

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



Berkeley
(VTT/)

Finland

Sodankylä

Rovaniemi

Oulu
Raahe

Kajaani

Kuopio
Jyväskylä

Tampere
Lappeenranta

Turku
Espoo
Rajamäki
Helsinki

Tokyo,
Japan

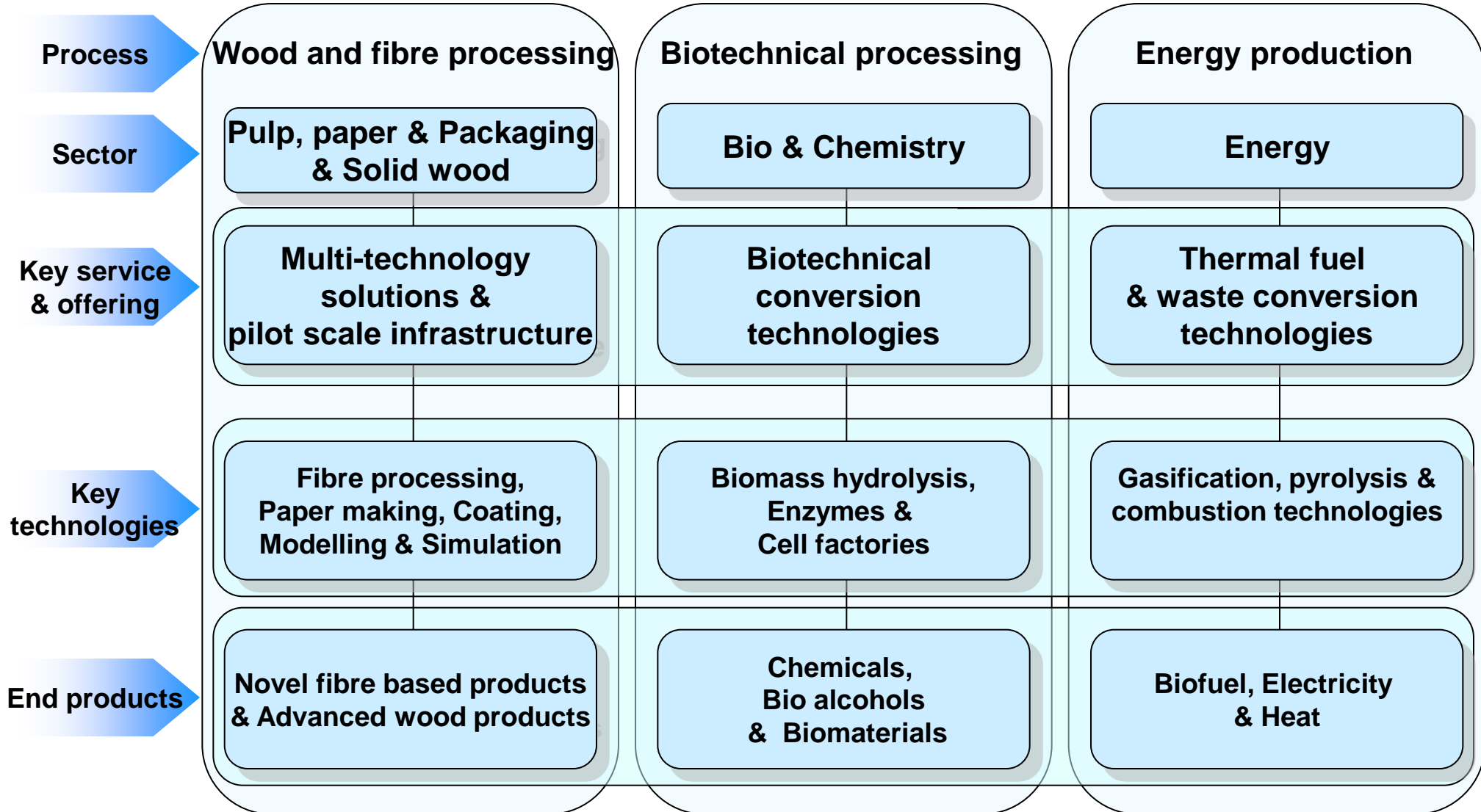
Australia



Assessments of new international projects

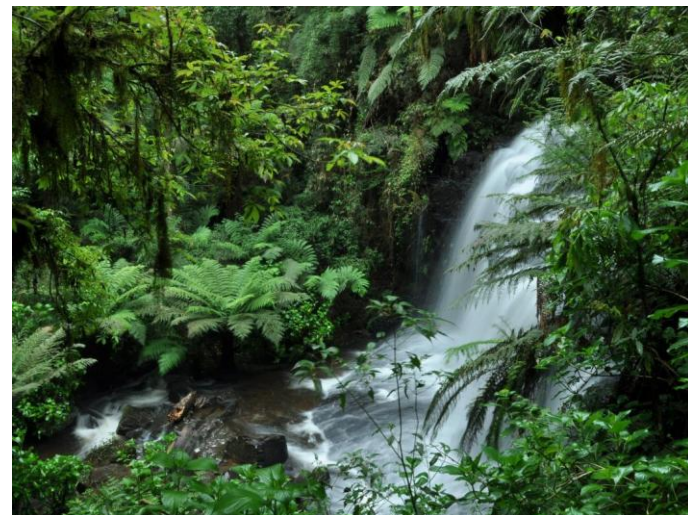
BIOREFINERY

Brazilian biomass raw material





BRAZIL



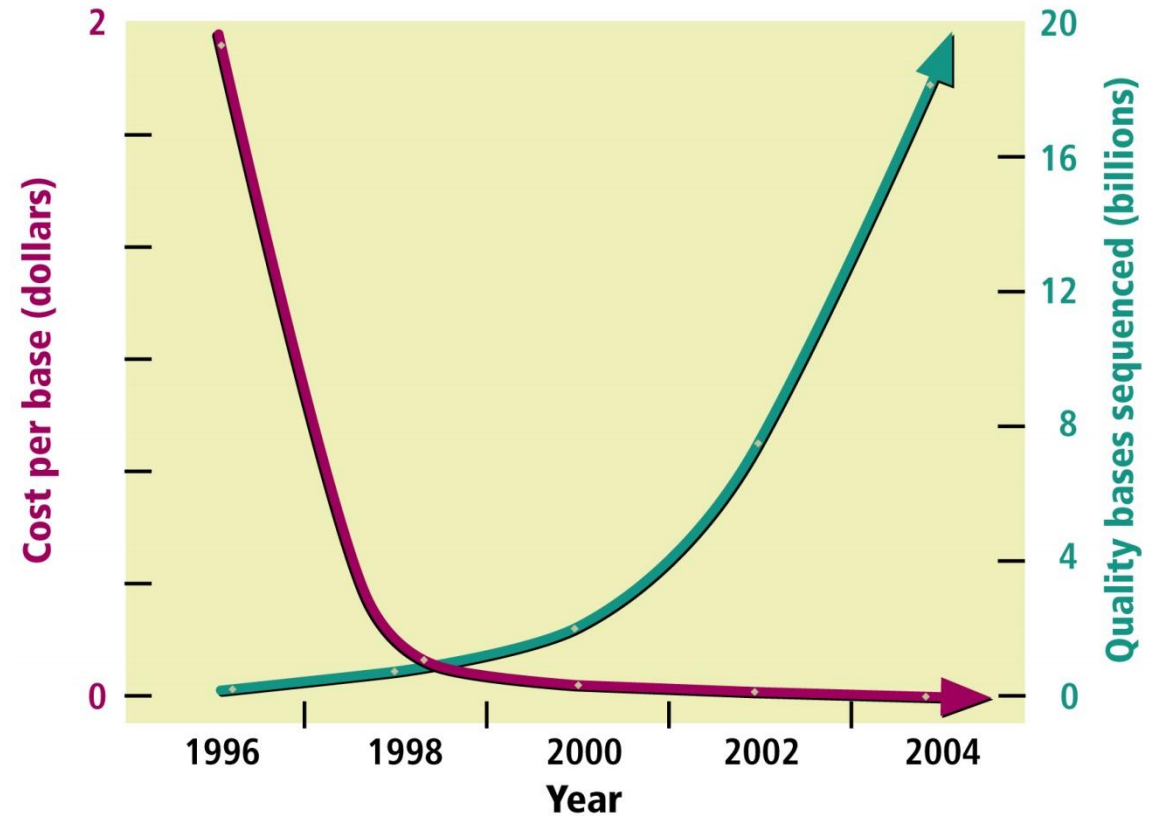
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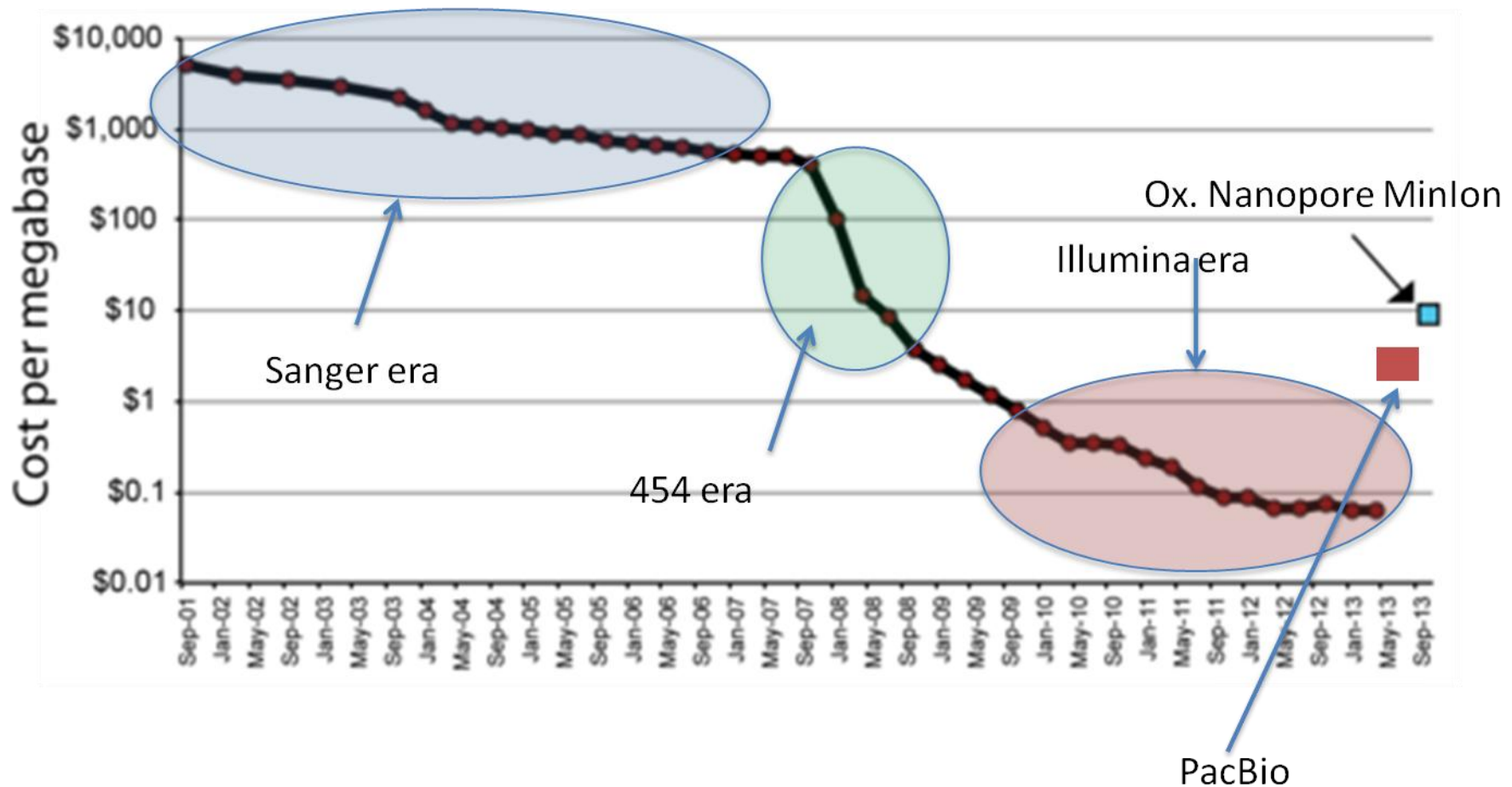
Human Genome Project



