ENZITEC 2016
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Caxias do Sul, RS

Enzymatic hydrolysis of lignocellulosic biomass: Second generation ethanol and beyond

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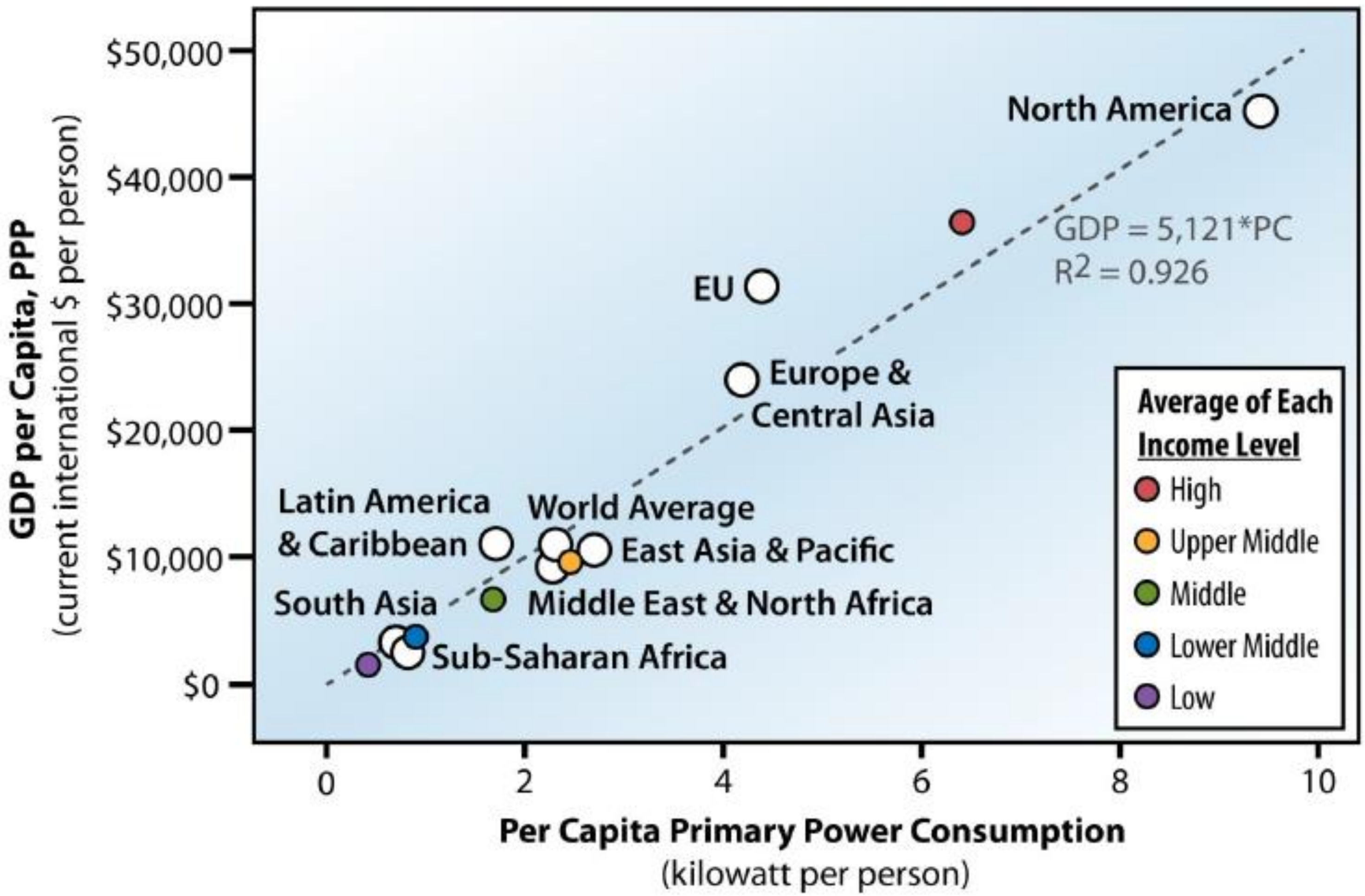
"The world …is getting **hot** (global warming); **flat** (the rise of high-consuming middle class all over the world), and **crowded** (on the track to adding roughly a billion people every thirteen years)."

"Hot, Flat and Crowded" Thomas L. Friedman

We are here

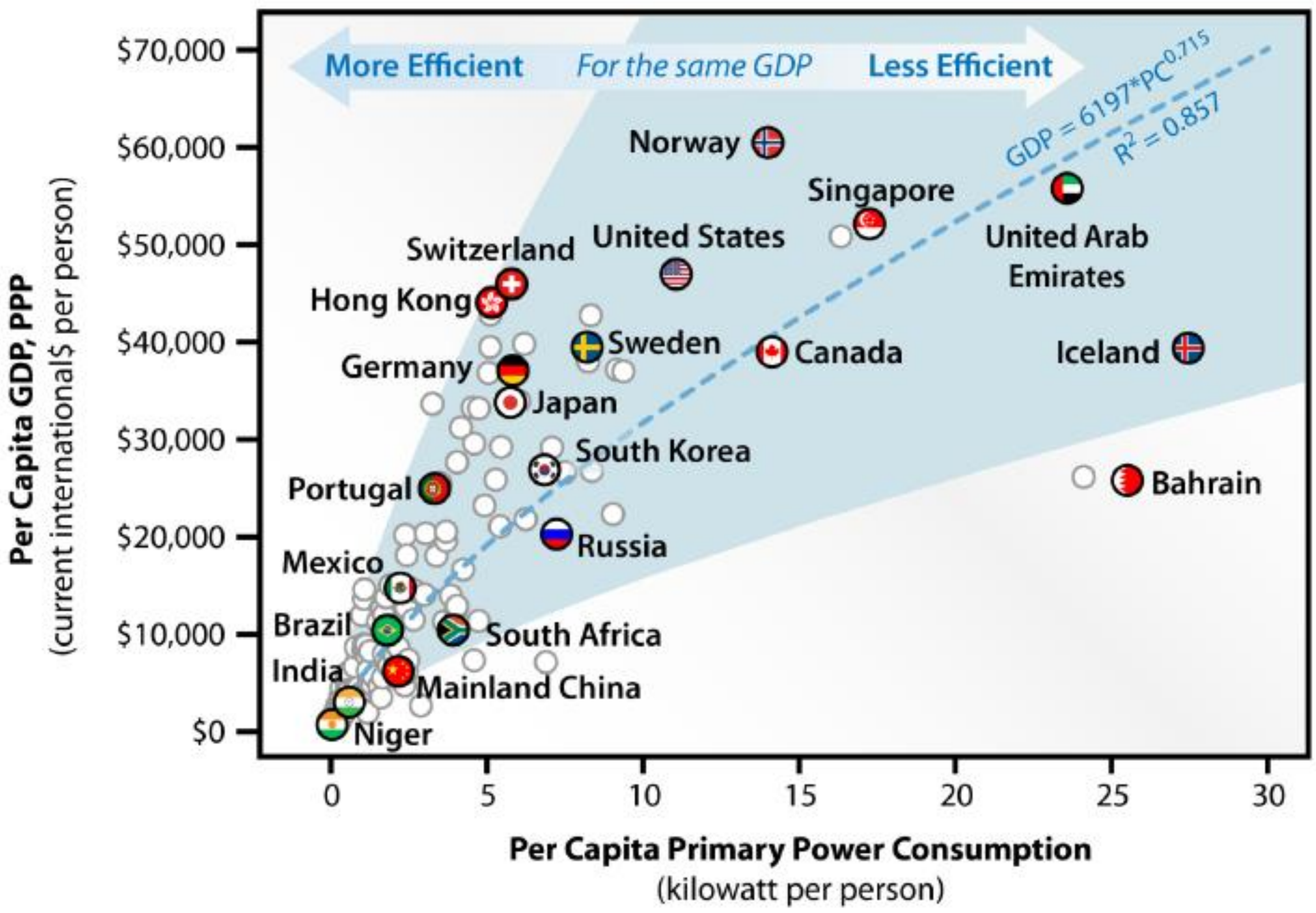
Current Earth population (2016): 7.4 billion people

Power Consumption and GDP (World Regions)



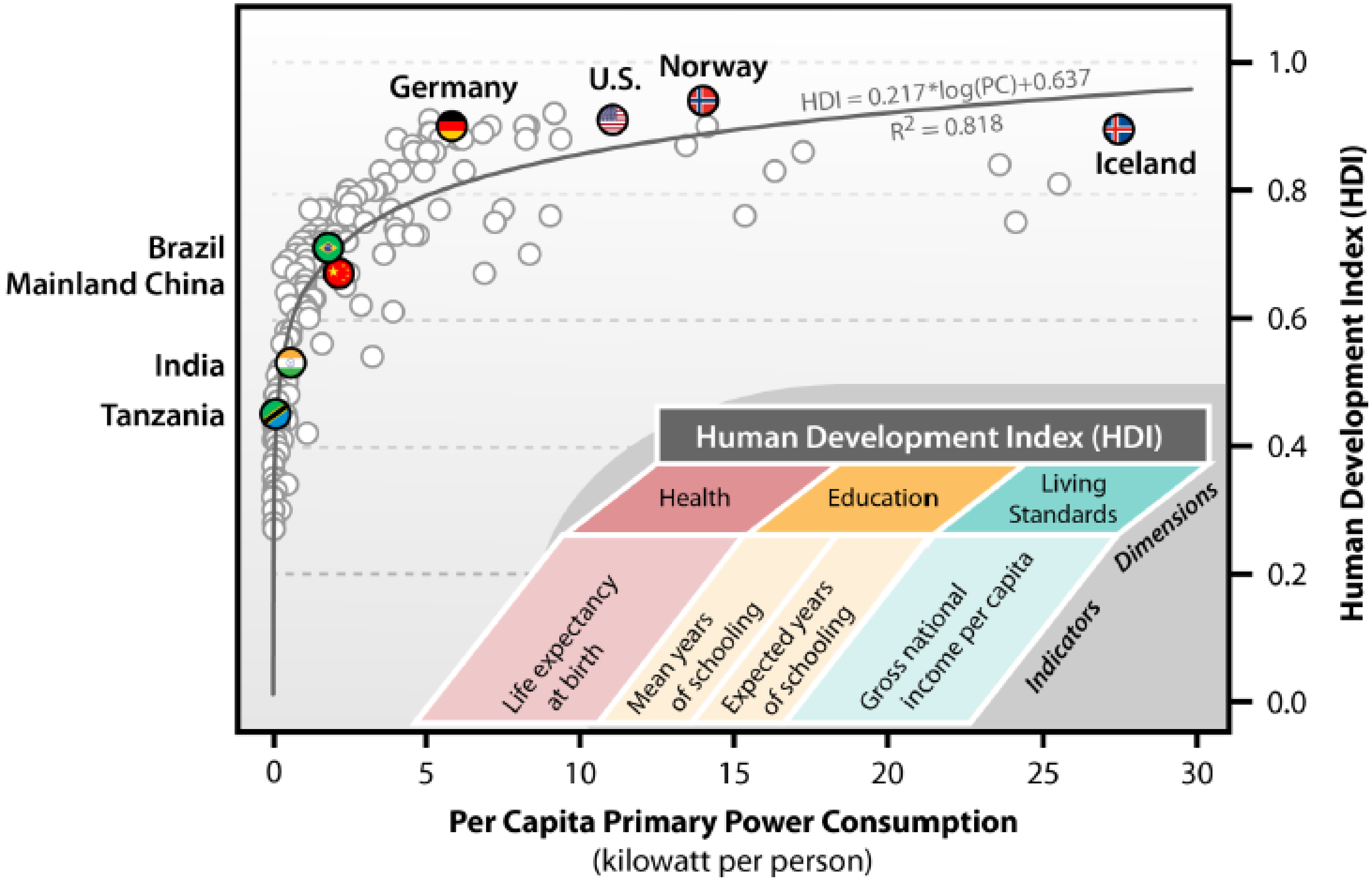
Courtesy of Prof. Bruce Dale

Energy Efficiency: Necessary but not Sufficient



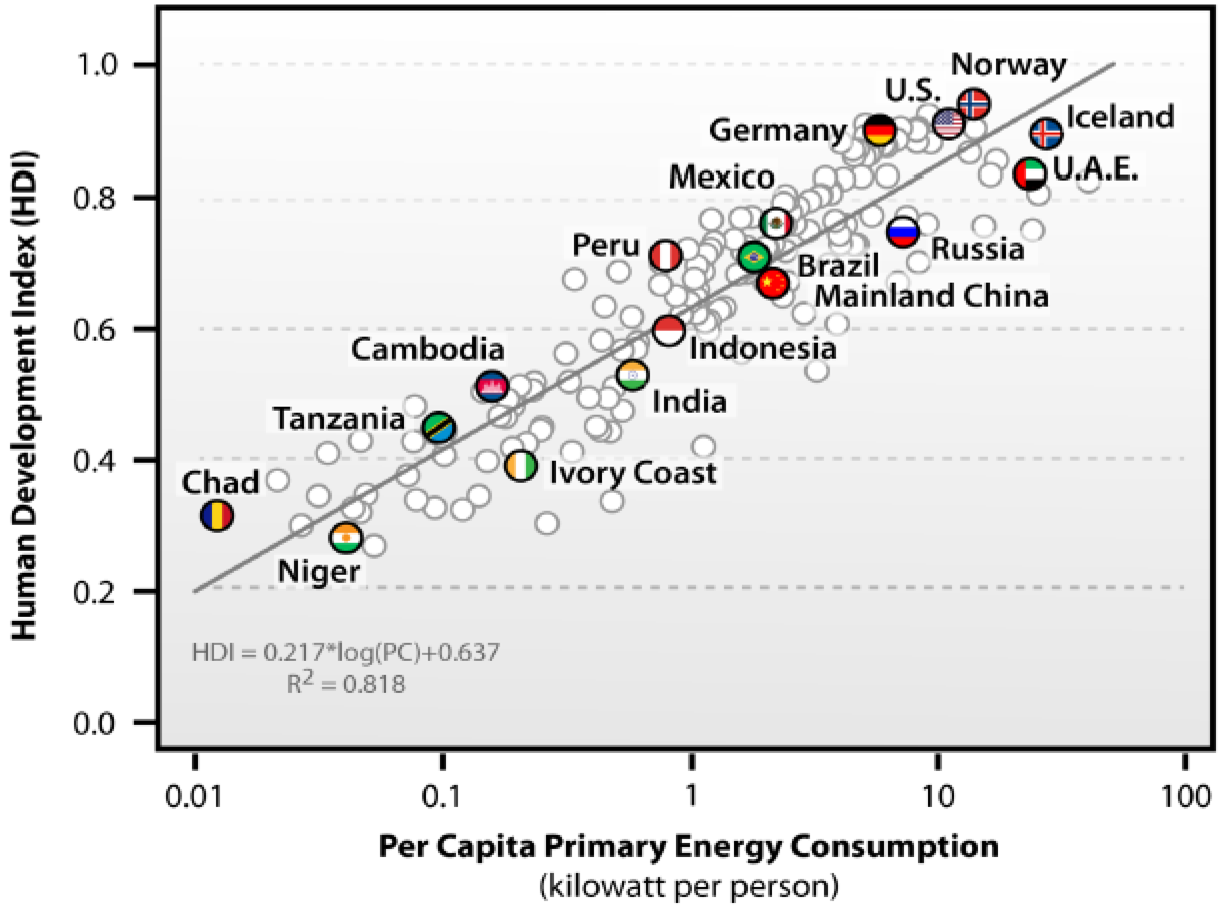
Courtesy of Prof. Bruce Dale

Energy Consumption & Human Well Being are Linked



Courtesy of Prof. Bruce Dale

Energy Consumption & Human Well Being are Linked: NO Countries have Both High HDI <u>and</u> Low Energy Use



Courtesy of Prof. Bruce Dale

- ✓ Rate of energy used and power consumed strongly affects (determines?) national wealth and human development
- ✓ All rich societies use a lot of energy (~33% oil)
- ✓ CO2 and methane emission: Climate change
- ✓ Energy and Food demand = Energy and Food Poverty
- ✓ ***Need of sustainable biofuels + green chemicals + renewable materials + food/feed – environmental impacts = Bioeconomy***

Research & Innovation
Bioeconomy

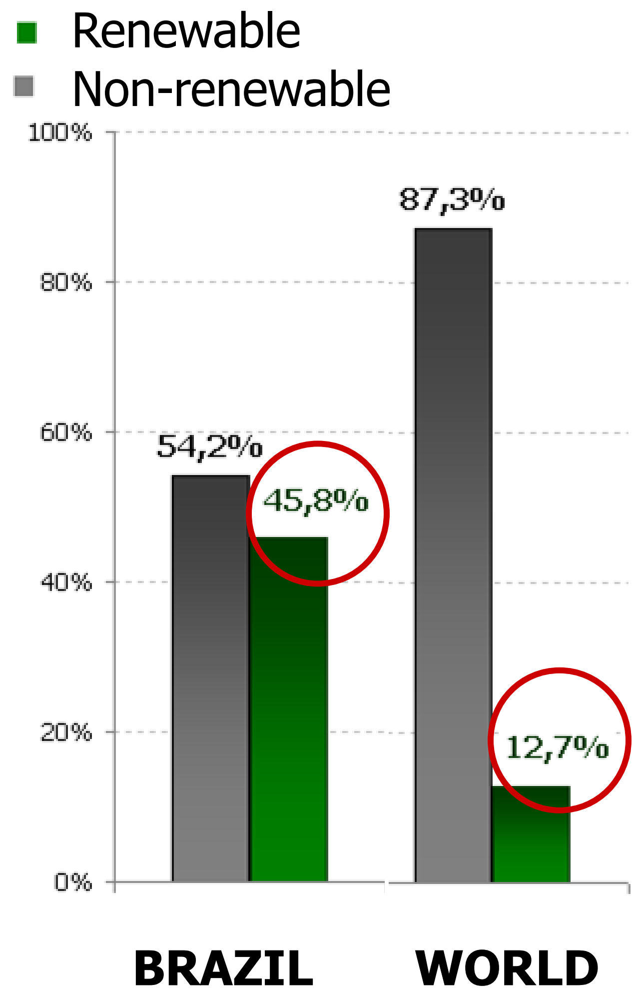
What is the bioeconomy?

The **bioeconomy** comprises those parts of the economy that use renewable biological resources from land and sea to produce food, materials and energy.

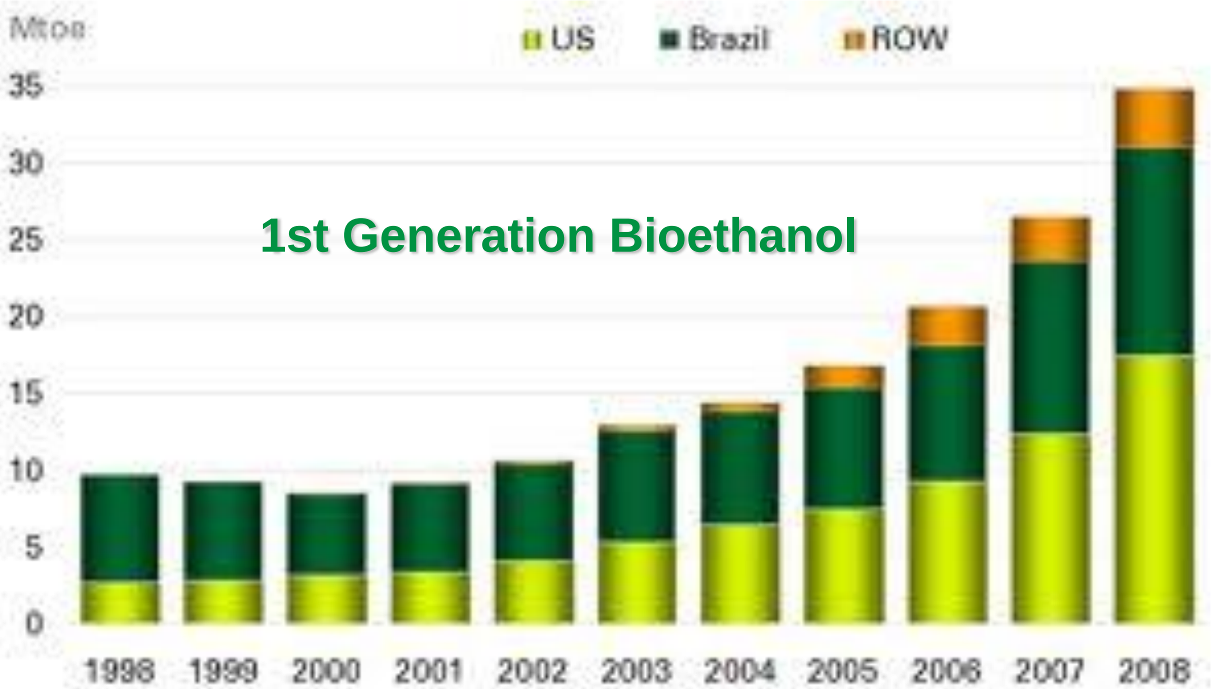
The evolution of the **biotechnology industry** and its application to **agriculture, health, chemical or energy industries** is a classic example of bioeconomic activity.

- **Brazilian Bioethanol**

World consumption



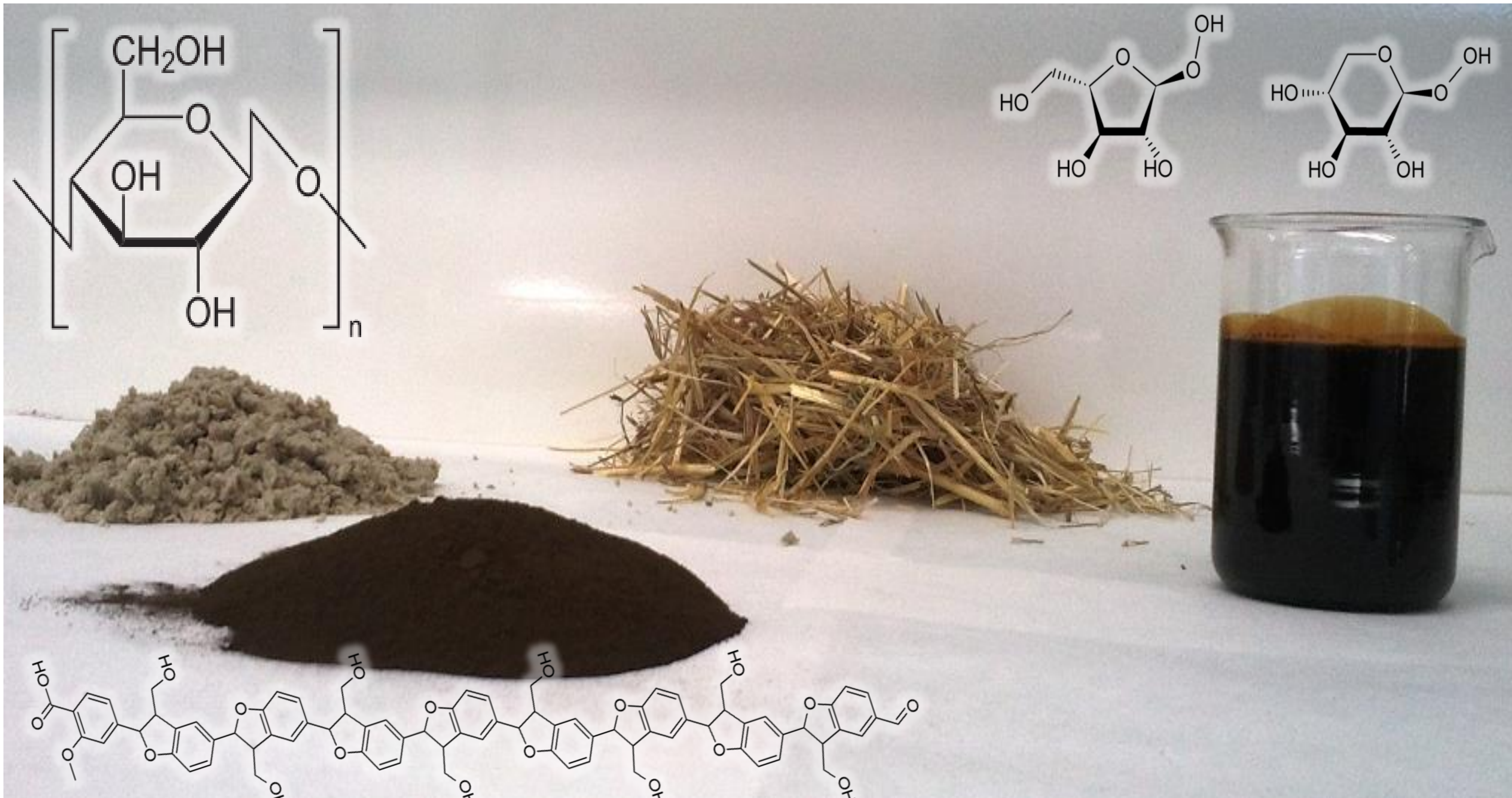
1st generation Bioethanol production in Brazil




Hélvio Romero/AE

Cellulose and glucose

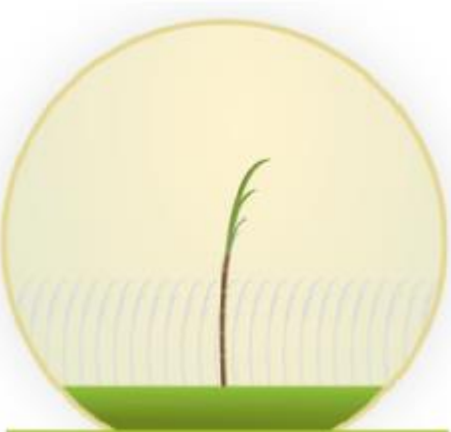
Hemicellulose and Pentose sugars



Lignins

GranBio

Primeira empresa a anunciar uma planta de biocombustíveis de segunda geração no Brasil, a GranBio passará a produzir etanol a partir da celulose em 2014. O projeto tem como sócio estratégico a usina Caeté, que pertence ao grupo Carlos Lyra, tradicional produtor de etanol no país.A fábrica de São Miguel dos Campos, em Alagoas, terá capacidade de produção de 82 milhões de litros por ano.Até 2020, a GranBio pretende ter capacidade instalada para produção de 1 bilhão de litros por ano de etanol 2G no Brasil.O etanol celulósico é um produto renovável e sustentável que não compete com a produção de alimentos.



Clique aqui para conhecer como a GranBio vai transformar resíduos agrícolas, como bagaço e palha de cana-de-açúcar, em combustível.

GranBio

Raízen = Shell (51%) + Cosan (49%)

RAIZEN: Sugar mill Costa-Pinto (Piracicaba, SP)

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CENTRO DE TECNOLOGIA CANAVIEIRA

A NOSSA CANA É O NOSSO MUNDO

EVENTOS

INSTITUCIONAL

MELHORAMENTO GENÉTICO

NOVAS TECNOLOGIAS

GESTÃO DE PESSOAS

:NTRAMOS NOSSA
IA NO FUTURO DA CANA

CONCE
ENERG

INOVAÇÃO PARA O FUTURO DA ENERGIA **ETANOL 2G** **NOVOS MÉTODOS DE PLANTIO** **LABORATÓRIO**

BIOMASSA **BIORREFINARIA** **GASEIFICAÇÃO**

ETANOL 2G

A produção de etanol é hoje estratégica, uma vez que se trata de um combustível de fonte limpa e renovável. O CTC está investindo no etanol celulósico, que é o etanol de 2ª geração, ou 2G, um combustível produzido a partir de materiais que contenham celulose, como o resultado do processamento da biomassa da cana: o bagaço e a palha. Essa tecnologia deverá permitir dobrar a quantidade de etanol produzida por unidade, em relação ao etanol de 1ª geração, sem necessidade de expandir a área plantada, e mantendo a autossuficiência energética industrial.

No CTC, os trabalhos para o desenvolvimento do etanol a partir de biomassa tiveram início em 2006, sendo a técnica escolhida a de hidrólise enzimática. O projeto, inovador, será totalmente integrado aos processos de geração de etanol já existentes nas usinas que operam no Brasil.

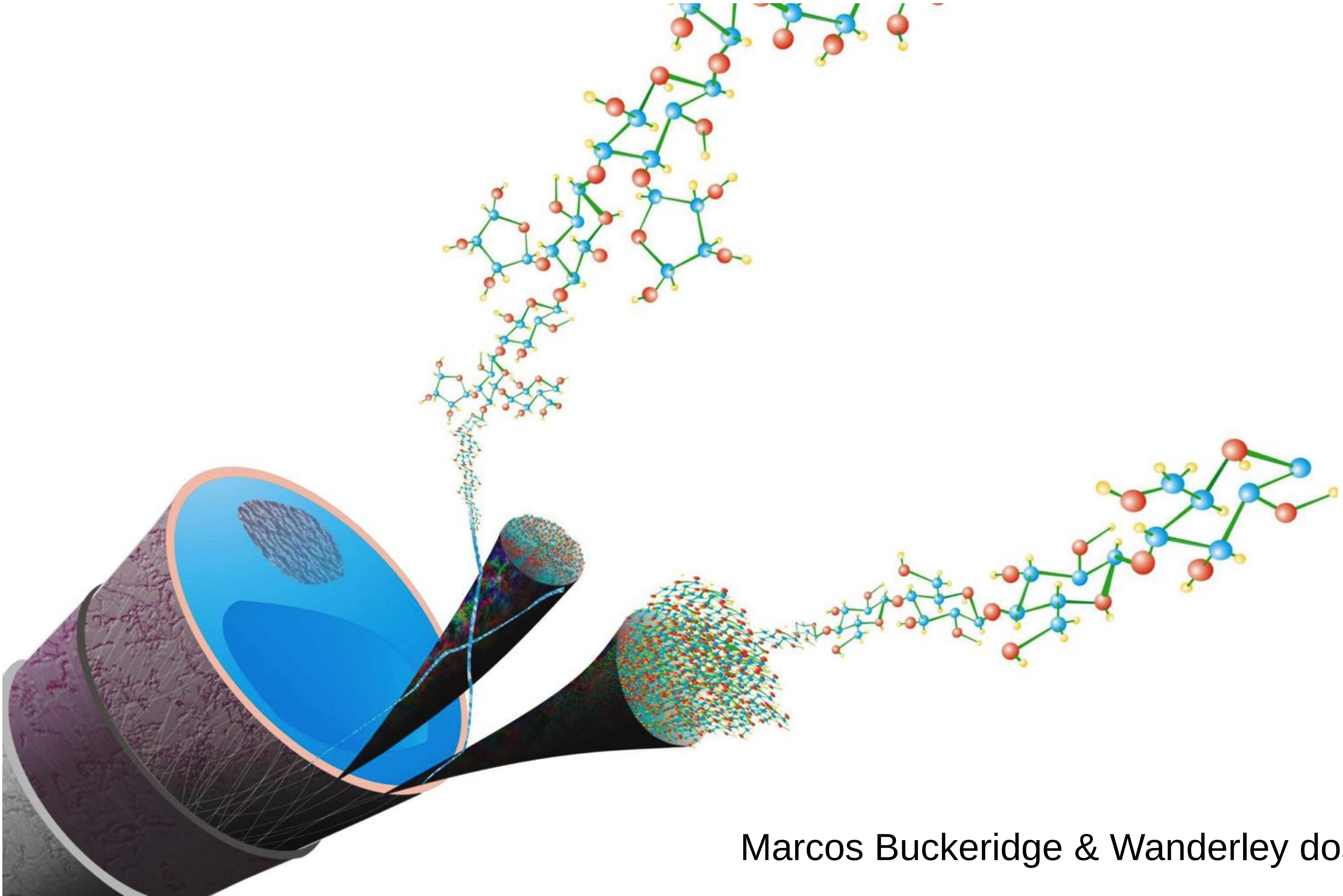
O grande diferencial dessa tecnologia no CTC reside no fato de ter sido projetada para permitir total integração com a estrutura existente nas usinas, visando à otimização dos custos de instalação e operação. Além disso, essa tecnologia está fundamentada no moderno conceito do aproveitamento de bagaço e palha, que resultará na implantação definitiva da colheita mecanizada, sem queima da palha da cana, e na utilização de caldeiras de alto desempenho.

