Business from technology

Enzymes and biotechnology: could we overcome modern challenges?

2nd International Conference on Genomics & Pharmacogenomics

Dr. Junio Cota
VTT Brasil LTDA

SUMMARY

- What is VTT?
- The OMICS Era
- New Enzyme Discovery
- Protein Engineering

VTT Group in brief

Turnover 292 M€ (2010) ■ Personnel 3,167 (1.1.2011)

Customer sectors

- Biotechnology, pharmaceutical and food industries
- Electronics
- Energy
- ICT
- Real estate and construction
- Machines and vehicles
- Services and logistics
- Forest industry
- Process industry and environment

Focus areas of research

- Applied materials
- Bio- and chemical processes
- Energy
- Information and communication technologies
- Industrial systems management
- Microtechnologies and electronics
- Services and the built environment
- Business research

VTT's operations

Research and Development ■ Strategic Research ■ Business Solutions ■ IP Business ■ Group Services

VTT's companies

VTT Expert Services Ltd (incl. Labtium Ltd, Enas Ltd) ■ VTT Ventures Ltd ■ VTT International Ltd ■ VTT Memsfab Ltd

VTT Group on the map

BIOREFINERY

Brazilian biomass raw material

Process	**Wood and fibre processing**	**Biotechnical processing**	**Energy production**
Sector	Pulp, paper & Packaging & Solid wood	Bio & Chemistry	Energy
Key service & offering	Multi-technology solutions & pilot scale infrastructure	Biotechnical conversion technologies	Thermal fuel & waste conversion technologies
Key technologies	Fibre processing, Paper making, Coating, Modelling & Simulation	Biomass hydrolysis, Enzymes & Cell factories	Gasification, pyrolysis & combustion technologies
End products	Novel fibre based products & Advanced wood products	Chemicals, Bio alcohols & Biomaterials	Biofuel, Electricity & Heat



BRAZIL



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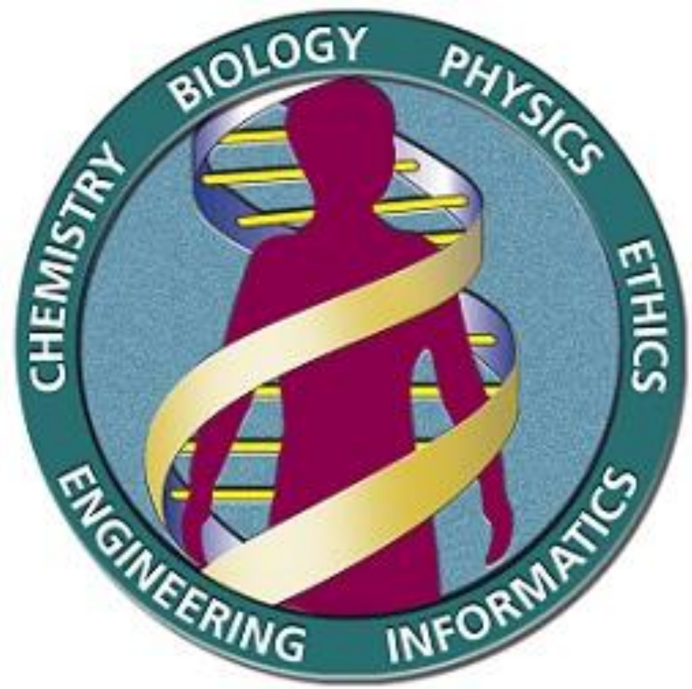
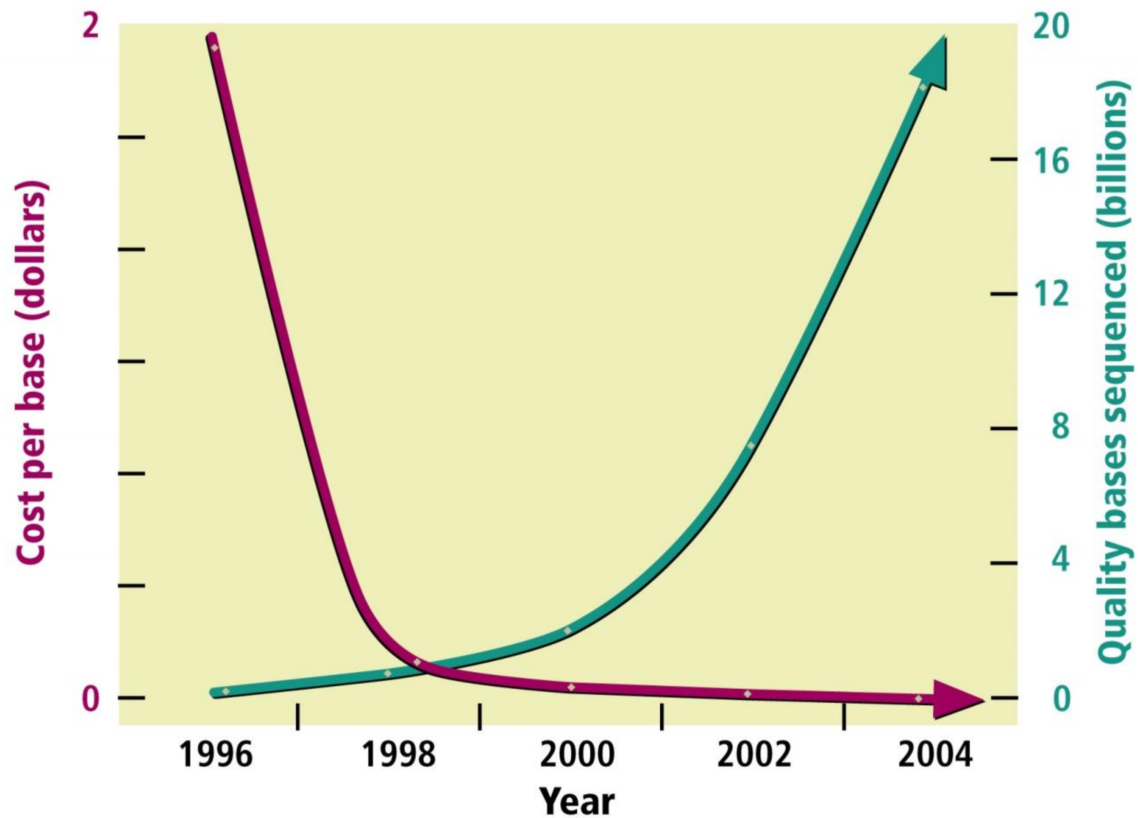
Human Genome Project