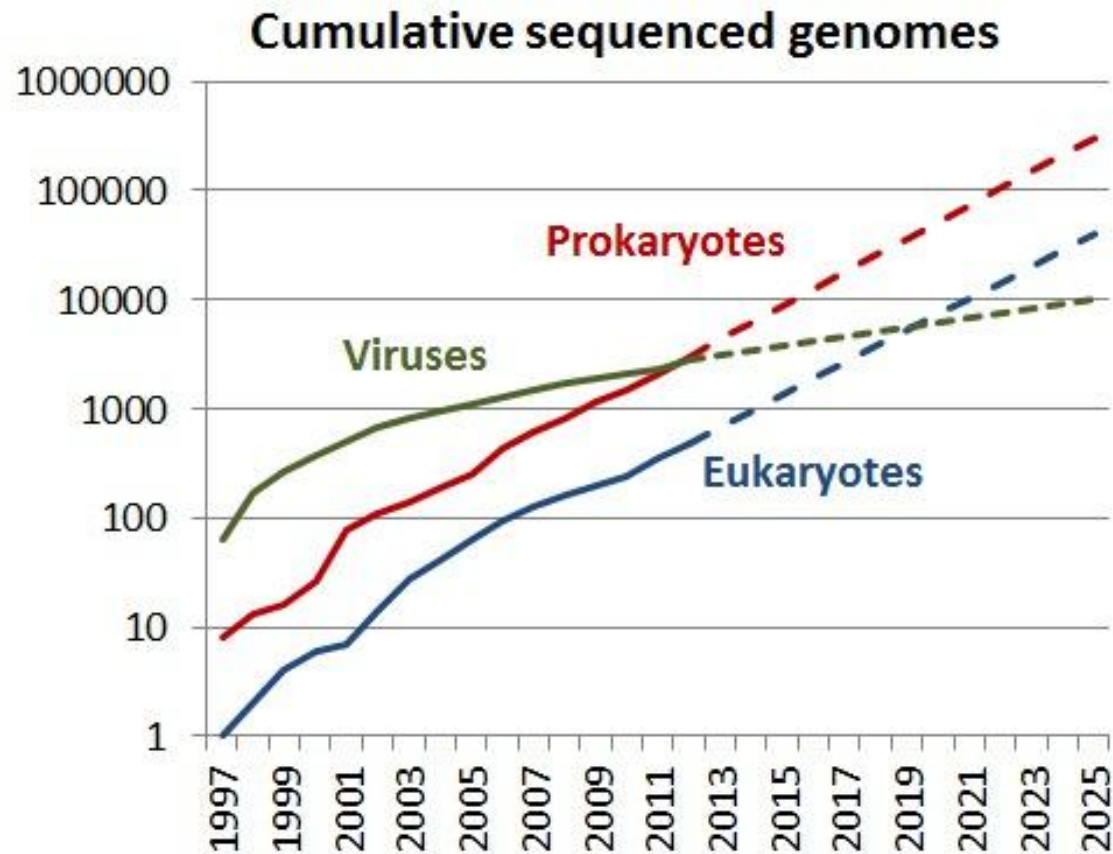
1990 - 2003



Evolution of Cost per Megabase



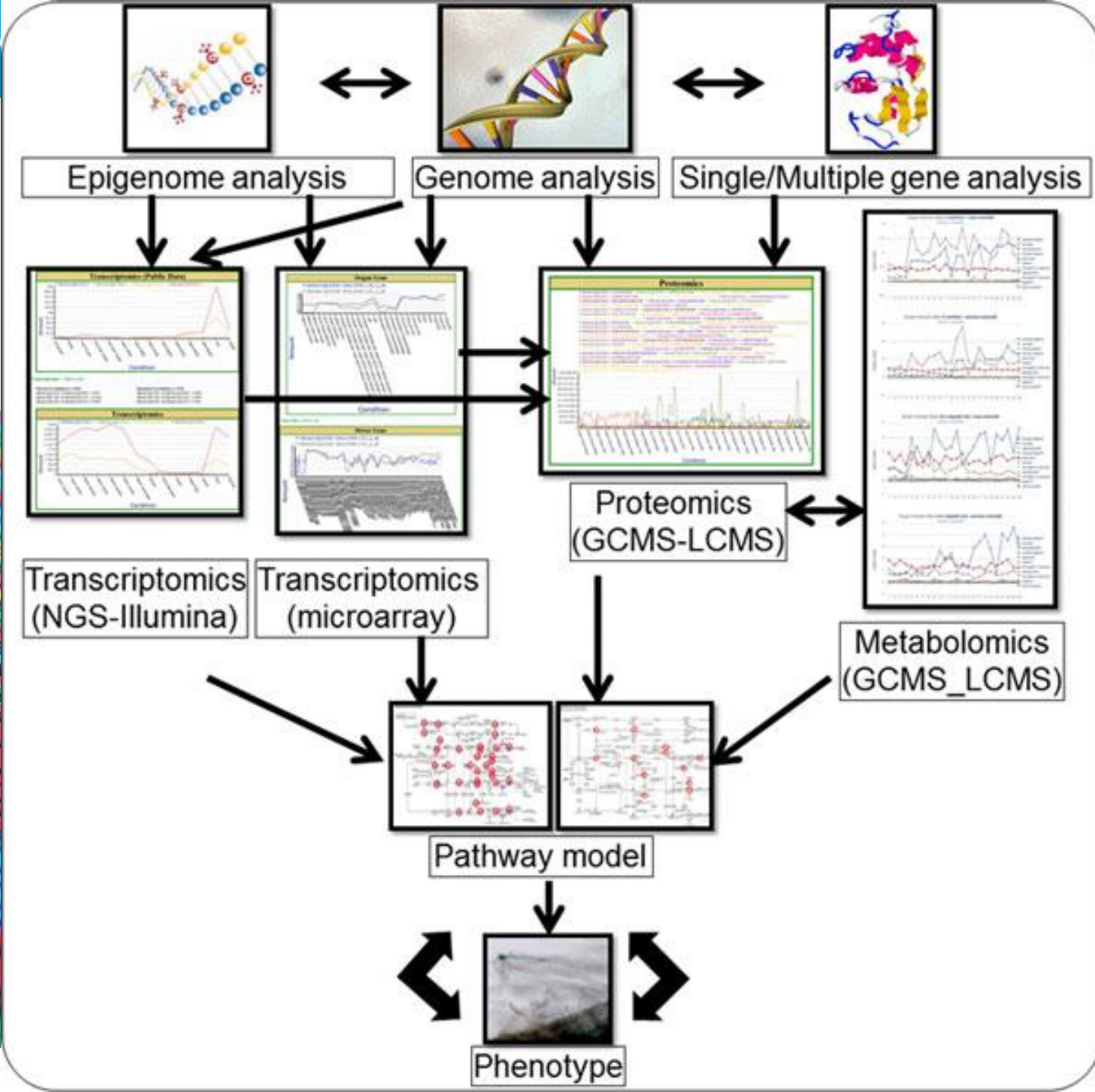
Evolution of Whole-Genome Sequencing



Big Data,

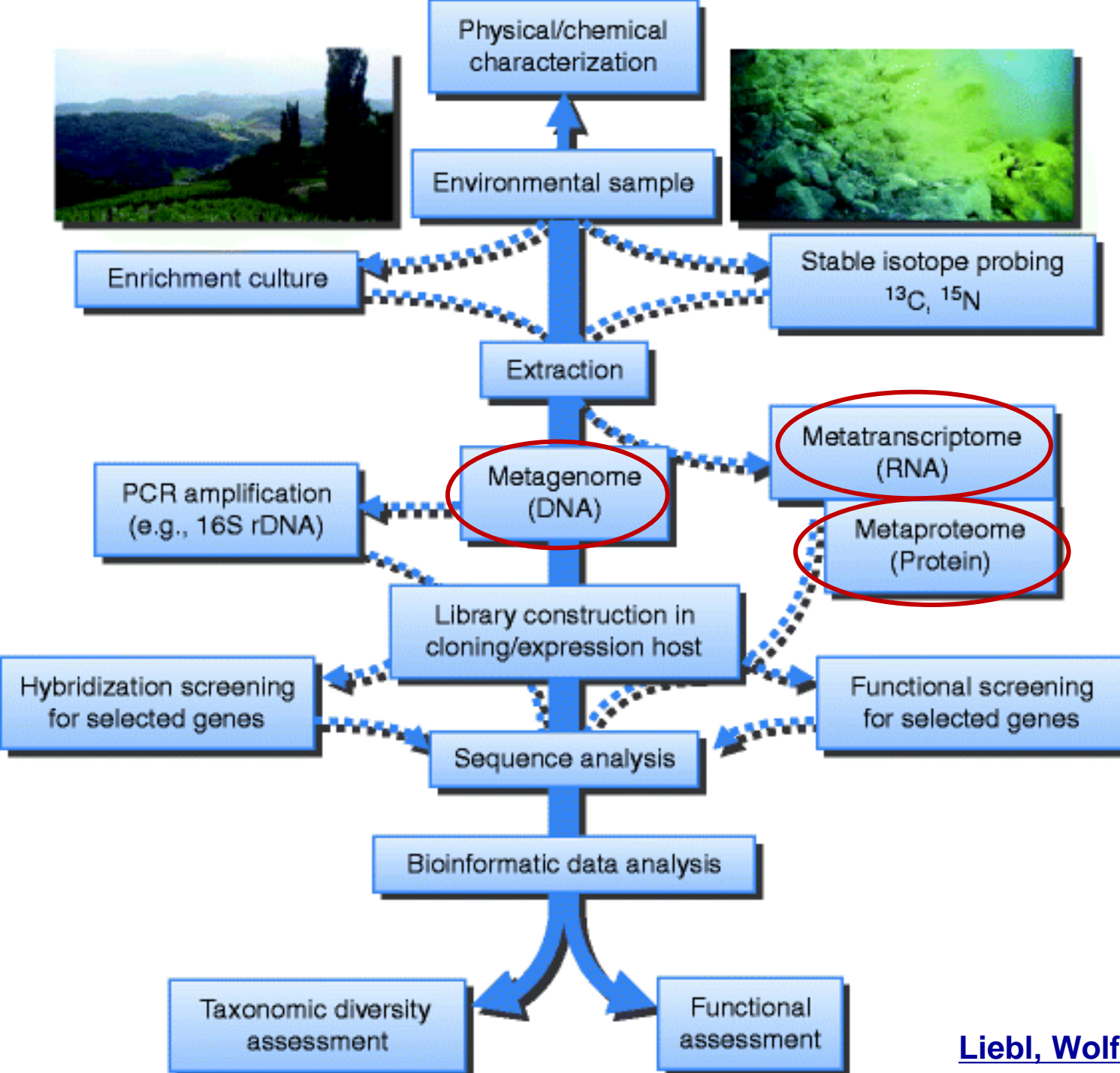


Modeling



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Enzyme discovery in the OMICS Era

