

Laccase *Trametes versicolor*: a versatile enzyme for the selective biocatalyzed C-C coupling of phenolic compounds

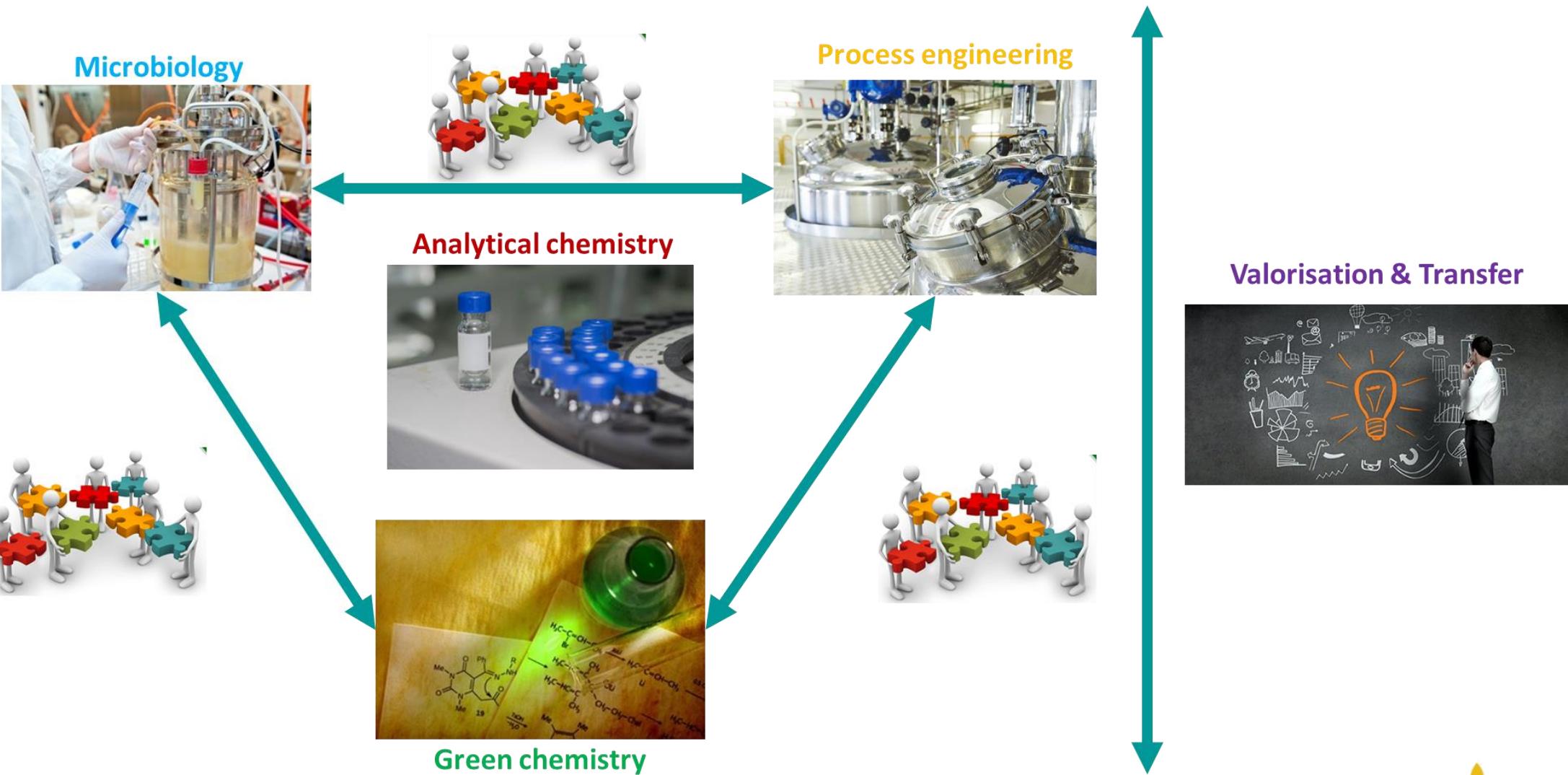
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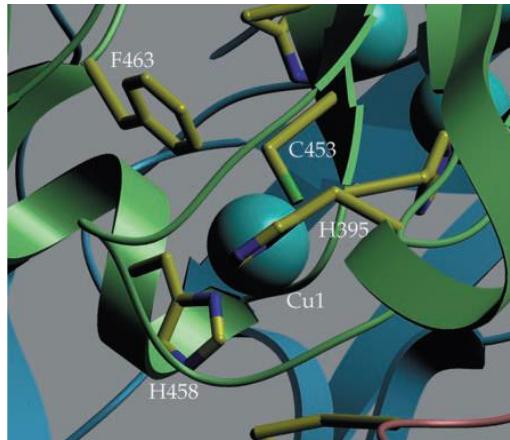
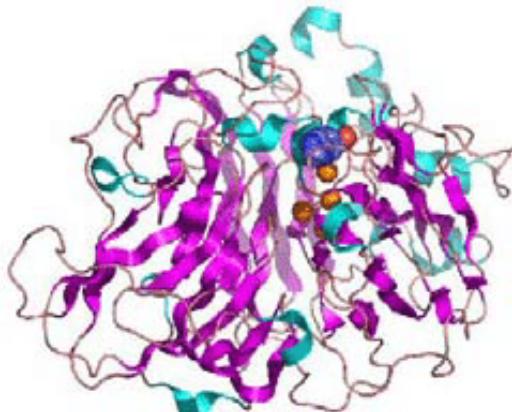
Amandine.flourat@agroparistech.fr