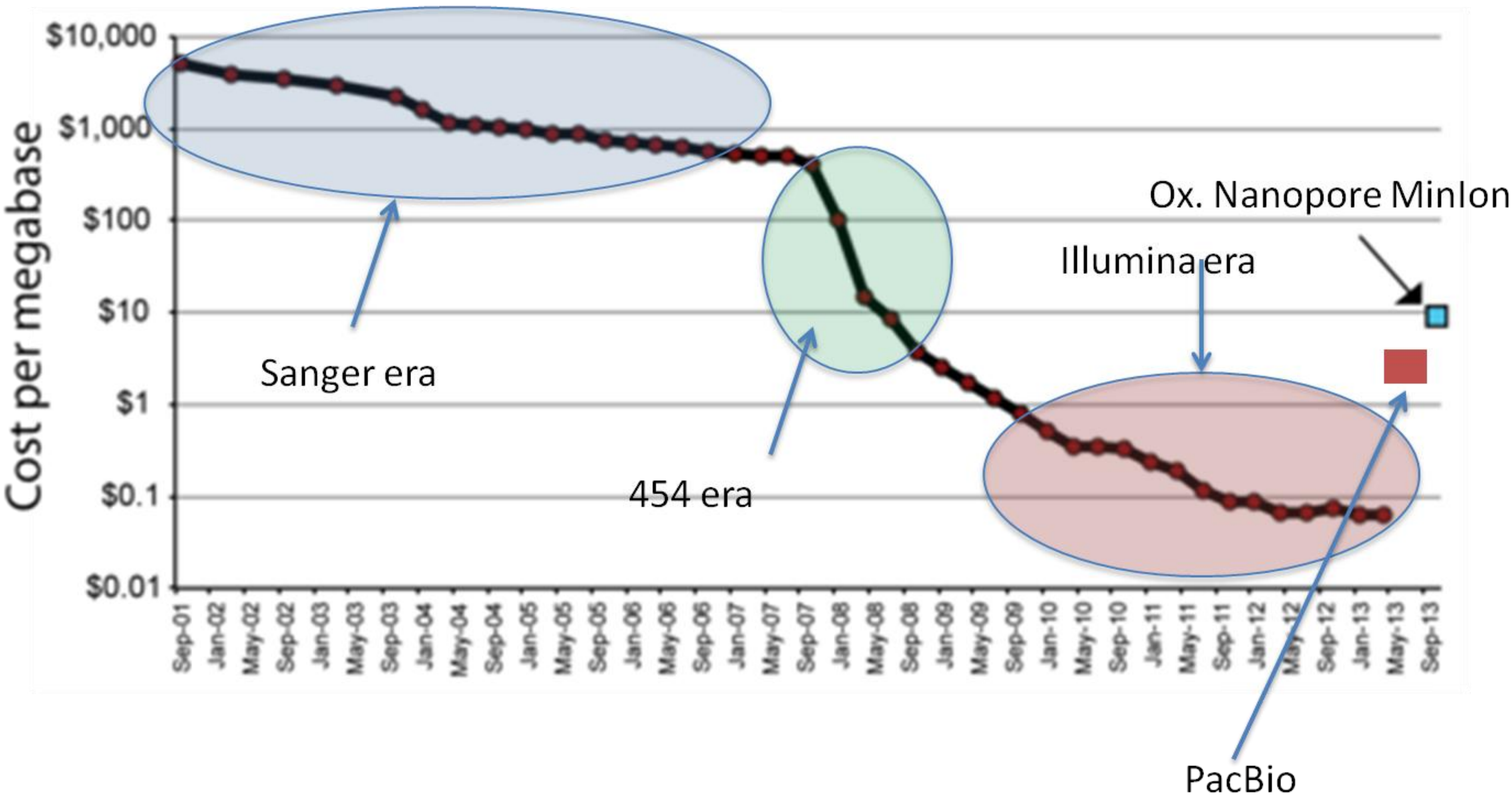




1990 - 2003

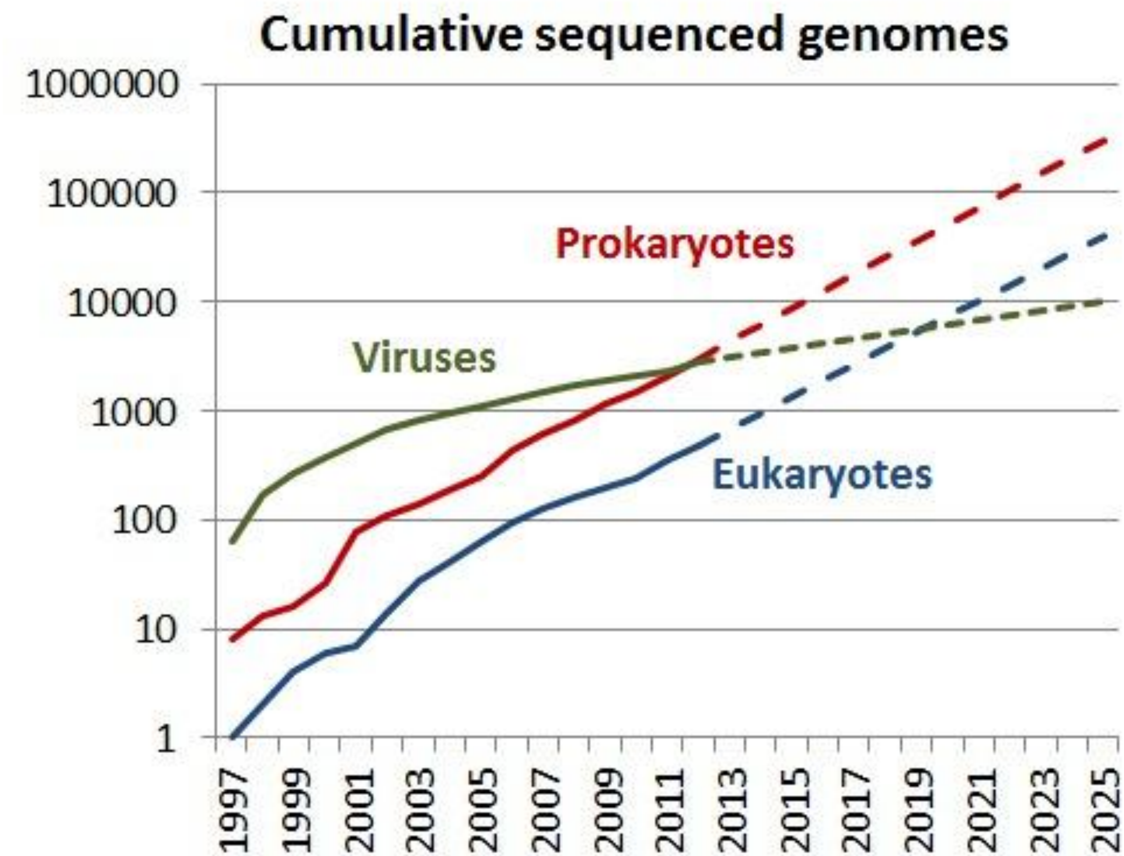
http://web.ornl.gov/sci/techresources/Human_Genome/project/whydoe.shtml

Evolution of Cost per Megabase



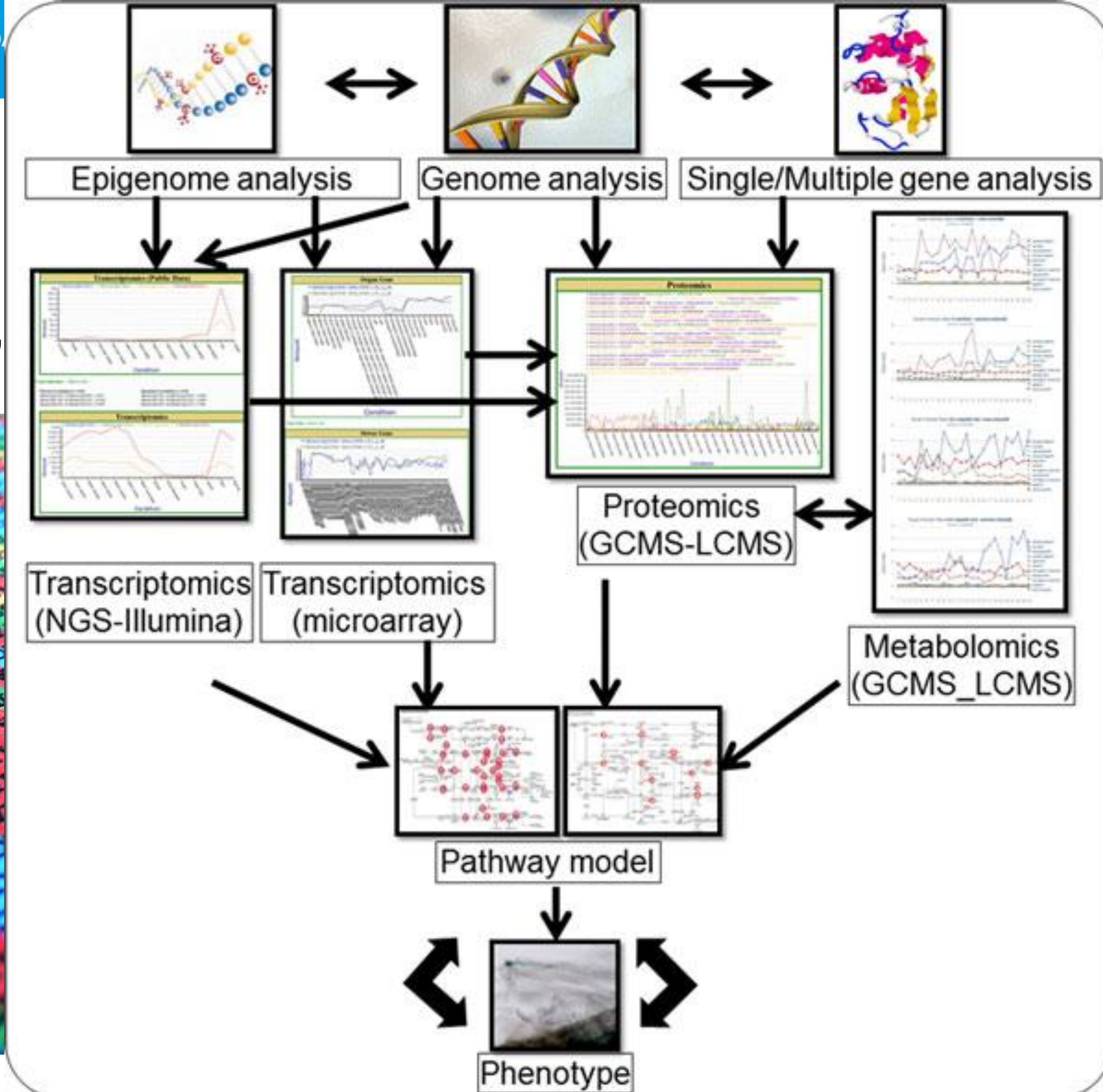
http://evomics.org/2014/01/sequencing-technology-wheres-my-minion/

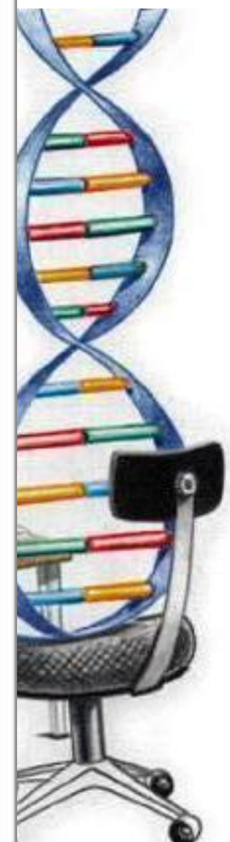
Evolution of Whole-Genome Sequencing



Su, Andrew (2013): Cumulative sequenced genomes. figshare.