Outra vantagem em relação ao etanol de 1ª geração, derivado do caldo de cana, ao derivado do milho: produzido a partir de palha e bagaço, não compete com um alimento. Outro aspecto da sustentabilidade da tecnologia é que ela requer pouca energia do petróleo e muita de um combustível limpo e renovável, resultando em menor emissão de gases do efeito estufa.

Este projeto está sendo desenvolvido por meio de parceria com as empresas Novozymes e Andritz, sendo

- **Biomass deconstruction**

Biomass is recalcitrant, but can be transformed into hexoses and pentoses in a technological process that evolves **pretreatment**, **enzymatic hydrolysis**, **fermentation** and distillation.

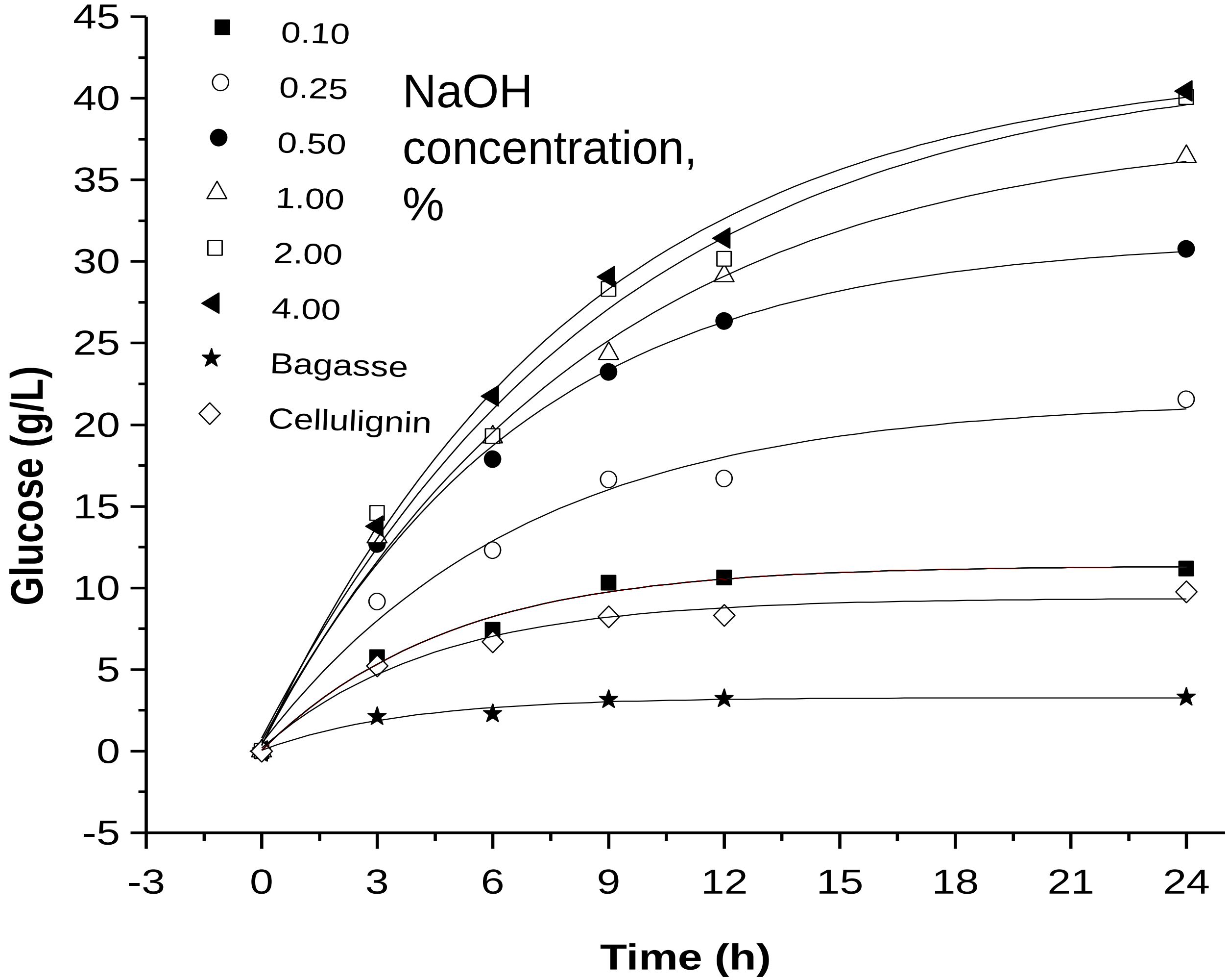


Marcos Buckeridge & Wanderley dos Santos

- **Biomass Pretreatment**

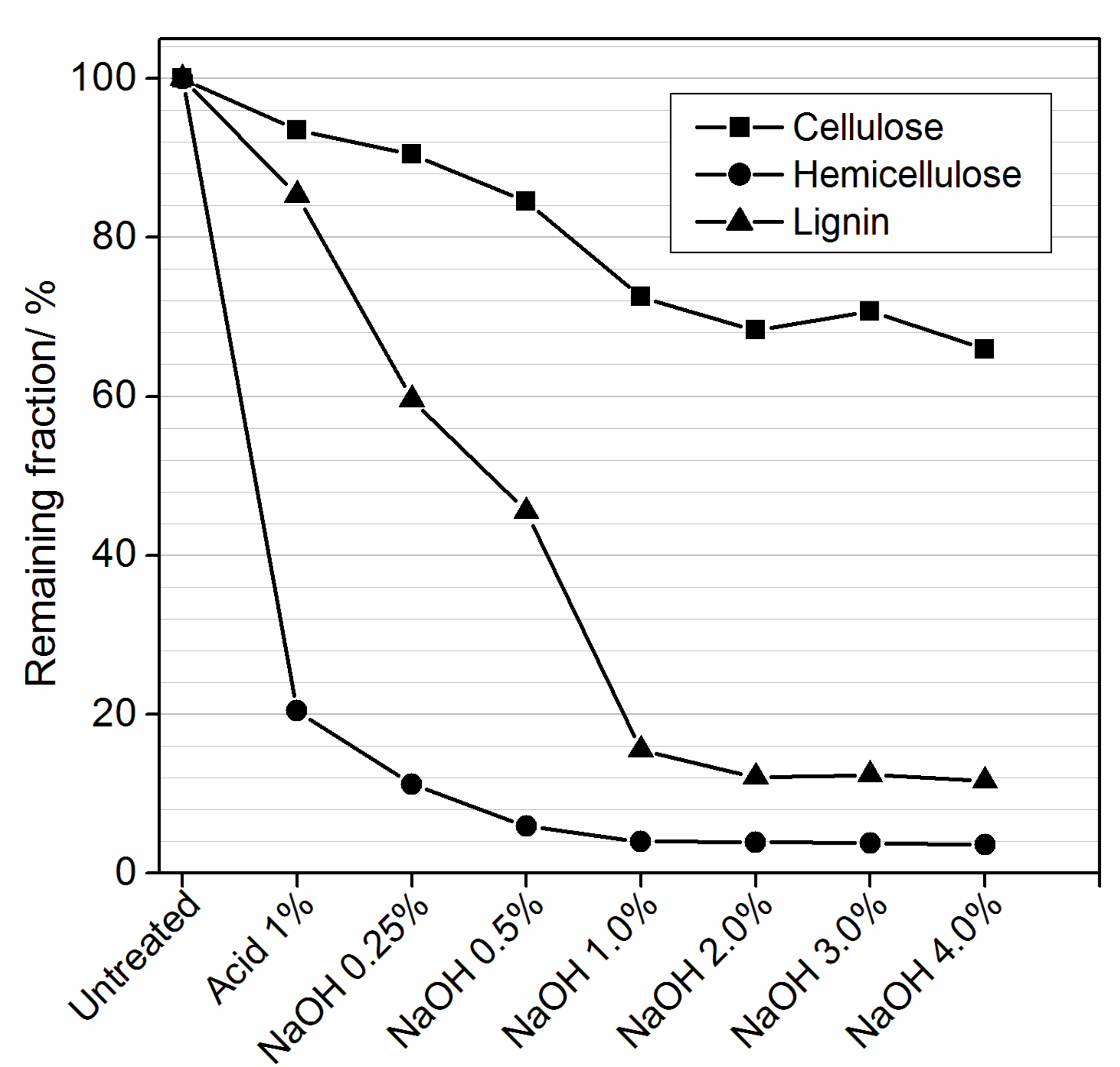
Efficiency of enzymatic hydrolysis of alkaline pretreated cellulignin increases with severity of pre-treatment

EFFICIENCY OF PRETREATED SUGAR CANE BAGASSE HYDROLYSIS



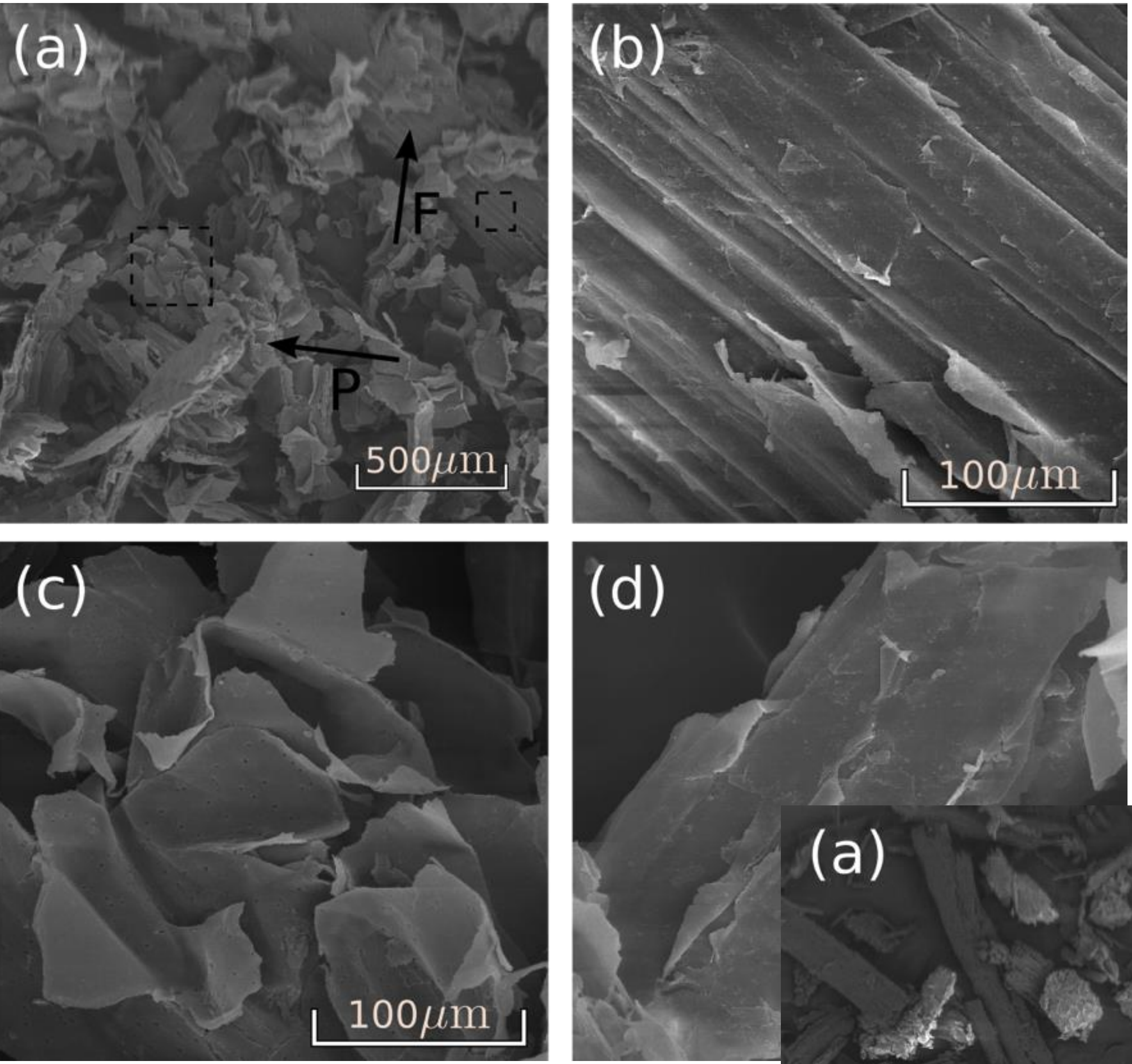
Maeda, Serpa, et al. (2011) Proc. Biochem. 46:1196 - 1201

Composition of bagasse samples after pretreatment steps



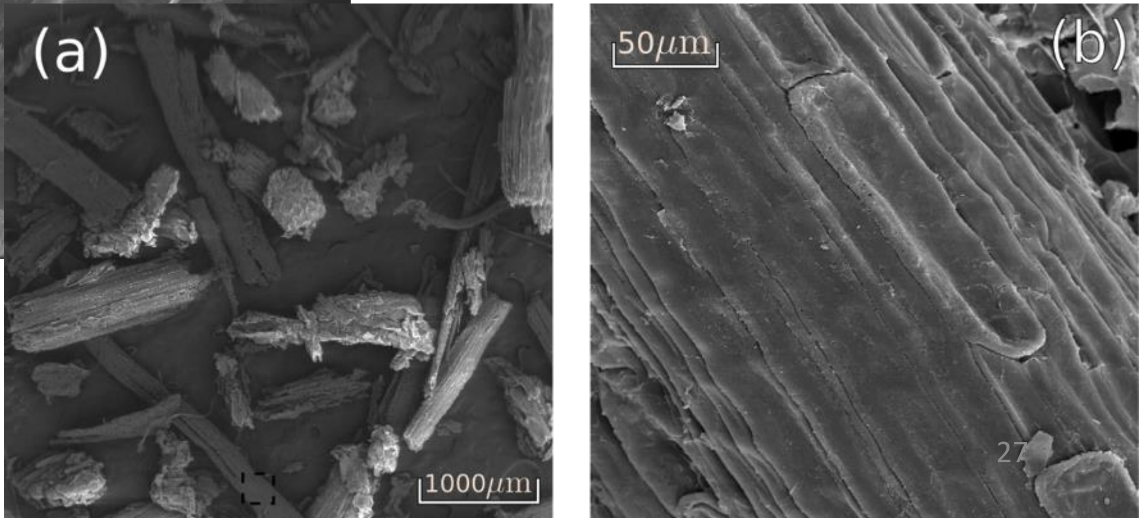
Rezende, et al., *Biotechnology for Biofuels* (2011) **4**:54

Morphology of untreated and acid pre-treated bagasse (SEM)

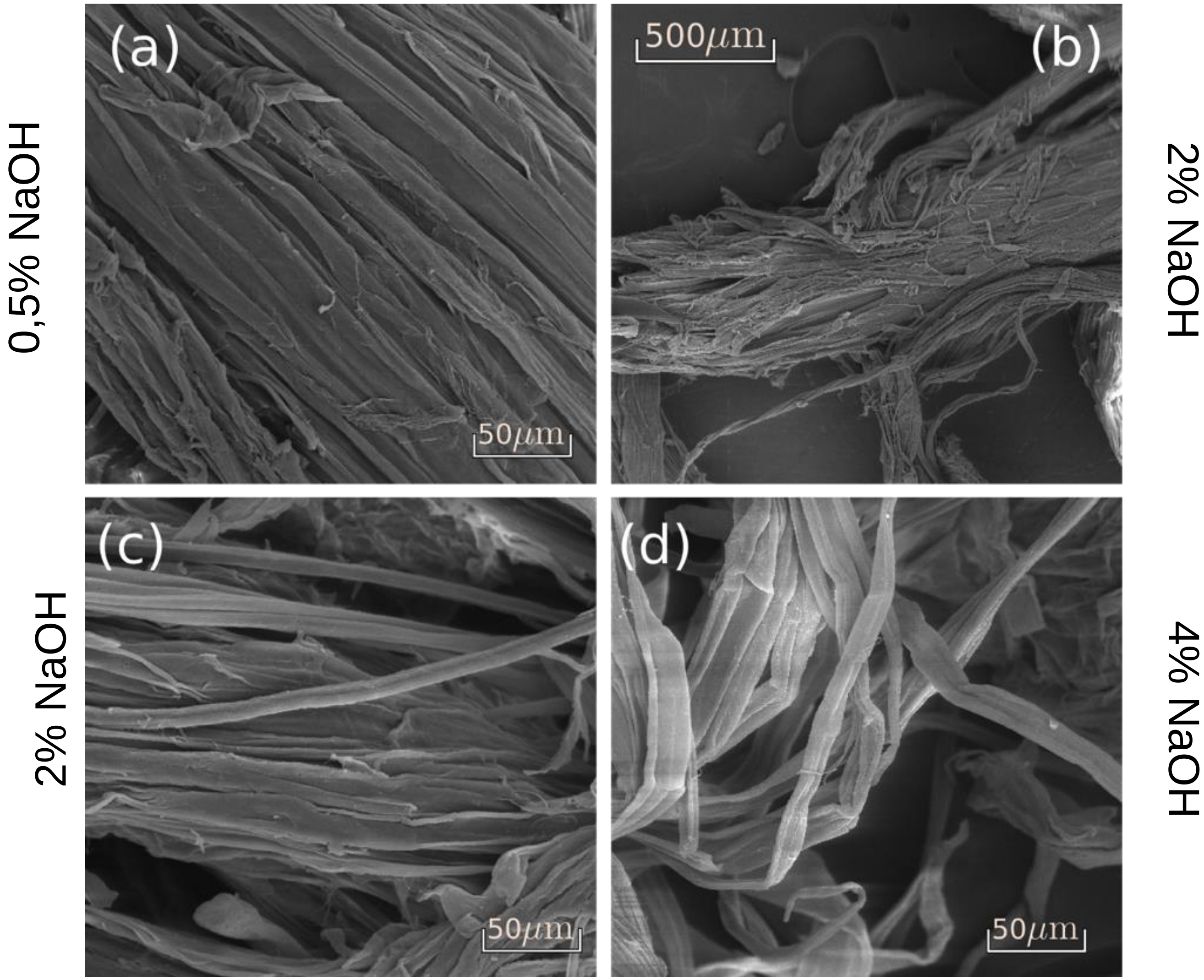


Untreated

Acid pre-treated



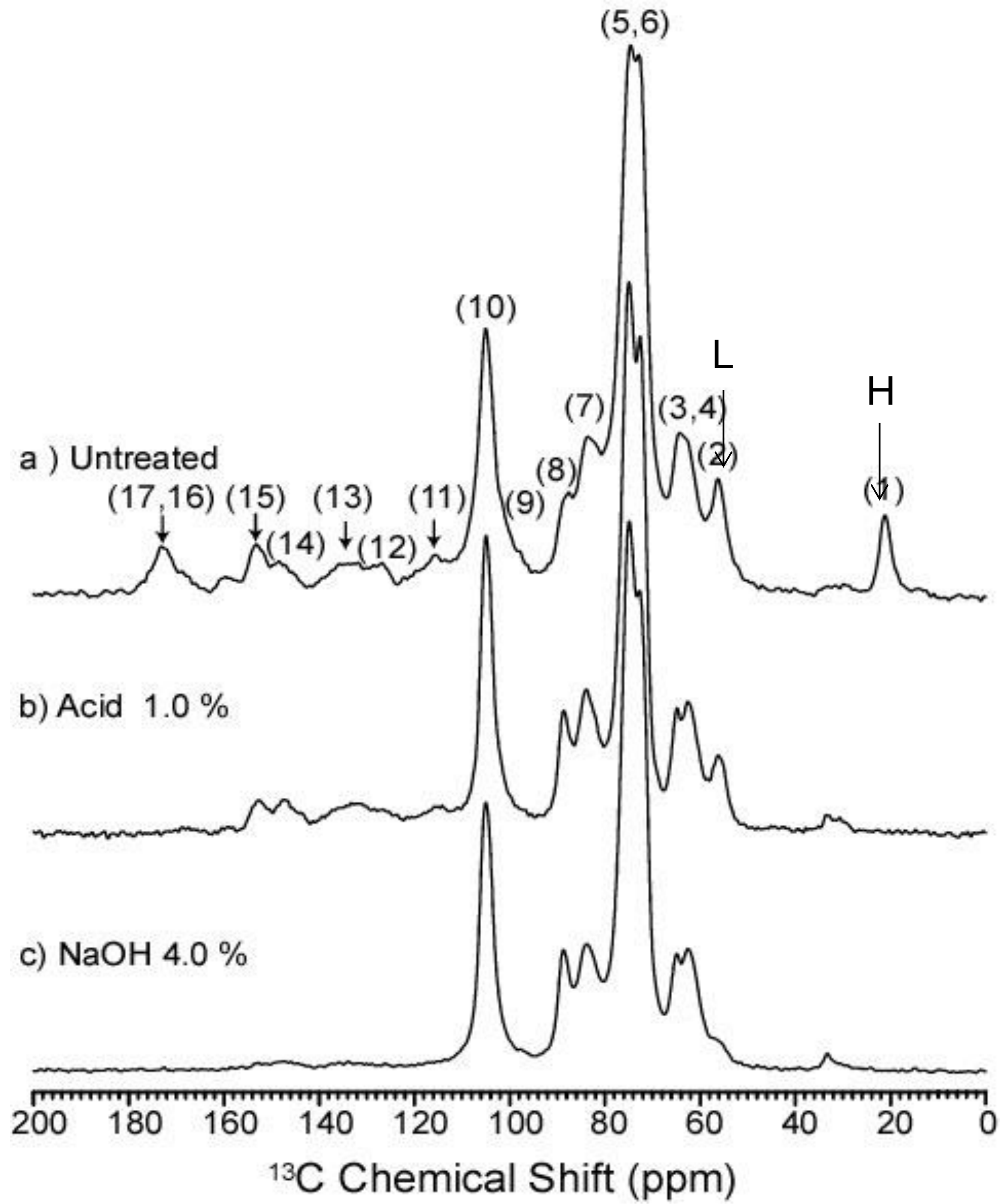
Morphology of acid+alkaline pre-treated bagasse



SEM surface images of the sugarcane bagasse sample treated with alkaline solutions: (a) NaOH 0.5% with bundles starting to come apart; (b) and (c) NaOH 2%, (unstructured and unattached bundles); and (d) NaOH 4%, (individual fibers).

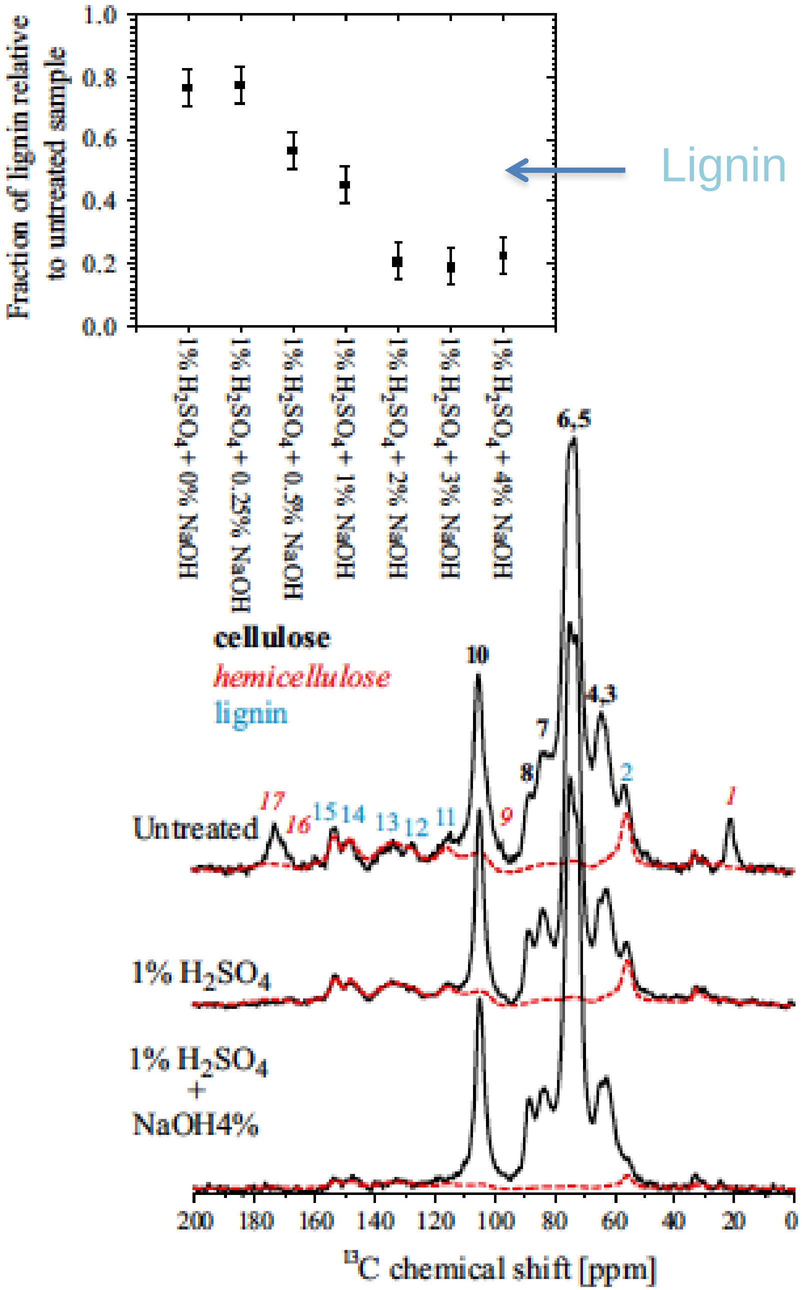
Rezende, et al., *Biotechnology for Biofuels* (2011) **4**:54

ssNMR



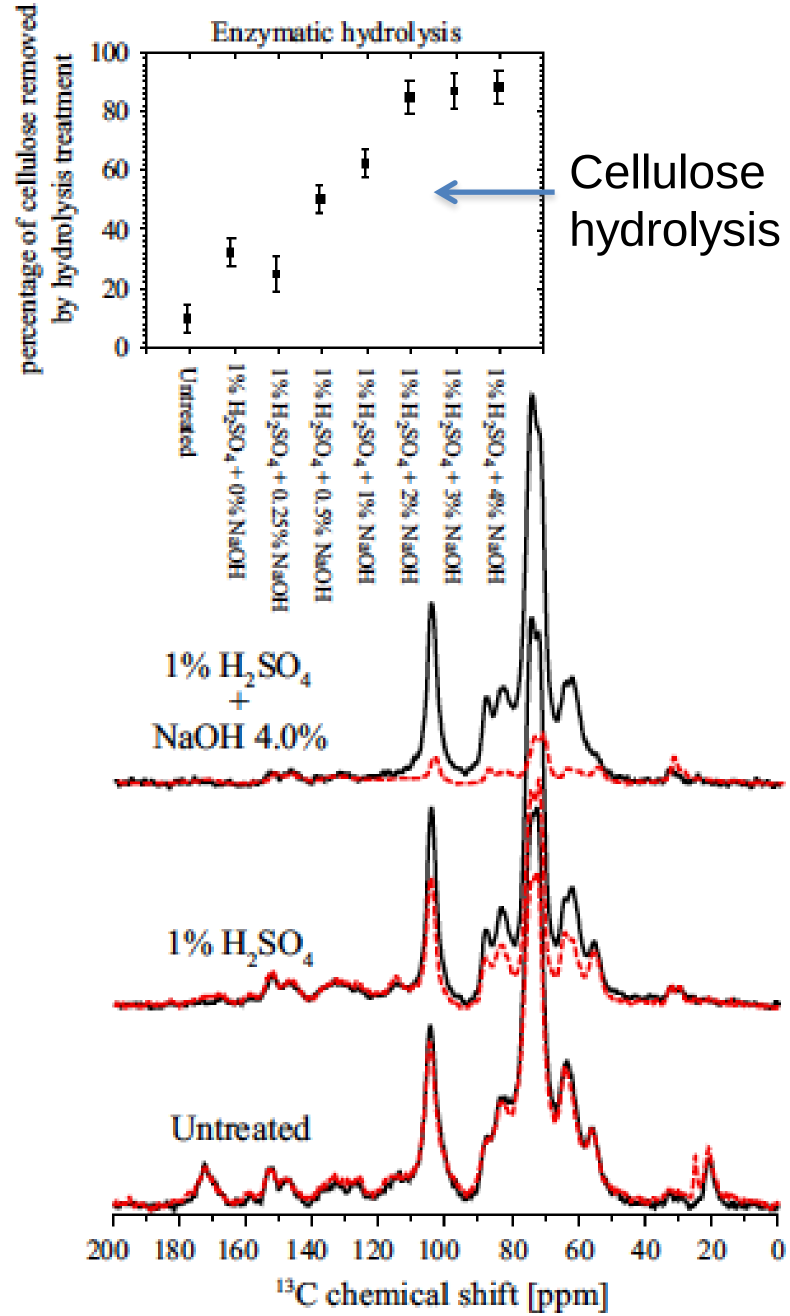
CPMAS-TOSS NMR spectra of sugarcane bagasse: (a) untreated; (b) bagasse treated with H2SO4 1.0% and (c) bagasse treated with acid and NaOH 4.0%. The spectra were normalized by the intensity of line 10 (C1 carbon of cellulose).