URD ABI : a pluridisciplinary team dedicated to biomass valorization



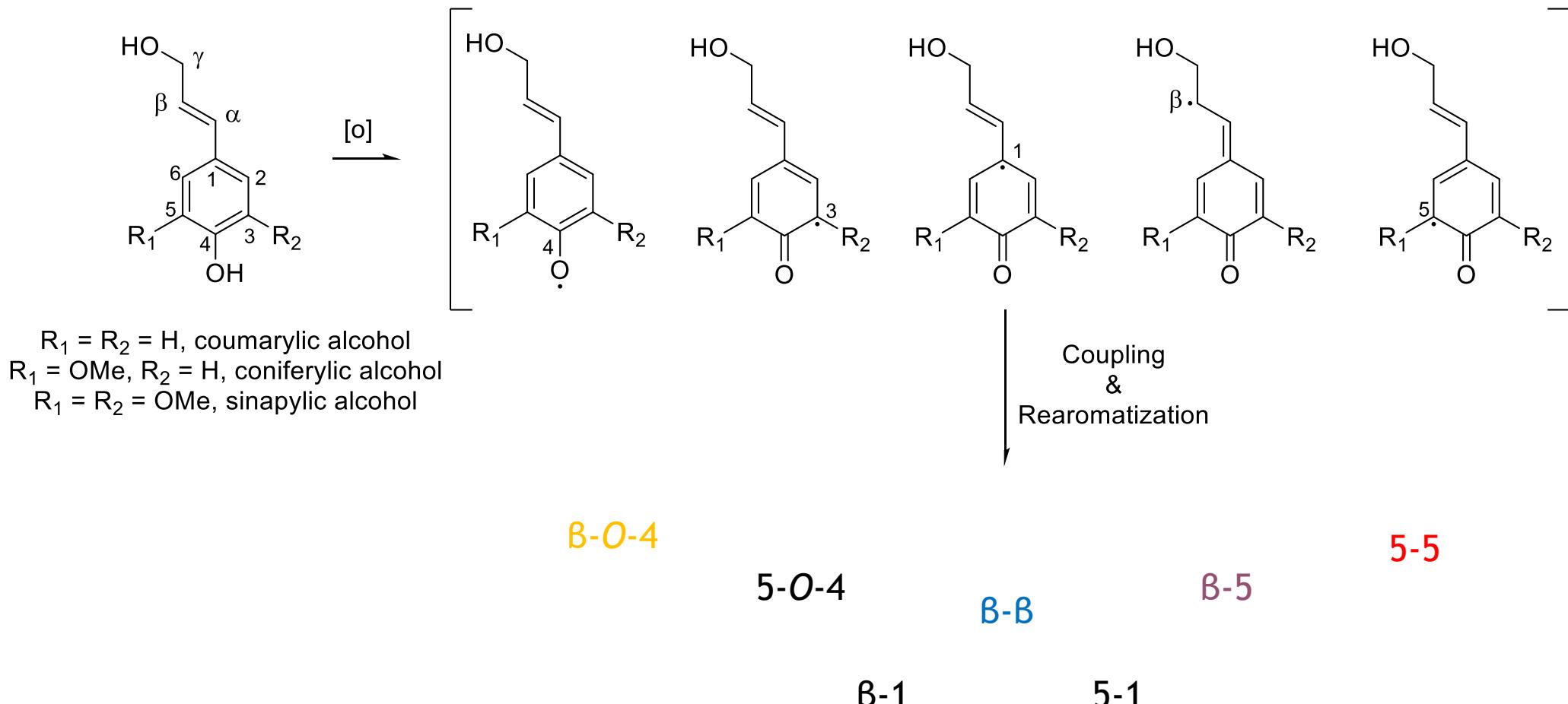
Laccase from *Trametes versicolor*

- ❖ Oxidase family -> need oxygen to be regenerated
- ❖ Stability:
 - Active between pH 3 - 8, preferred pH 5 - 6
 - Active between 10 - 50 °C, preferred 30 °C
- ❖ Catalytic site
 - One Copper (I) which oxidize the phenolic moiety with two histidine residues
 - Three Copper (II) which regenerate the active site



(14) (PDF) Degradation of dyes with microorganisms : studies with ascomycete yeasts
(researchgate.net) <https://doi.org/10.1016/j.biortech.2008.01.084>

Lignin nomenclature



Laccase-mediated 5-5 dimerization

↗ Substrate Vanillin : a single favorable position

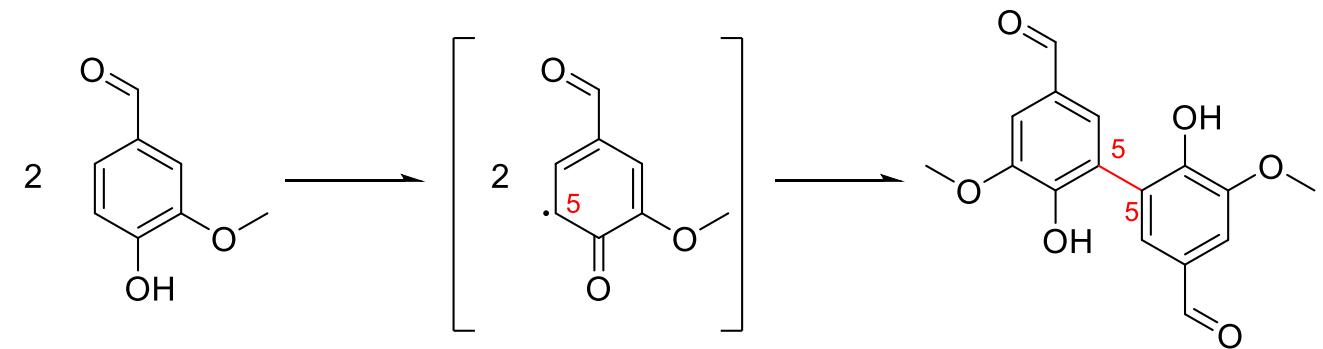
↗ Optimum conditions

- Temperature 42 °C
- pH 6
- [vanillin] = 0.1 mol.L-1
- Enzyme/Substrate 4.6 U.mmol