New Enzymes for Biofuels: GH 10 Xylanase

OPEN ACCESS Freely available online

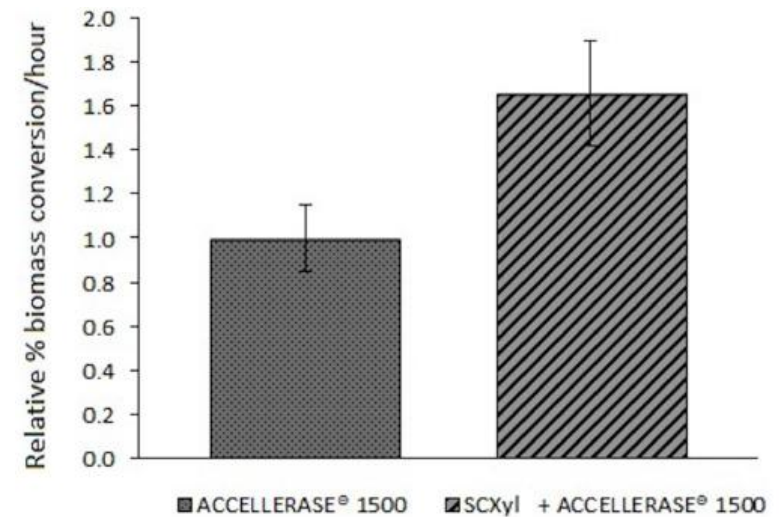
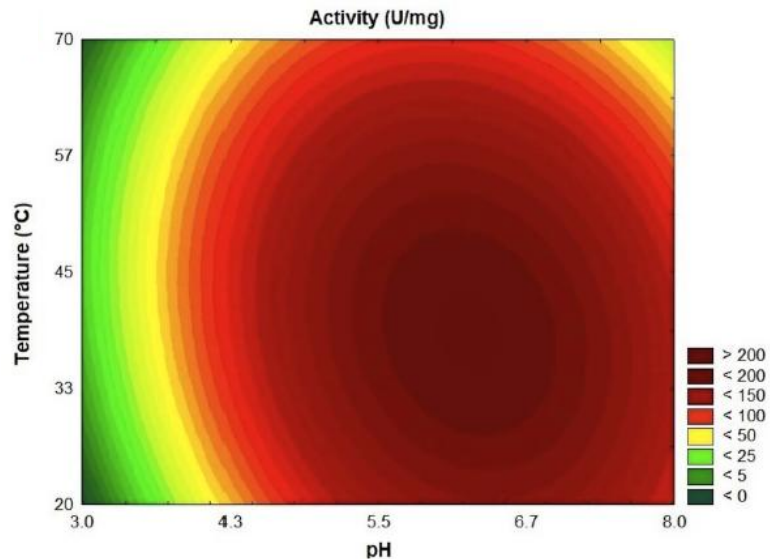
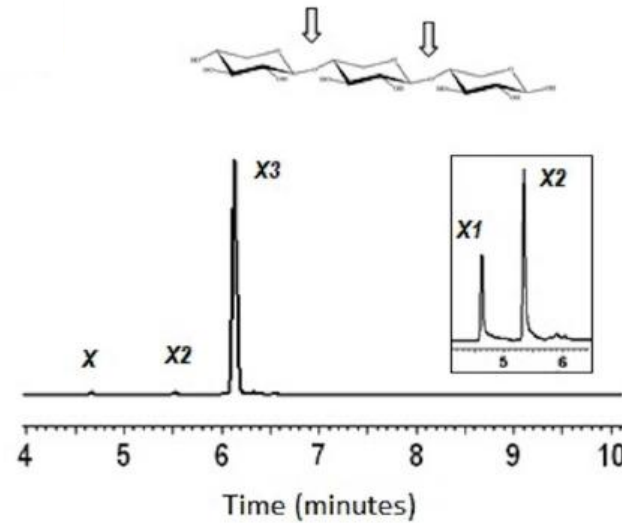
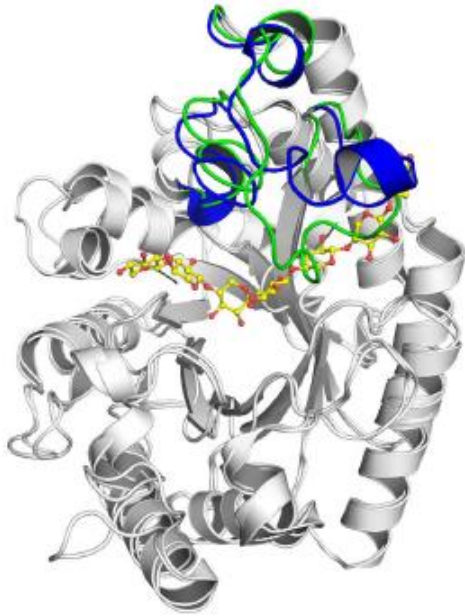


