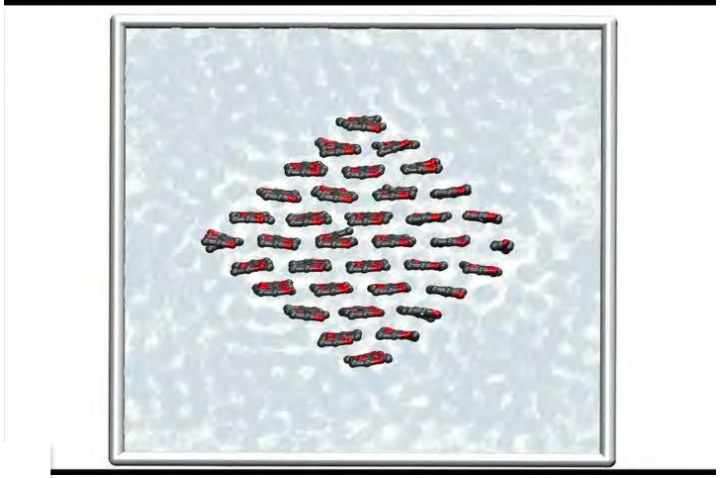
Computational studies of Lignocellulose deconstruction

Gnana S. Gnanakaran

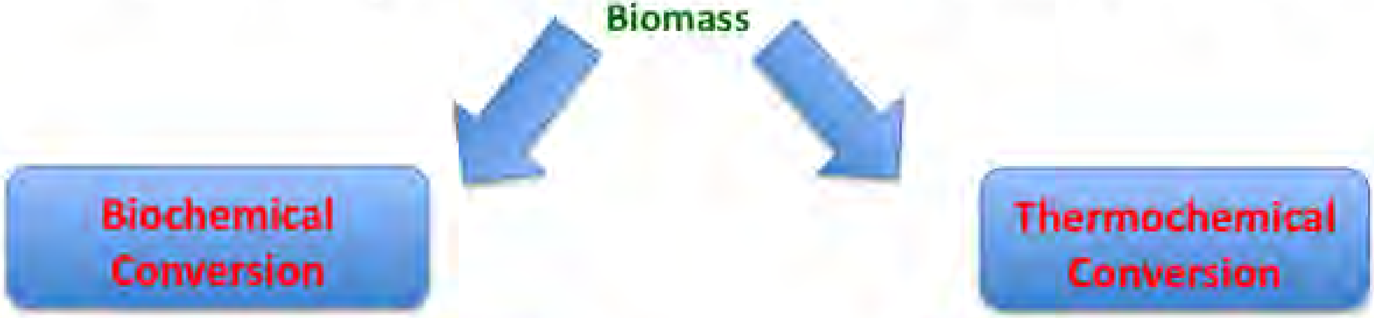
Los Alamos National Labs

Ohm Muruga



I. Thermal decomposition of biomass
II. Cellulose Properties responsible for recalcitrance to digestion
III. Alter cellulose substrate for efficient digestion
IV. Mechanistic kinetic model for Cellulases

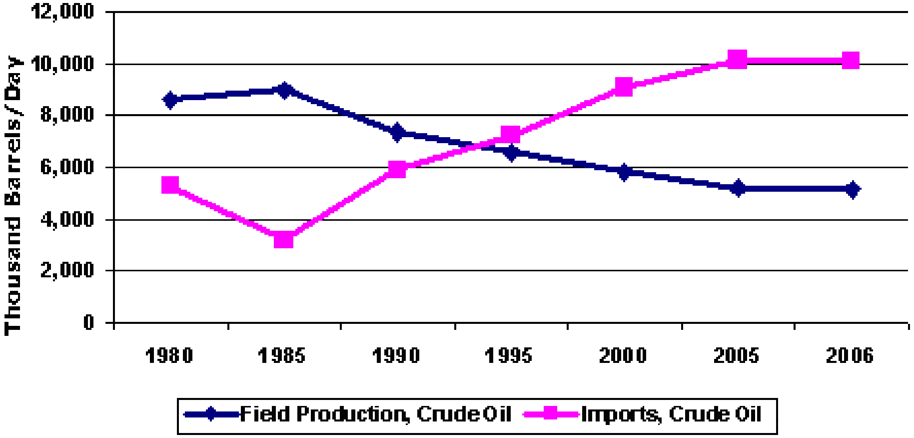
Summary – Biofuel Theoretical Efforts


Biomass

Biochemical Conversion

Thermochemical Conversion

National Energy Security



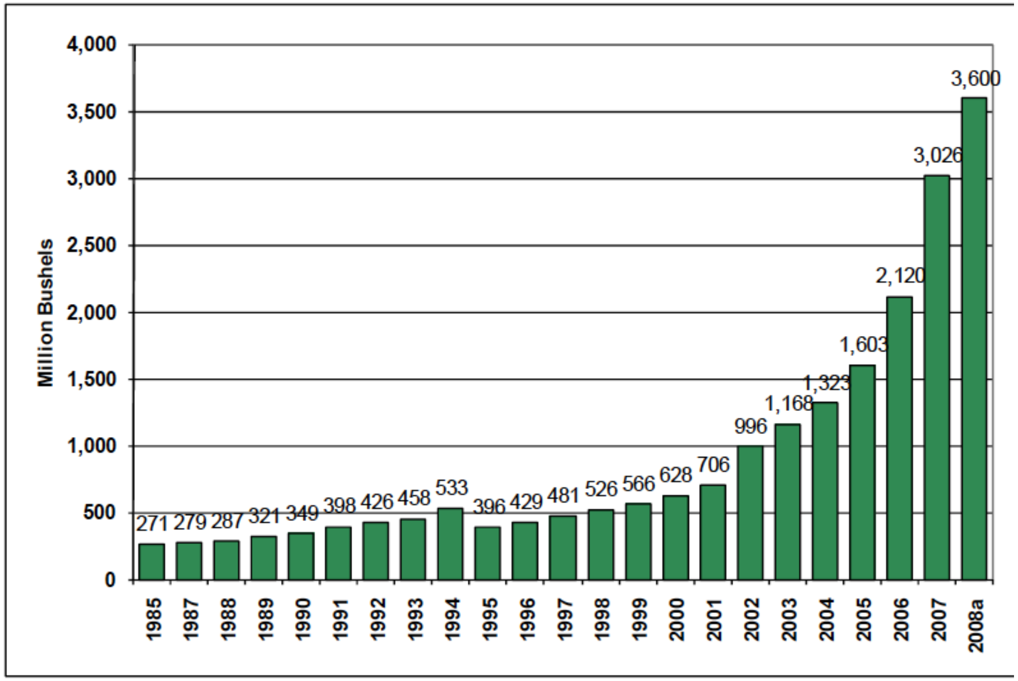
U.S. economy is heavily dependent on oil imports.

U.S. has 4% of the world's population but consumes 25% world's oil production.

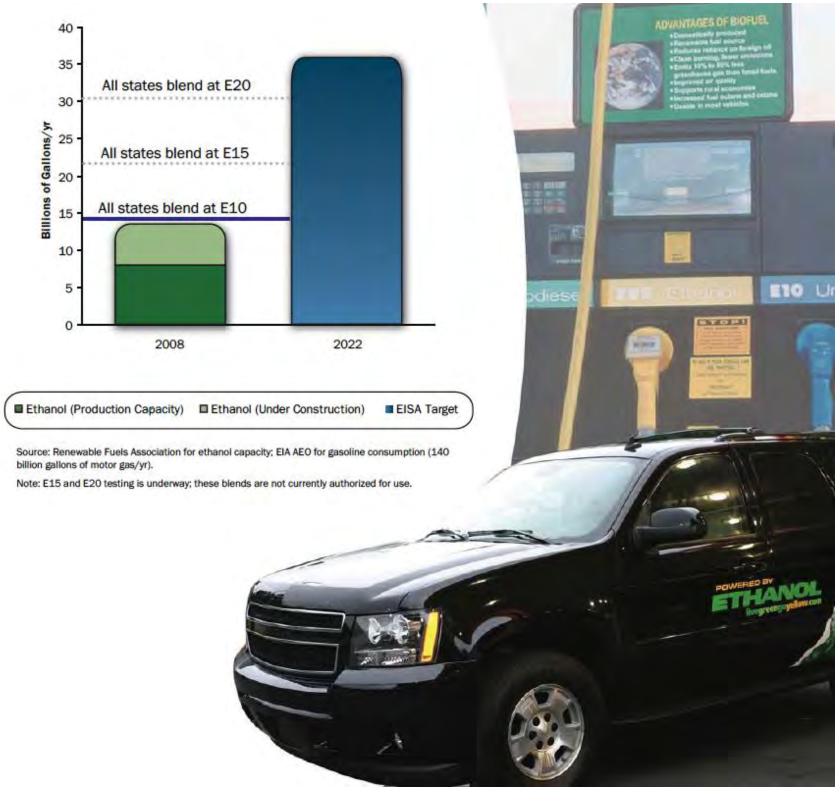
Domestic production of crude oil has declined but the dependence on imported oil has increased and accounts for 65% of crude oil supplies.

Biofuels are an alternative to conventional energy sources that increase our Nation's energy security by dramatically reducing our dependence on imported oil.

Currently, our Nation's growing bioethanol industry is largely based on the use of starch in grains such as corn, that are also needed for food.



Source:
National Corn Growers Association, *The World of Corn,* 2009 and previous annual editions,



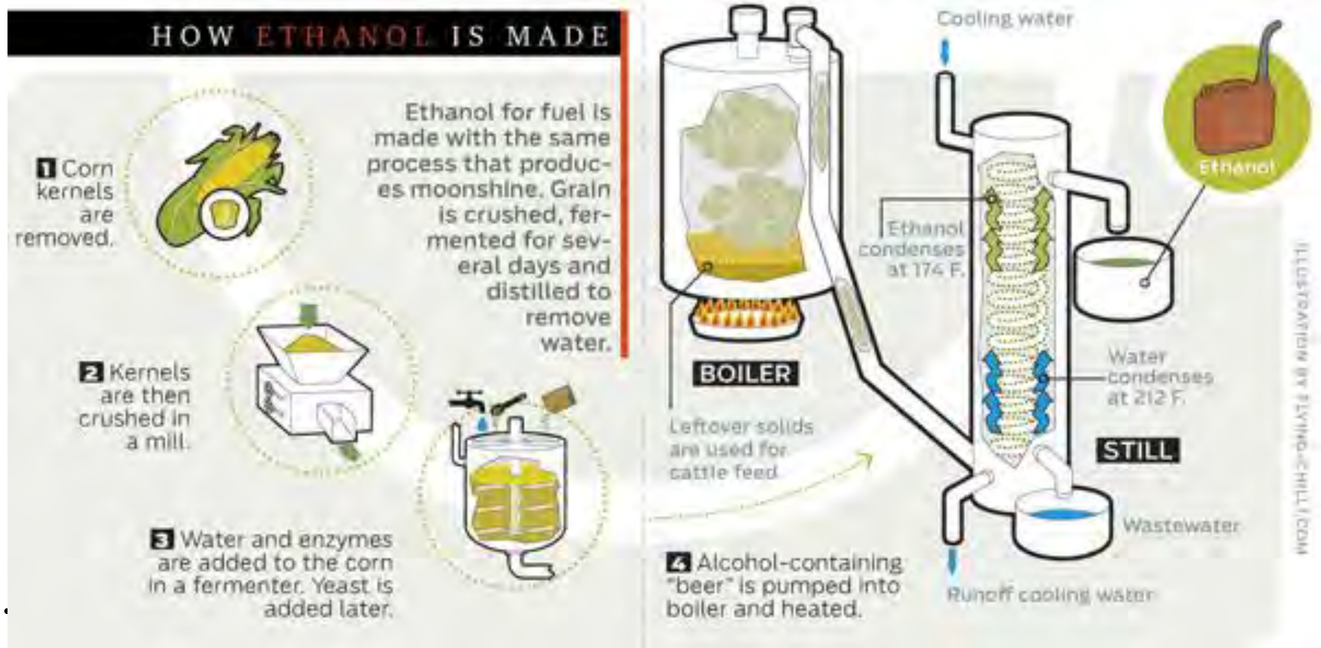
Source: Renewable Fuels Association for ethanol capacity; EIA AEO for gasoline consumption (140 billion gallons of motor gas/yr).

Note: E15 and E20 testing is underway; these blends are not currently authorized for use.

Corn for ethanol production has increased by nearly 5- fold from 2000 to 2008

Corn based Ethanol

More than 130 ethanol plants are now in operation.



HOW ETHANOL IS MADE

Ethanol for fuel is made with the same process that produces moonshine. Grain is crushed, fermented for several days and distilled to remove water.

1 Corn kernels are removed.

2 Kernels are then crushed in a mill.

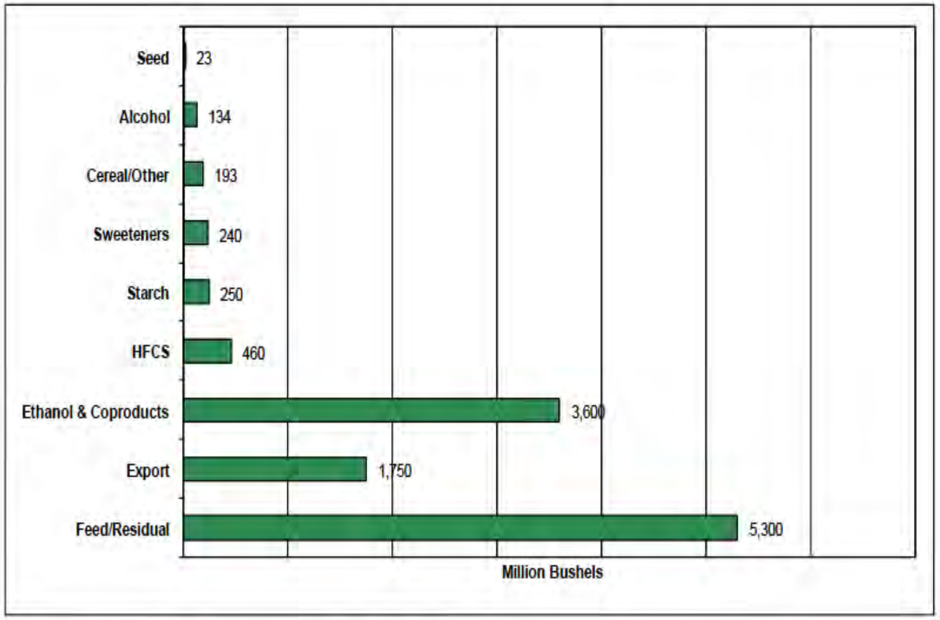
3 Water and enzymes are added to the corn in a fermenter. Yeast is added later.

4 Alcohol-containing "beer" is pumped into boiler and heated.

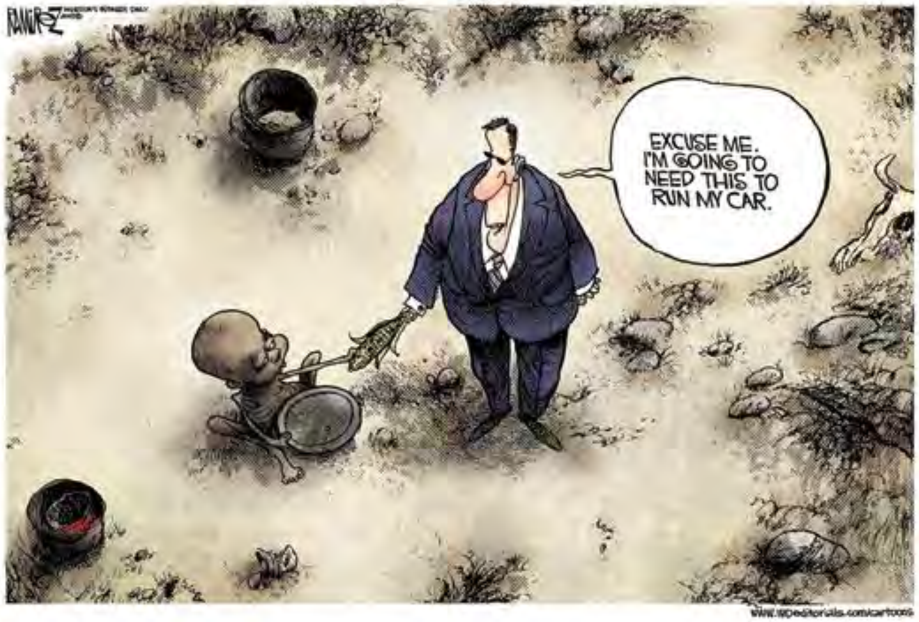
Leftover solids are used for cattle feed

Process is similar to making "moonshine" which initially became popular during prohibition

Where will the food come from?



Source:
National Corn Growers Association, *The World of Corn*, 2009



Energy Independence &Security Act of 2007

36 billion gallons of annual renewable fuel use by 2022 and required that 60 % to be met by advanced biofuels, including cellulosic ethanol

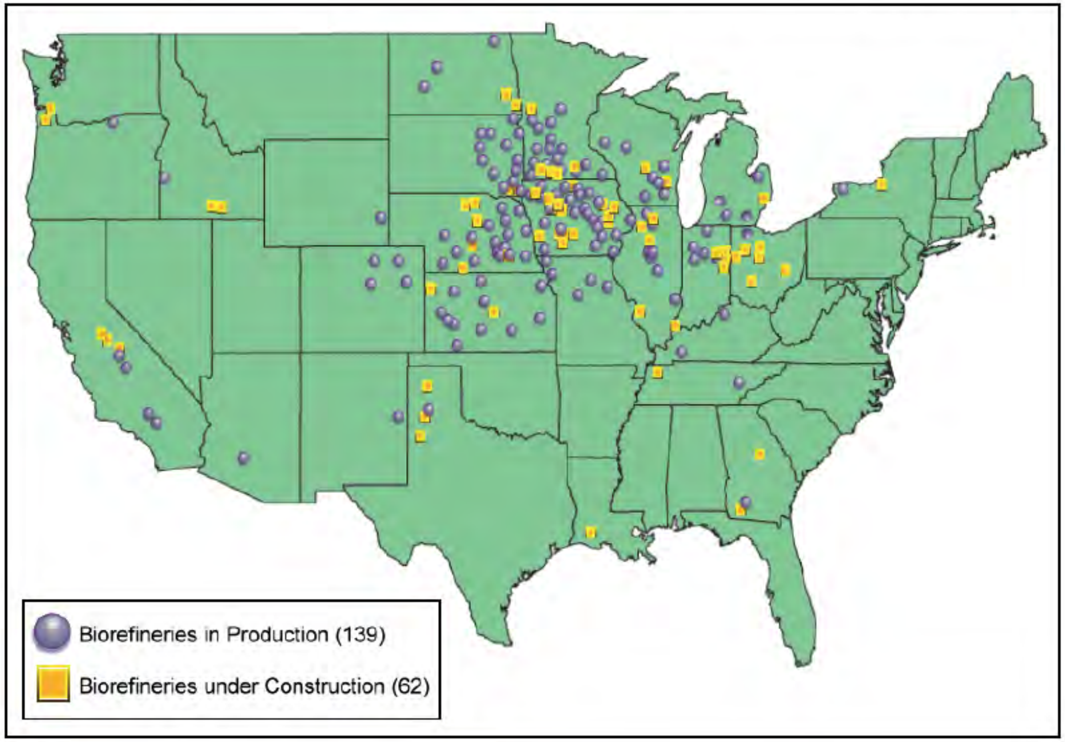
Lignocellulosic biomass, the inedible fibrous material from wood and plant stems, is an abundant alternative source.

corn stalks are more challenging!

Biofuels need to be made from a wide range of hardy and fast-growing plants, such as switchgrass--which is a perennial native to American prairies. It requires about a quarter of the irrigation and fertilization of row crops.

Cellulosic-ethanol Refineries

Ethanol Production Facilities Current and Under Construction, January 24, 2008



Source:
Renewable Fuels Association, http://www.ethanolrfa.org/.

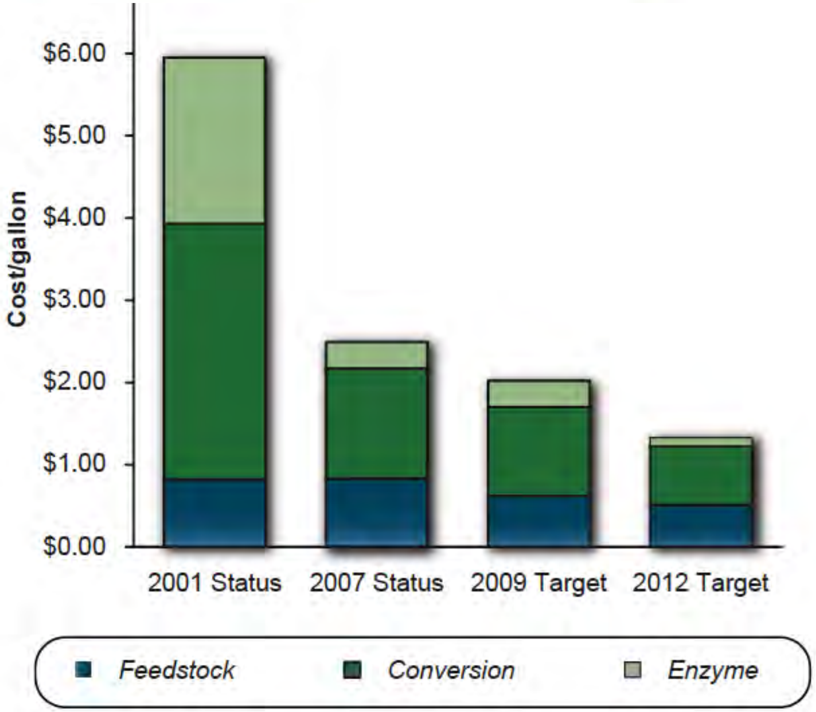
Company	Location	Size (Millions of gallons per year)	Feedstock	Completio date
Broin	Emmetsburg, Iowa	31	Corn stover (cobs and stalks)	2009
BlueFire Ethanol	Southern California	19	Waste wood	2009
Alico	La Belle, Florida	20	Wood, ag waste	2010
Abengoa Bioenergy	Colwich, Kansas	11.4	Corn stover, wheat straw, etc.	2011
Iogen Biorefinery	Shelley, Idaho	18	Ag waste	2010
Range Fuels	Soperton, Georgia	50	Waste wood, energy crops	2011

e winners. The U.S. Department of Energy recently backed six cellulosic-ethanol refineries.

Industry has been slow to explore the potential of lignocellulosic biomass because so far it is more difficult and costly than starch to convert to ethanol.

Biomass Conversion

Main problem:
complex structure of plant
Cell wall, impedes efficient
Conversion into sugars that
can be fermented to ethanol



Source: DOE EERE Office of the Biomass Program, Multi-year Program Plan, Appendix C.

Biomass recalcitrance

Biomass Production

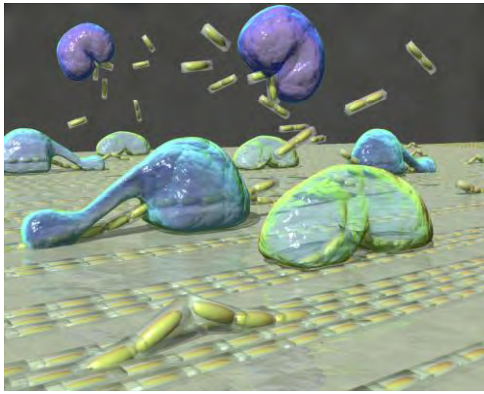
Solids: cellulose
hemicellulose, lignin

Chemicals

Pretreatment

Cellulose

Cellulases

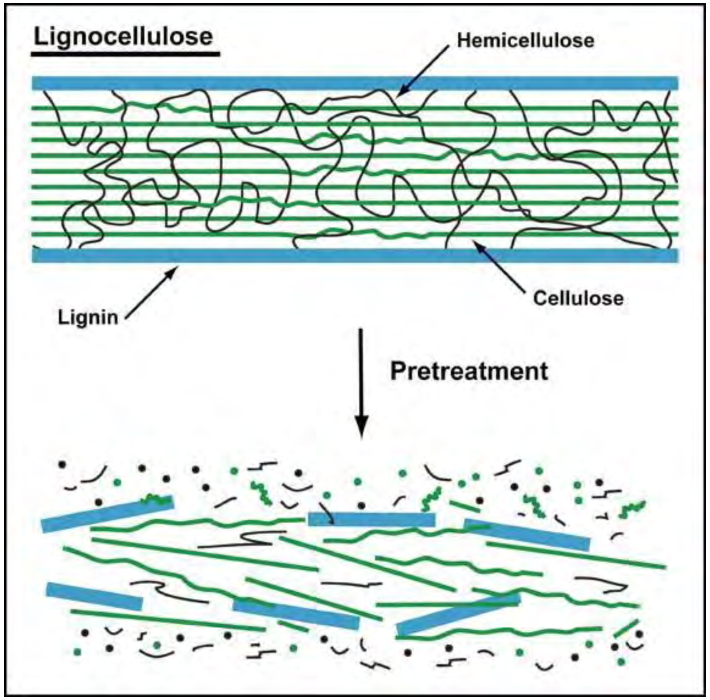


Enzymatic Hydrolysis

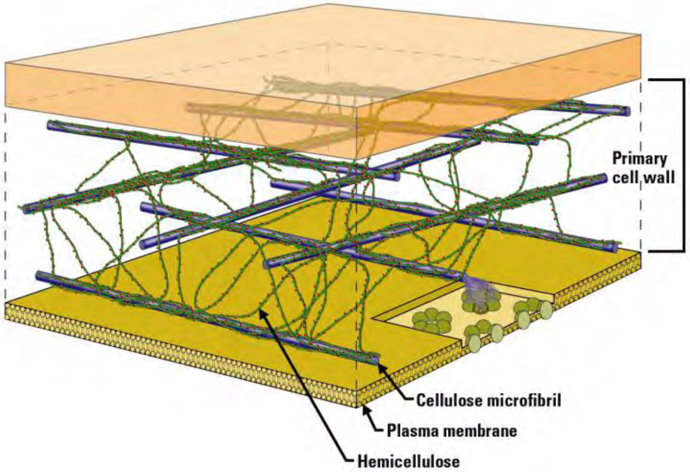
Dissolved sugars

Fermentation of Sugars

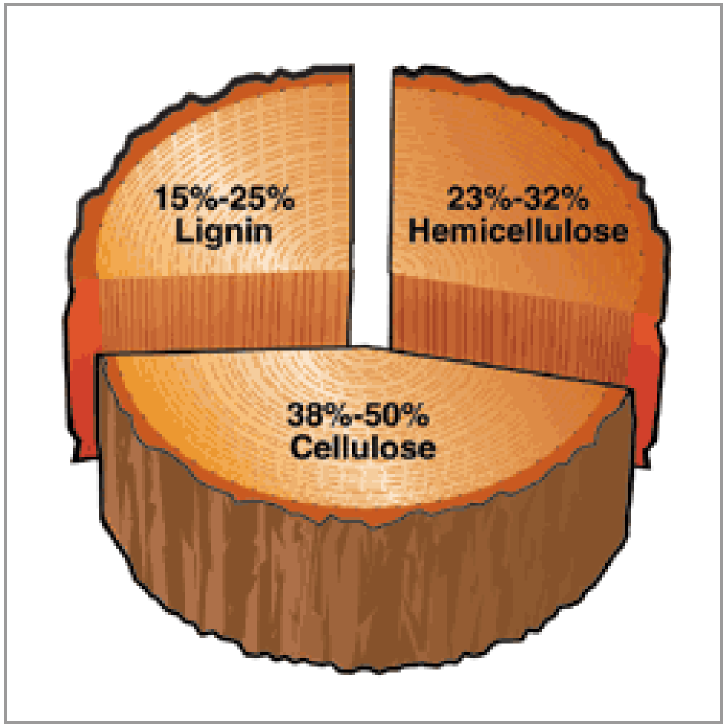
Effect of Pretreatment

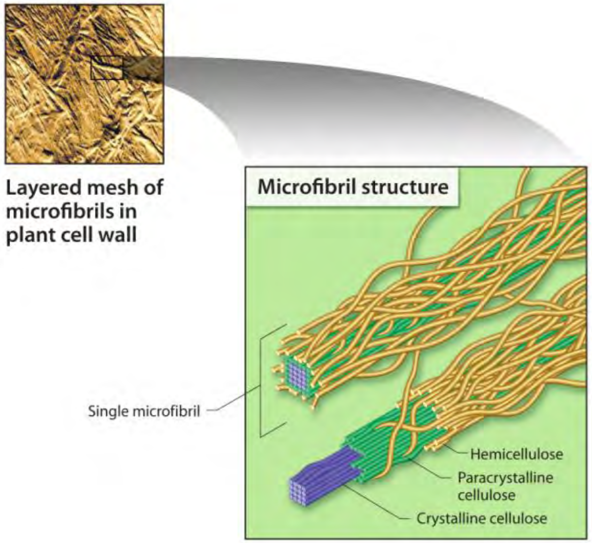


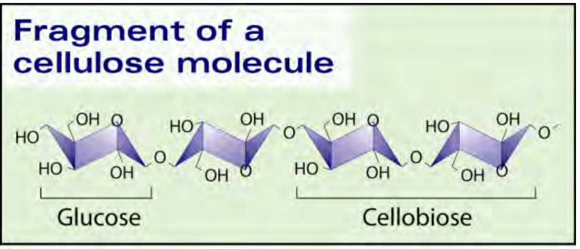
Biomass



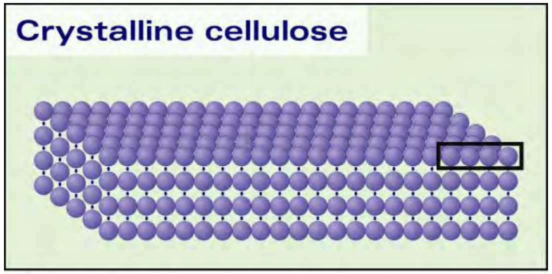
Plant cell walls contain cellulose microfibrils, hemicelluloses & lignins



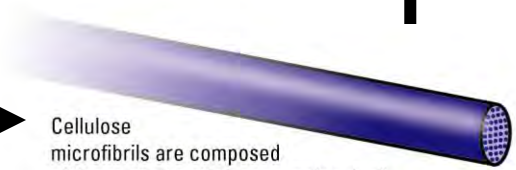




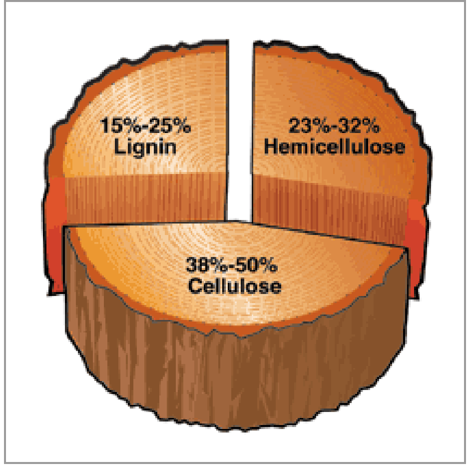
Alternating glucose residues are in an inverted orientation so the cellobiose (a disaccharide) is the repeating structural unit.