↗ Yield 96%

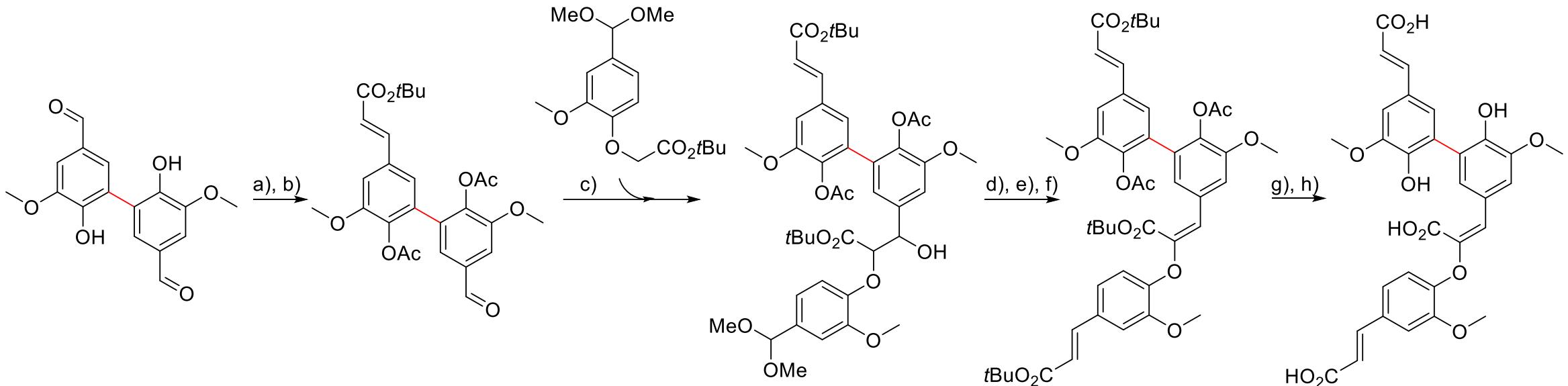
↗ Product recovery through filtration

↗ Scale-up until 50 g



Usage of bisvanillin

Synthesis of naturally occurring lignan oligomers



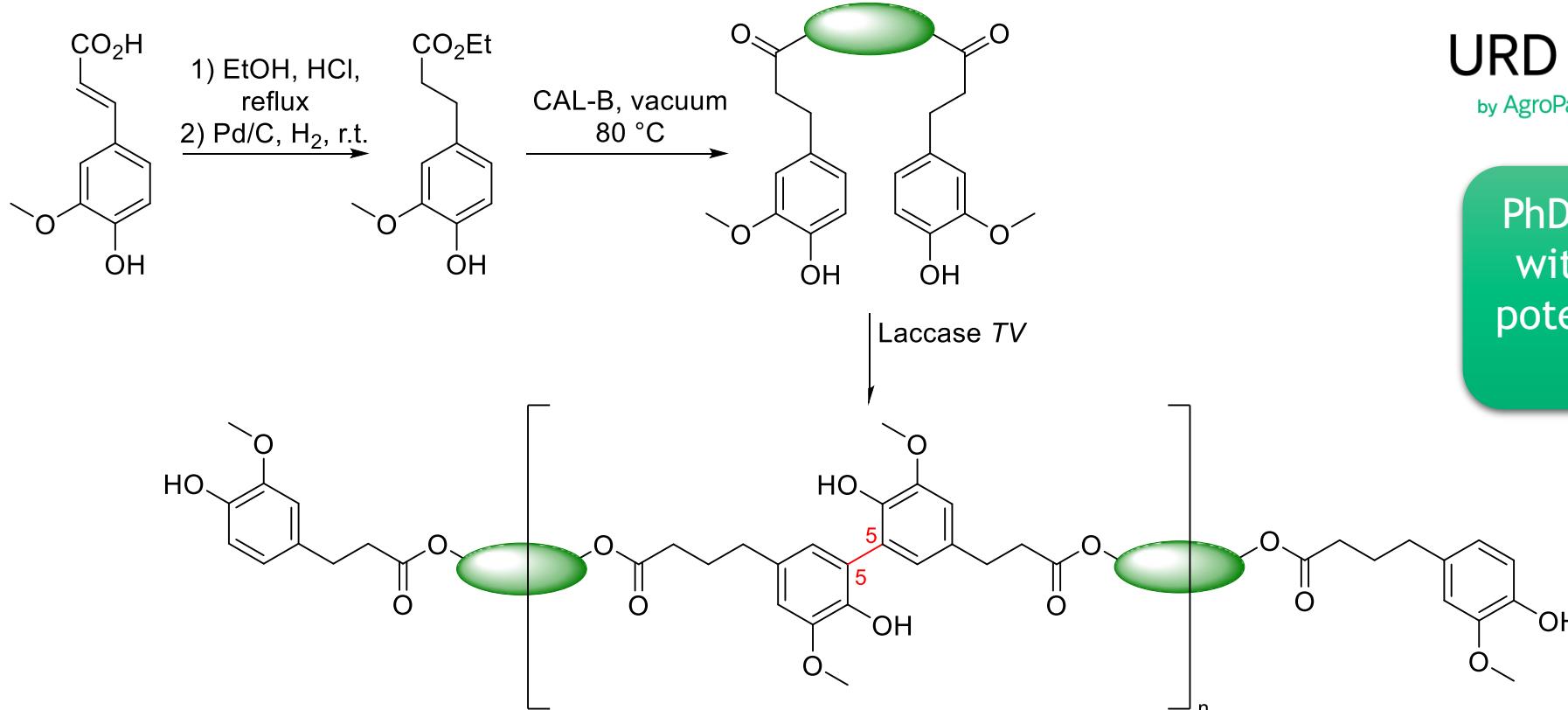
Synthesis of polymers

- Polyacetal with free phenolic moieties = anti-oxidant polymers

Mouterde *et al.* Eur. J. Org. Chem. 2013, 1, 173-179
Allais, F. *et al.* Patent WO 2018211213

Laccase-mediated 5-5 oligomerization

From macrobisphenols



M_n up to 10 kg.mol⁻¹
285 °C < T_d (5%w) < 340 °C
30 °C < T_g < 95 °C

7



PhD will start, in collaboration with URCOM, to evaluate the potential of such compounds in cosmetic formulations