Development and Biotechnological Application of a Novel Endoxylanase Family GH10 Identified from Sugarcane Soil Metagenome

Thabata M. Alvarez^{1,2}, Rosana Goldbeck¹, Camila Ramos dos Santos³, Douglas A. A. Paixão¹, Thiago A. Gonçalves^{1,2}, João Paulo L. Franco Cairo^{1,2}, Rodrigo Ferreira Almeida¹, Isabela de Oliveira Pereira¹, George Jackson¹, Junio Cota¹, Fernanda Büchli^{1,2}, Ana Paula Citadini¹, Roberto Ruller¹, Carla Cristina Polo³, Mario de Oliveira Neto⁴, Mário T. Murakami^{3*}, Fabio M. Squina^{1*}

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

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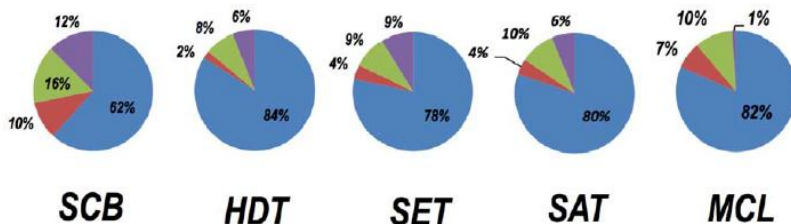


The *Penicillium echinulatum* Secretome on Sugar Cane Bagasse

Daniela A. Ribeiro¹, Júnio Cota¹, Thabata M. Alvarez¹, Fernanda Brüchli¹, Juliano Bragato¹, Beatriz M. P. Pereira¹, Bianca A. Pauletti¹, George Jackson¹, Maria T. B. Pimenta¹, Mario T. Murakami², Marli Camassola³, Roberto Ruller¹, Aldo J. P. Dillon³, Jose G. C. Pradella¹, Adriana F. Paes Leme¹, 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 Sul, Brazil

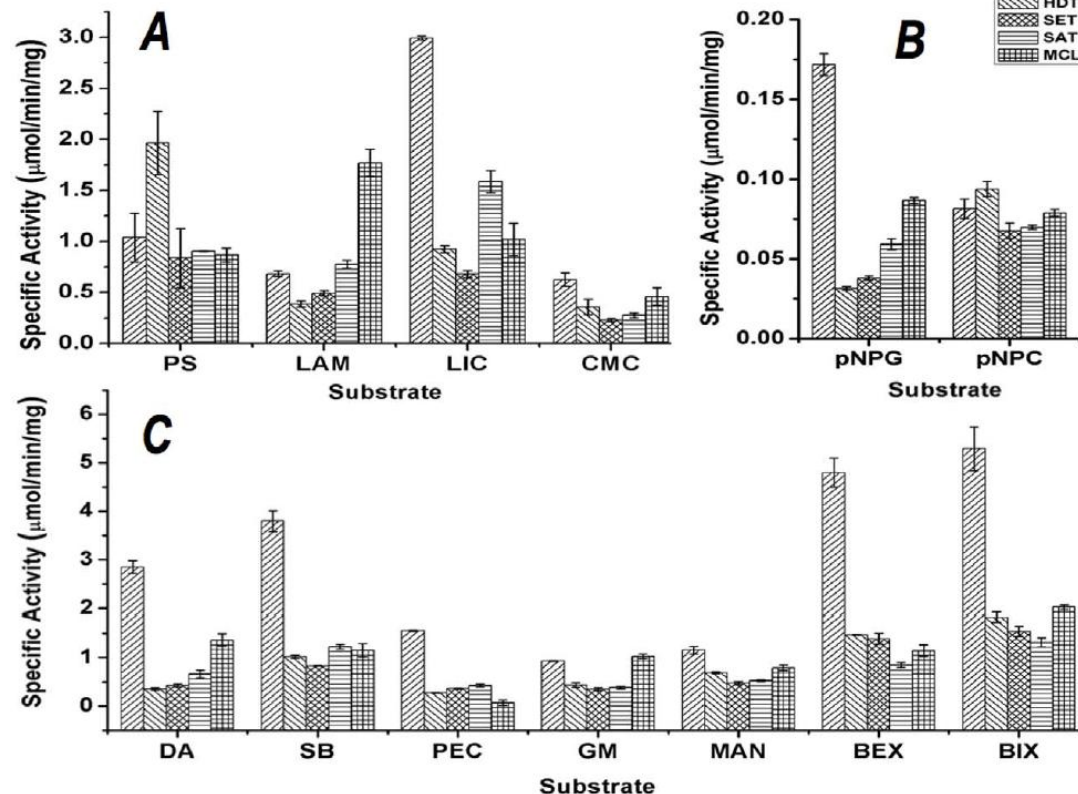
Proteomics: Secretome of *Penicillium equinulatum*



■ Cellulases
 ■ Other GH
 ■ Non GH
 ■ Hypothetical Proteins

	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



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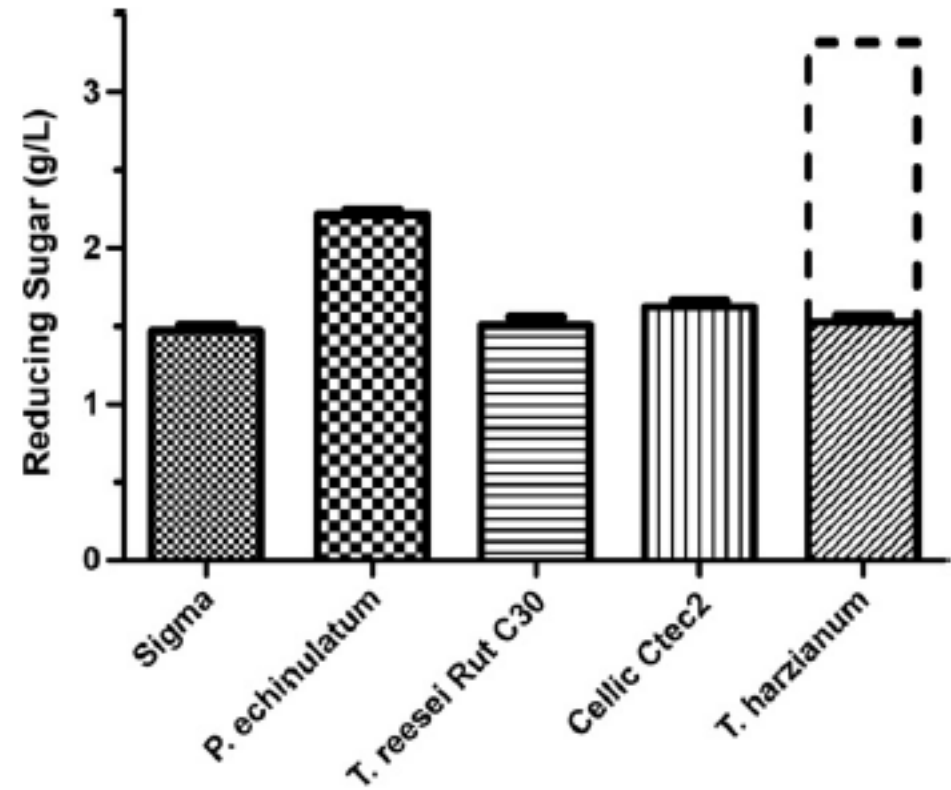
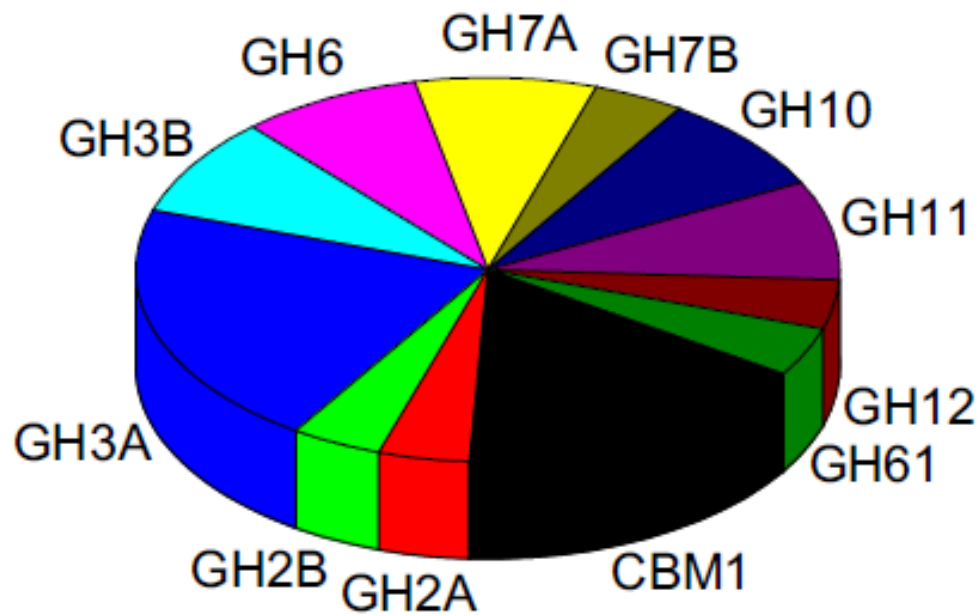
Understanding the cellulolytic system of *Trichoderma harzianum* P49P11 and enhancing saccharification of pretreated sugarcane bagasse by supplementation with pectinase and α -L-arabinofuranosidase

