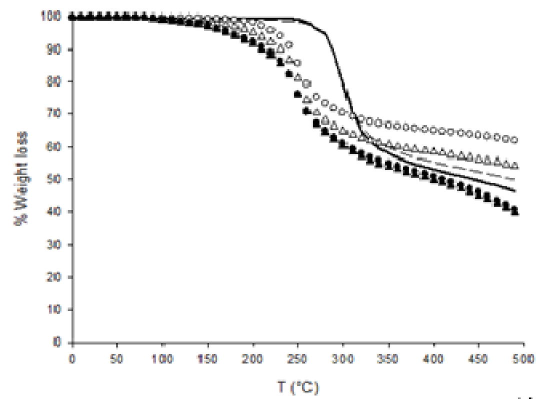
Photoluminiscent

Nabid *et al.,* Macromolecular Research 19, 280-285, 2011.

Grafting of gallate esters onto biopolymers (HRP)

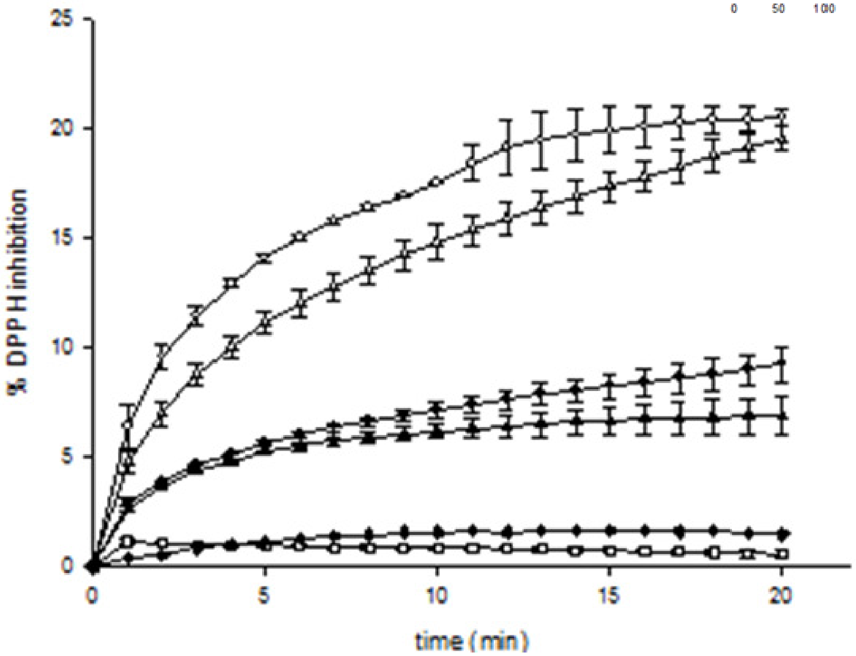


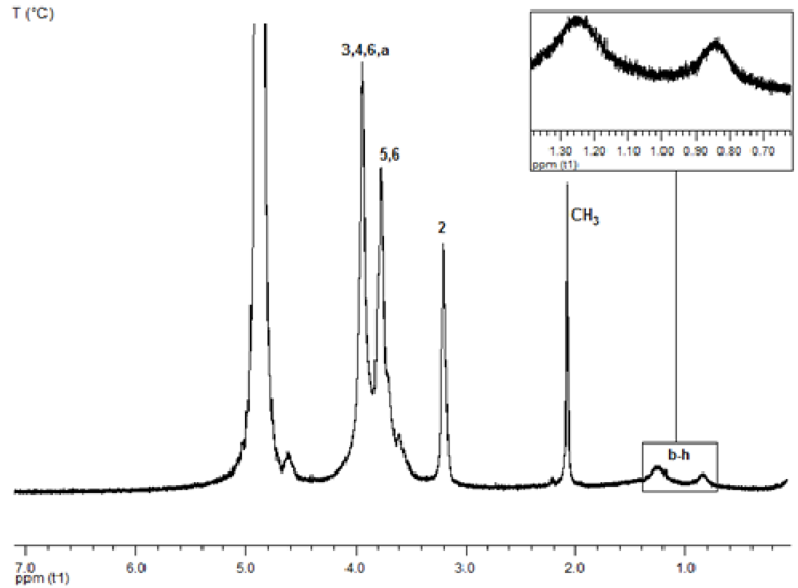
Antimicrobial