Allais, F. et al. WO2015055936
Reano, A. F. et al. *Green Chem.* 2016, 18, 3334

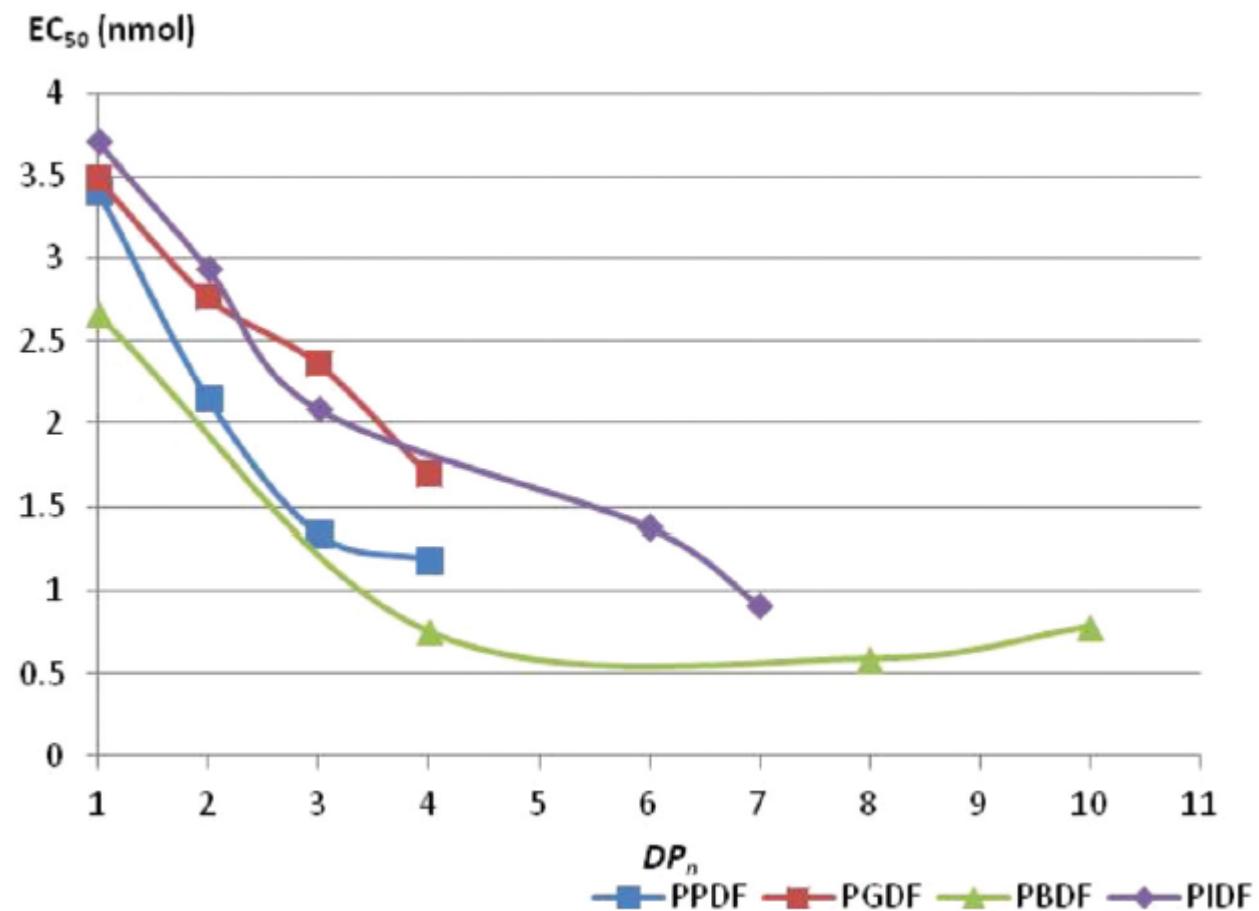
Properties of 5-5 oligomers

✓ Tunable size of oligomers

- Temperature
- Solvent
- Enzymatic loading
- Ratio Solvent/Buffer

✓ High anti-radical efficiency

- Low EC₅₀ compare to BHT (8.9 or BHA (7.7)



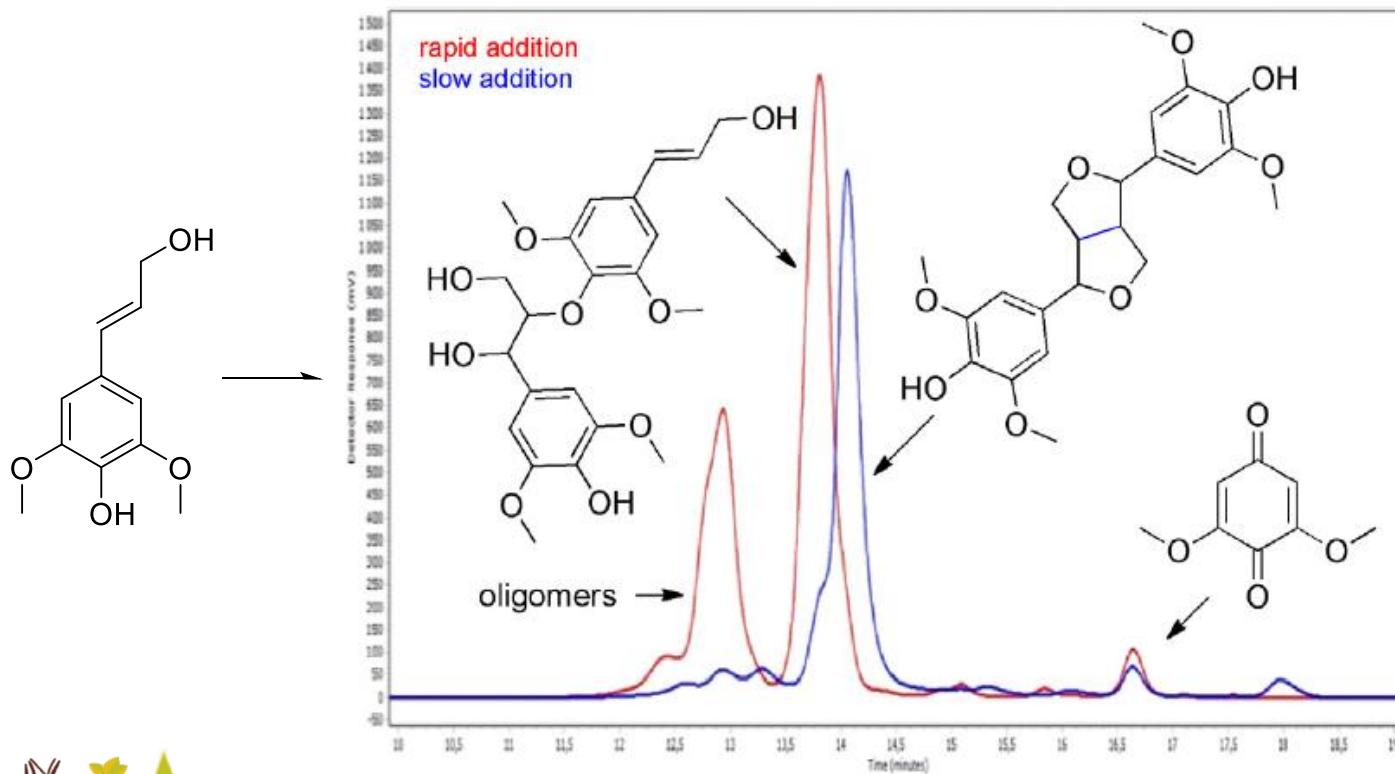
Allais, F. et al. WO2015055936

Reano, A. F. et al. *Green Chem.* **2016**, *18*, 3334

Laccase mediated β - β dimerization

Case of sinapyl alcohol

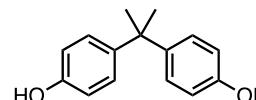
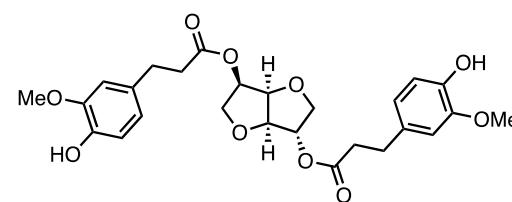
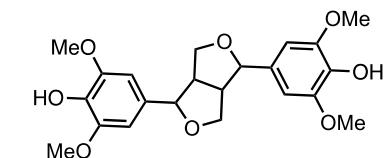
- Reverse selectivity depending of experimental conditions
 - Different mechanism depending of the amount of radical species at « t » time



After optimisation
93% yield, no need of silica gel purification

- ↓
- Synthesis of polymers
- Polyolefins
 - Epoxy-amine resins
 - Poly(hydroxy)urethanes (NIPUs)
 - Thiol-ene polymers

Syringaresinol : Bisphenol A substitute?

