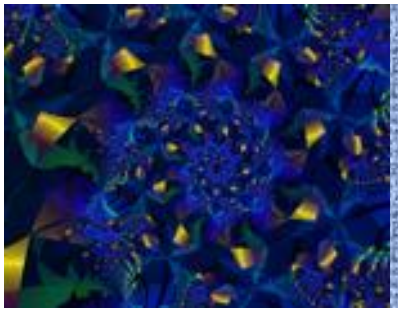


Theoretical Chemistry at UNICAMP
Molecular Dynamics / Skaf Group

Hydrolytic Enzymes for Biomass Transformation into Second Generation Biofuels: Structure & Dynamics Studies

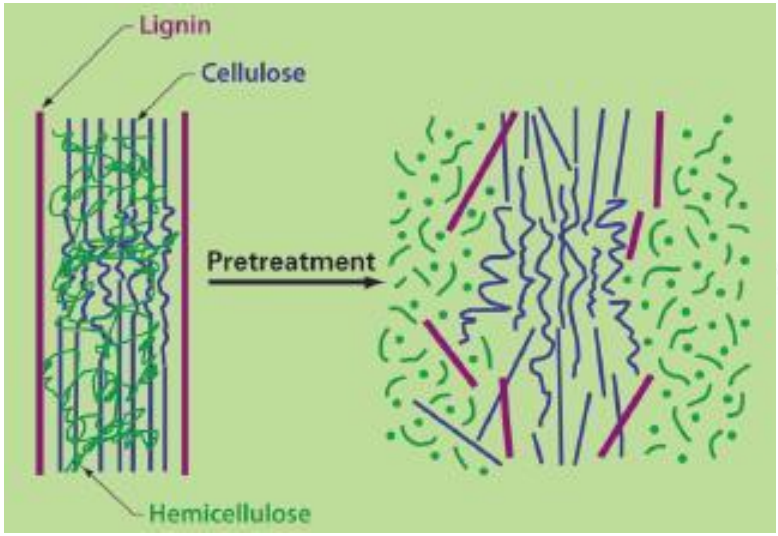
Munir S. Skaf
skaf@iqm.unicamp.br

Institute of Chemistry / State University of Campinas

1st Brazilian BioEnergy Science and Technology Conference
Campos do Jordão - August - 14 to 18, 2011

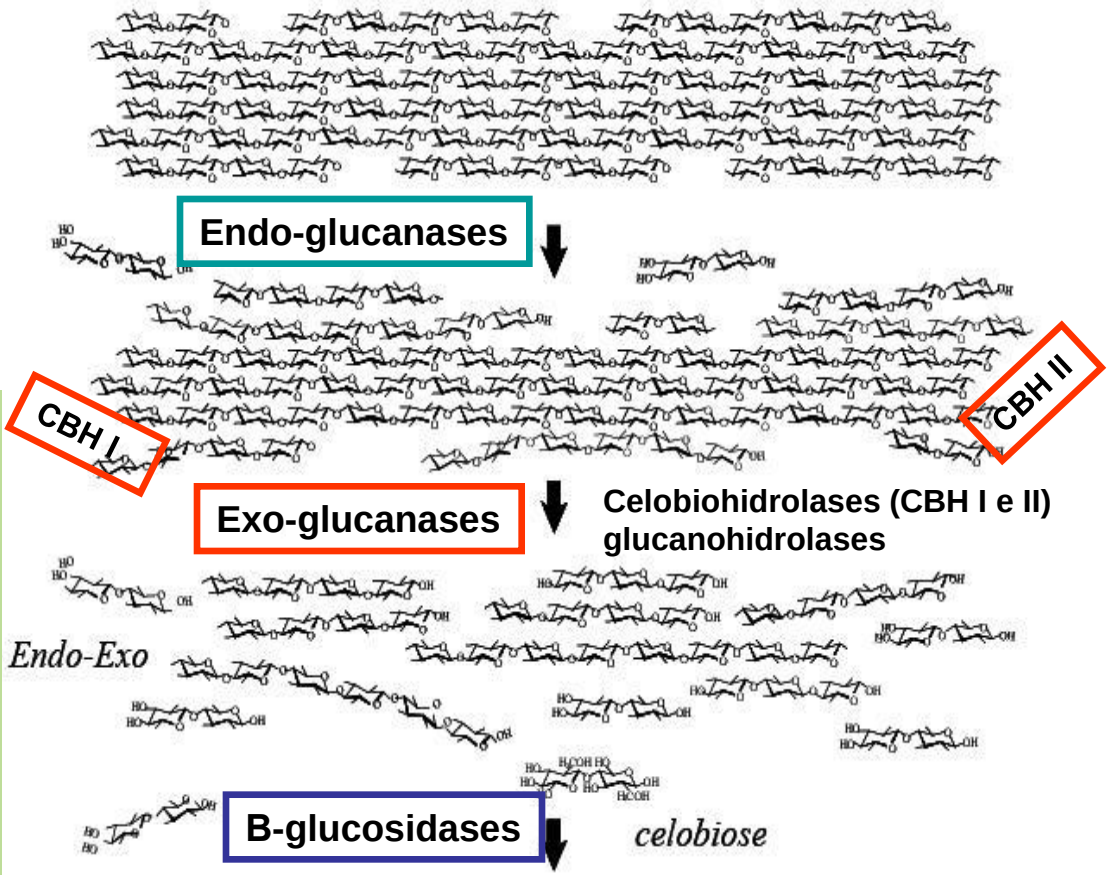
Sugar-cane bagasse ...

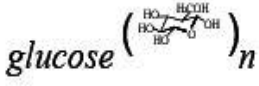
Cell wall



Lignin: 20-30%
Hemicellulose: 20-25%
Cellulose: 35-40%

Enzymatic complex





Glucose to ethanol...

- **Recalcitrance: efficiency of enzymatic hydrolysis is very low without biomass pre-treatment --- challenges & opportunities for chemistry**

- **Reaction product inhibit enzymatic activity of EG**

- **Enzymes are expensive and slow [recovery (?); kinetics; thermochemically stable]**

Structural Studies of Glucosyl Hydrolases

Watanabe, L. et al. & Polikarpov, I. (2010) ***J. Struct. Biol.*** 169: 226-242

Kim, K.-Y., Nascimento, A.S. et al. (2008) ***BBRC*** 371:600-605

Zamorano, L.S.et al. (2008) ***Biochimie*** 90: 1737-1749.

Nascimento, A.S. et al., & Polikarpov, I. (2008) ***J. Mol. Biol.*** 382:763-778

Rojas, A.L. et al. & Polikarpov, I. (2005) ***Biochemistry*** 44, 15578-15584.

Golubev, A.M. et al., and Polikarpov, I. (2004) ***J. Mol. Biol.*** 339, 413-422.

Nagem, R.A.P. et al. & Polikarpov, I. (2004) ***J. Mol. Biol.*** 344, 471-480.

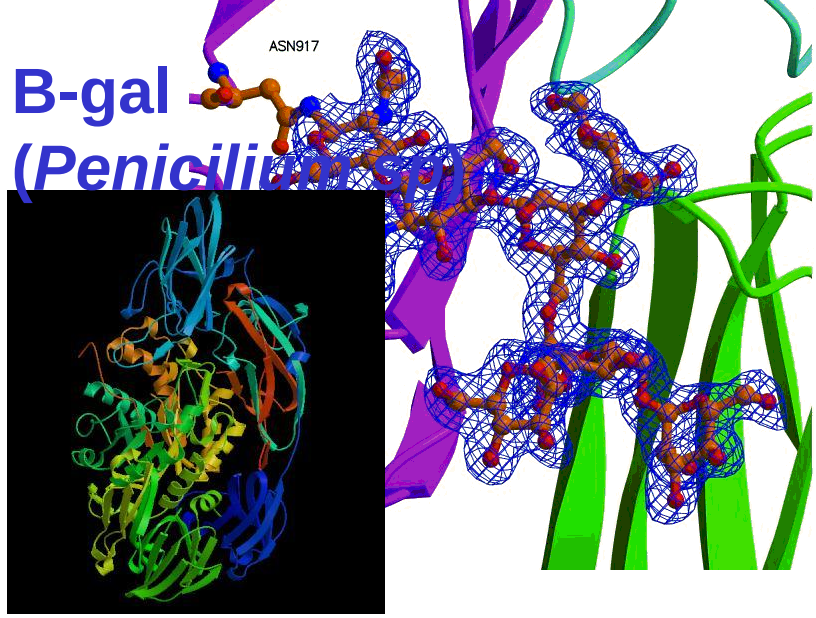
Rojas, A.L., Nagem, R.A.P. *et al.*, & Polikarpov, I. (2004) ***J. Mol. Biol.*** **343**, 1281-1292.

Aparicio, I. et al. & Polikarpov, I. (2002) ***Biochemistry*** 41, 9370-9375.