Big Data, … Modeling





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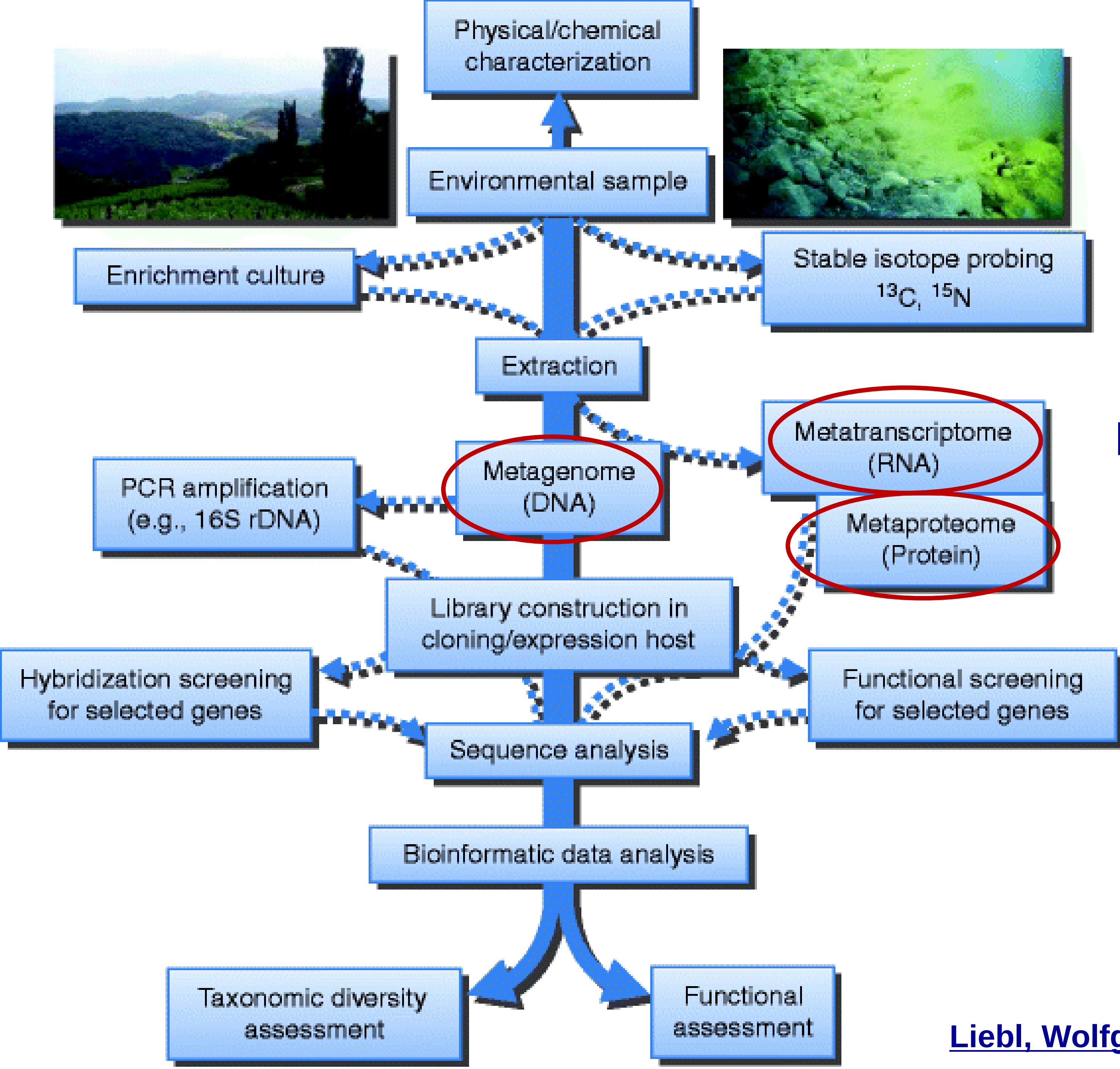
Enzyme discovery in the OMICS Era



Liebl, Wolfgang (2011): Metagenomics

New Enzymes for Biofuels: GH 10 Xylanase

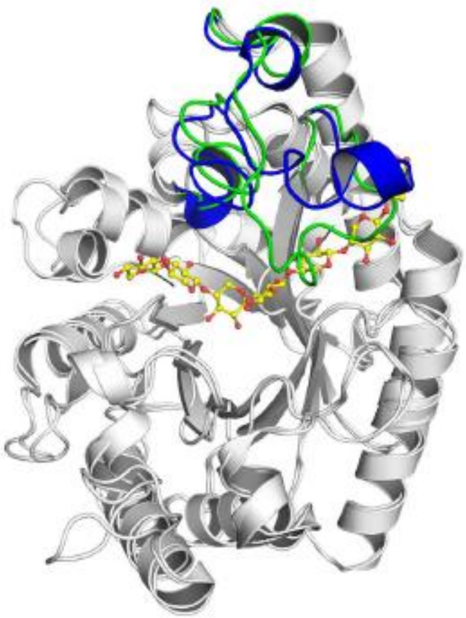
OPEN ACCESS Freely available online PLOS ONE

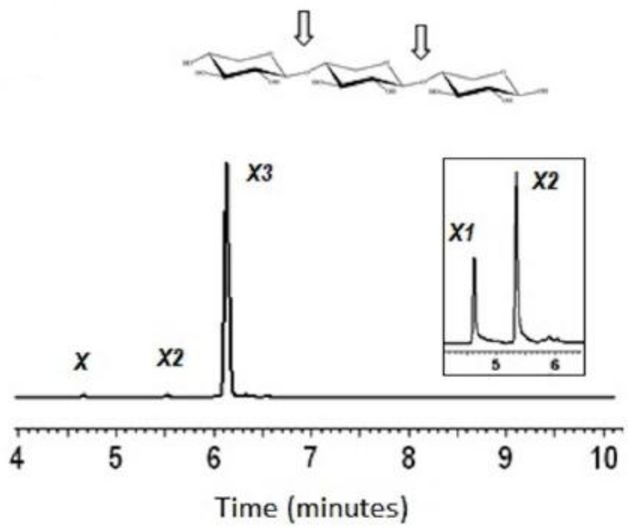
Development and Biotechnological Application of a Novel Endoxylanase Family GH10 Identified from Sugarcane Soil Metagenome

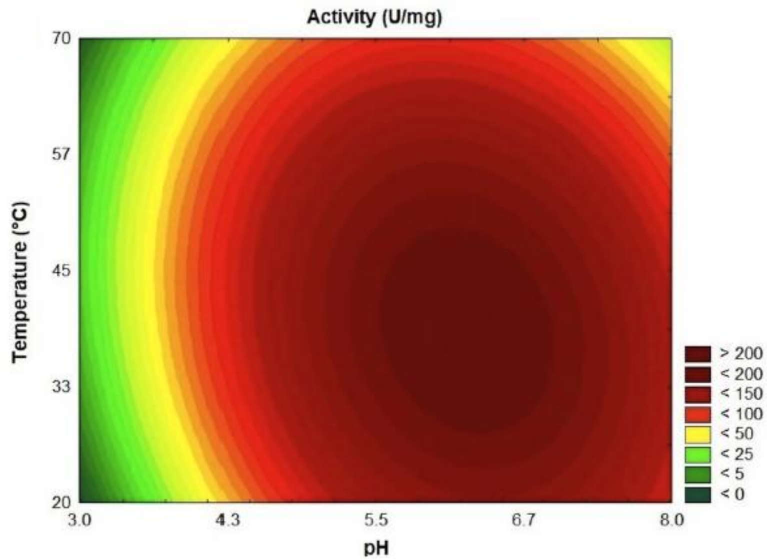
Thabata M. Alvarez[1,2], Rosana Goldbeck[1], Camila Ramos dos Santos[3], Douglas A. A. Paixão[1], Thiago A. Gonçalves[1,2], João Paulo L. Franco Cairo[1,2], Rodrigo Ferreira Almeida[1], Isabela de Oliveira Pereira[1], George Jackson[1], Junio Cota[1], Fernanda Büchli[1,2], Ana Paula Citadini[1], Roberto Ruller[1], Carla Cristina Polo[3], Mario de Oliveira Neto[4], Mário T. Murakami[3]*, Fabio M. Squina[1]*

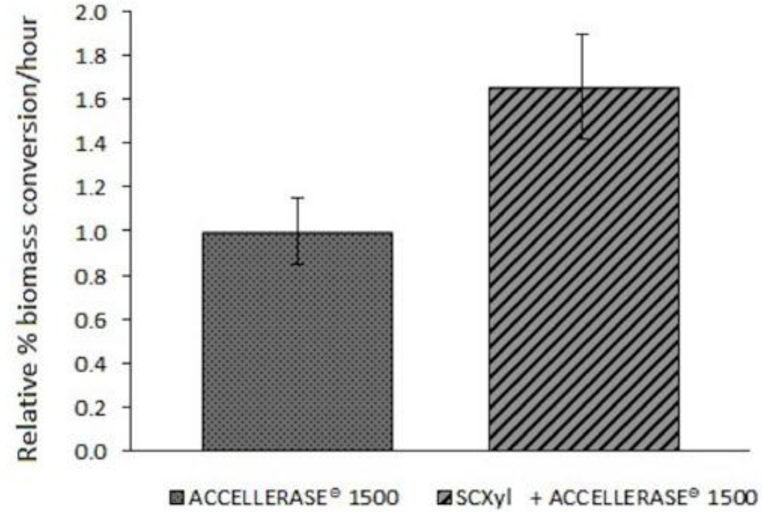
1 Laboratório Nacional de Ciência e Tecnologia do Bioetanol (CTBE), Centro Nacional de Pesquisa em Energia e Materiais (CNPEM), Campinas, São Paulo, Brasil, **2** Departamento de Bioquímica, Instituto de Biologia (IB), Universidade Estadual de Campinas (UNICAMP), Campinas, São Paulo, Brasil, **3** Laboratório Nacional de Biociências (LNBio), Centro Nacional de Pesquisa em Energia e Materiais (CNPEM), Campinas, São Paulo, Brasil, **4** Departamento de Física e Biofísica, Instituto de Biociências, Universidade Estadual Paulista (UNESP), Botucatu, São Paulo, Brasil

Enzymes for Biofuels: GH 10 Xylanase









Proteomics: Secretome of *Penicillium equinulatum*

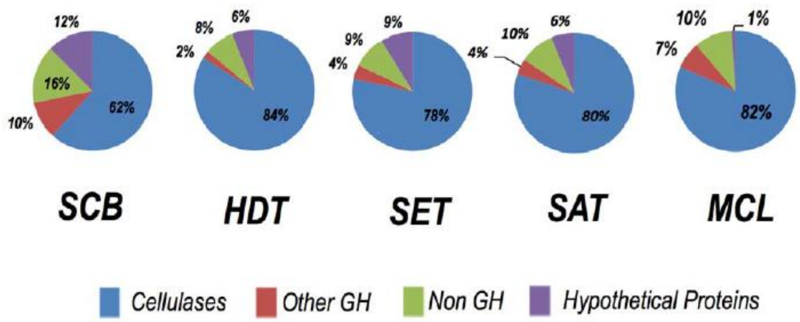
OPEN ACCESS Freely available online PLOS ONE