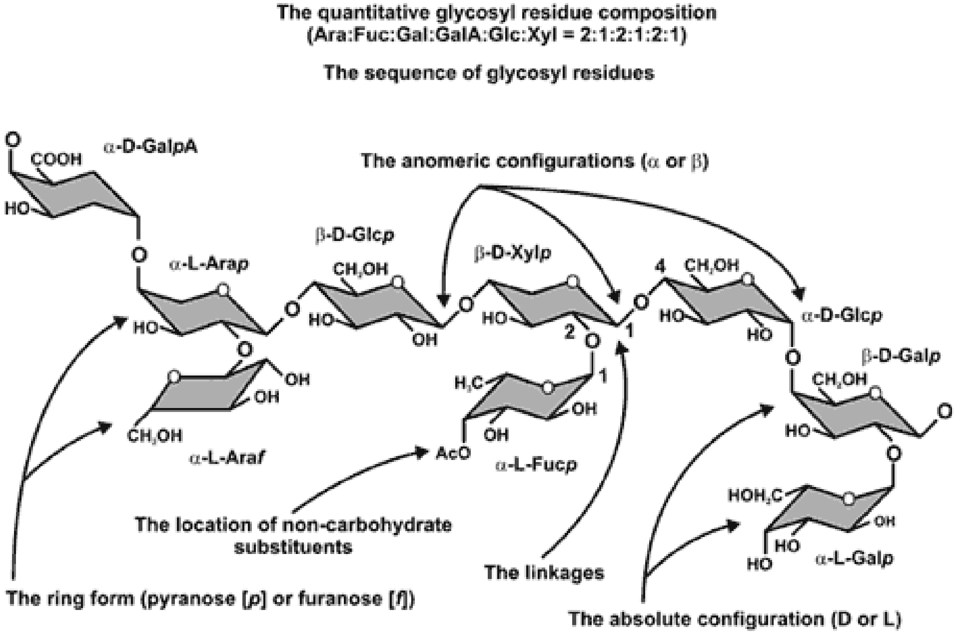
The glucan chains contain thousands of glucose residues.

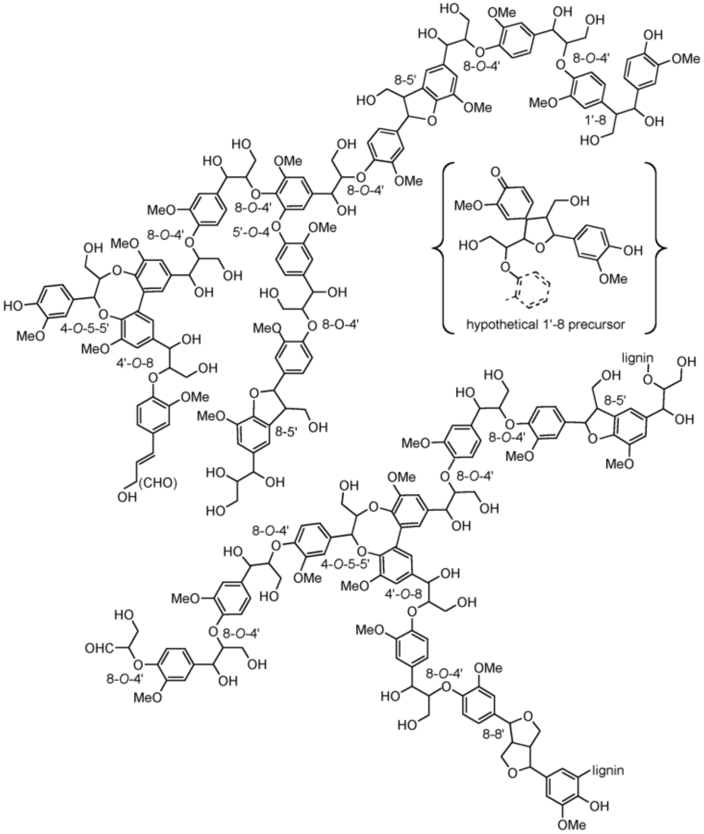


Cellulose microfibrils are composed of linear chains of glucose molecules* that hydrogen bond to form the microfibrils.



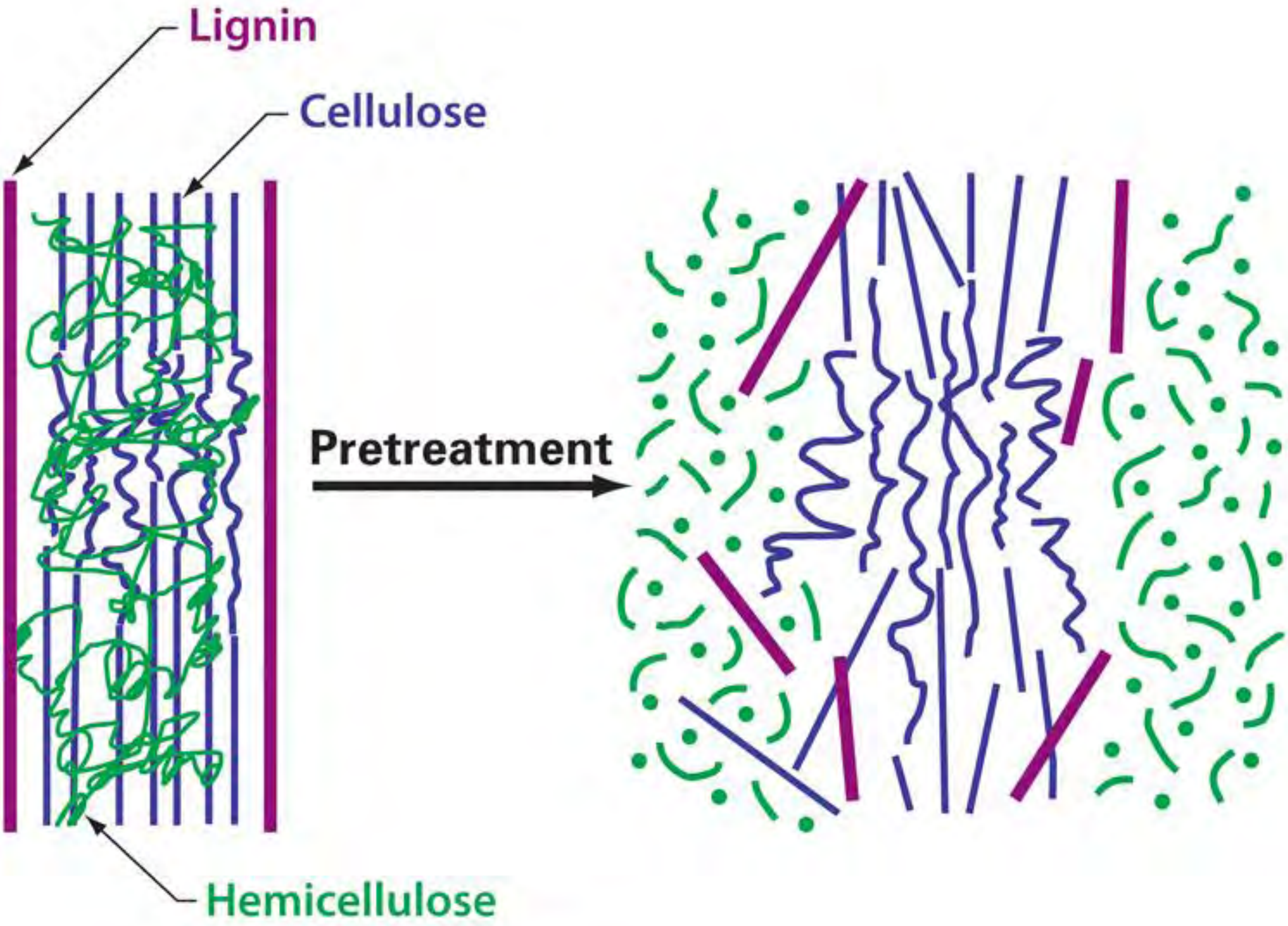
Hemicellulose
- Xylose
- Polymer of 5- and 6- carbon sugars





Lignin
- Complex aromatic structure
- High Energy Content
- Resists biochemical conversion

Pretreatment

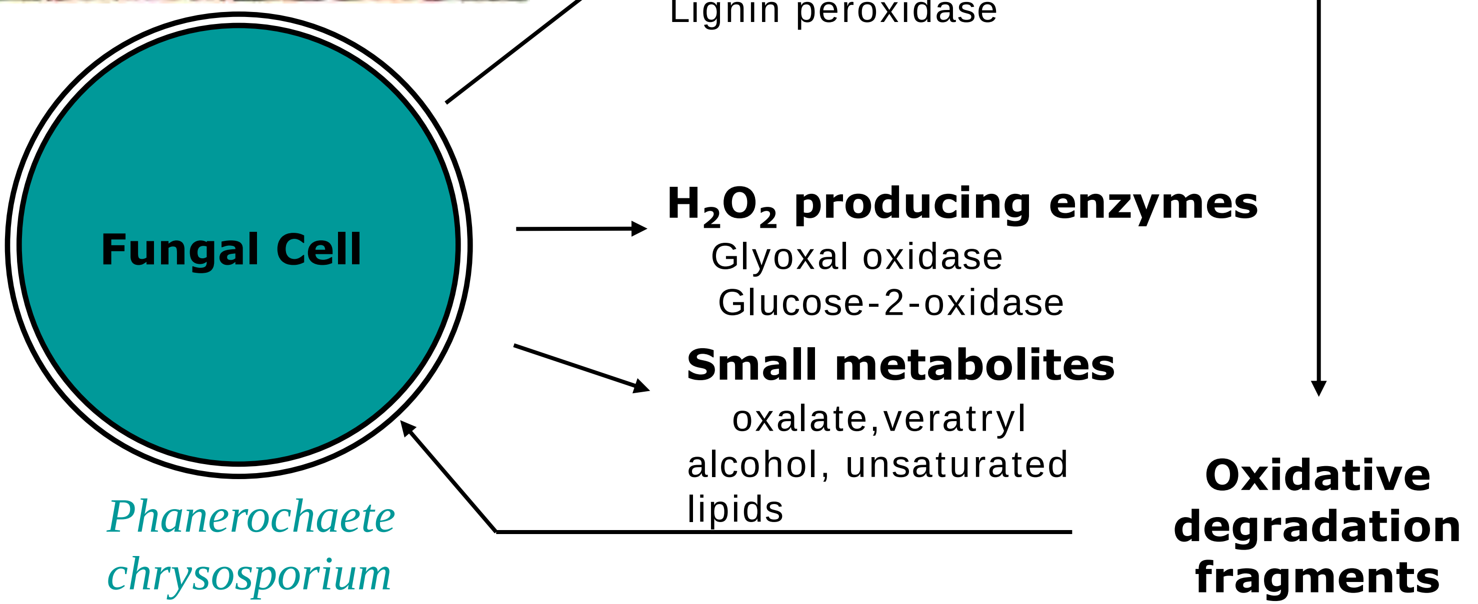


LIGNIN DEGRADATION

Microorganisms :

Wood rotting fungi – depolymerization of lignins by C-C bond cleavage

Part of the few microorganisms capable of a complete lignin degradation : lignin $CO_2 + H_2O$ →



Polymeric lignin

Lignin Degrading Enzymes

Manganese peroxidase

Lignin peroxidase

H_2O_2 producing enzymes

Glyoxal oxidase

Glucose-2-oxidase

Small metabolites

oxalate, veratryl alcohol, unsaturated lipids

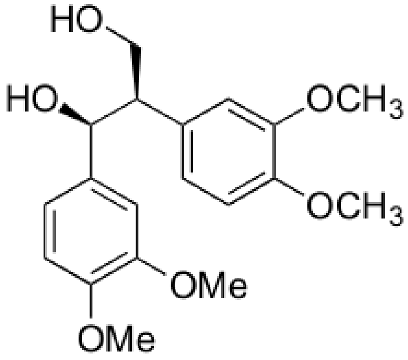
Oxidative degradation fragments

Fungal Cell

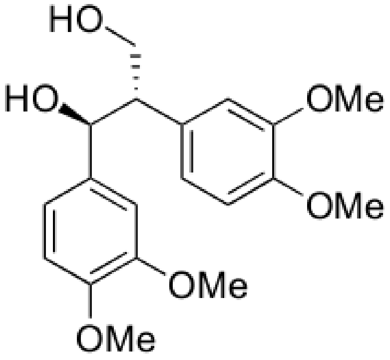
Phanerochaete chrysosporium

Slide from M. Gold

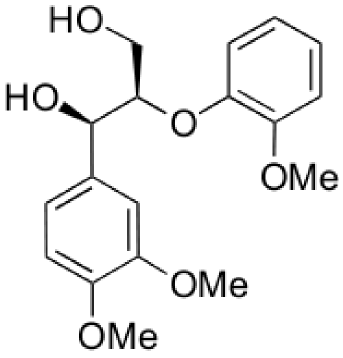
QM Studies on Lignin Models



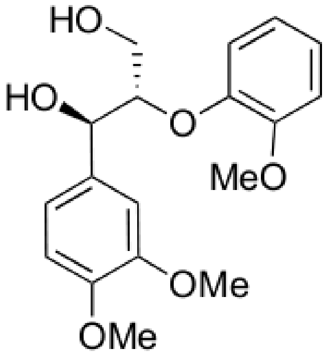
β-1 Erythro



β-1 Threo



β-O-4 Erythro



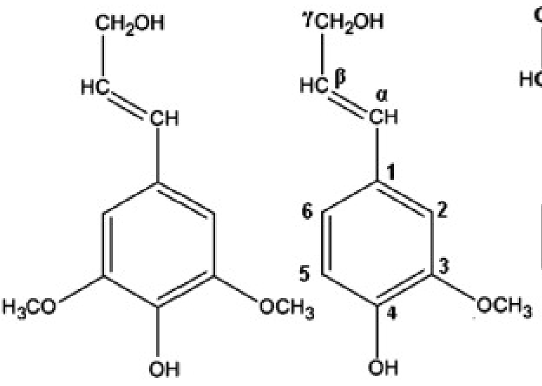
β-O-4 Threo

D. W. Cho, R. Parthasarathi, A. S. Pimentel, G. D. Maestas, H. J. Park, U. C. Yoon, D. Dunaway-Mariano, S. Gnanakaran, P. Langan, P. S. Mariano, The Nature and Kinetic Analysis of Carbon-Carbon Bond Fragmentation Reactions of Cation Radicals Derived from SET-Oxidation of Lignin Model Compounds, *J. Org. Chem* 75 (2010) 6549–6562.

- Radical cationic state playing a essential part in Lignin fragmentation
- Evaluate relative chemical reactivity trends from Ionization potentials
- Identify weak C—C bond fragmentation in lignin compounds
- Recognize the potential reactive sites

Study of Diverse Linkages in Lignin

Monomeric precursors of lignin

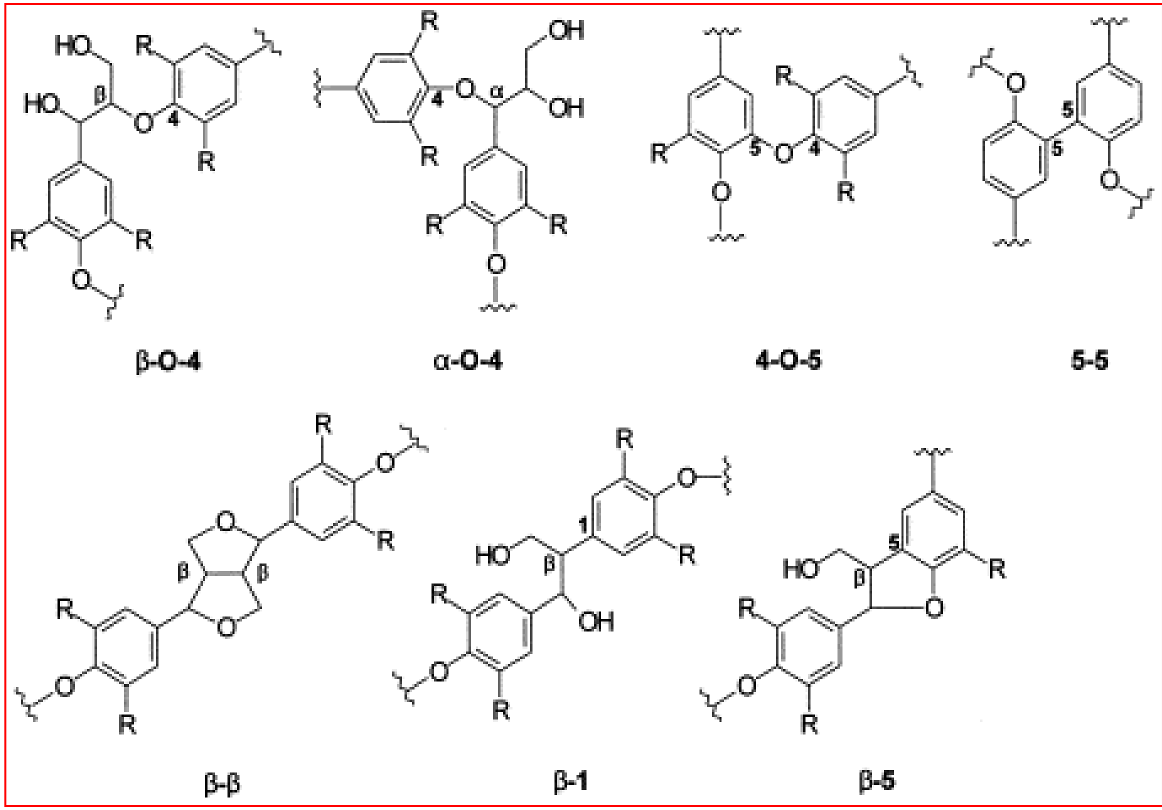


syringyl (S)

guaiacyl (G)

***p*-hydroxyphenylpropane (H)**

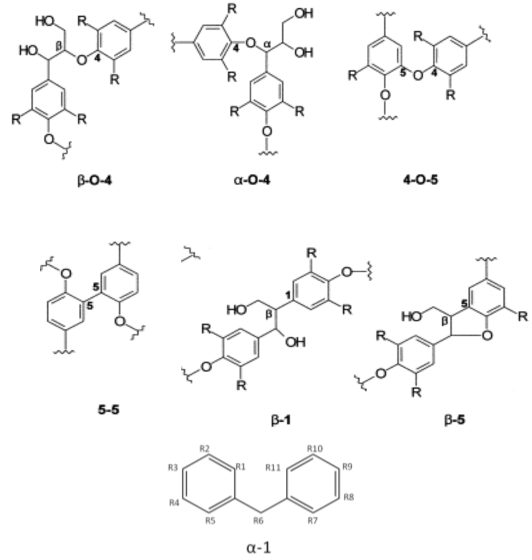
Common linkages in lignin



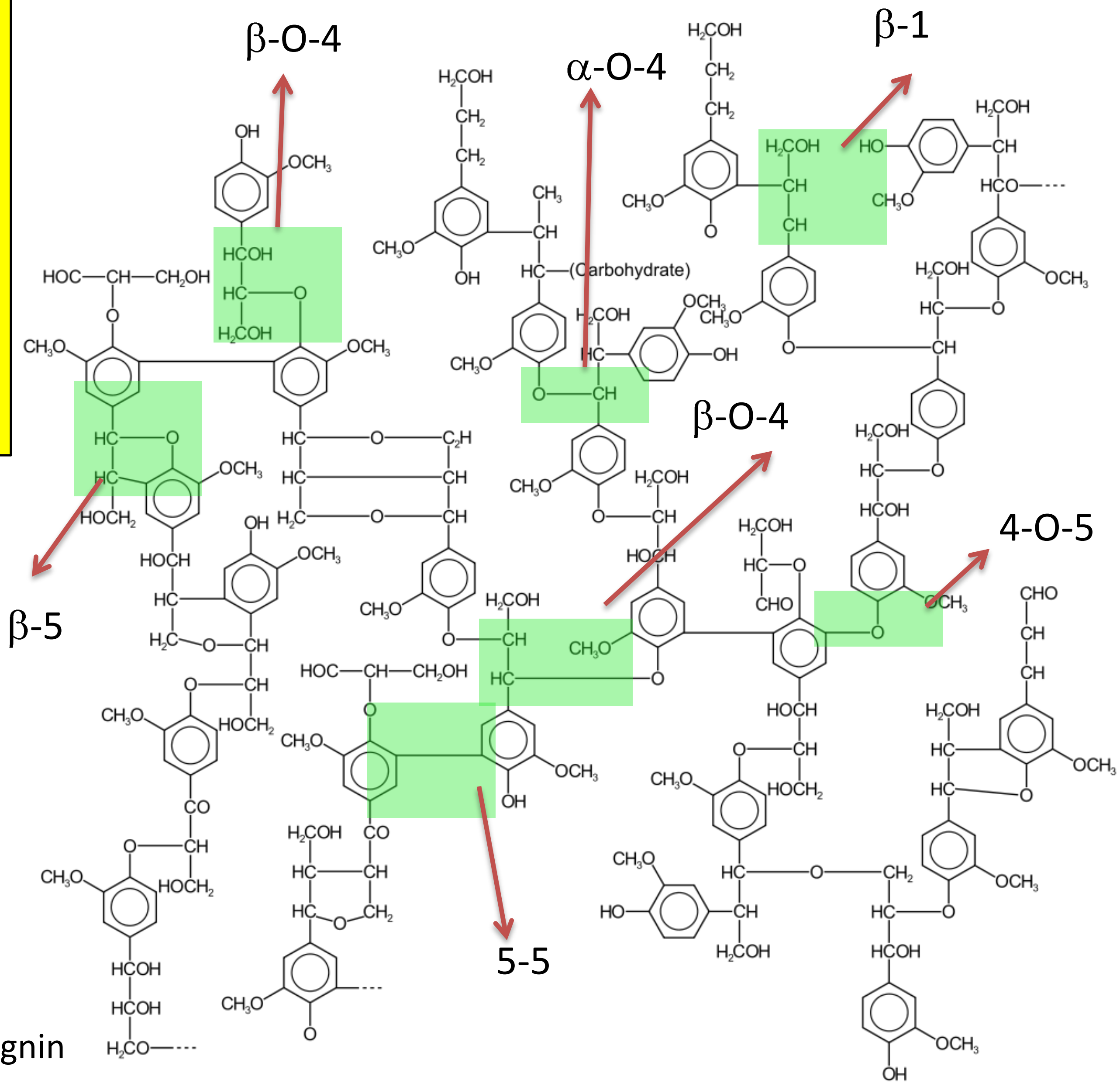
R. Parthasarathi, R. A. Romero, A. Redondo, S. Gnanakaran, *Theoretical Study of the Remarkably Diverse Linkages in Lignin*, ***J. Phys. Chem. Letters.*** 2011. 2, 2660–2666.

NABC National Advanced Biofuels Consortium
Biofuels for Advancing America

THEORETICAL Study: DIVERSITY OF LIGNIN LINKAGES

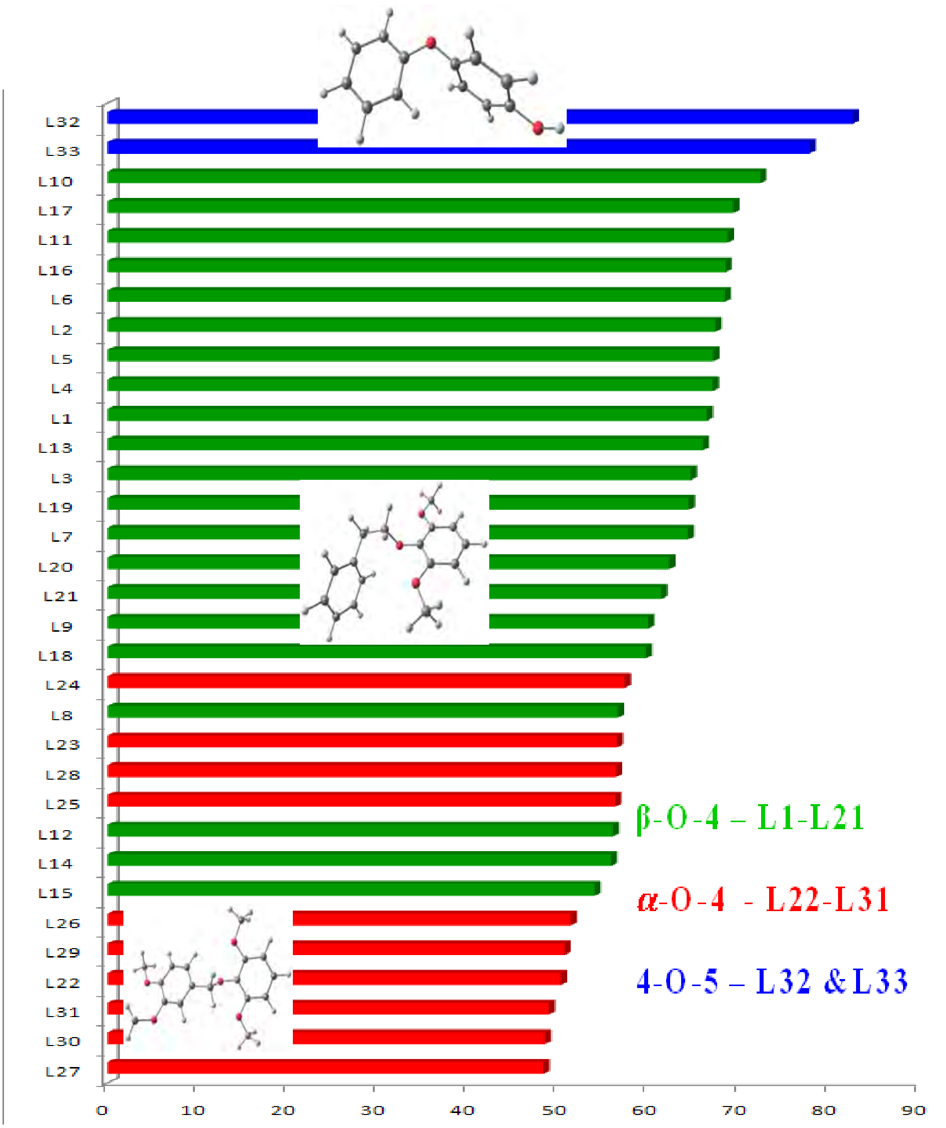


The most dominant linkages in lignin

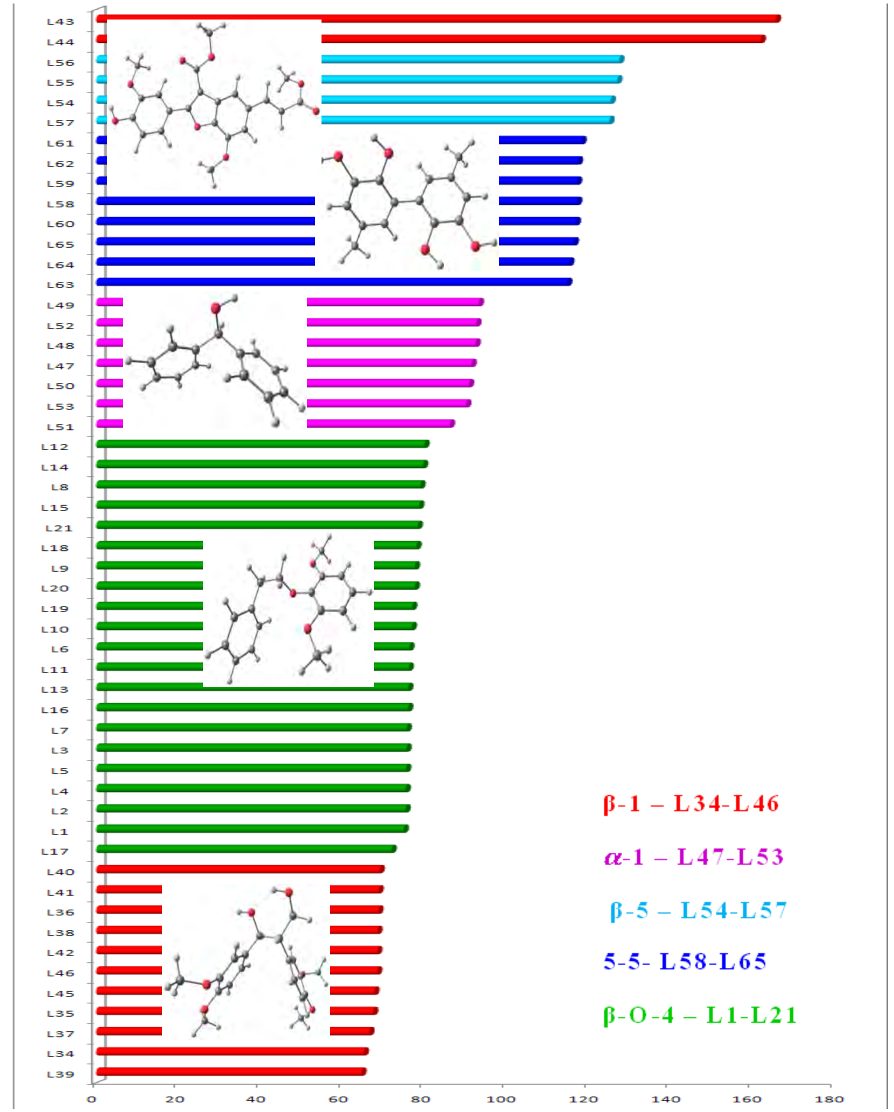


Density Functional Calculations of bond fragmentation in 65 distinct Lignin Model Compounds

