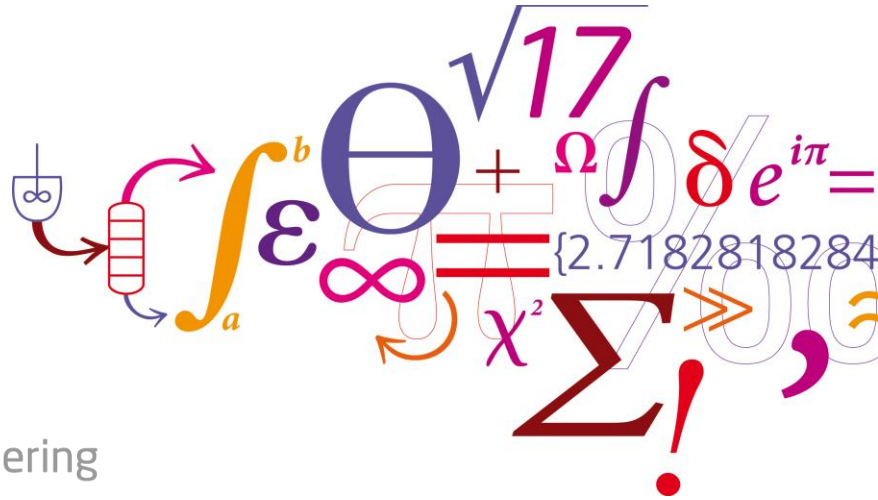
Paris, 2016 Conversion of Biomass: Green Chemistry and Innovative Processes

L'Institut Francais du Danemark

Biocatalytic enzyme processes for CO_2 conversion and lignin modification



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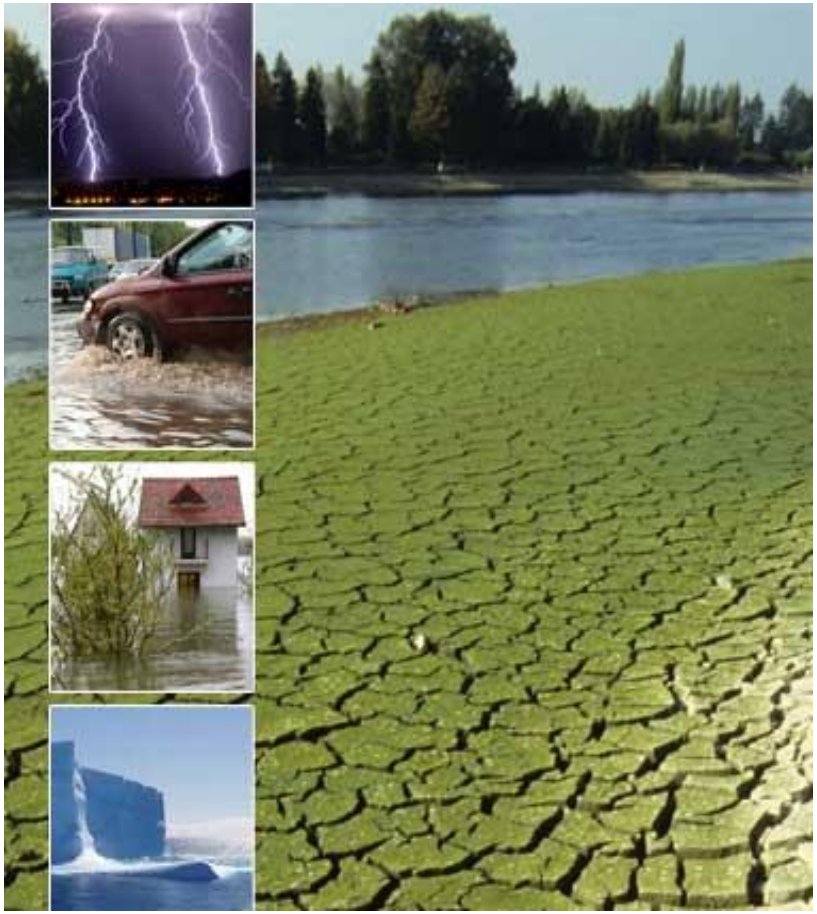
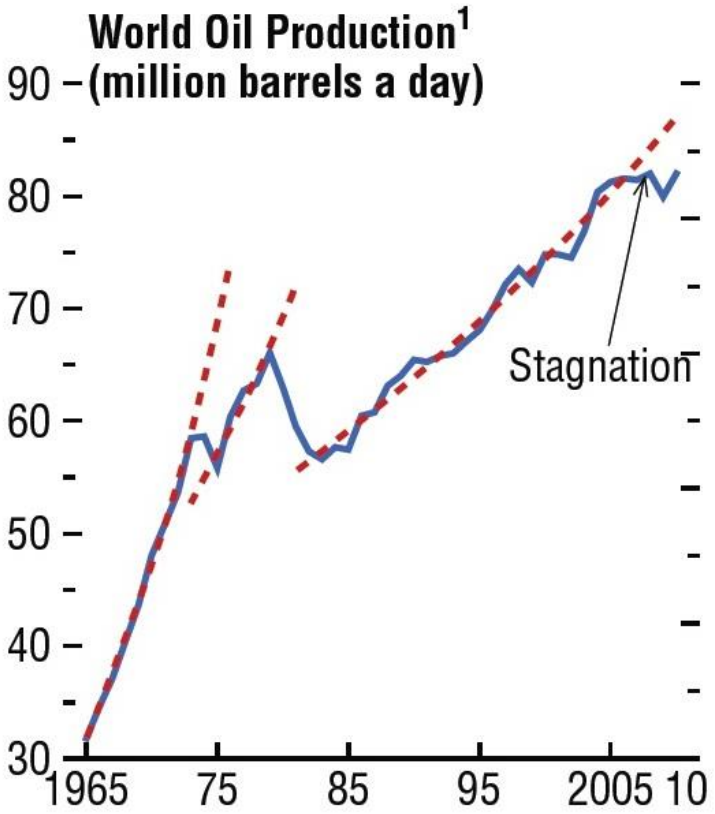
New challenges: Global challenges

- Biocatalytic conversion of CO_2
- Enzymatic upgrading of lignin





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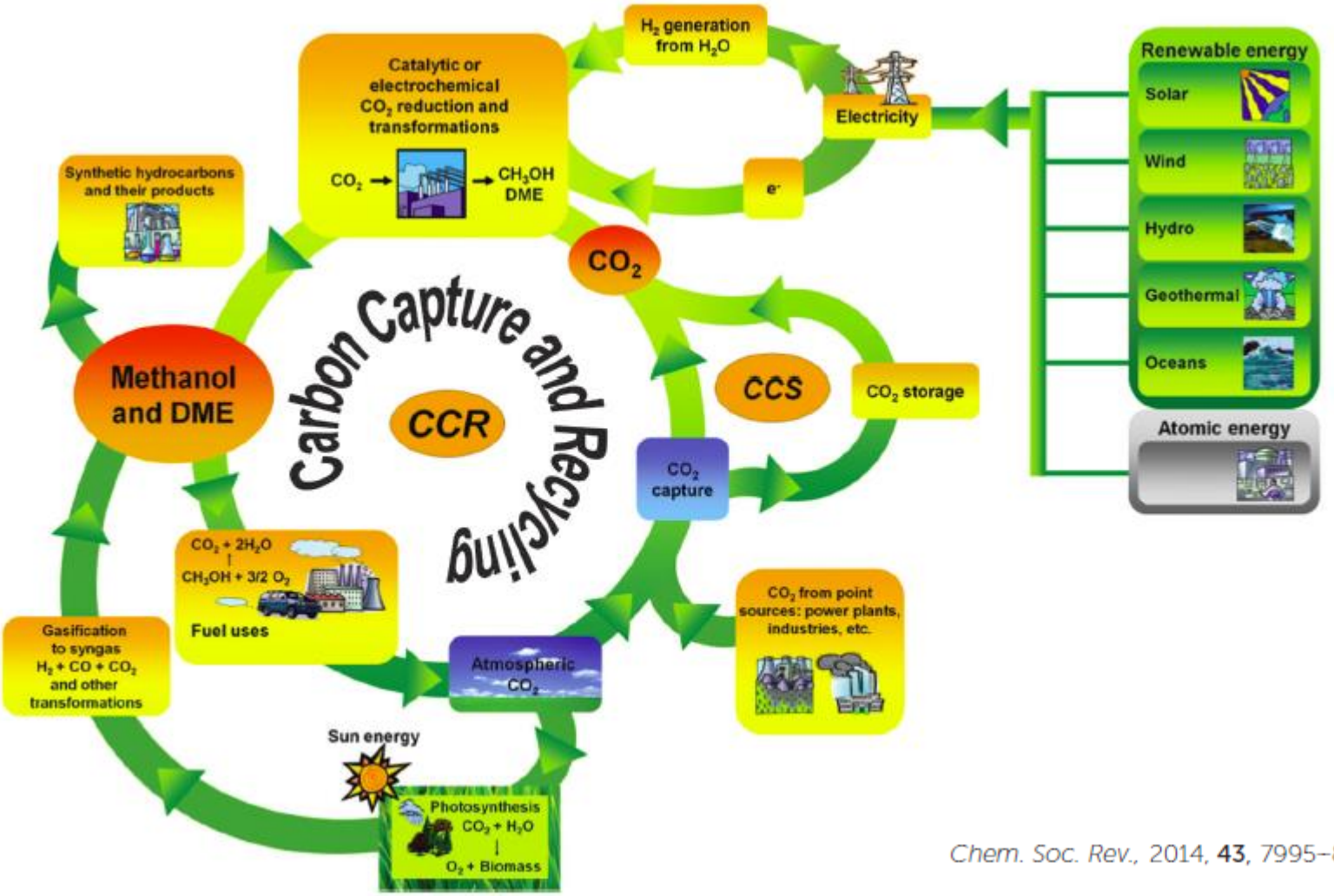
Carbon cycle within the ”Methanol Economy”