The *Penicillium echinulatum* Secretome on Sugar Cane Bagasse

Daniela A. Ribeiro[1], Júnio Cota[1], Thabata M. Alvarez[1], Fernanda Brüchli[1], Juliano Bragato[1], Beatriz M. P. Pereira[1], Bianca A. Pauletti[1], George Jackson[1], Maria T. B. Pimenta[1], Mario T. Murakami[2], Marli Camassola[3], Roberto Ruller[1], Aldo J. P. Dillon[3], Jose G. C. Pradella[1], Adriana F. Paes Leme[1], Fabio M. Squina[1]*

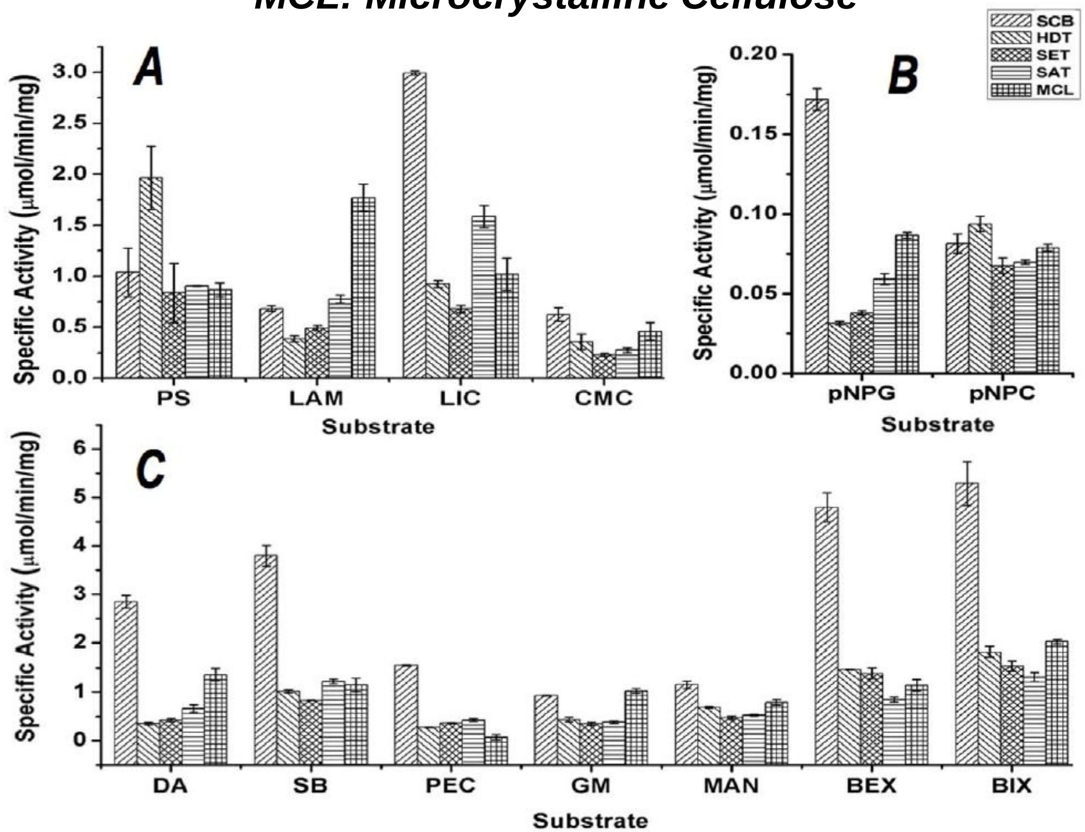
1 Laboratório Nacional de Ciência e Tecnologia do Bioetanol (CTBE), Centro Nacional de Pesquisa em Energia e Materiais, Campinas, (CNPEM), Campinas, São Paulo, Brazil, **2** Laboratório de Espectrometria de Massas, Laboratório Nacional de Biociências (LNBio), Centro Nacional de Pesquisa em Energia e Materiais, Campinas, (CNPEM), Campinas, São Paulo, Brazil, **3** Instituto de Biotecnologia, Universidade de Caxias do Sul (UCS), Caxias do Sul, Rio Grande do Sol, Brazil

Proteomics: Secretome of *Penicillium equinulatum*



	SCB	HDT	SET	SAT	MCL
GH3	4	0	0	0	3
GH5	29	26	28	25	31
GH6	16	20	22	21	18
GH7	22	38	30	30	25
GH12	5	0	0	0	2
GH17	2	0	0	1	1
GH61	1	0	0	0	0
GH10	3	0	3	1	0
GH11	2	0	1	0	0
GH62	2	1	0	0	0
GH43	2	0	0	0	0
PL4	2	0	0	0	0
CE1	1	0	0	0	0
GH18	1	0	0	2	4
GH20	0	0	0	0	2
GH13	0	1	0	0	0
GH65	0	0	0	1	0
GH92	0	0	0	0	1
PF	20	8	9	9	10
HYP	15	5	9	5	1

SCB: Sugar Cane Bagasse
HDT: Hydrothermal Treatment
SET: Steam Explosion Treatment
SAT: Sulfuric Acid Treatment
MCL: Microcrystalline Cellulose



Proteomics: Secretome of *Trichoderma harzianum*

Bioresource Technology 131 (2013) 500–507

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

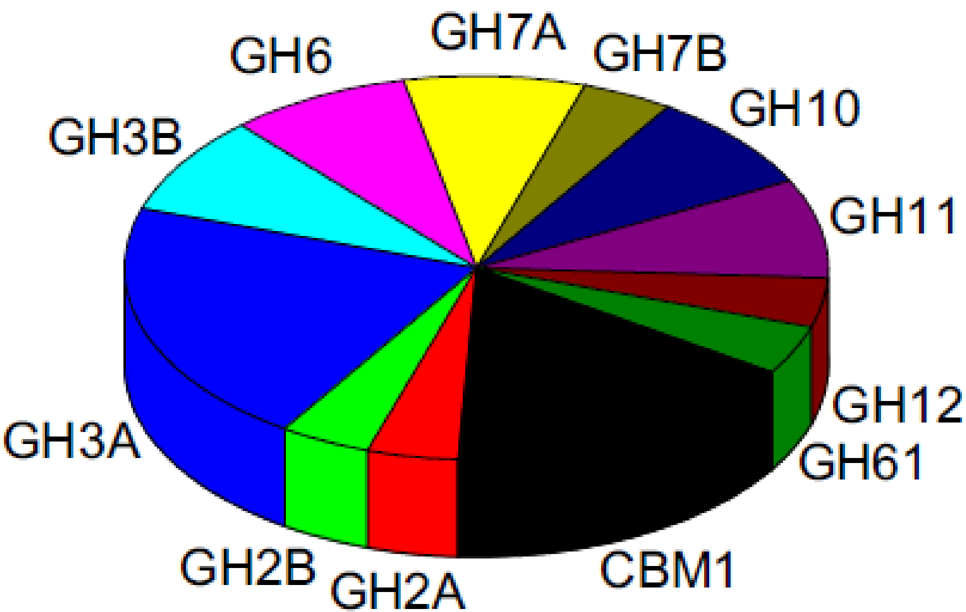
Understanding the cellulolytic system of *Trichoderma harzianum* P49P11 and enhancing saccharification of pretreated sugarcane bagasse by supplementation with pectinase and α-L-arabinofuranosidase

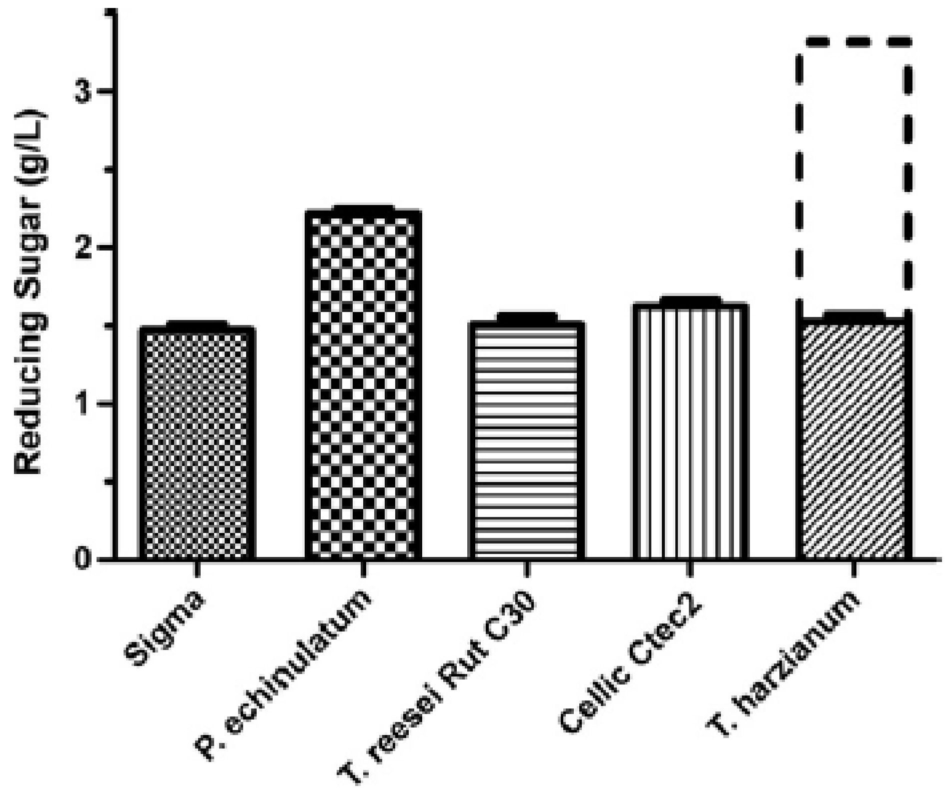
Priscila da Silva Delabona [a,*], Júnio Cota [a], Zaira Bruna Hoffmam [a], Douglas Antonio Alvaredo Paixão [a], Cristiane Sanchez Farinas [b], João Paulo Lourenço Franco Cairo [a], Deise Juliana Lima [a], Fábio Marcio Squina [a], Roberto Ruller [a], José Geraldo da Cruz Pradella [a]

[a] *Brazilian Bioethanol Science and Technology Laboratory – CTBE, Rua Giuseppe Maximo Scolfaro 10000, Pólo II de Alta Tecnologia, Caixa Postal 6192, CEP 13083-970, Campinas, São Paulo, Brazil*

[b] *Embrapa Instrumentation, Rua XV de Novembro 1452, CEP 13560-970, São Carlos, São Paulo, Brazil*

Proteomics: Secretome of *Trichoderma harzianum*





SUMMARY

- What is VTT?
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- Protein Engineering

Protein Engineering is a tailor-made process

THE SCIENCE OF WHAT'S POSSIBLE.™

What's on your mind?