Bond dissociation energies (kcal/mol)



Ether bond linkages

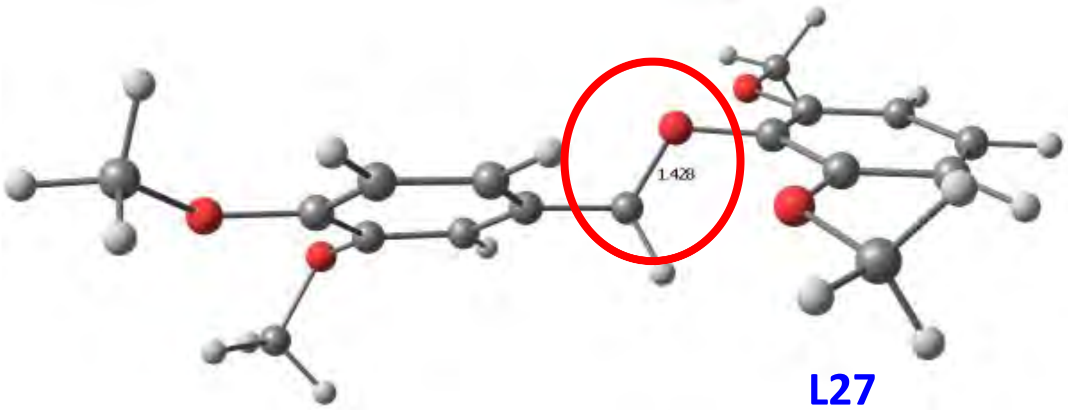


C-C bond linkages

Weakest lignin linkage

Ether linkage - α-O-4

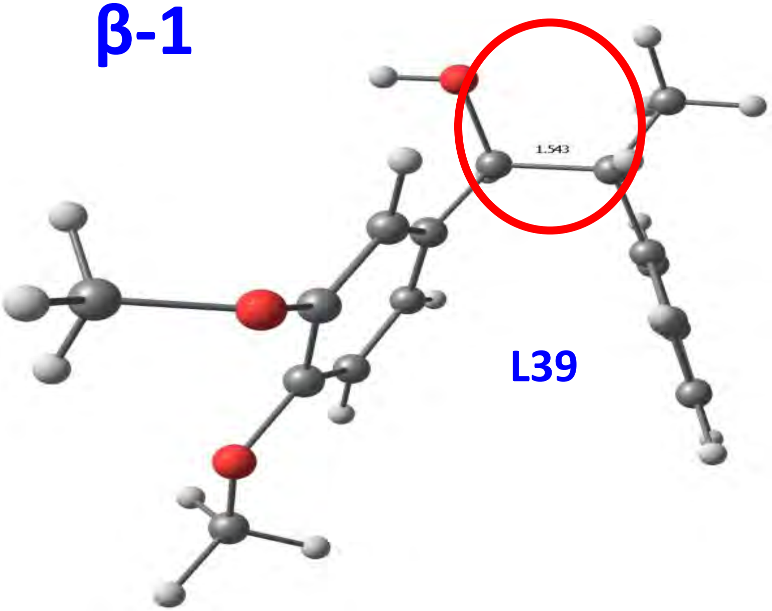
BDE= *48.31* kcal/mol



L27

C-C linkage - β-1

BDE= 64.7 kcal/mol

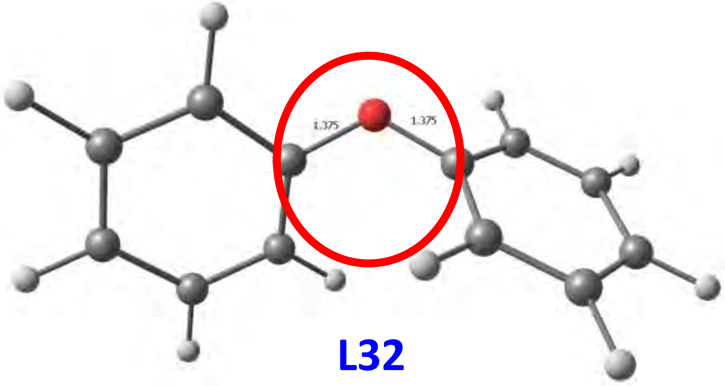


L39

Strongest lignin linkage

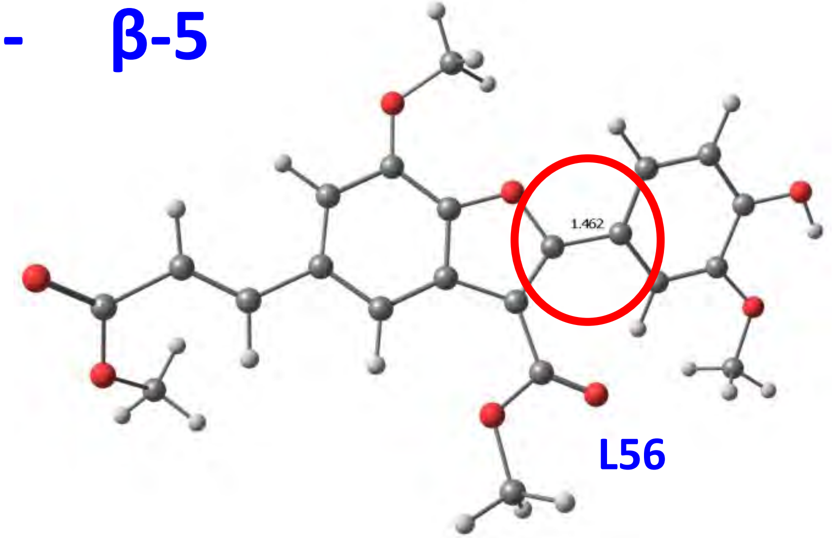
Ether linkage - 4-O-5

82.54 kcal/mol



L32

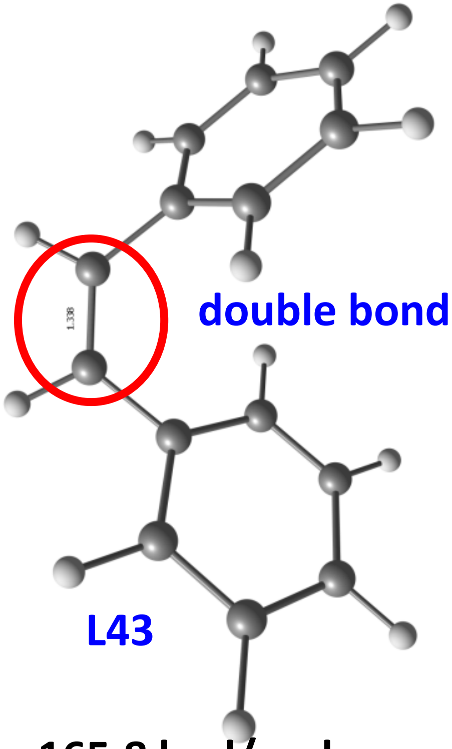
C-C linkage - β-5



L56

127.6 kcal/mol

β-1



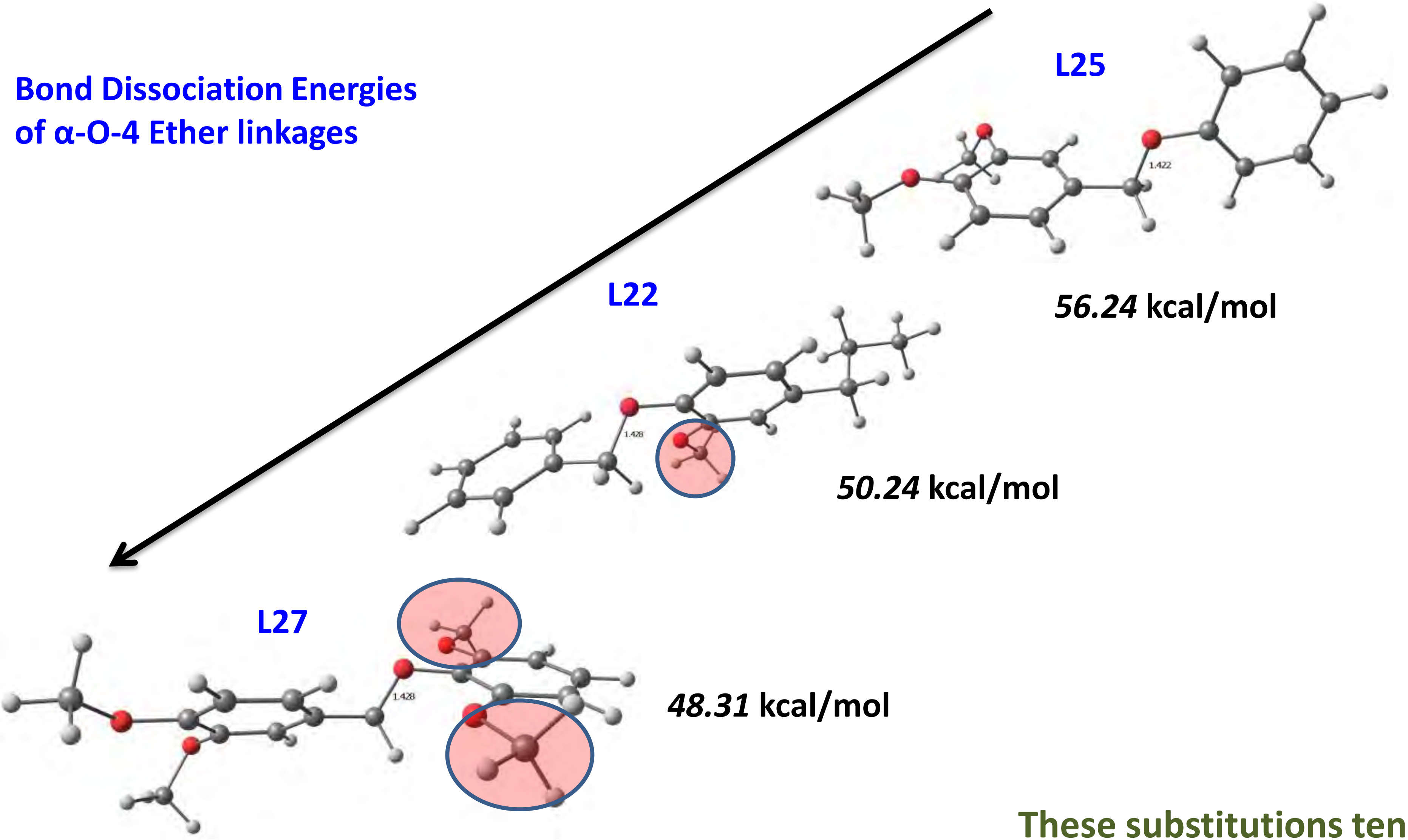
double bond

L43

165.8 kcal/mol

Influence of *ortho* methoxy group substitutions

Bond Dissociation Energies
of α-O-4 Ether linkages



L25

56.24 kcal/mol

L22

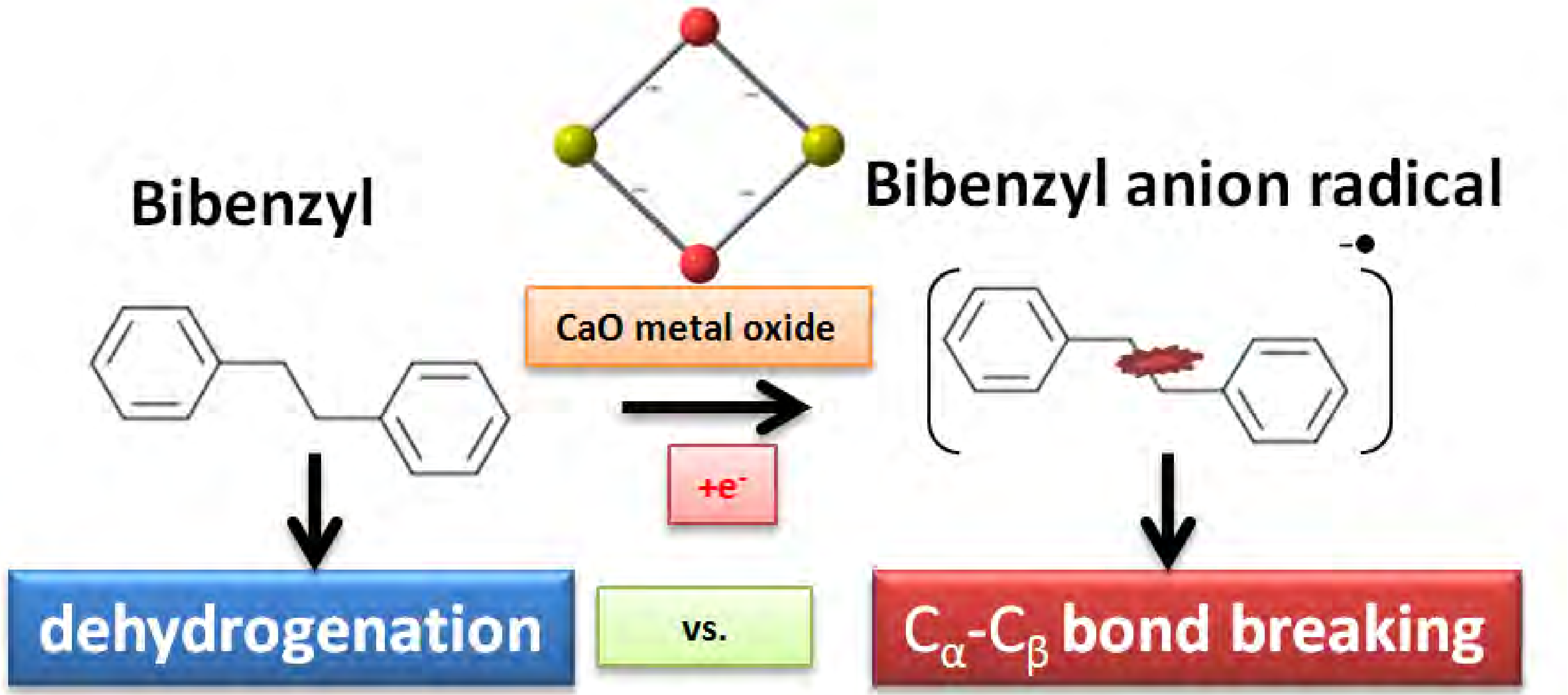
50.24 kcal/mol

L27

48.31 kcal/mol

These substitutions tend
to lower the strength of
β-O-4 linkages as well

Metal oxides as catalysts for fast pyrolysis



Bibenzyl

CaO metal oxide

+e$^-$

Bibenzyl anion radical

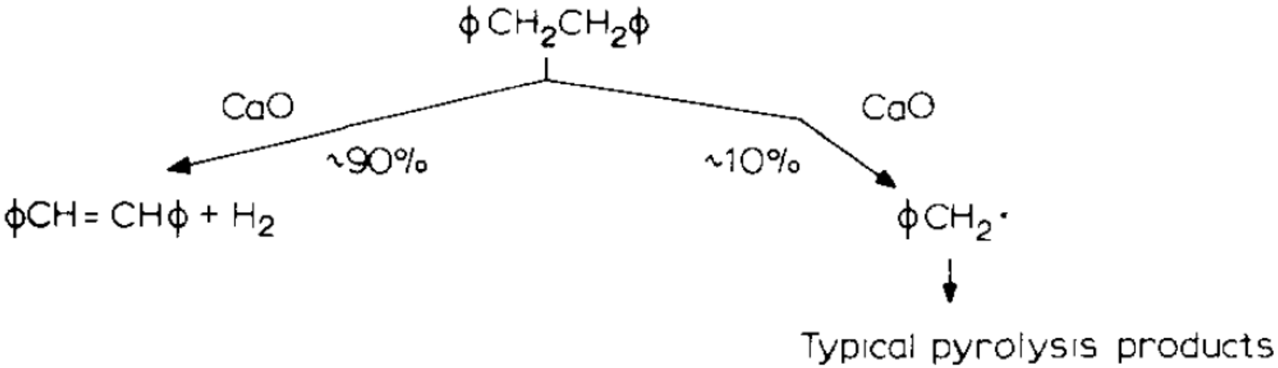
dehydrogenation

vs.

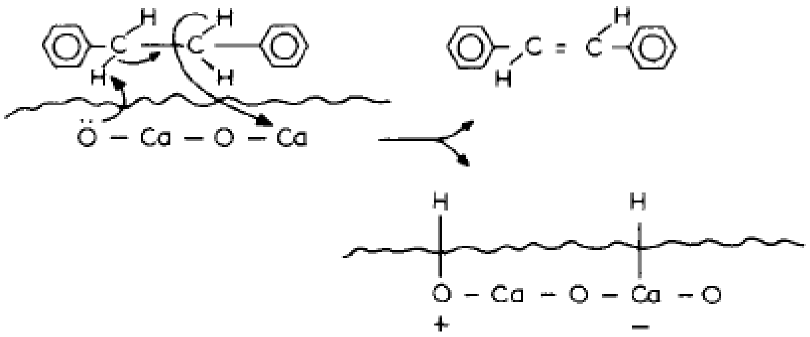
C_α-C_β bond breaking

Collaboration with Tom Elder (USDA)

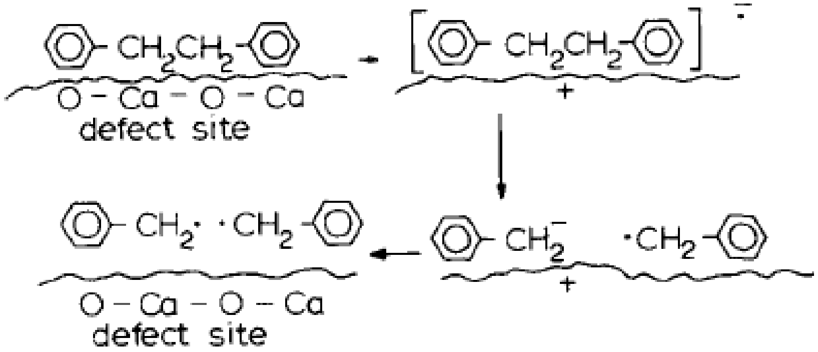
Catalytic Pyrolysis of Bibenzyl



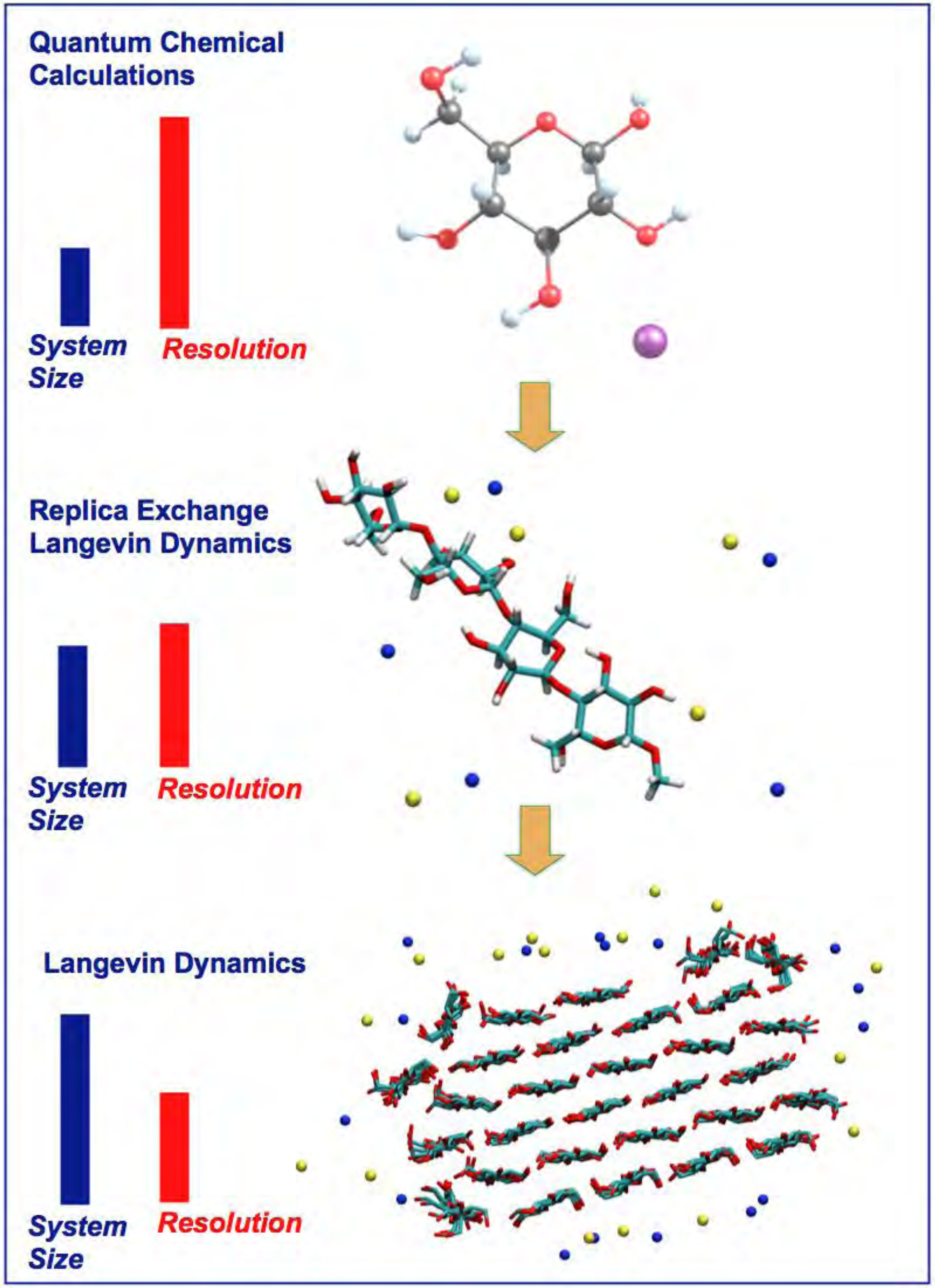
Dehydrogenation – stilbene
Favorable – lower temperature



CH2-CH2 cleavage – facilitated by CaO



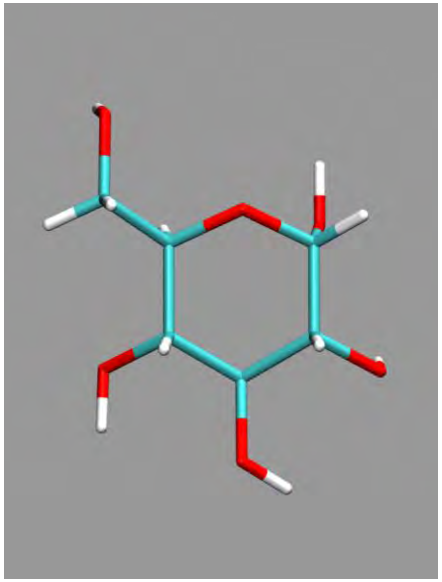
Dehydrogenation > Electron transfer



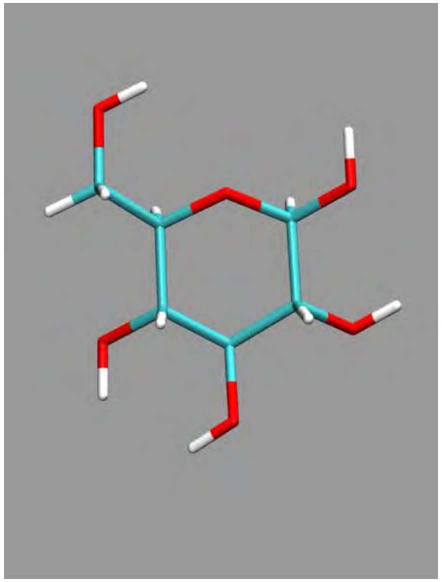
Exploiting quantum and classical computational methods to study the atomistic details of the interactions of NaCl with β-glucose, oligomeric and crystalline fibrillar cellulose

Simulation Details

- System
  - Aqueous α-glucose (α-D-Glc)
  - Aqueous β-glucose (β-D-Glc)
- Replica exchange molecular dynamics (REMD) simulations
  - Control (H2O, 0% NaCl, Sol)
  - 5.0% wt. NaCl (0.9 M)
  - 10.5% wt. NaCl (2 M)
- NVT REMD simulations
  - Temperature range 275 ~ 505 K
  - Used Glycam06 for glucose
  - Ion force field from Joung & Cheatham, JPCB 2008, 112, 9020

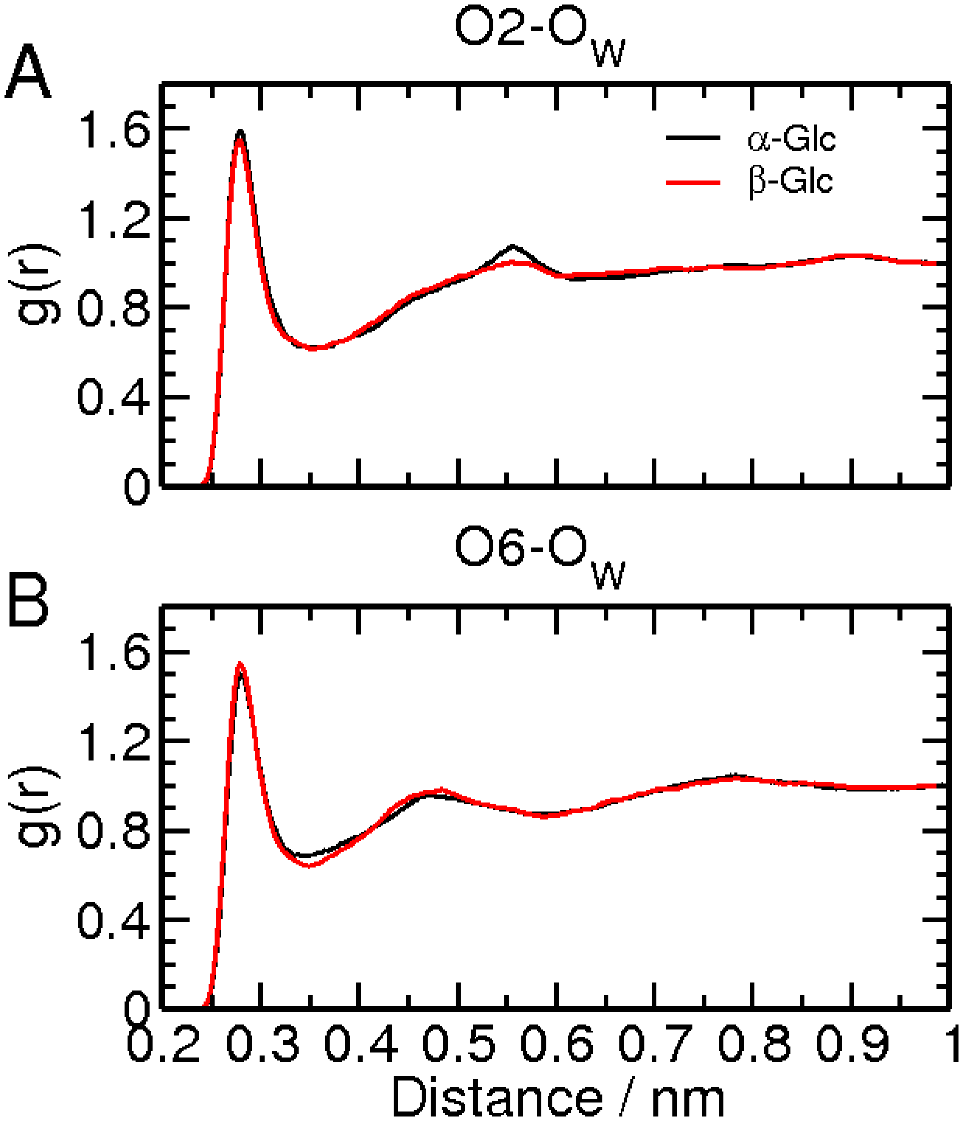


α-glucose



β-glucose

Radial distribution function –Solvation by Water



No Significance difference between α-glucose and β-glucose

Influence of Alkali Metal on stereoisomers of glucose

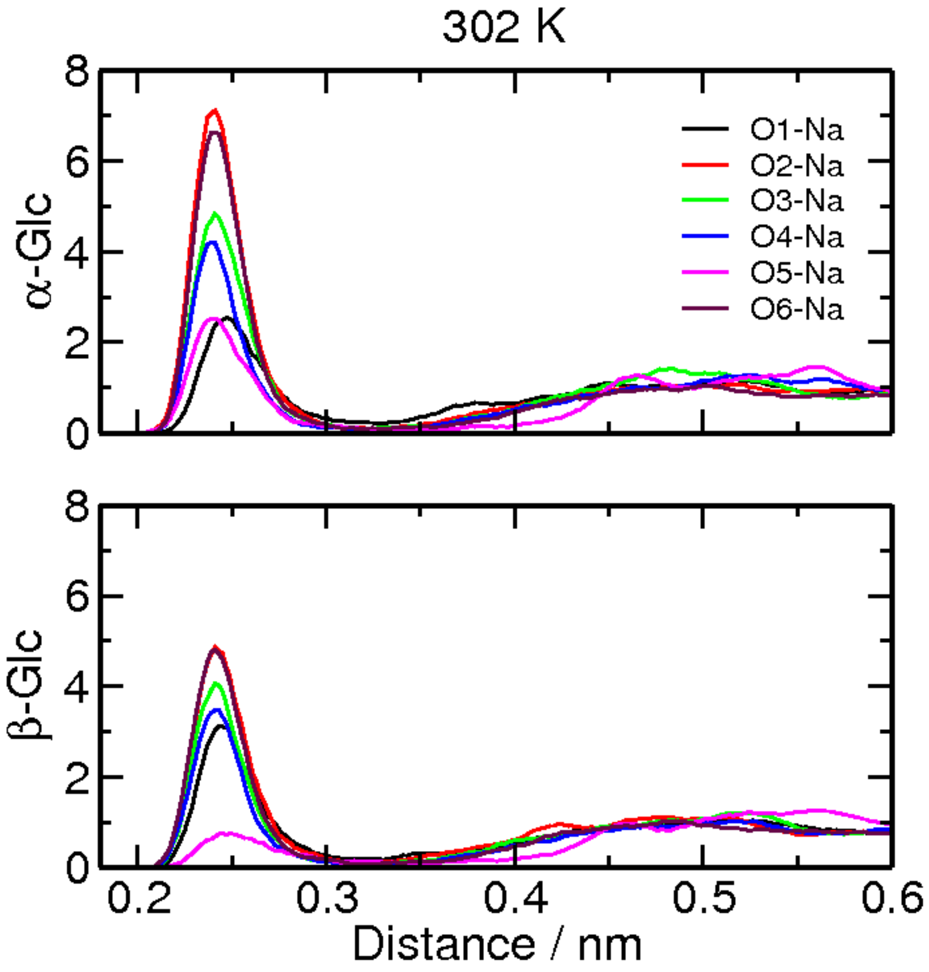
Alkali metal effects that depend on stereochemistry