Quantitative 13C ssNMR as a tool for evaluation of cellulose crystallinity directly within biomass

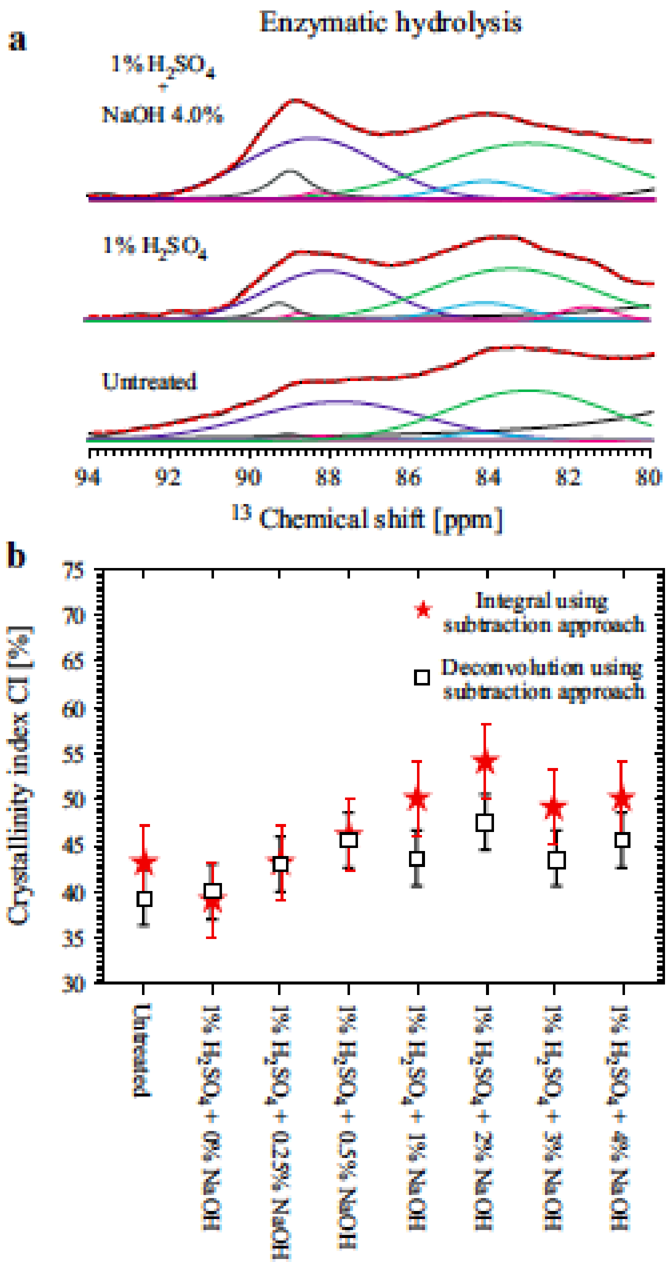


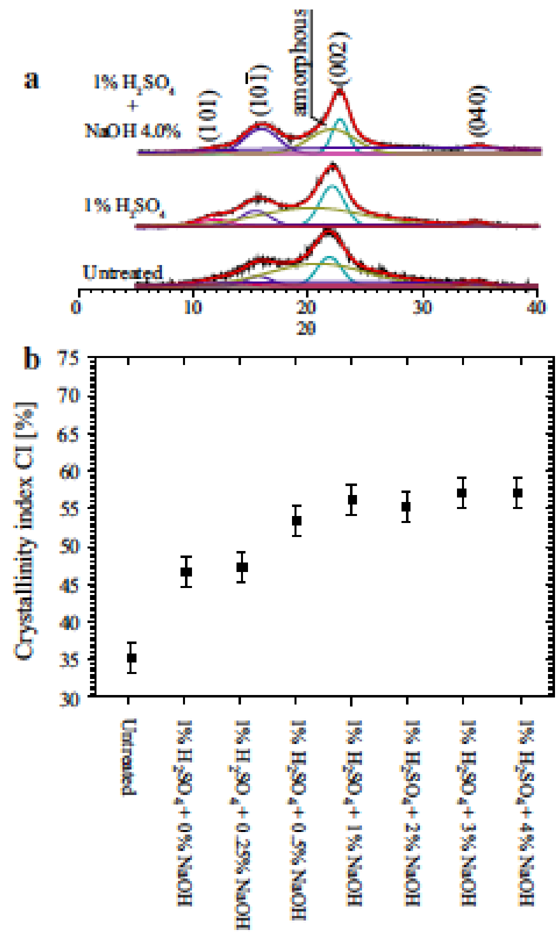
Lignin

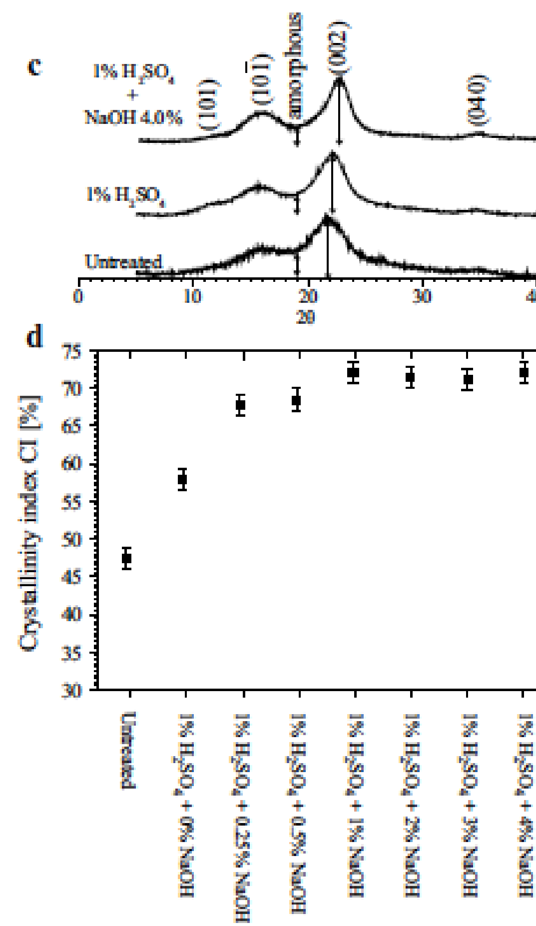
Cellulose hydrolysis



Quantitative 13C ssNMR as a tool for evaluation of cellulose crystallinity directly within biomass

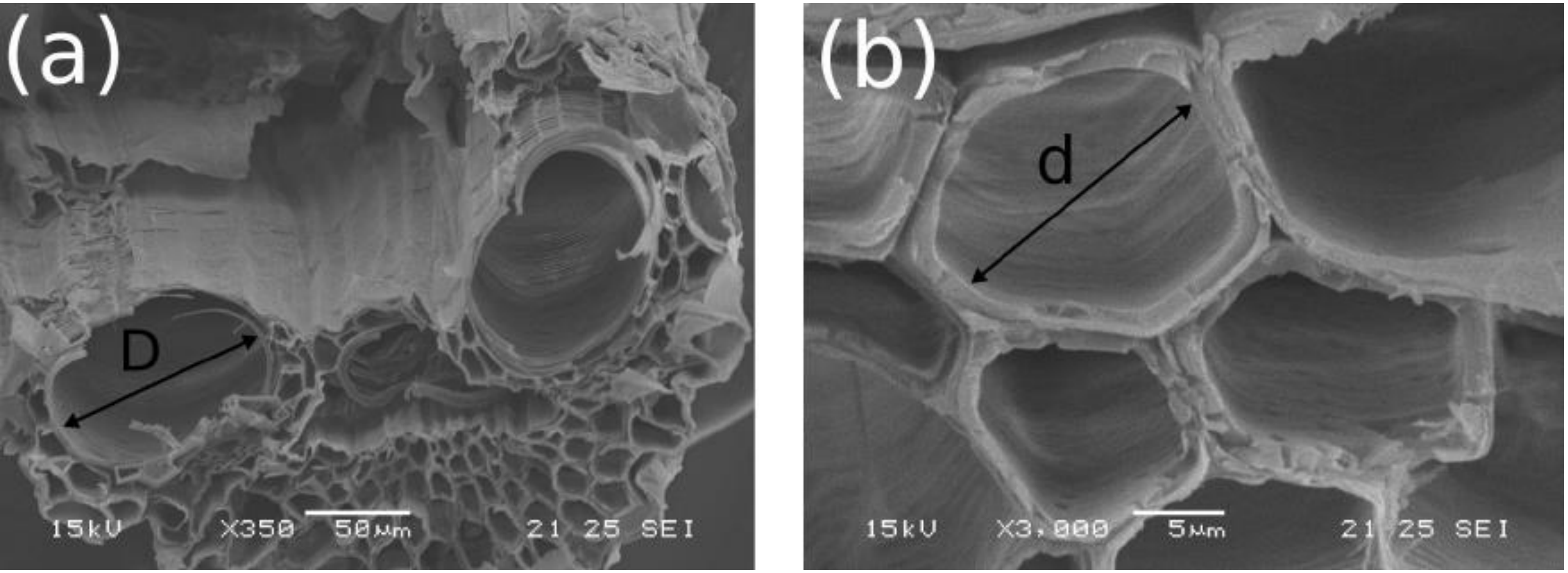




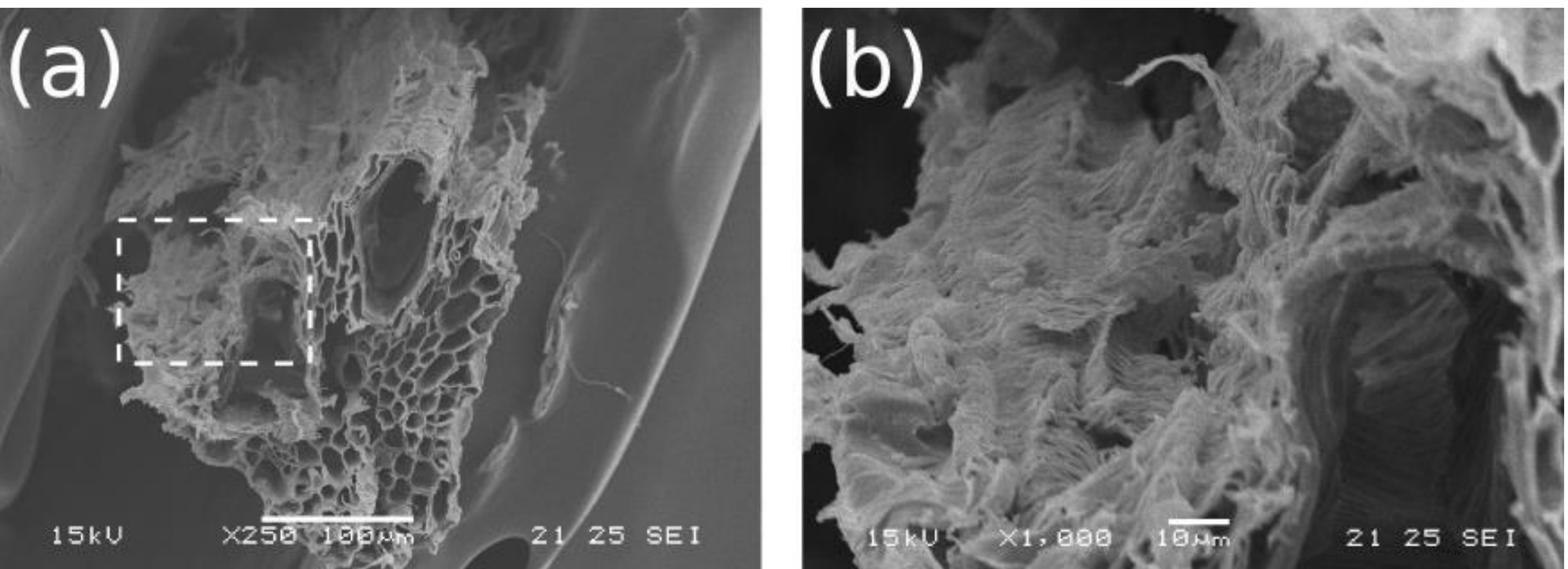


Bernardelli et al., *Biotechnology for Biofuels* (2015) **8**:110

Porosity: Nuclear magnetic resonance investigation of water accessibility in cellulose of pretreated sugarcane bagasse



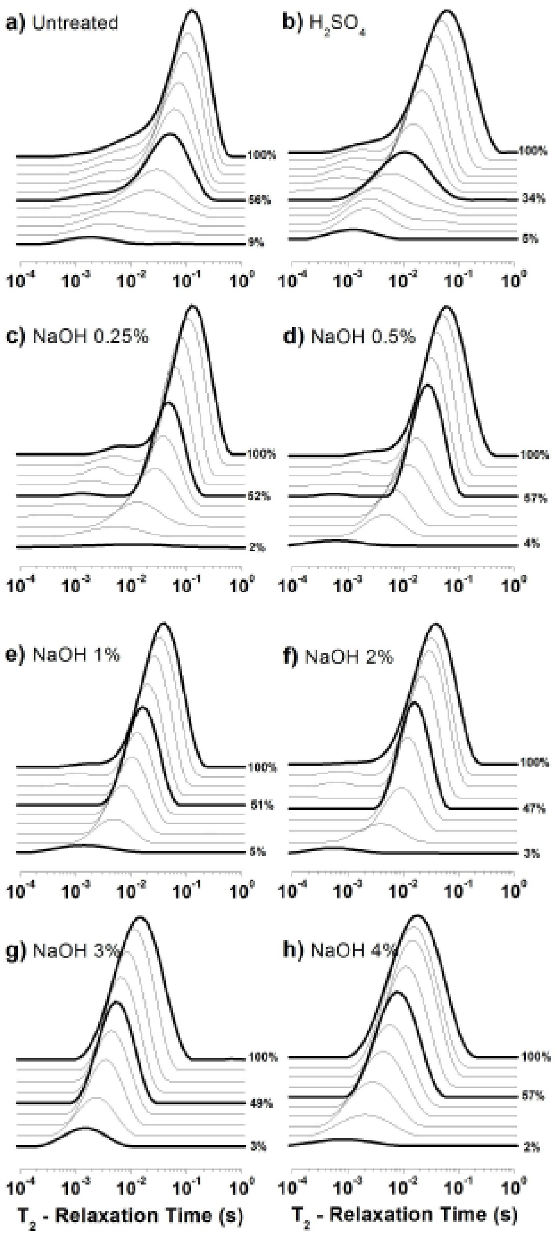
Untreated bagasse
Wide lumen
D=70um;
d=10um

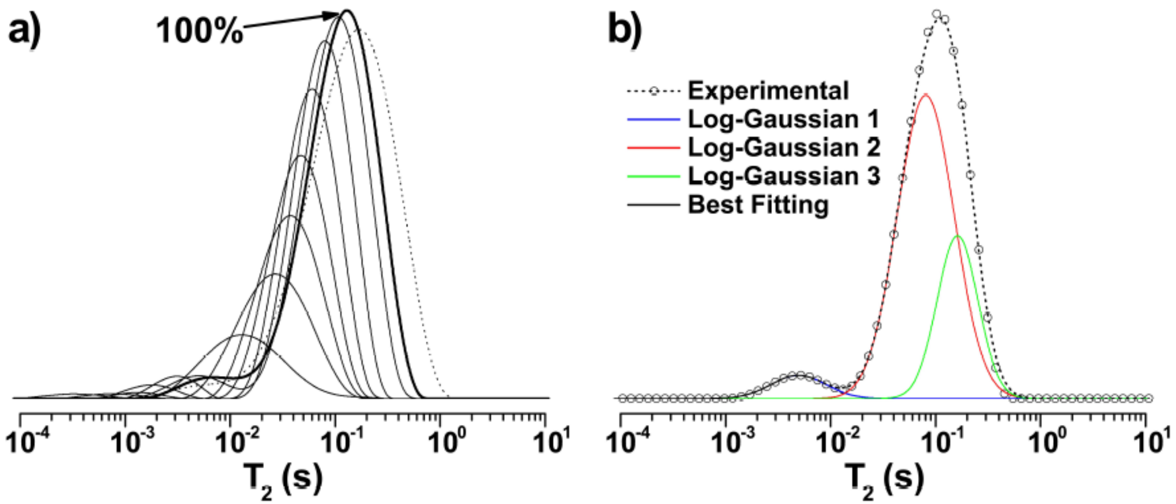


Pretreated bagasse;
2 steps:
1% H_2SO_4 +
1% NaOH

Tsuchida et al., *Biotechnology for Biofuels* (2014) **7**:127

Transverse relaxation times T2 of water molecules defines their mobility: the longer T2, the higher mobility



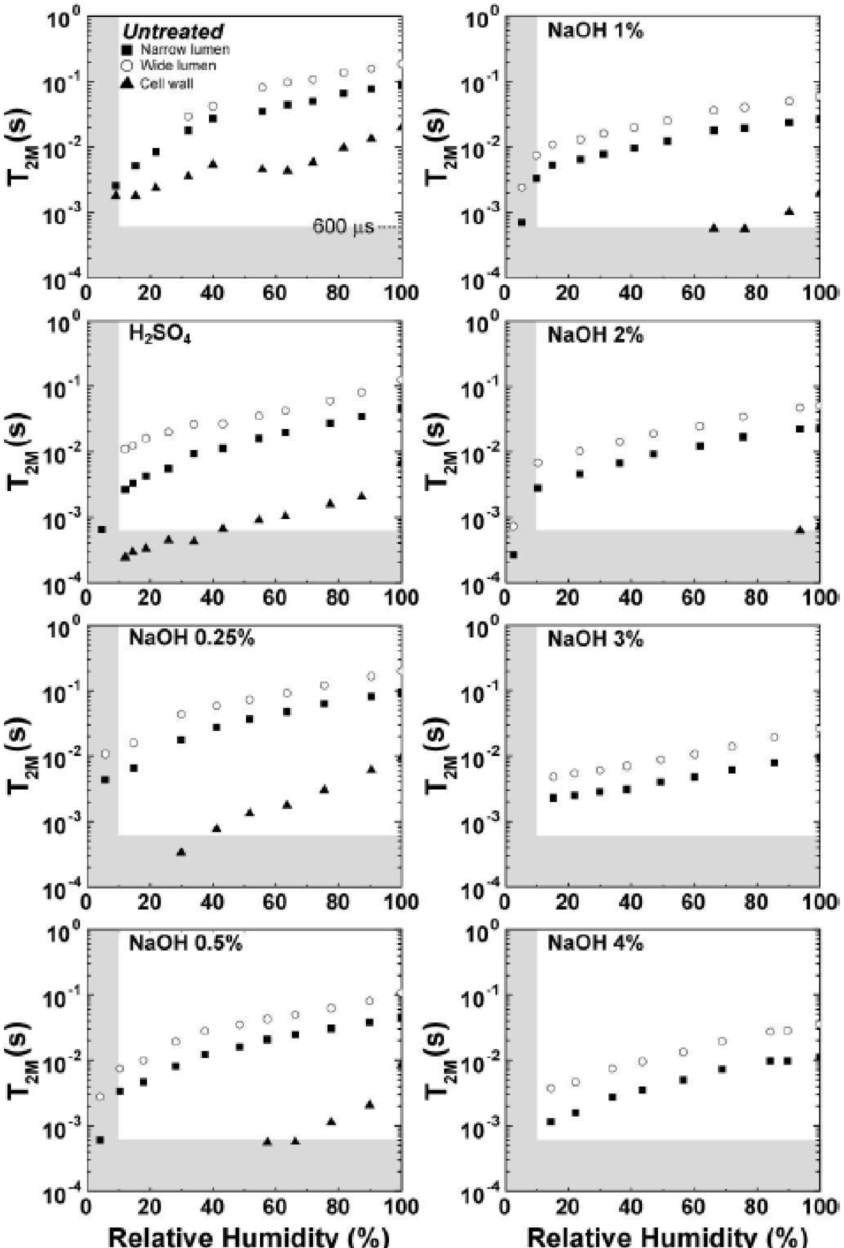


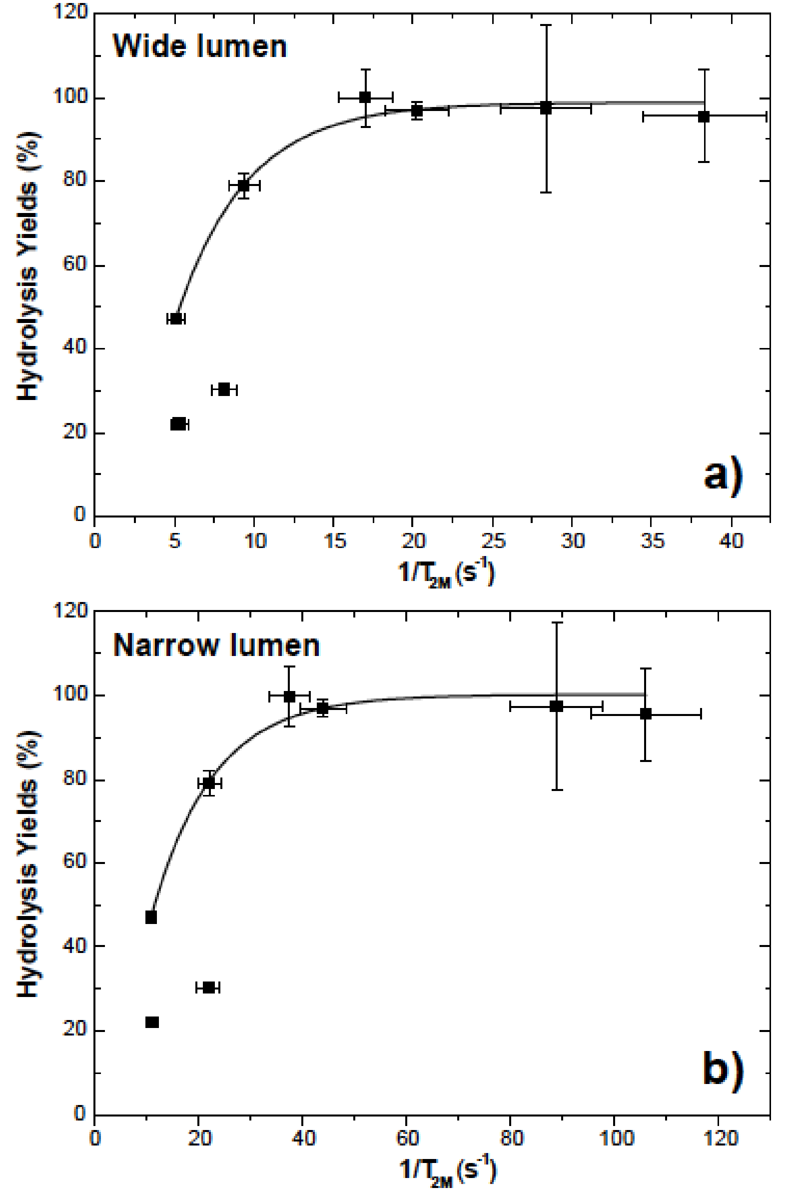
In non-pretreated or mildly pretreated samples three different characteristic T2 times can be observed

In heavily pretreated samples only 2 characteristic T2 times can be quantified

Tsuchida et al., *Biotechnology for Biofuels* (2014) **7**:127

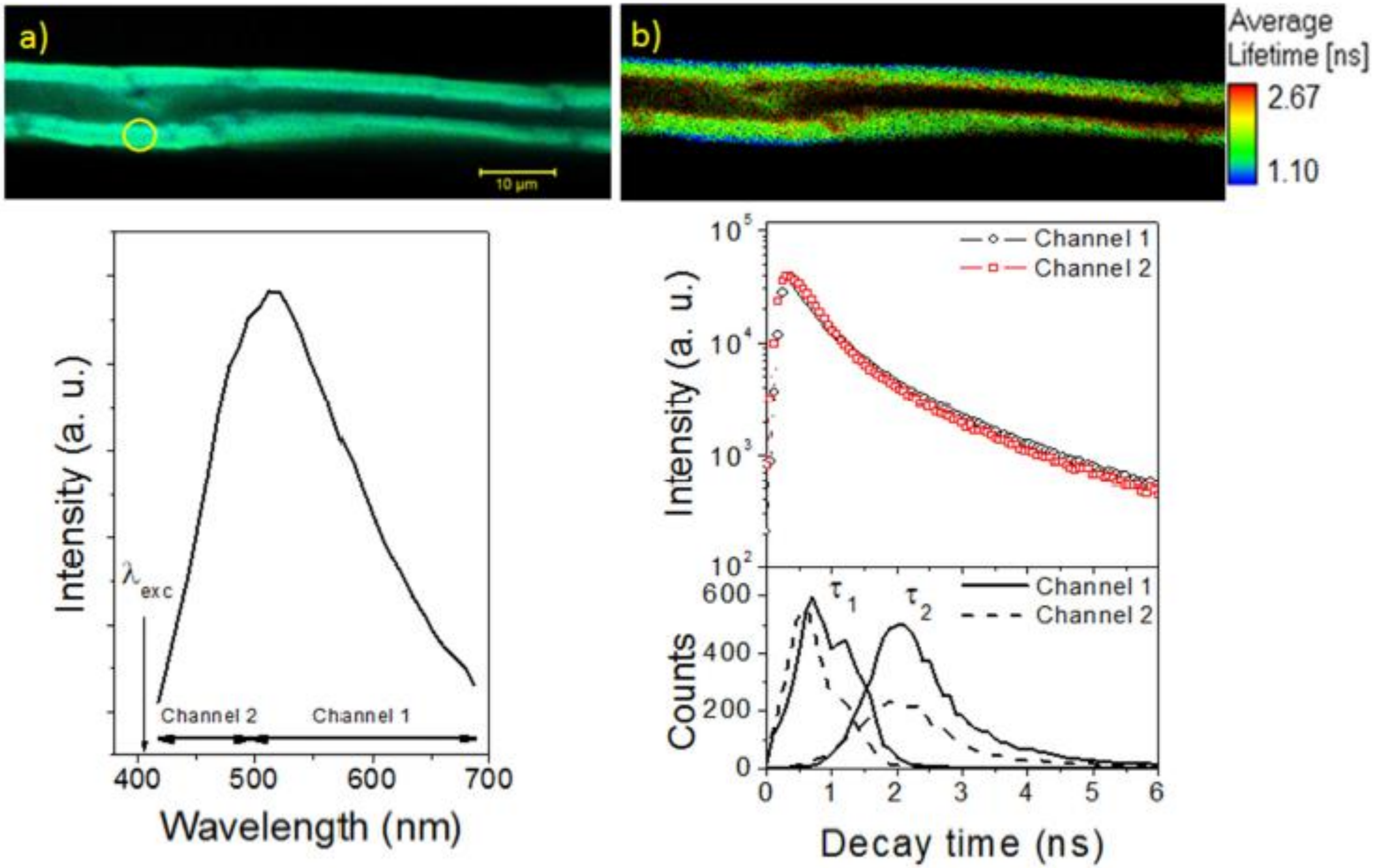
T2 relaxation times are related to the efficiency of enzymatic hydrolysis





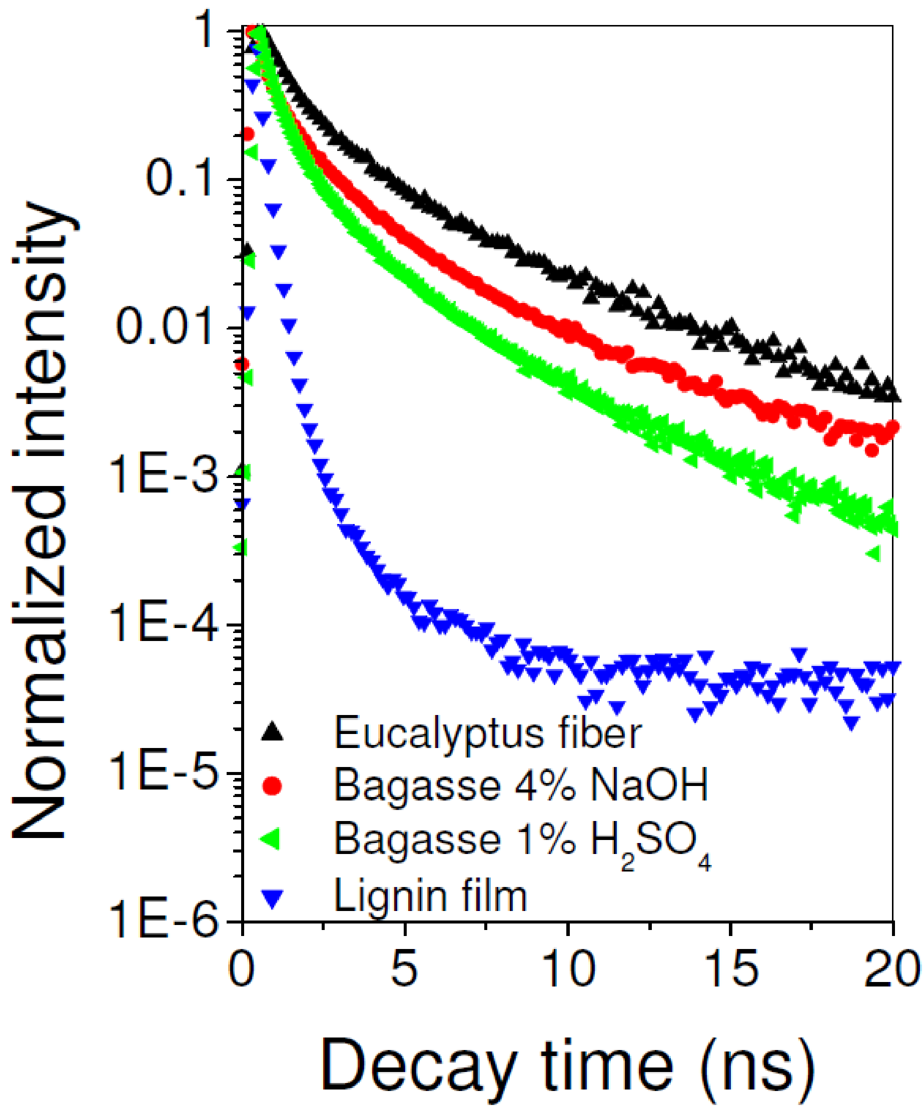
Tsuchida et al., *Biotechnology for Biofuels* (2014) **7**:127

Mapping the lignin distribution in pretreated sugarcane bagasse by confocal and fluorescence lifetime imaging microscopy



a) Spectral confocal image of a single bagasse fiber treated with NaOH 0.5% excited at λ_{exc}= 405 nm (continuous wave). The spectrum below corresponds to the emission evaluated at the yellow spot of the cell wall. b) The corresponding FLIM image and the associated decay features detected from channels 1 and 2. The figure below shows the decay time distributions for τ_1 and τ_2 evaluated from the FLIM image for channel 1 (solid lines) and channel 2 (dashed lines).