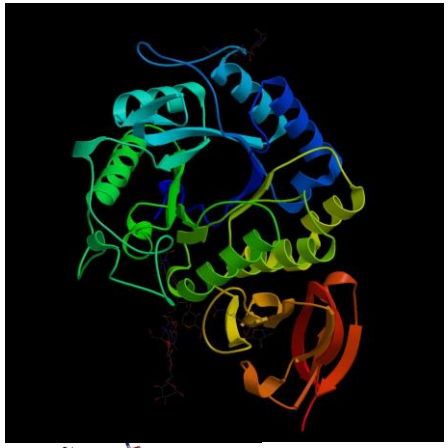
Igor Polikarpov IPolikarpov@if.sc.usp.br

UNIVERSIDADE DE SÃO PAULO
Instituto de Física de São Carlos

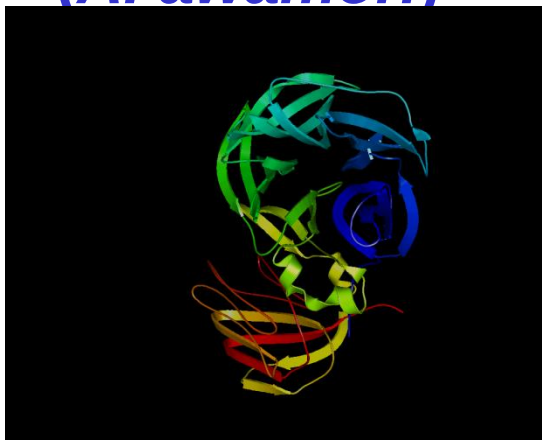
B-gal (*Penicillium sp.*)



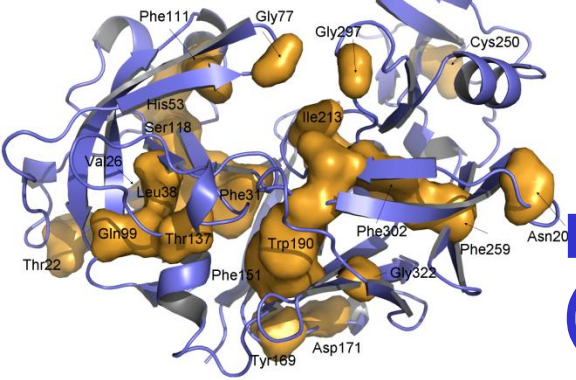
A-gal (*T. reesei*)

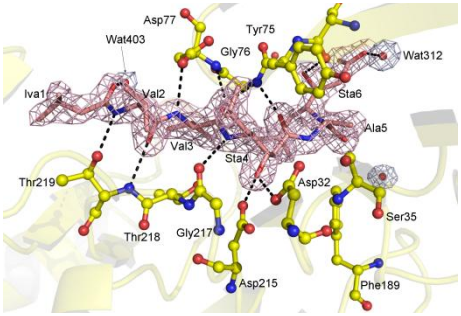


Exo- Inulinase (*A. awamori*)

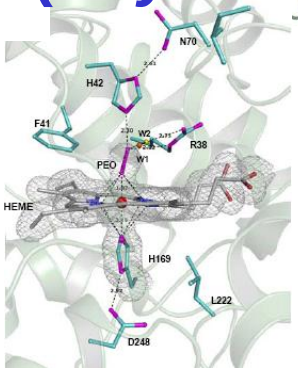


TrAsP (*T. reesei*)

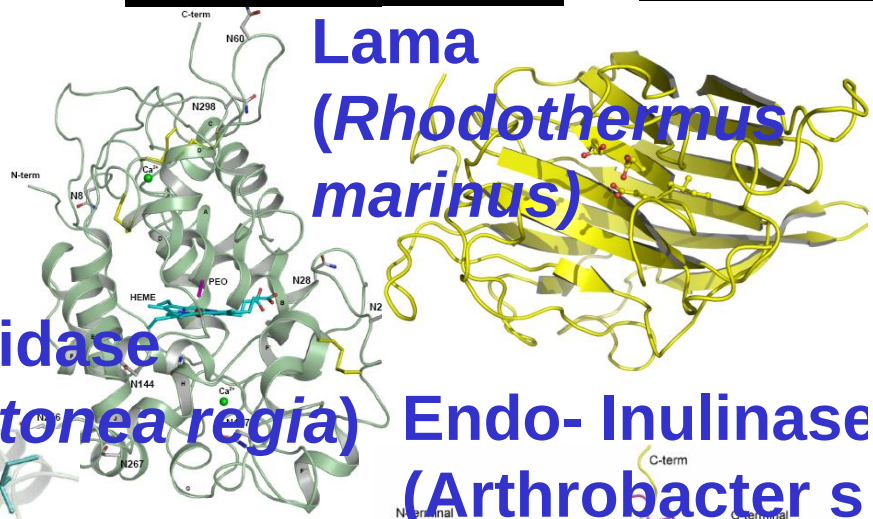




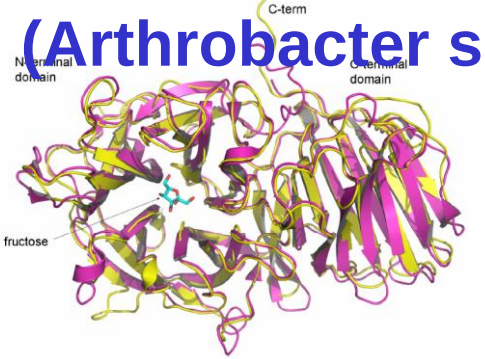
Peroxidase (*Roystonea regia*)



Lama (*Rhodothermus marinus*)



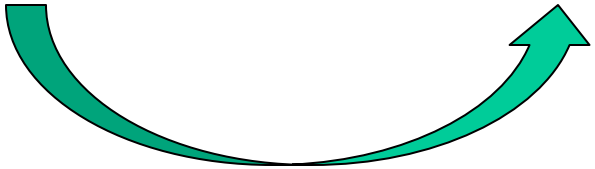
Endo- Inulinase (Arthrobacter s



B-man (*T. reese*

The Holy Grail of Structural Molecular Biology

Protein structure  **Biological function**

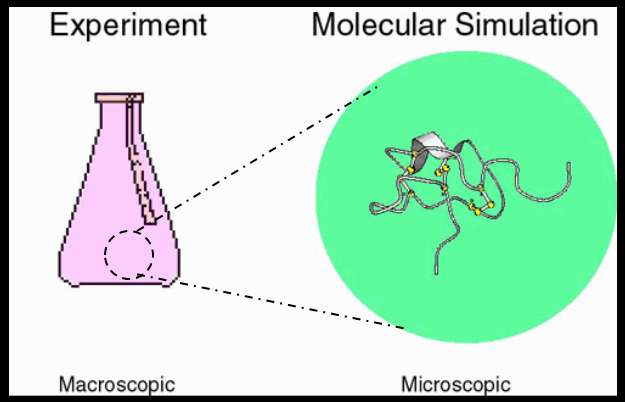


Dynamics

Understanding how molecules move requires knowledge of interactions at the atomic level