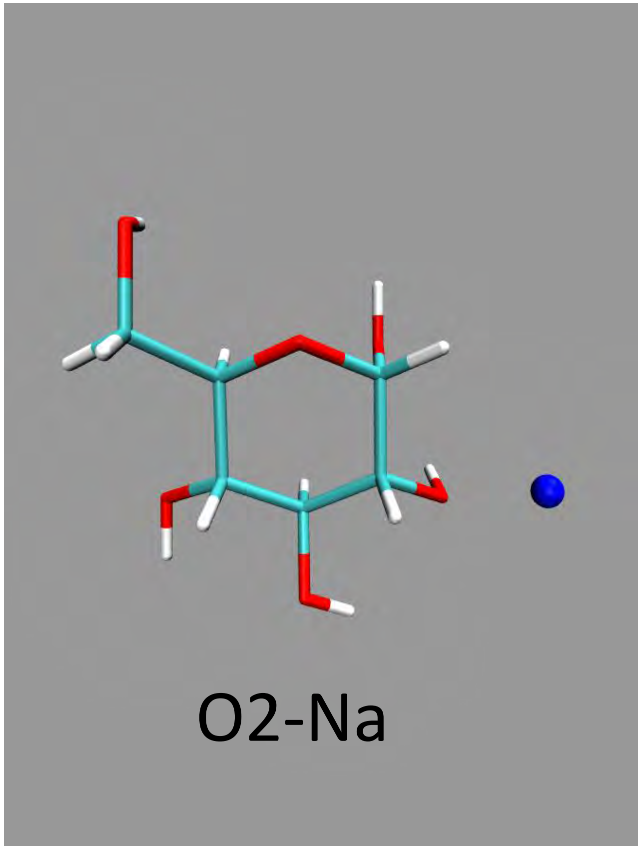
- Coordination with Alkali Metals

Alkali metal effects that didn't depend on stereochemistry

- Conformations of Glucose

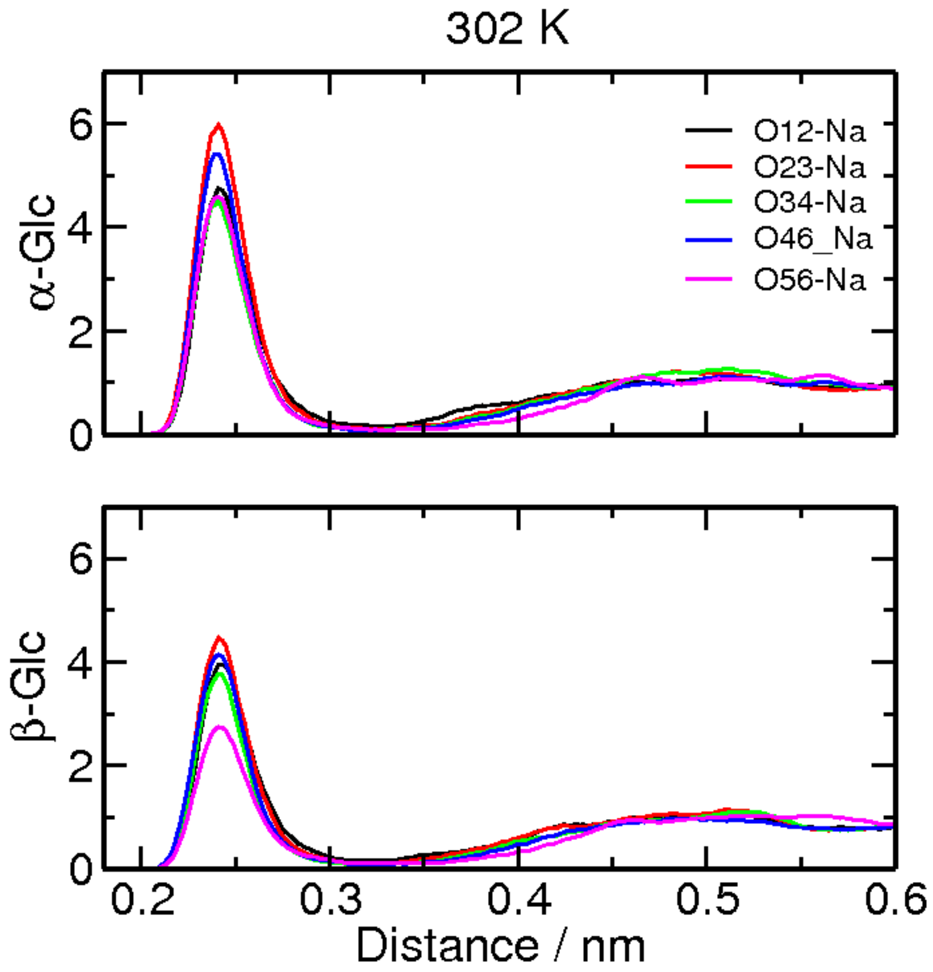
Single Coordination to Alkali Metal

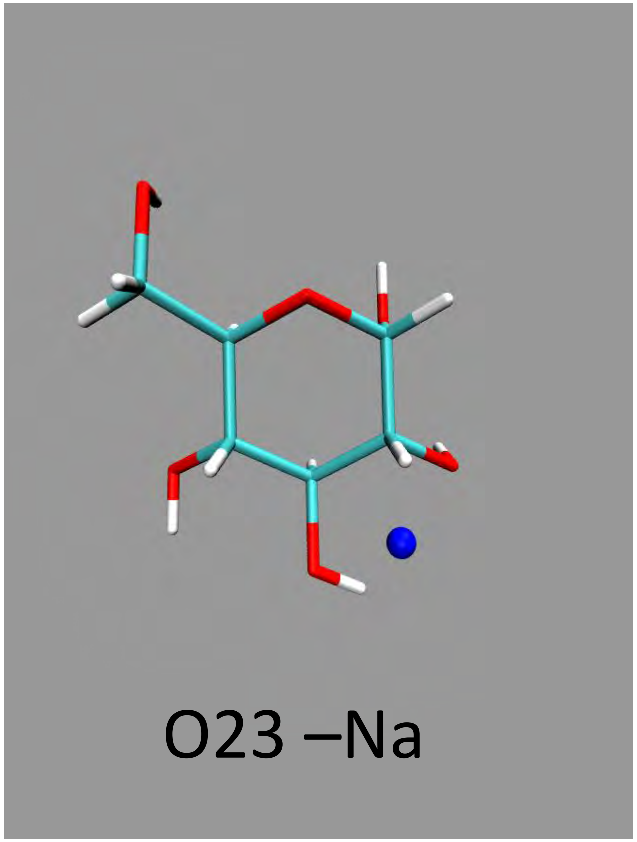




- **Affinity of Na to hydroxyl or hydroxymethyl oxygen: O2>O6>O3**
- **Affinity for β-glucose is lower, and the order is slightly different: O2≈O6>O3**

Bridged Coordination to Alkali Metal





- **Several bridging coordination positions with Na+ possible**
- **However, probabilities are lower for β-glucose**

Summary – Part I

- Reactivity trends of diverse linkages and substitutions of lignin model compounds

Ongoing Work

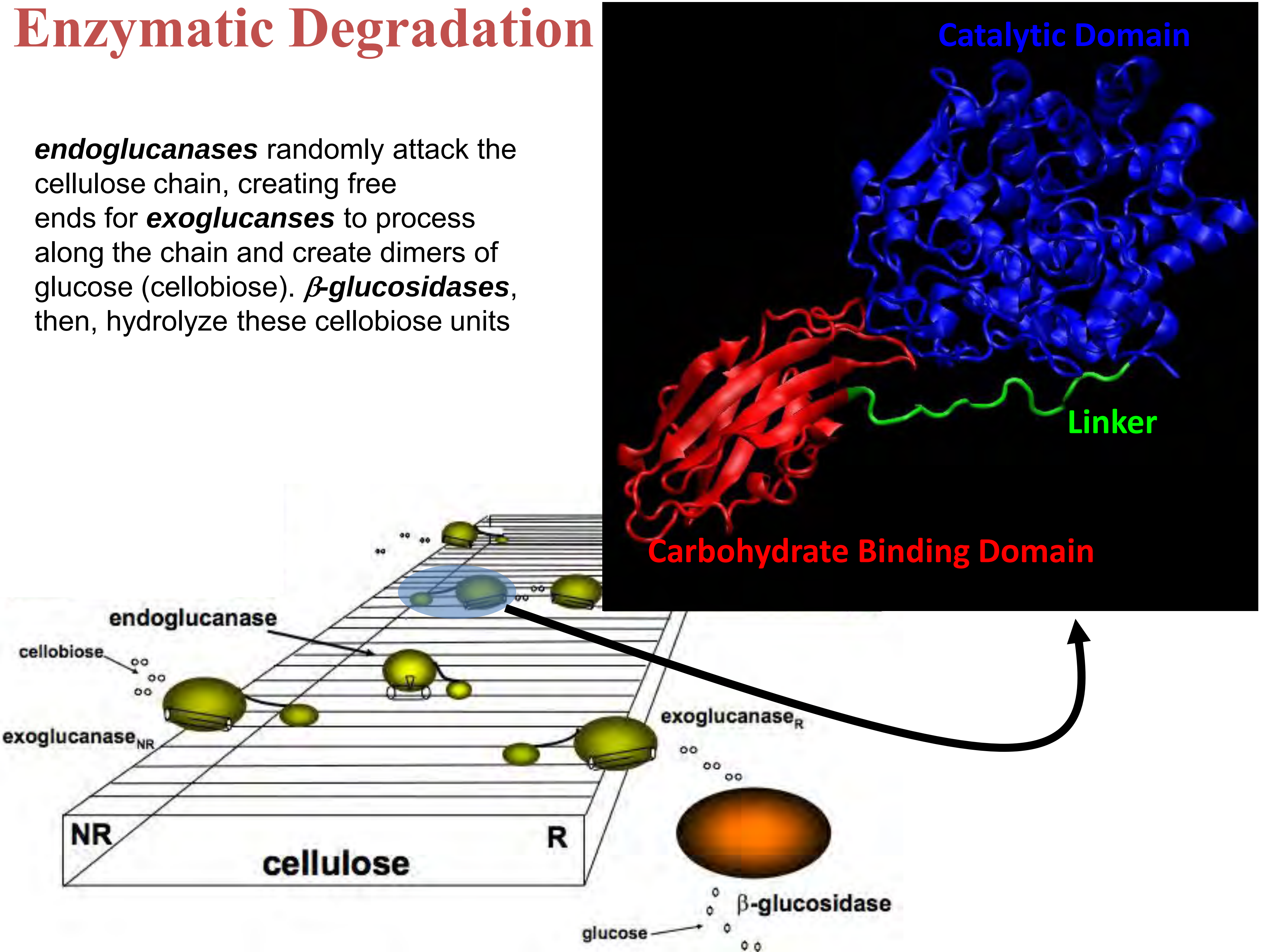
- Interactions of bi-benzyl with $(CaO)_2$ metal oxide have been studied using DFT approach to understand mechanistic insights involved in the catalytic pyrolysis reactions

- Acid Catalyzed reactions of beta5 model compounds

- Effect of Na+ on cellulose pyrolysis

Enzymatic Degradation

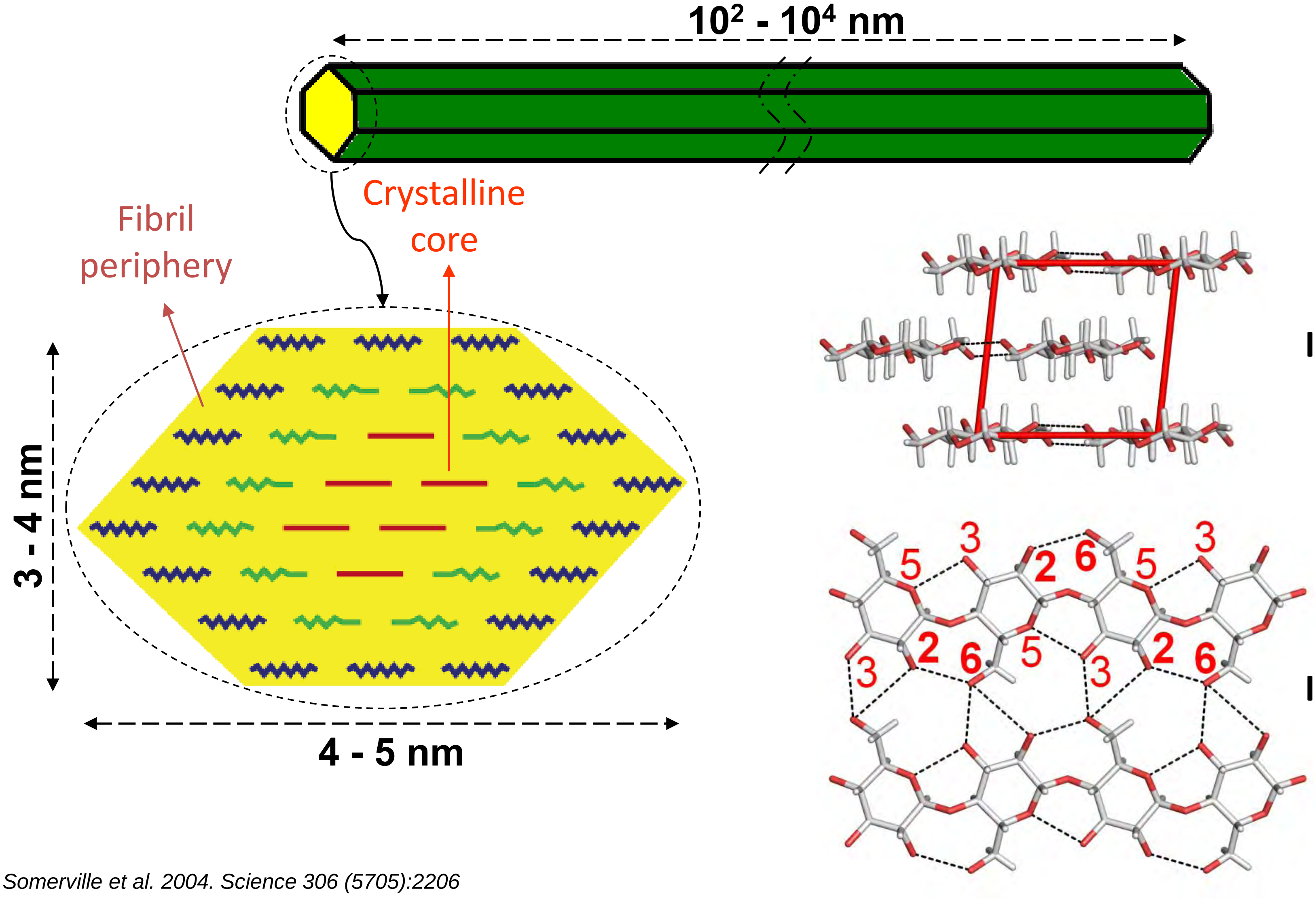
endoglucanases randomly attack the cellulose chain, creating free ends for ***exoglucanses*** to process along the chain and create dimers of glucose (cellobiose). ***β-glucosidases***, then, hydrolyze these cellobiose units



PART II.

Cellulose Properties responsible for Recalcitrance to digestion

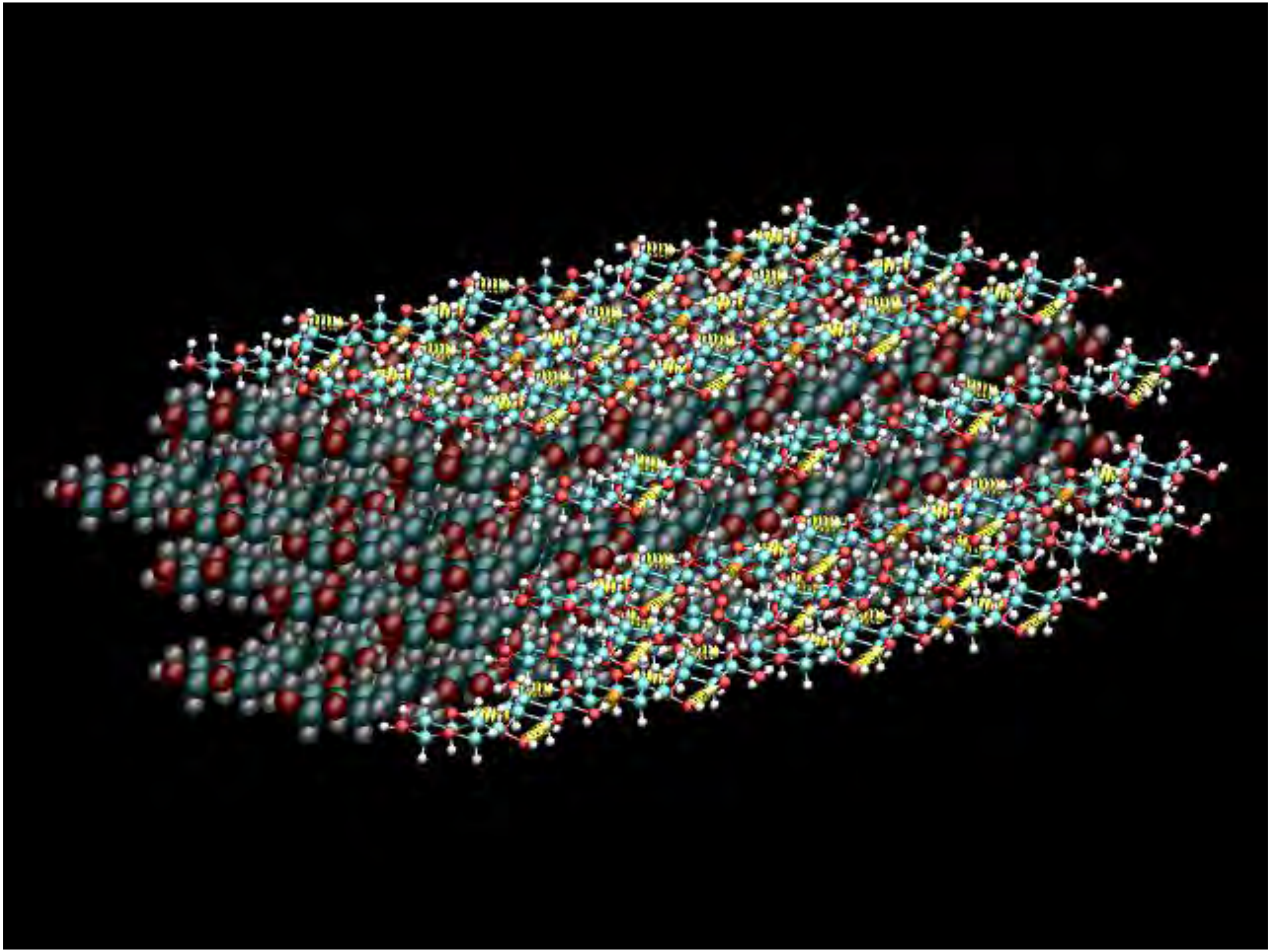
Native plant-derived cellulose I microfibrils



Cellulose I_{β}

Somerville et al. 2004. Science 306 (5705):2206
Chundawat et al., 2011, J Am Chem Soc, 133, 11163
Ding et al., 2006, J Agric Food Chem, 54, 597

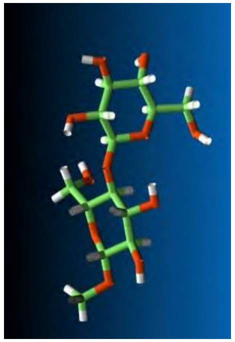
Cellulose – A Dynamical System

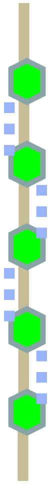


Movie:
Al French
(USDA)

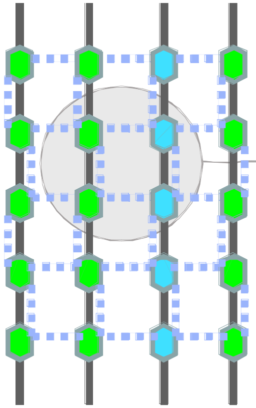
Giovanni Bellesia, Andrea Asztalos, Tongye Shen, Paul Langan, Antonio Redondo and S. Gnanakaran. Computational studies of crystalline cellulose and its degradation by enzymes. Acta Crystal. D. (2010).

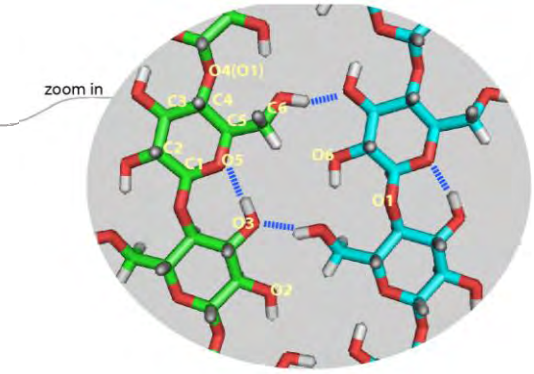
Multiscale modeling of cellulose assembly/disassembly



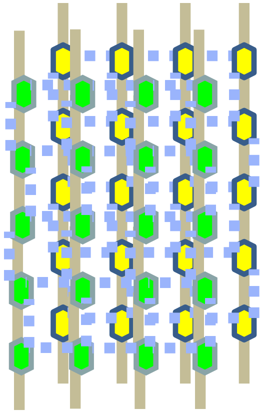


intra-strand H-bonding:
All-atom MD Simulation
Enhanced Sampling

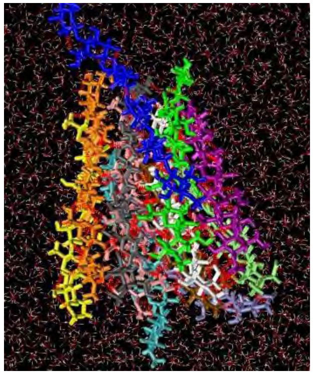


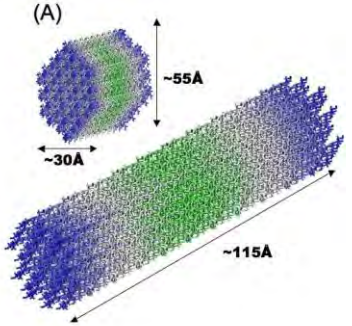


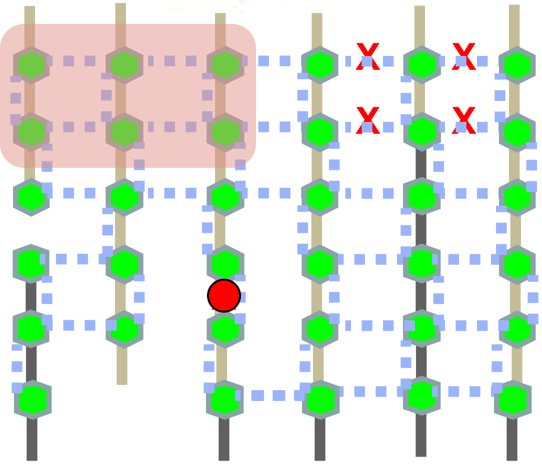
2D sheet formation:
Inter vs intra H-bonding
Coarse-grained Model



3D fiber formation:
Replica Exchange
MD Simulations







Enzymatic degradation:
All-atom MD simulations
Agent Based Modeling

Initial Studies

Coarse-grained Hydrogen Bond Network Model for Crystalline Cellulose

The multiplicity in H-bond network and switching between different H-bond networks with increasing temperature make the cellulose assembly very stable

Biophysical Journal

Volume 96
Number 8
April 22, 2009

www.biophysj.org

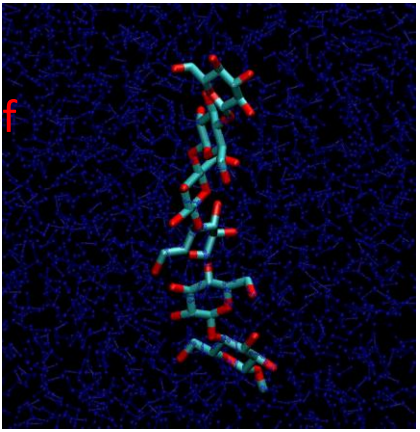
Biophysical Society

Published by Cell Press
for the Biophysical Society

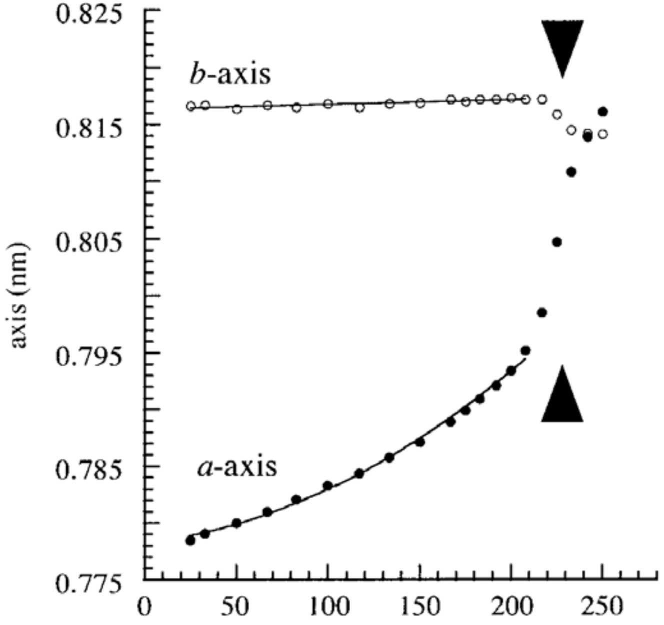
"The stability of cellulose: a statistical perspective from a coarse grain model of hydrogen bonding"
Shen, T., Gnanakaran, S. Biophysical Journal 96, 3032-3040 (2009).

All-atom molecular dynamics of Cellulose Oligomers and their assembly

- Effective sampling of structural properties cellulose including ring flips
- Mechanical properties- chains are getting less flexible with increasing # of monomers



Conformational flexibility of soluble cellulose oligomers: Chain Length & T-dependence. Tongye Shen, Paul Langan, Alfred D. French, Glenn P. Johnson and S. Gnanakaran. J. Am. Chem. Soc., 131: 14786 (2009).

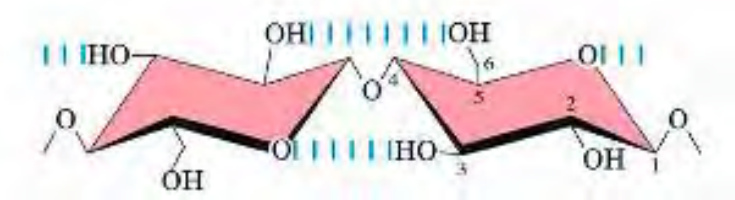


M. Wada, JPSB (2002)

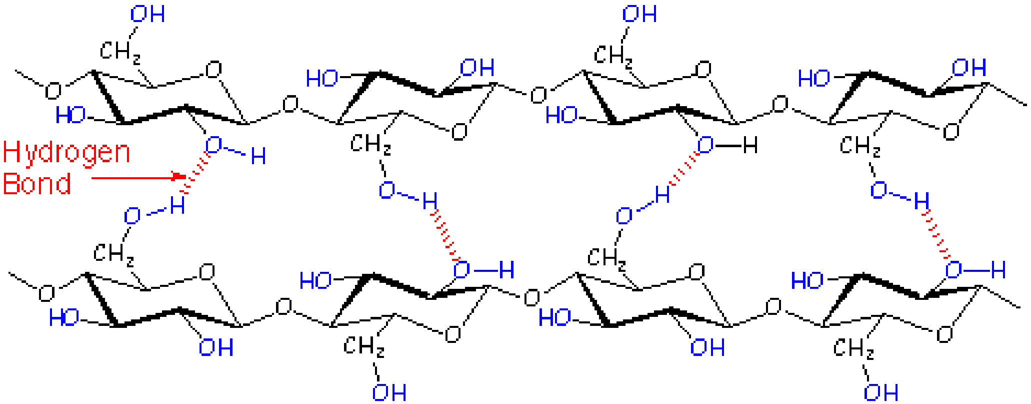
Why is cellulose so stable?