Chem. Soc. Rev., 2014, **43**, 7995–8048

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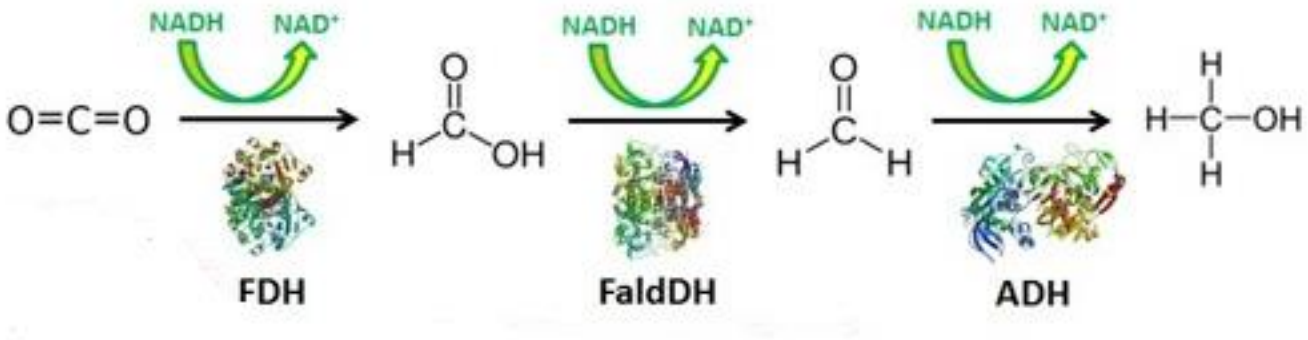
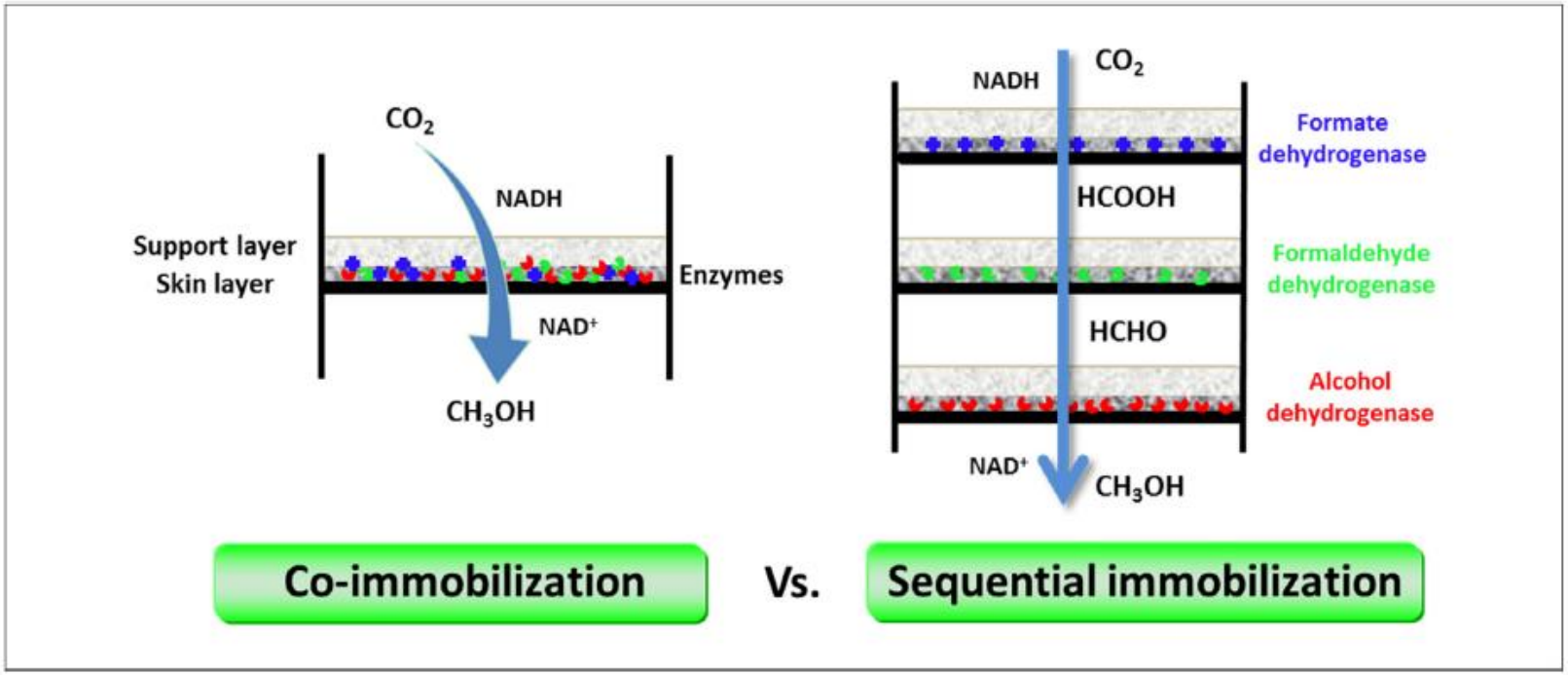
Biocatalytic conversion of CO_2 to methanol





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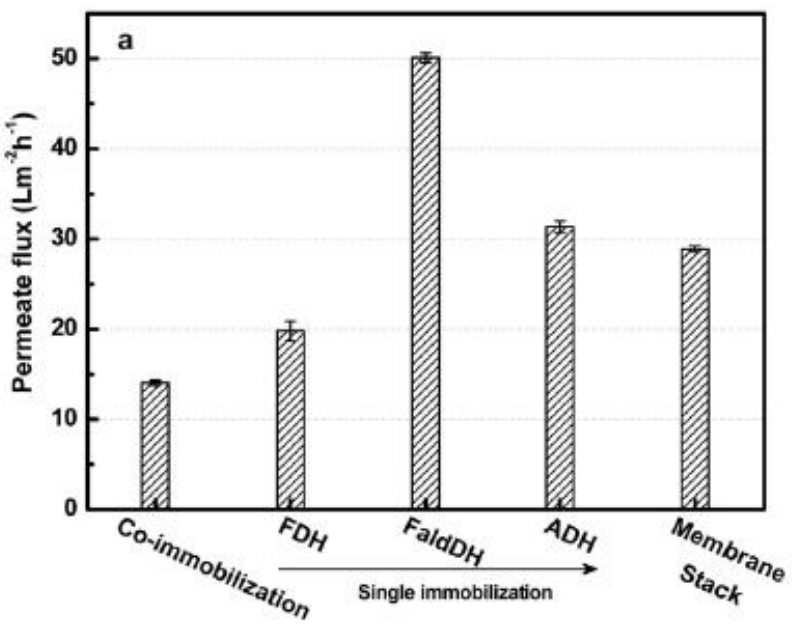
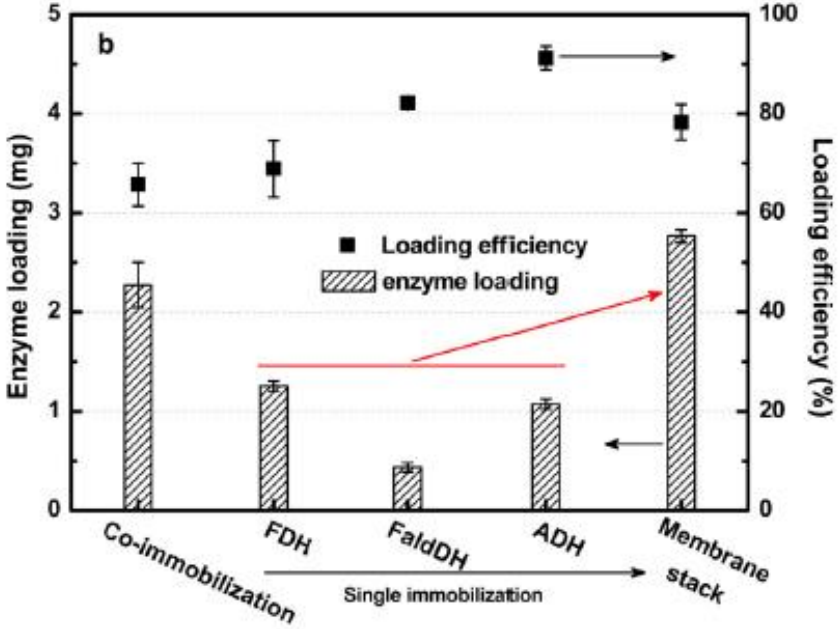
New Biotechnol 2015 32, 319-327

CO_2 as a substrate to produce methanol





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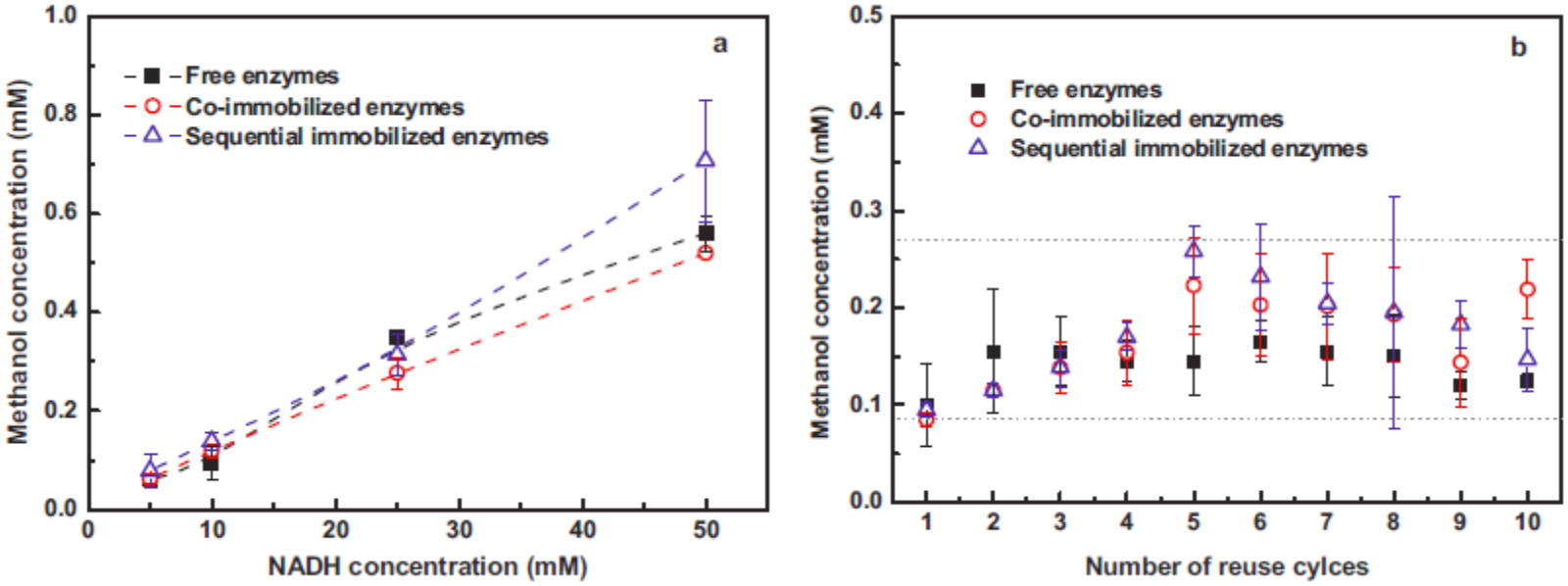
It works! But methanol conc. is low



(a) Methanol production at different NADH concentrations with free and immobilized enzymes and **(b)** with recycling and reusing of free and immobilized enzymes (NADH = 10 mM). Reaction time = 30 min. Enzyme and NADH concentrations were same for free and immobilized enzyme systems; in order to reuse free enzymes, an enzymatic membrane reactor equipped with 10 kDa regenerated cellulose membrane (skin layer facing feed) was used.