	Bisphenol-A (BPA)	Isosorbide diferulate (IDF)	Syringaresinol (SYR)	
				
Sourcing	<input checked="" type="checkbox"/> Oil	<input checked="" type="checkbox"/> Ferulic acid	<input checked="" type="checkbox"/> Sinapic acid	
Toxicity	<input checked="" type="checkbox"/> Endocrine disruptor	<input checked="" type="checkbox"/> No endocrine activity	<input checked="" type="checkbox"/> No endocrine activity	
EPOXY-AMINE RESINS	Mechanical properties	<input checked="" type="checkbox"/> $T_g = 150 \text{ }^\circ\text{C}$ <input checked="" type="checkbox"/> $T_\alpha = 166 \text{ }^\circ\text{C}$ <input checked="" type="checkbox"/> $T_{deg} = 326 \text{ }^\circ\text{C}$	$T_g = 85 \text{ }^\circ\text{C}$ $T_\alpha = 99 \text{ }^\circ\text{C}$ $T_{deg} = 295 \text{ }^\circ\text{C}$	<input checked="" type="checkbox"/> $T_g = 126 \text{ }^\circ\text{C}$ <input checked="" type="checkbox"/> $T_\alpha = 157 \text{ }^\circ\text{C}$ <input checked="" type="checkbox"/> $T_{deg} = 298 \text{ }^\circ\text{C}$
Degradation		<input checked="" type="checkbox"/> No degradation (NaOH nor HCl)	<input checked="" type="checkbox"/> Degradable (NaOH and HCl)	<input checked="" type="checkbox"/> No degradation (NaOH nor HCl)



Laccase mediated β - β dimerization

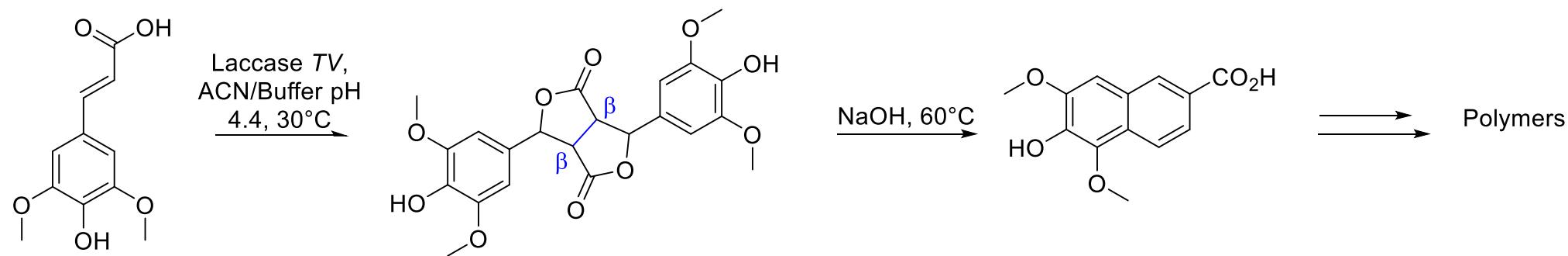
Case of sinapic acid

- Concentration 0.4 mol.L⁻¹
- Enzyme/Substrate 7 U.mmol⁻¹
- 30 °C
- pH 4.4

Total selectivity, high yield (>90%)