Mapping the lignin distribution in pretreated sugarcane bagasse by confocal and fluorescence lifetime imaging microscopy

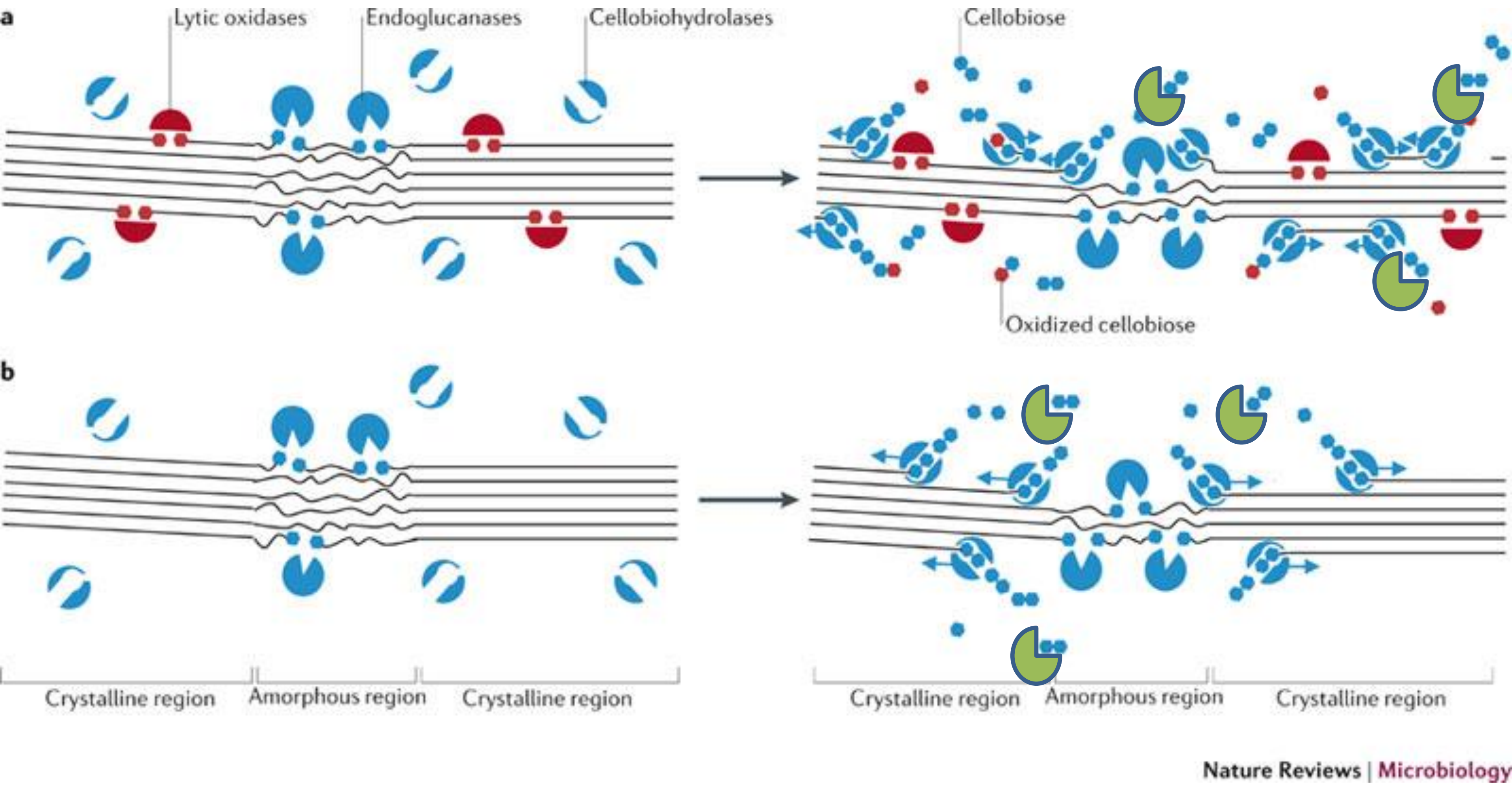


Fluorescence decay behavior for lignin in different systems
Comparison among the fluorescence decay dependences evaluated from single fiber FLIM images of bagasse treated with H_2SO_4 1%, bagasse treated with NaOH 4%, eucalyptus fiber and lignin film.

Coletta et al., *Biotechnology for Biofuels* (2013) **6**:43

- **Enzymatic Hydrolysis**

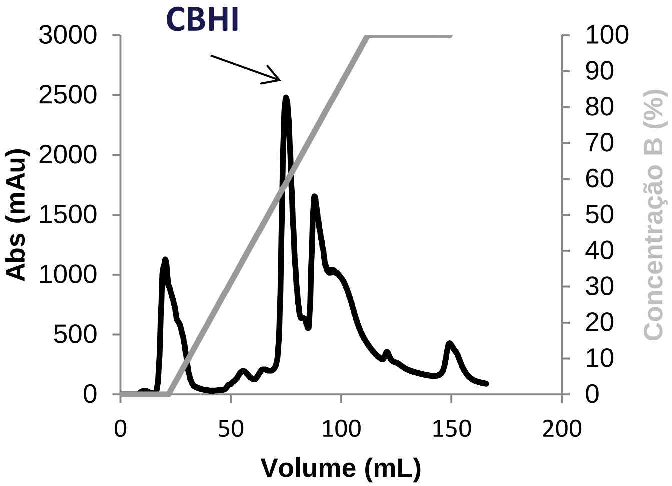
Enzymatic hydrolysis of cellulose

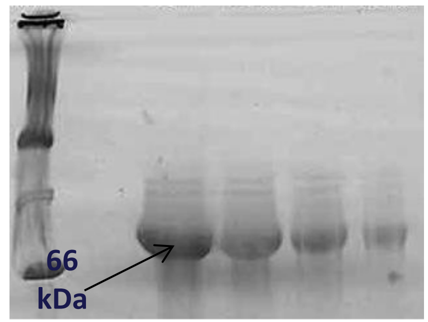


Adapted from Medie, F.M., Davies, G.J., Drancourt, M. & Henrissat, B. Nature Reviews Microbiology (2012) 10, 227-234.

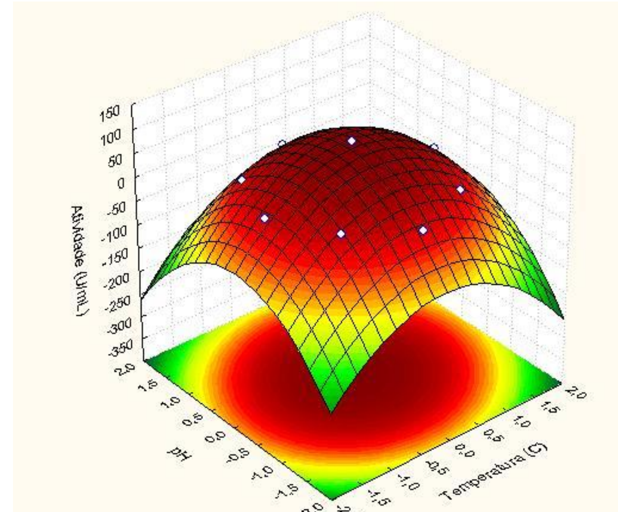
- **Exoglucanases (*T. harzianum* CBHI/Cel7A)**

Trichoderma harzianum CBHI

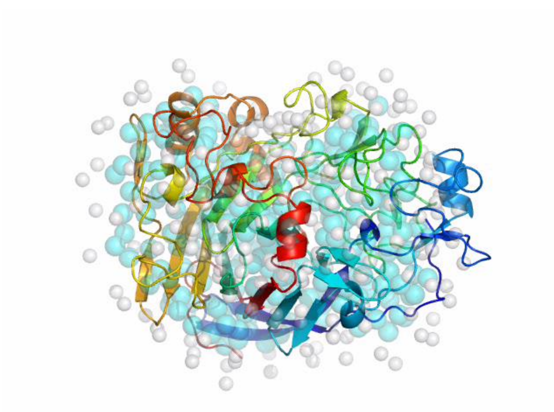


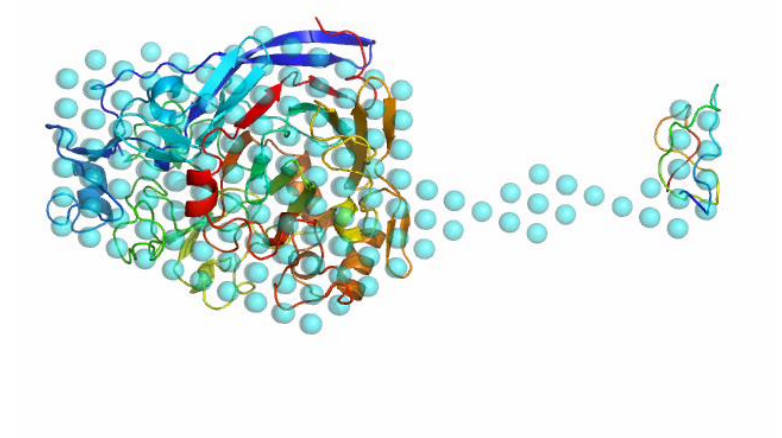


Native gel electrophoresis of CBHI (6, 3, 1.5 e 1 mg/mL)

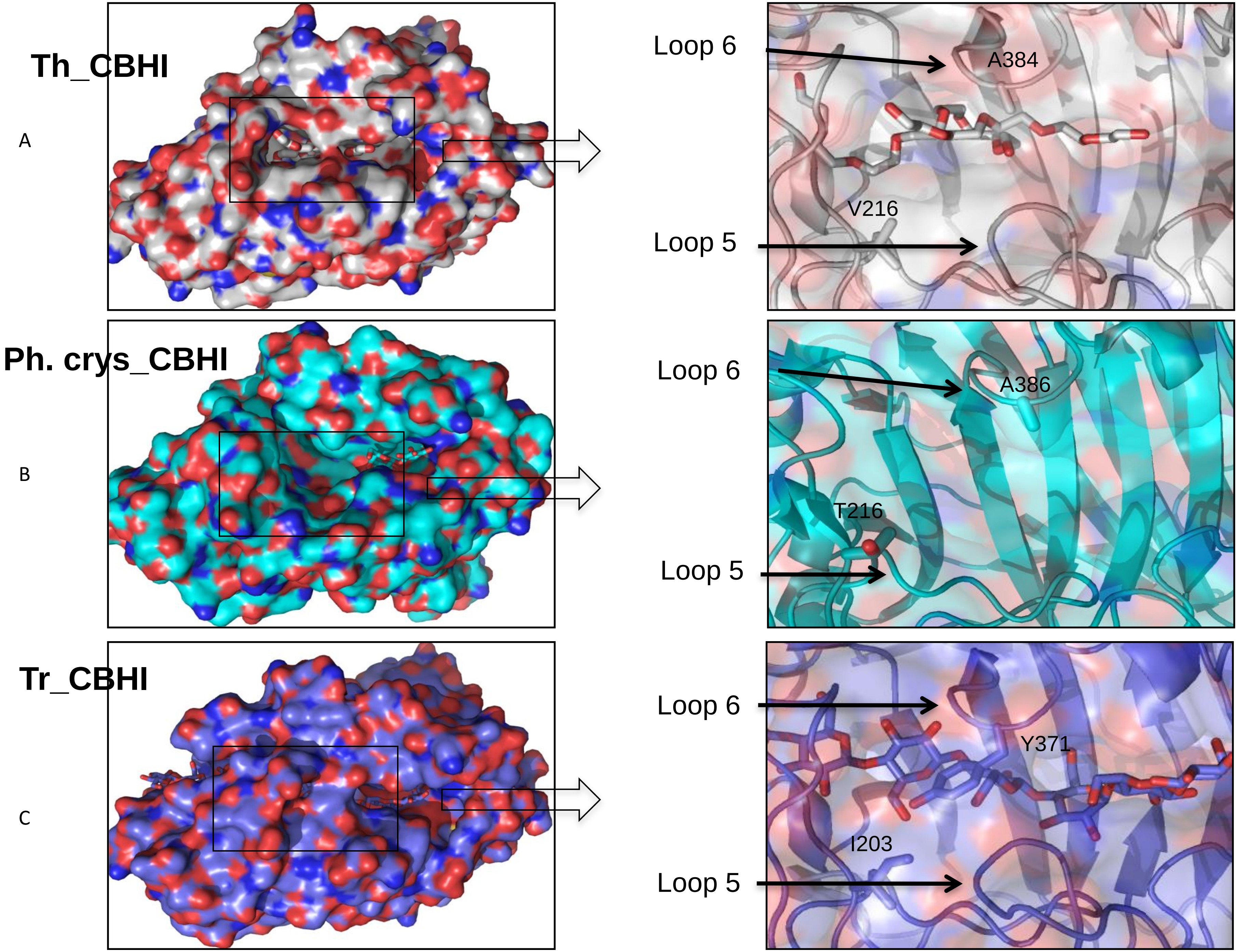


Topt=50ºC, pHopt=5



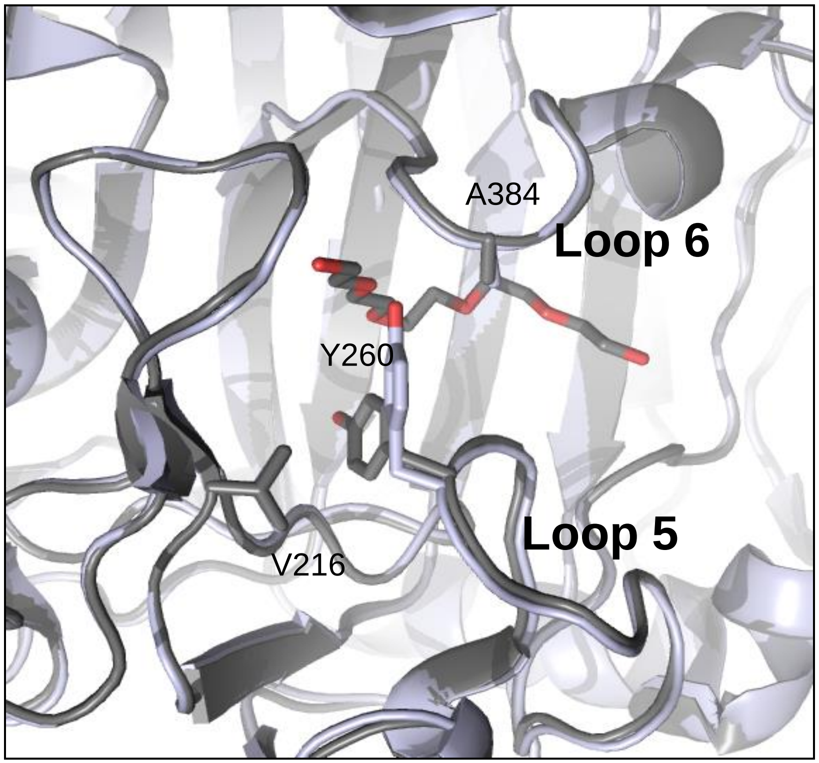


Colussi, F., Textor, L.C., et al. *J. Microbiol. Biotech.* (2011) 21: 808–817


Th_CBHI
A
Loop 6
A384
V216
Loop 5
Ph. crys_CBHI
B
Loop 6
A386
T216
Loop 5
Tr_CBHI
C
Loop 6
Y371
I203
Loop 5

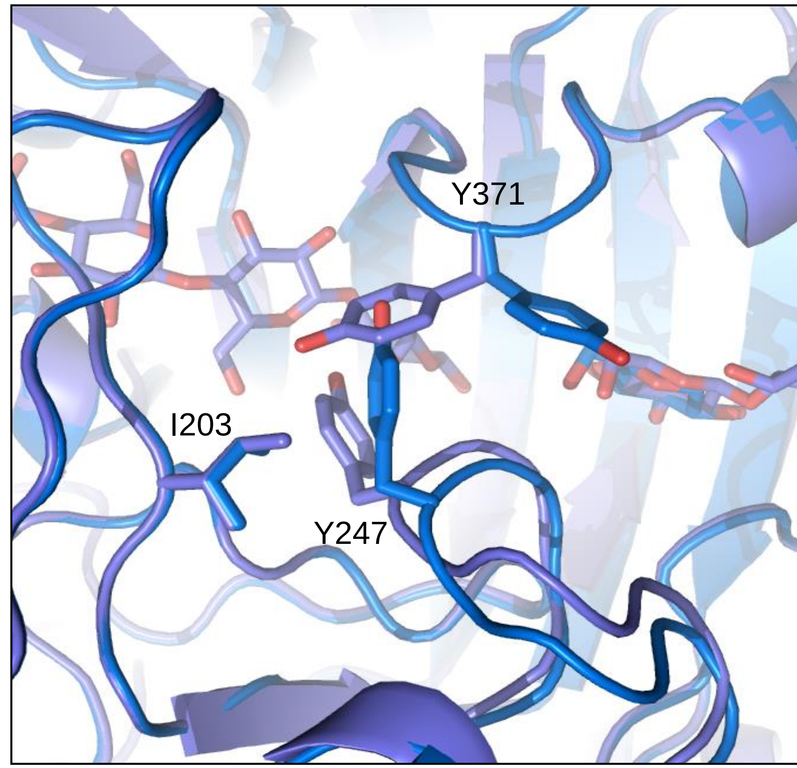
A

T. harzianum CBHI



T. reesei CBHI

B



Textor et al., *FEBS J.* (2013) 280(1): 56-69.

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T_harzianum      18  EQVCTQQAETHPPLTWQKCTA.S.GCTPQQGSVVLDANWRWTHDTKSTTN
T_emersonii       1  EQAGTATAENHPPLTWQECTA.PGSCTTQNGAVVLDANWRWVHDVNGYTN
P_chrysosporium   1  EQAGTNTAENHPQLQSQQCTT.SGGCKPLSTKVVLDSNWRWVHSTSGYTN
T_reesei          1  ESACTLQSETHPPLTWQKCSSGG.TCTQQTGSVVIDANWRWTHATNSSTN

                                                                    L3
T_harzianum      66  CYDGNTWSSTLCPDDATCAKNCCLDGANYSGTYGVTTSGDALTLQFVTA.
T_emersonii      50  CYTGNTWDPTYCPDDETCAQNCALDGADYEGTYGVTSSGSSLKLNFVTG.
P_chrysosporium  50  CYTGNEWDTSLCPDGKTCAANCALDGADYSGTYGITSTGTALTLKFVTG.
T_reesei         50  CYDGNTWSSTLCPDNETCAKNCCLDGAAYASTYGVTTSGNSLSIDFVTQS

                     L3
T_harzianum     115  ..SNVGSRLYLMANDSTYQEFTLSGNEFSFDVDVSQLPCGLNGALYFVSM
T_emersonii      99  ..SNVGSRLYLLQDDSTYQIFKLLNREFSFDVDVSNLPCGLNGALYFVAM
P_chrysosporium  99  ..SNVGSRVYLMADDTHYQLLKLLNQEFTFDVDMSNLPCGLNGALYLSAM
T_reesei        100  AQKNVGARLYLMASDTTYQEFTLLGNEFSFDVDVSQLPCGLNGALYFVSM

T_harzianum     163  DADGGQSKYPGNAAGAKYGTGYCDSQCPRDLKFINGQANVEGWEPSSNNA
T_emersonii     147  DADGGVSKYPNNKAGAKYGTGYCDSQCPRDLKFIDGEANVEGWQPS.snn
P_chrysosporium 147  DADGGMSKYPGNKAGAKYGTGYCDSQCPKDIKFINGEANVGNWTETGSN.
T_reesei        150  DADGGVSKYPTNTAGAKYGTGYCDSQCPRDLKFINGQANVEGWEPSSN .

                                                                 L5
T_harzianum     213  N.TGVGGHGSCCSEMDIWEANSISEALTPHPCETVGQTMCSGDSCGGTYS
T_emersonii     196  anTGIGDHGSCCAEMDVWEANSISNAVTPHPCDTPGQTMCSGDDCGGTYS
P_chrysosporium 196  ..TGTGSYGTCCSEMDIWEANNDAAAFTPHPCTTTGQTRCSGDDCA....
T_reesei        200  N.TGIGGHGSCCSEMDIWQANSISEALTPHPCTTVGQEICEGDGCGGTYS

                     L5
T_harzianum     262  NDRYGGTCDPDGCDWNPYRLGNTSFYGPGSSFALDTTKKLTVVTQFAT..
T_emersonii     246  NDRYAGTCDPDGCDFNPYRMGNTSFYGPG..KIIDTTKPFTVVTQFLTDD
P_chrysosporium 240  ..RNTGLCDGDGCDFNSFRMGDKTFLGKG..MTVDTSKPFTVVTQFLTND
T_reesei        249  DNRYGGTCDPDGCDWNPYRLGNTSFYGPGSSFTLDTTKKLTVVTQFET..

T_harzianum     310  ......DGSISRYYVQNGVKFQQPNAQVGSYS.GNTINTDYCAAEQTAFG
T_emersonii     294  GTDTGTLSEIKRFYIQNSNVIPQPNSDISGVT.GNSITTEFCTAQKQAFG
P_chrysosporium 286  NTSTGTLSEIRRIYIQNGKVIQNSVANIPGVDPVNSITDNFCAQQKTAFG
T_reesei        297  ......SGAINRYYVQNGVTFQQPNAELGSYS.GNELNDDYCTAEEAEFG

                                                    A384
T_harzianum     353  .GTSFTDKGGLAQINKAFQGGMVLVMSLWDDYAVNMLWLDSTYPTNATAS
T_emersonii     343  DTDDFSQHGGLAKMGAAMQQGMVLVMSLWDDYAAQMLWLDSDYPTDADPT
P_chrysosporium 336  DTNWFAQKGGLKQMGEALGNGMVLALSIWDDHAANMLWLDSDYPTDKDPS
T_reesei        340  .GSSFSDKGGLTQFKKATSGGMVLVMSLWDDYYANMLWLDSTYPTNETSS

T_harzianum     402  TPGAKRGSCSTSSGVPAQVEAQSPNSKVIYSNIRFGPIGSTGGntgsn
T_emersonii     393  TPGIARGTCPTDSGVPSDVESQSPNSYVTYSNIKFGPINSTFTas
P_chrysosporium 386  APGVARGTCATTSGVPSDVESQVPNSQVVFSNIKFGDIGSTFSGTS
T_reesei        389  TPGAVRGSCSTSSGVPAQVESQSPNAKVTFSNIKFGPIGSTGNPSG
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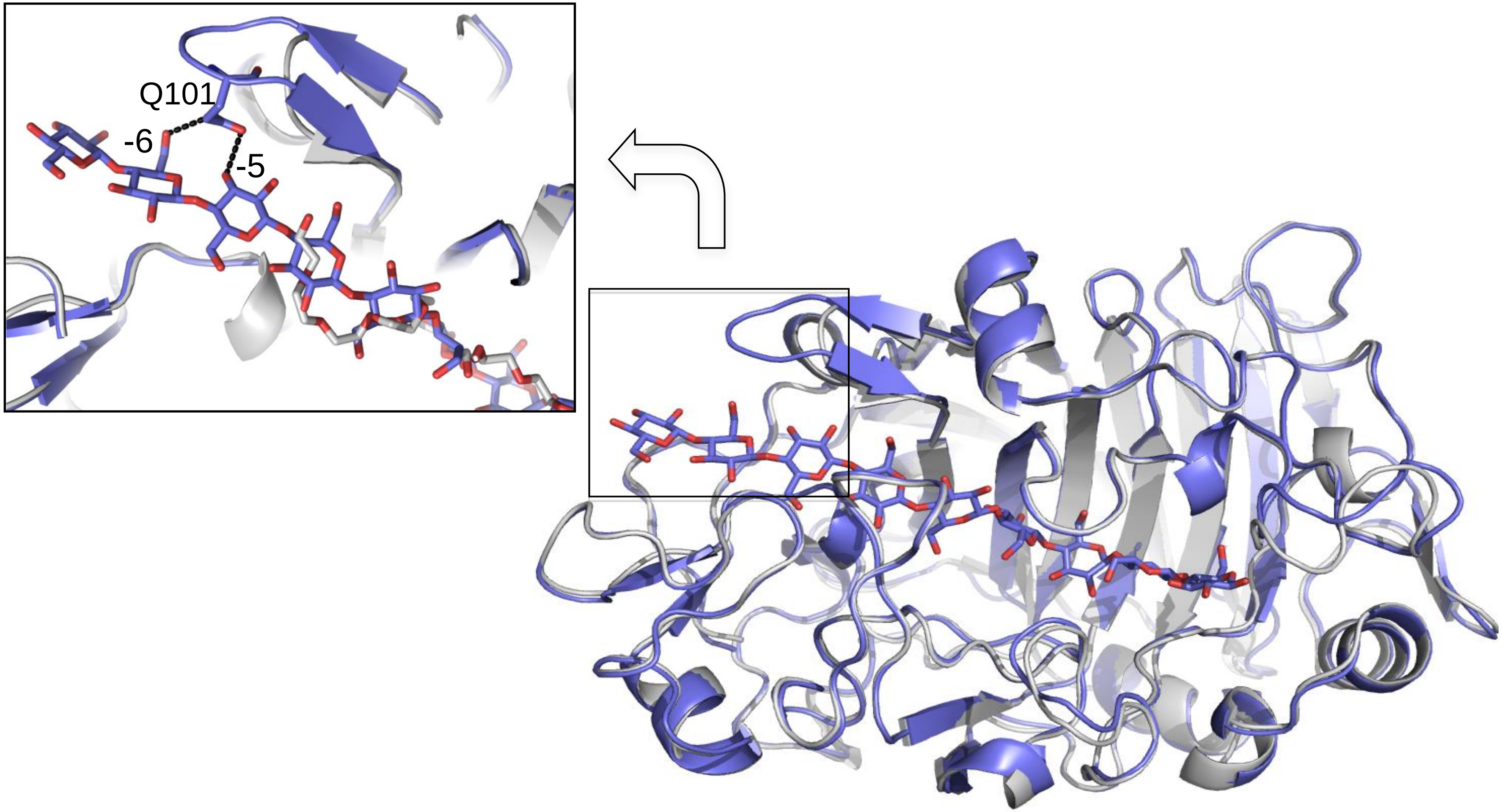
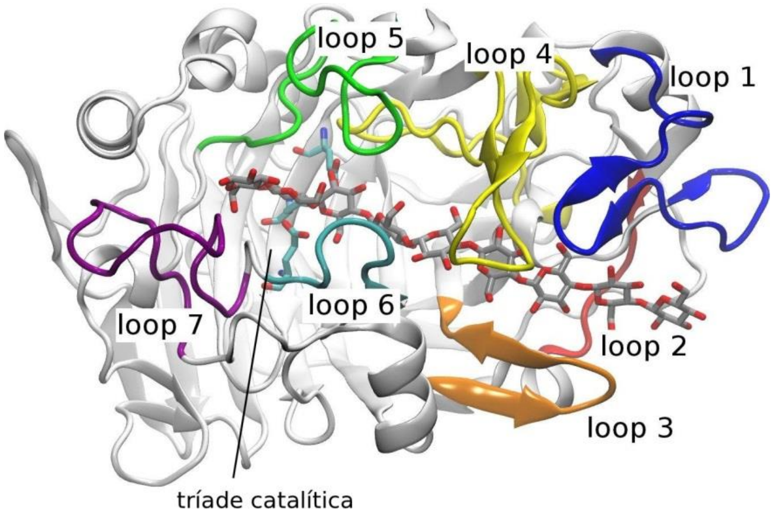
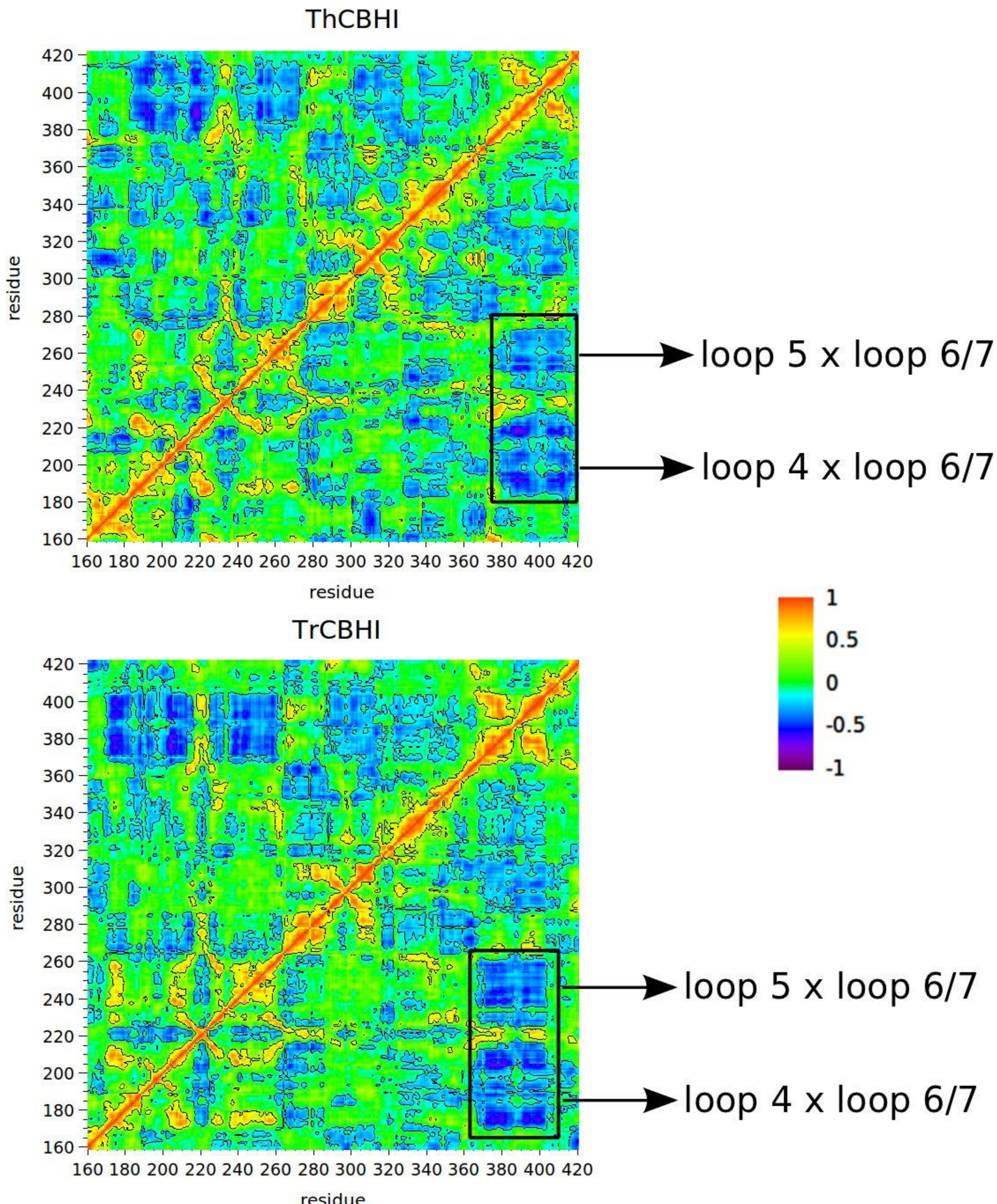


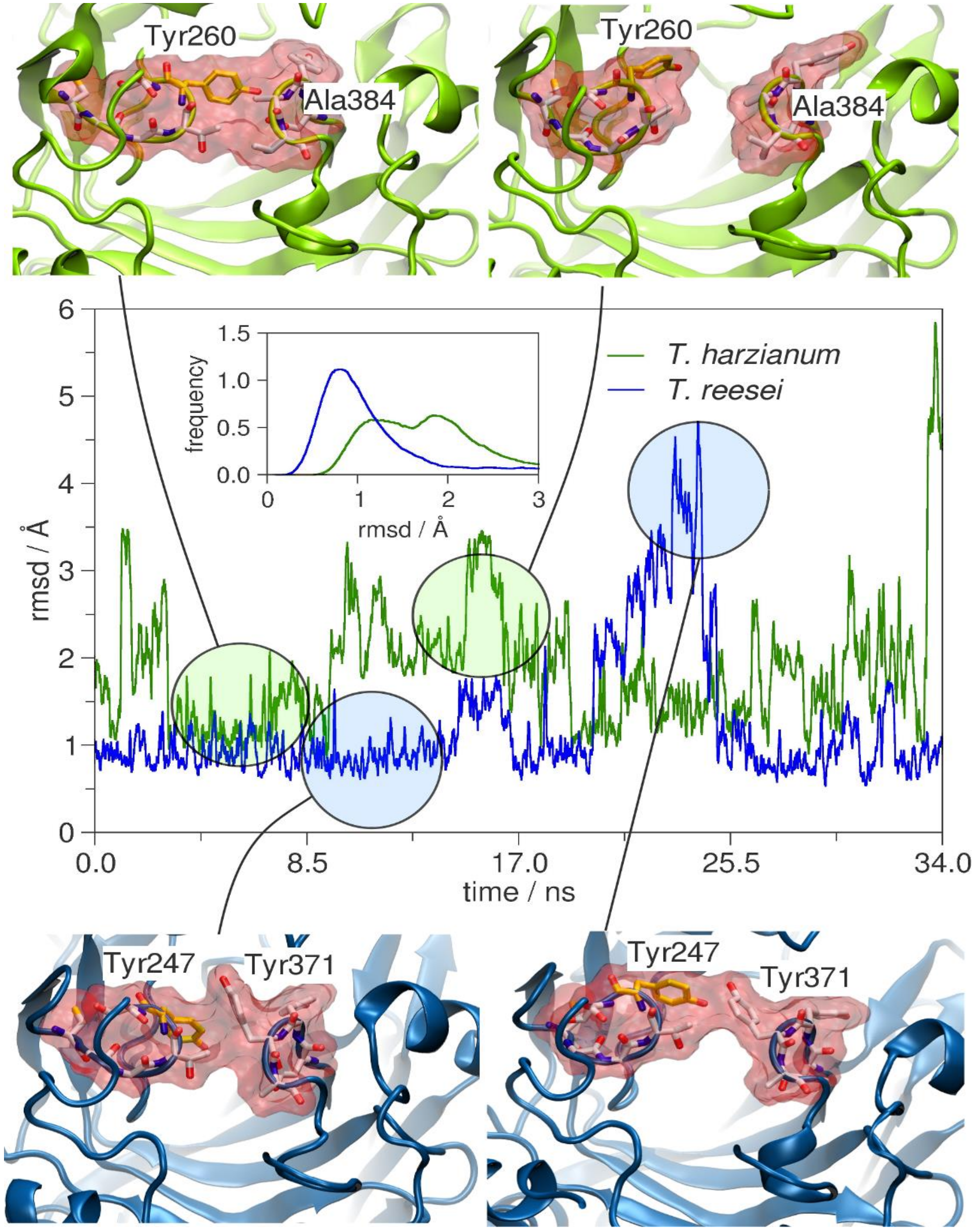
Textor et al., *FEBS J.* (2013) 280(1): 56-69.