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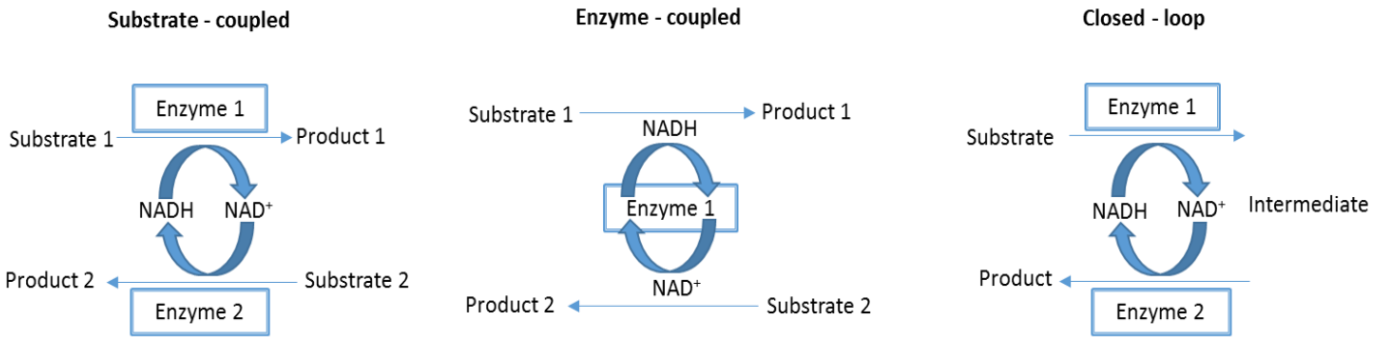
The bottleneck

Kinetic parameters of enzymes for different reactions.			
Enzyme/reaction		K_m (mM)	V_{max} (mM min^{-1})
Formate dehydrogenase	$CO_2 \rightarrow HCOOH$[a]	30–50	0.002
	$HCOOH \rightarrow CO_2$	3.3	0.02
Formaldehyde dehydrogenase	$HCOOH \rightarrow HCHO$[b]	NA	NA
	$HCHO \rightarrow HCOOH$	0.06	0.01
Alcohol dehydrogenase	$HCHO \rightarrow CH_3OH$	17.5	0.3
	$CH_3OH \rightarrow HCHO$	275	0.5×10^{-3}



More efficient enzymes are needed

We currently work on the co-factor regeneration system

Concept CO_2 to Methanol works!

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New Biotechnol 2015 32, 319-327

Vision: CO_2 conversion at exhaust site

The biocatalytic CO_2 technologies are genuinely disruptive

Unique green technology that have potential for export globally

Huge financial potential for European companies

Very interested in collaboration in this area

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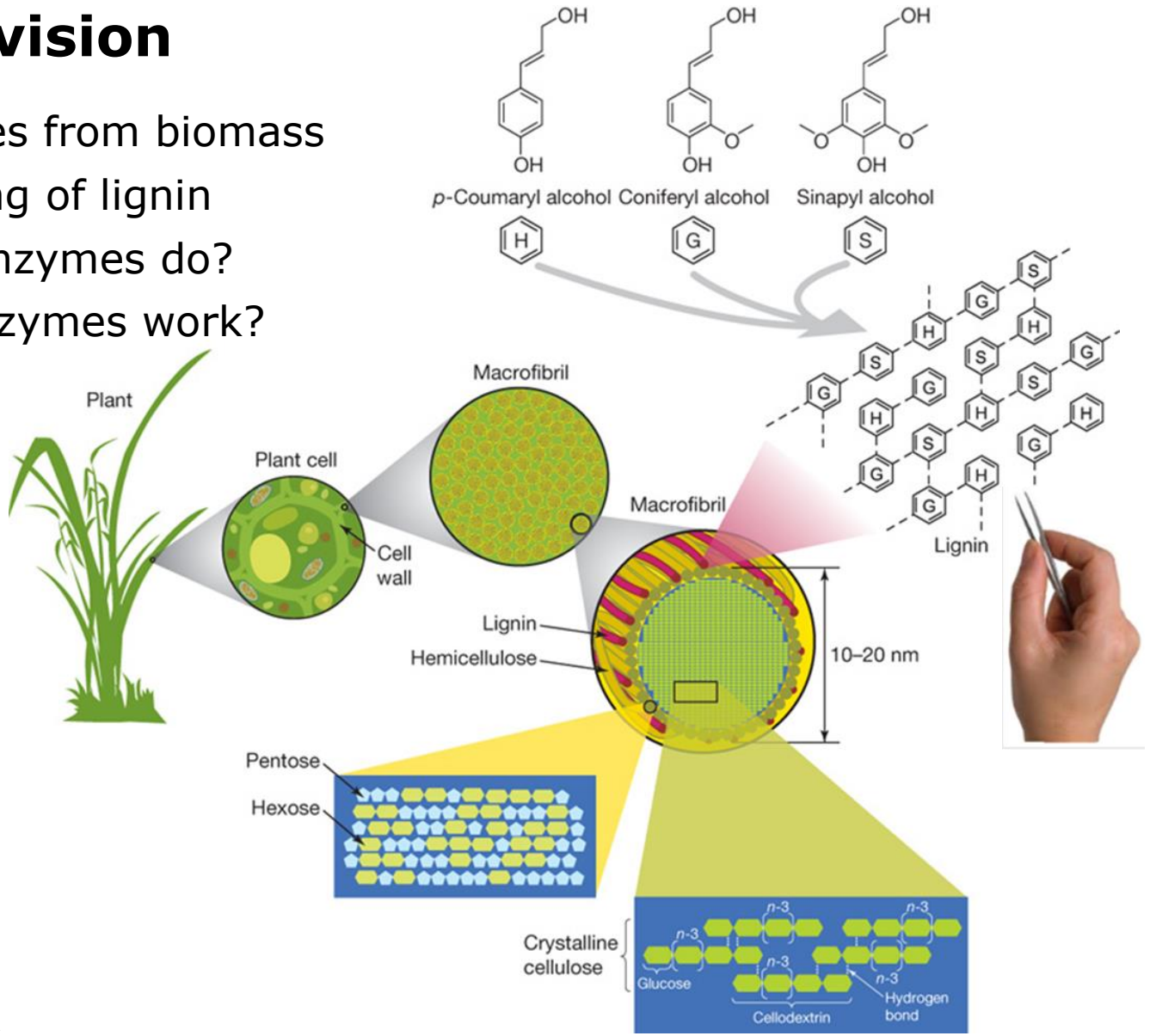
Enzymatic lignin modification

Biorefinery vision

- Useful molecules from biomass
- Useful upgrading of lignin
- **What** do the enzymes do?
- **How** do the enzymes work?



White rot fungi grow on lignocellulose

Ganoderma lucidum

Phanerochaete chrysosporium

Trametes versicolor

"The *G. lucidum* genome encodes one of the richest sets of wood degradation enzymes among all sequenced basidiomycetes" (..and the set includes laccases)

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Genome sequence of the model medicinal mushroom *Ganoderma lucidum. Nature Comm.* 3:913 | DOI: 10.1038/ncomms1923

Enzymatic modification of lignin by laccases?

Laccase EC 1.10.3.2, AA1

4 benzenediol + O_2 ⇄ 4 benzosemiquinone + 2 H_2O

or: 4 monophenols + O_2 ⇄ 4 quinones + 2 H_2O

Role of laccases in nature ?

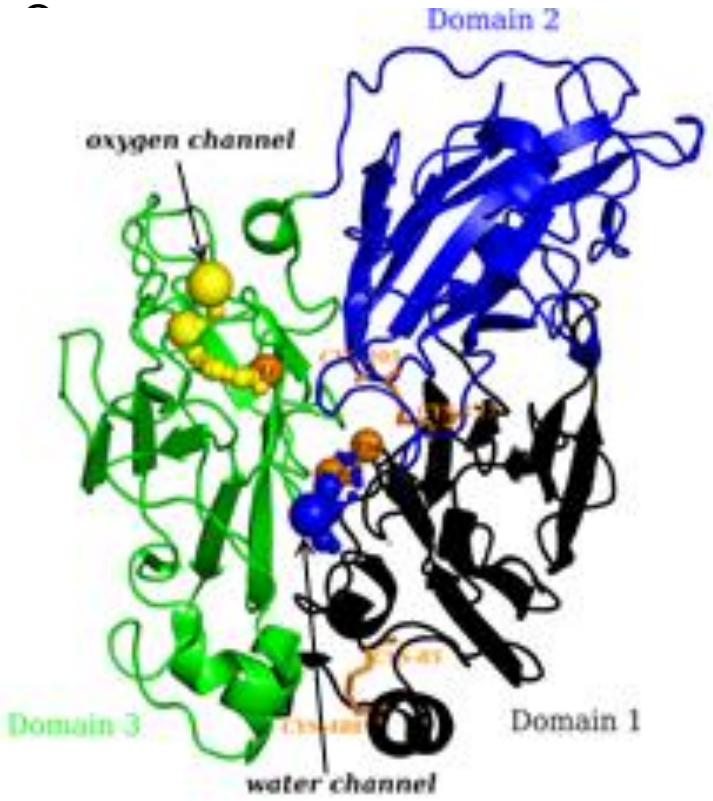
Lignification, delignification

Fungal virulence?

Plant laccases: ”Stress management”

Fungal laccases: 520-550 aa

60-70 kDa, pI ~ 4



Trametes versicolor laccase

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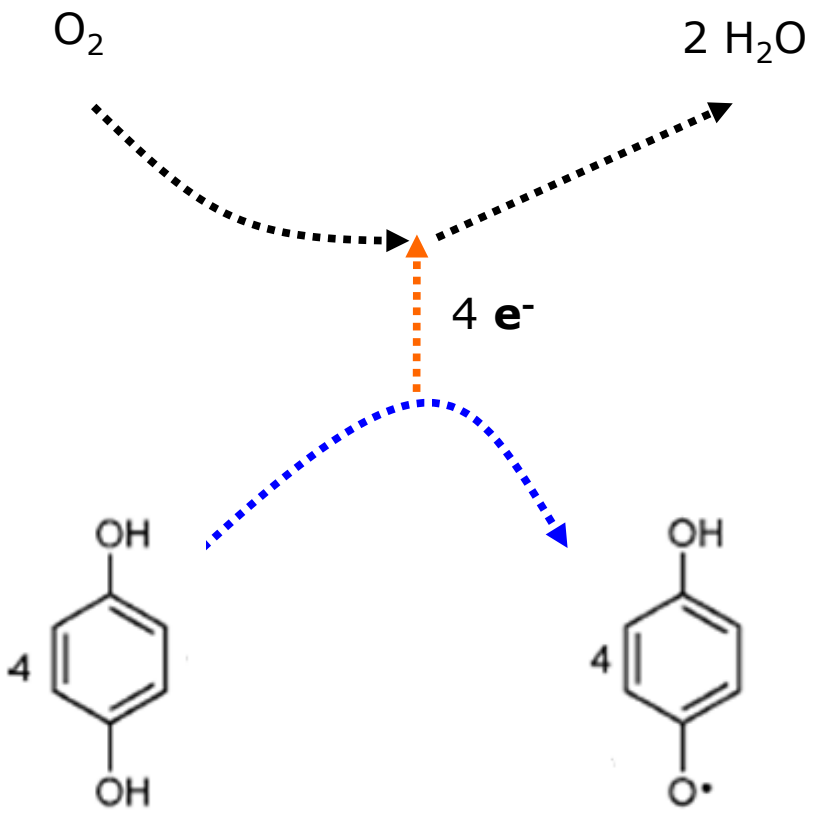
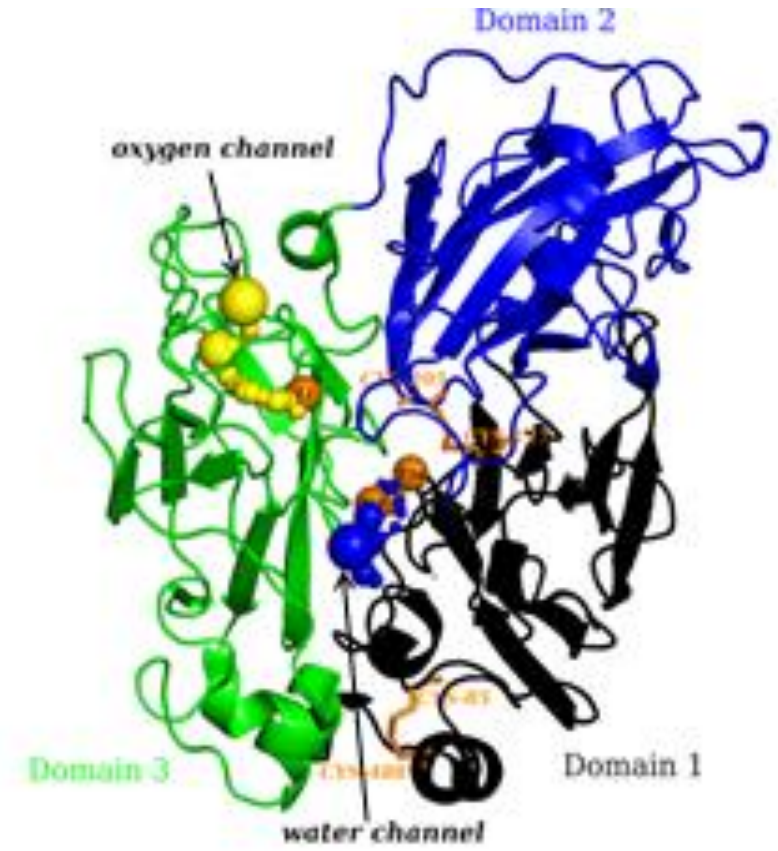
Laccase