Application

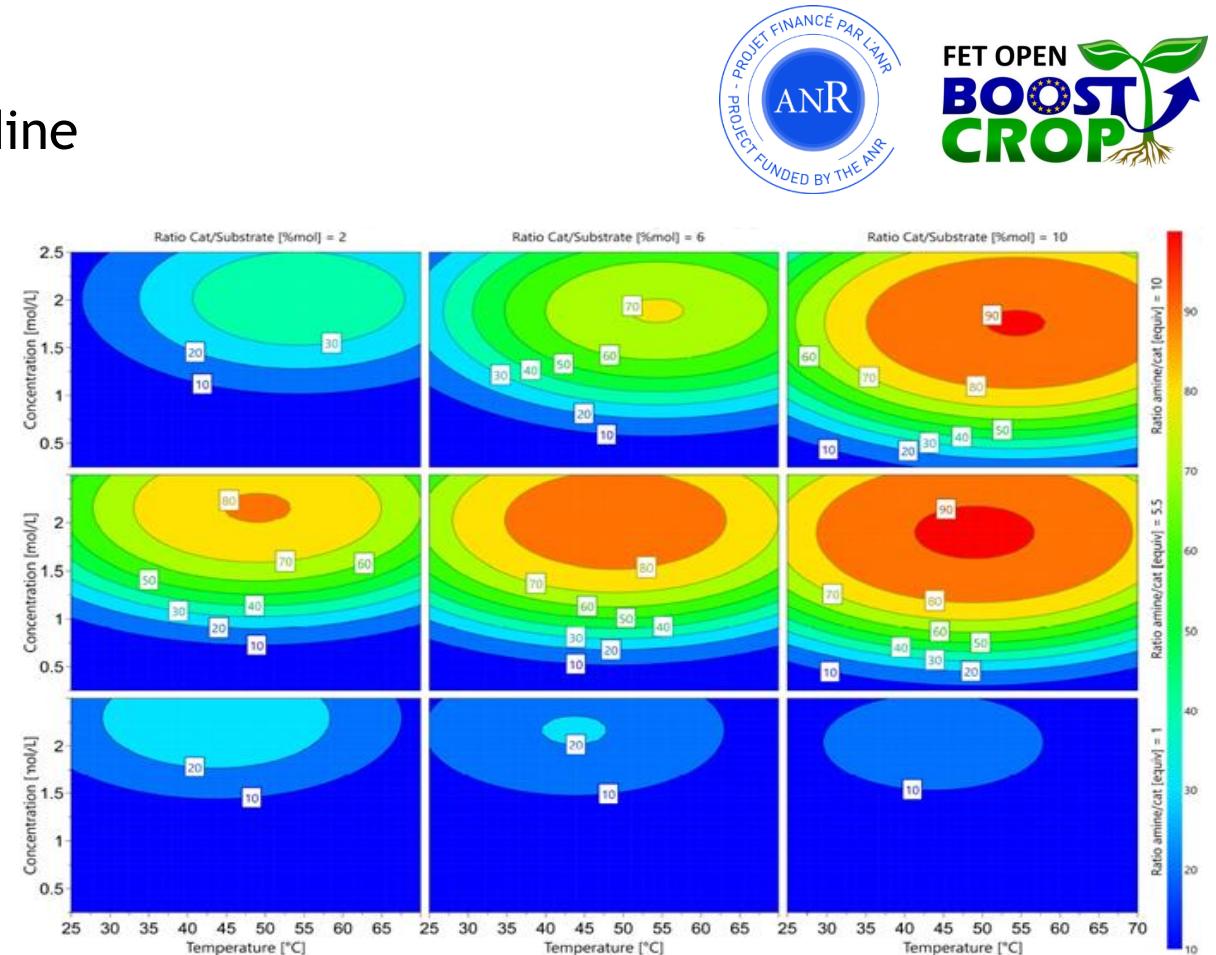
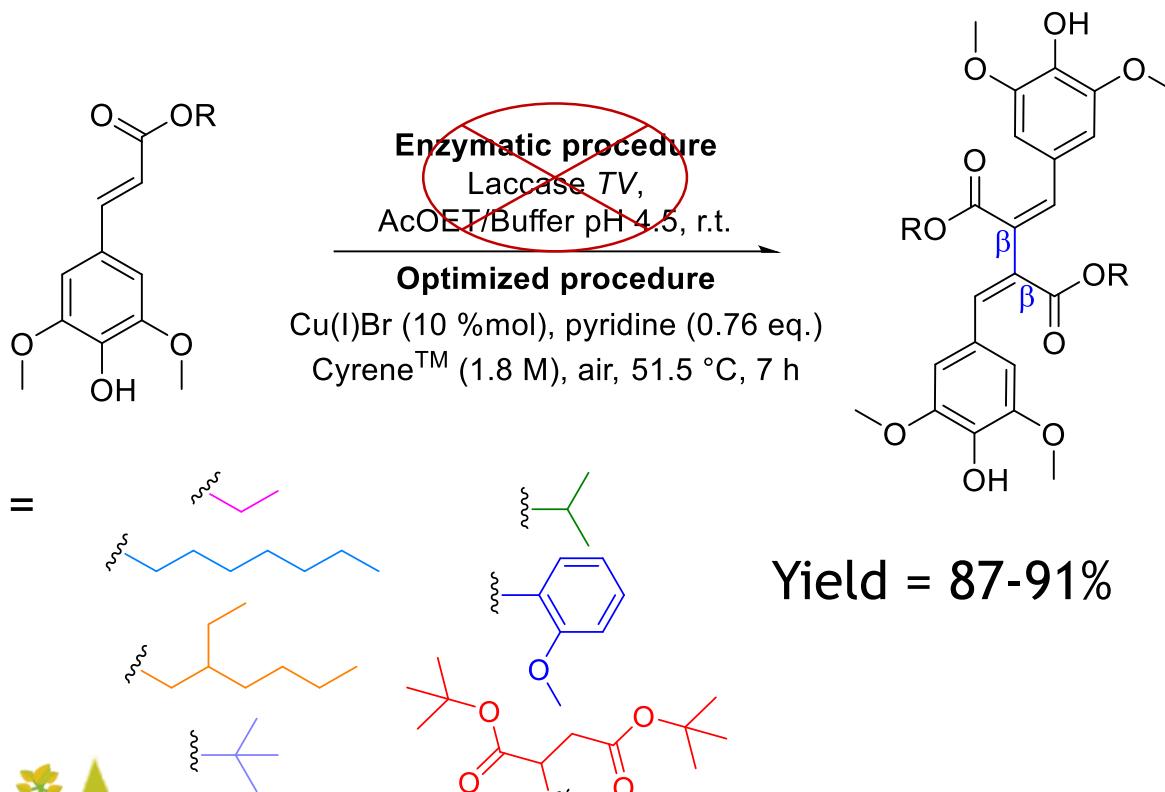
- Synthesis of dimethyl naphtanoic acid
 - Precursor for polymers
 - Potential substituent for petro-sourced 2-naphtanoic acid



Laccase mediated β - β dimerization

Case of sinapic esters

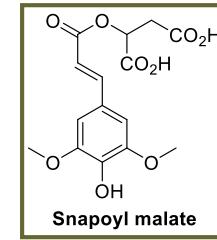
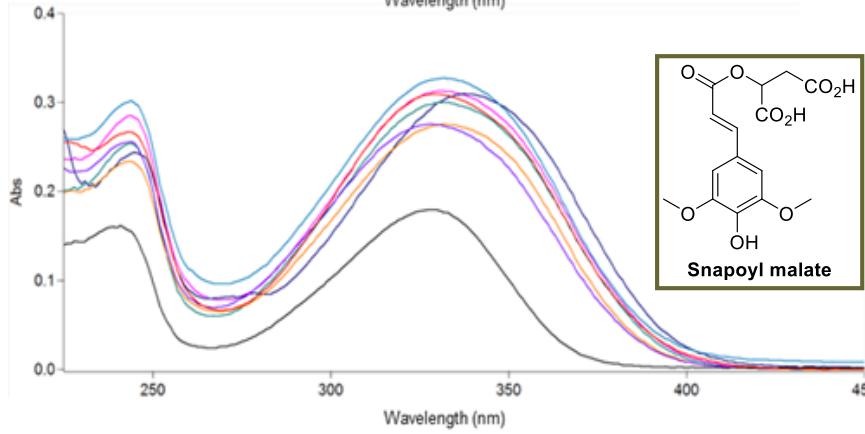
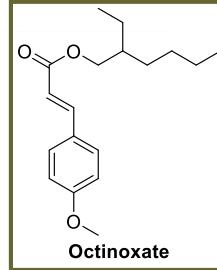
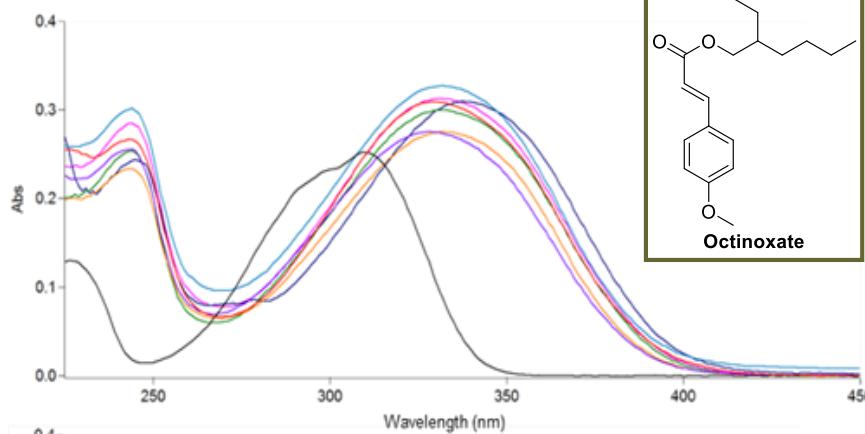
- No selectivity and majority of β -O-4
- Mimic of laccase using Cu(I)Br and pyridine