What's your need?

Protein Engineering: Rational or Non-rational Design?

Current Paradigms

Mechanism-based (Rational) Detailed structural analysis	**X**	Empiricism-based (Non-rational) Libraries based

Building a Xylanase – Lichenase Chimera

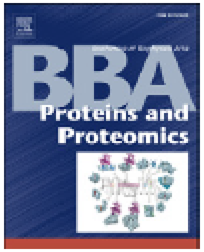
Biochimica et Biophysica Acta 1834 (2013) 1492–1500

ELSEVIER

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

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



Assembling a xylanase–lichenase chimera through all-atom molecular dynamics simulations

CrossMark

Junio Cota [a,d,1], Leandro C. Oliveira [a,b,1], André R.L. Damásio [a], Ana P. Citadini [a], Zaira B. Hoffmam [a], Thabata M. Alvarez [a], Carla A. Codima [a], Vitor B.P. Leite [b], Glaucia Pastore [c], Mario de Oliveira-Neto [d], Mario T. Murakami [e], Roberto Ruller [a], Fabio M. Squina [a,*]

[a] *Laboratório Nacional de Ciência e Tecnologia do Bioetanol — CTBE/CNPEM, Campinas, SP, Brazil*
[b] *Departamento de Física, IBILCE, Universidade Estadual Paulista - UNESP, São José do Rio Preto, SP, Brazil*
[c] *Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas, Campinas, SP, Brazil*
[d] *Departamento de Física e Biofísica, Instituto de Biociências, UNESP, Botucatu, São Paulo, Brazil*
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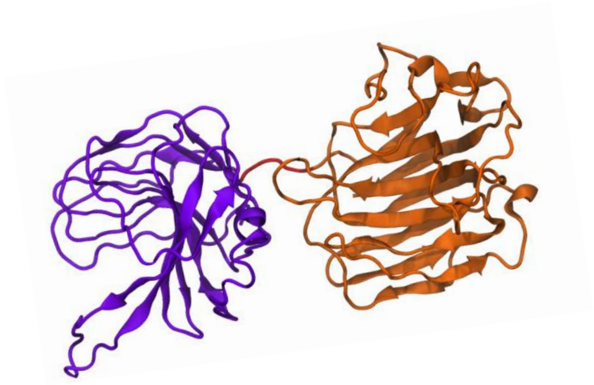
Chimeras: Multidomain Proteins

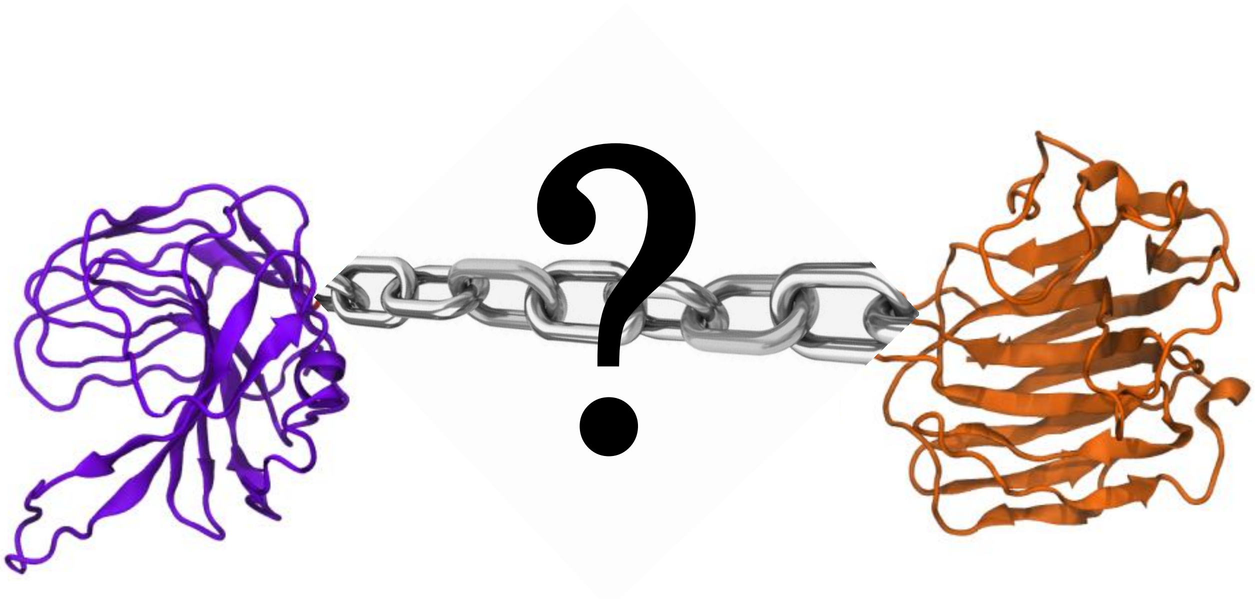
- ✓ Multidomain/multifunctional proteins can reduce costs with enzyme load;
- ✓ End-to-end fusion between the N and C termini of the parental enzymes can result in nonfunctional chimeras.



S.Y. Hong et al., Biotechnology Letters, 29, 931-936 (2007)

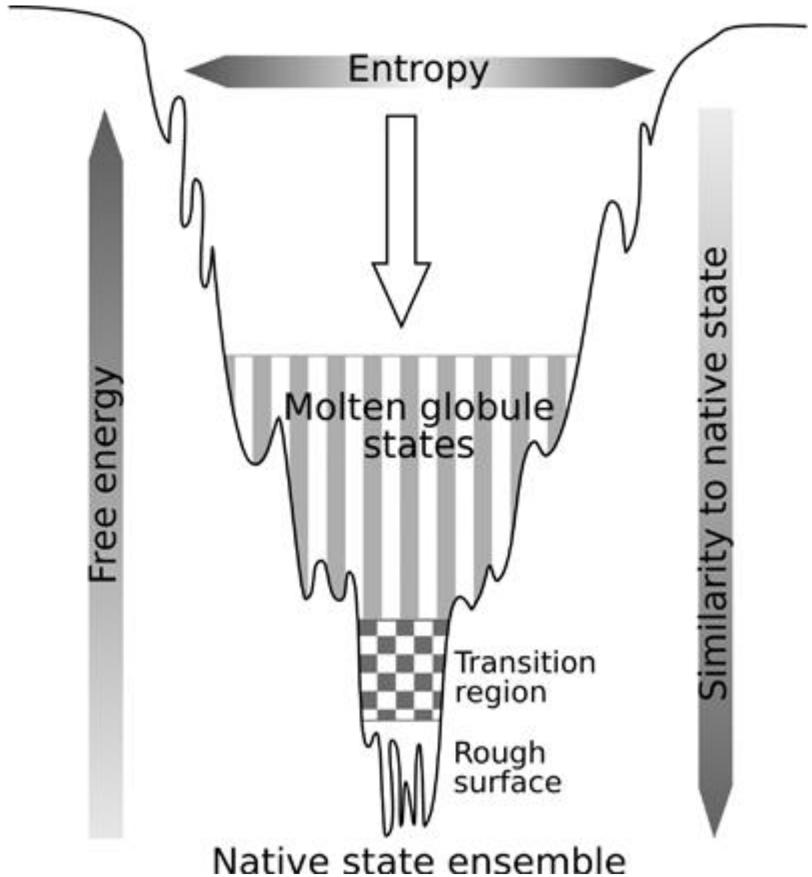
Chimeras: Multidomain Proteins

- ✓ The selection of the linker sequence is particularly important for the construction of functional fusion proteins