- Broad electron donor substrate specificity
- Oxygen in, water out, hence 4 e^- transfer





Trametes versicolor laccase

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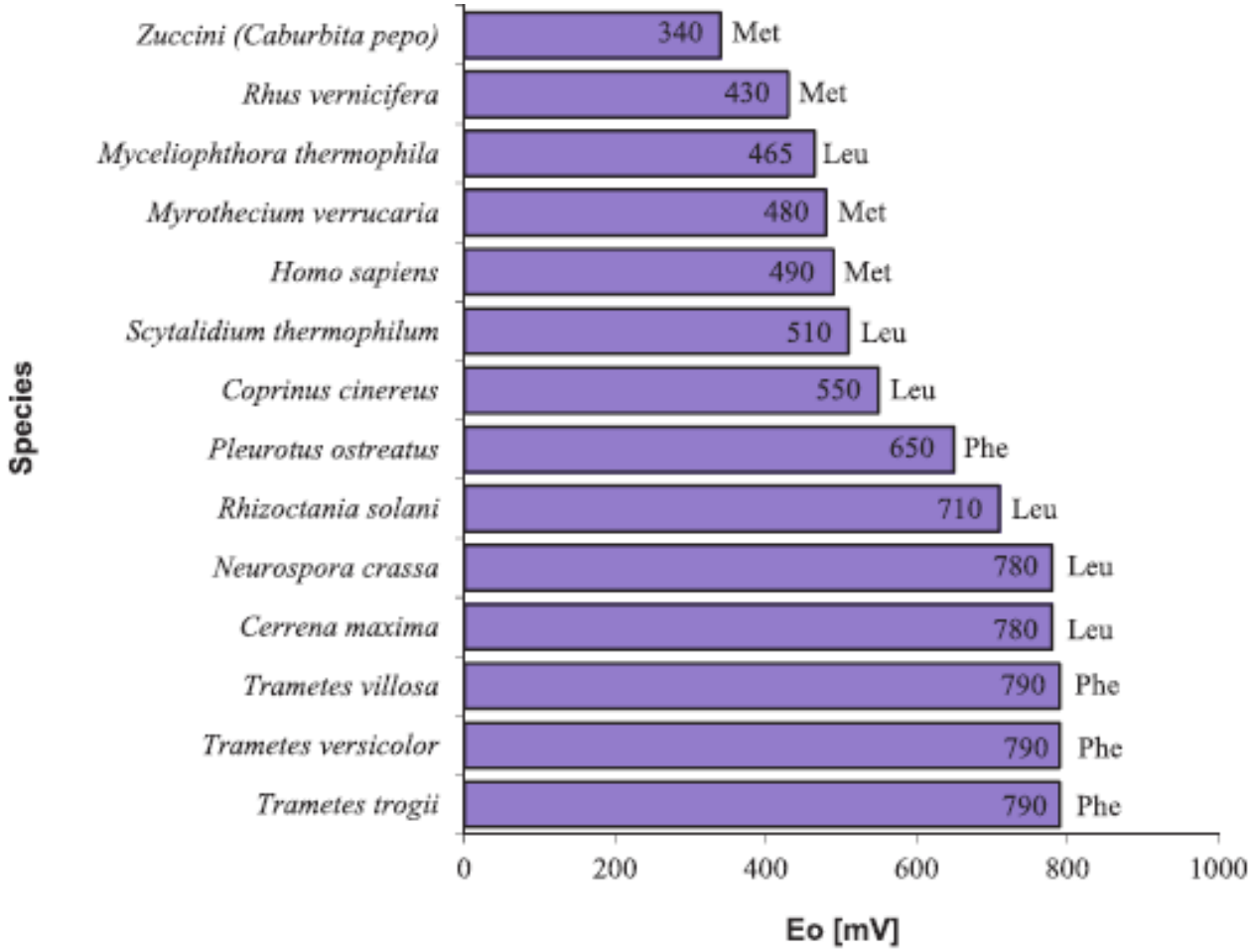
Fungal laccases high redox potential



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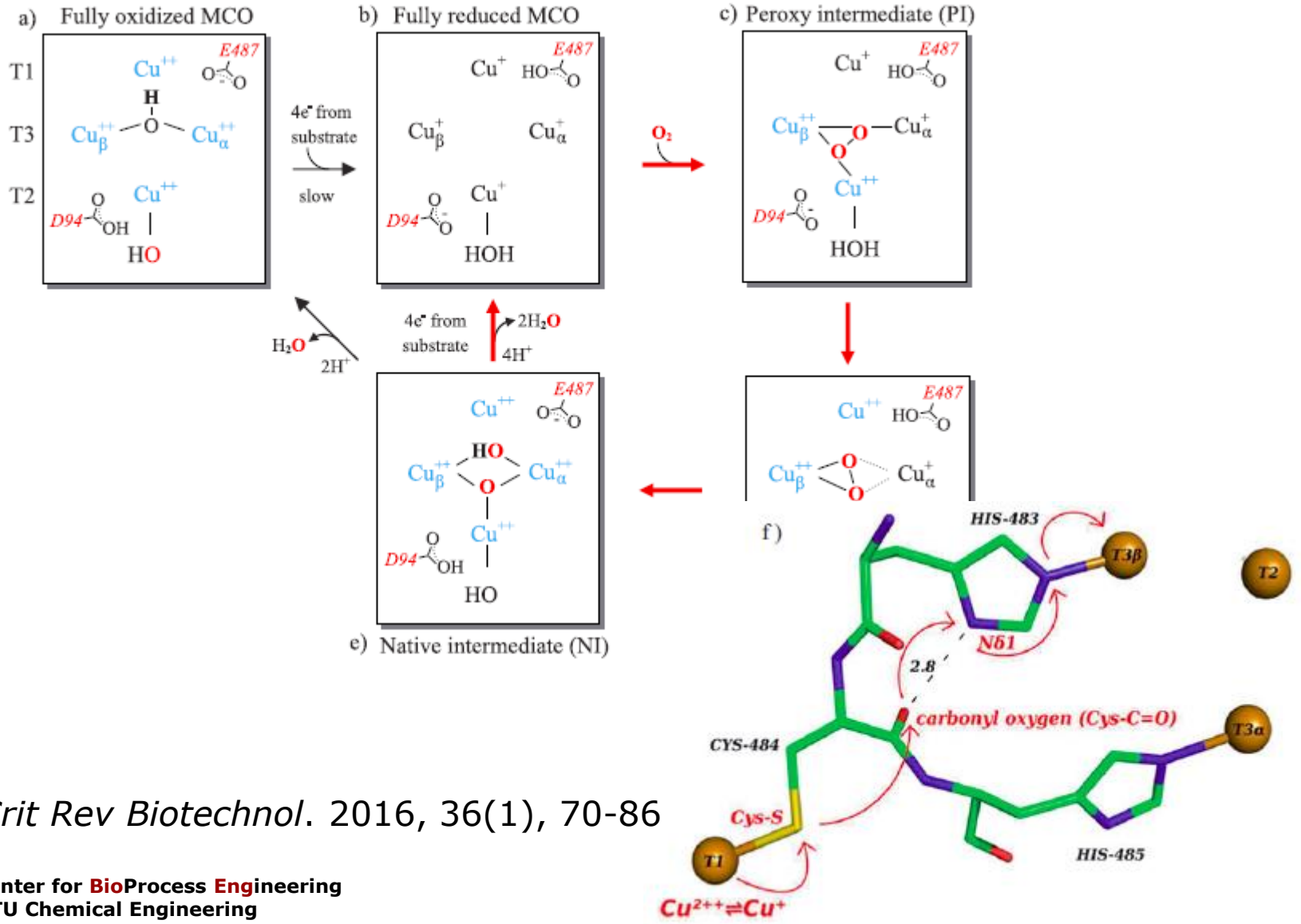
Crit Rev Biotechnol. 2016, 36(1), 70-86

Laccase catalytic mechanism (amazing!)



Crit Rev Biotechnol. 2016, 36(1), 70-86

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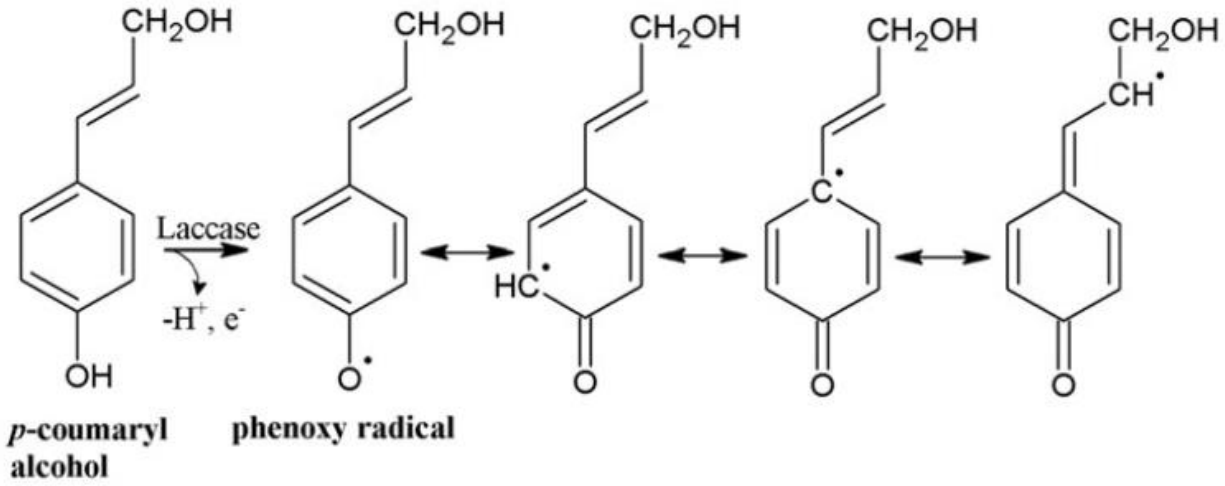
Enzymatic modification of lignin by laccases?