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*



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- 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



Contents lists available at [SciVerse ScienceDirect](http://SciVerse.ScienceDirect.com)

Biochimica et Biophysica Acta

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



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/CNPEN, Campinas, SP, Brazil

^b Departamento de Física, IBILCE, Universidade Estadual Paulista - UNESP, São José do Rio Preto, SP, Brazil

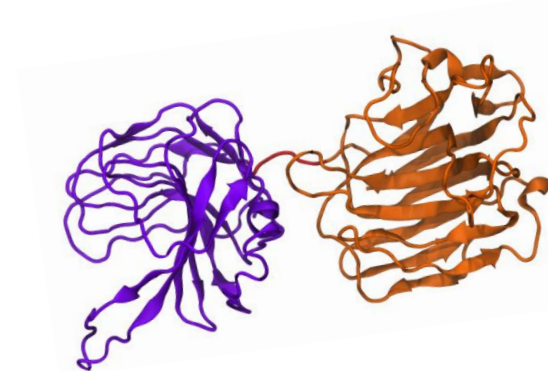
^c Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas, Campinas, SP, Brazil

^d Departamento de Física e Biofísica, Instituto de Biociências, UNESP, Botucatu, São Paulo, Brazil

^e Laboratório Nacional de Biociências – LNBio/CNPEN, Campinas, SP, Brazil

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.