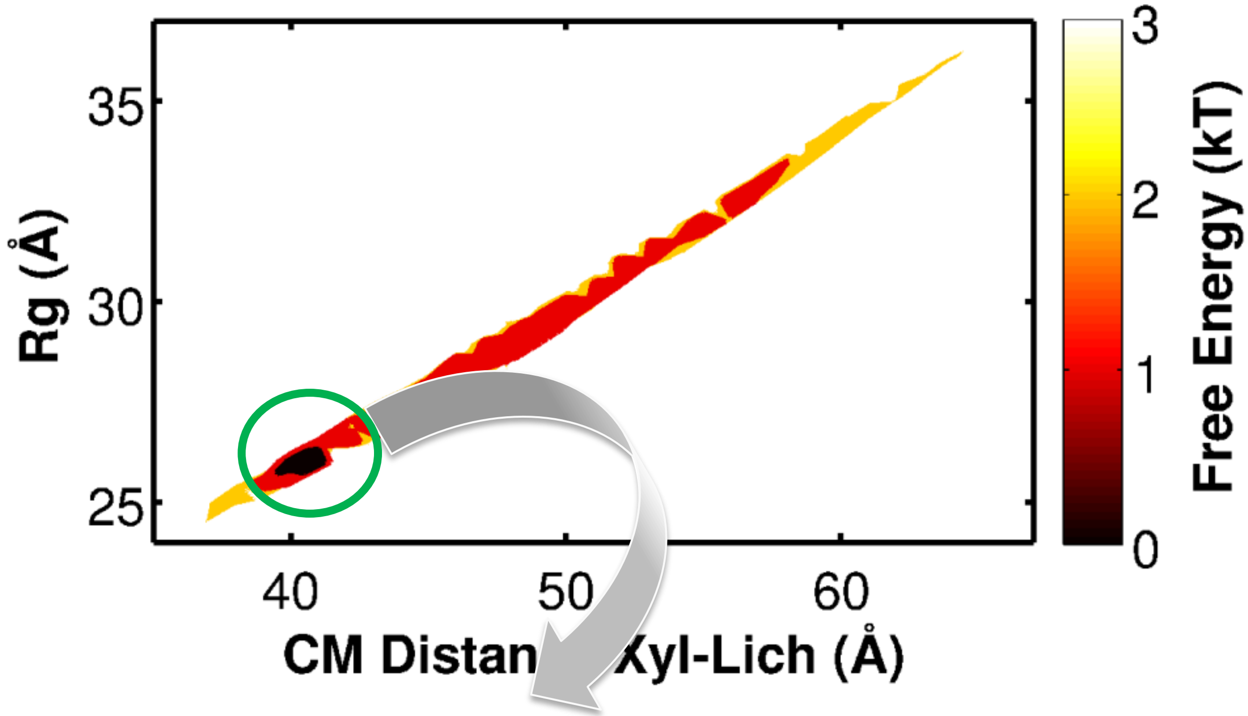
Building Chimeras: Molecular Dinamics

- ✓ Energy Landscape Theory
- ✓ Structure Based Models
- ✓ The topology could drives the protein folding
- ✓ Save computational time



X. L. Ji, and S. Q. Liu. J. Biomol Struct Dyn 28, 621-623 (2011)

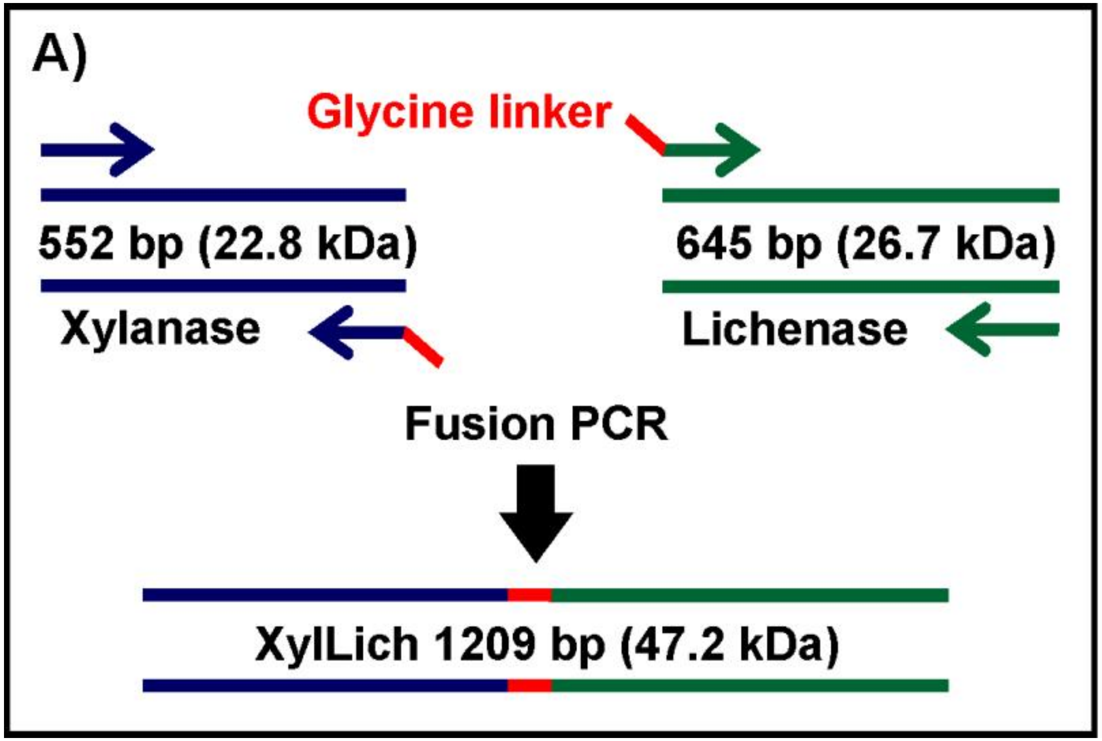
Structure Based Models (SB)



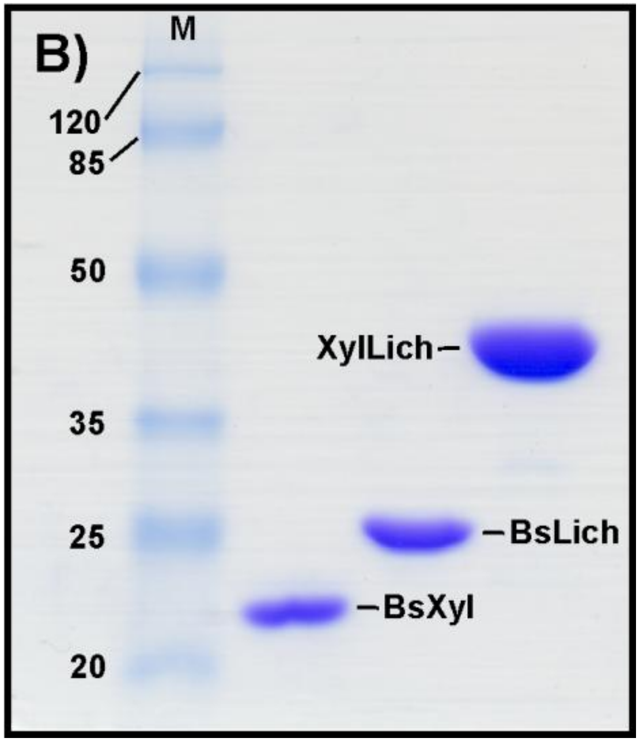
The unique Free Energy basin suggests a group of structures candidates: simulations are mainly driven by the entropy of the system

Building Chimera

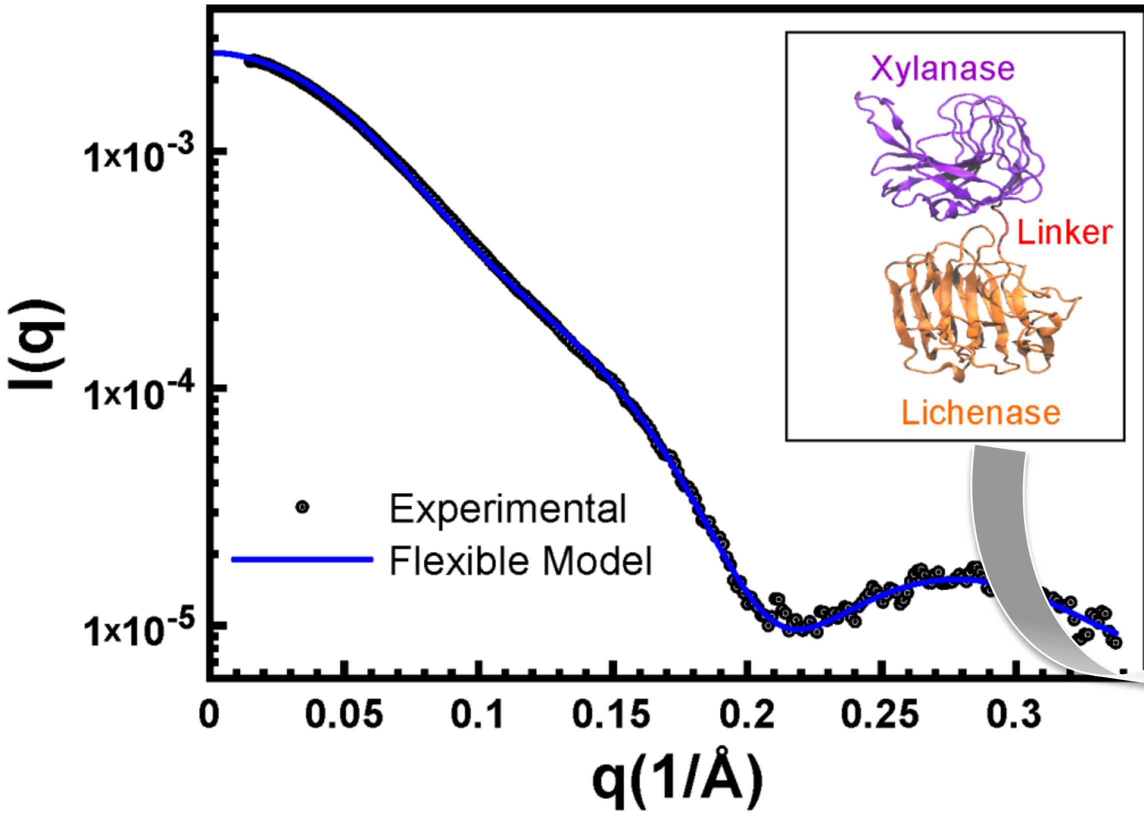
Overlap PCR



SDS-PAGE



SAXS experimental and theoretical curves

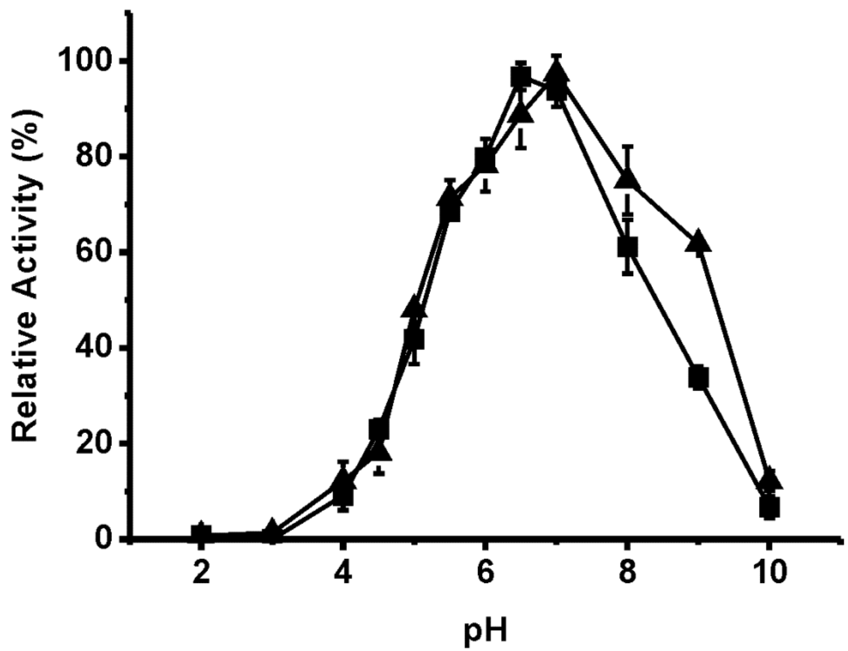


Representative structure taken from the Free Energy basin with χ^2=2.80, *Rg*=26.0Å and *CM* distance=40.7Å inset