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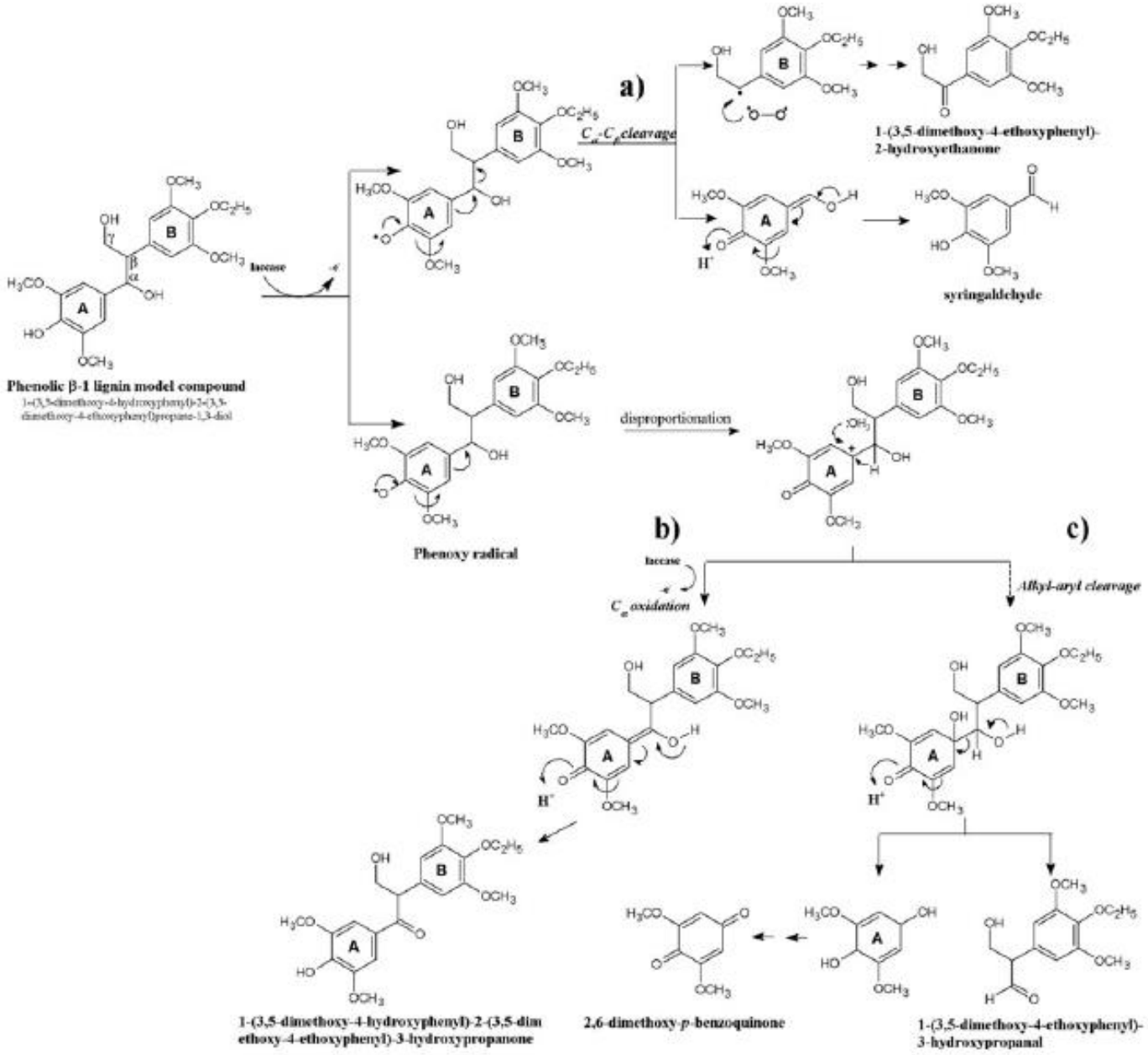
Various theories built on MODEL COMPOUNDS



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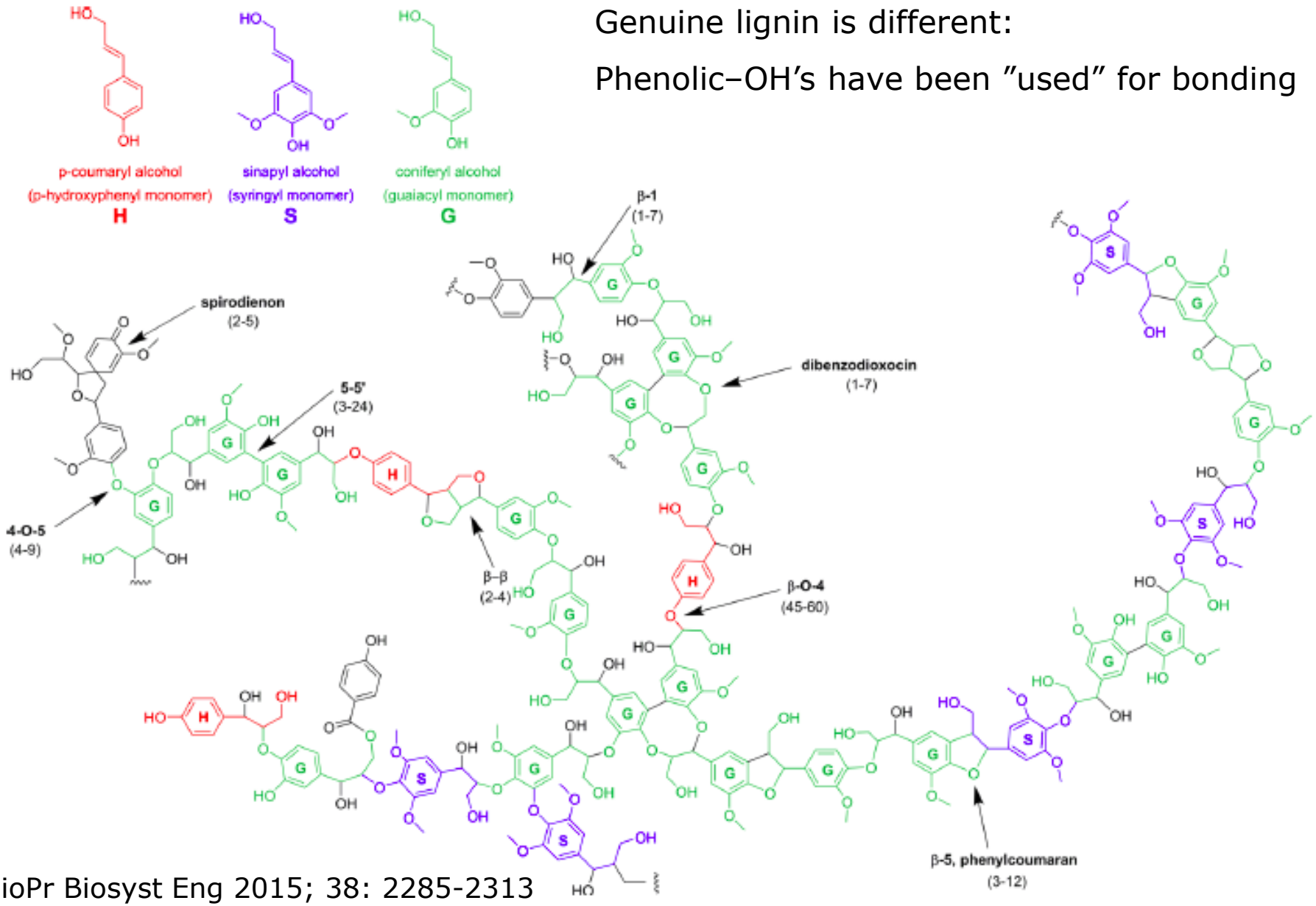
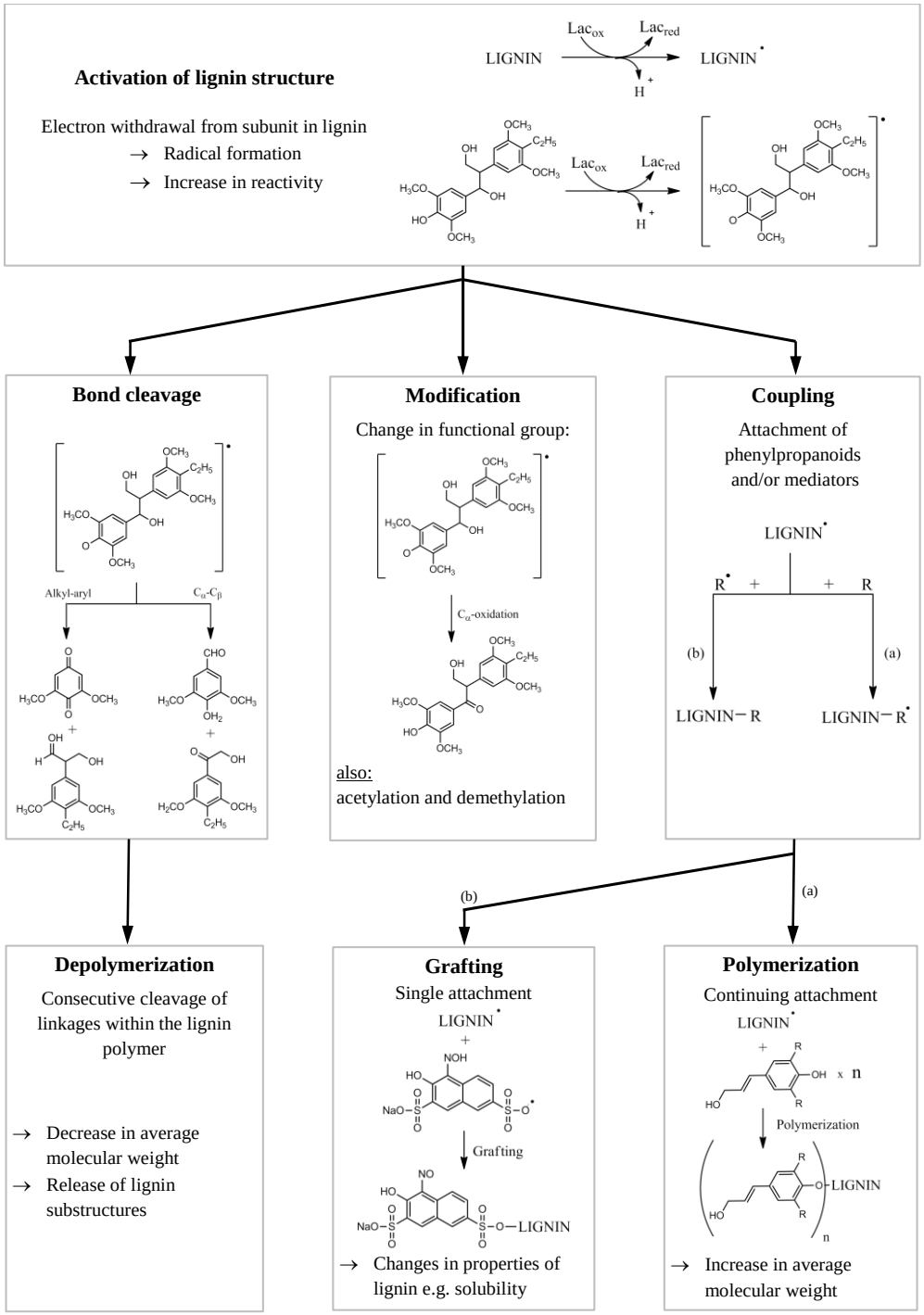
Lignin

Genuine lignin is different:

Phenolic–OH's have been "used" for bonding



BioPr Biosyst Eng 2015; 38: 2285-2313



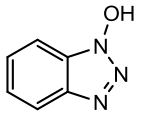
Cleaves lignin model compounds

Laccase **cannot** act on lignin without a mediator

With presence of a mediator different reactions with lignin may occur :
New upgrading routes!