MD simulation is a powerful tool for studying atomic motions

MD Simulations in a Nutshell



Initial Configuration: $\mathbf{R}^N$, $\mathbf{V}^N$ ← PDB

↓

Save atomic positons

↓

Potential $V(\mathbf{r}_i)$ at i

↓

Newton's Second Law

$$\mathbf{a}_i(t) = -\frac{1}{m_i}\frac{\partial V}{\partial \mathbf{r}_i}$$

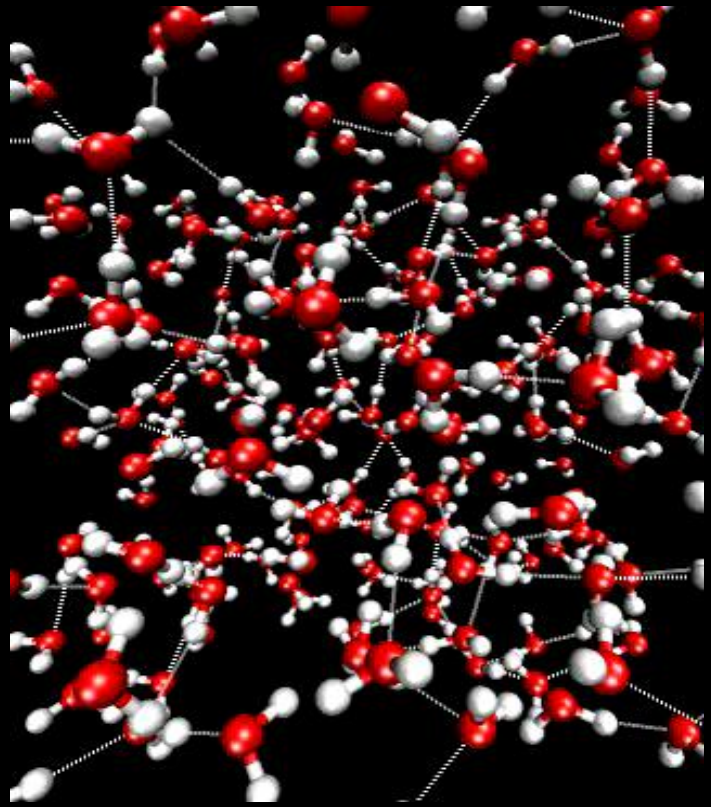
↓

Integrations of the Equations of Motion

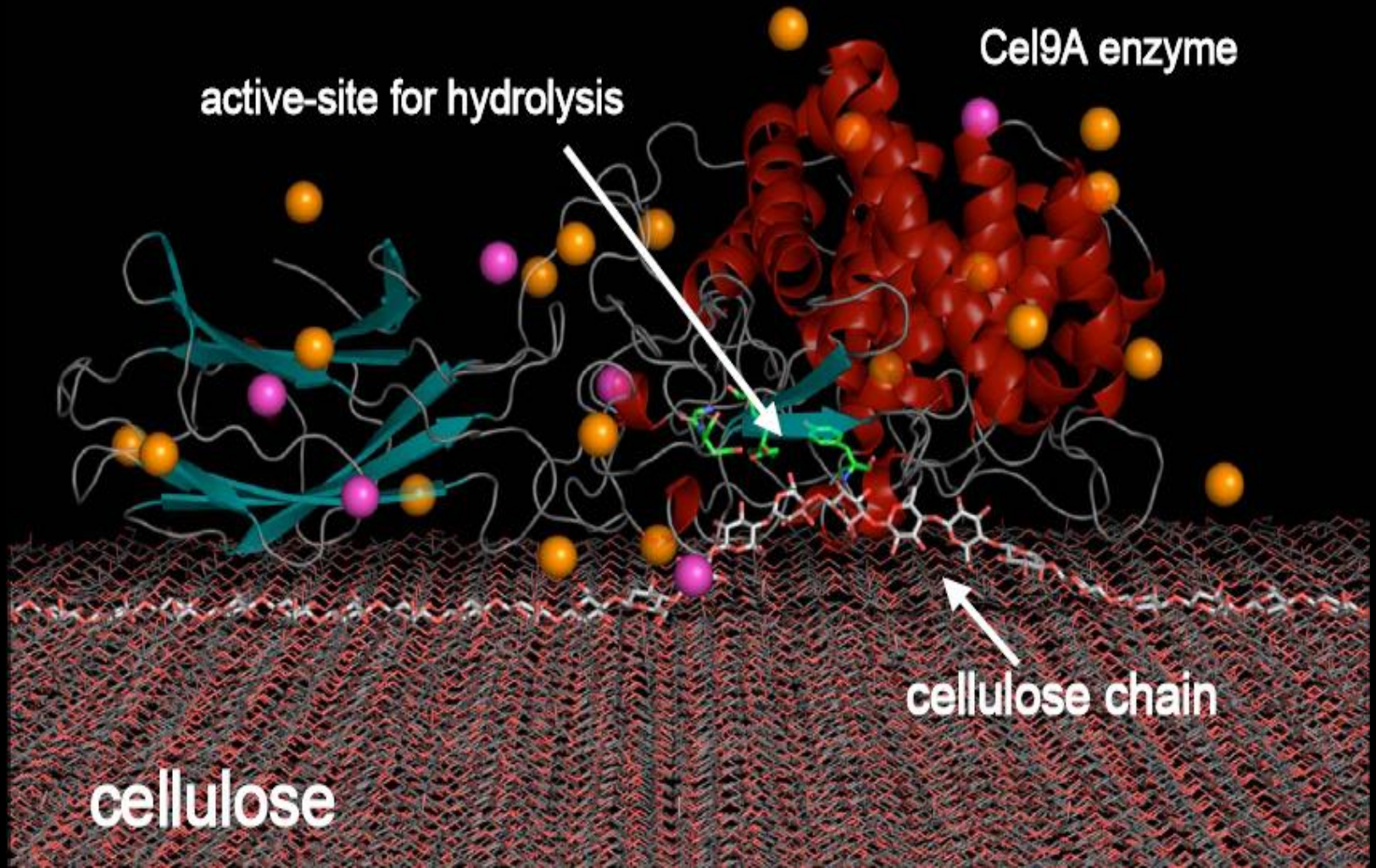
$$\mathbf{r}_i(t+\Delta t) = \mathbf{r}_i(t) + \mathbf{v}_i(t)\Delta t + \frac{\mathbf{a}_i(t)\Delta t^2}{2}$$

$$\mathbf{v}_i(t+\Delta t) = \mathbf{v}_i(t) + \mathbf{a}_i(t)\Delta t$$

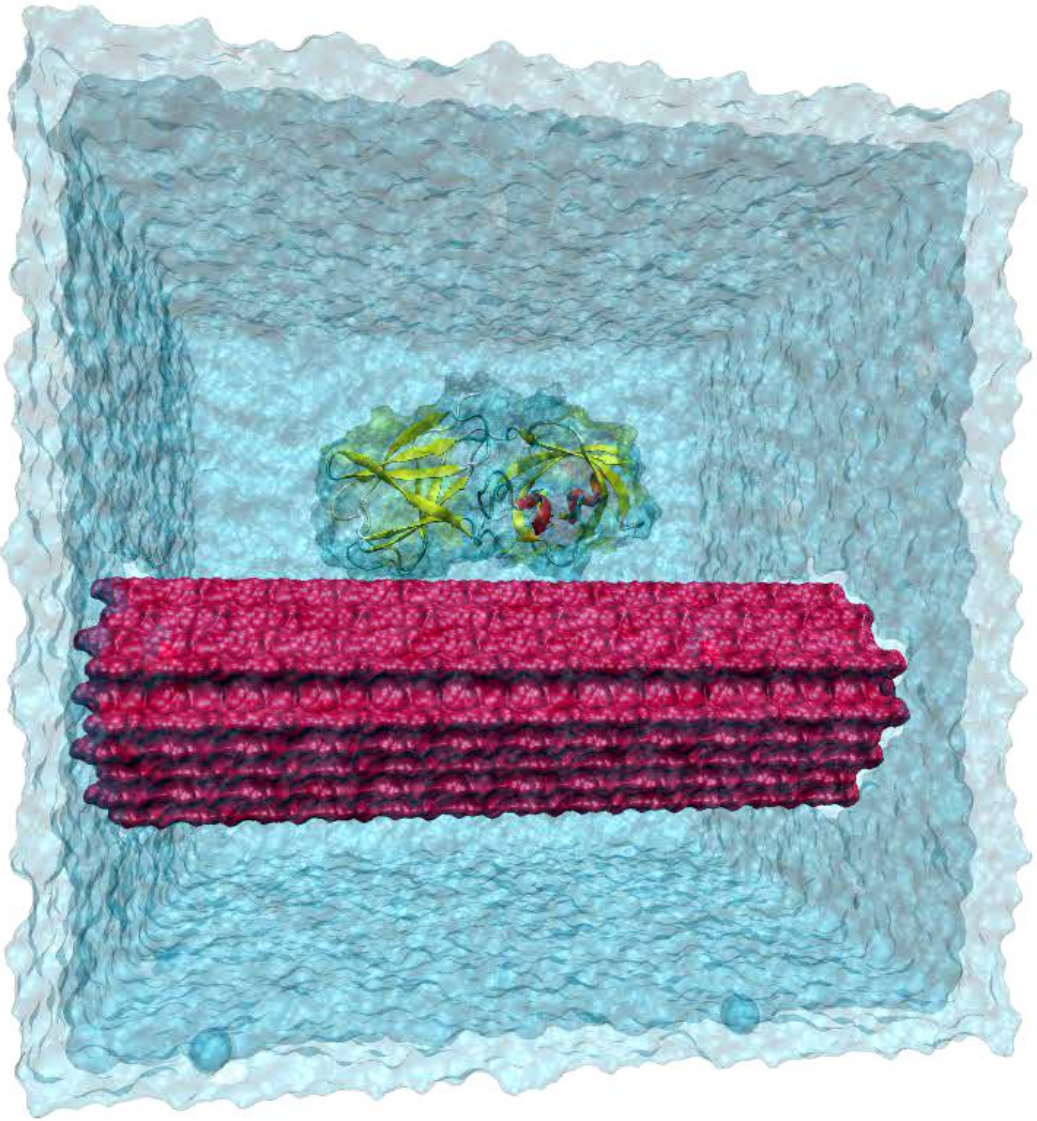
iterate



Atomistic MD studies of GH



Binding to cellulose microfribils (200,000 atoms)



Report on Two Cases

- Laminarinase from *R. marinus* (hyperthermophilic EG)