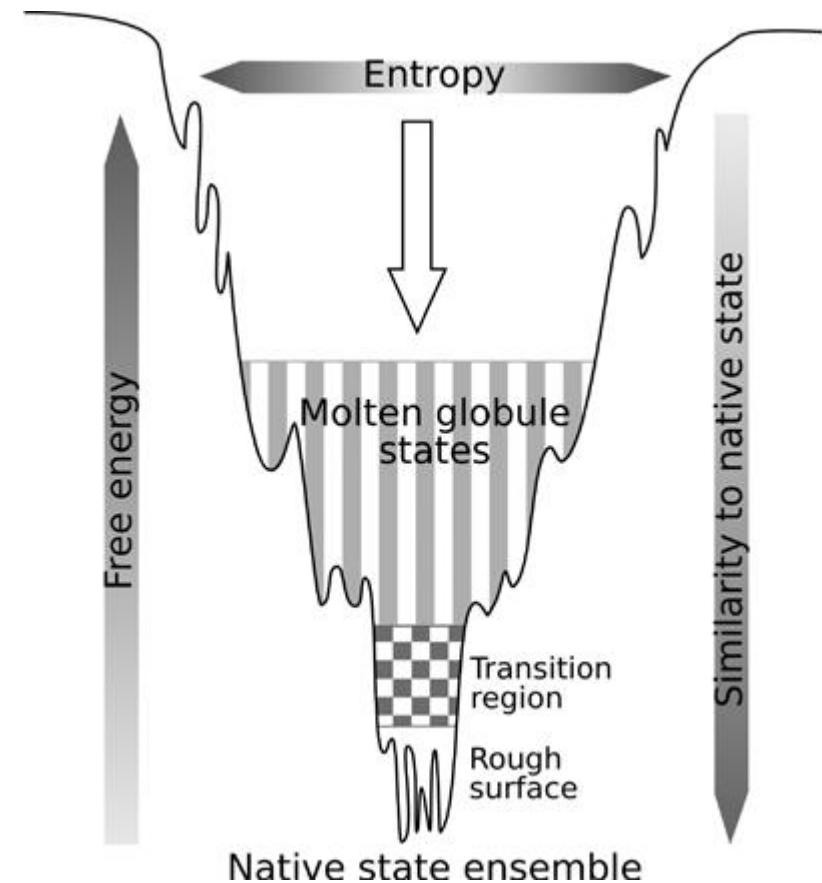
Chimeras: Multidomain Proteins

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

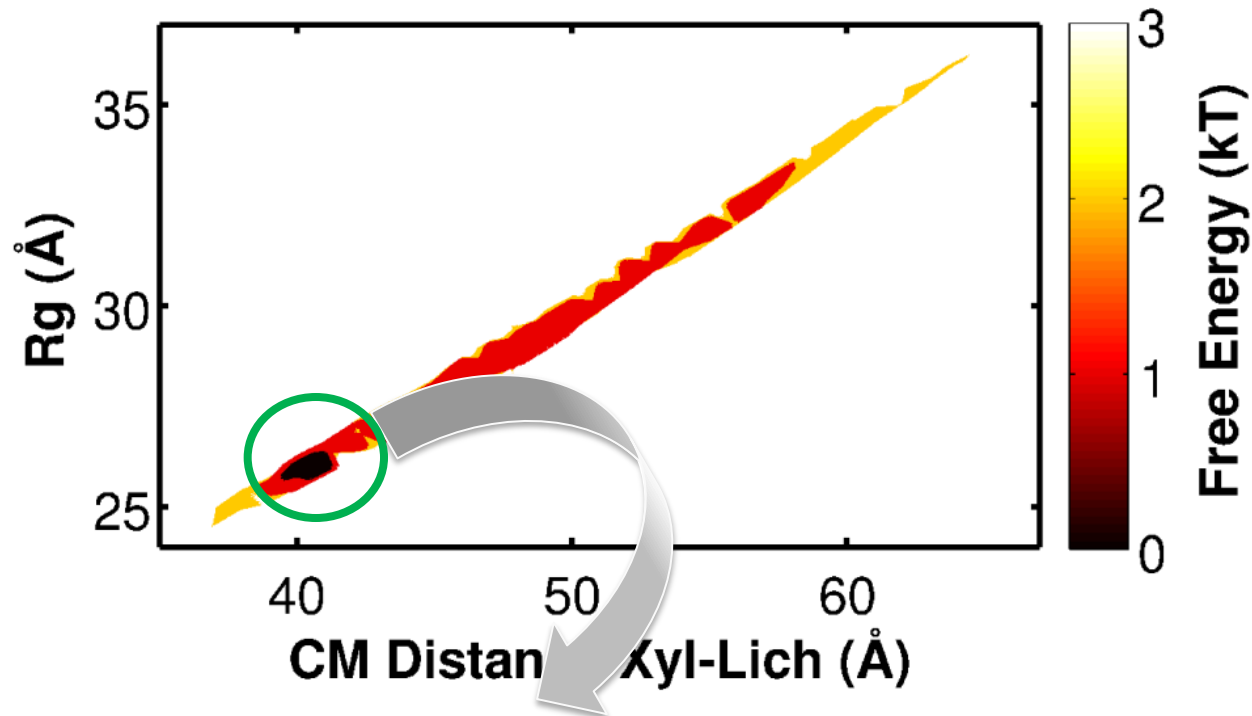


Building Chimeras: Molecular Dynamics

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



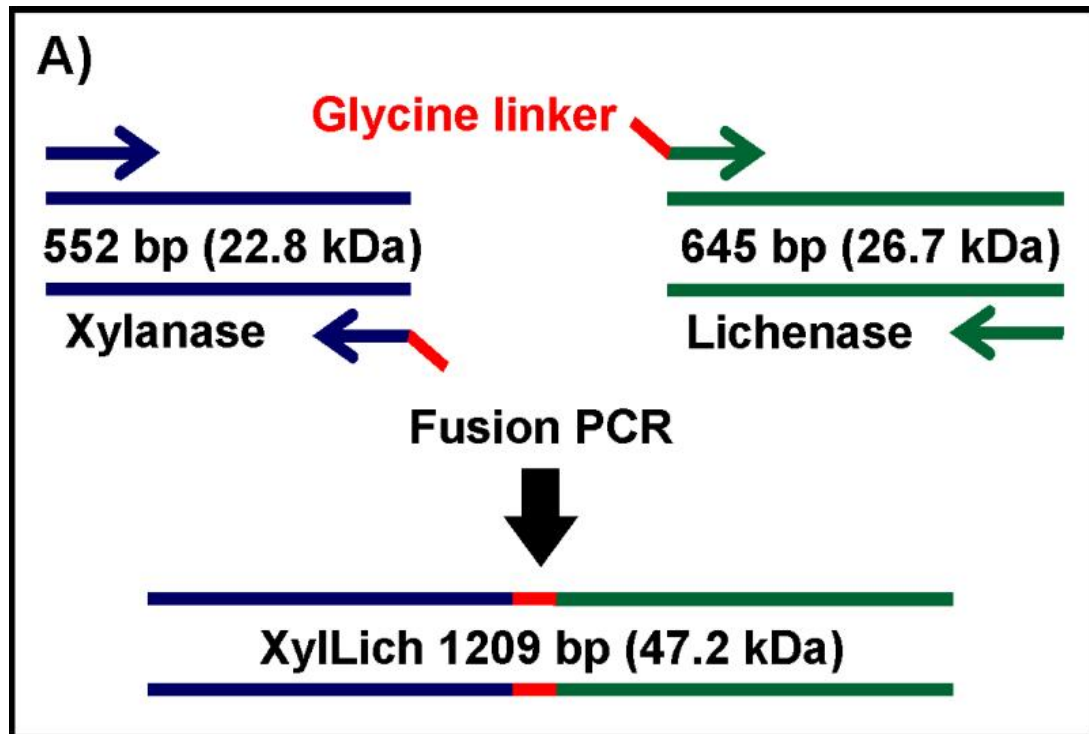
Structure Based Models (SB)



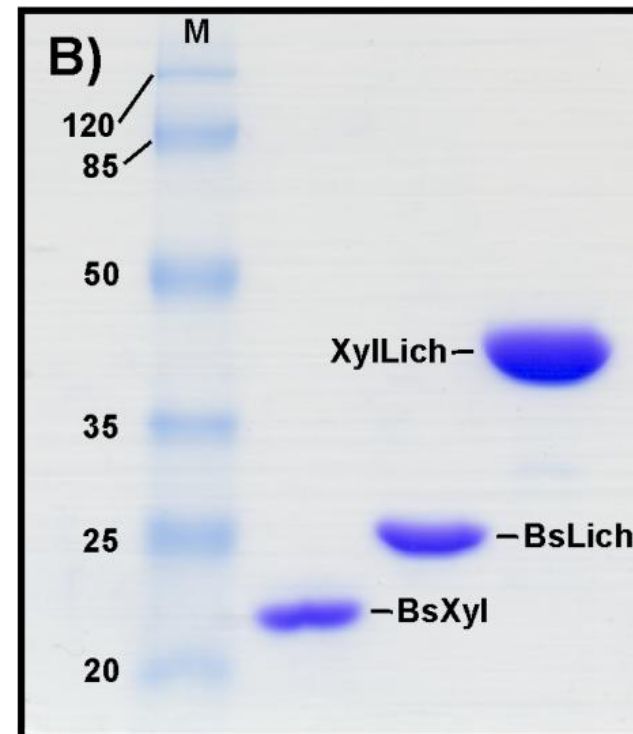
The unique Free Energy basin suggests a group of structures candidates: simulations are mainly driven by the entropy of the system

Building Chimera

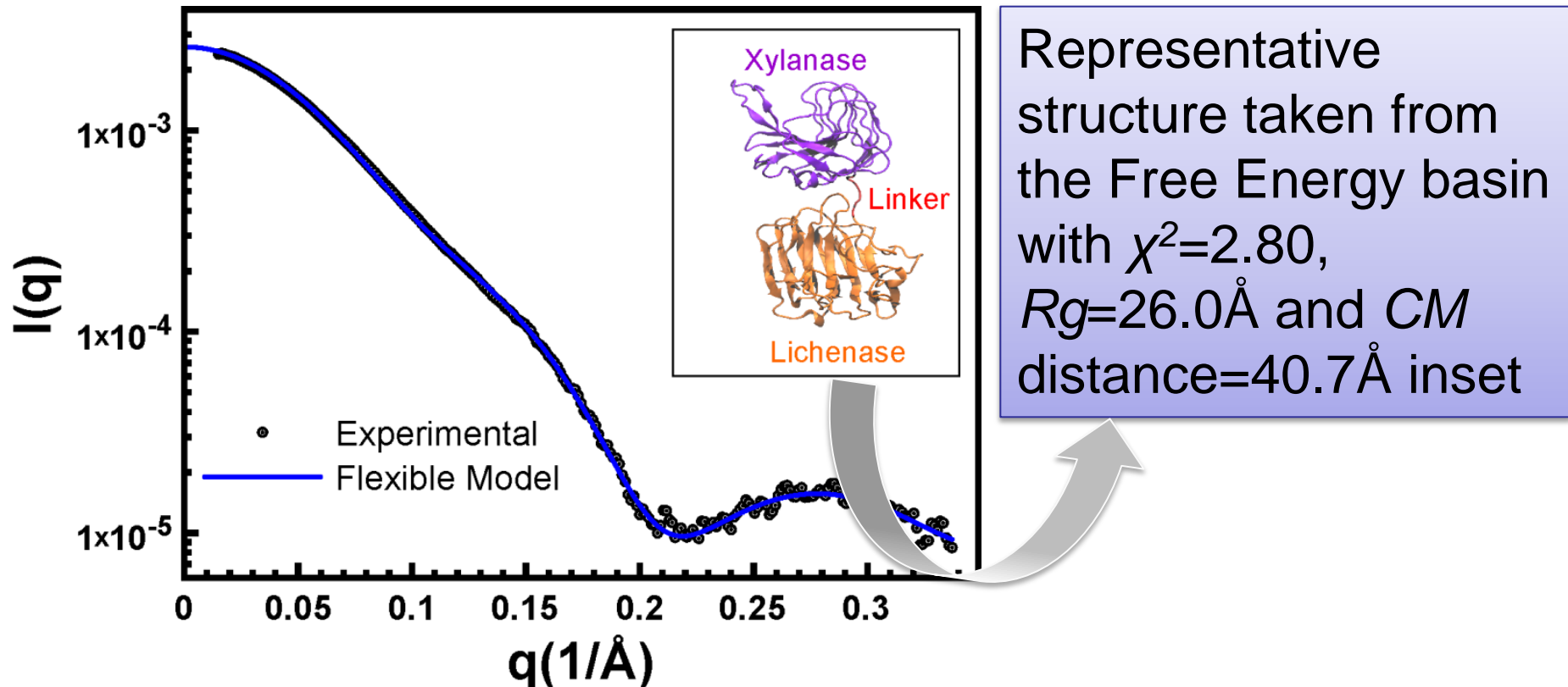
Overlap PCR



SDS-PAGE



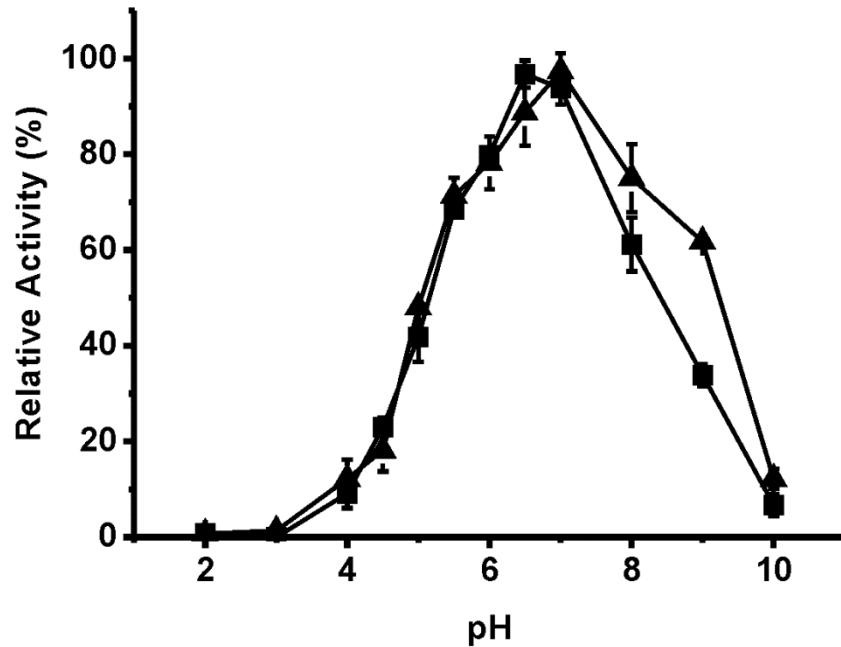
SAXS experimental and theoretical curves