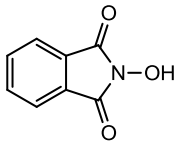
Can laccases catalyze bond cleavage in lignin? *Biotechnol Adv*, 2015, 33:13-24

Laccase-oxidation with mediators



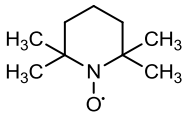
HBT

1-hydroxybenzotriazole



HPI

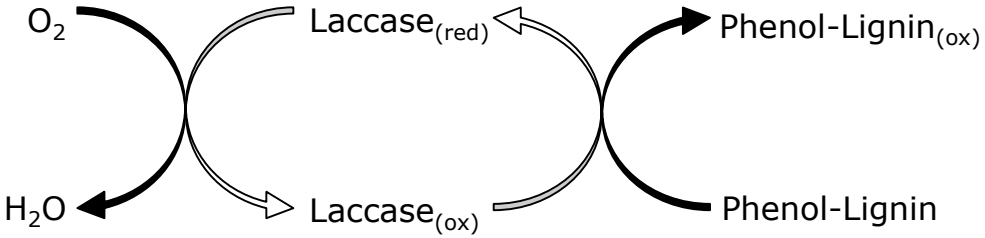
N-hydroxyphthalimide



TEMPO

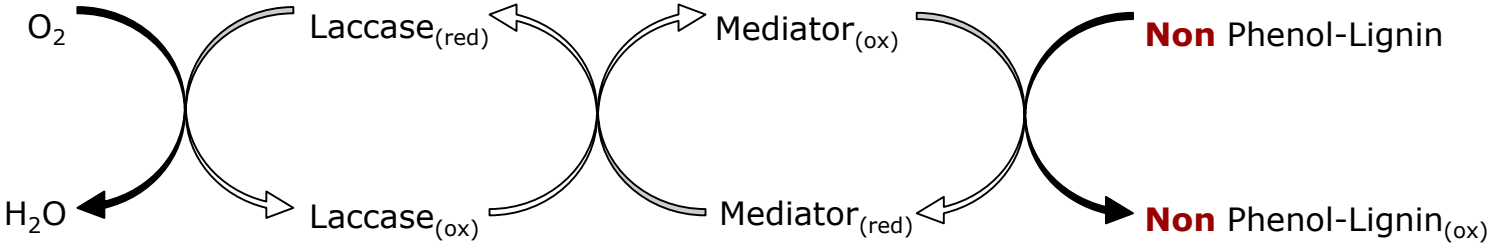
2,2,6,6-tetramethyl-Piperidine1-yloxy

Laccase oxidation



(1-hydroxybenzotriazole)

Laccase-mediator system oxidation



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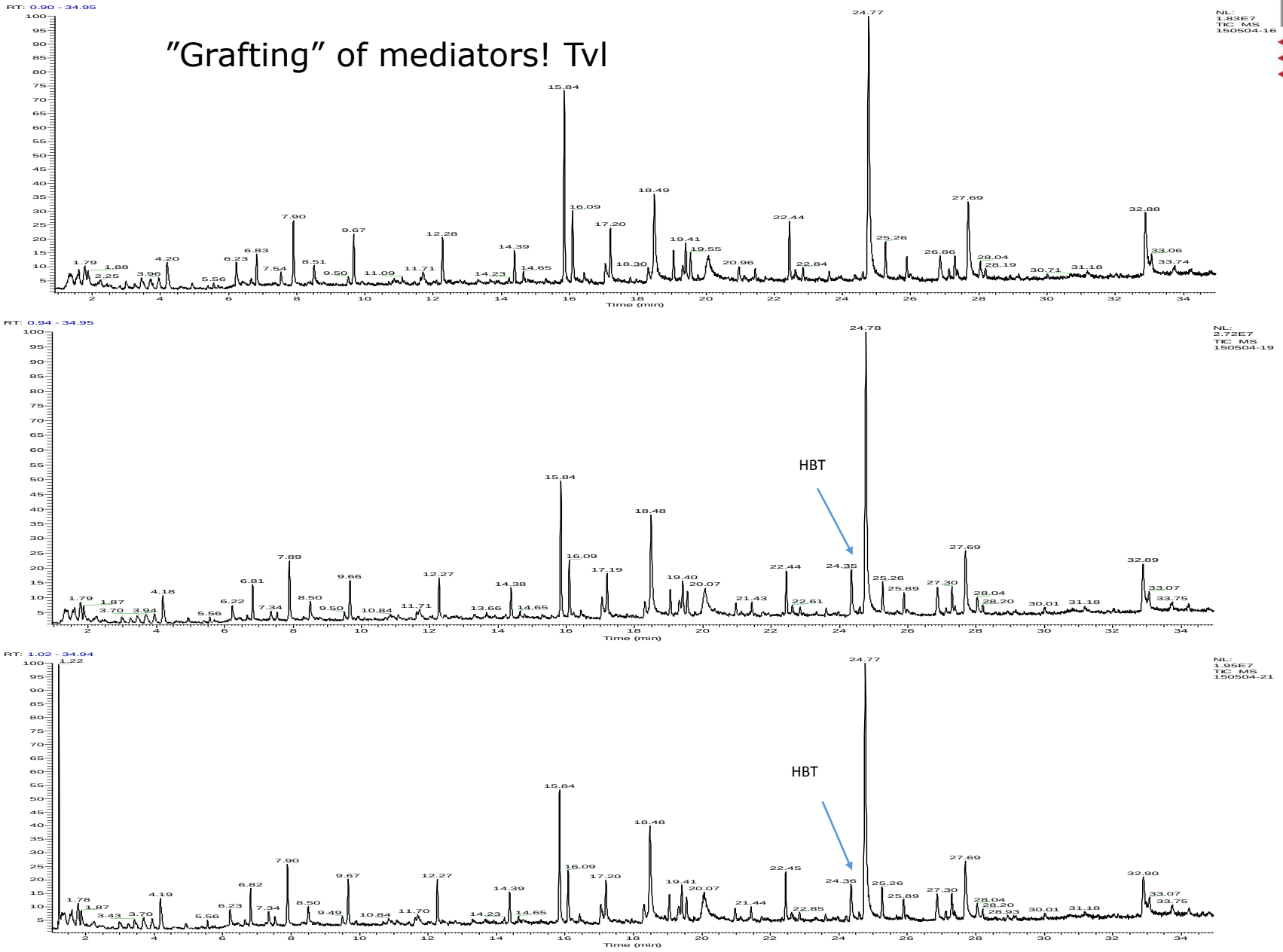
”Grafting” of mediators! Tvl



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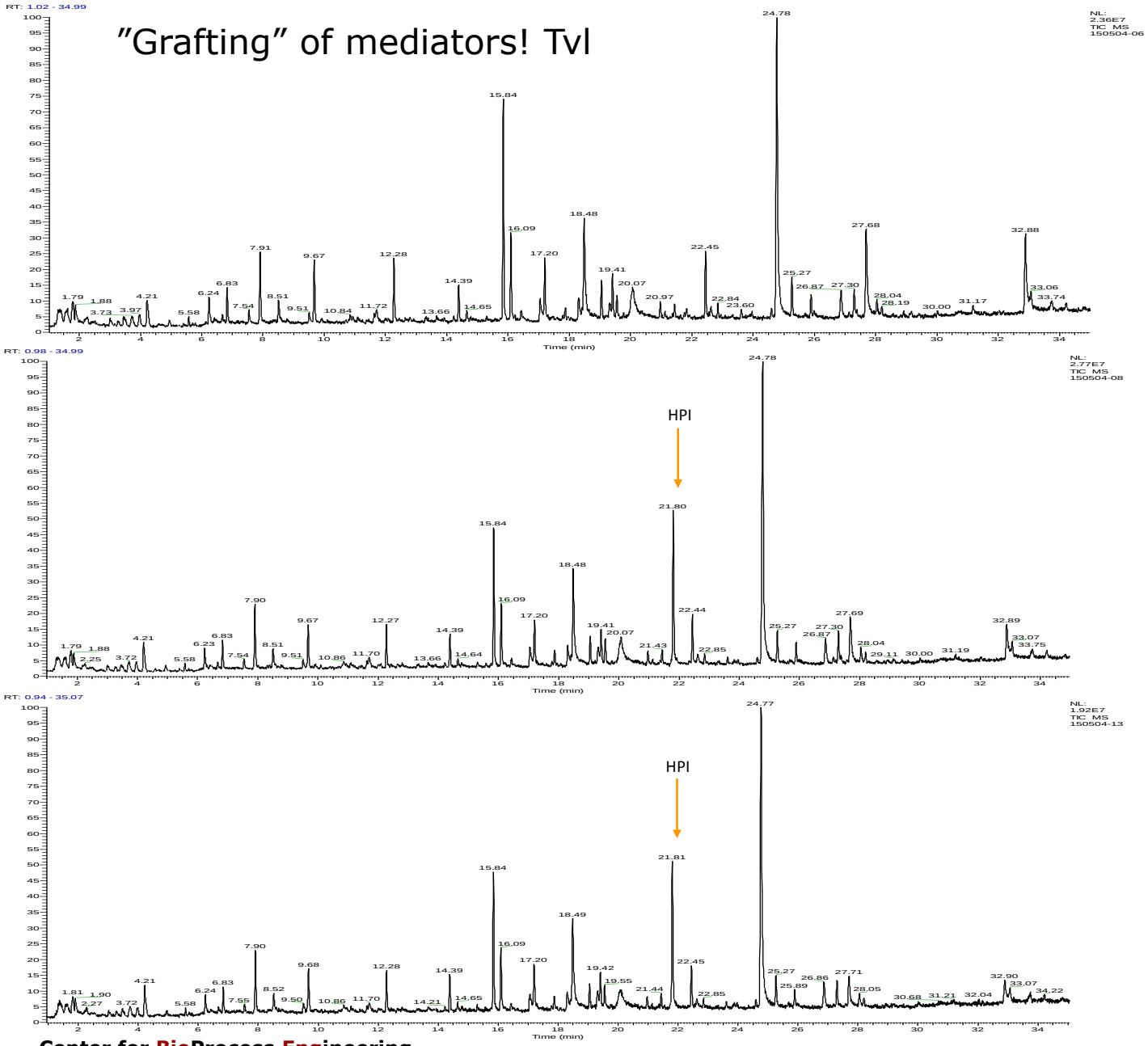
Ongoing work