+

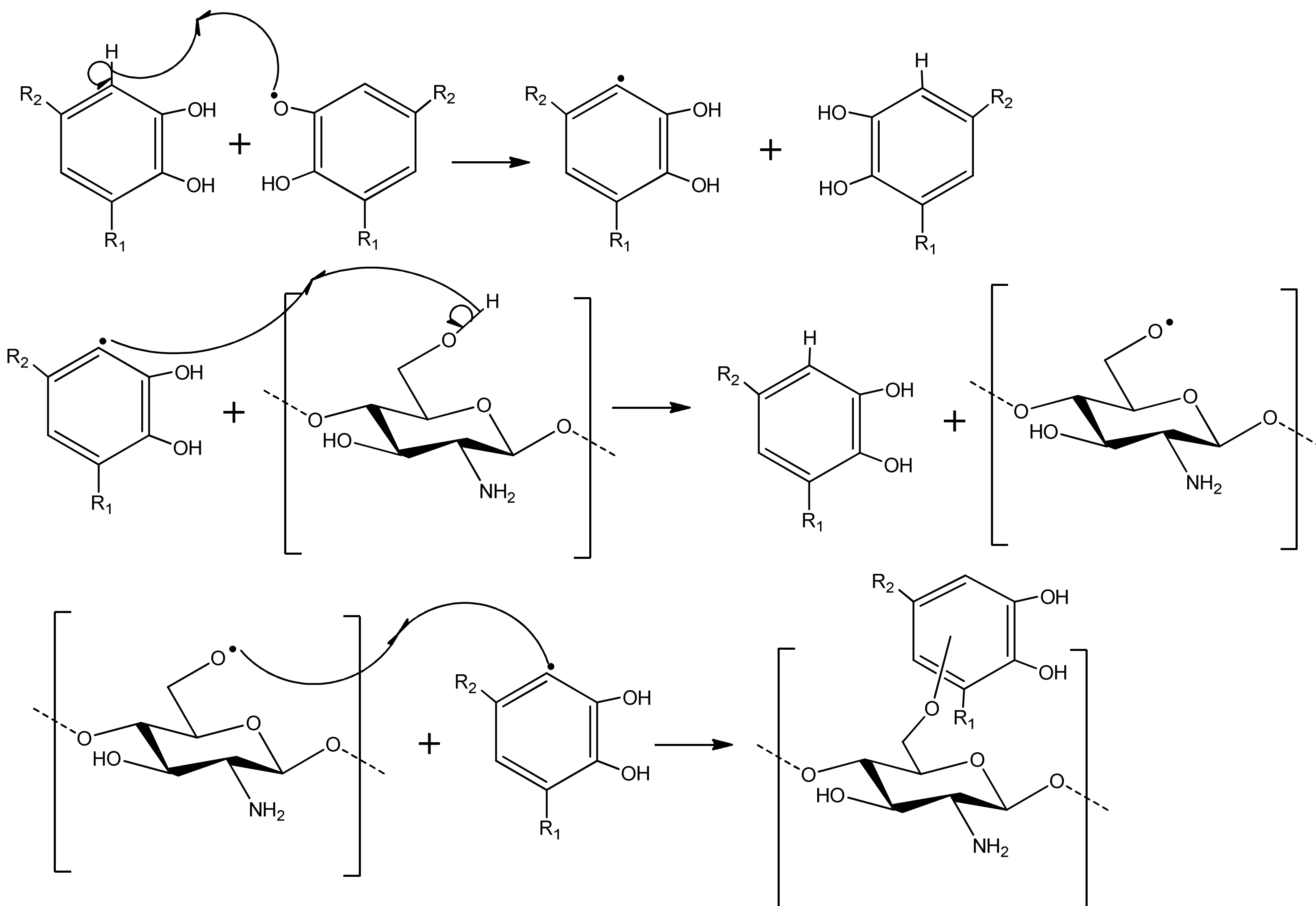
Antioxidant





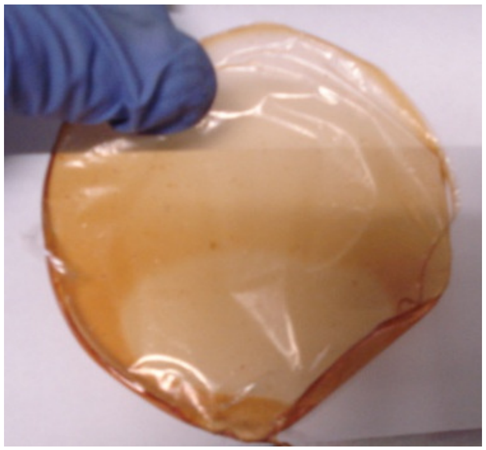
Itzincab-Mejía *et al.* *International Journal of Food Science and Technology*, 48, 2034-2041, 2013
Zavaleta-Avejar *et al.* *Food Hydrocolloids* 39, 113-119, 2014