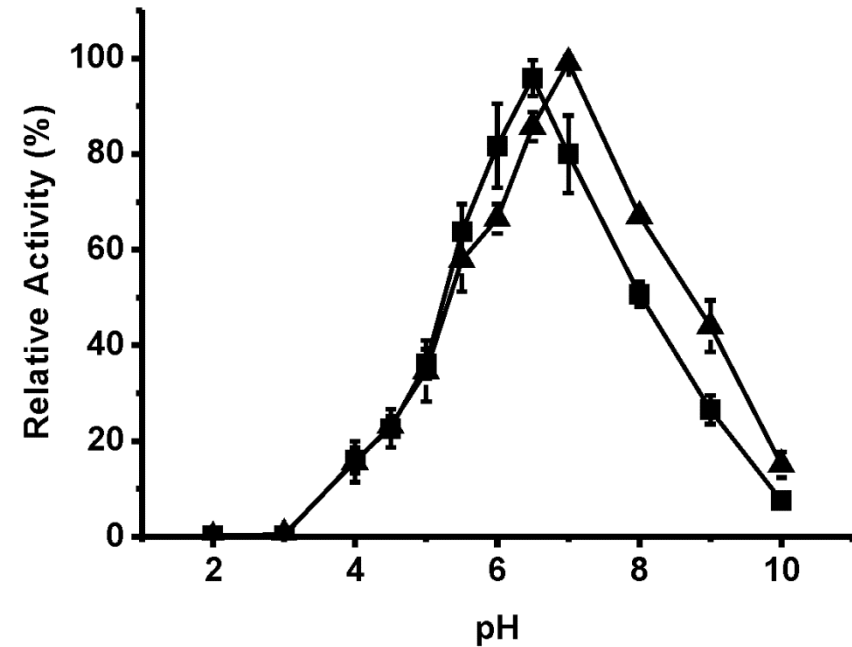
Theoretical scattering curve was generated in CRY SOL
and the representation in VMD