”Grafting” of mediators! Tvl



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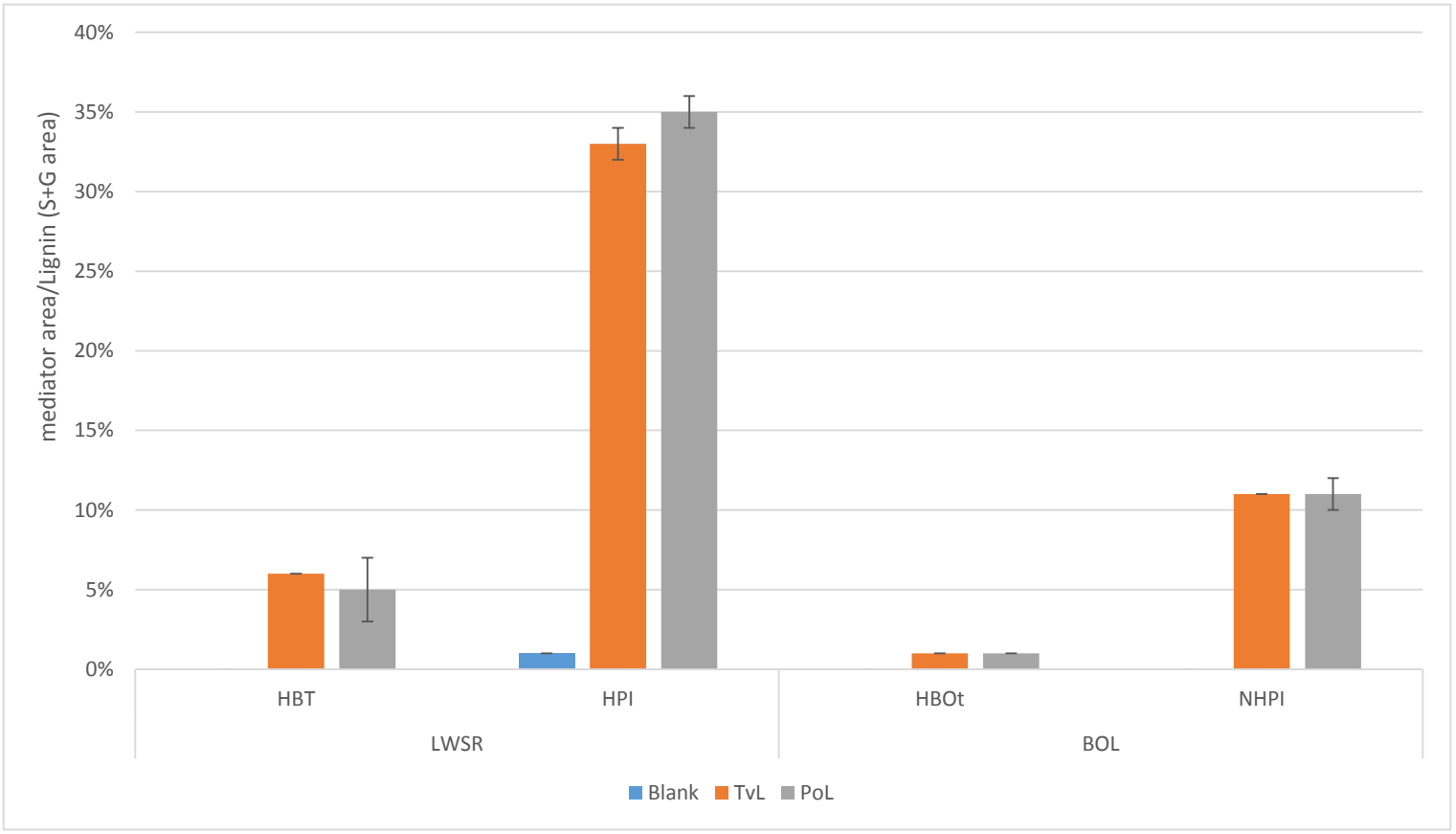
Ongoing work

HPI grafts more than HBT



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Ongoing work

Laccase discovery for lignin modification

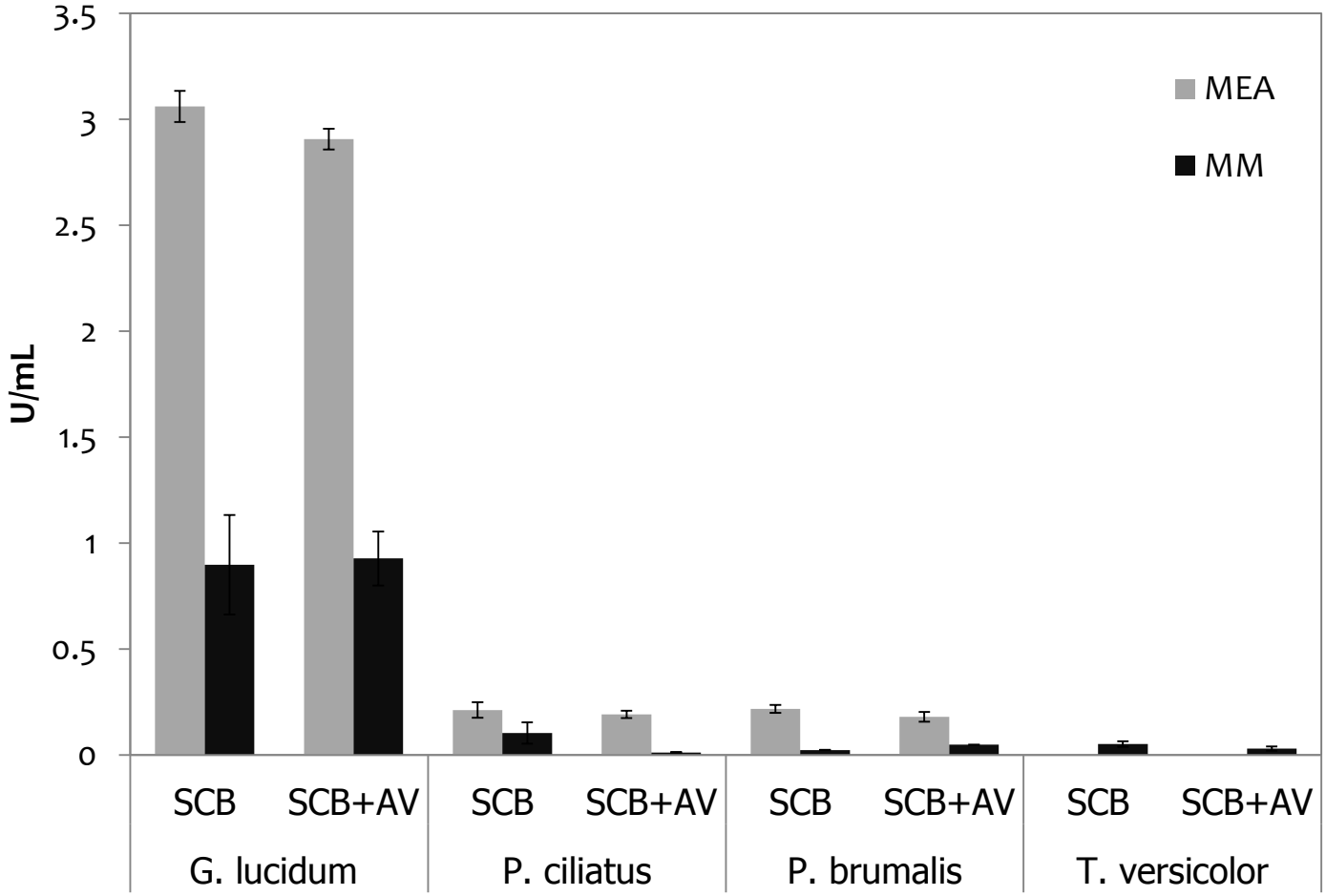
44 white rot fungi, 4 could grow on lignin

Fungal growth

Cultivation media		*Ganoderma lucidum*	*Polyporus brumalis*	*Polyporus ciliatus*	*Trametes versicolor*
Lignin	MM	+	±	±	±
	MEA	++	-	-	-
SCB	MM	++	+	+	+
	MEA	+++	+	+	+

Identification of a laccase fom *Ganoderma lucidum* CBS229.93 for enhancing cellulase catalyzed lignocellulose degradation
EMT, 2013, 53: 378-385

Did these fungi produce laccase?



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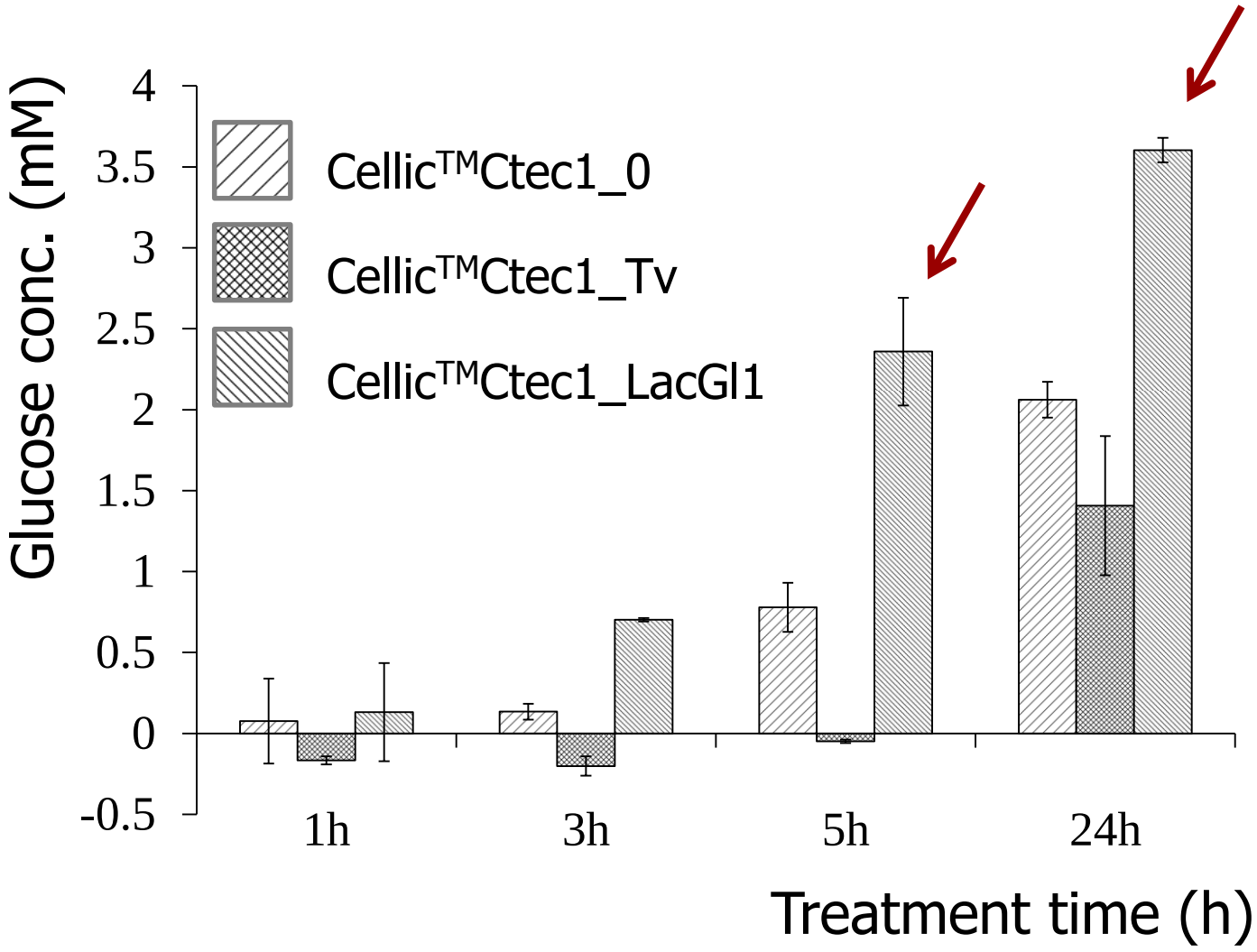
Identification of a laccase fom *Ganoderma lucidum* CBS229.93 for enhancing cellulase catalyzed lignocellulose degradation
EMT, 2013, 53: 378-385