DYNAMIC CROSS-CORRELATION MATRIX & ESSENTIAL DYNAMICS

Catalytic side loops movements are strongly anticorrelated!



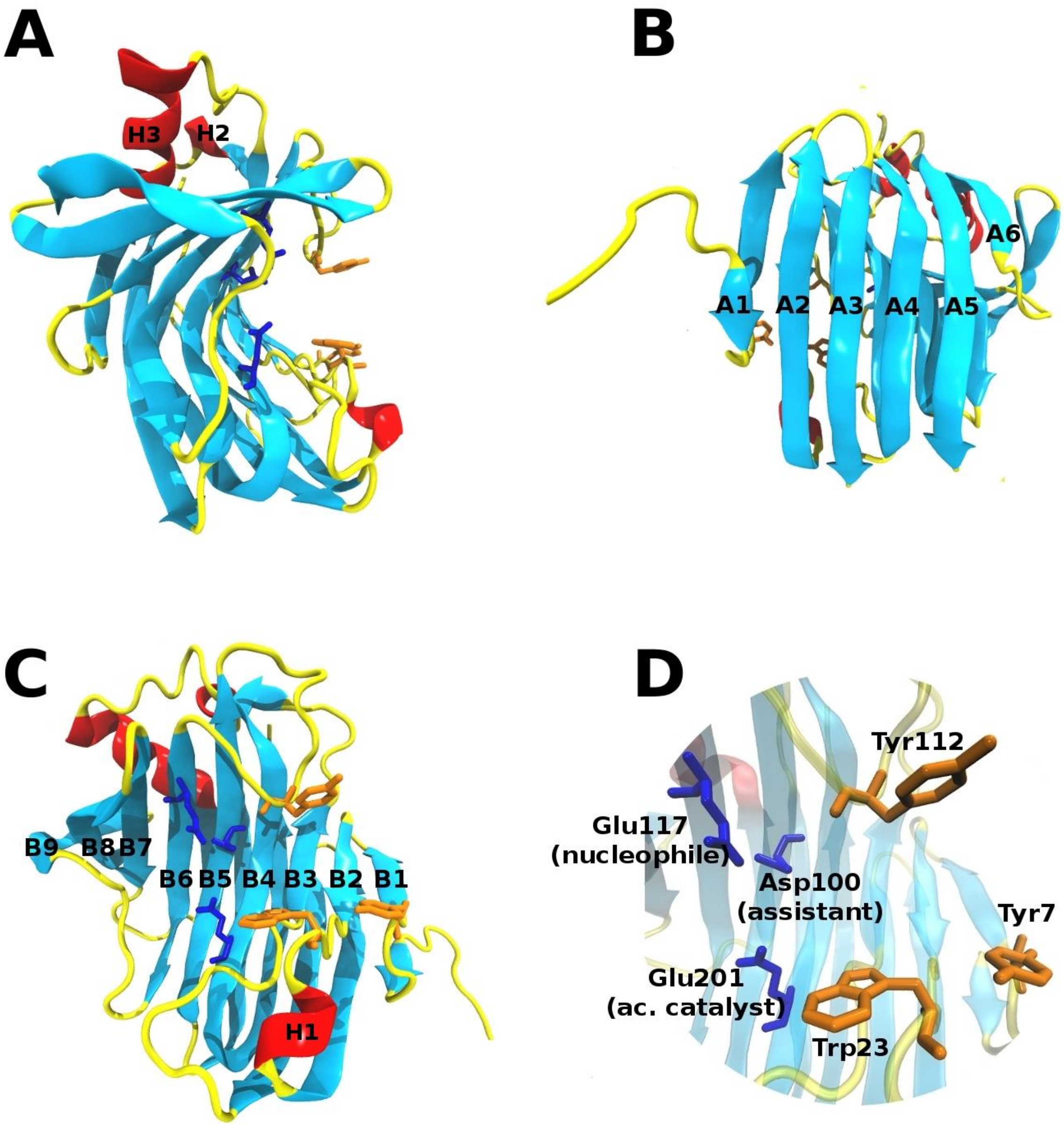




Textor et al., *FEBS J.* (2013) 280(1): 56-69.

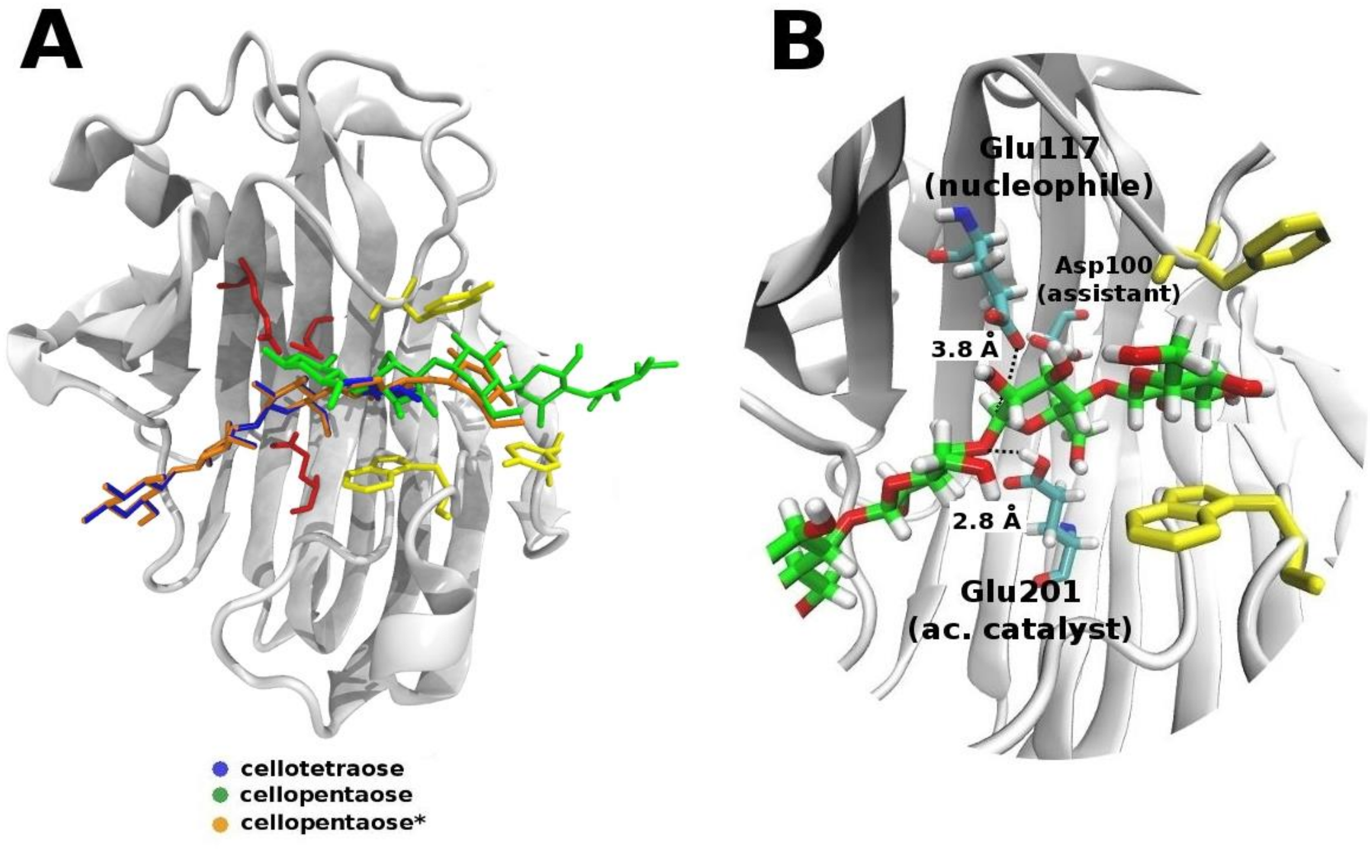
- **Endoglucanases (*T. harzianum* EG3/Cel12)**

3D structure of EG3 (Cel12, T. harzianum): A cellulase without CBM



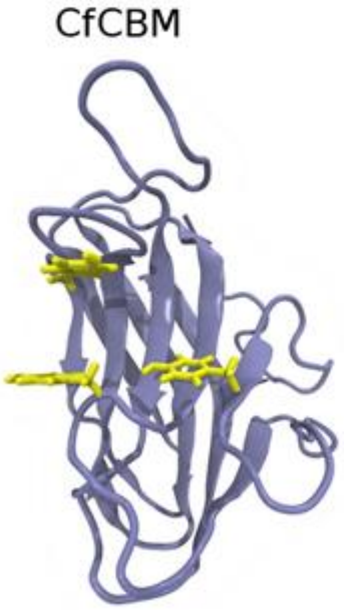
Prates et al., PLoS One (2013) 8(3): e59069

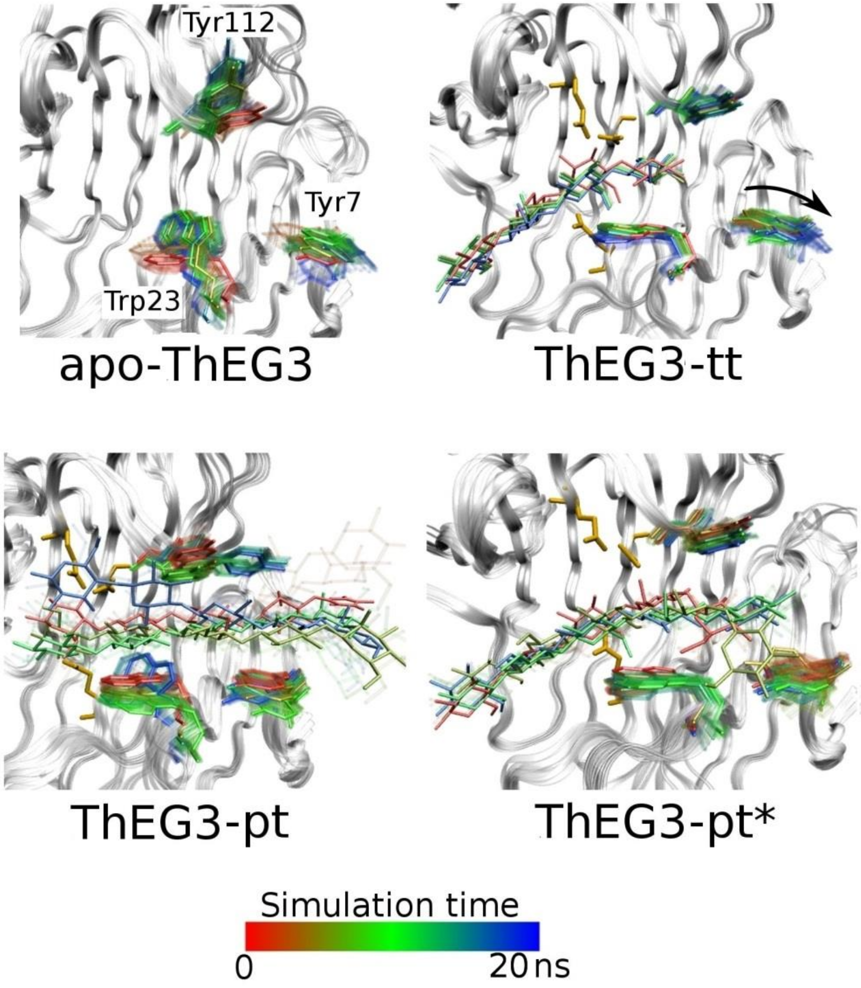
Substrate Binding Cleft



Prates et al., PLoS One (2013) 8(3): e59069

Comparison between Celulomonas fimi endoglucanase C and ThEG3



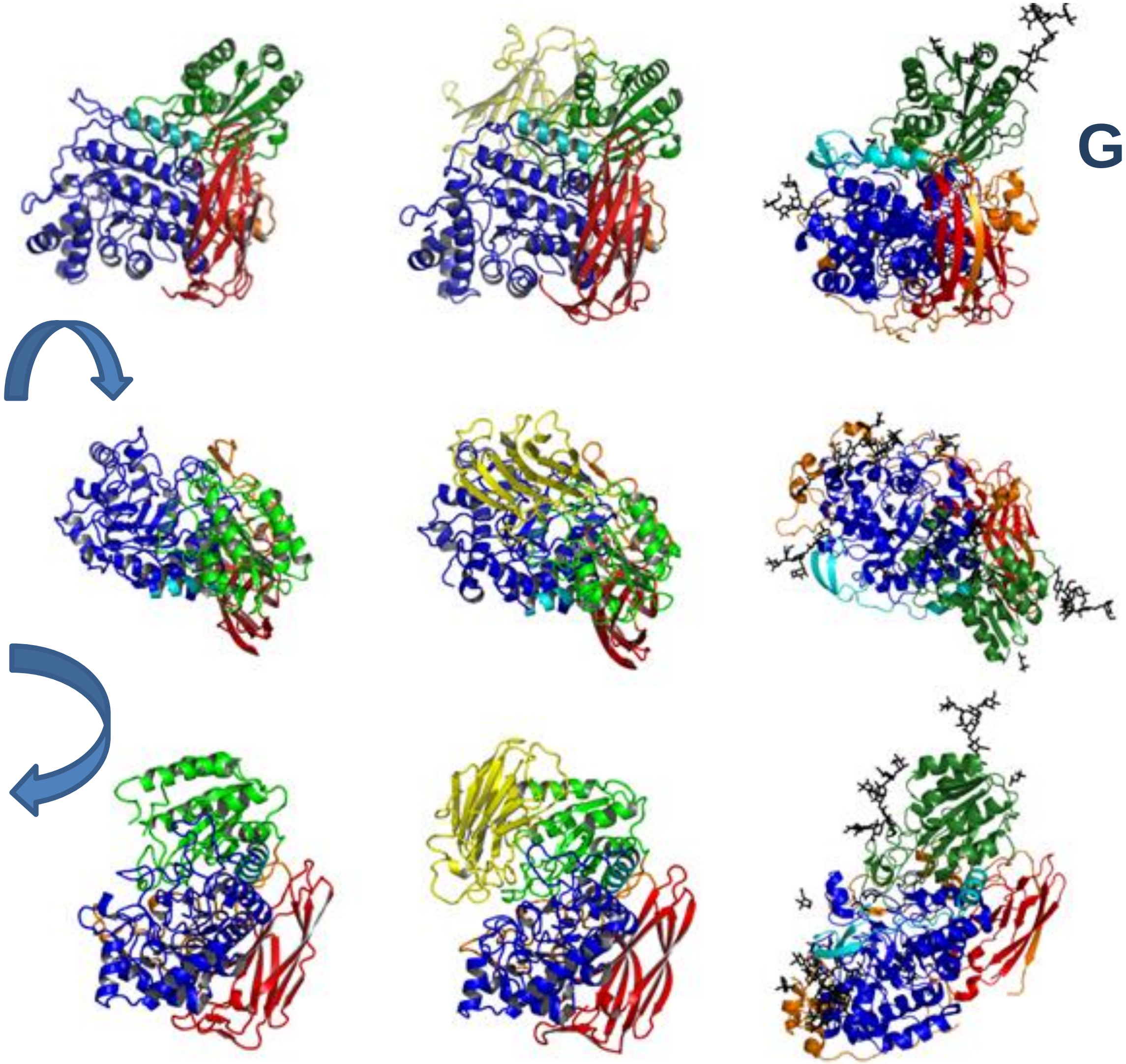


Prates et al., PLoS One (2013) 8(3): e59069

- **Beta-glucosidases**

Beta-glucosidases belong to the CAZy families GH3 and GH1

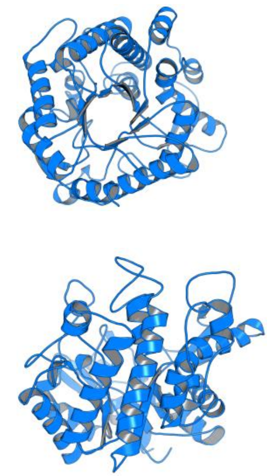
GH3 *vs* **GH1**



Thermotoga neapolitana Bgl3

Kluyeromices marxianus Bgl1

Aspergillus aculeatus Bgl1

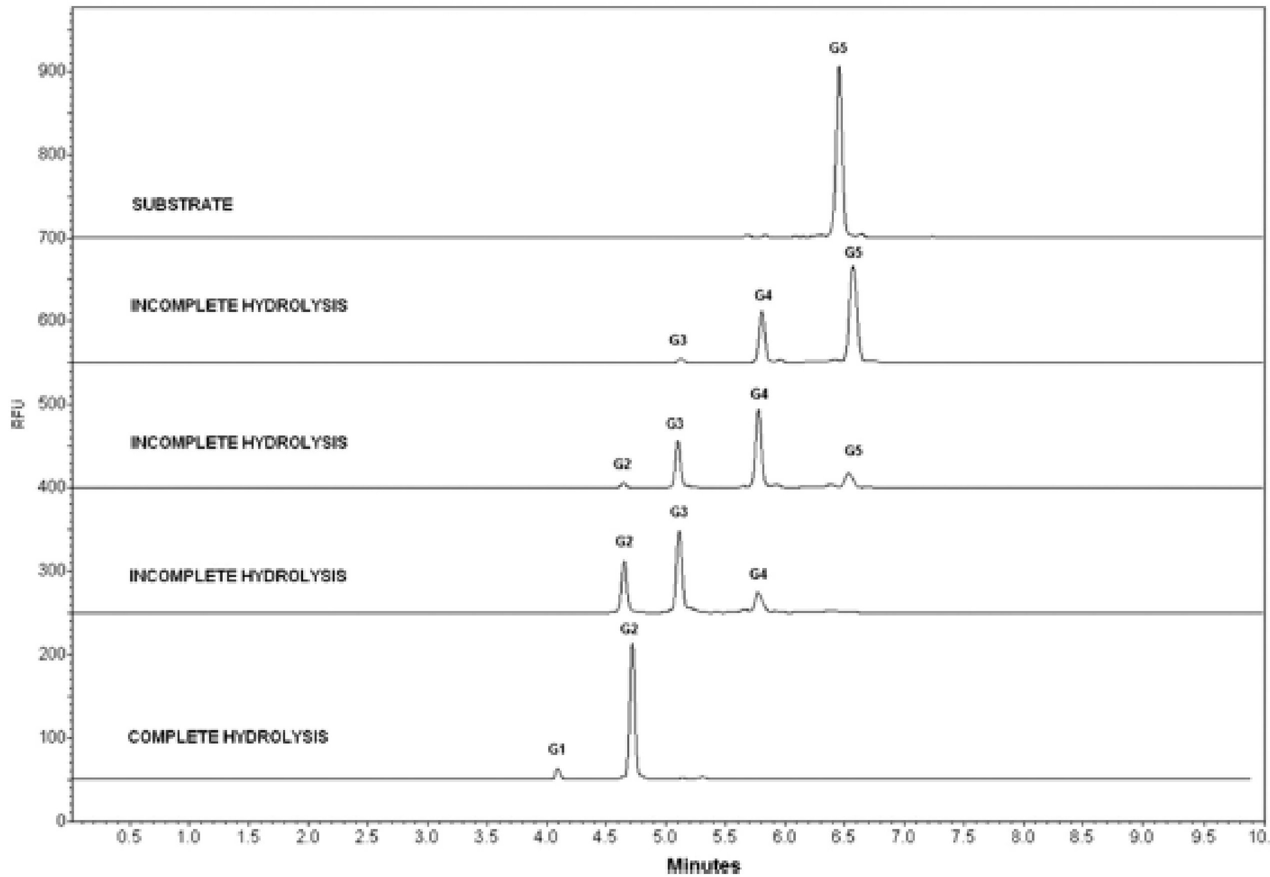


T. harzianum Bgl1

A. niger beta-glucosidase (GH3)

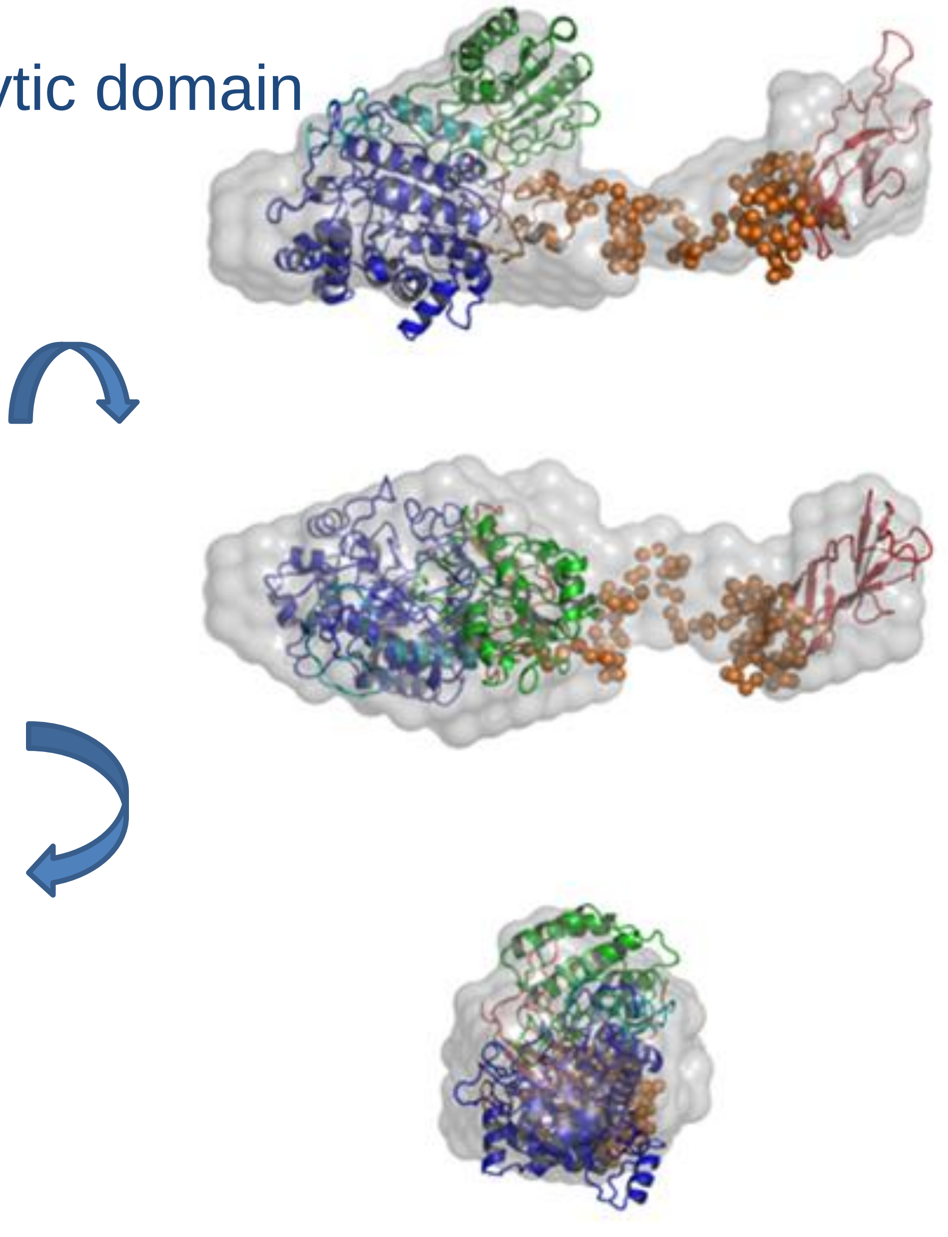
NOVOZYM 188

Purified B-glucosidase



Lima, et al., (2013) J. Biol. Chem. 288: 32991-33005

A. niger beta-glucosidase (GH3) has a cellulase-like tadpole shape



Lima, et al., (2013) J. Biol. Chem. 288: 32991-33005

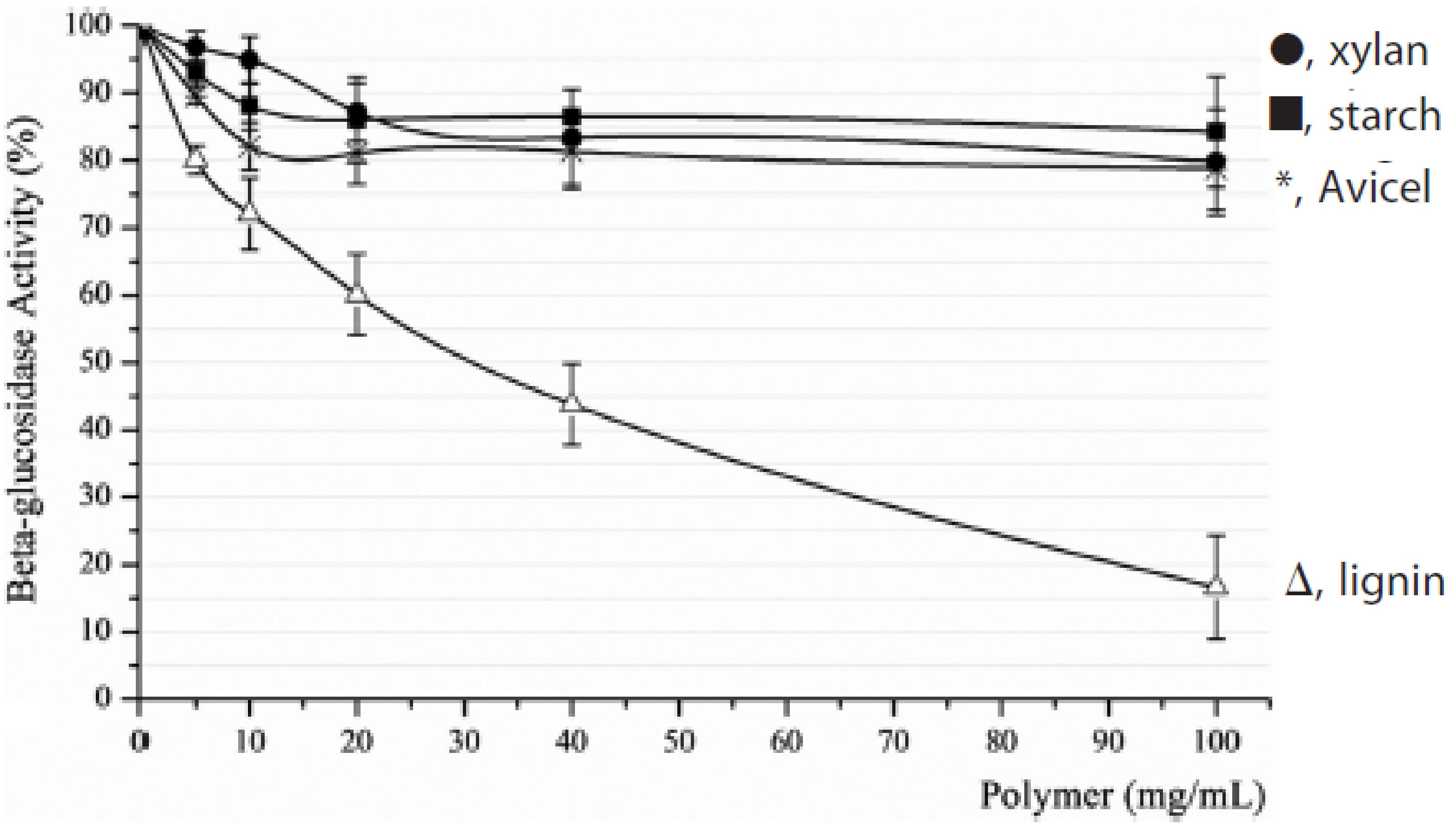
A. niger beta-glucosidase (GH3) hydrolase only cellobiose

Substrate specificity of purified *An*Bgl1

Substrates	Relative activity[a]
	%
Cellobiose	100
Debranched arabinan	0.03
Linear arabinan	0
Sugar beet	0
Galactomannan	0
β-1,4-Mannan	0
Rye arabinoxylan	0
Xylan birchwood	0.24
Xyloglucan	0
Xylan oat spelt	0.99
Laminarin	1.02
Carboxymethylcellulose	0
β-Glucan	0
Lichenan	1.01
Avicel PH101	1.57
Sigma Cellulose	1.94
Microcrystalline Cellulose	1.27

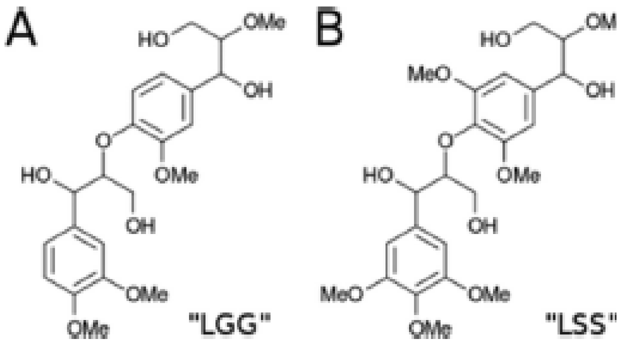
[a] The relative activities are expressed as percentage by normalizing to the cellobiose specific activity (98.7 units/mg).