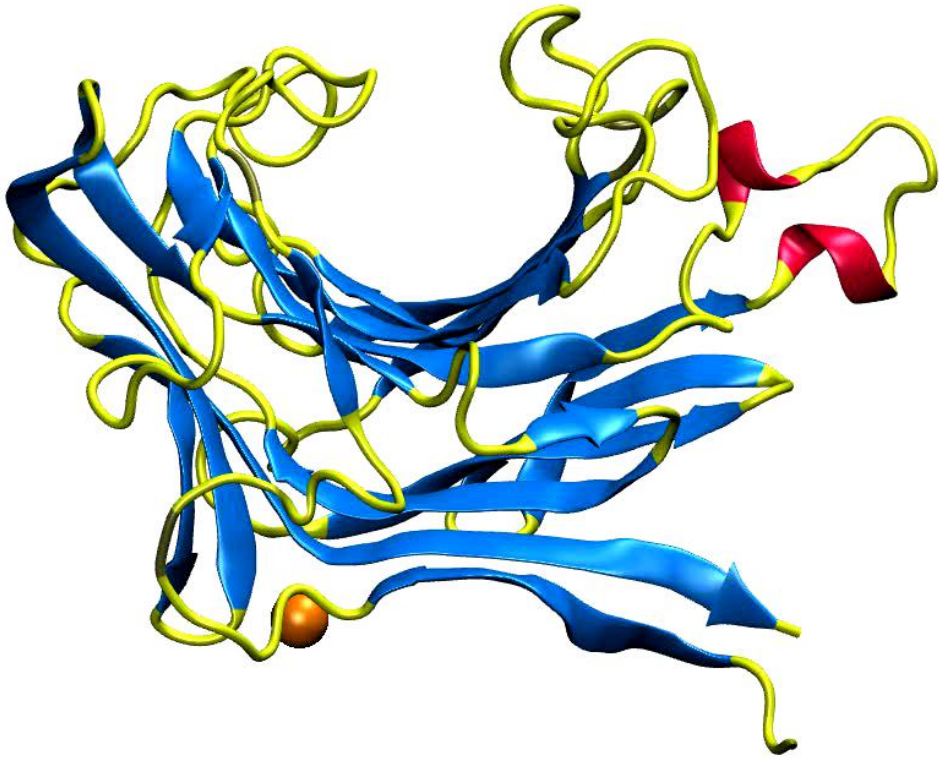
- CBH1 (CCD) from *T. harzianum*

EG3 from *T. harzianum*

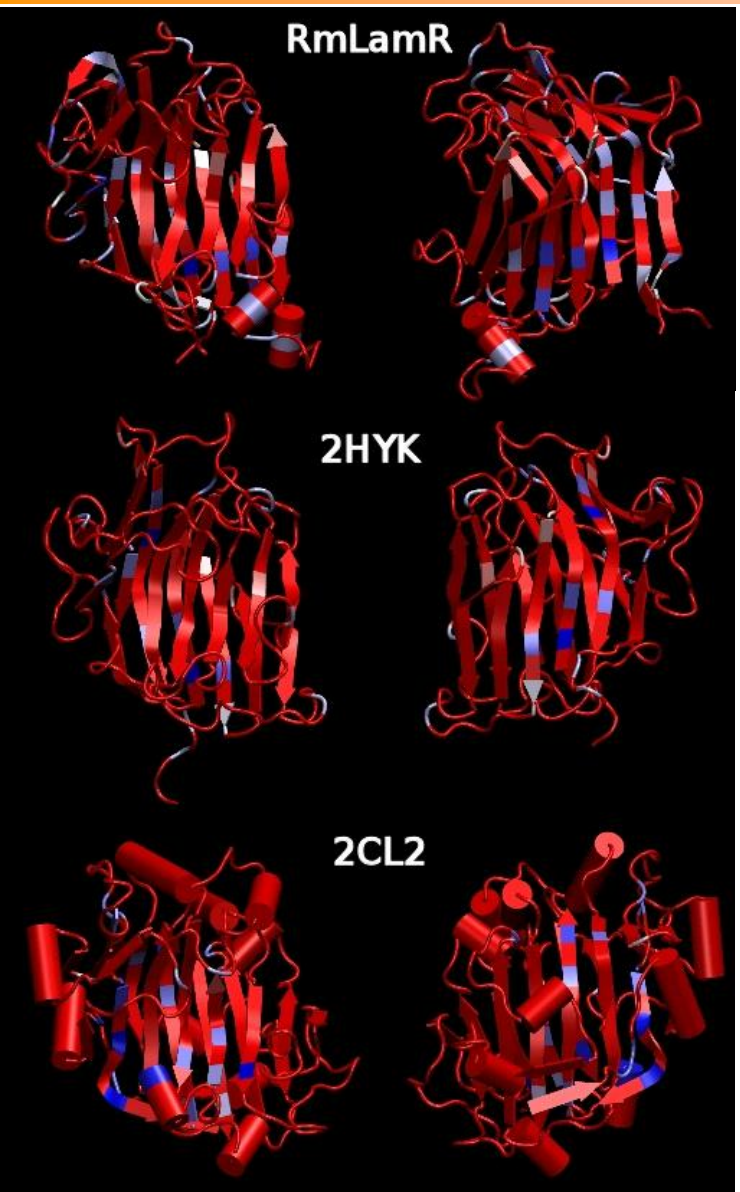
Molecular basis of the thermostability and termophilicity of laminarinases from *R. marinus* (GH16; endoglucanase)

J. Phys. Chem. B, 115, 7940 (2011).

X-ray: 1.95 A Resolution (I. Polikarpov)



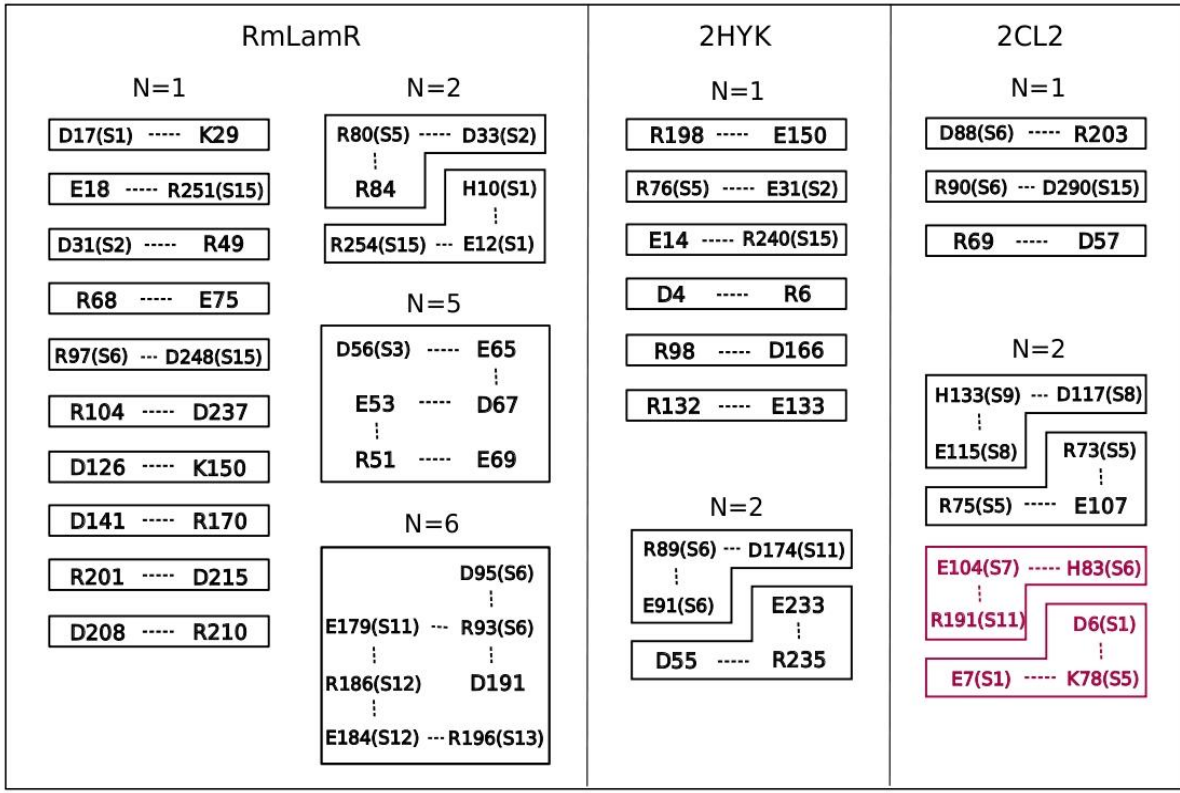
Thermostability of Rhodothermus marinus β-1,3-glucanase (laminarinase) vs alkaliphilic Nocardiopsis sp.strain F96 Lam & Phanerochaete chrysosporium Lam



Number of salt-bridges:

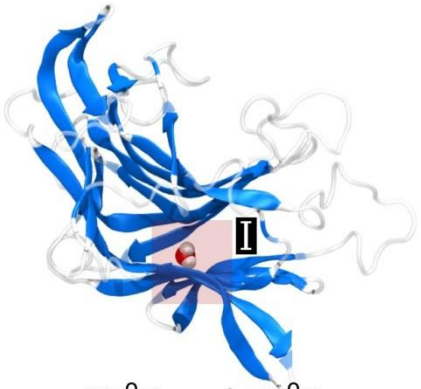
Rodothermus-Lam:	**24**
Nocardiopsis-Lam:	**9**
P. chrysosporium-Lam:	**11**