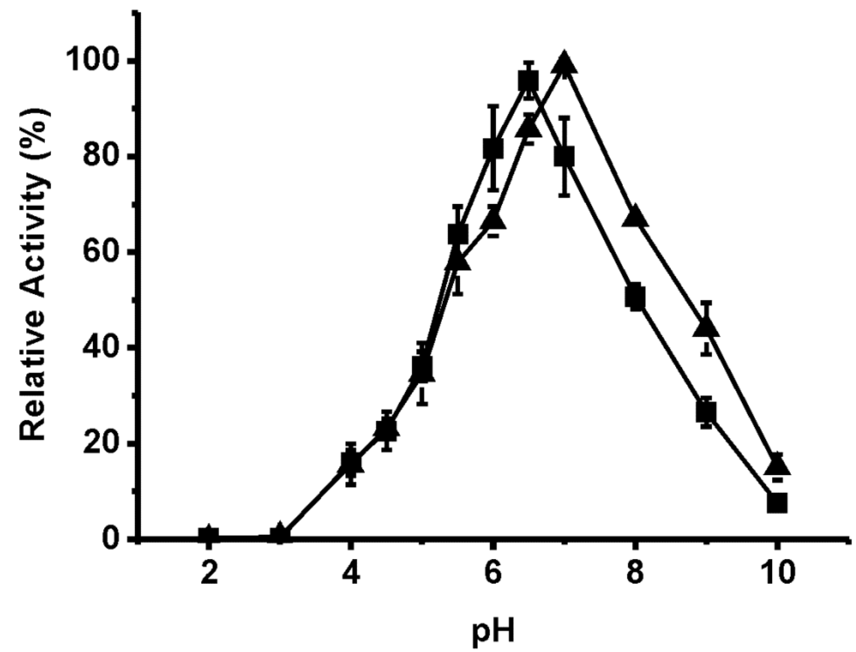
Theoretical scattering curve was generated in CRYSOL and the representation in VMD