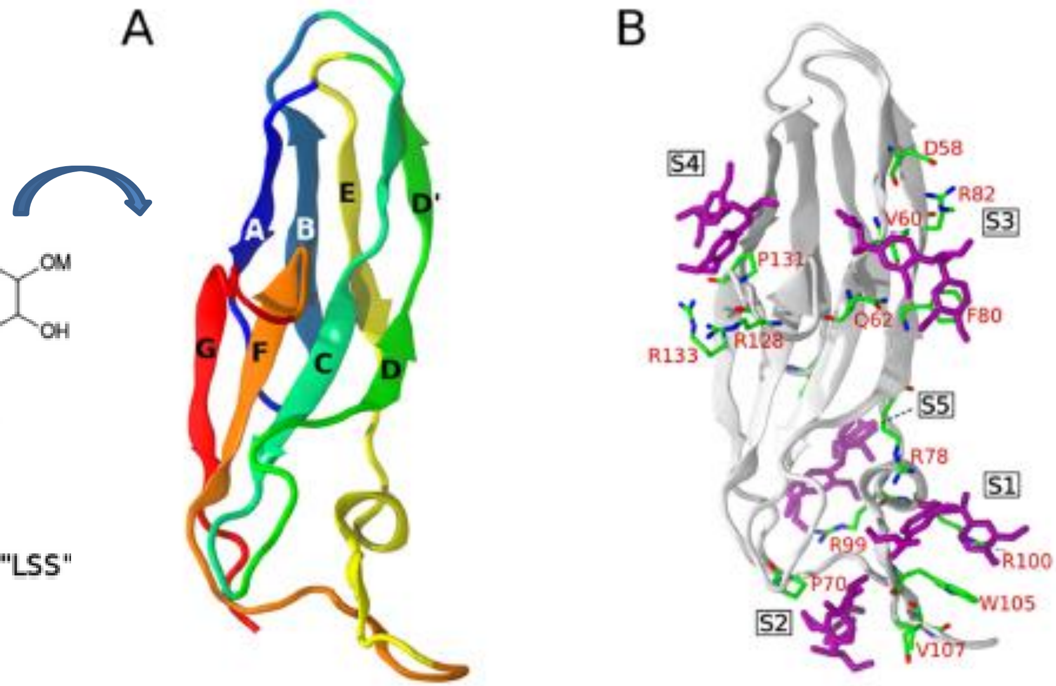
A. niger beta-glucosidase (GH3) binds lignin

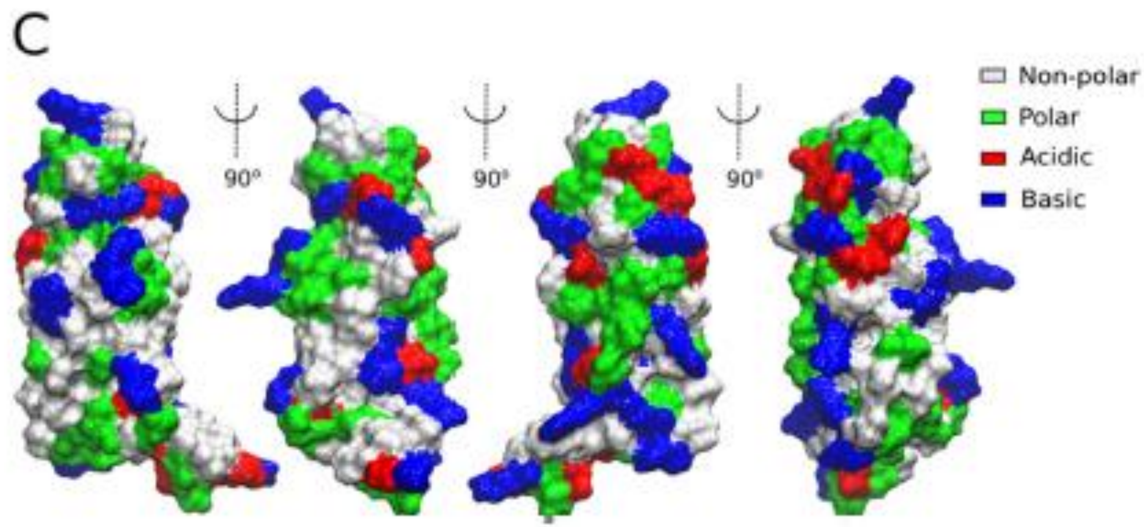


Lima, et al., (2013) J. Biol. Chem. 288: 32991-33005

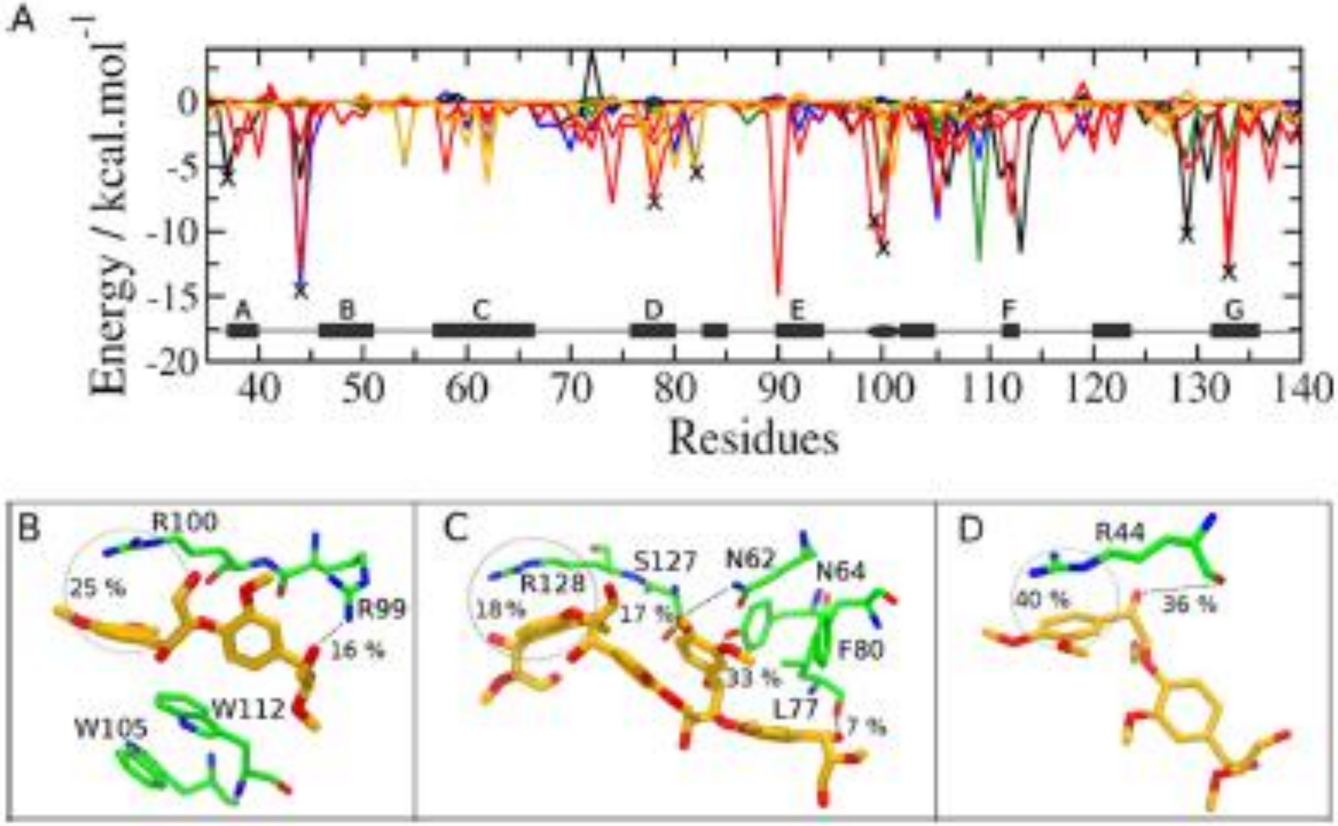
A. niger beta-glucosidase FnIII domain has lignin binding sites







Binding of lignin to A. niger beta-glucosidase FnIII domain



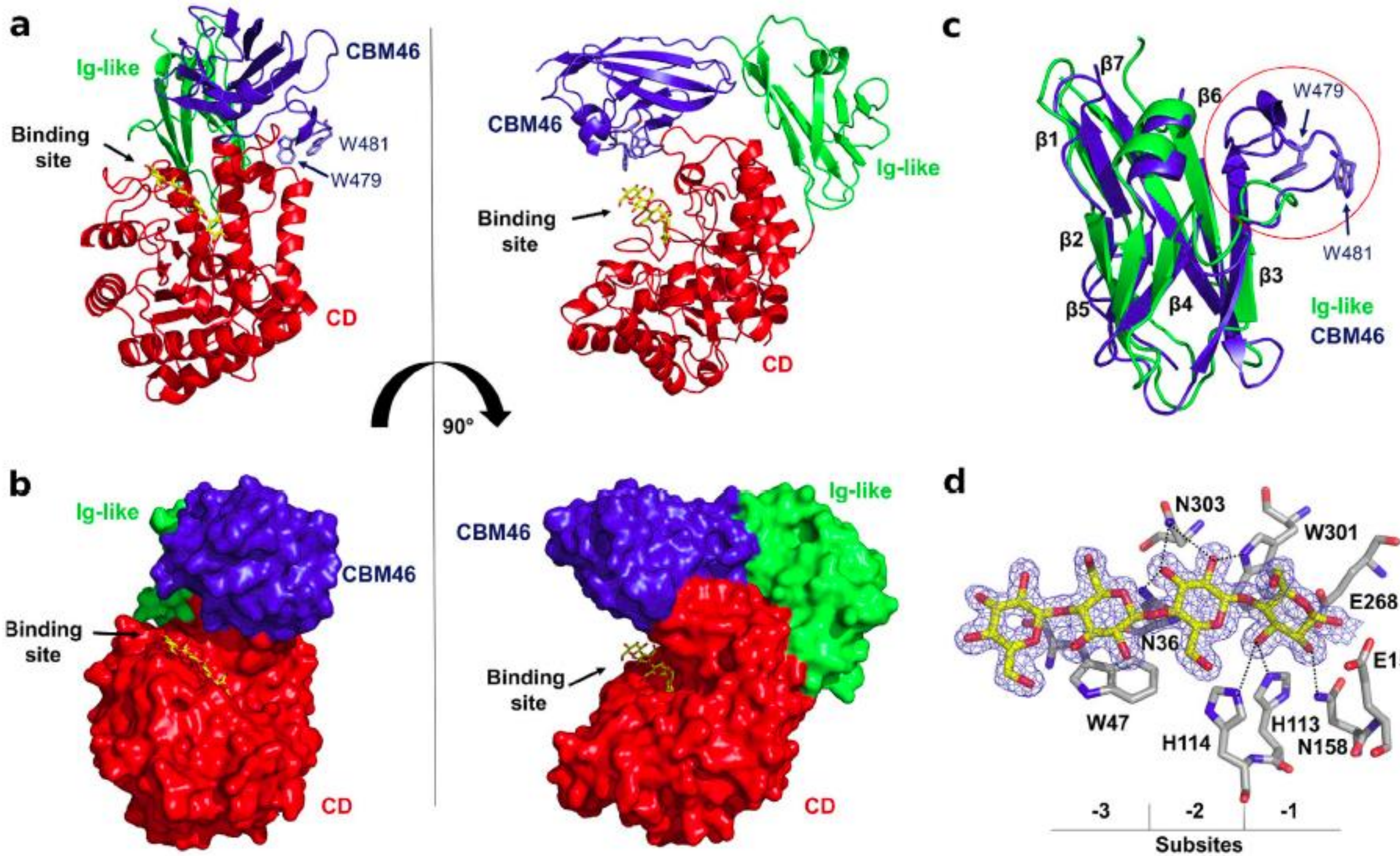
Adsorption of beta-glucosidases in two commercial preparations onto pretreated biomass and lignin

Biotechnology for Biofuels 2013, **6**:165 doi:10.1186/1754-6834-6-165

Mai Østergaard Haven (maope@dongenergy.dk) & Henning Jørgensen (hnj@ign.ku.dk)

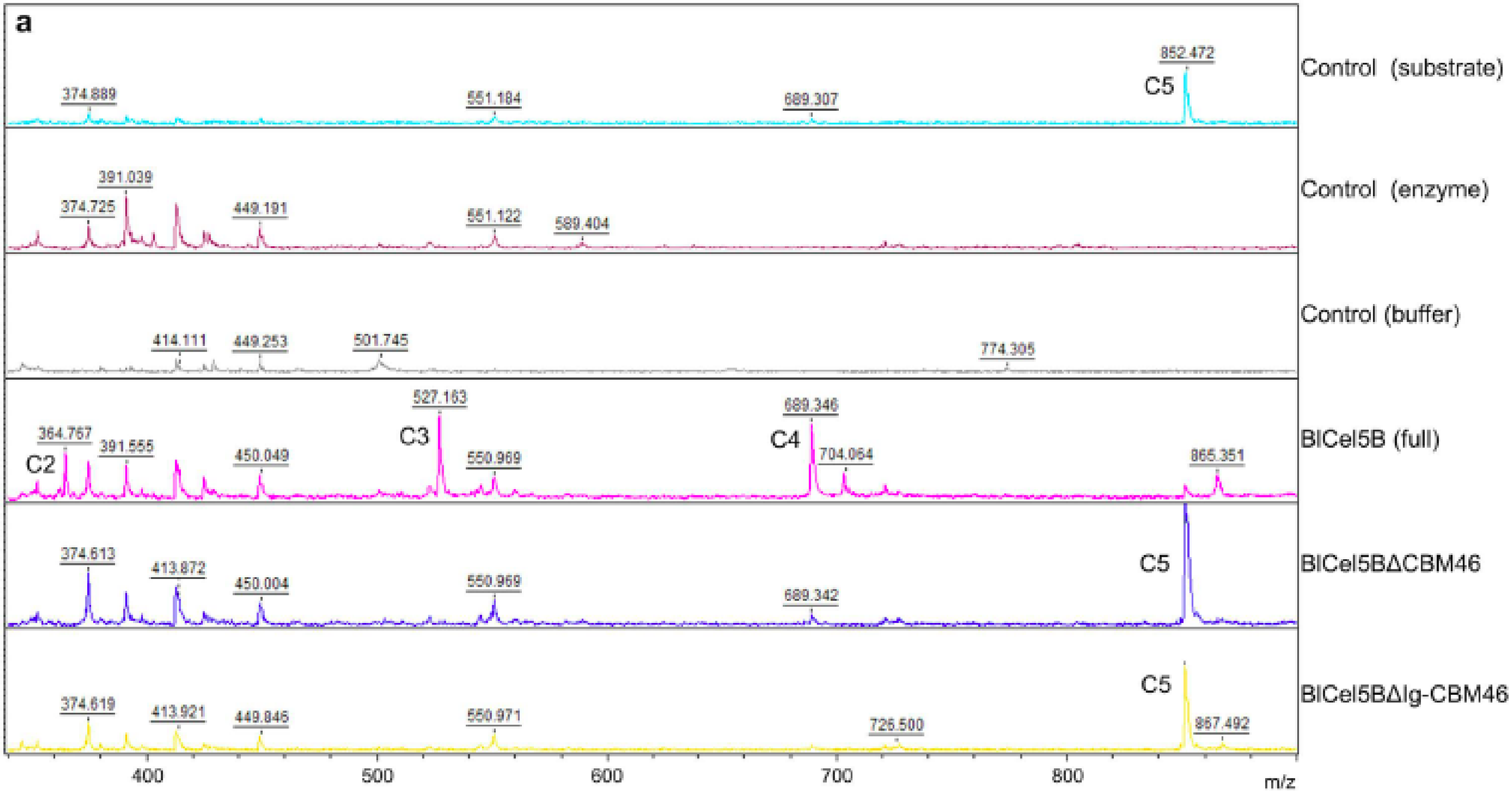
- **Induced-fit mechanism of activity for GHs**

GH5 subfamily 4 enzyme from Bacillus licheniformis (BlCel5B)



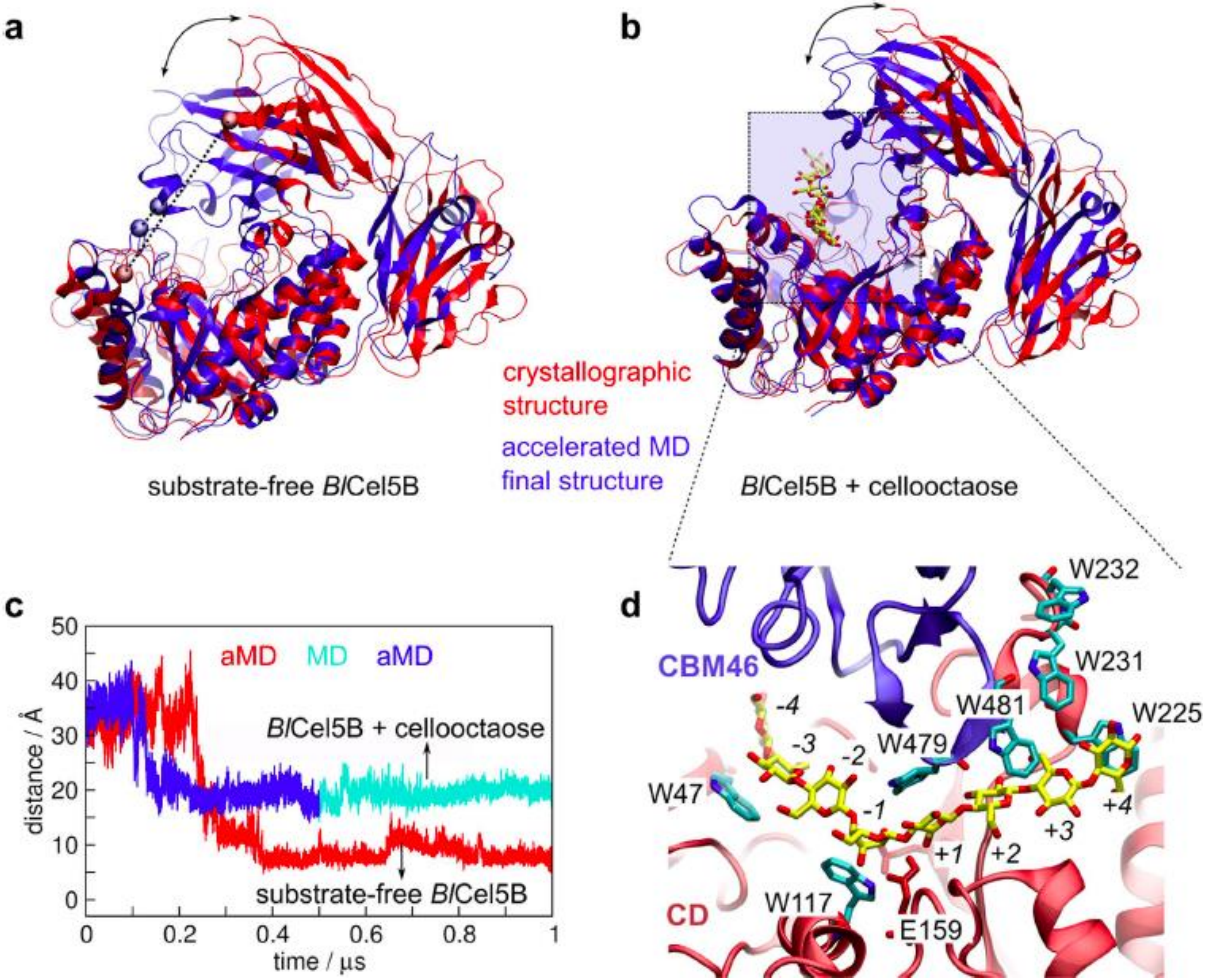
Liberato et al. *Scientific Reports* (2016) 6: 23473

BlCel5B enzymatic digestion of cellopentaose (C5) does not occur without CBMs



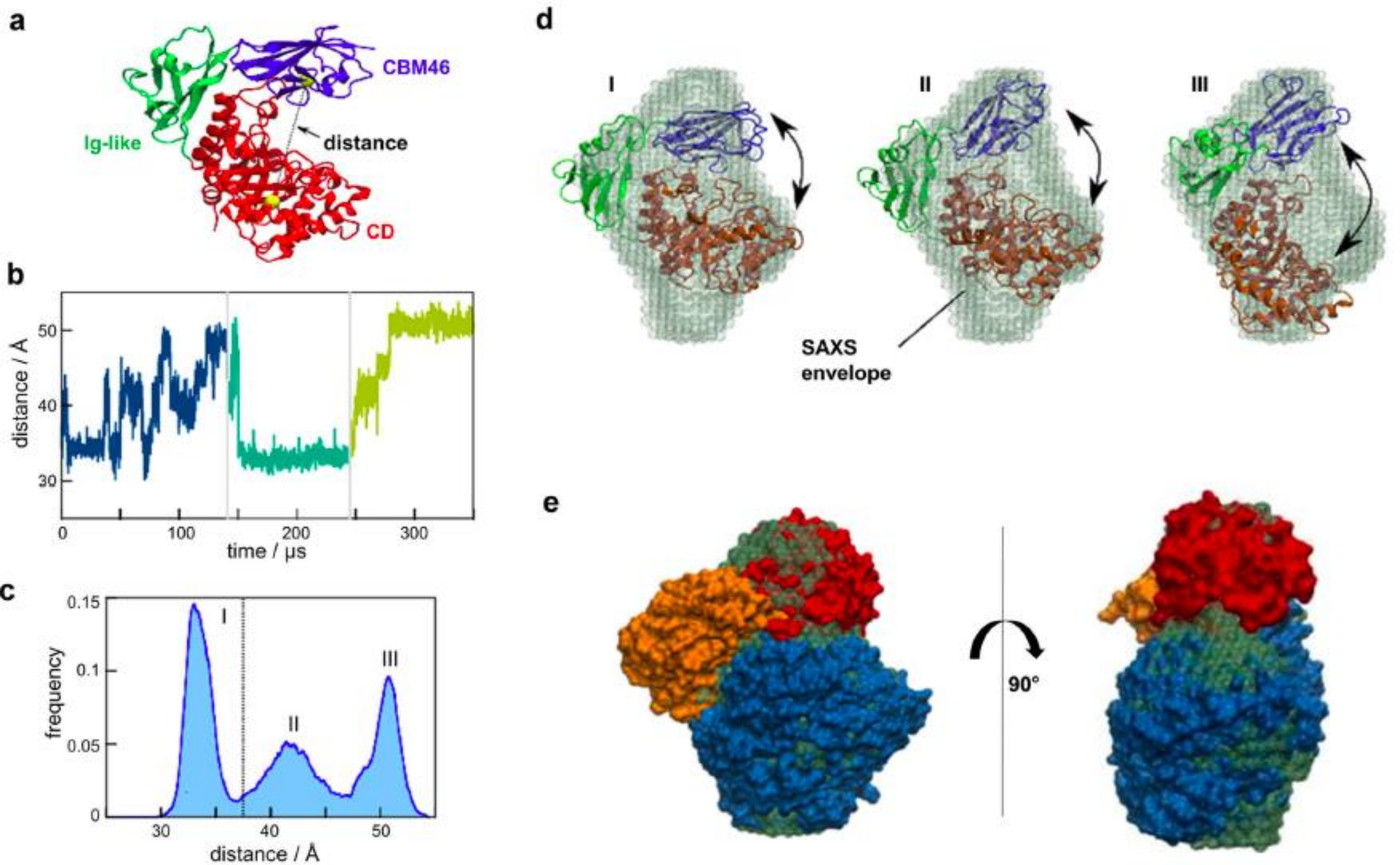
Liberato *et al. Scientific Reports* (2016) 6: 23473

MD simulations of BlCel5B ligand-induced open-closed transition



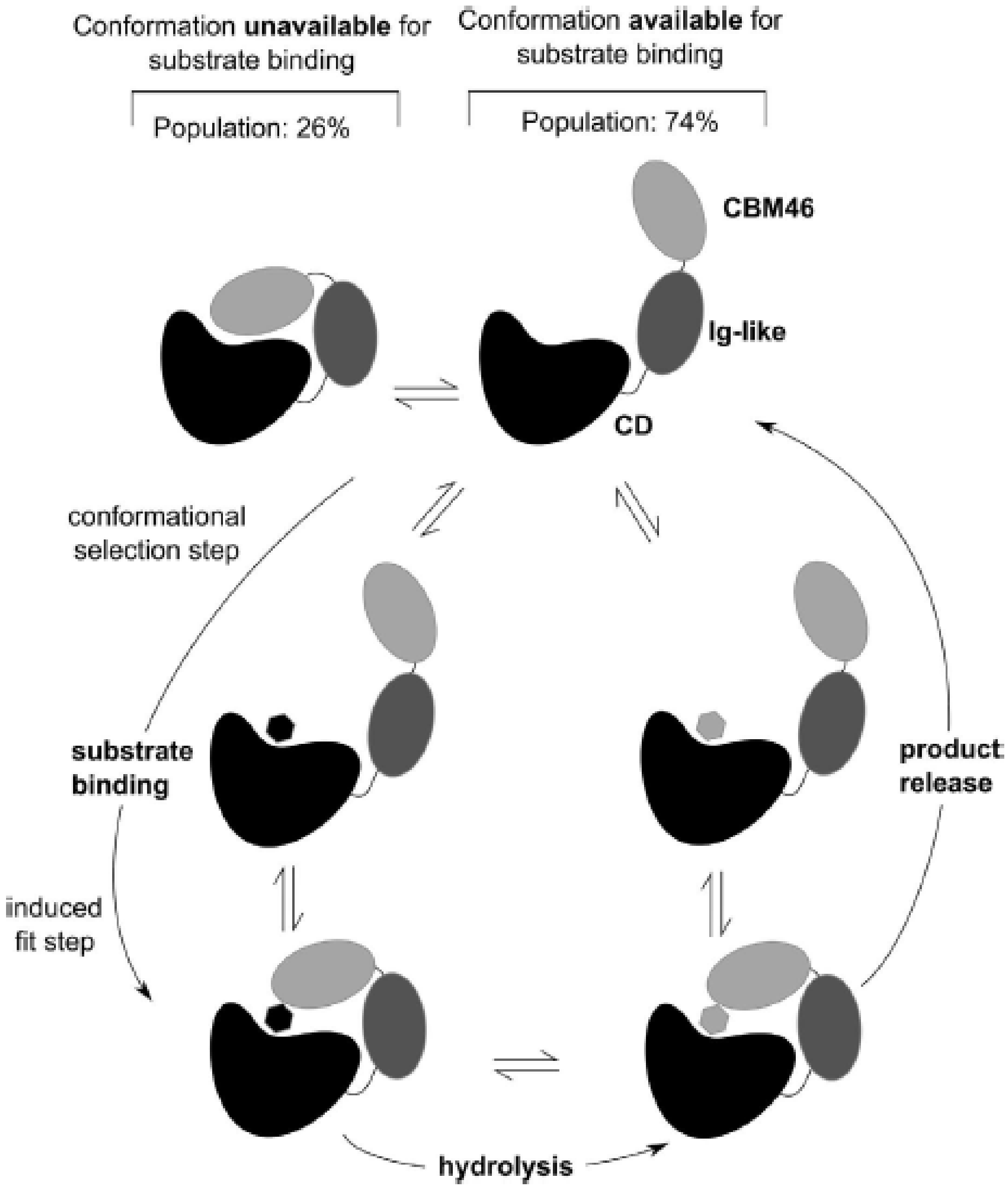
Liberato et al. *Scientific Reports* (2016) 6: 23473

BlCel5B MD simulated conformations and their fit into SAXS model



Liberato et al. *Scientific Reports* (2016) 6: 23473

Molecular mechanism of BlCel5B confomational selection



Liberato et al. *Scientific Reports* (2016) 6: 23473

- **New Ingredients: Expansins, CBMs and LPMOs**

Family 1 CBMs enhance saccharification rates

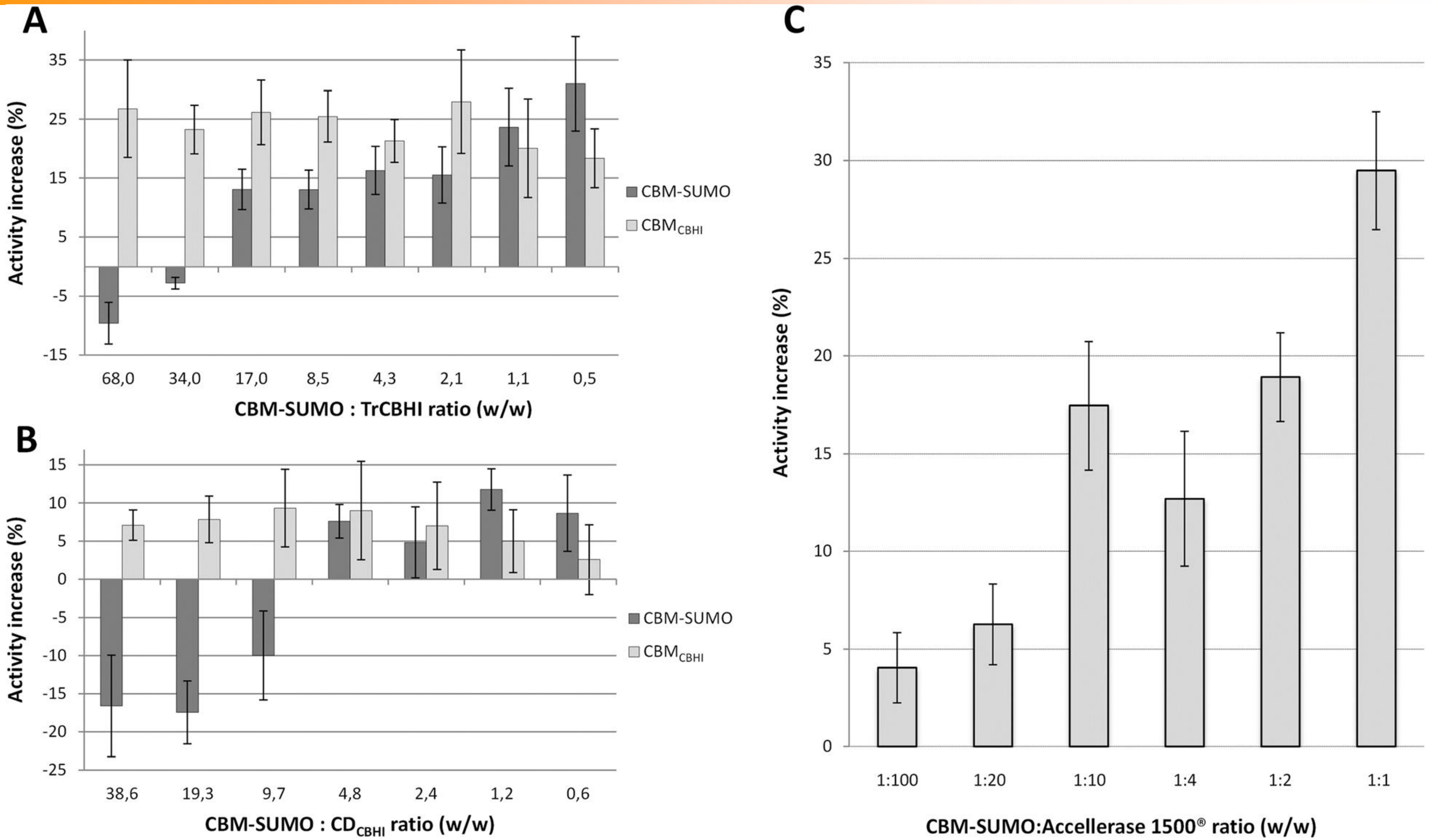
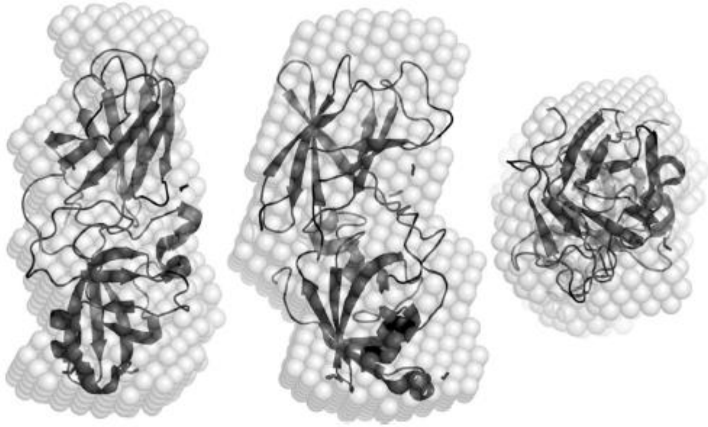
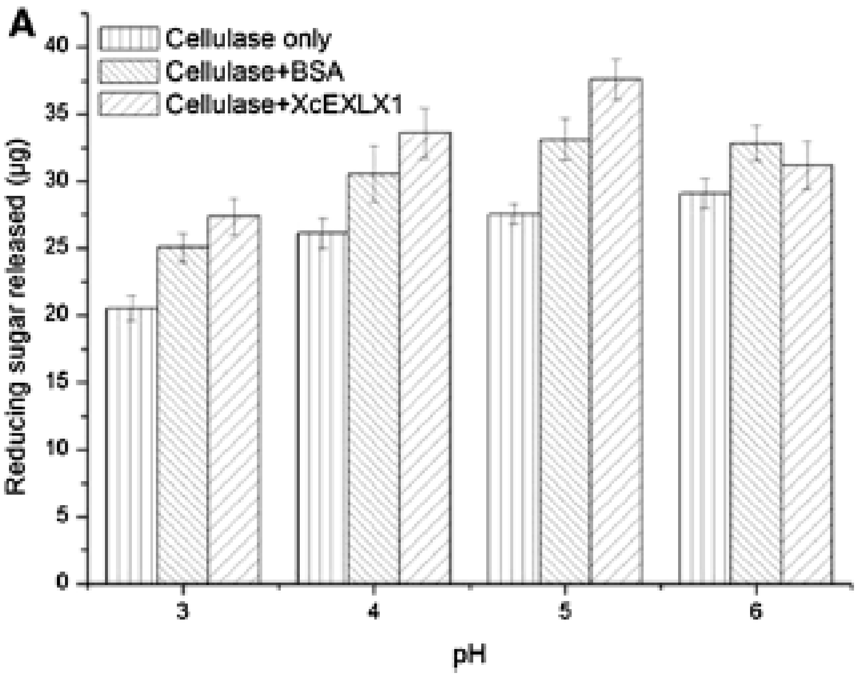


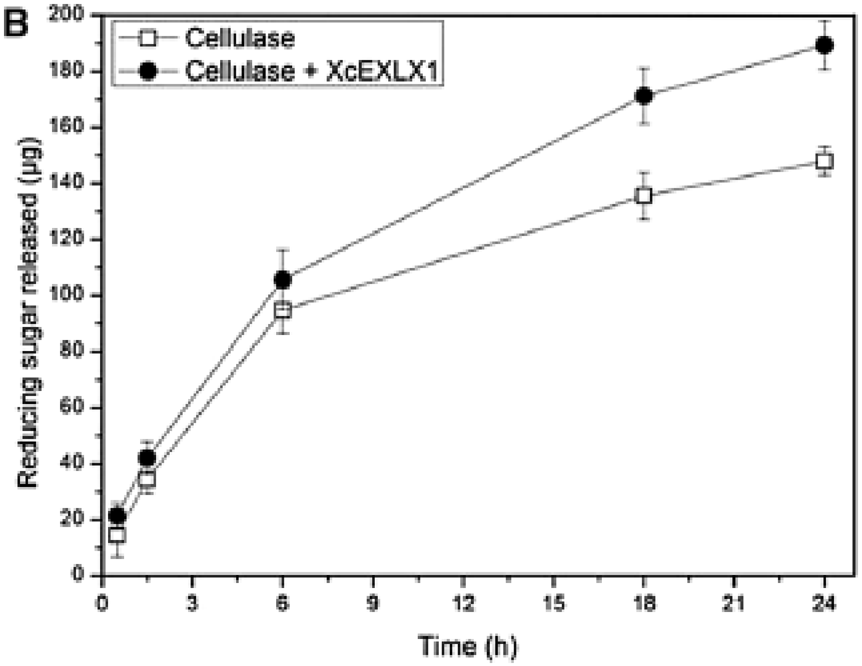
Figure: Effect of increasing amounts of CBM-SUMO or CBM-CBHI on filter paper hydrolysis. The ratios of CBM-SUMO to enzyme varied from 70:1 to 1:100 (w/w).