And the salt-bridges are interconnected forming clusters:



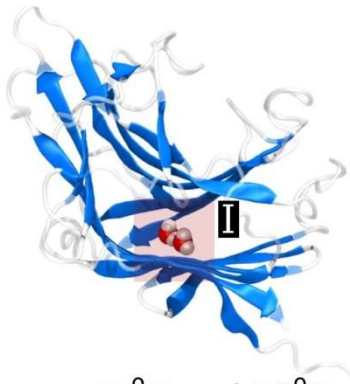
Water penetrates the hydrophobic core in mesophilic, but not in thermophilic and hyperthermophilic laminarinase

A) RmLamR



25°C and 90°C

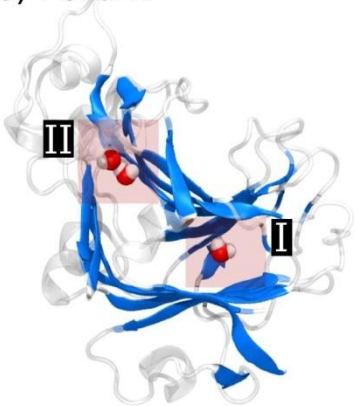
B) aNLam



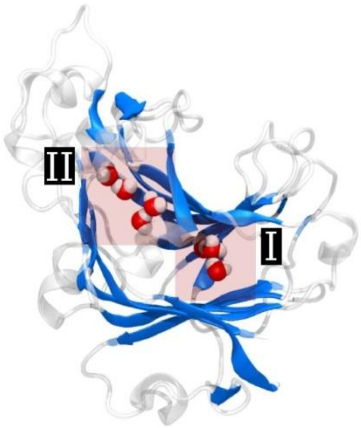
25°C and 90°C

Thermophilic and hyperthermophilic

C) PcLam

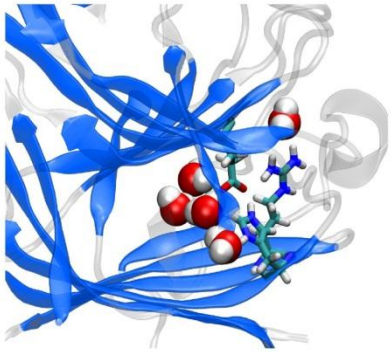


25°C



90°C

D)