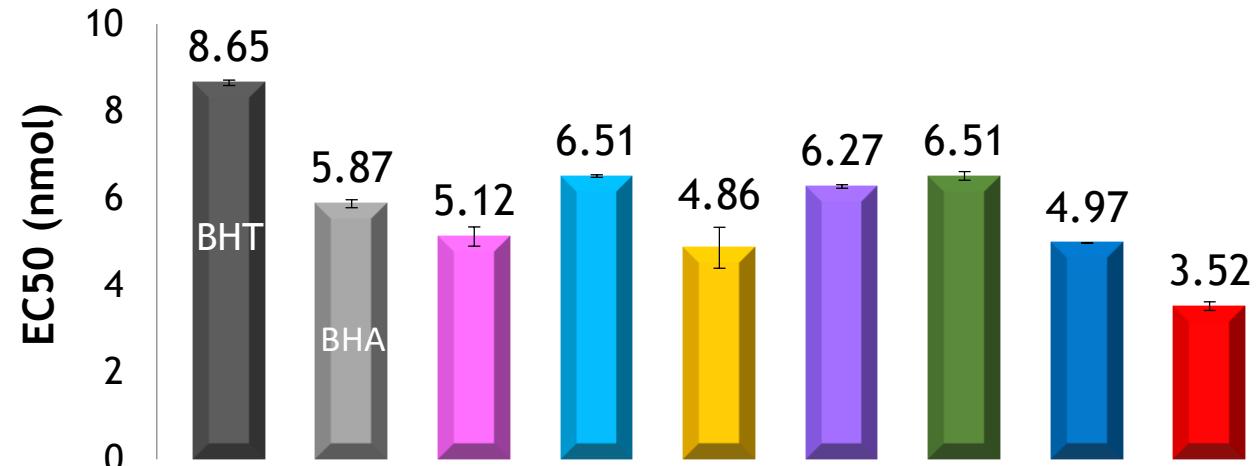
Mention, M. et al. *Green Chem.* 2020

Properties of β - β' sinapate dimers

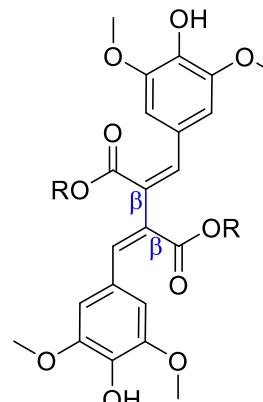
Anti-UV activity



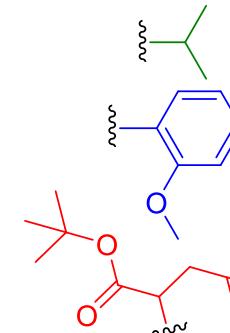
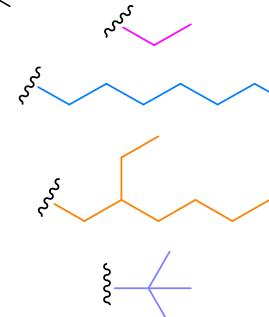
Antiradical activity



Antiradical properties from the ABTS^{•+} assay: benchmarking of the sinapate esters β - β' dimers against BHT and BHA



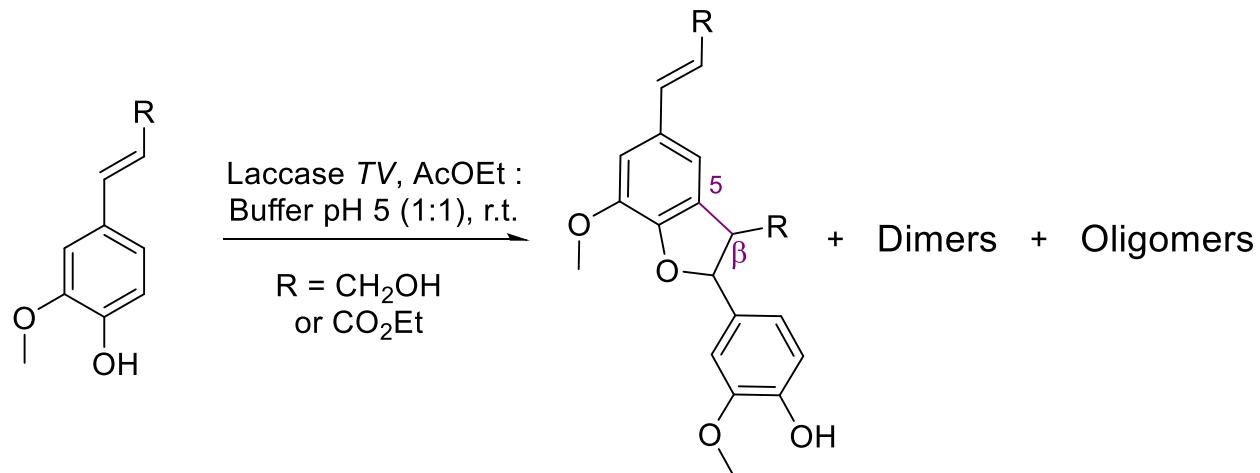
R =



Laccase-mediated β -5 dimerization

Starting material ethyl ferulate or coniferyl alcohol

- 5 positions for radical coupling (3 favorables) => β -5, β - β , β -O-4, 5-O-4, 5-5
- Oligomerization