Mello and Polikarpov *AMB Express* 2014, 4:36

Expansins increase enzymatic hydrolysis rate of cellulose (20% to 35%)

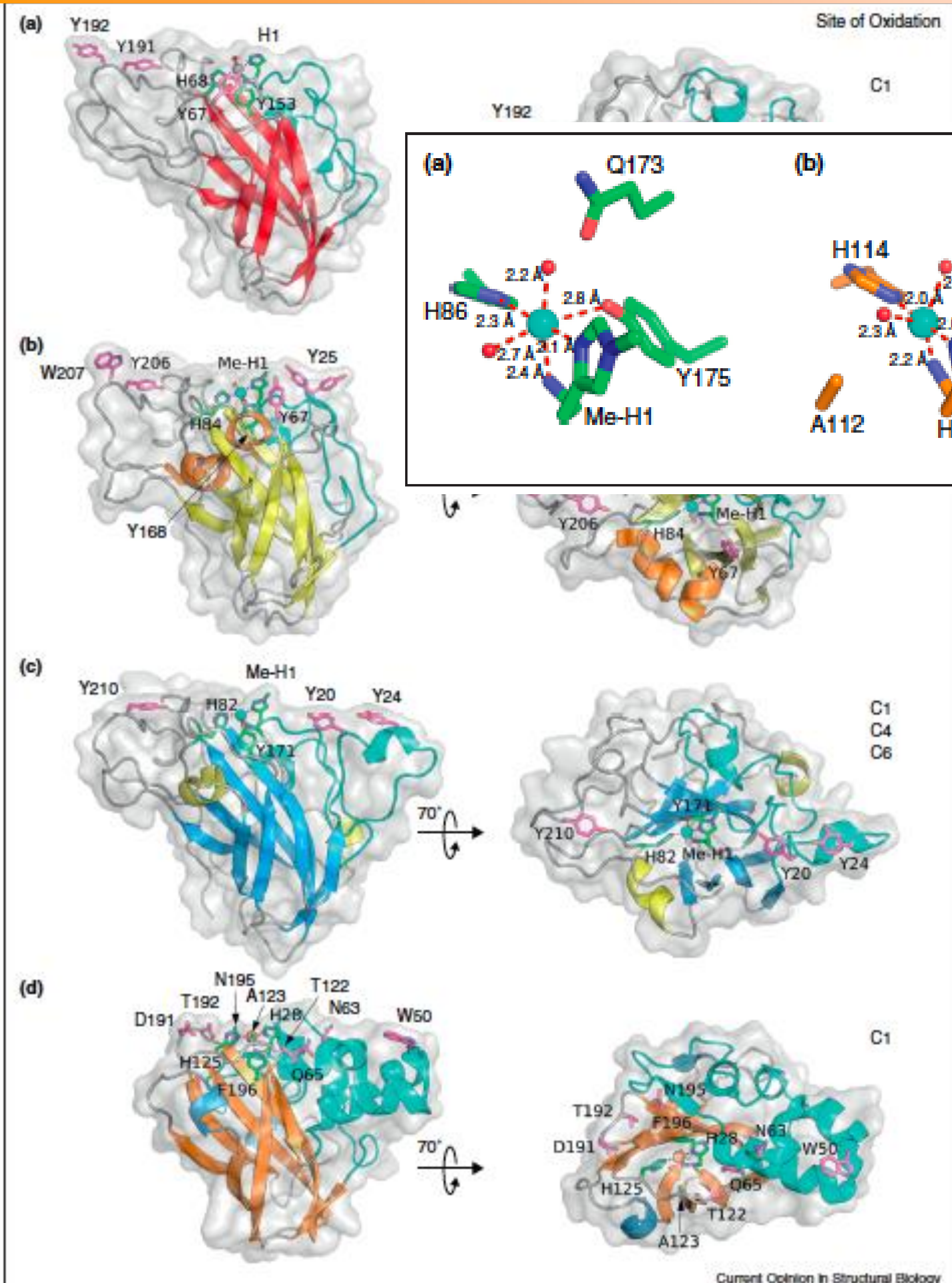


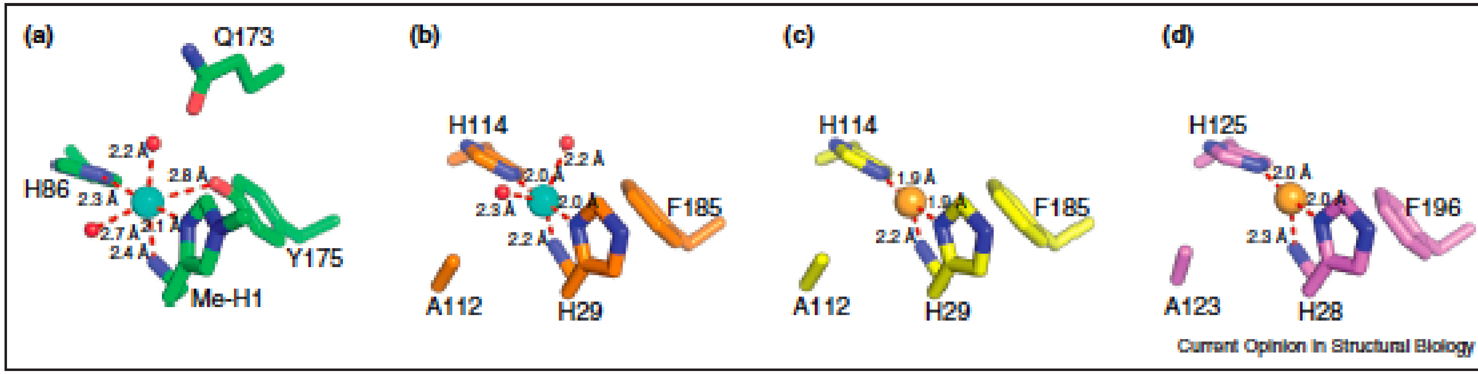


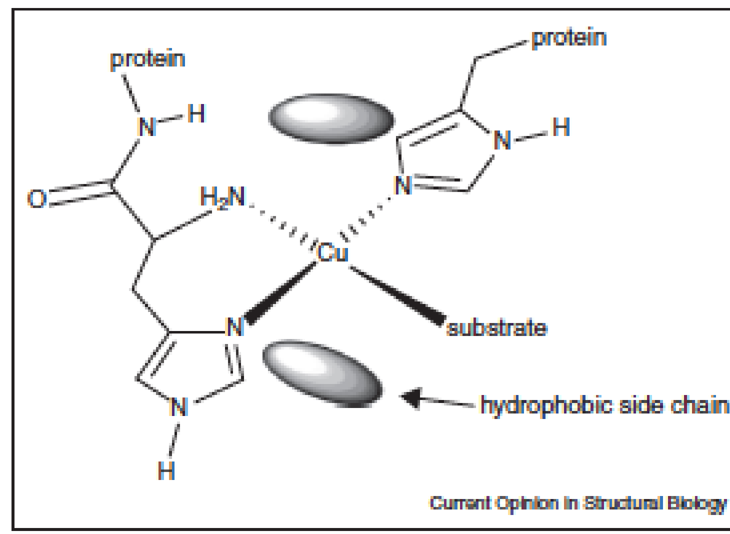


Tomazzini et al., *Biotech. Lett*. (2015) 37:2419-2426

Lytic polysaccharide monooxigenases (LPMOs)

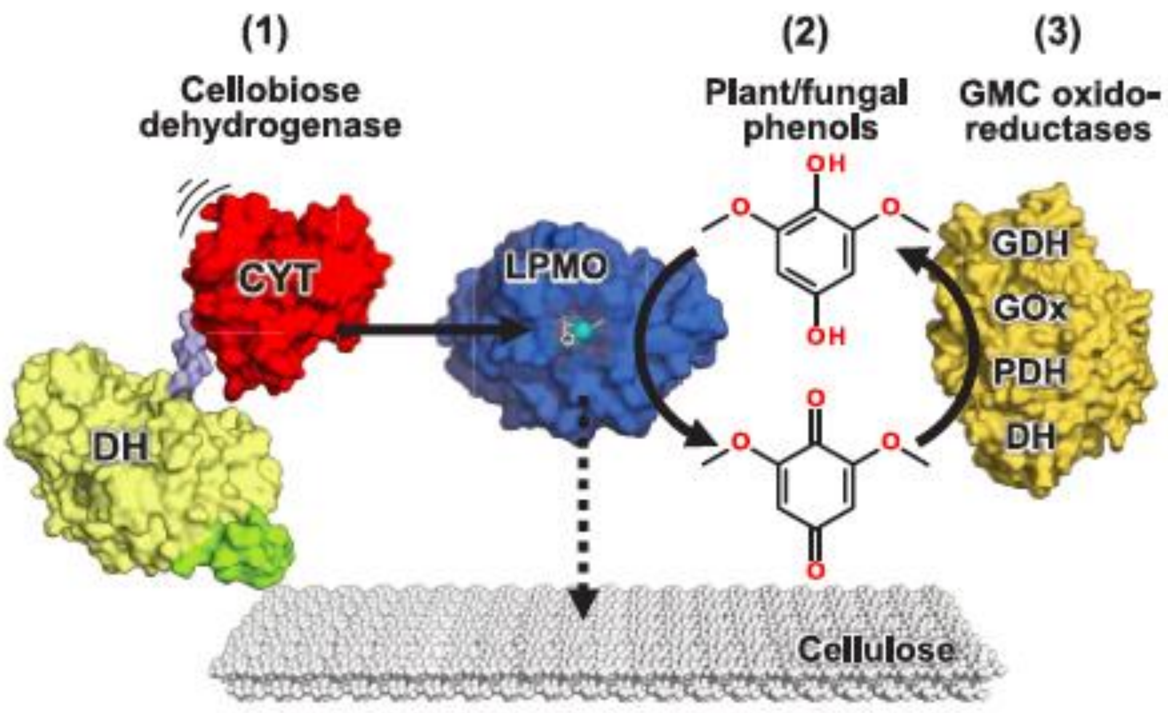






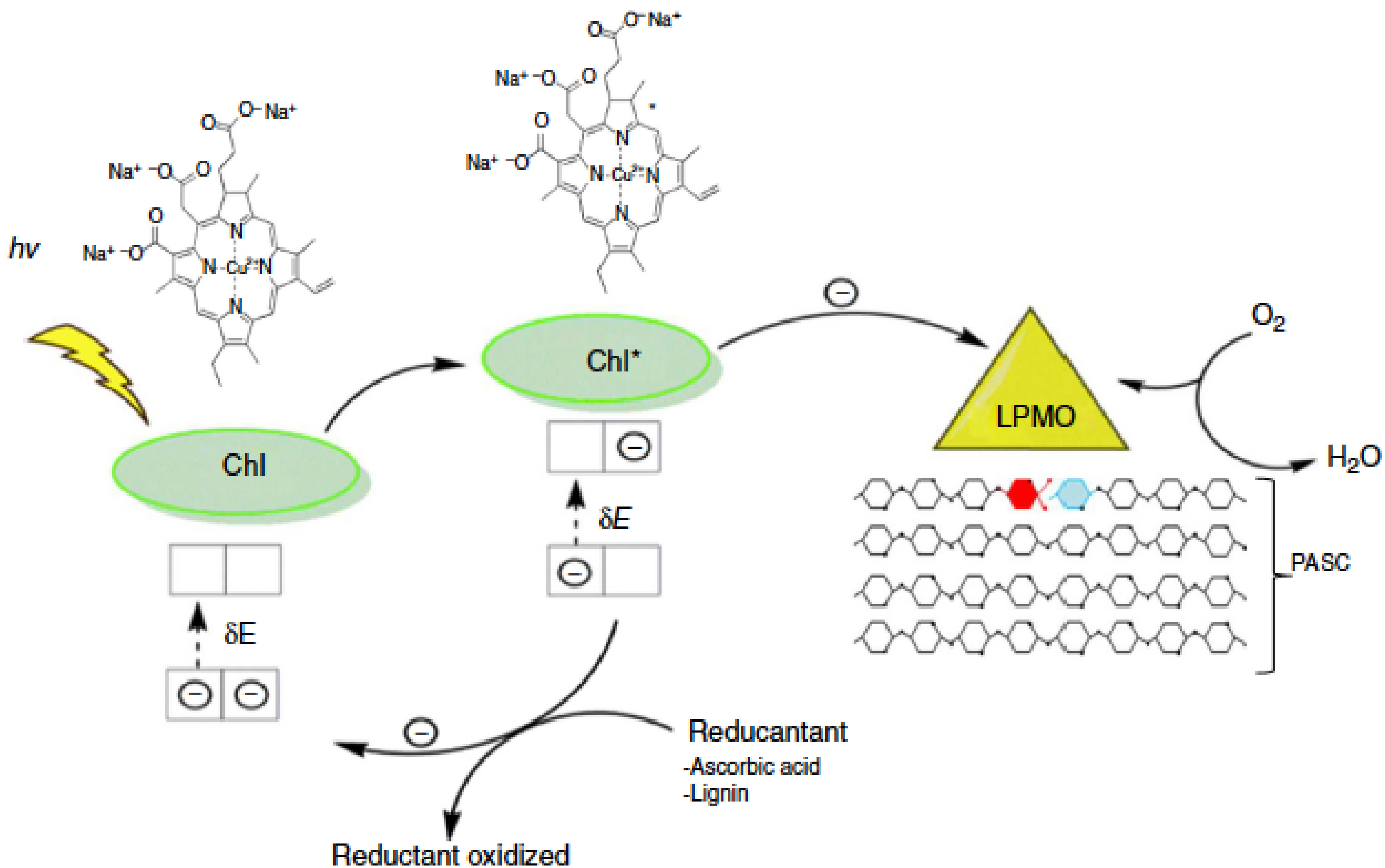
Hemsworth, Davies and Walton, *Curr. Opin. Struct. Biol.* (2013) 23: 660-8

Three electron transfer systems reduce the LPMO active-site copper to initiate cellulose attack



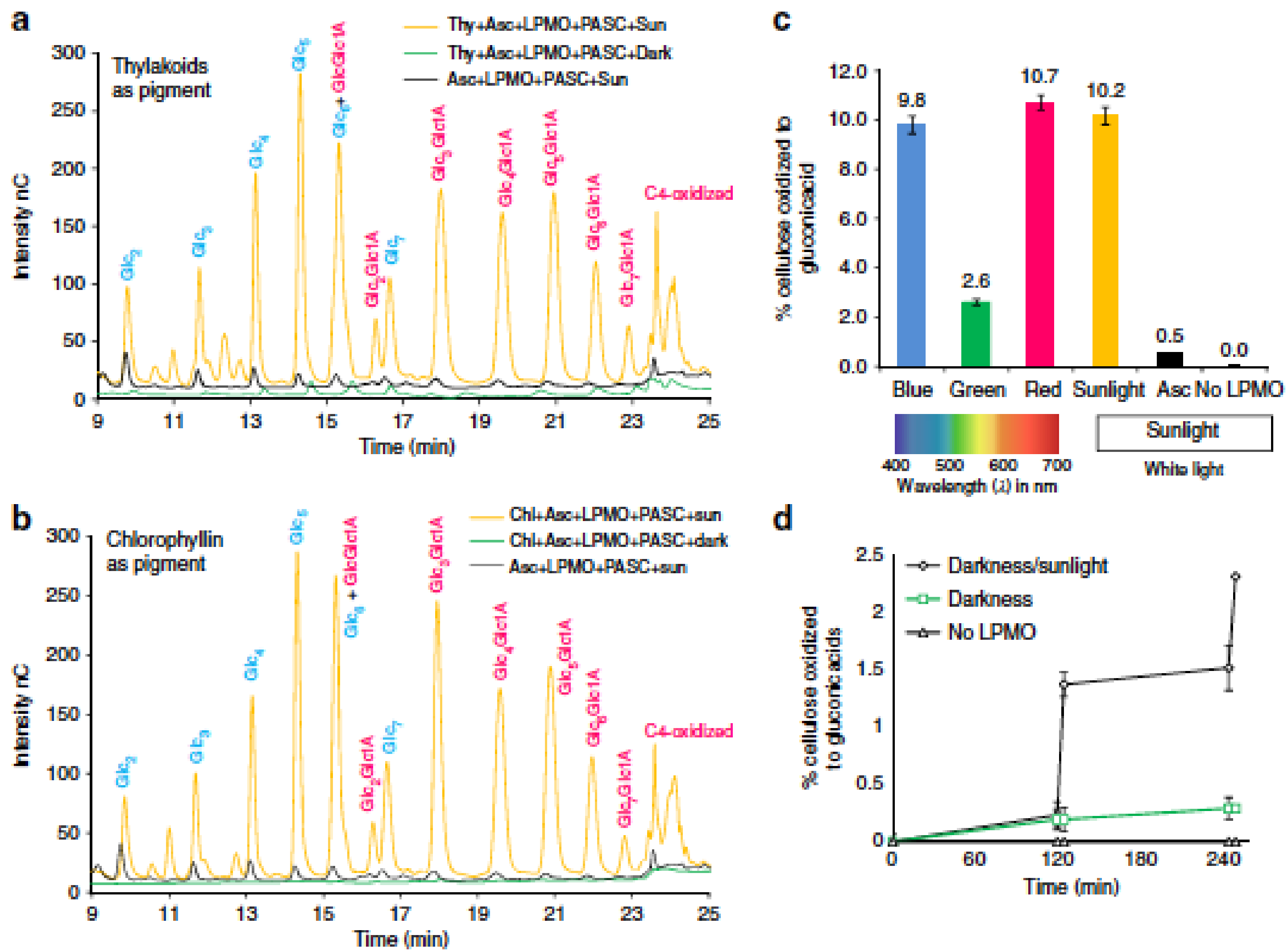
Kracher, et al., (2016) *Science* **352**: 1098-1101

Light driven activation of LPMOs mediated by chlorophyllin



Cannella, et al. *Nature Communications* (2016) **7**:11134

Light driven activation of LPMOs: Up to 100x increase in catalytic activity



Cannella, et al. *Nature Communications* (2016) **7**:11134

- **Fermentation**

- **C5 fermenting strains adapted to Brazilian ethanol plants operating conditions and procedures**

- ***What´s next?***

Biopolymers for renewable functional materials

Review

Cell PRESS

Applications of functionalized and nanoparticle-modified nanocrystalline cellulose

Edmond Lam, Keith B. Male, Jonathan H. Chong, Alfred C.W. Leung and John H.T. Luong

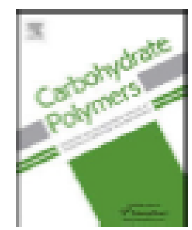
Biotechnology Research Institute, National Research Council Canada, 6100 Royalmount Avenue, Montreal, H4P 2R2, Canada

Carbohydrate Polymers 87 (2012) 963–979

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol



Review

Green composites from sustainable cellulose nanofibrils: A review

H.P.S. Abdul Khalil*, A.H. Bhat, A.F. Ireana Yusra

School of Industrial Technology, Universiti Sains Malaysia, 11800 Penang, Malaysia

Biopolymers for renewable functional materials

SCIENTIFIC REPORTS

OPEN

Cellulose Nanofiber Paper as an Ultra Flexible Nonvolatile Memory

SUBJECT AREAS:
INFORMATION STORAGE
ELECTRONIC PROPERTIES AND MATERIALS

Kazuki Nagashima[1], Hirotaka Koga[1], Umberto Celano[2,3], Fuwei Zhuge[1], Masaki Kanai[1], Sakon Rahong[1], Gang Meng[1], Yong He[1], Jo De Boeck[2], Malgorzata Jurczak[2], Wilfried Vandervorst[2,3], Takuya Kitaoka[4], Masaya Nogi[1] & Takeshi Yanagida[1]

Received
3 April 2014

Accepted
13 June 2014

[1]The Institute of Scientific and Industrial Research, Osaka University, 8-1 Mihogaoka Ibaraki, Osaka, 567-0047, Japan, [2]IMEC, Kapeldreef 75, B-3001 Heverlee (Leuven), Belgium, [3]KU Leuven, Department of Physics and Astronomy (IKS), Celestijnenlaan 200D, 3001 Leuven, Belgium, [4]Department of Agro-environmental Sciences, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Fukuoka, 812-8581, Japan.

Biopolymers for health, food and feed

World J Microbiol Biotechnol (2011) 27:1119–1128
DOI 10.1007/s11274-010-0558-5

ORIGINAL PAPER

Functional oligosaccharides: production, properties and applications

Seema Patel · Arun Goyal

Xylooligo-saccharides: manufacture and applications

M.J. Vázquez, J.L. Alonso, H. Domínguez and J.C. Parajó*

Departamento de Enxeñería Química, Universidade de Vigo (Campus Ourense), Edificio Politécnico, As Lagoas, 32004 Ourense, Spain (tel: +34-9-8838-7047; fax: +34-988-387001; e-mail: jcparajo@uvigo.es)

Trends in Food Science & Technology 11 (2000) 387–393

TRENDS IN FOOD SCIENCE & TECHNOLOGY

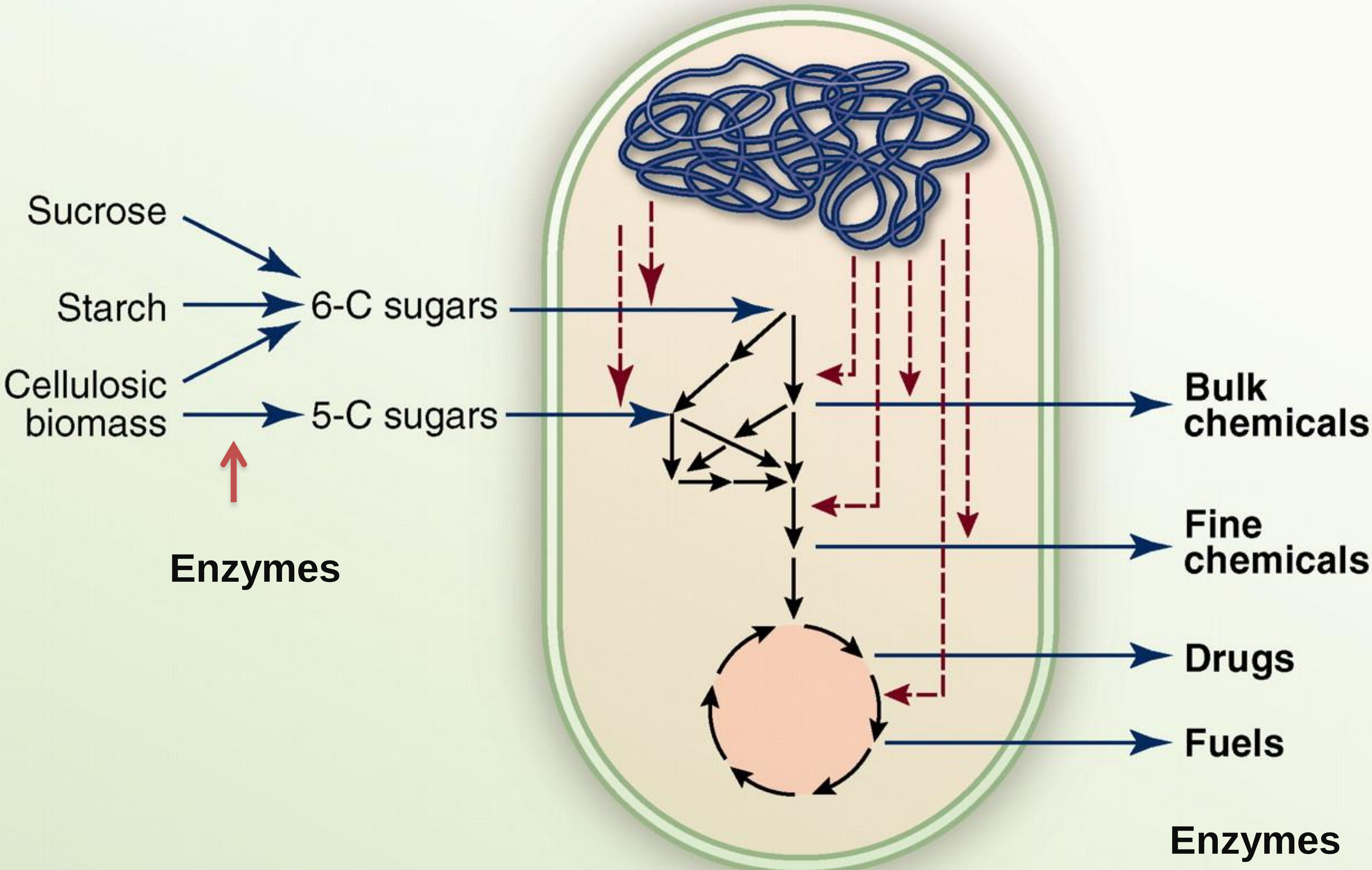
Comprehensive REVIEWS In Food Science and Food Safety

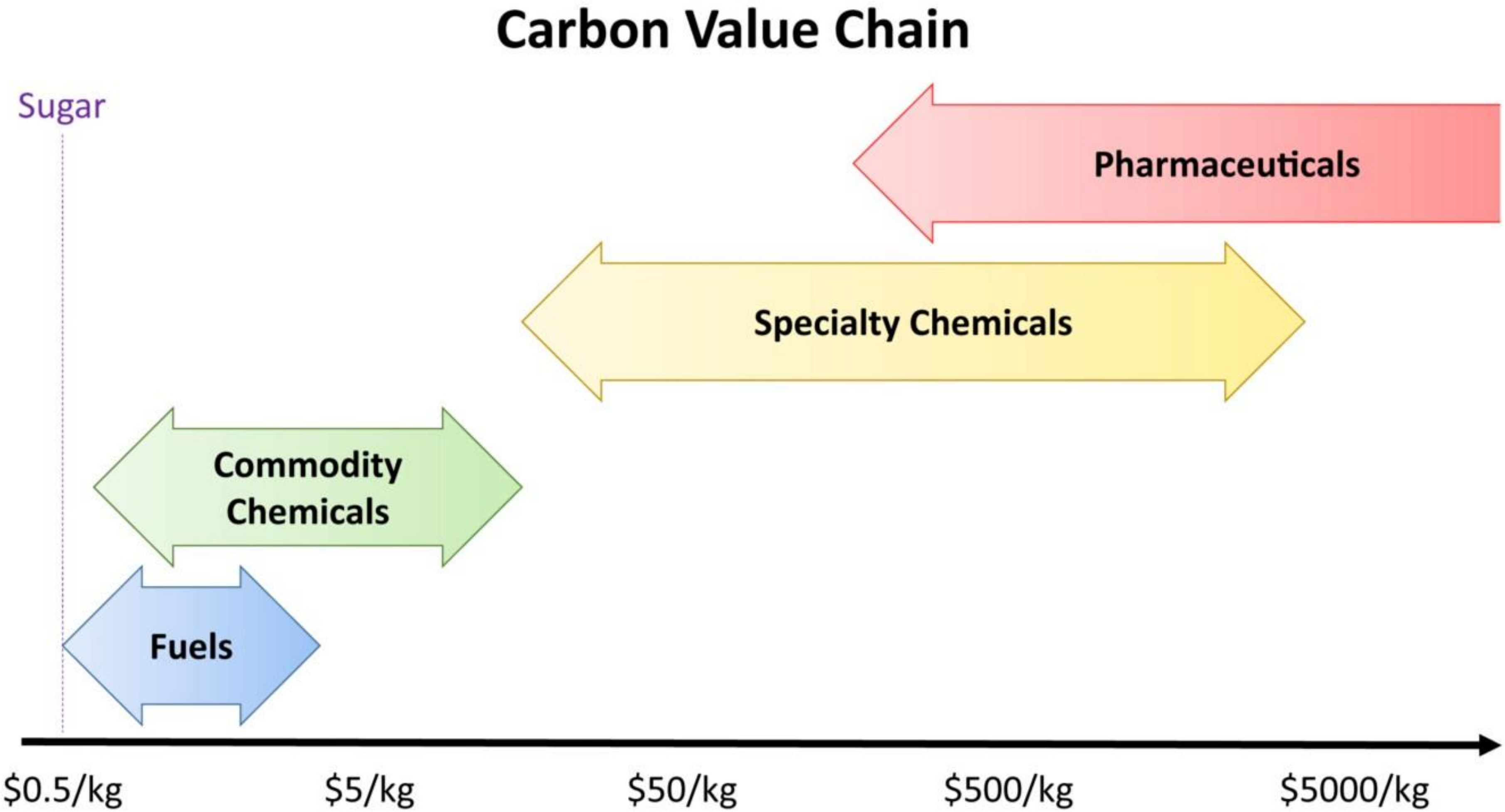
Xylooligosaccharides (XOS) as an Emerging Prebiotic: Microbial Synthesis, Utilization, Structural Characterization, Bioactive Properties, and Applications