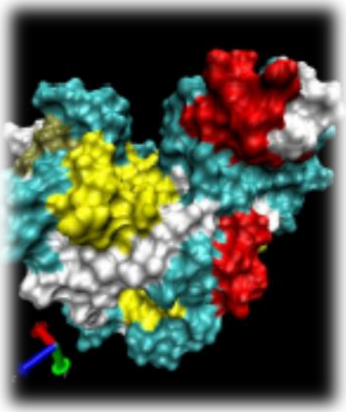


Mesophilic: destabilization at 90 °C

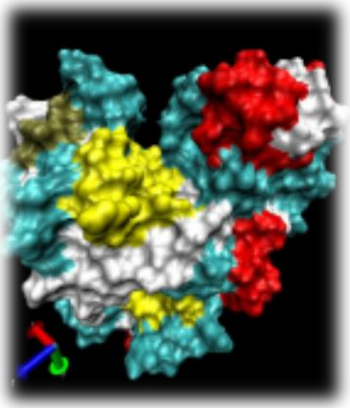
Molecular basis for thermophilicity

RmLamR: substrate binding channel is preserved

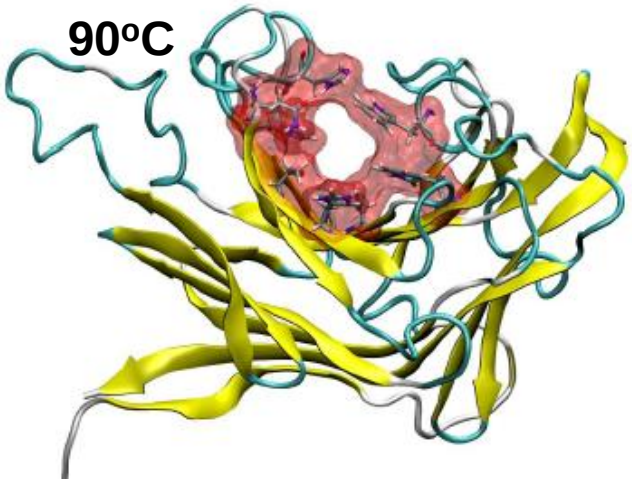
25°C



90°C

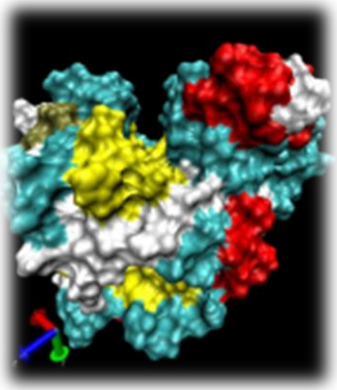


90°C

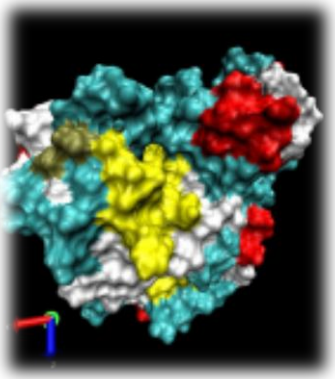


2CL2: substrate binding channel is obstructed at 90°C

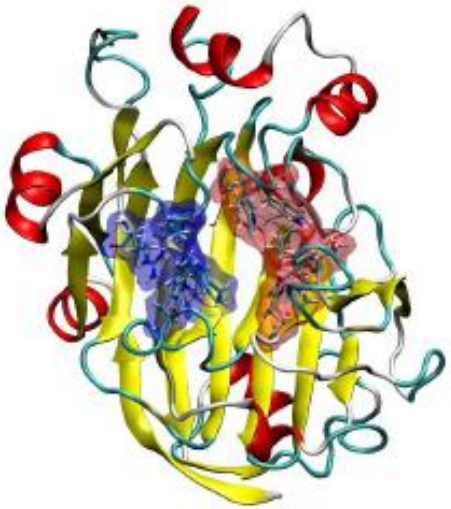
25°C



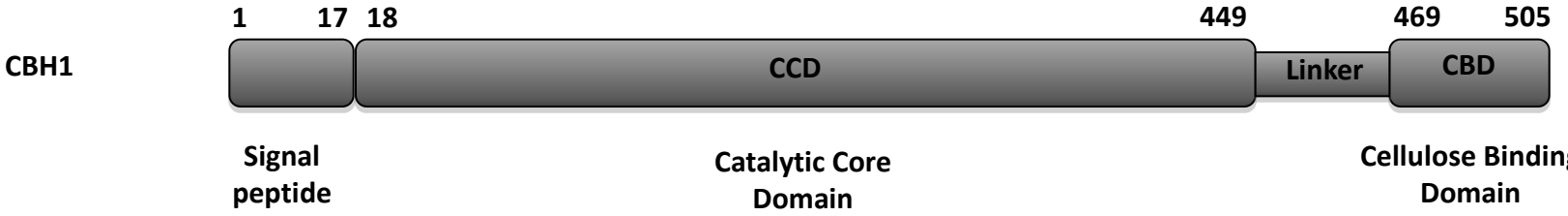
90°C



90°C

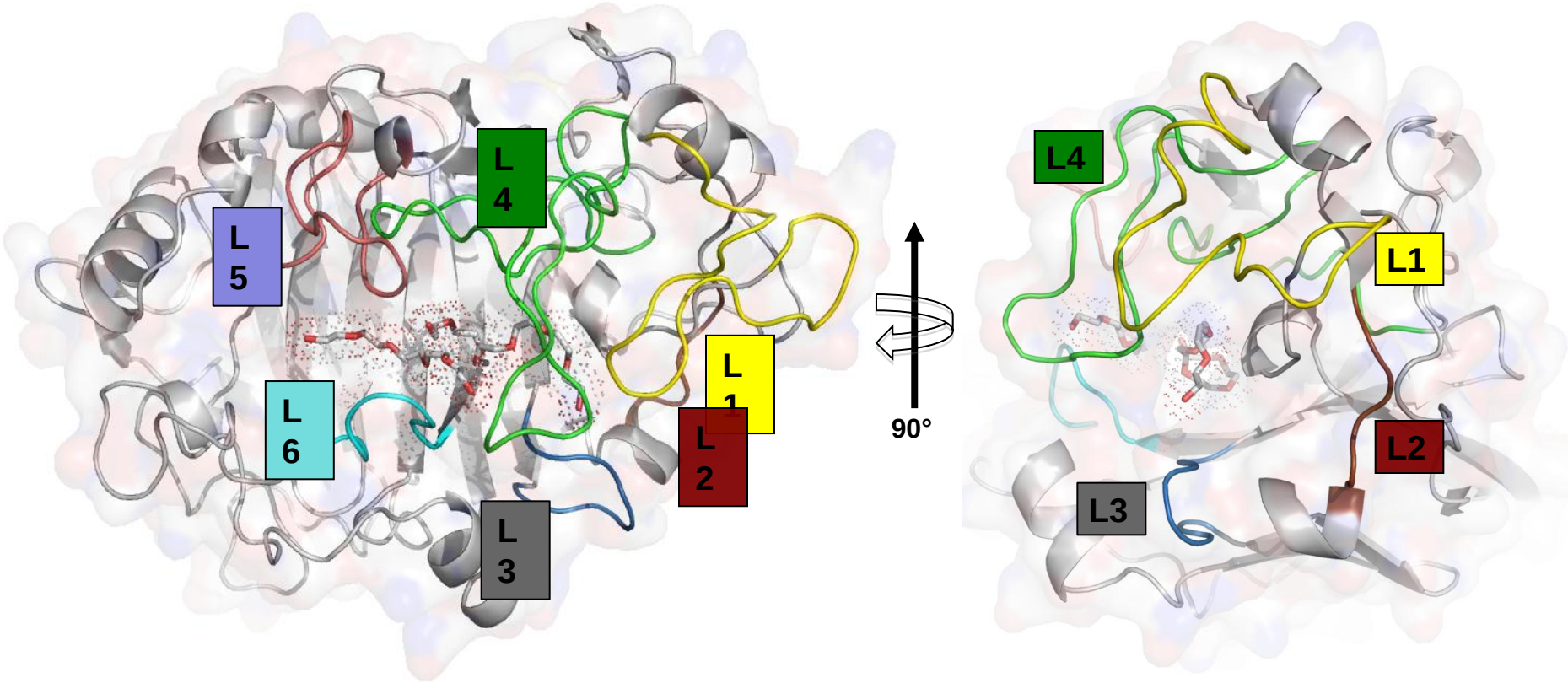


X-ray structure of the CCD of CBH1 from T. harzianum

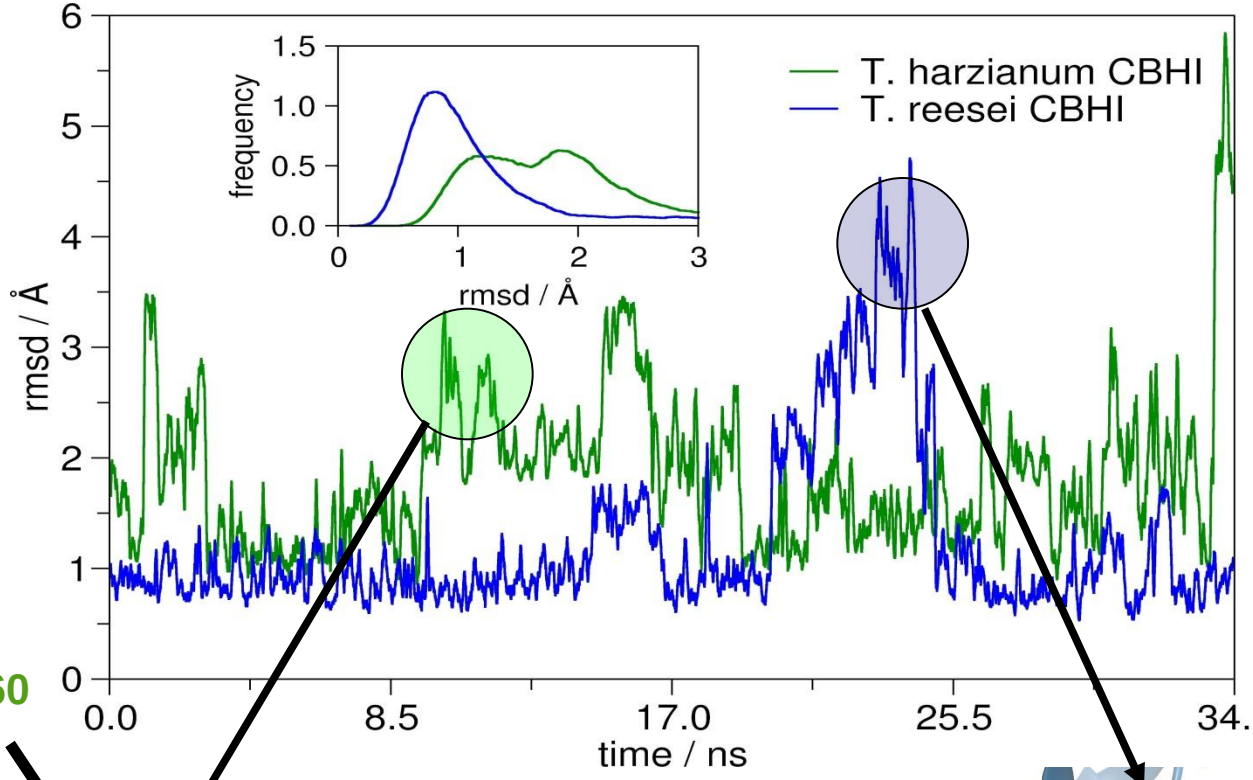


(B)

(C)