Biphasic media at room temperature, pH 5 and 16 U. mmol^{-1} of laccase and slow addition (9 mL.h $^{-1}$)

- Selectivity toward 8-5
- Yield up to 43%

Laccase-mediated resveratrol oxidation

Production of resveratrol dimers

- Literature reports only δ -viniferine through enzymatic procedure with a maximum of 33% yield
- Modification of experimental conditions allows to achieve 25% of labruscol
- Cosmetic and medicinal fields



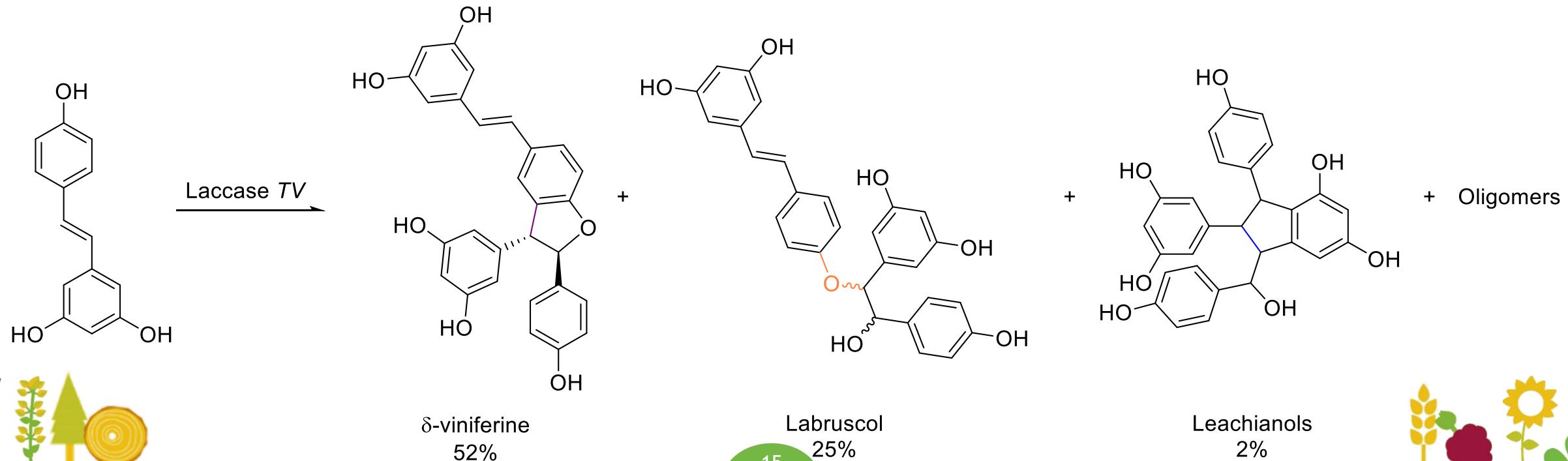
Glycostil

noveal
INGREDIENTS FOR BEAUTY

RIBP
Résistance Induite et
Bio-protection des Plantes

MEDyC

ICMR
Institut de Chimie Moléculaire de Reims
UMR CNRS 7312

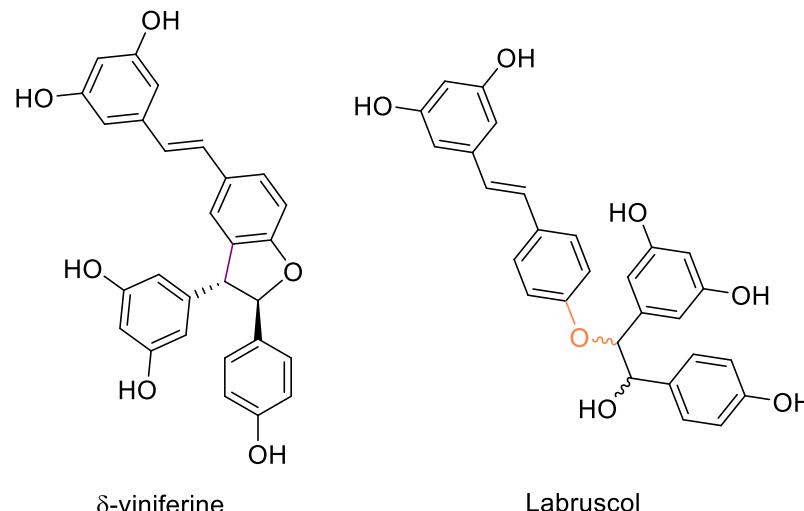
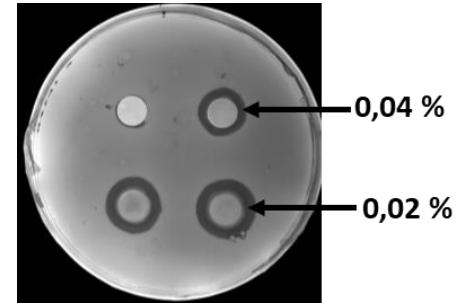


Laccase-mediated resveratrol oxidation

Properties of resveratrol dimers

- Antiradical activity through DPPH scavenging
- Antimicrobial activities

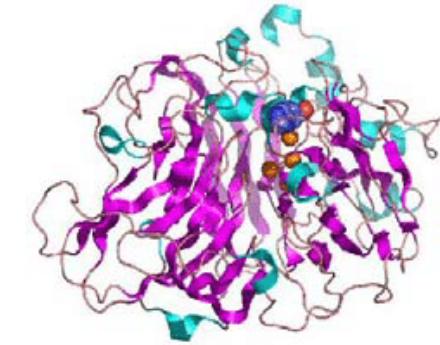
Compound	Ec50 (nmol)	Antimicrobial	Antifungal
δ-viniferin	6.3	+++	-
Labruscol	14.1	++	-



Take home messages

✓ Laccase is a versatile enzyme

- Middle conditions (15-50 °C)
- With co-solvent (up to 70%)

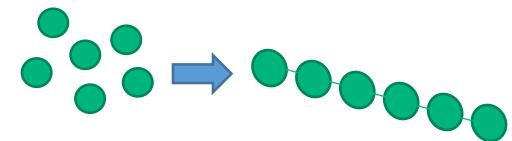


✓ Experimental conditions can reverse selectivity

- Ratio of radical species at anytime
- Temperature

✓ Access to valuable compounds

- Antiradical activity
- Anti-UV activity
- Antimicrobial activity
- Monomers for bio-based polymers



Thanks



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Chargée de recherches en chimie verte

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