Proposed mechanism



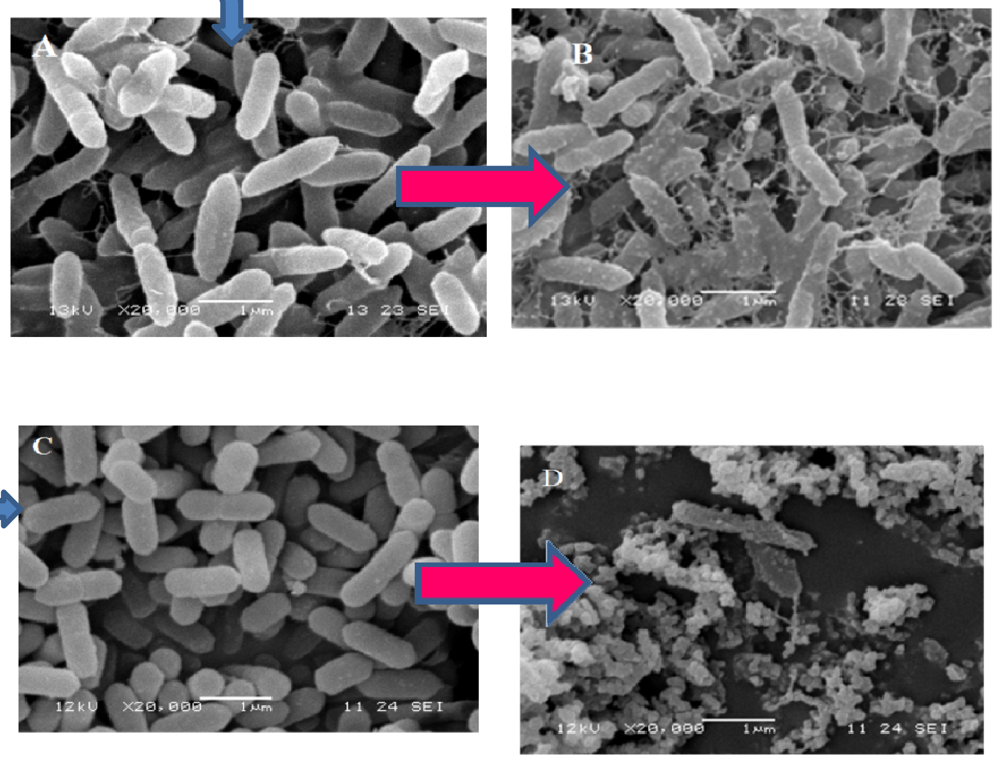
Antimicrobial activities

Film production



E. coli

L. monocytogenes



Research facilities at campus UNAM Faculty of Chemistry

Thank you!

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