MD of CBH1: *T. reesei* vs. *T. harzianum*



T. harzianum

Improved substrate accessibility

↓

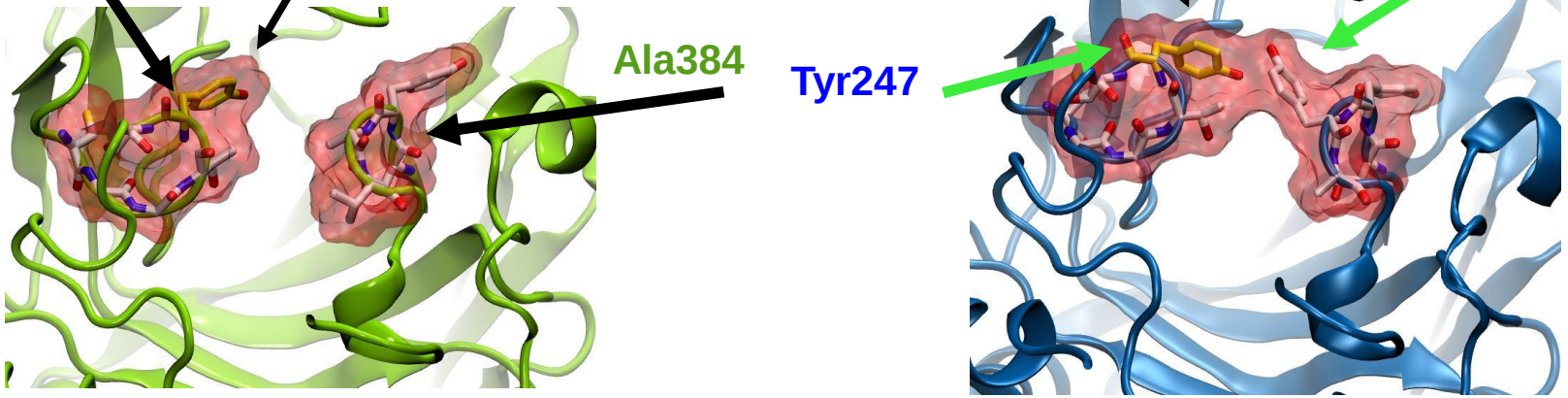
Better catalytic efficiency

Tyr260

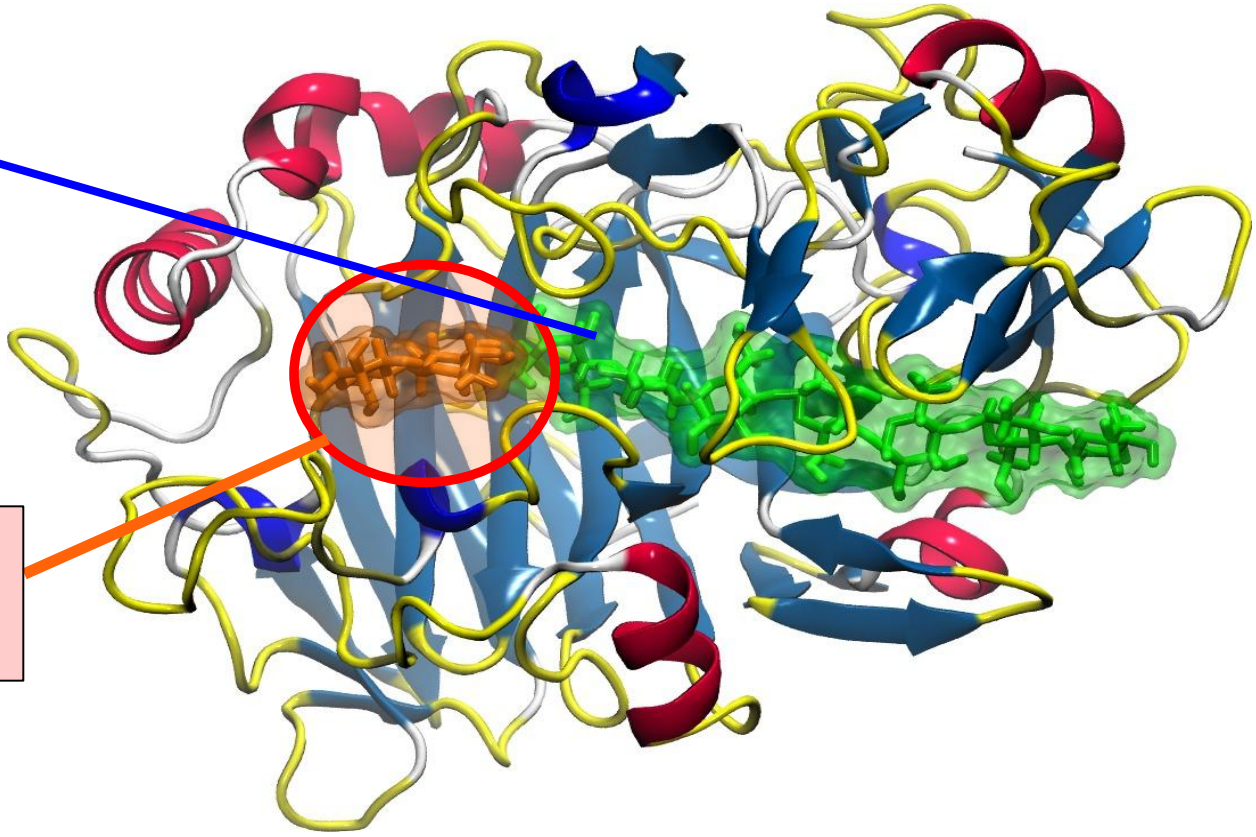
Ala384

Tyr247

Tyr371

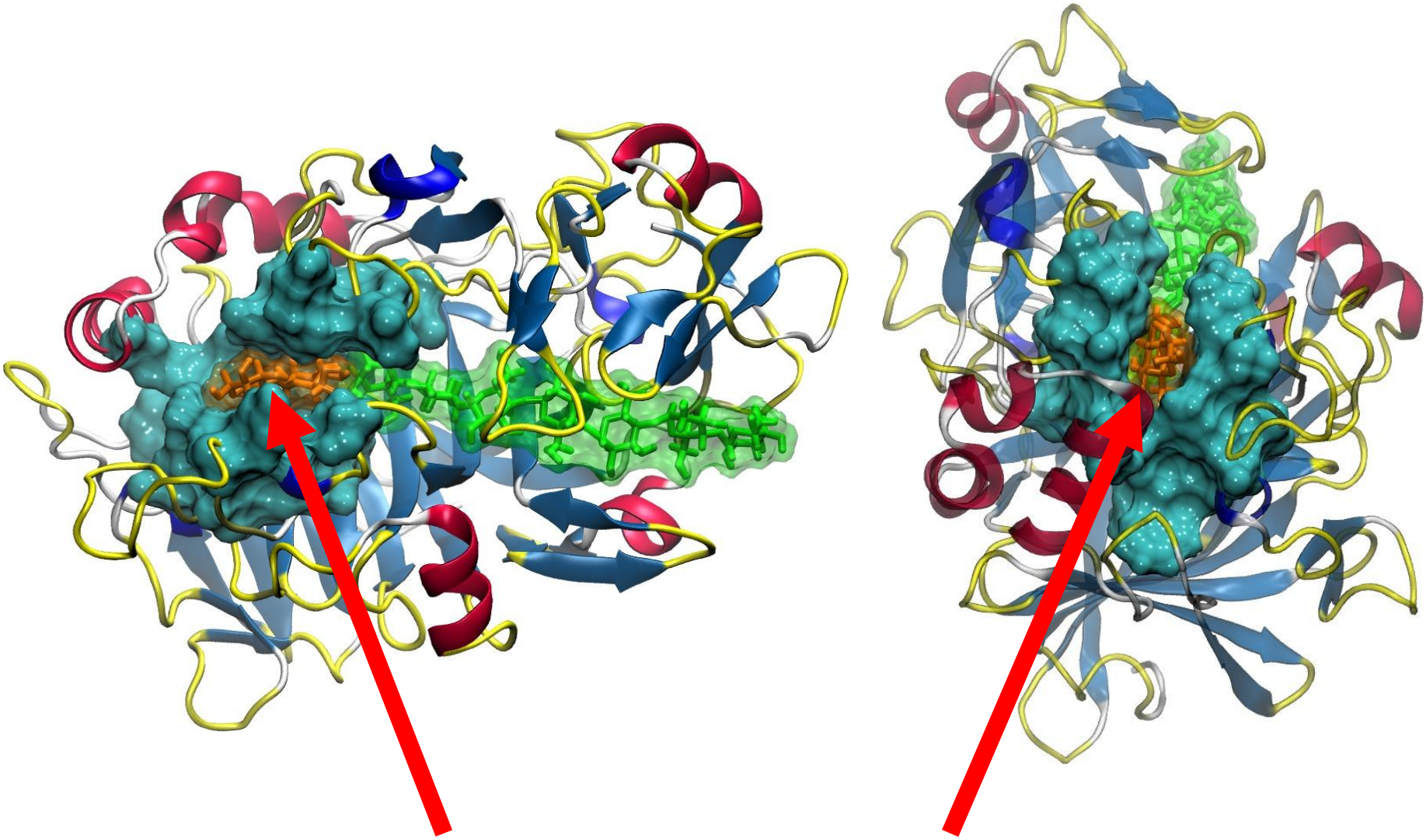


MD study of CBH1 from *T. reesei*
Product inhibition



Cellobiose is an inhibitor of CBH1

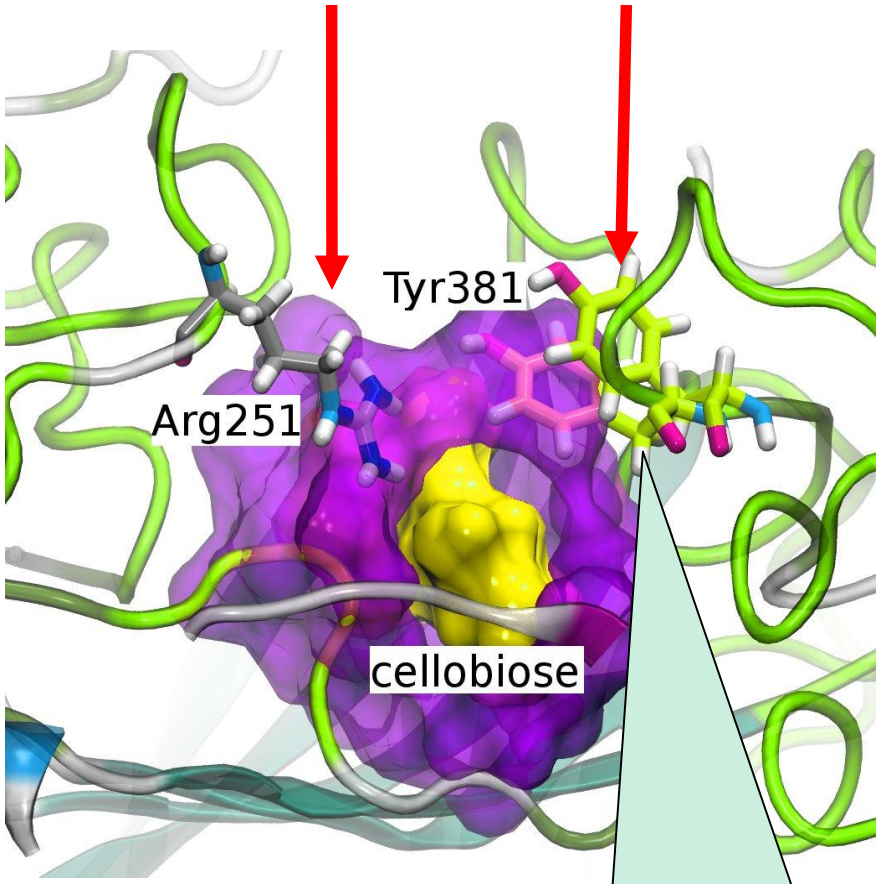
Why does cellobiose inhibit the enzymatic activity?