Stability of cellulose fibers is due to

- ***Intra***-molecular hydrogen bonding

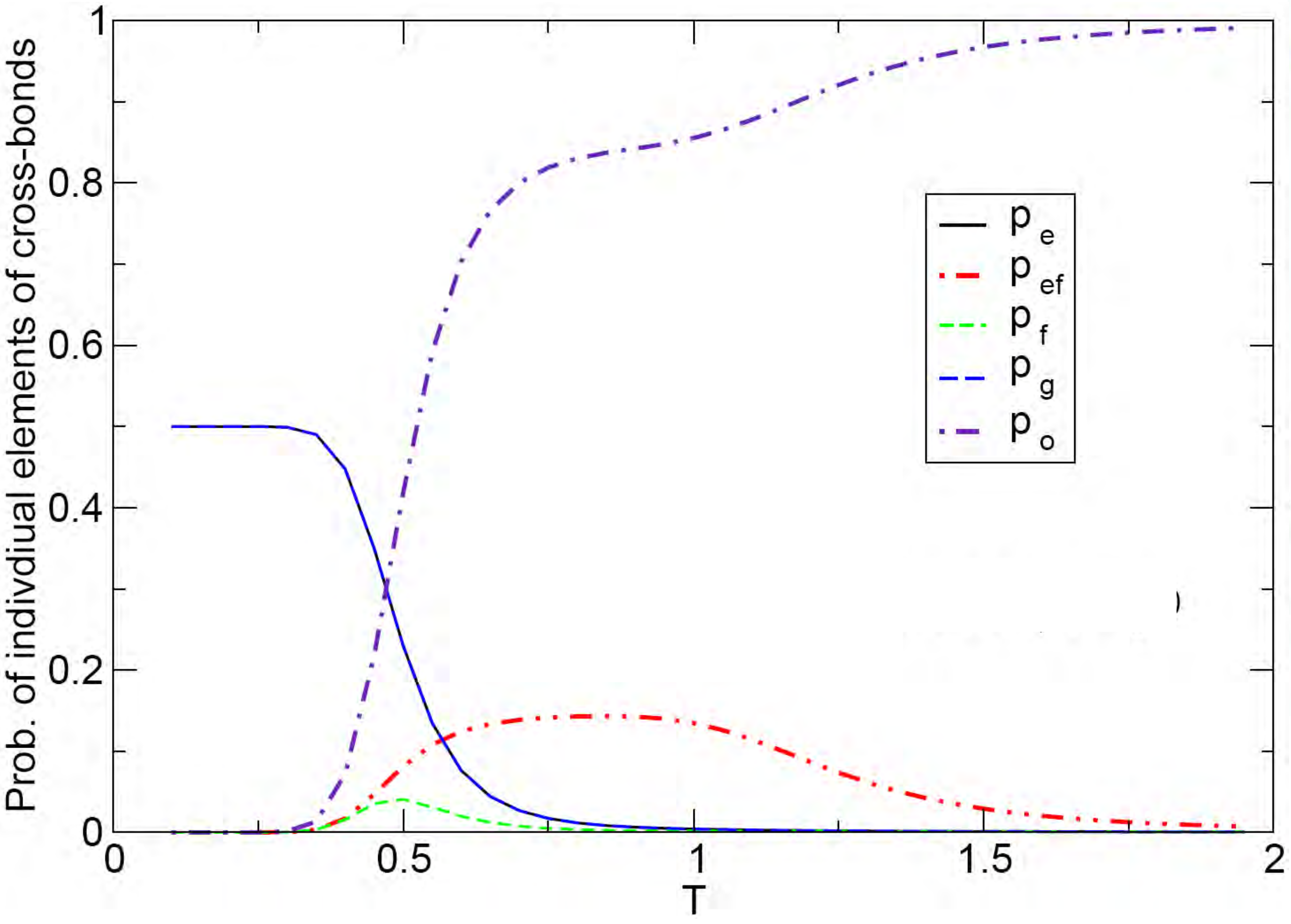


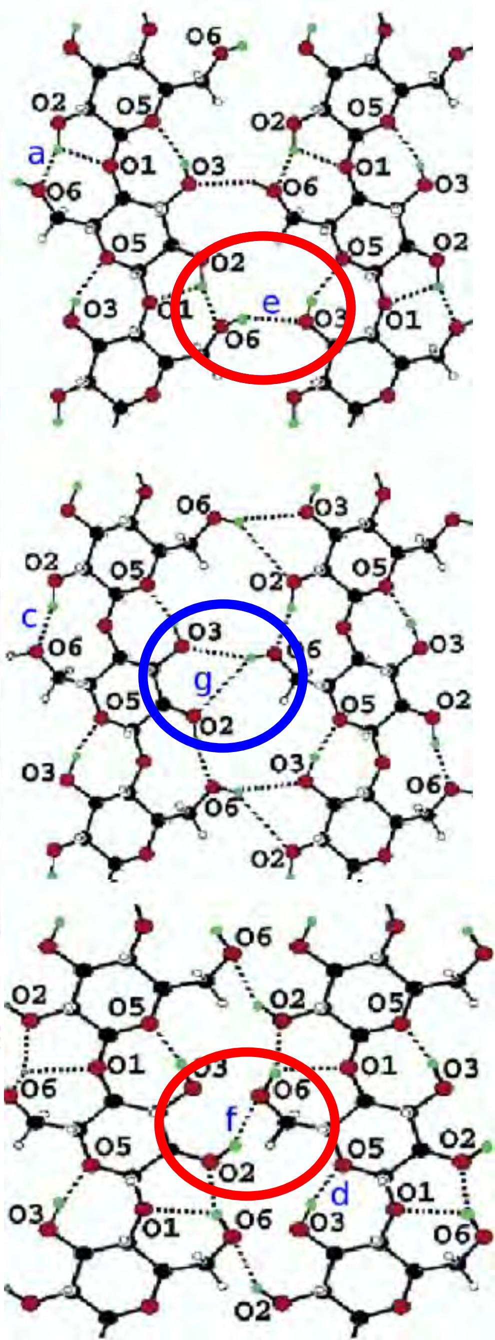
- ***Inter***-molecular hydrogen bonding



Cross-strand H-bonding elements

Total 5 cross-strand bonding states: ø_, e, f, g, e+f

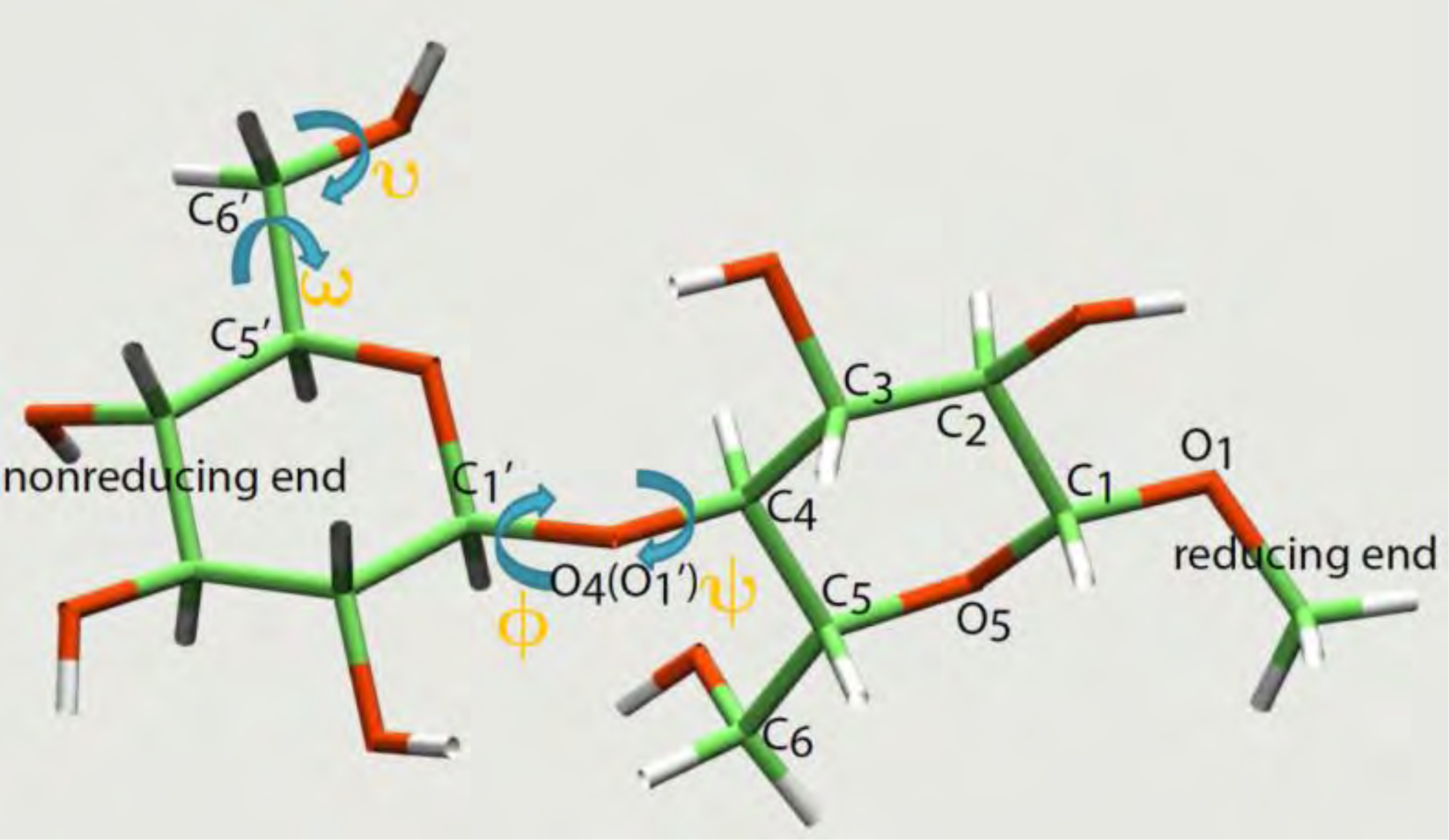


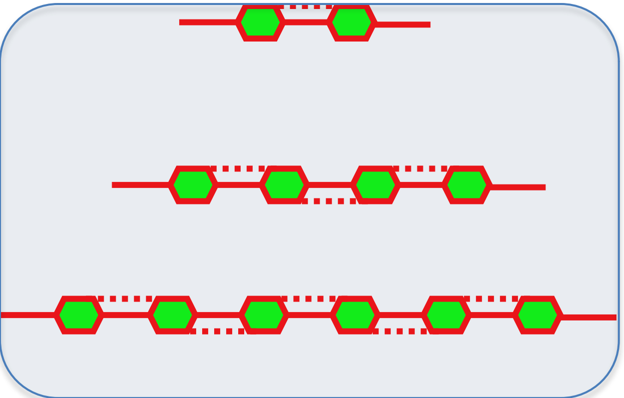


At low T, either O3….HO6 or [O3….HO6, O2….HO6]

As T increases, a switch to O3….HO6 & O6….HO2

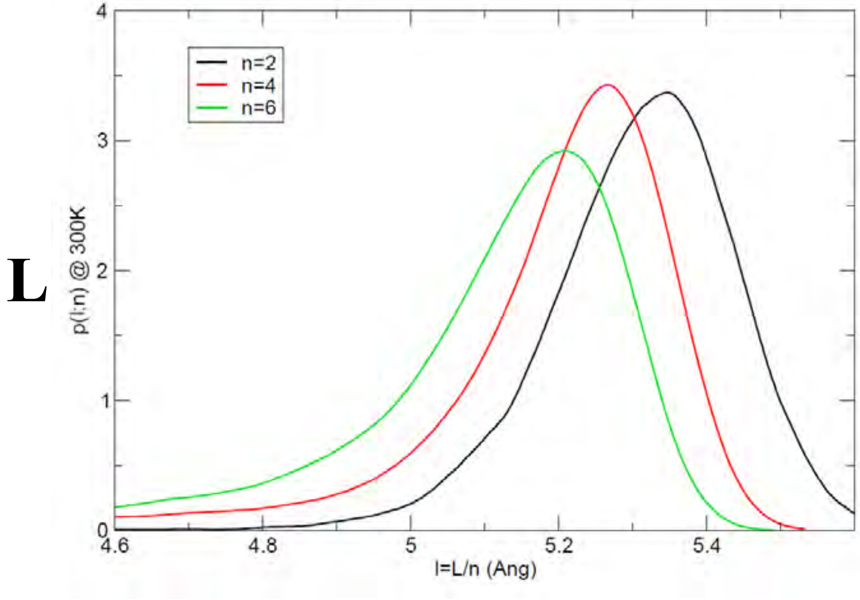
"The stability of cellulose: a statistical perspective from a coarse grain model of hydrogen bonding" Shen, T., Gnanakaran, S. Biophysical Journal 96, 3032-3040 (2009).

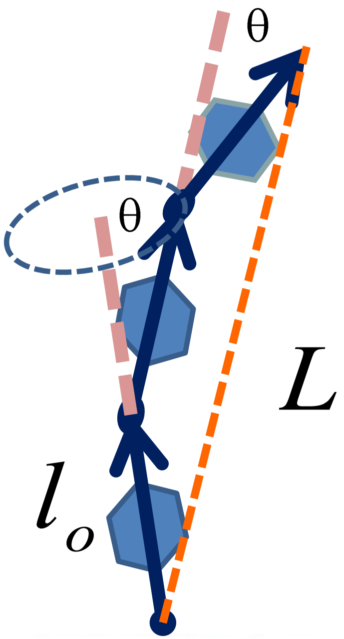




Single chains (2mer, 4mer, 6mer):
Torsions
Flexibility
Ring flips

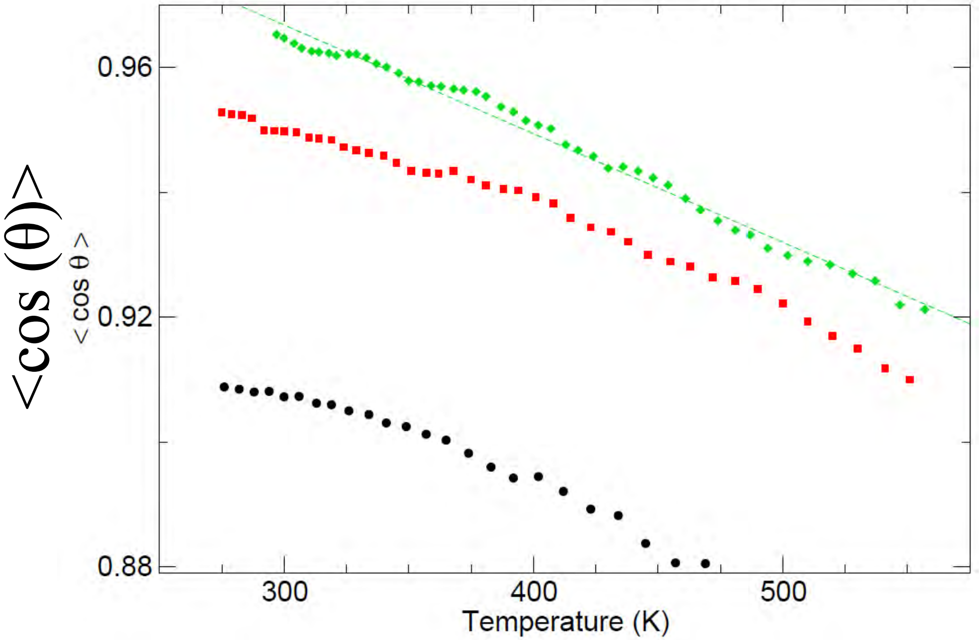
Chains (2mer, 4mer, 6mer): End-to-end distances and Flexibilities





Freely Rotating
Chain Model

Rigidity:
6mer > 4mer > 2mer

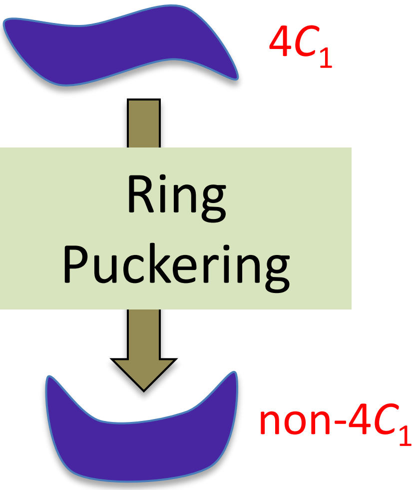


$$\langle \cos q \rangle (T) \gg 1 - k_B T / c$$

$$c = 47.8 \quad kJ/mol$$

Persistence length, $x = l_o \times c / k_B T \gg 10.5\,nm$

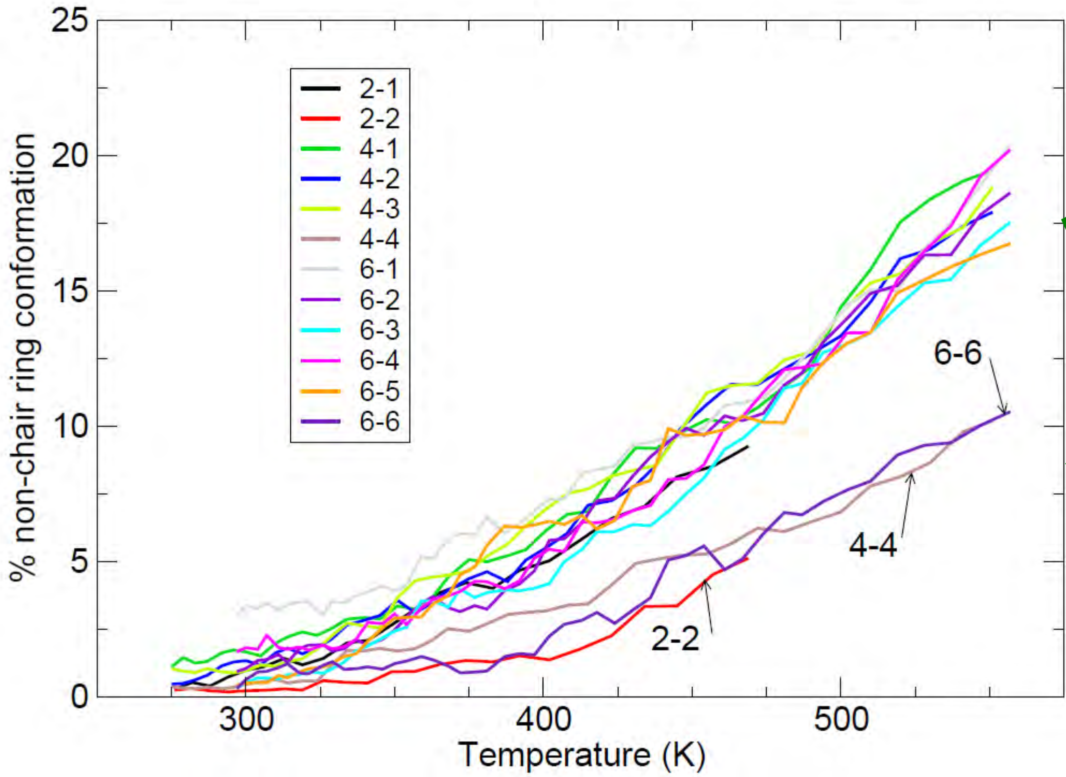
Ring Flipping Tendencies in Cellulose Oligomers

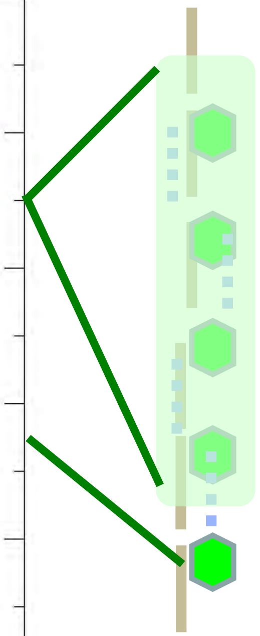


$4C_1$

Ring Puckering

non-$4C_1$

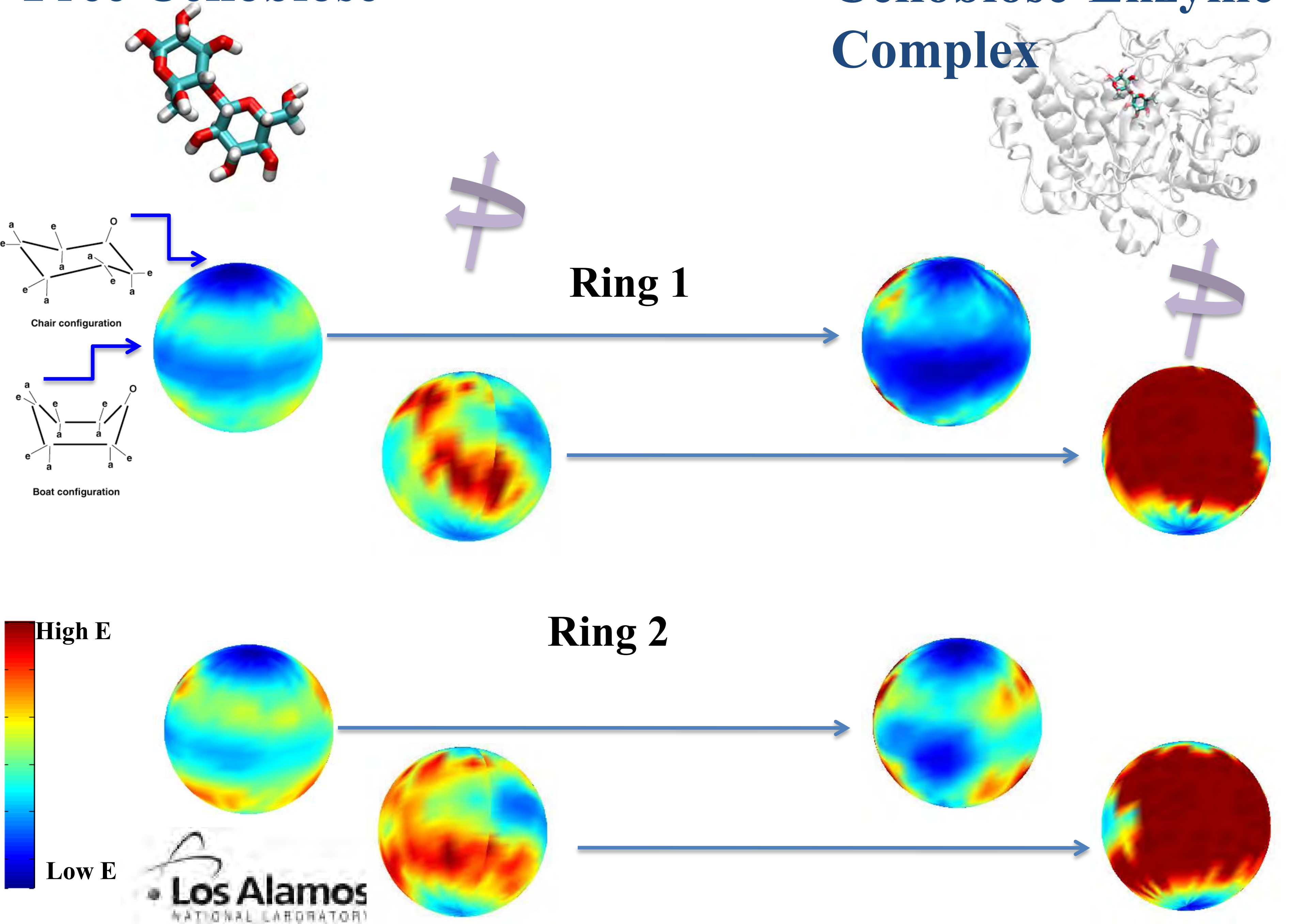




Rings at the non-reducing end is more stable

Free Cellobiose

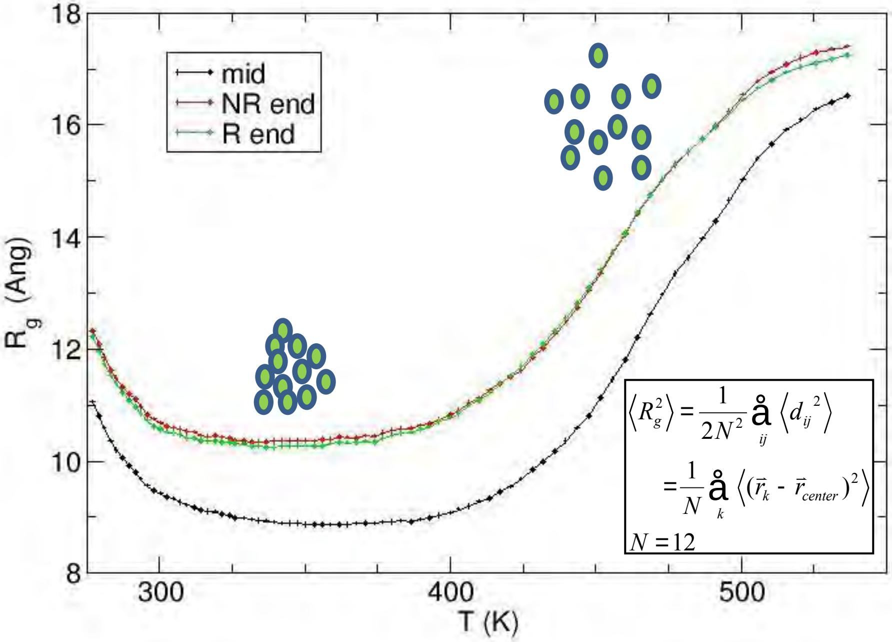
Cellobiose-Enzyme Complex



Ring 1

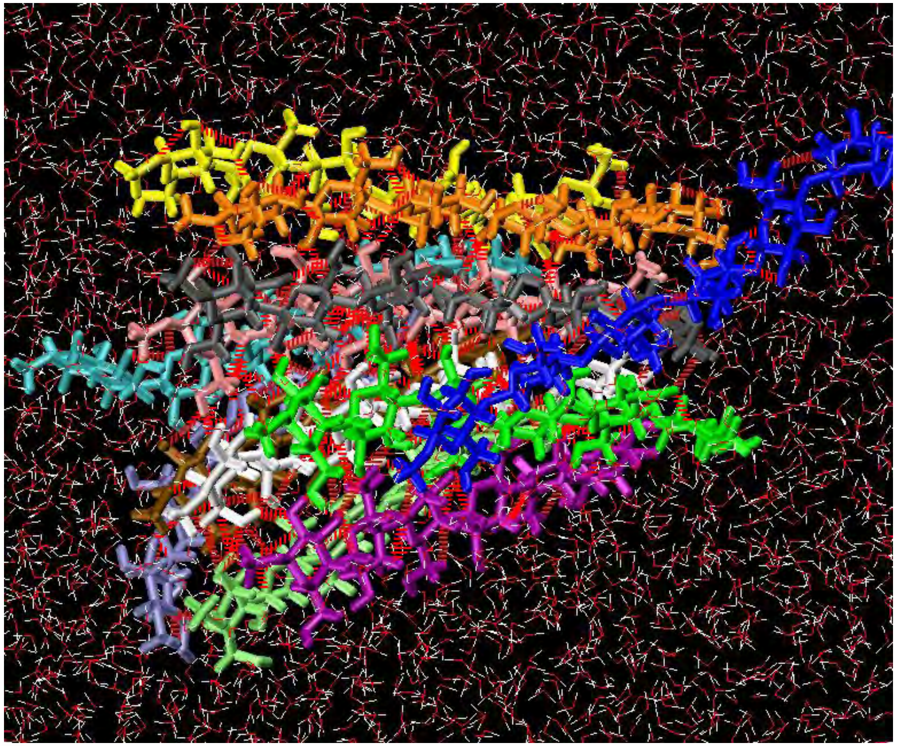
Ring 2

“Fiber Formation”



$$\langle R_g^2 \rangle = \frac{1}{2N^2} \sum_{ij} \langle d_{ij}^{\,2} \rangle = \frac{1}{N} \sum_{k} \langle (\vec{r}_k - \vec{r}_{center})^2 \rangle$$

$N = 12$

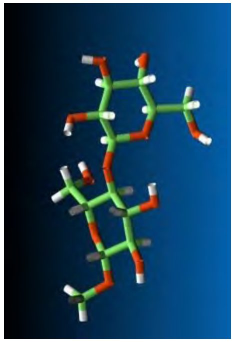


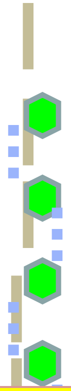
- At high T (>460K), the system is in a random configuration with little order; while at lower T, the system is in a much more ordered state.

- At low T (<310K), all factors indicate the system is going to a different state.