Ayyappan Appukuttan Aachary and Siddalingaiya Gurudutt Prapulla

- **Green chemicals: Synthetic Biology**

Synthetic Biology



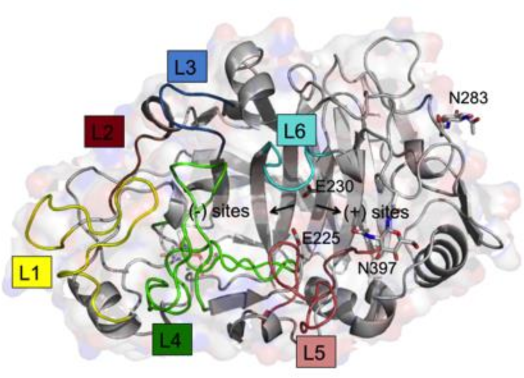

Carbon Value Chain
Sugar
Pharmaceuticals
Specialty Chemicals
Commodity Chemicals
Fuels
$0.5/kg
$5/kg
$50/kg
$500/kg
$5000/kg

Enzymes for agriculture, health, green chemistry & bioenergy, synthetic biology and metabolic engineering

Bioinformatics:
genes selection

Cloning & Expression:
appropriate enzymes in appropriate hosts

Fermentation & Separation:
final product (enzyme, chemical, fuel)



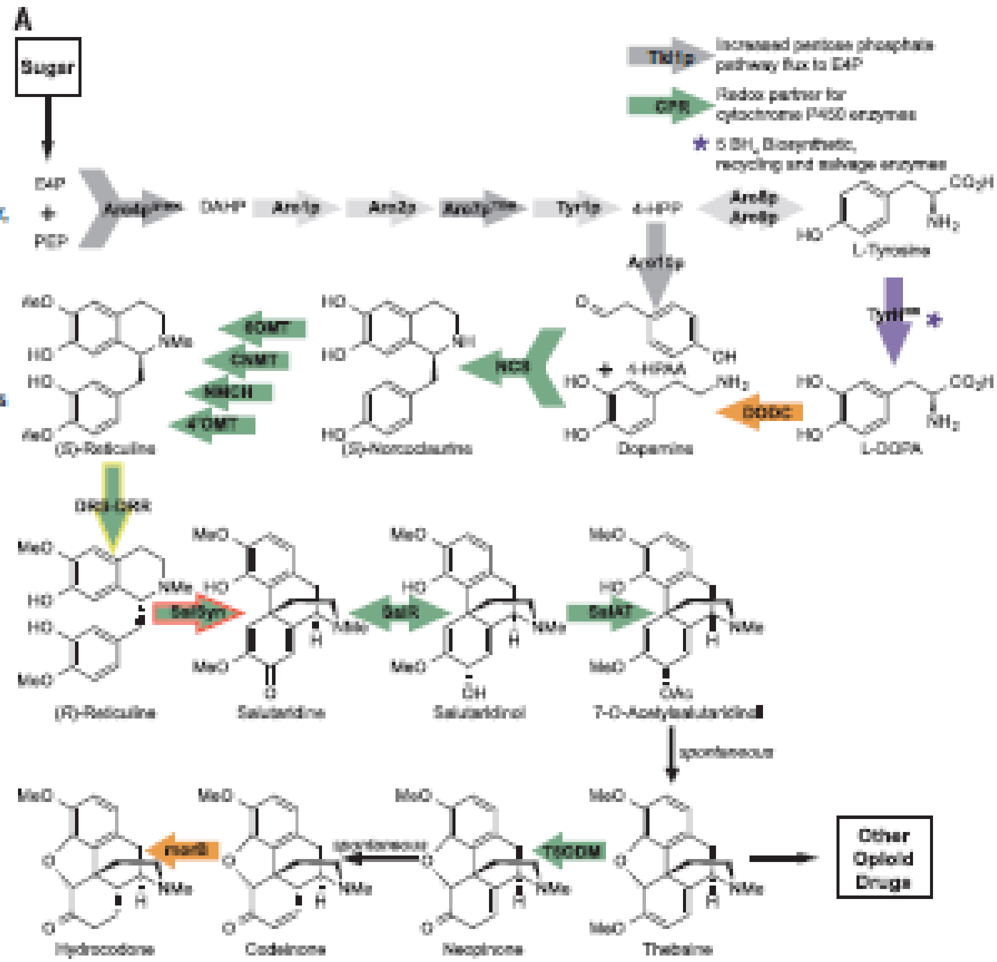
4 SEPTEMBER 2015 • VOL 349 ISSUE 6252 sciencemag.org SCIENCE

SYNTHETIC BIOLOGY

Complete biosynthesis of opioids in yeast

Stephanie Galanie,[1] Kate Thodey,[2] Isis J. Trenchard,[2] Maria Filsinger Interrante,[2] Christina D. Smolke[2]*

Opioids are the primary drugs used in Western medicine for pain management and palliative care. Farming of opium poppies remains the sole source of these essential medicines, despite diverse market demands and uncertainty in crop yields due to weather, climate change, and pests. We engineered yeast to produce the selected opioid compounds thebaine and hydrocodone starting from sugar. All work was conducted in a laboratory that is permitted and secured for work with controlled substances. We combined enzyme discovery, enzyme engineering, and pathway and strain optimization to realize full opiate biosynthesis in yeast. The resulting opioid biosynthesis strains required the expression of 21 (thebaine) and 23 (hydrocodone) enzyme activities from plants, mammals, bacteria, and yeast itself. This is a proof of principle, and major hurdles remain before optimization and scale-up could be achieved. Open discussions of options for governing this technology are also needed in order to responsibly realize alternative supplies for these medically relevant compounds.



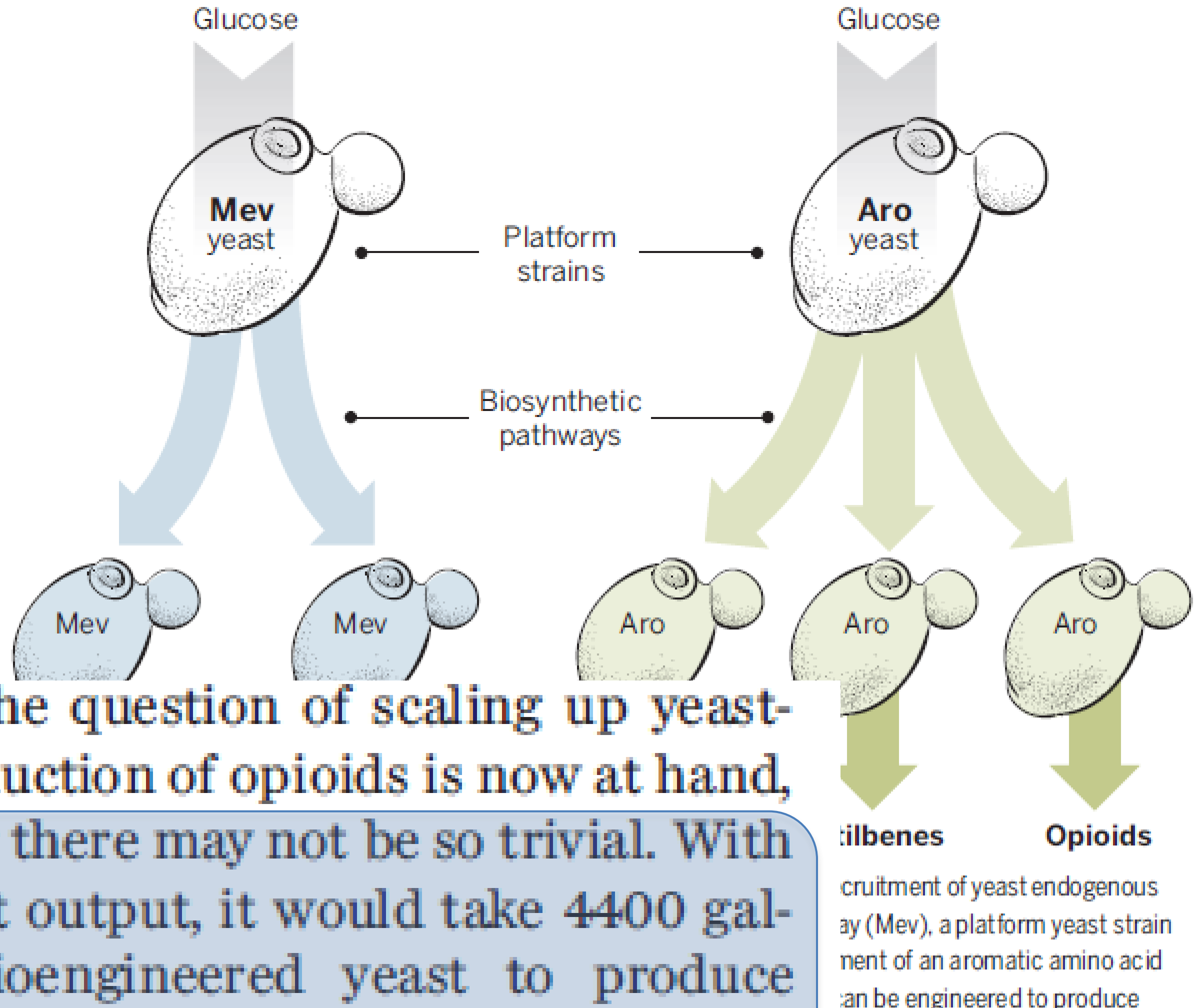
21/23 enzyme pathways for opioids (thebaine/paramorphine & hydrocodone) biosynthesis

BIOENGINEERING

Yeast cell factories on the horizon

Metabolic engineering in yeast gets increasingly more versatile

By Jens Nielsen[1,2,3]



The question of scaling up yeast-based production of opioids is now at hand, but getting there may not be so trivial. With the current output, it would take 4400 gallons of bioengineered yeast to produce a single therapeutic dose for pain relief (10). Scaling up the production of artemisinin, for example, took more than 5 years of investigation and required investments exceeding $50 million.

Aromatic amino acid pathway (ARO)

Enzymes for agriculture, health, green chemistry, bioenergy & synthetic biology

Fermentation & Separation

Industrial enzymes for cellulosic ethanol are being produced at 100g/L yields!

- **Synthetic Biochemistry for Green chemicals and Food/Feed production**

OPEN ACCESS Freely available online

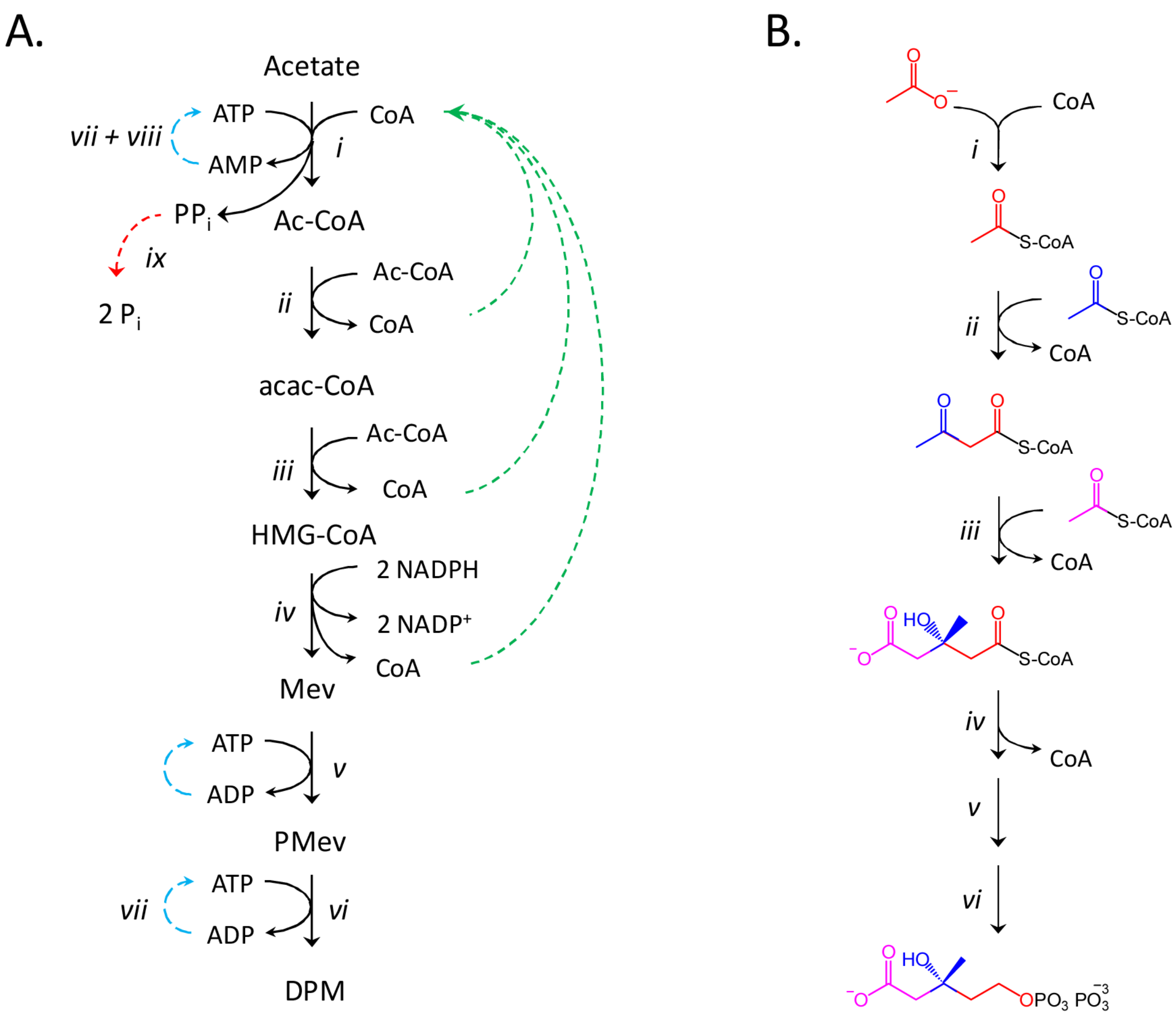
PLOS | ONE

An Enzymatic Platform for the Synthesis of Isoprenoid Precursors

Sofia B. Rodriguez, Thomas S. Leyh*

The Department of Microbiology & Immunology, The Albert Einstein College of Medicine, Bronx, New York, United States of America

PLOS ONE | www.plosone.org August 2014 | Volume 9 | Issue 8 | e105594

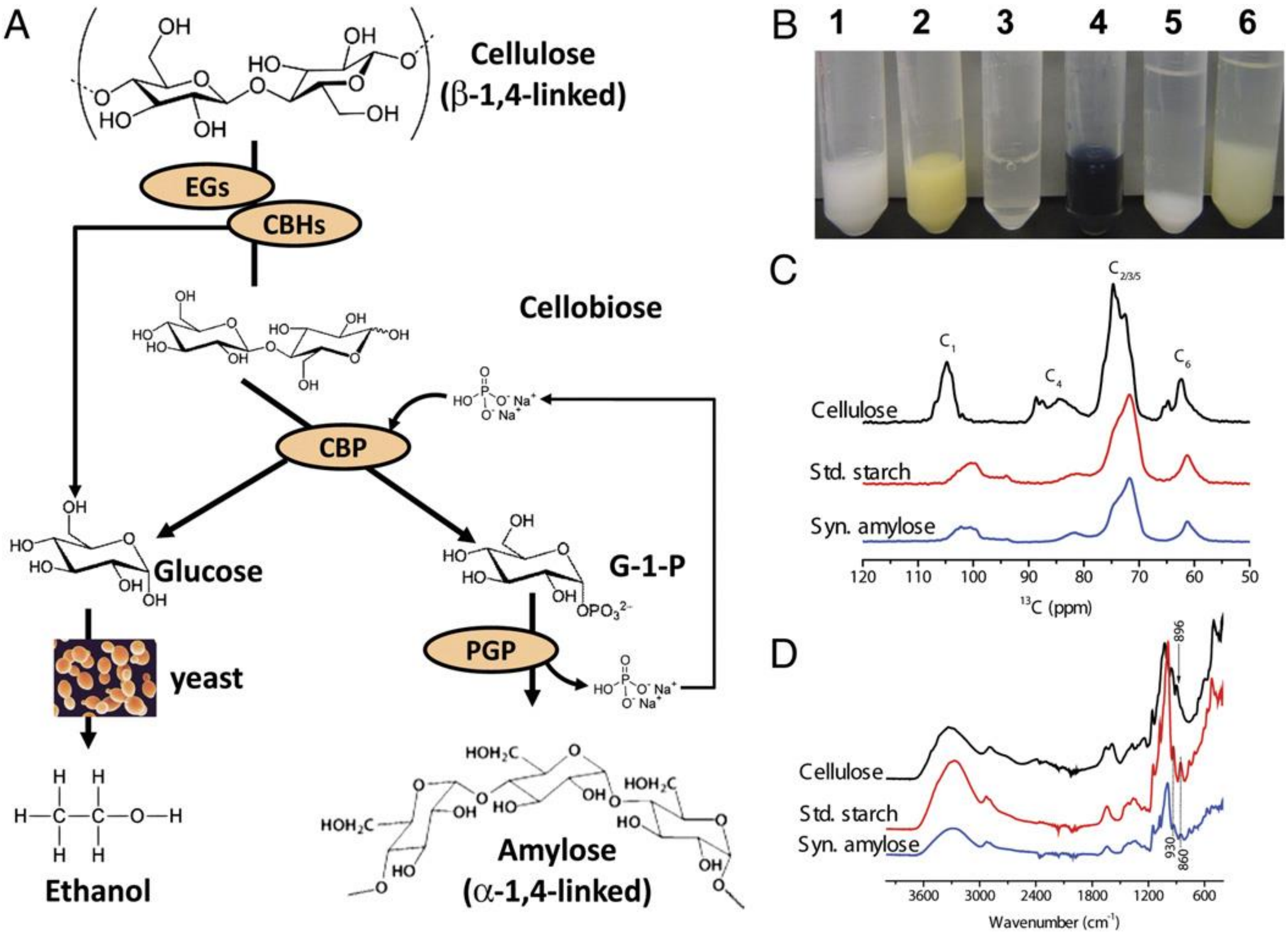


10 enzymes in single-pot synthesis for high-yield (**>85%**) of diphosphomevalonate (DPM)

Enzymatic transformation of nonfood biomass to starch

Chun You[a,1], Hongge Chen[a,b,1], Suwan Myung[a,c], Noppadon Sathitsuksanoh[a,c], Hui Ma[d], Xiao-Zhou Zhang[a,d], Jianyong Li[e], and Y.-H. Percival Zhang[a,c,d,f,g,2]

7182–7187 | PNAS | April 30, 2013 | vol. 110 | no. 18



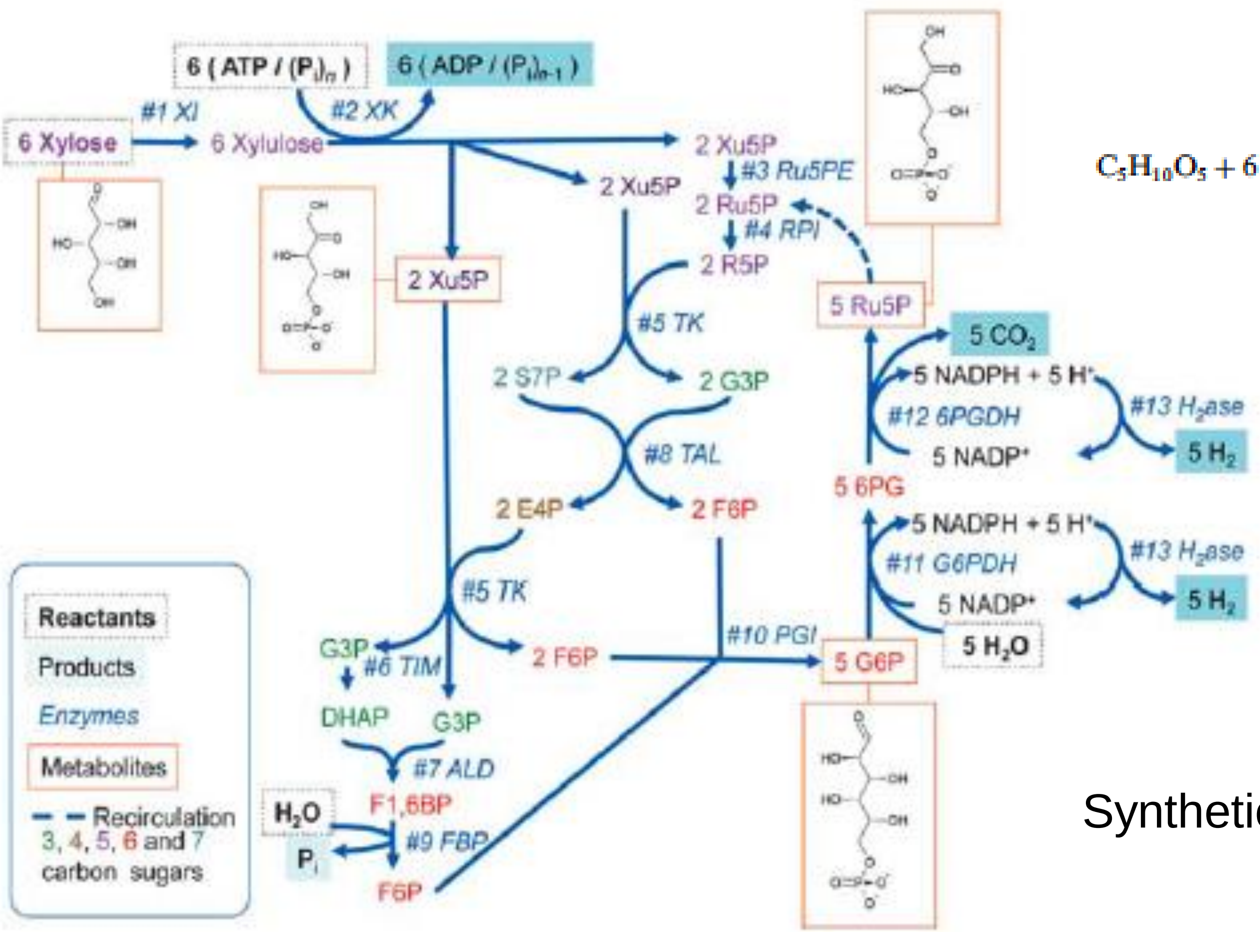
Biocatalysis

Angewandte Chemie International Edition

DOI: 10.1002/anie.201300766

High-Yield Production of Dihydrogen from Xylose by Using a Synthetic Enzyme Cascade in a Cell-Free System**

*Julia S. Martín del Campo, Joseph Rollin, Suwan Myung, You Chun, Sanjeev Chandrayan, Rodrigo Patiño, Michael WW Adams, and Y.-H. Percival Zhang**



$$C_5H_{10}O_5 + 6\,H_2O + (P_i)_n \rightarrow 10\,H_2 + 5\,CO_2 + (P_i)_{n-1} + P_i$$

Overall H2 yield is 96% of theoretical maximum: 9,6 moles of H2 per 1 mole of xylose

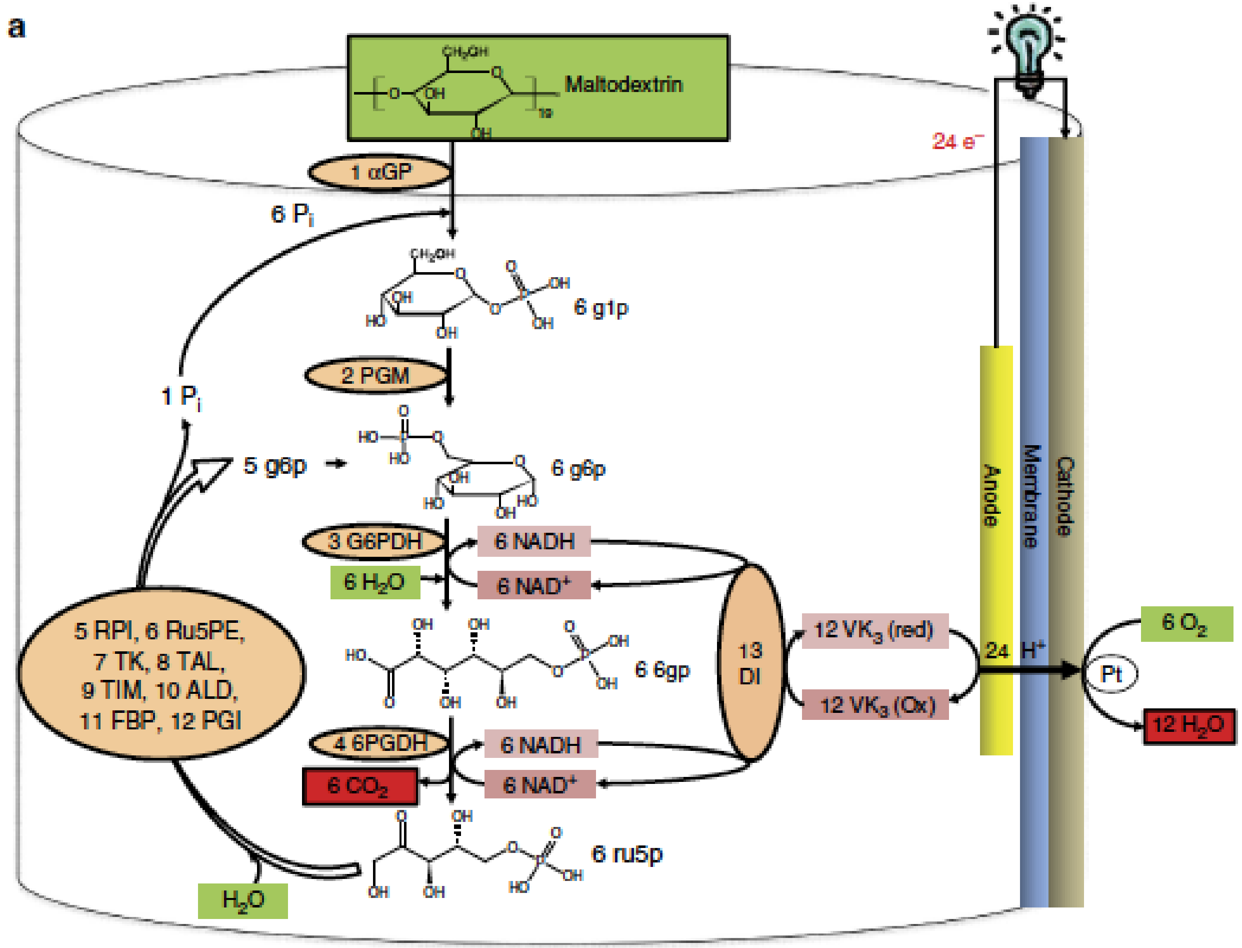
Synthetic pathway of 13 enzymes

Received 9 Jul 2013 | Accepted 26 Nov 2013 | Published 21 Jan 2014 DOI: 10.1038/ncomms4026

A high-energy-density sugar biobattery based on a synthetic enzymatic pathway

Zhiguang Zhu[1,2], Tsz Kin Tam[2], Fangfang Sun[2], Chun You[1] & Y.-H. Percival Zhang[1,2,3]

NATURE COMMUNICATIONS | 5:3026 | DOI: 10.1038/ncomms4026 | www.nature.com/naturecommunications



13 enzymes in a synthetic pathway: 24 e- per glucose molecule

Received 9 Jul 2013 | Accepted 26 Nov 2013 | Published 21 Jan 2014

DOI: 10.1038/ncomms4026

nature COMMUNICATIONS

A high-energy-density sugar biobattery based on a synthetic enzymatic pathway

Zhiguang Zhu[1,2], Tsz Kin Tam[2], Fangfang Sun[2], Chun You[1] & Y.-H. Percival Zhang[1,2,3]

NATURE COMMUNICATIONS | 5:3026 | DOI: 10.1038/ncomms4026 | www.nature.com/naturecommunications

High-energy-density, green, safe batteries are highly desirable for meeting the rapidly growing needs of portable electronics. The incomplete oxidation of sugars mediated by one or a few enzymes in enzymatic fuel cells suffers from low energy densities and slow reaction rates. Here we show that nearly 24 electrons per glucose unit of maltodextrin can be produced through a synthetic catabolic pathway that comprises 13 enzymes in an air-breathing enzymatic fuel cell. This enzymatic fuel cell is based on non-immobilized enzymes that exhibit a maximum power output of 0.8 $mW\,cm^{-2}$ and a maximum current density of 6 $mA\,cm^{-2}$, which are far higher than the values for systems based on immobilized enzymes. Enzymatic fuel cells containing a 15% (wt/v) maltodextrin solution have an energy-storage density of 596 $Ah\,kg^{-1}$, which is one order of magnitude higher than that of lithium-ion batteries. Sugar-powered biobatteries could serve as next-generation green power sources, particularly for portable electronics.

CONCLUSIONS

- For Brazil bioeconomy is not a choice, it´s a necessity.
- Currently Brazil has well-established 1st generation ethanol production facilities and those generate large amounts of biomass that can be used for 2nd generation ethanol (2GE) production.
- Several industrial and pilot-scale facilities for 2GE have been already launched in Brazil.
- Pretreatment protocols, enzymatic mixtures and fermentation procedures still have to be optimized to make Brazilian 2GE production sustainable.
- A solid scientific and technological base and established 1GE agro-industrial sector will (hopefully) make Brazilian bioeconomy a reality.


Thank you!
Obrigado!