Crystallographic structures show a completely open cellobiose binding site

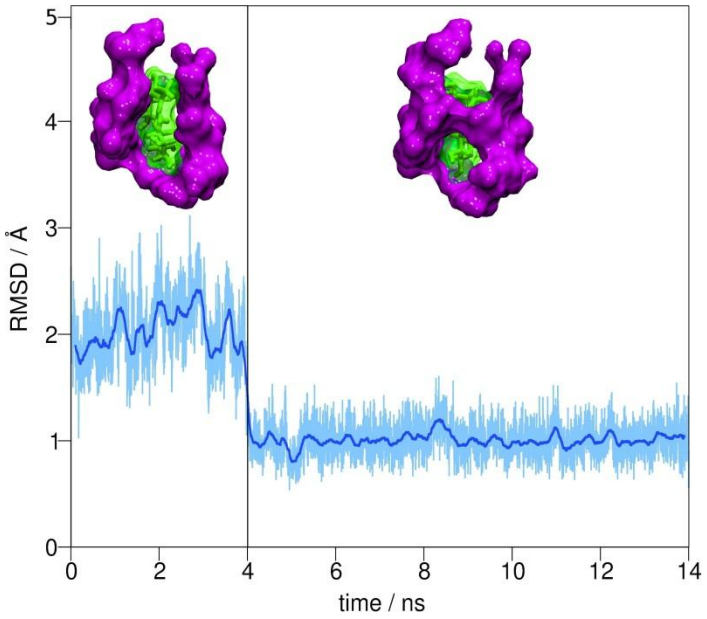
MD simulations

A tunnel is formed by residues Arg251 and Tyr381 at the product binding site

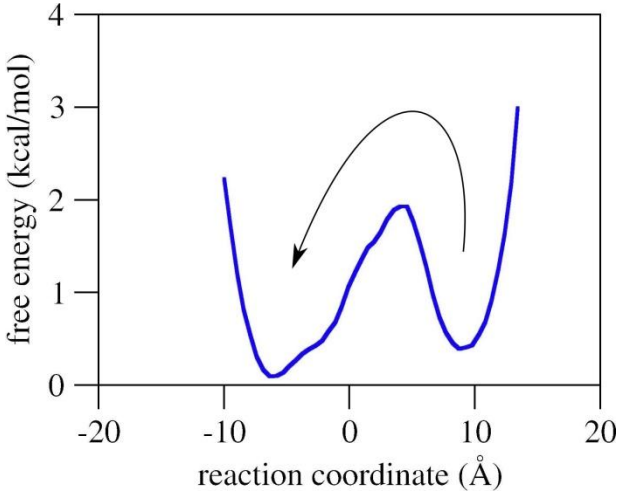


Tyr381 undergoes a conformational change

Cellobiose gets trapped in the CCD

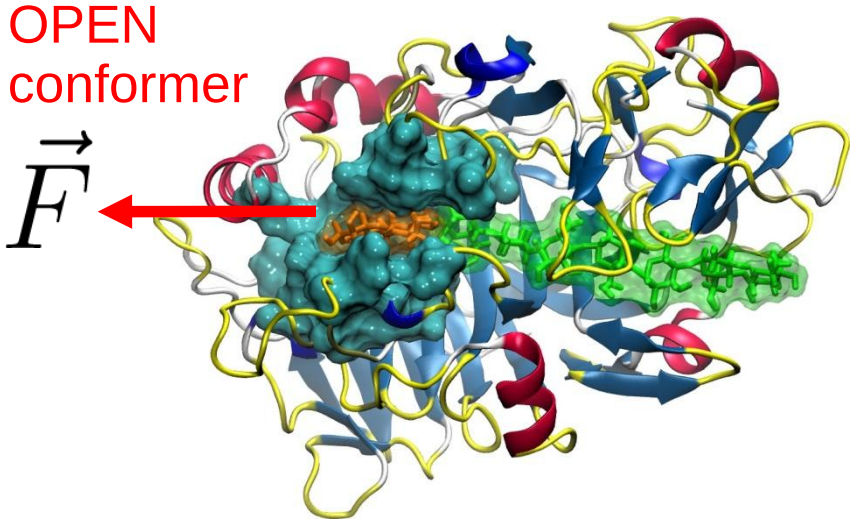


Transition between two minima: G(q)= - kT ln P(q)

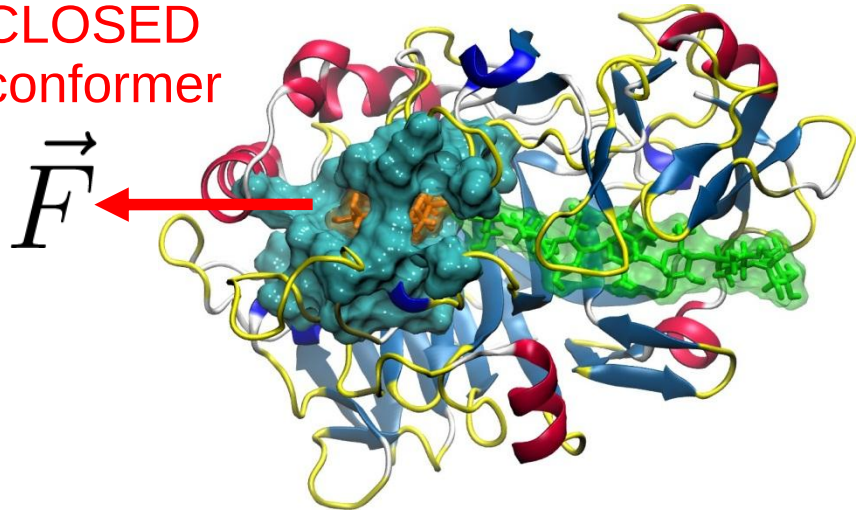


Cellobiose-CCD Unbinding Steered MD simulations

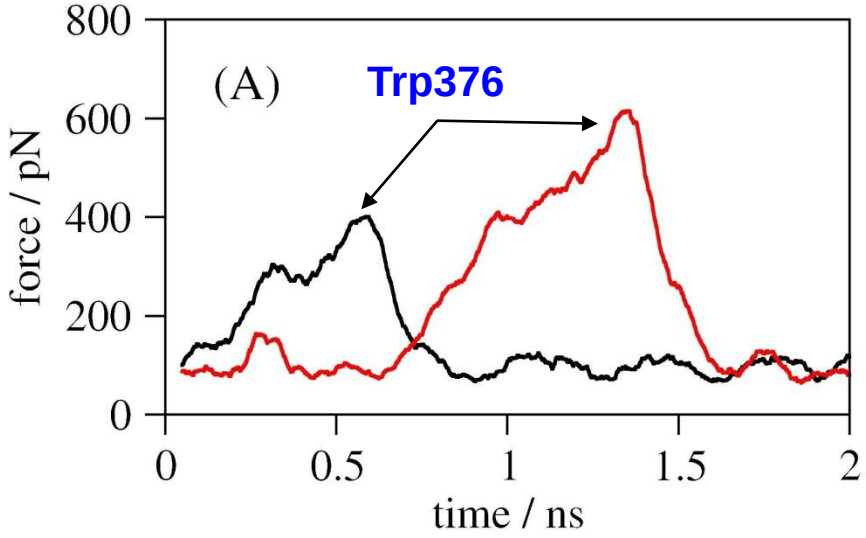
OPEN conformer



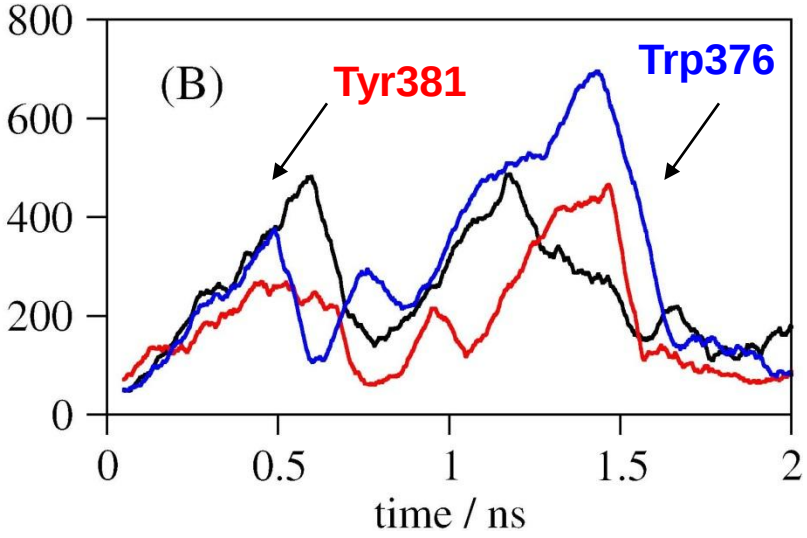
CLOSED conformer



Just one barrier for product expulsion



Extra barrier for product expulsion due to Tyr381



CONCLUSIONS & ACKNOWLEDGEMENTS

- **X-ray diffraction analysis and MD simulations of hyperthermostable *Rhodothermus marinus* β-1,3-glucanase indicate molecular basis of thermostability of the enzyme.**

- **Crystallographic structure and MD simulations of *T. harzianum* CBHI shed light on putative structural reasons of its specificity toward different substrates.**

ACKNOWLEDGEMENTS

Igor Polikarpov – IFSC-USP/São Carlos
Lucas Bleicher / Alessandro Nascimento / Larissa Textor / Francieli Colussi

Fabio Squina CCTB - LNLS
Leandro Martínez – IFSC-USP/São Carlos

Rodrigo Silveira
Erica Prates / Thiago Gomes

Funding: Fapesp & CNPq