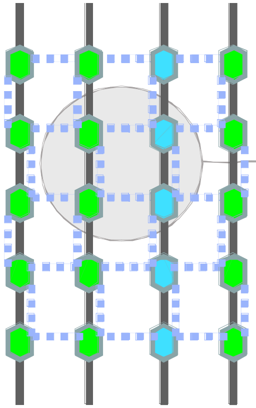
Summary PART I I

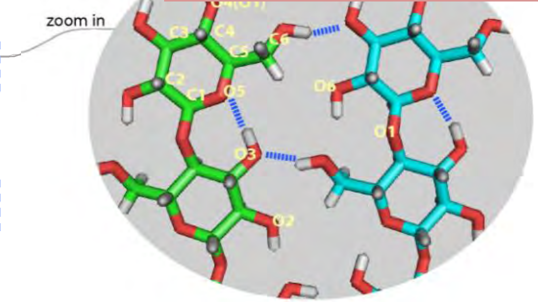
Multiscale models of cellulose



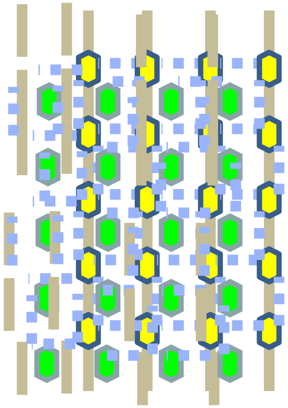


Rigidity increases as chain length increases & Ring Flipping Tendencies

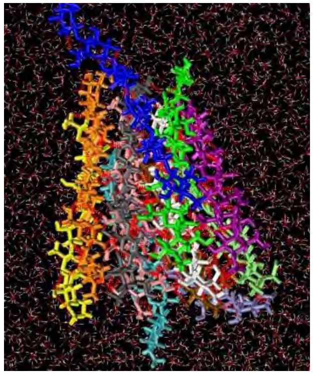


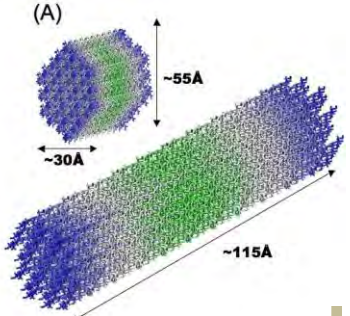


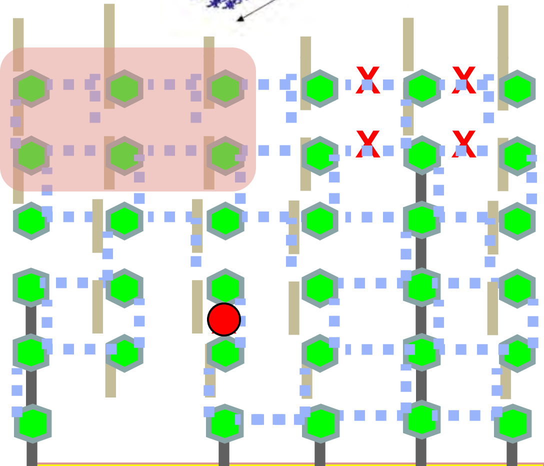
Ability to form Multiple Hydrogen Bond Networks



Self assembly of chains





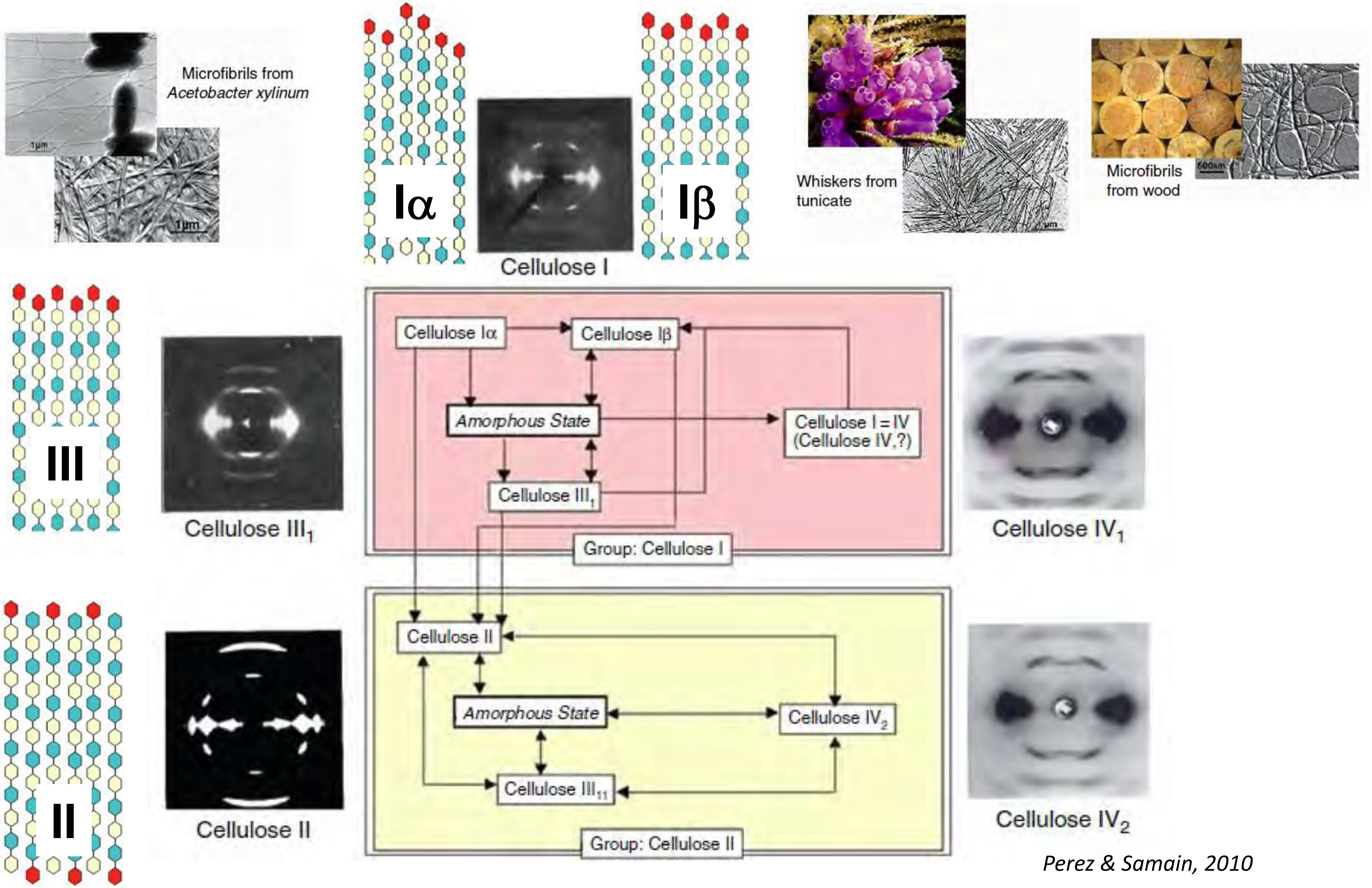


Capturing synergy of enzyme cocktails with Rule-based Model

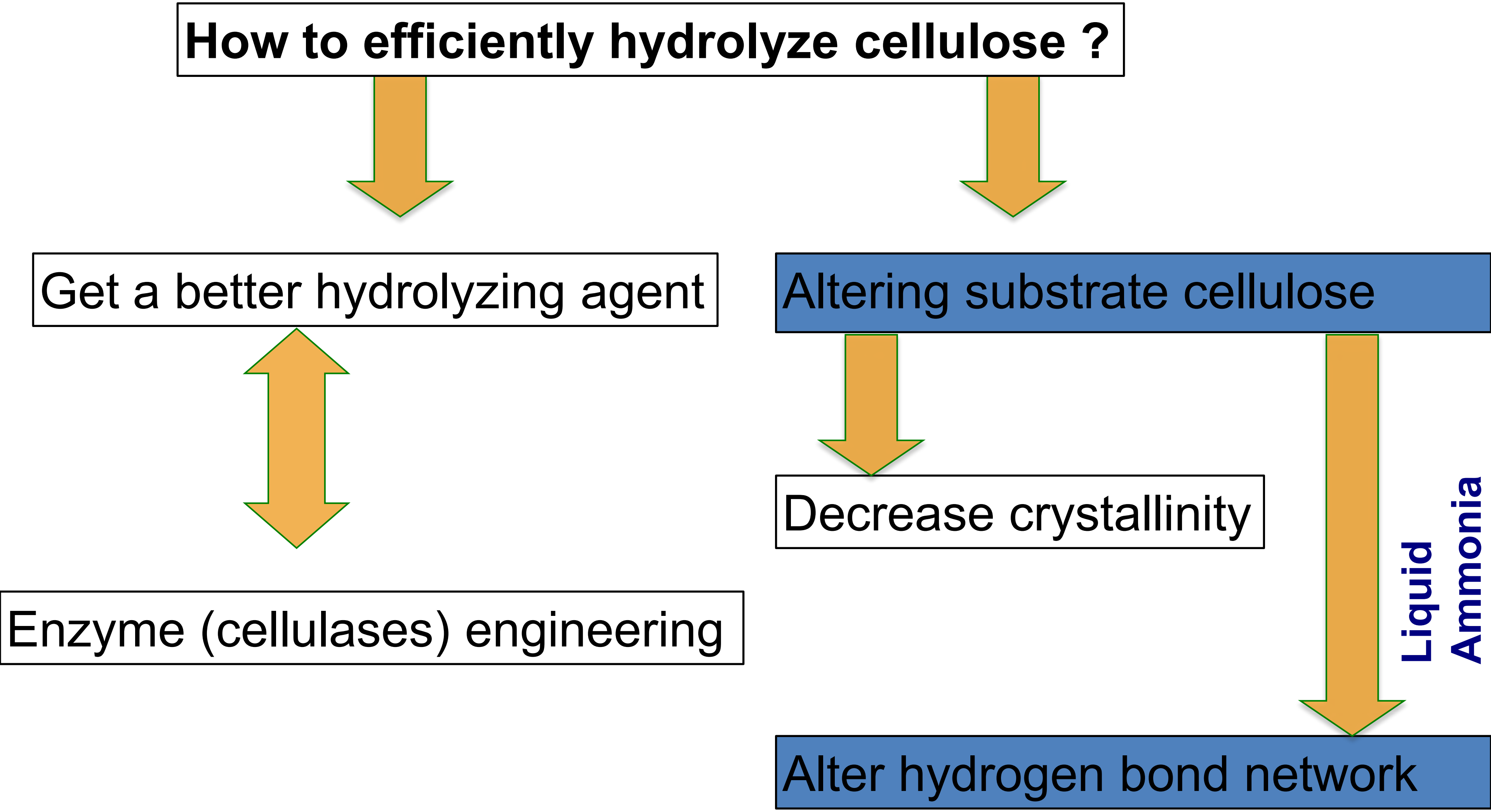
PART III.

Alter cellulose substrate for efficient digestion

Cellulose can exist in various crystalline forms



Perez & Samain, 2010



How to efficiently hydrolyze cellulose ?

Get a better hydrolyzing agent

Enzyme (cellulases) engineering

Altering substrate cellulose

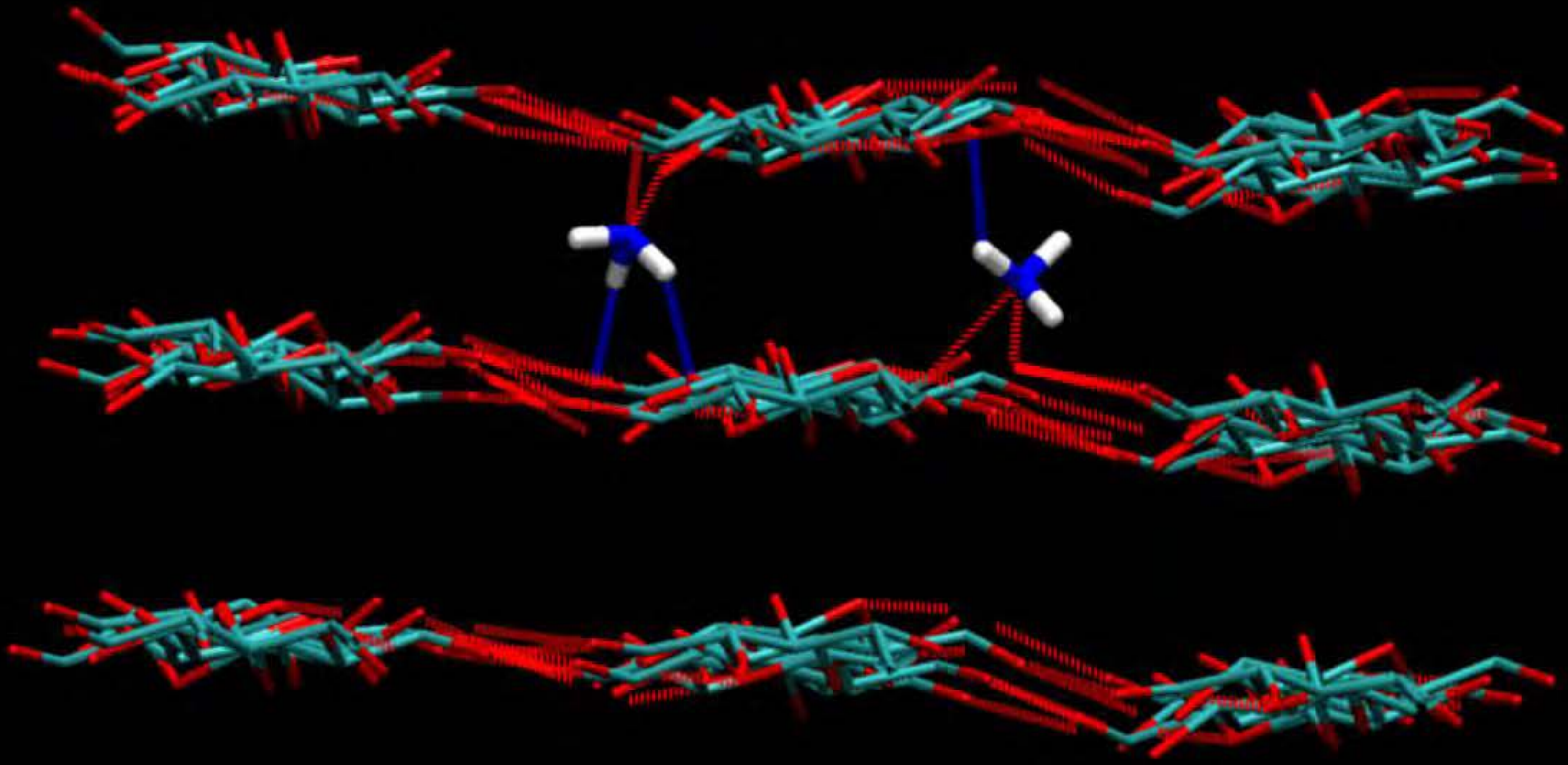
Decrease crystallinity

Liquid Ammonia

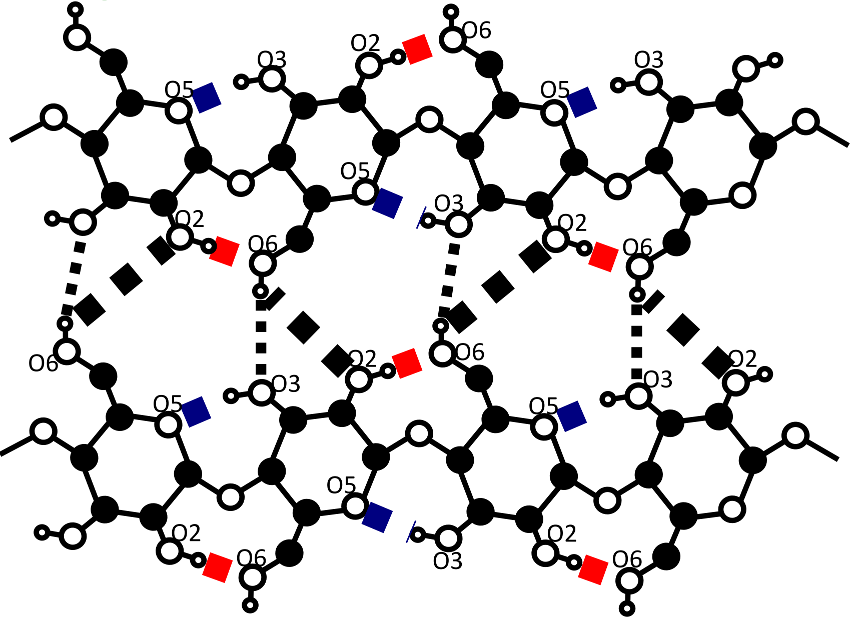
Alter hydrogen bond network

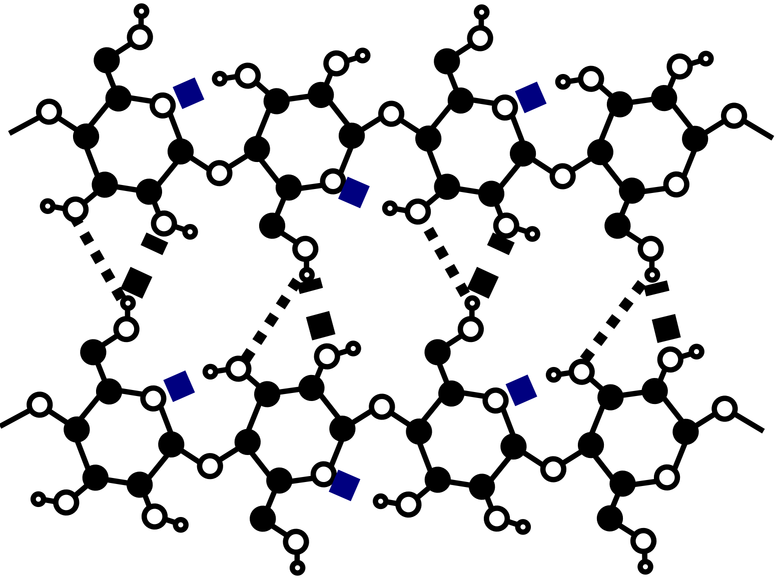
Rearrangement of H-bonding during treatment of cellulose with NH_3

Masahisa Wada, Yoshiharu Nishiyama, Giovanni Bellesia, Trevor Forsyth, S. Gnanakaran and Paul Langan. Neutron crystallographic and molecular dynamics studies of the structure of ammonia-cellulose I: rearrangement of hydrogen bonding during the treatment of cellulose with ammonia. Cellulose. 18, **2011**, 191-206, 9488-5

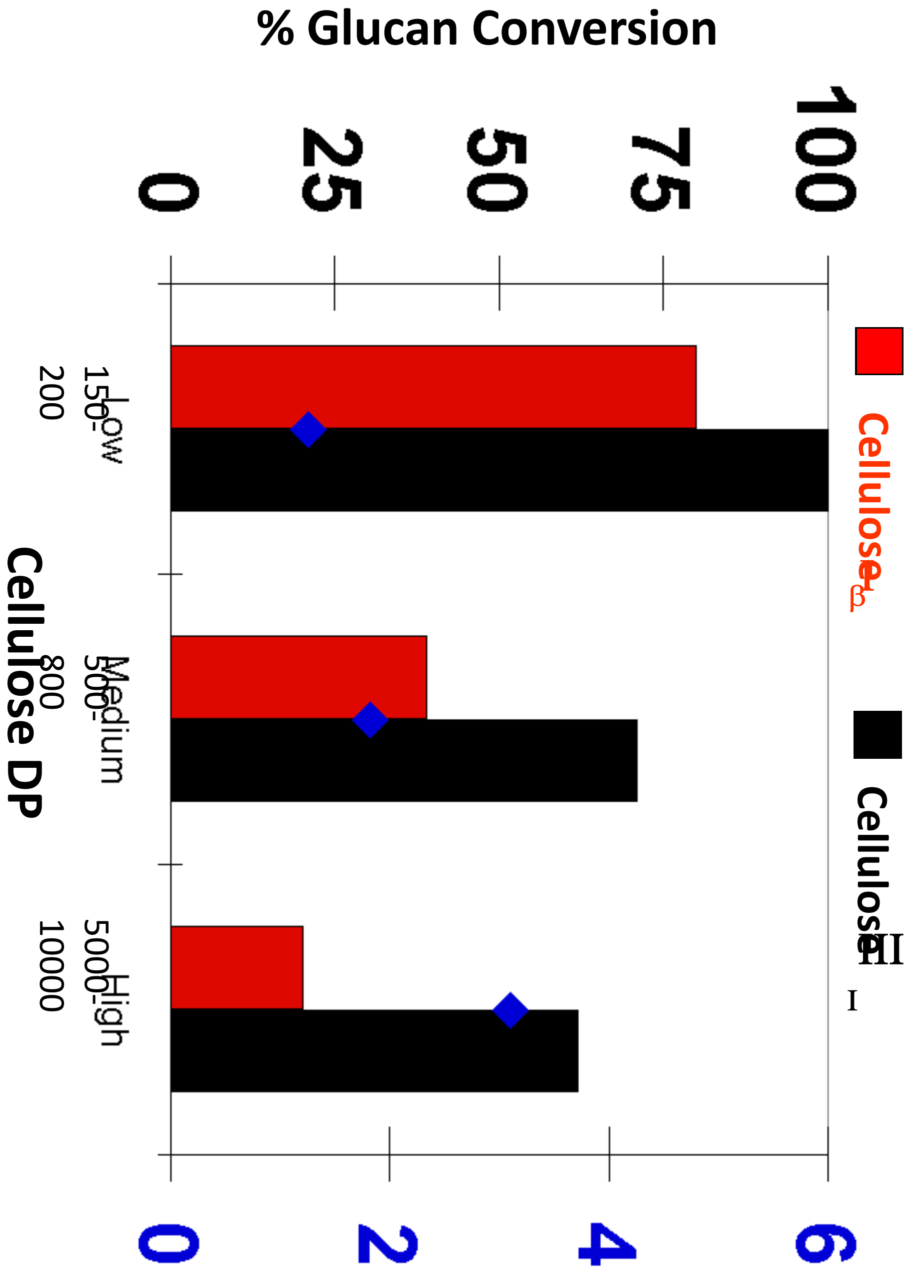


Liquid Ammonia Pretreatment



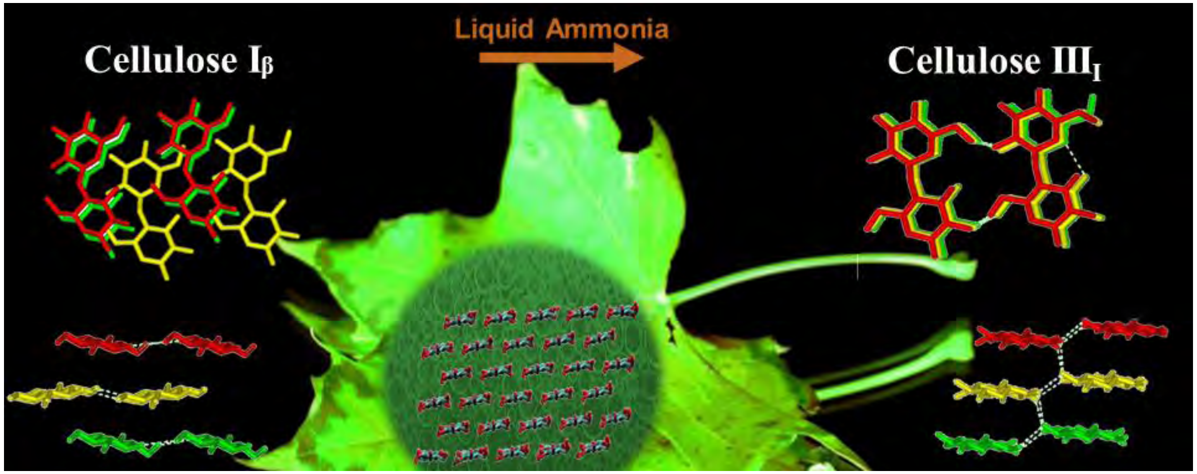


Nishiyama et al., Biomacromolecules 9(11), 3133-3140 (2008)



DOE Great Lakes Bioenergy Center

Why is it easier to digest cellulose III?



RESTRUCTURING CELLULOSE HYDROGEN BOND NETWORK

Chemical Properties:

Quantum Mechanical Calculations

(JPC A , **2011**, 115 pp. 14191-14202)

Dynamical Properties:

All-atom Molecular Dynamics

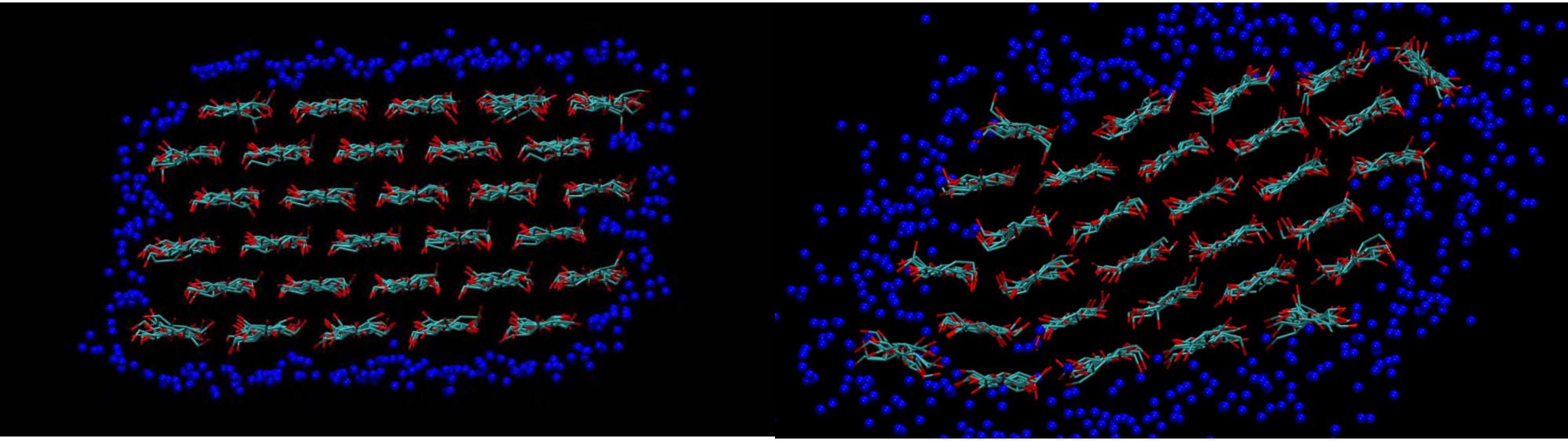
(JACS, **2011**, 133 pp. 11163-11174)
(JPC B , **2011**, 115 pp. 9782-9788)

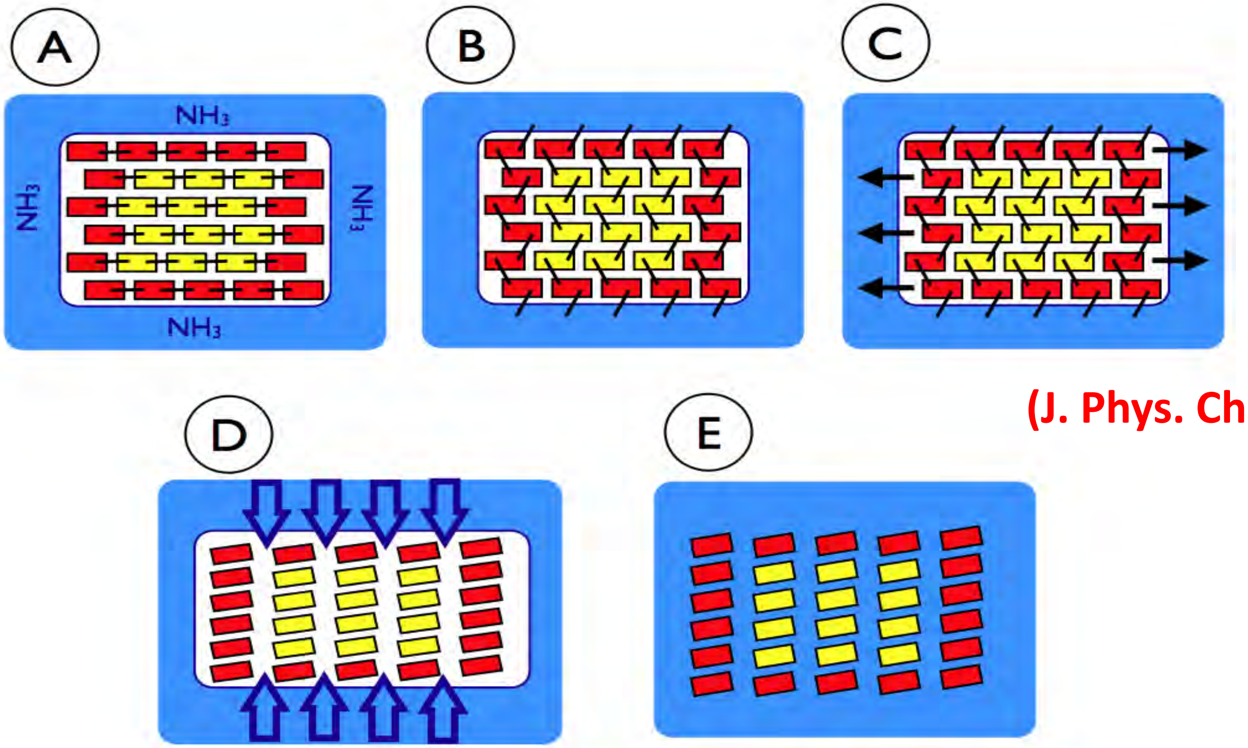
Bulk Properties:

Coarse-grained Description

(JPC B , **2012**, PMID: 22712833)

Initial Molecular Events Associated with Liquid NH_3 Treatment

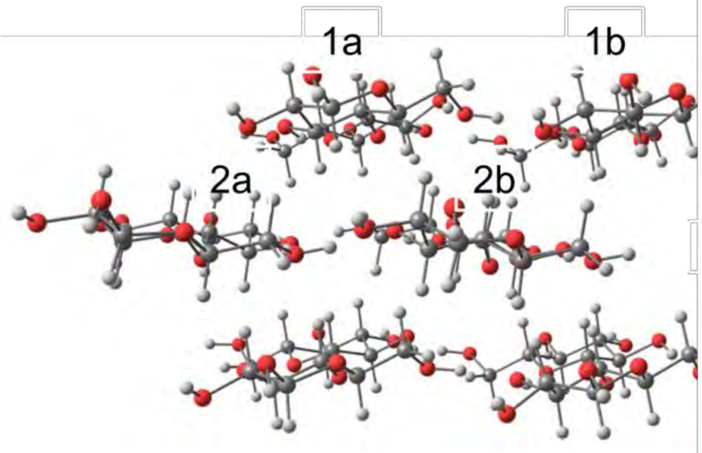


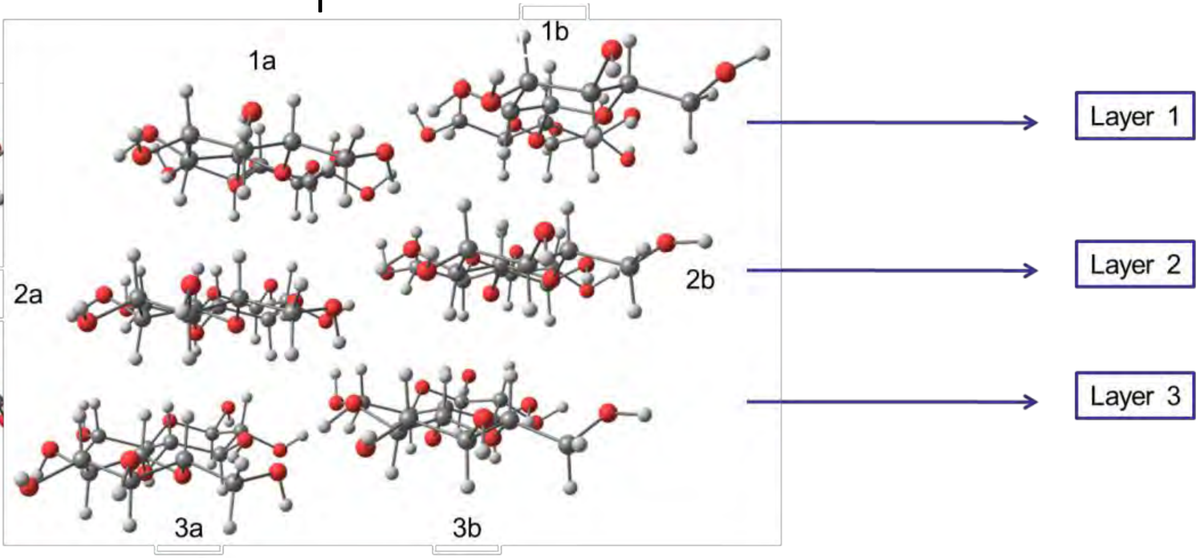


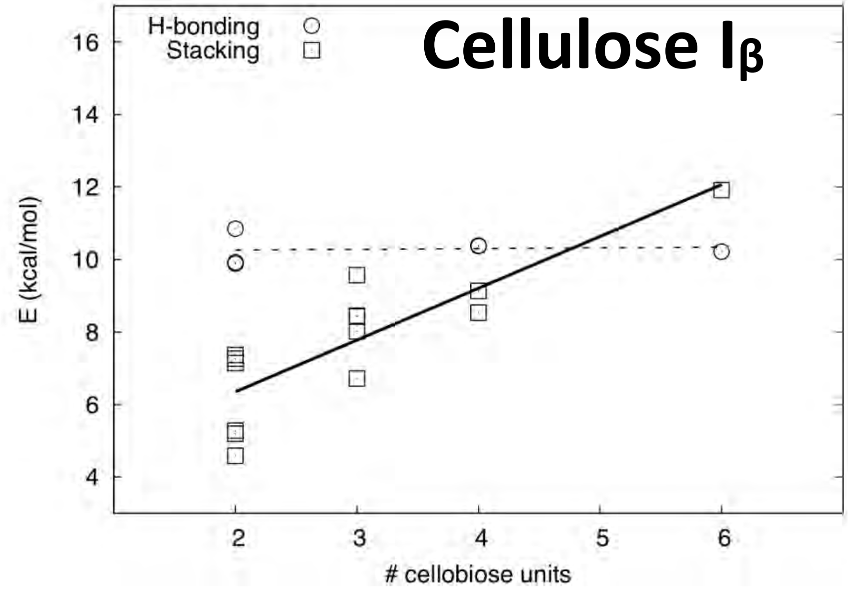
(J. Phys. Chem. B, 2011, 115 pp. 9782-9788)