Boosting of cellulases? SC-Bagasse



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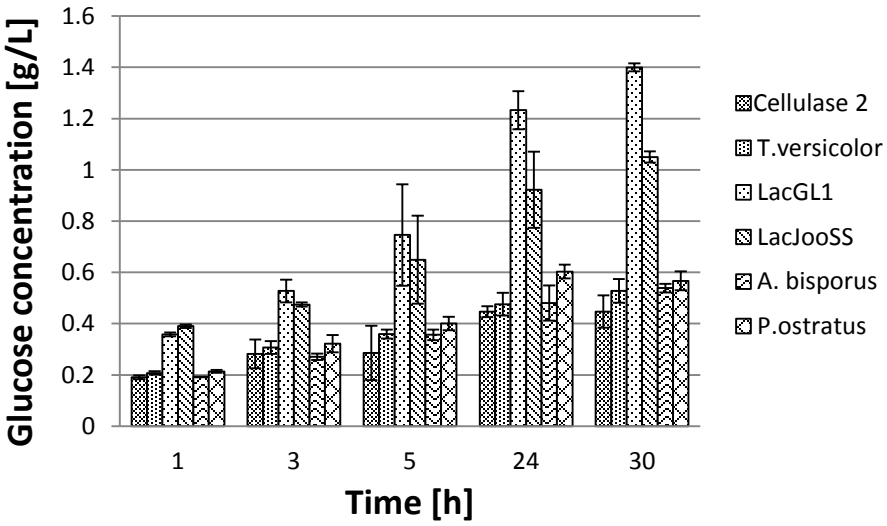
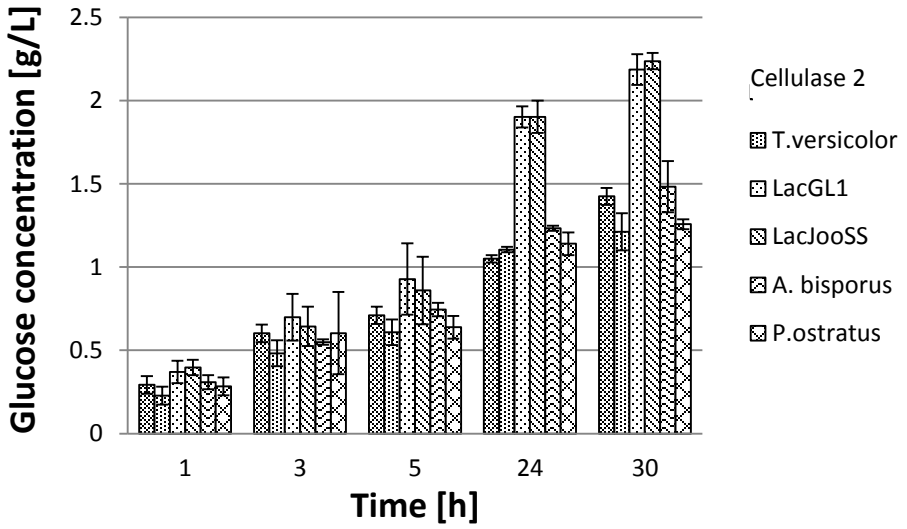
Identification of a laccase fom *Ganoderma lucidum* CBS229.93 for enhancing cellulase catalyzed lignocellulose degradation
EMT, 2013, 53: 378-385

LacGL1 on barley straw and wheat straw

Barley straw



Wheat straw



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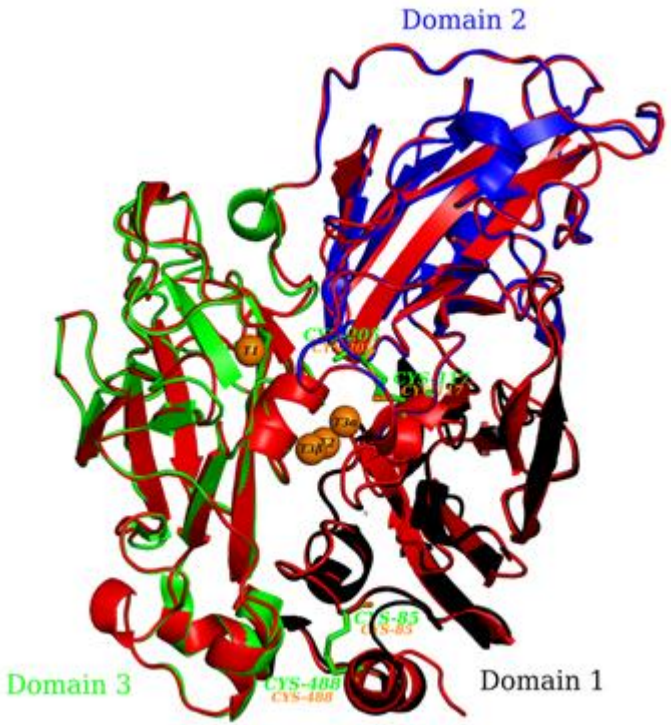
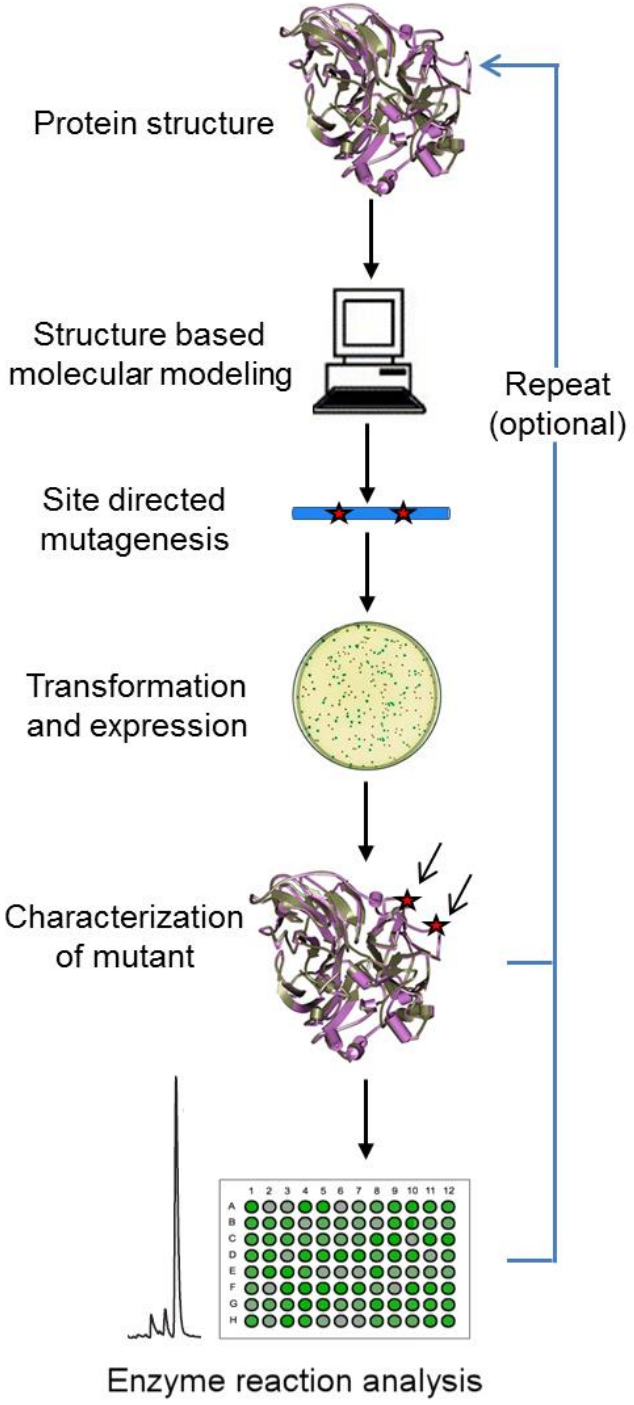
WO 2014/041030 A1

Enzyme engineering





Structure, functionality, and tuning up of laccases for lignocellulose and other industrial applications. Crit. Rev. Biotechnol. DOI:10.3109/07388551

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Enzymatic lignin modification

- Laccases with mediators can modify lignin, but cannot without mediators
- A novel laccase from *Ganoderma lucidum* improves cellulase can catalyze improved glucose release from biomass
- The *G. lucidum* laccase investigated/engineered to understand mechanism
- Interested in collaboration to understand function of fungal laccases ***in nature***

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Arjen Punt (WUR)

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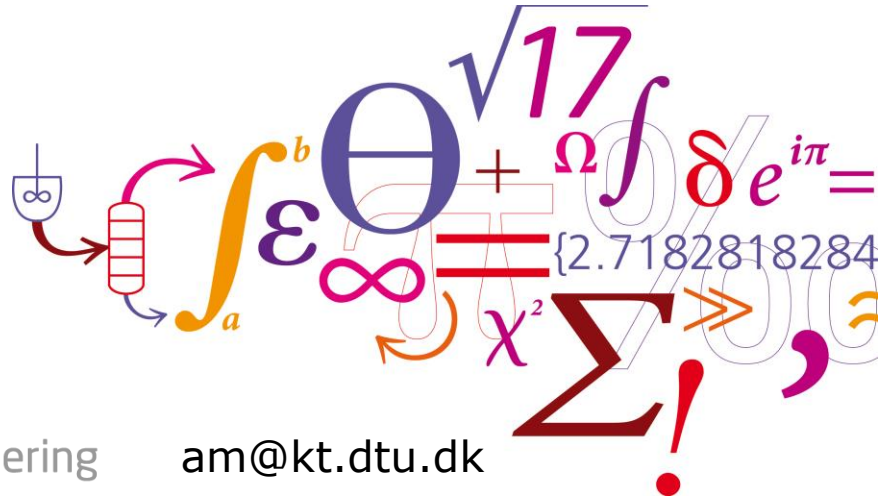

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