Optimal pH

Xylan



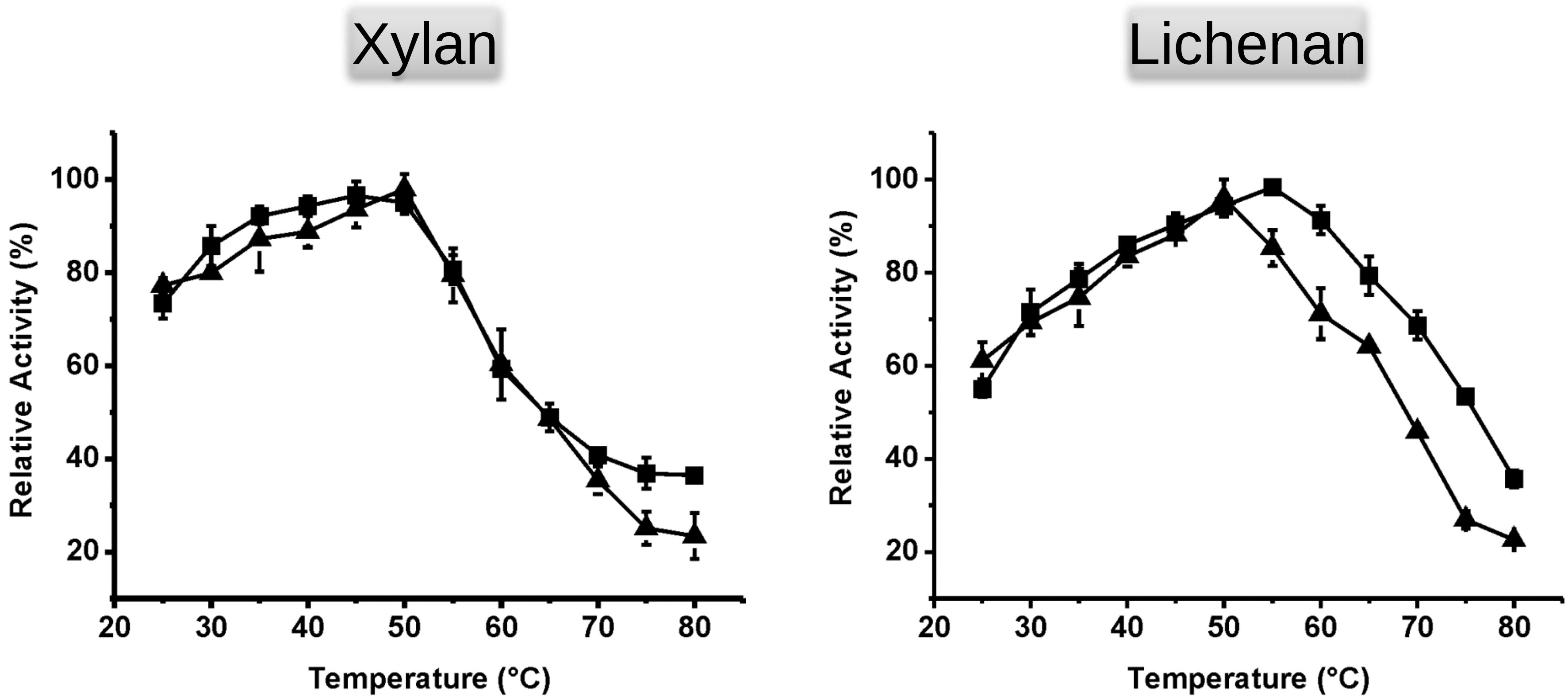
Lichenan



▲ Parental enzyme

■ Chimera

Optimal Temperature



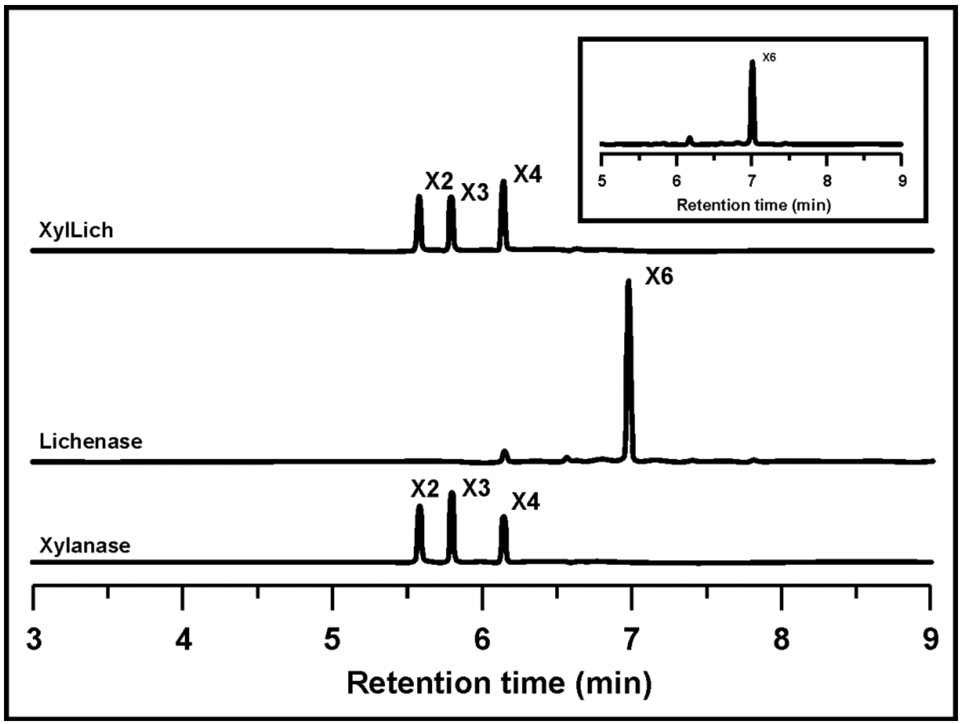
▲ Parental enzyme
■ Chimera

Substrate Specificity

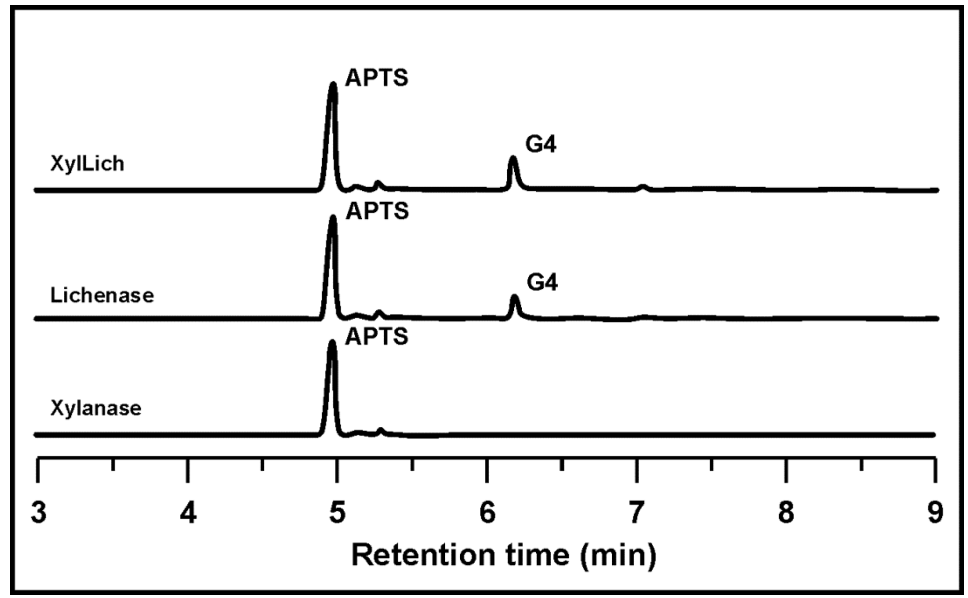
	Specific activity (U/nmol)		
Substrate	Xylanase	Lichenase	XylLich
Birchwood Xylan	3.73 ± 0.29	ND	2.71 ± 0.13
Beechwood Xylan	3.17 ± 0.07	ND	2.87 ± 0.08
Rye Arabinoxylan	3.73 ± 0.14	ND	3.03 ± 0.15
Wheat Arabinoxylan	1.36 ± 0.12	ND	0.88 ± 0.07
Oat Spelt Xylan	3.28 ± 0.27	ND	2.15 ± 0.06
Lichenan	ND	3.65 ± 0.29	3.85 ± 0.16
β-Glucan	ND	5.03 ± 0.20	5.11 ± 0.07
Laminarin	ND	ND	ND
Xyloglucan	ND	ND	ND
Glucomannan (Konjac)	ND	ND	ND

Capillary Electrophoresis

Xylohexaose

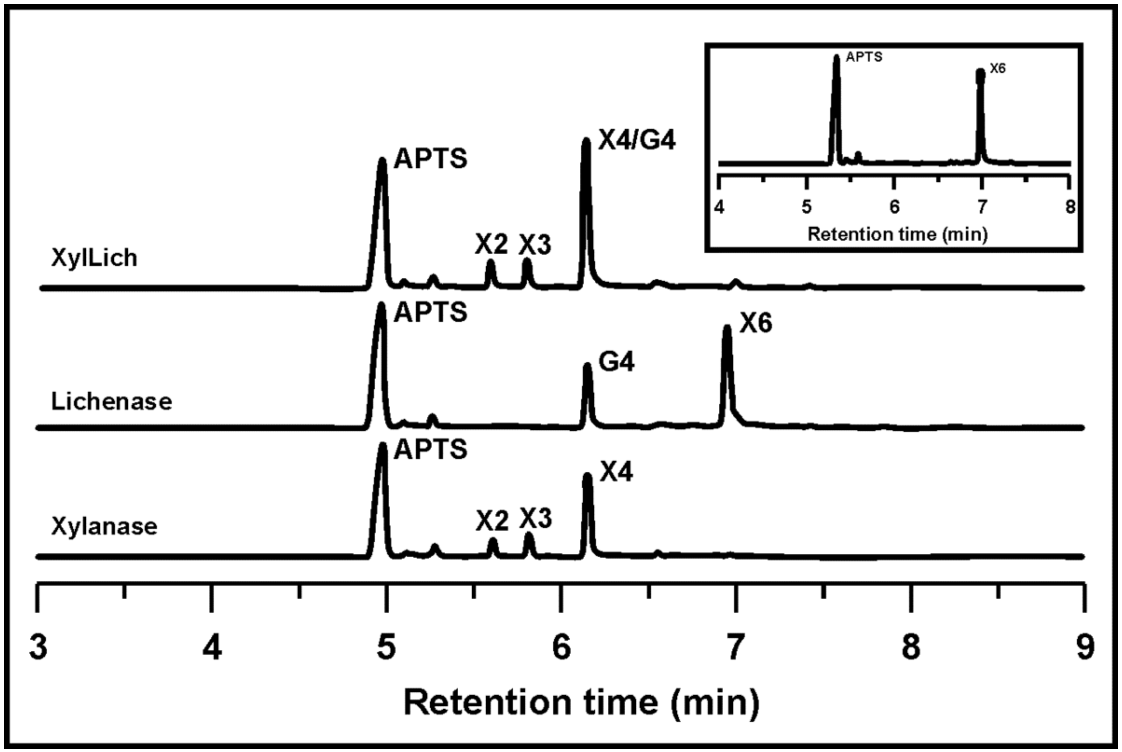


Lichenan

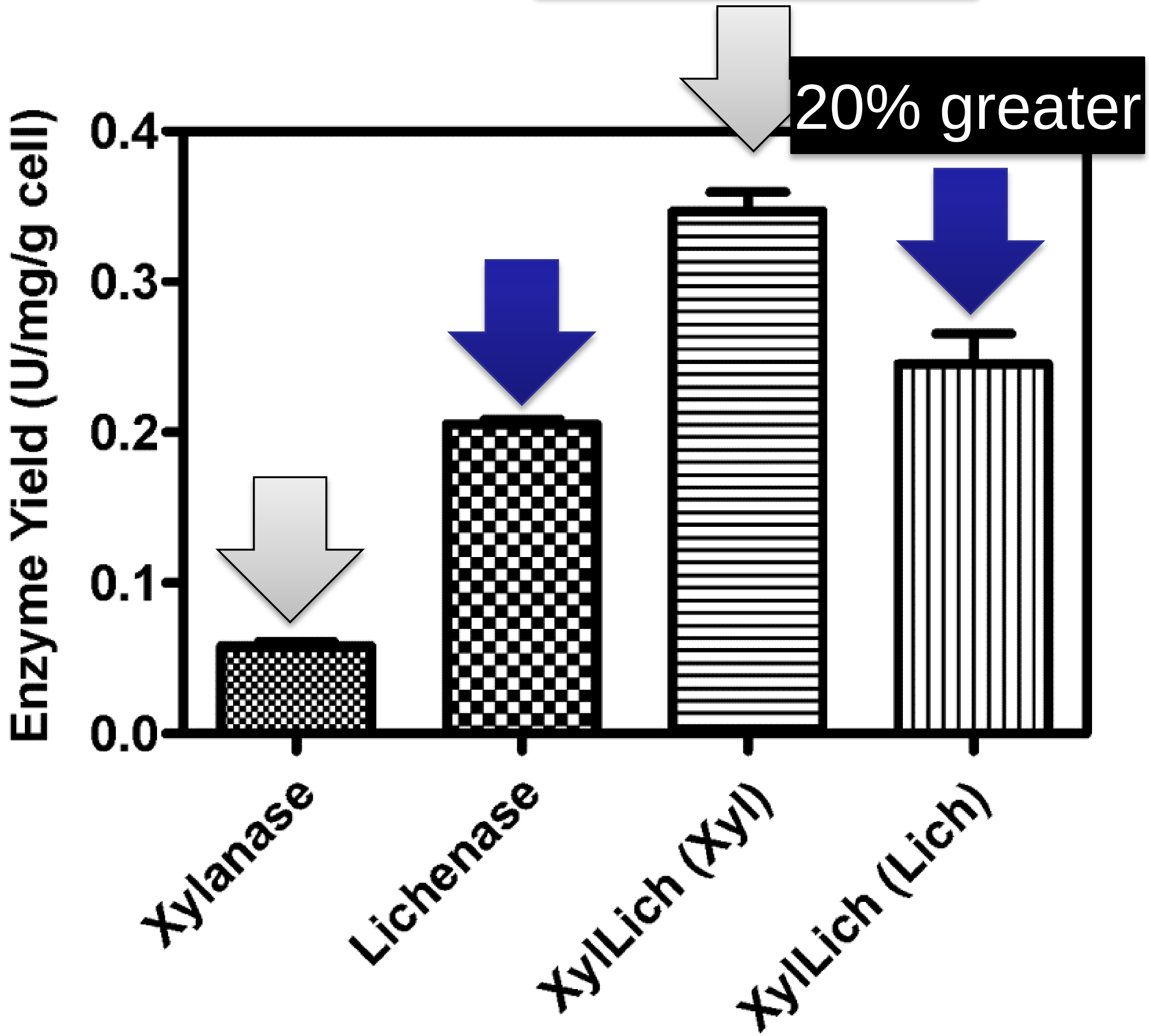


Capillary Electrophoresis

Xylohexaose + Lichenan



Enzym 6 times greater



Conclusions

- ✓ This work presented a novelty way to predict the disposal of chimera domains in solution before experimental assays;
- ✓ A potential tool for screening and development of enzyme cocktails for second generation biofuels;
- ✓ The expansion of hydrolase activities in an unique protein could be a route for increase cost-effective of biomass saccharification;
- ✓ Enzyme production data suggests an advantage on producing the fused protein instead the wild type ones separated.

Protein Engineering: Typical Challenges

- Design proteins with certain function;
- Design proteins which bind novel ligands;
- Alter binding affinity and specificity of proteins;
- Increase activity of enzymes;
- Change thermal tolerance, pH stability;
- Alter allosteric regulation;
- Decrease inhibition of enzymes;
- Increase protease resistance;
- Reactivity in nonaqueous solvents;
- Eliminate cofactor requirement.

Acknowledgements

VTT BRASIL LTDA

CTBE
Centro de Ciência e Tecnologia do Bioetanol

UNICAMP

CNPq

FAPESP

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