Hydrogen Bonding .vs. Stacking Interactions

Cellulose Iβ and Cellulose III$_I$ Model conformations






Cellulose Iβ

All calculations were carried out using hybrid DFT m062X/6-31+G(d,p) using G09

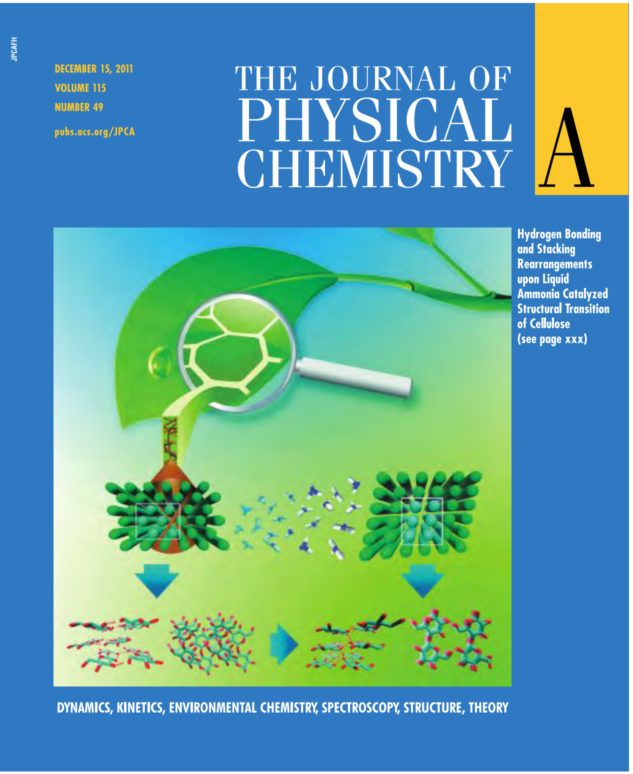
Interplay between H-bonding and Stacking

Cellulose I_{β}

- Strong Hydrogen bonding interactions within a sheet
- Highly cooperative stacking between sheets

Cellulose III_{1}

- Slightly weaker hydrogen bonding but cooperative
- Non-cooperative stacking interactions



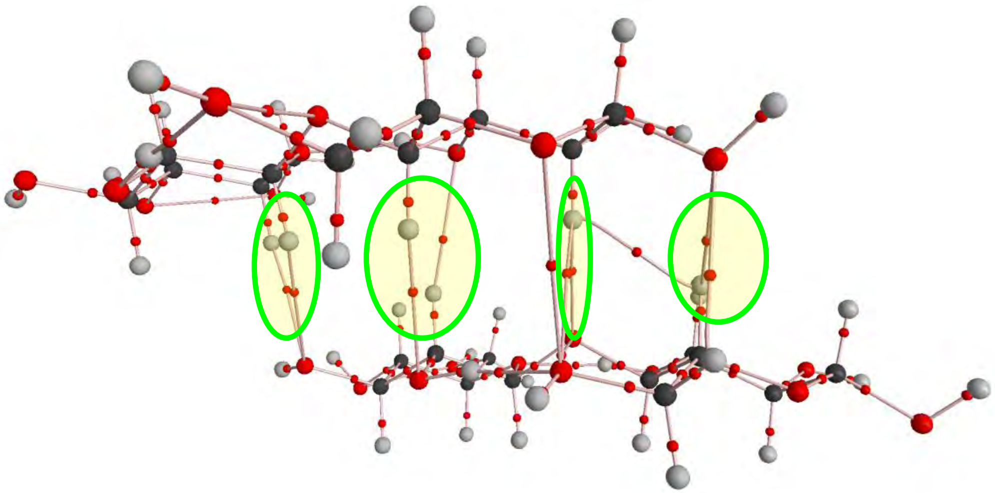
JPCAFH

DECEMBER 15, 2011
VOLUME 115
NUMBER 49
pubs.acs.org/JPCA

THE JOURNAL OF PHYSICAL CHEMISTRY A

Hydrogen Bonding and Stacking Rearrangements upon Liquid Ammonia Catalyzed Structural Transition of Cellulose (see page xxx)

DYNAMICS, KINETICS, ENVIRONMENTAL CHEMISTRY, SPECTROSCOPY, STRUCTURE, THEORY

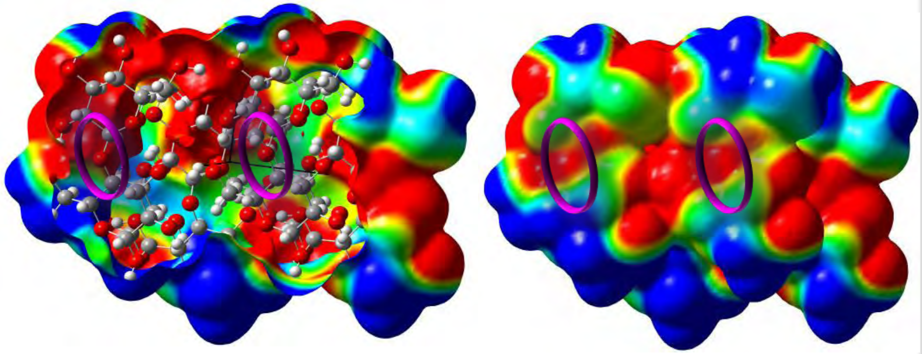


R. Parthasarathi, G. Bellesia, S.P.S. Chundawat, B. E. Dale, P. Langan, and S Gnanakaran. *The Journal of Physical Chemistry A* , 115:14191-202 (2011) – Highlighted in Cover.

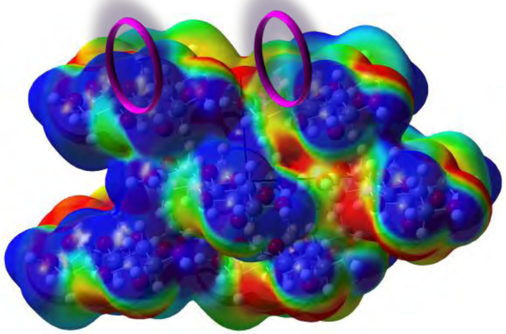
Molecular Electrostatic Potential Map

Cellulose I$_\beta$

Side view

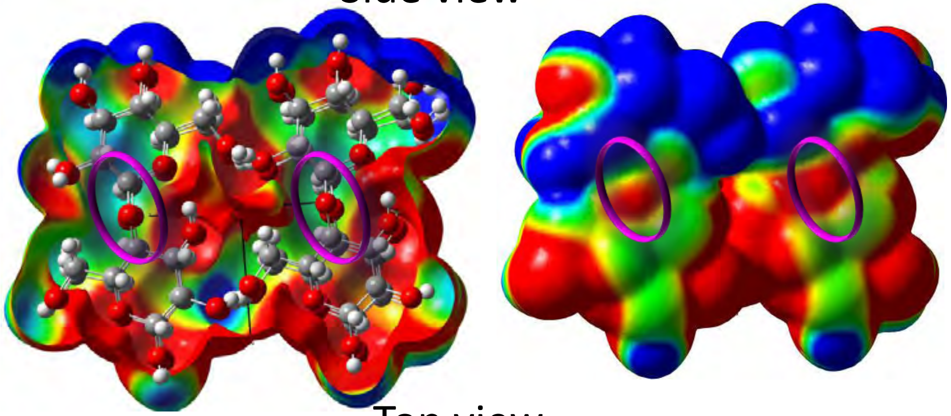


Top view

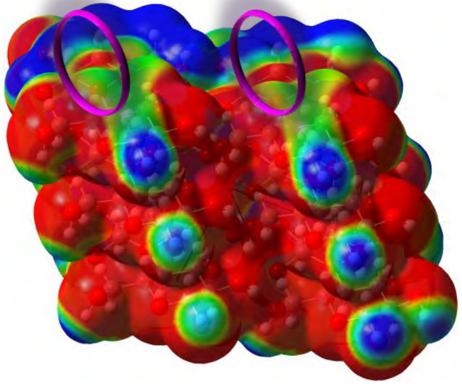


Cellulose III$_I$

Side view



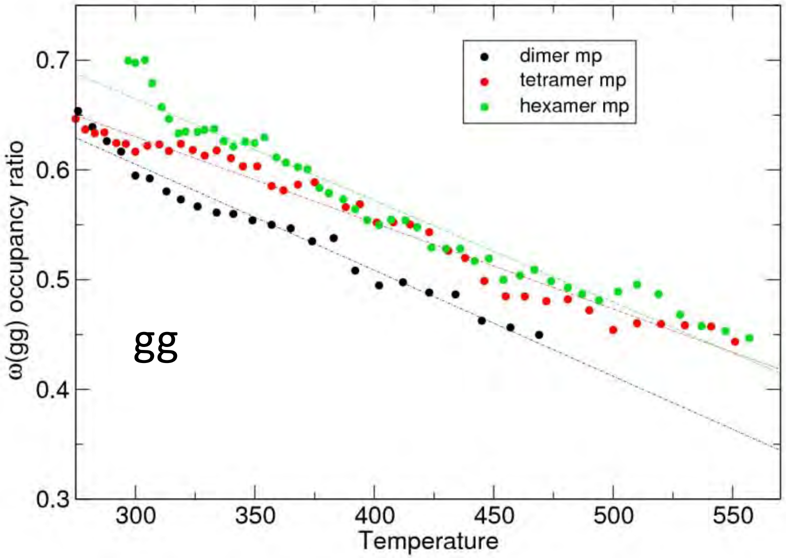
Top view

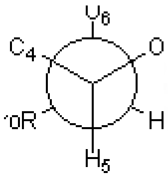


Improved exposure of the hydroxyl groups and glycosidic oxygens in cellulose III$_I$

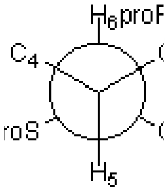
The color scale indicates the charges on the atoms: red = most negative, green = neutral, blue = most positive charge: ±0.04 au isosurface.

O6 side chain based Conformational Variability

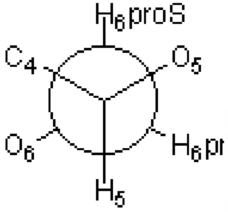




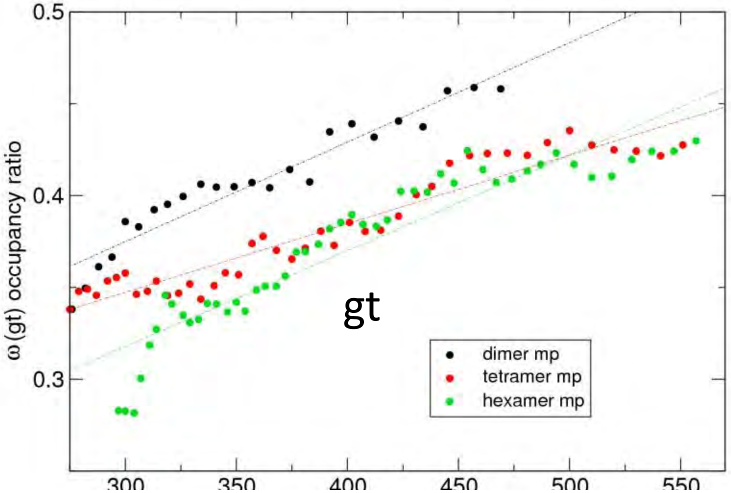
gg

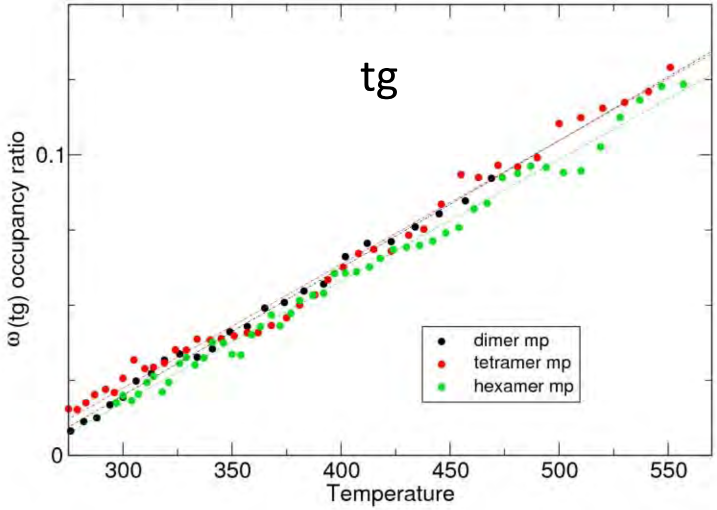


gt



tg



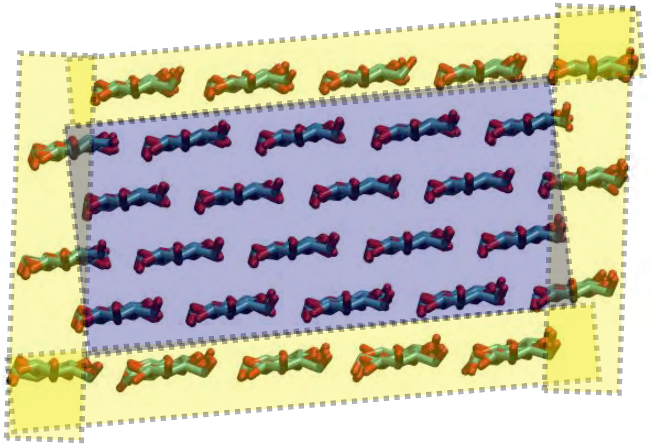


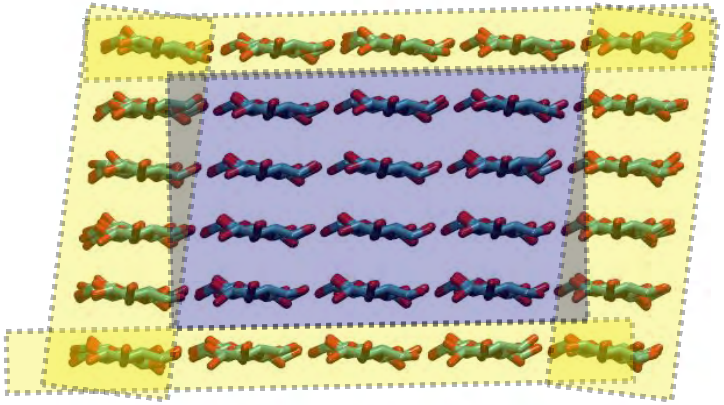
***gg* is preferred for solvated oligomers**

No *tg* of Cellulose Ib & Ia crystals!

No *gt* of dimer, trimer & tetramer x-ray structures

FIBRIL SURFACE BEHAVES DIFFERENTLY FROM THE CRYSTALLINE CORE


Cellulose I$_\beta$

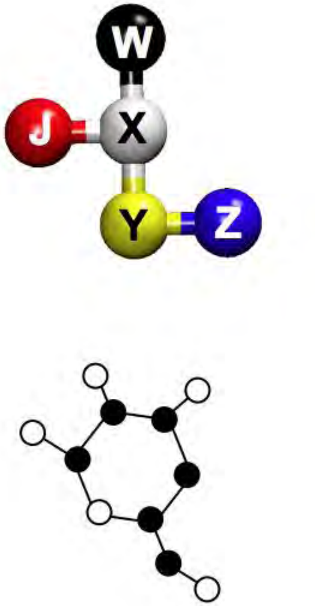

Cellulose III$_I$

(1) more flexibility
(2) higher hydration
(3) lower percentage of the *tg* rotational state

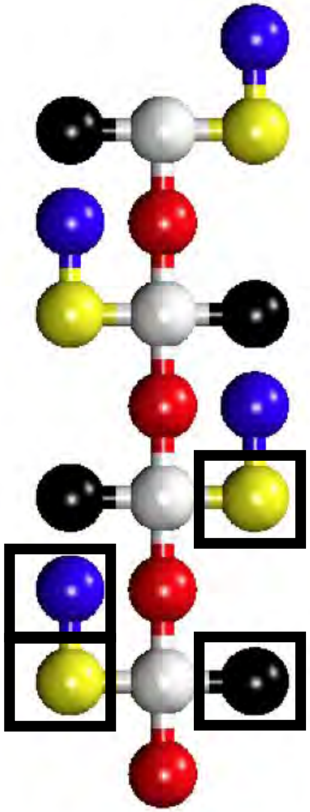
Overall, cellulose III$_I$ surface is more “similar” to amorphous cellulose

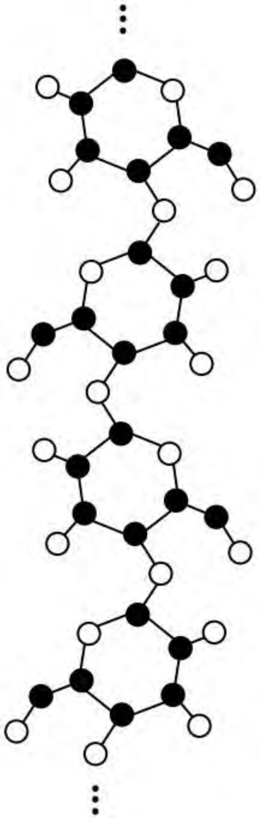
(J. Am. Chem. Soc., 2011, 133 pp. 11163-11174)

COARSE-GRAINED MODEL (II)



glucose unit





cellulose chain

$$H=\sum_{i\neq j}4\epsilon\left[\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12}-\lambda_{ij}\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{6}\right]+$$

$$\sum_{bonds}\frac{K_b}{2}(r_{ij}-r_0)^2+$$

$$\sum_{angles}\frac{K_\theta}{2}(\theta_{ij}-\theta_0)^2+$$

$$\sum_{dihe}C\cos(\gamma-\boxed{\delta})$$

Cellulose I_β $\delta=180^\circ$ **tg**

Cellulose III_I $\delta=130^\circ$ **gt**

● ● $\sigma=2.0,\lambda=1,\epsilon=2.0$

~~● ● $\sigma=3.5,\lambda=1,\epsilon=2.0$~~

? ? $\sigma=2.0,\lambda=0,\epsilon=1.0$

Cellulose I_β ● ● $\sigma=4.0,\lambda=1,\epsilon=2.0$

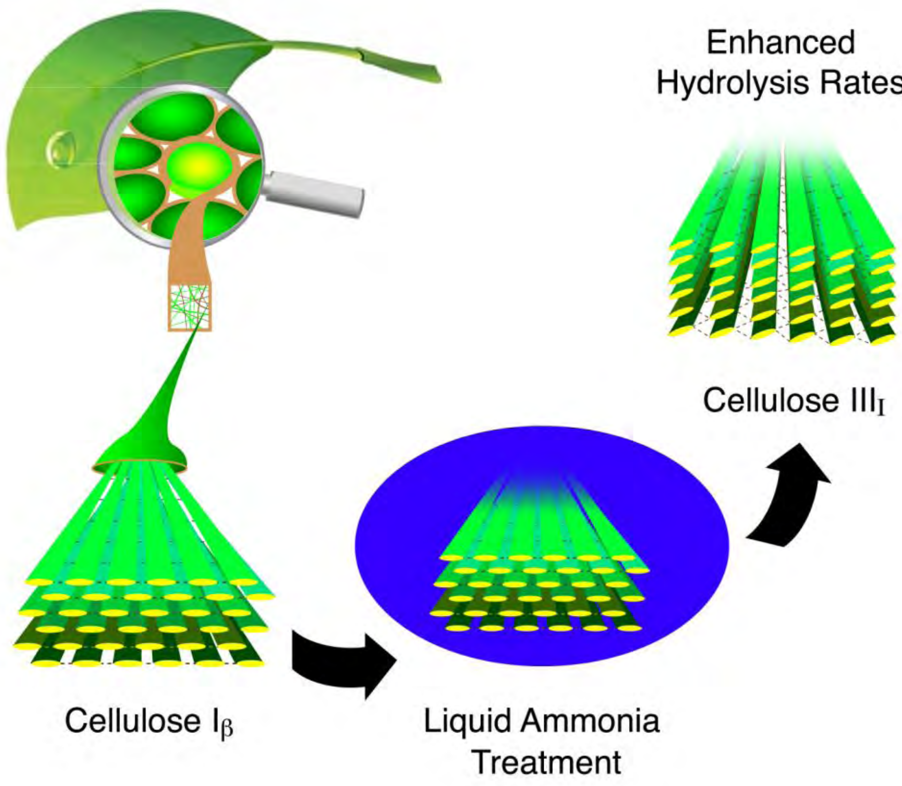
Cellulose III_I ● ● $\sigma=3.0,\lambda=1,\epsilon=2.0$

Summary II.
Differences between cellulose I and III

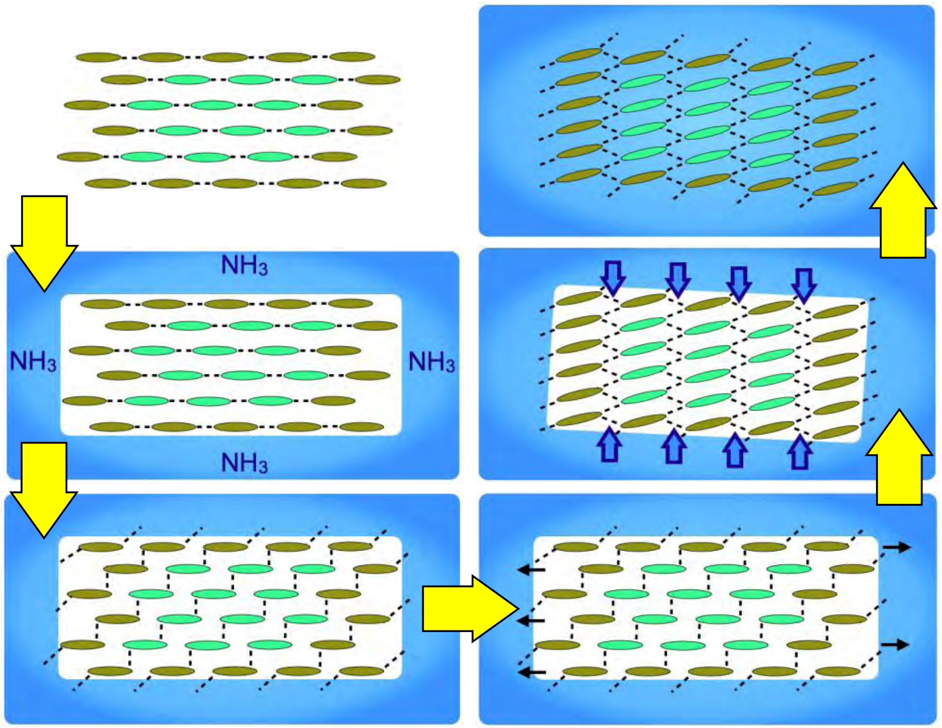
Cellulose I_β	**Cellulose III_1**
Strong Hydrogen bonding interactions within sheet	Slightly weaker hydrogen bonding but cooperative
Cooperative stacking	Non-cooperative stacking
Less structural flexibility	More structural flexibility
Low hydration	High hydration
More stable	Less stable
Ideal packing	Loosely packed

Summary III

Molecular Level Understanding
Liquid Ammonia Pretreatment



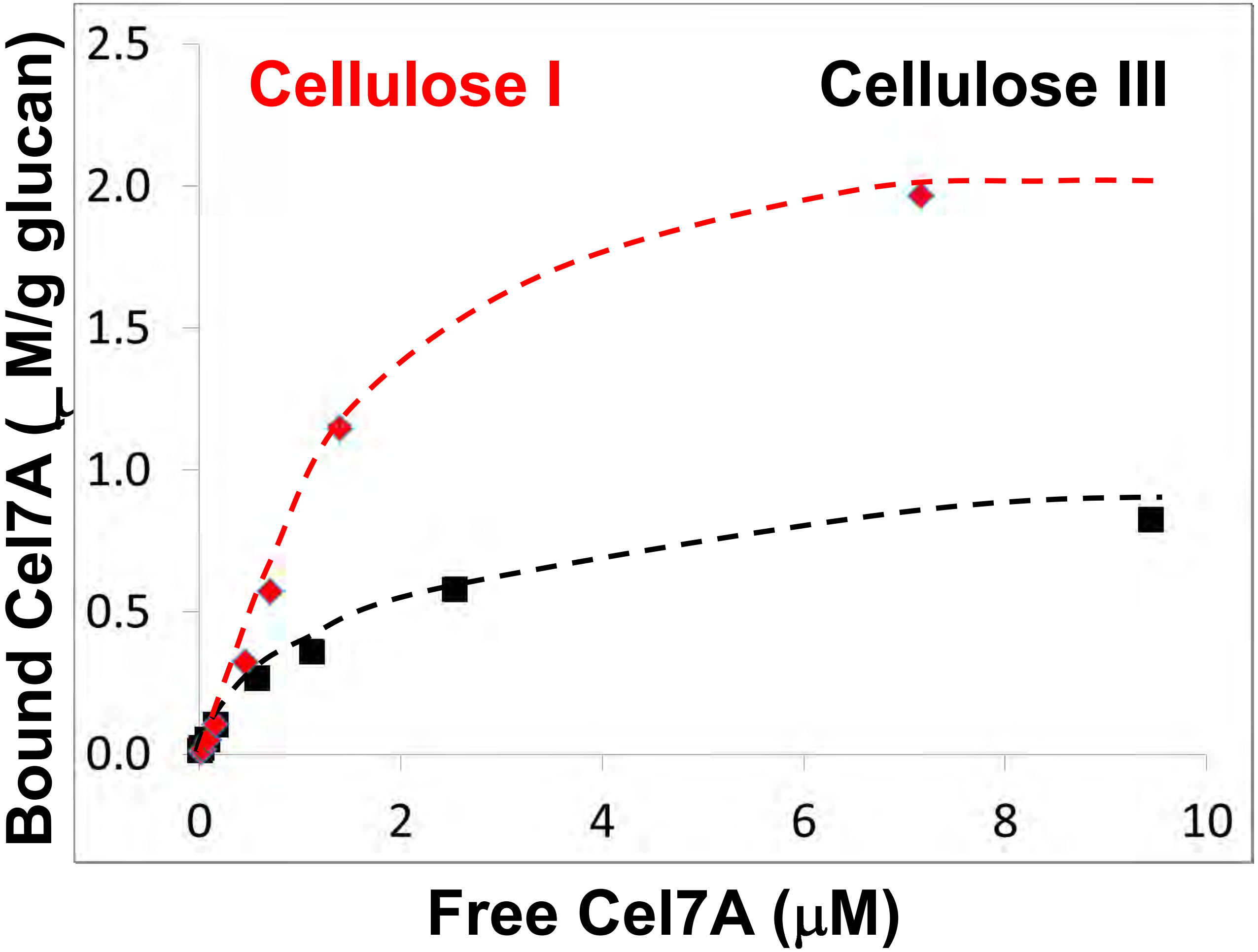
I. Rapid formation of an extended H-bond network between surface chains and NH3.
II. Relative shifting of the layers that in turn leads to the formation of channels orthogonal to the (100) and (-100)
III. These channels allow NH3 to penetrate into the cellulose fibril



PART IV.