Optimal pH

Xylan



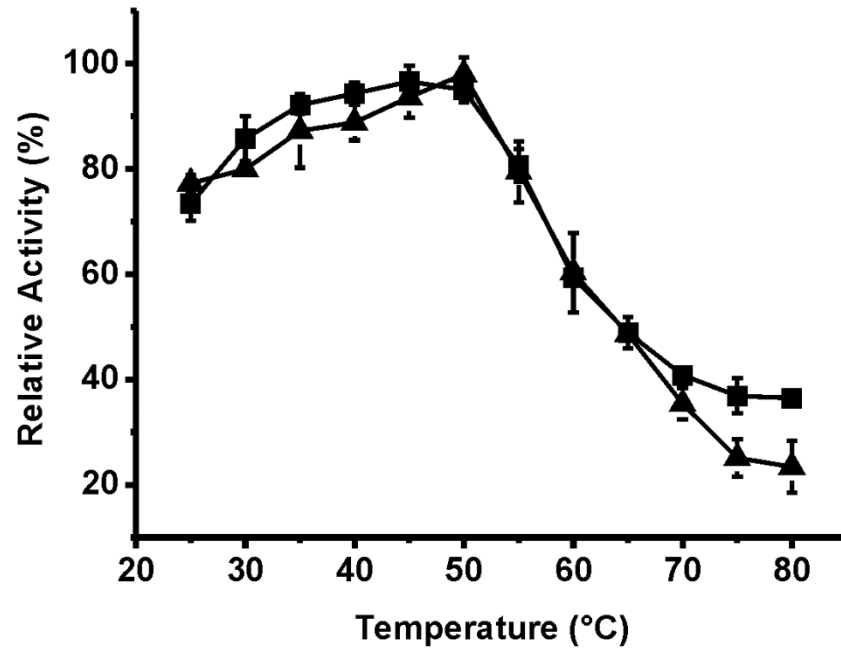
Lichenan



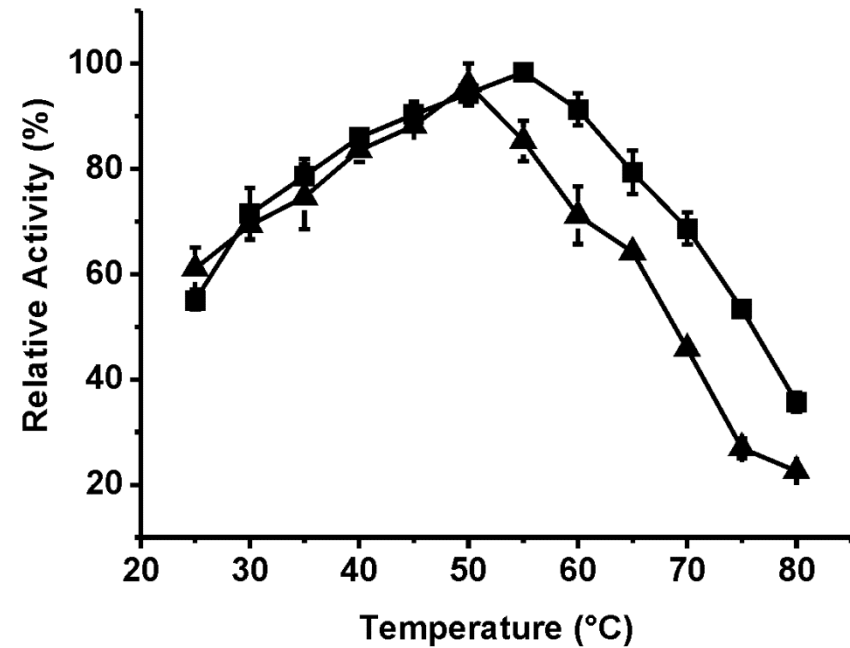
- ▲ Parental enzyme
- Chimera

Optimal Temperature

Xylan



Lichenan



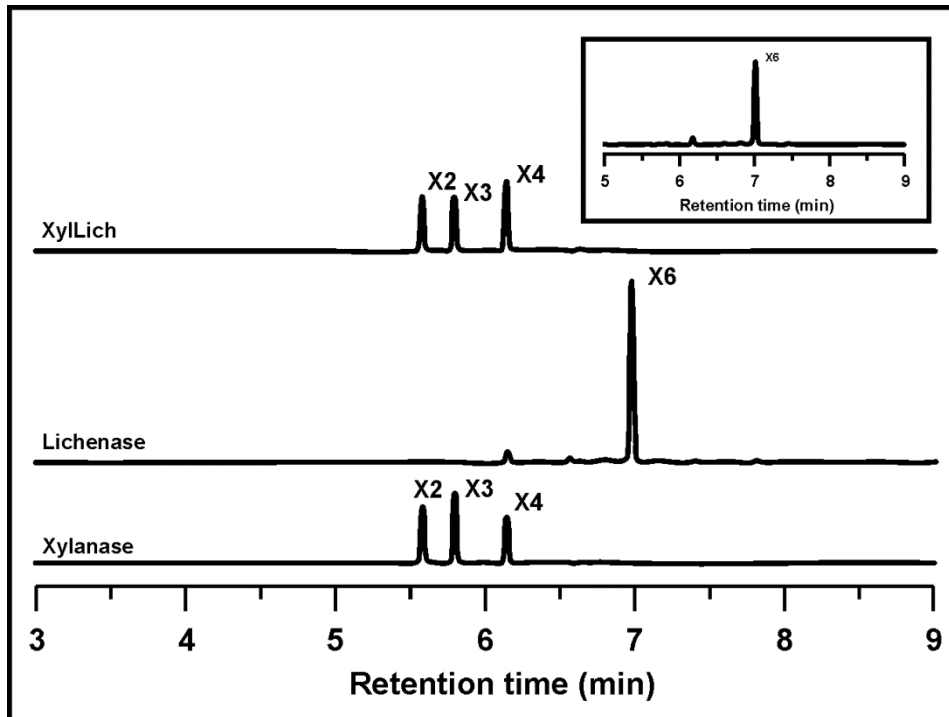
- ▲ Parental enzyme
- Chimera

Substrate Specificity

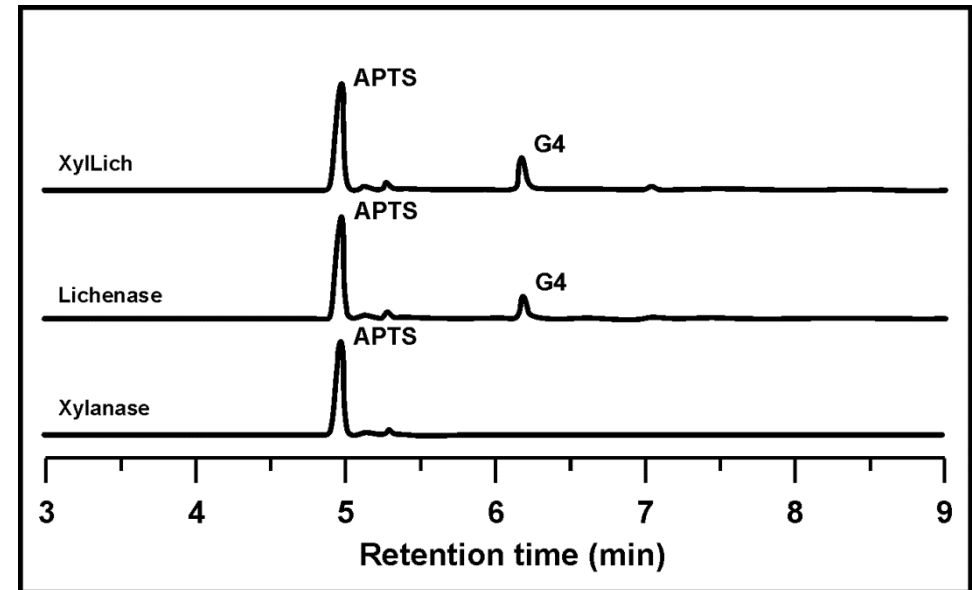
Substrate	Specific activity (U/nmol)		
	Xylanase	Lichenase	XylLich
Birchwood Xylan	3.73 ± 0.29	ND	2.71 ± 0.13
Beechwood Xylan	3.17 ± 0.07	ND	2.87 ± 0.08
Rye Arabinoxylan	3.73 ± 0.14	ND	3.03 ± 0.15
Wheat Arabinoxylan	1.36 ± 0.12	ND	0.88 ± 0.07
Oat Spelt Xylan	3.28 ± 0.27	ND	2.15 ± 0.06
Lichenan	ND	3.65 ± 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

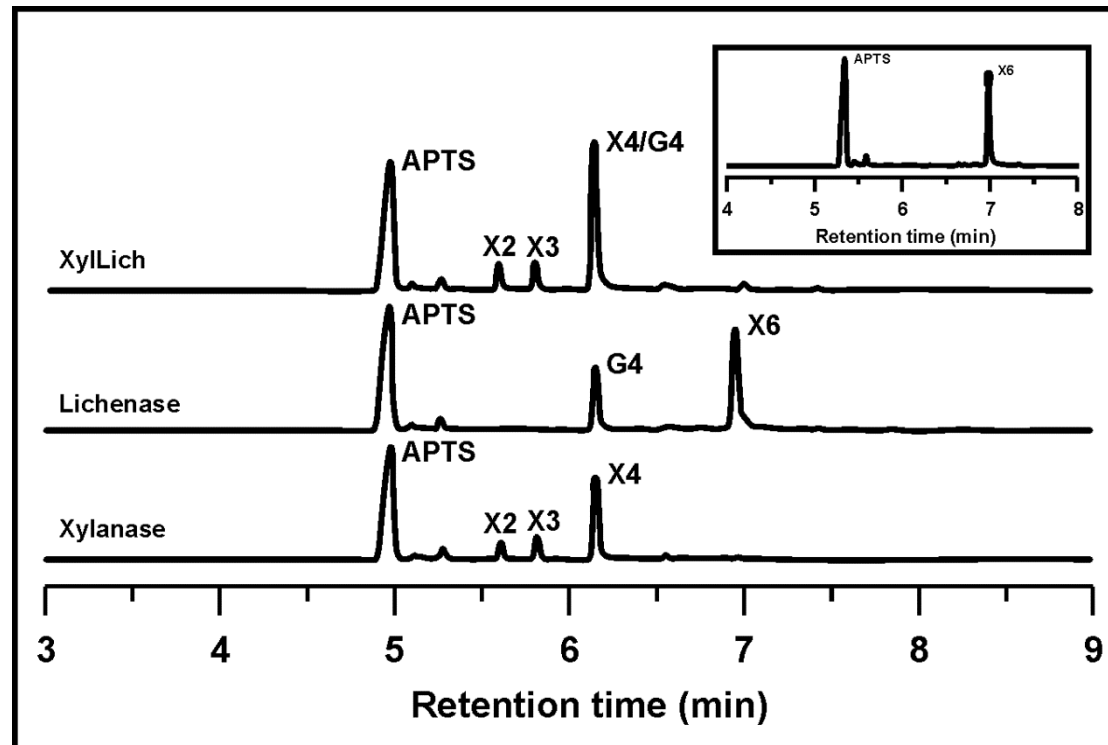


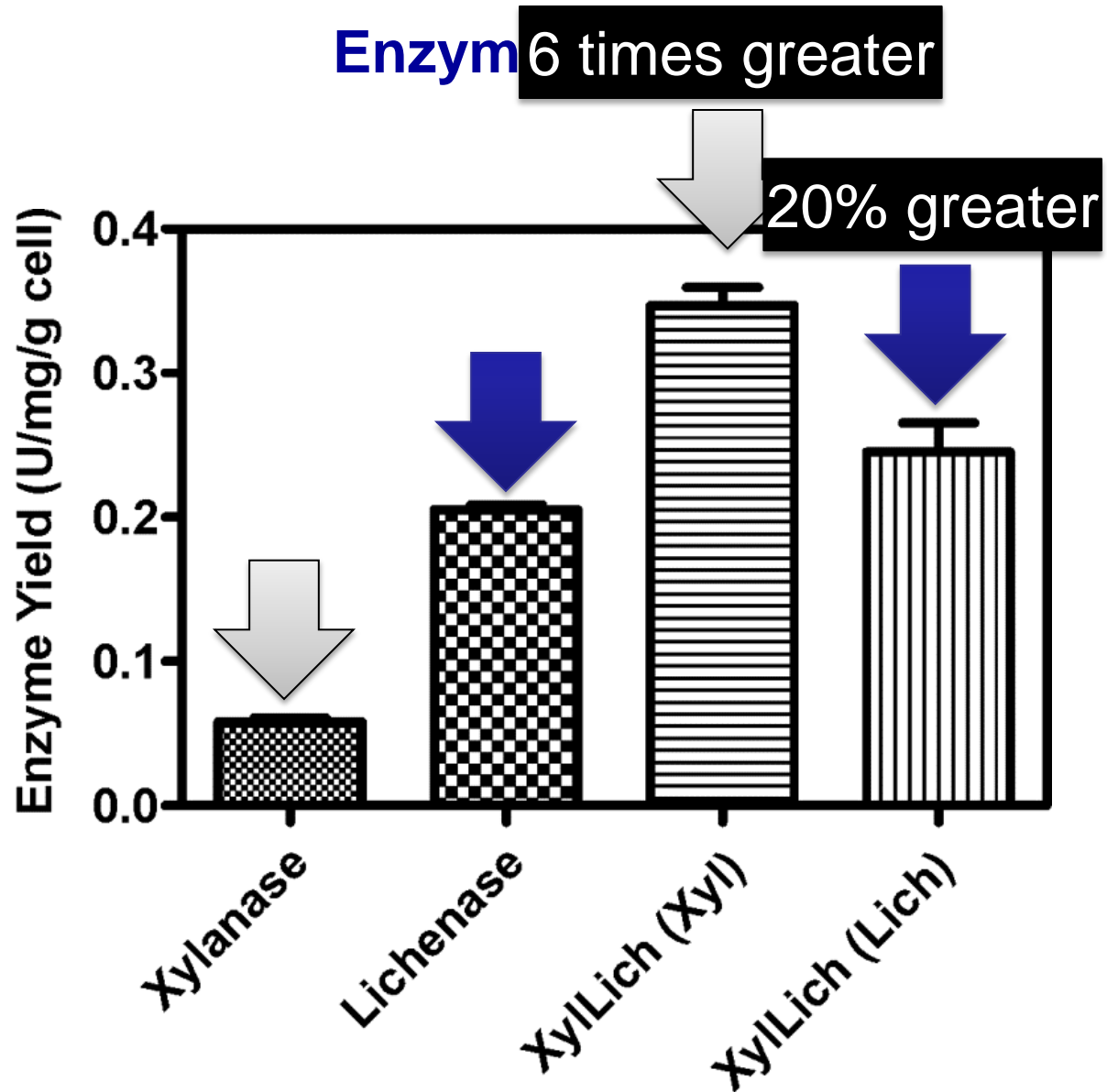
Lichenan



Capillary Electrophoresis

Xylohexaose + Lichenan





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.





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