Mechanistic kinetic model for Cellulases

But something odd was noticed about cellulase binding



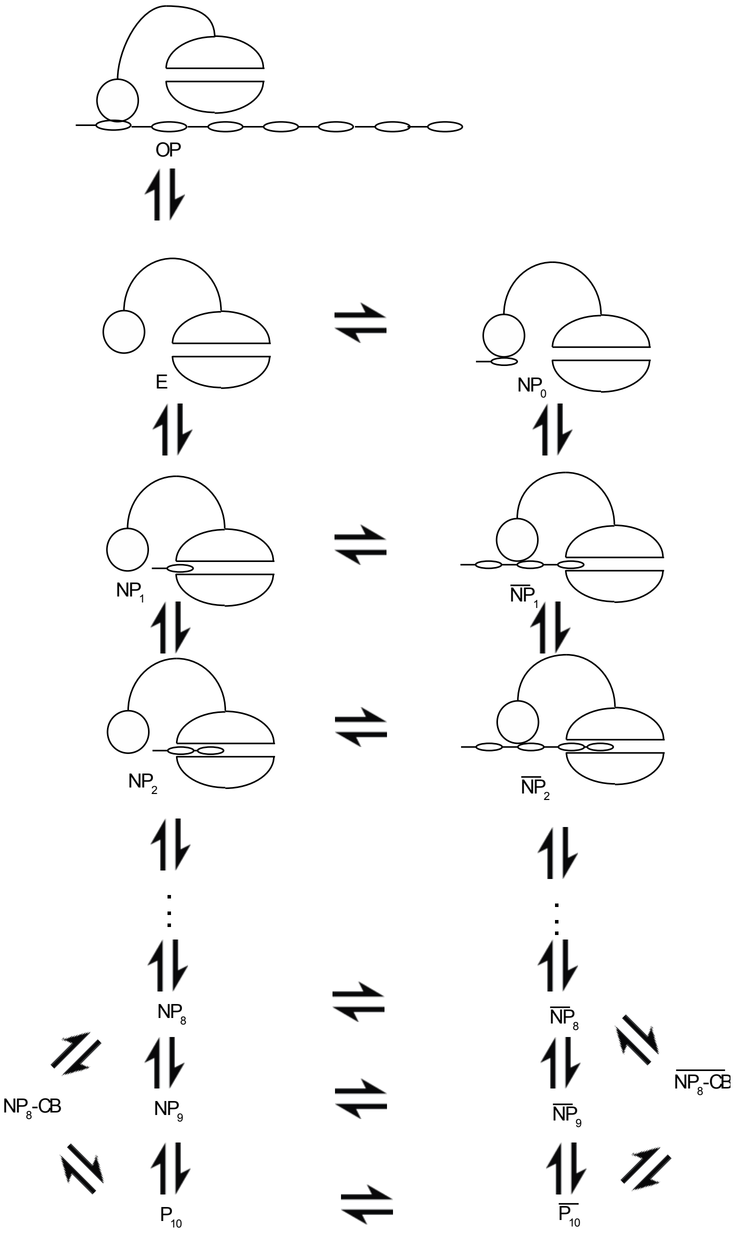
4 Degrees C, 2 hrs equilibration time

Shishir Chundawat, GLBRC

How does lower enzyme binding in Cellulose III lead to increase in catalytic activity?

"Increased enzyme binding to substrate is not necessary for more efficient cellulose hydrolysis,”
D. Gao, S.P.S. Chundawat, A. Sethi, V. Balan, S. Gnanakaran and Bruce E Dale
(under review)

Overview of kinetic model for CBH1



This model takes into account adsorption of carbohydrate binding module (CBM) and catalytic domain (CD) individually to the cellulose surface.

In addition to chain ends, CBM can bind to other regions of the cellulose as well (CD cannot do this).

Once CD is adsorbed to the cellulose surface, the cellulose chain can slide into the active site a single glucose unit at a time.

Once a productive enzyme (10 glucose units in active site) is formed, the protein can hydrolyze the cellulose to form product-bound enzyme (P10).

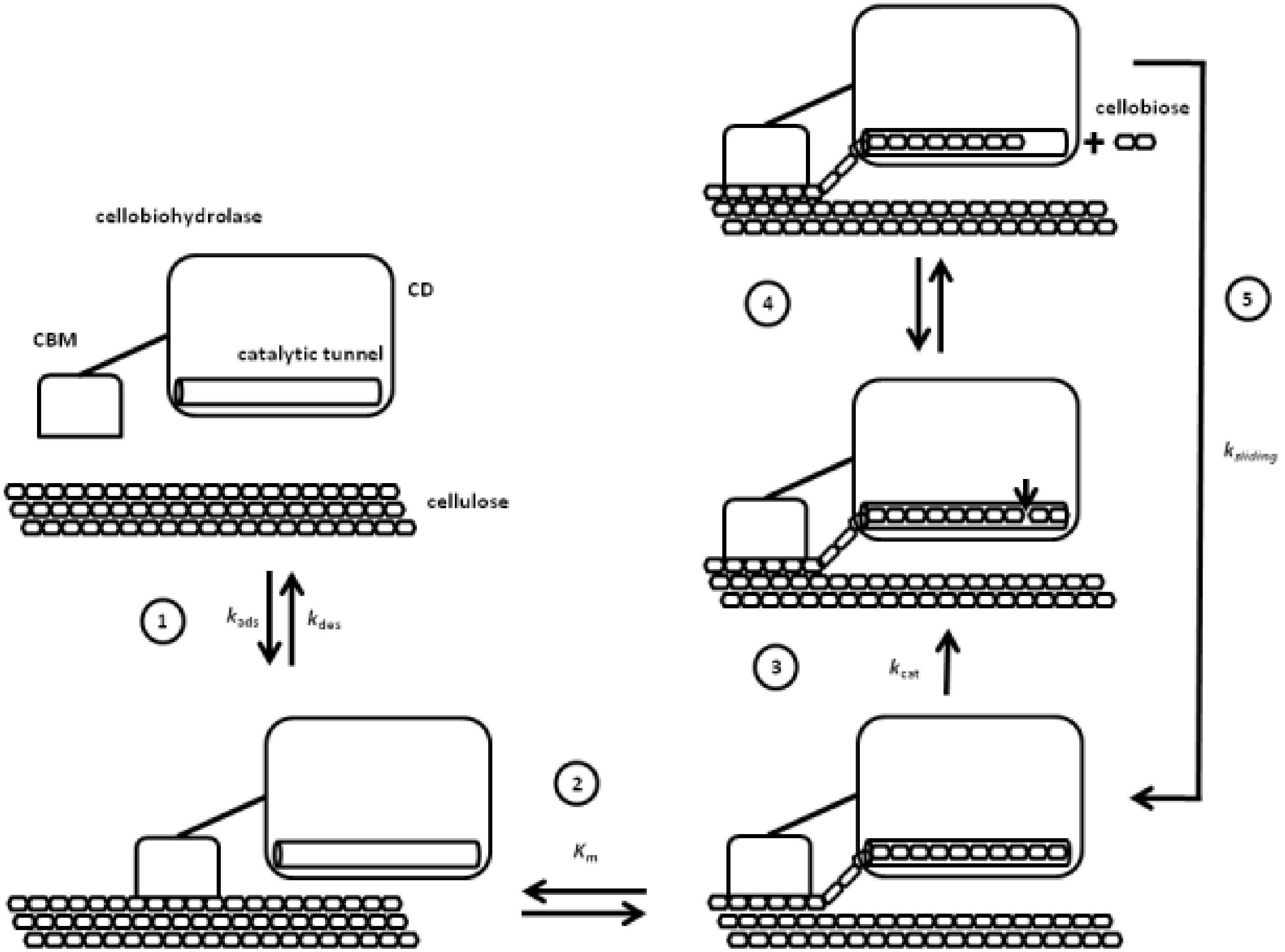
Cellobiose can then undock from the enzyme to form the enzyme bound substrate with 8 glucose units remaining within CD (NP8-CB).

Key Steps in the Model

Binding

Sliding

Hydrolysis

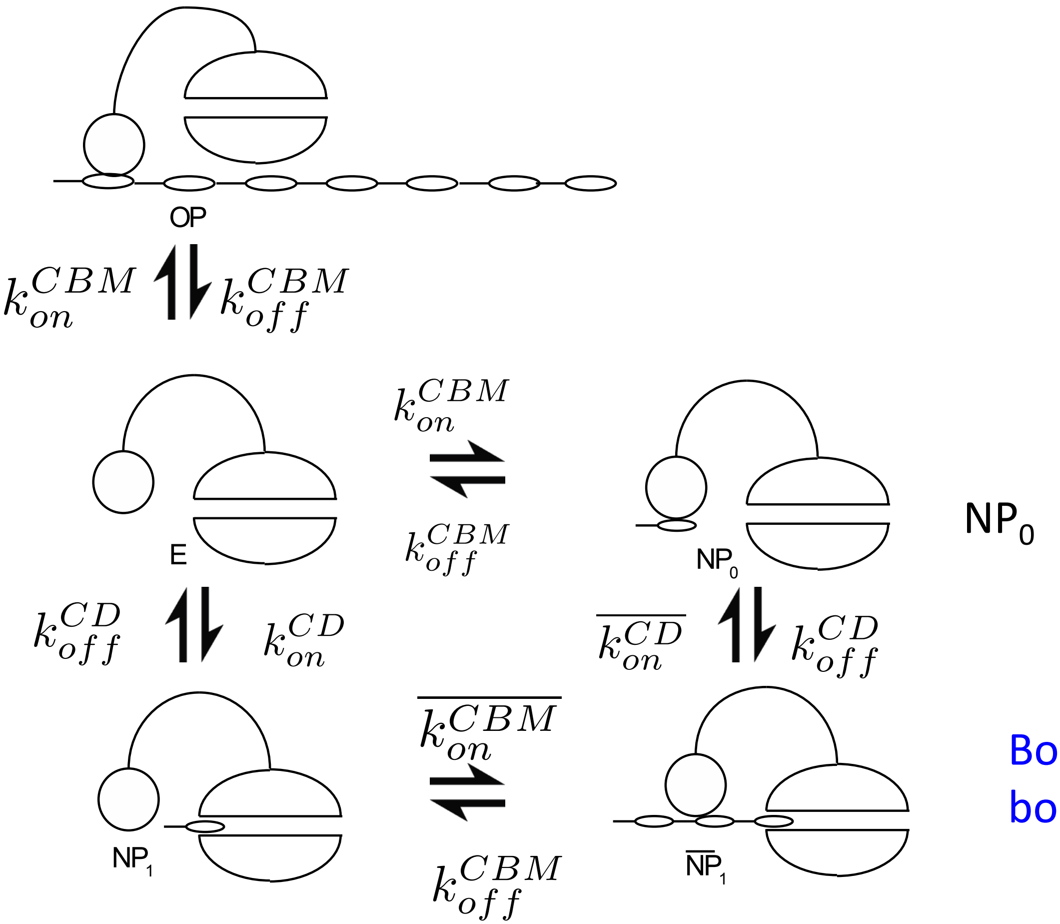


Binding of enzyme

Off-pathway nonproductive complex OP

apo Enzyme E

NP$_1$ (1 glucose unit in CD active site)



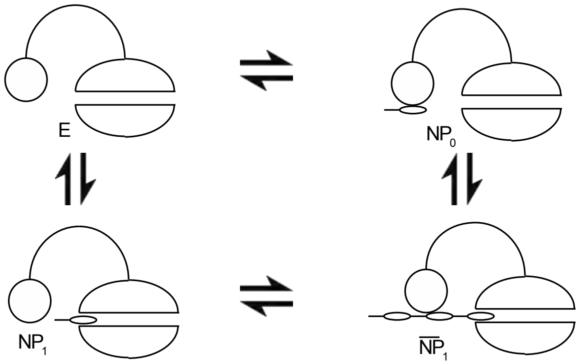
Both domains bound to cellulose

$$\frac{d[E]}{dt} = -k_{on}^{CBM}[E][C_{fe}] + k_{off}^{CBM}[NP_0] - k_{on}^{CD}[E][C_{fe}] + k_{off}^{CD}[NP_1] - k_{on}^{CBM}[E][C_{inner}] + k_{off}^{CBM}[OP]$$

$$\frac{d[OP]}{dt} = k_{on}^{CBM}[E][C_{inner}] - k_{off}^{CBM}[OP]$$

$$\frac{d[NP_0]}{dt} = k_{on}^{CBM}[E][C_{fe}] - k_{off}^{CBM}[NP_0] + k_{off}^{CD}[\overline{NP_1}] - \overline{k_{on}^{CD}}[NP_0]$$

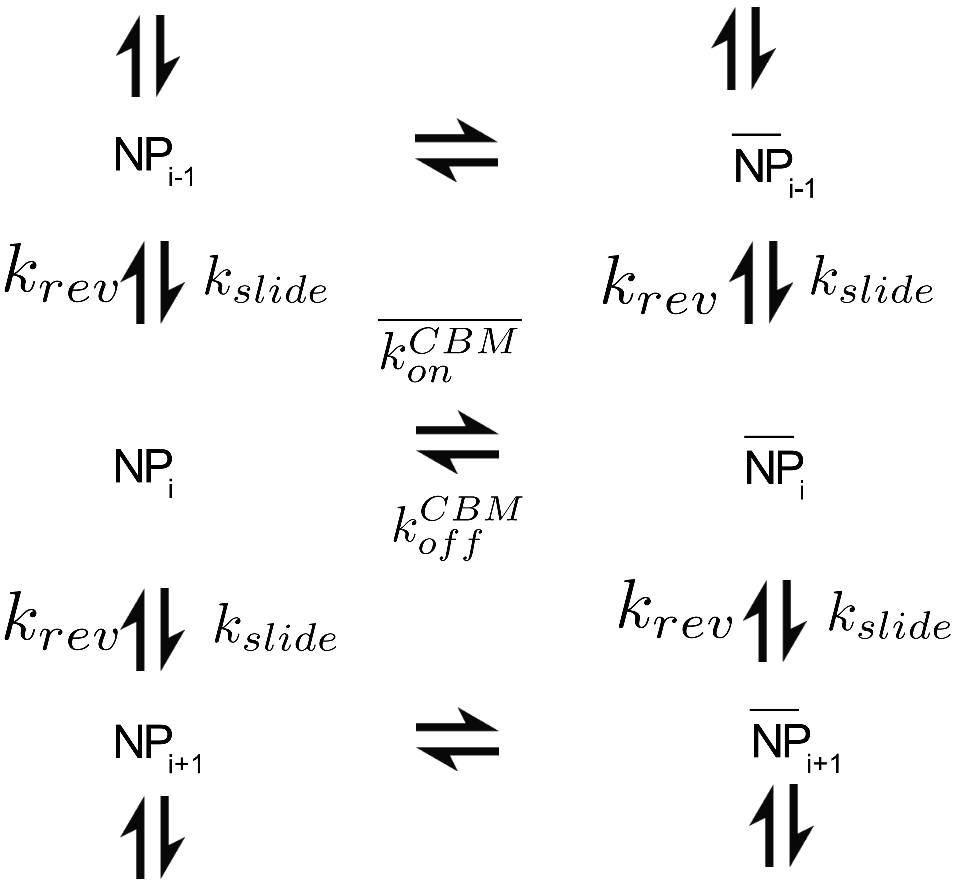
OP



Intramolecular binding constants can be upto 100 times greater

A. Sethi, B. Goldstein and S. Gnanakaran. Quantifying intramolecular binding in multivalent interactions. ***PLoS Comput. Biol.****, 2011.(10):e1002192.*

Sliding of enzyme



i refers to number of glucose units inside the catalytic domain of the protein.

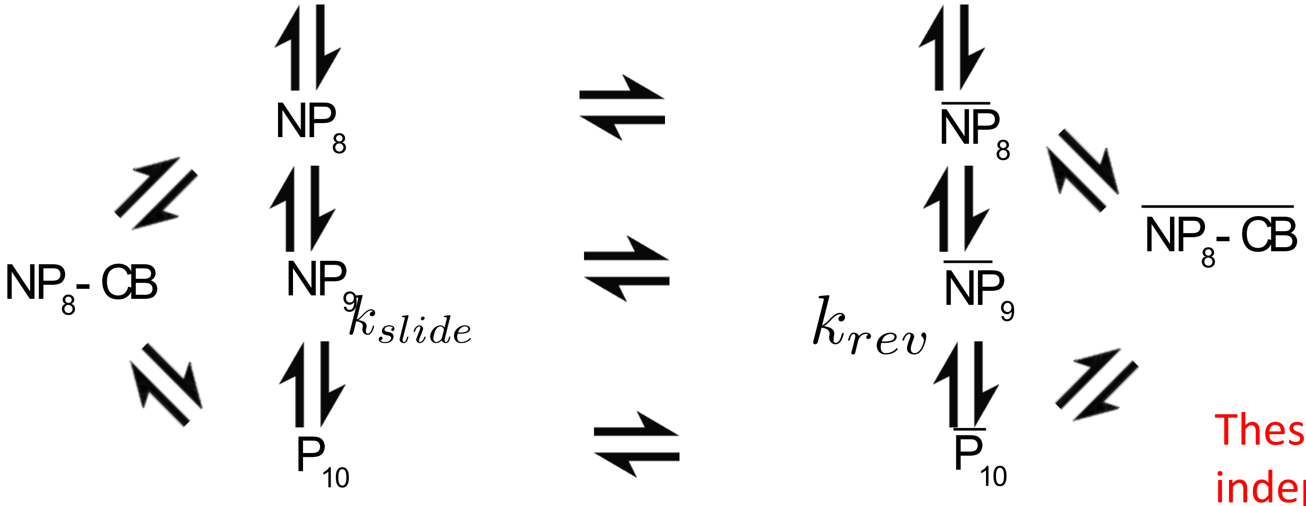
The cellulose chain can slide in one glucose unit at a time into the CD.

i=2 to 7

$$\frac{d[NP_i]}{dt} = k_{slide}[NP_{i-1}] - k_{rev}[NP_i] + k_{rev}[NP_{i+1}] - k_{slide}[NP_i] + k_{off}^{CBM}[\overline{NP_i}] - \overline{k_{on}^{CBM}}[NP_i]$$

$$\frac{d[\overline{NP_i}]}{dt} = k_{slide}[\overline{NP_{i-1}}] - k_{rev}[\overline{NP_i}] + k_{rev}[\overline{NP_{i+1}}] - k_{slide}[\overline{NP_i}] + \overline{k_{on}^{CBM}}[NP_i] - k_{off}^{CBM}[\overline{NP_i}]$$

Catalytic activity by CBH`



NP_8

NP_9

P_{10}

NP_8- CB

k_{slide}

k_{rev}

$\overline{NP}_8$

$\overline{NP}_9$

$\overline{P}_{10}$

$\overline{NP_8\text{- CB}}$

k_b^{CB} k_u^{CB}

Binding and undocking rate of cellobiose

k_h k_b

Forward and backward catalytic rates by enzyme.

These four parameters are independent of cellulose crystal and depend on enzyme.

$$\frac{d[NP_8]}{dt} = k_{slide}[NP_7] - k_{rev}[NP_8] + k_{rev}[NP_9] - k_{slide}[NP_8] \\ +k_{off}^{CBM}[\overline{NP_8}] - \overline{k_{on}^{CBM}}[NP_8] + k_u^{CB}[NP_8 - CB] - k_b^{CB}[CB][NP_8]$$

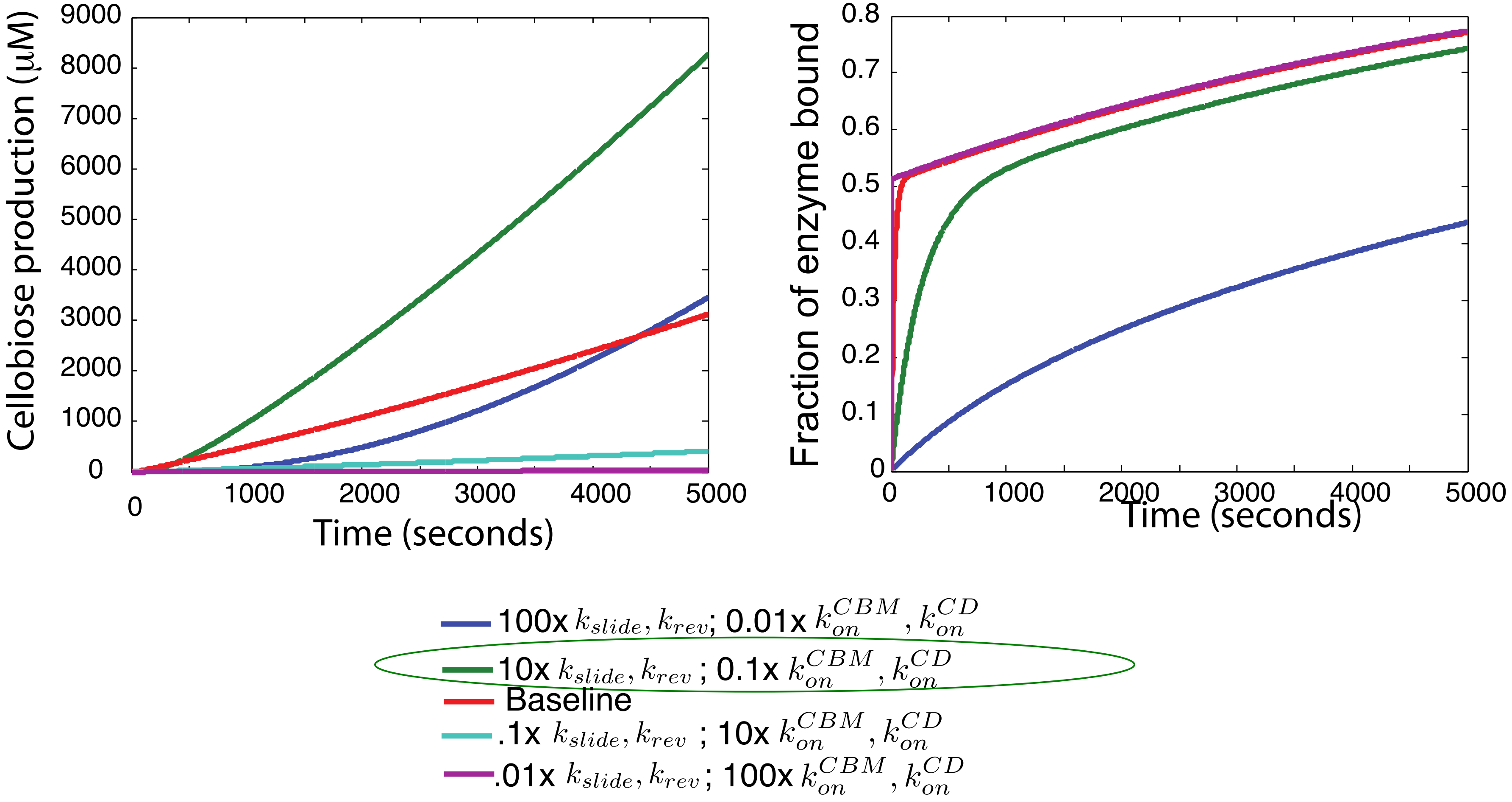
$$\frac{d[P_{10}]}{dt} = k_{slide}[NP_9] - k_{rev}[P_{10}] + k_{off}^{CBM}[\overline{P_{10}}] \\ -\overline{k_{on}^{CBM}}[P_{10}] + k_b[NP_8 - CB] - k_h[P_{10}]$$

$$\frac{d[NP_8 - CB]}{dt} = k_b^{CB}[NP_8][CB] - k_u^{CB}[NP_8 - CB] + +k_h[P_{10}] - k_b[NP_8 - CB] \\ k_{off}^{CBM}[\overline{NP_8 - CB}] - \overline{k_{on}^{CBM}}[NP_8 - CB]$$

Consider different scenarios for combination of factors that could lead to increase of hydrolysis rate of enzyme simultaneously with decrease in amount of bound enzyme as seen in experiments.

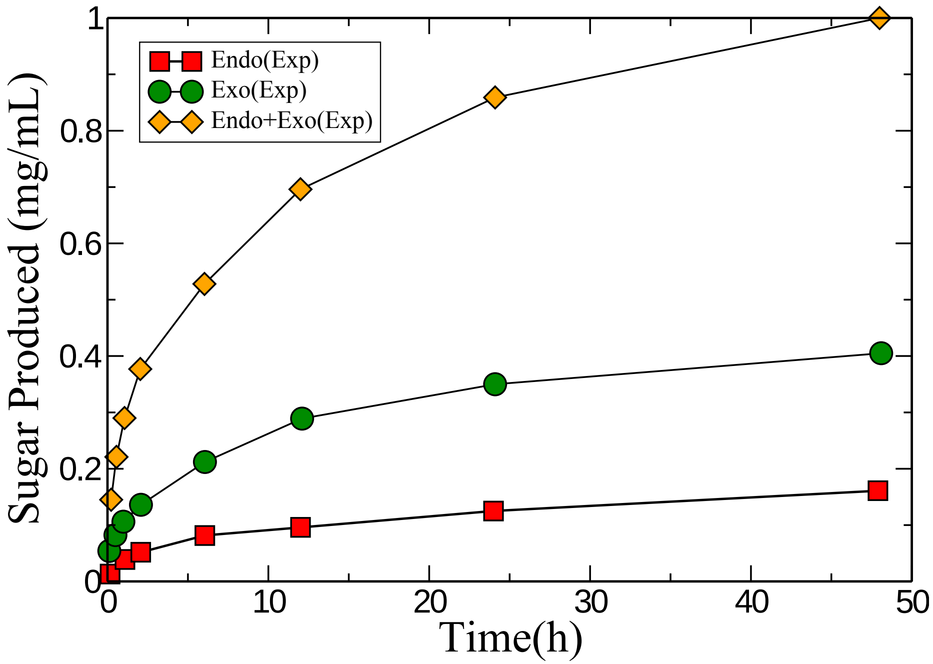
only one scenario explains observations

Higher Sliding & Lower Binding Rates in Cellulose III



Only increase in sliding rate with decrease in binding rates of both domains to the surface of cellulose reproduces the observations

Can we implement a more complex kinetic model?



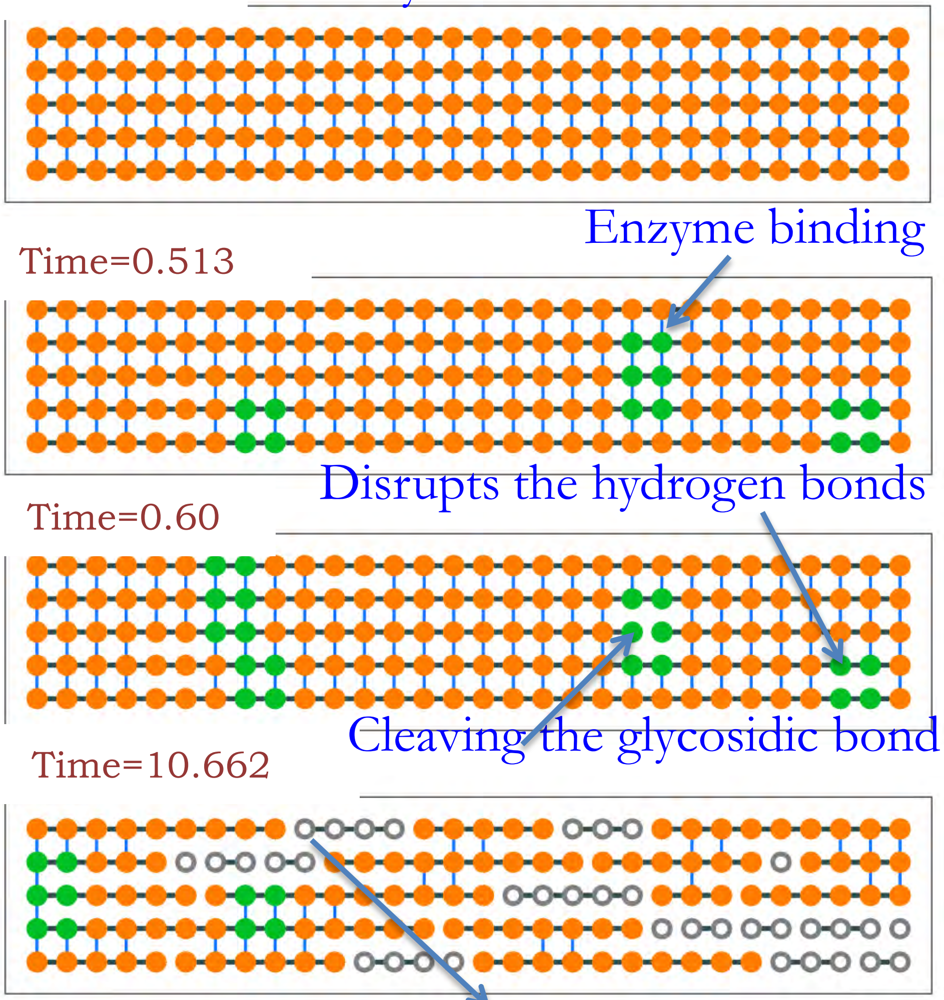
[Eriksson et al., *Applied Biochem. and Biotech.* 101:41 (2002)]

1) How does the substrate change during hydrolysis?

2) What cellulase cocktails would result in an efficient cellulose degradation?

Coarse-grained Dynamical Models – Enzymatic Degradation

Samples from Agent-based Simulations



Time=0.00

Crystalline cellulose surface

Enzyme binding

Time=0.513

Disrupts the hydrogen bonds

Time=0.60

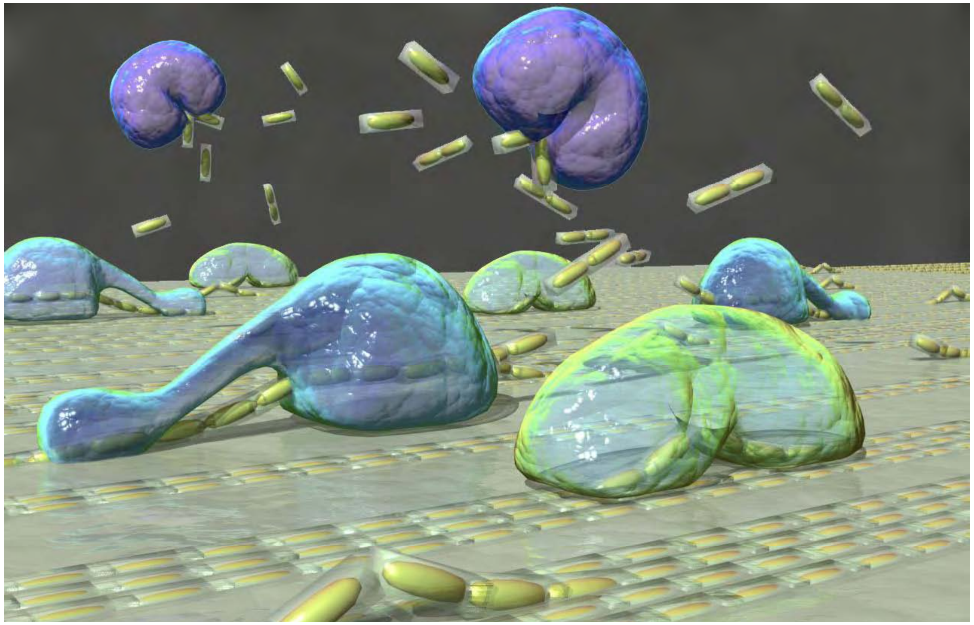
Cleaving the glycosidic bond

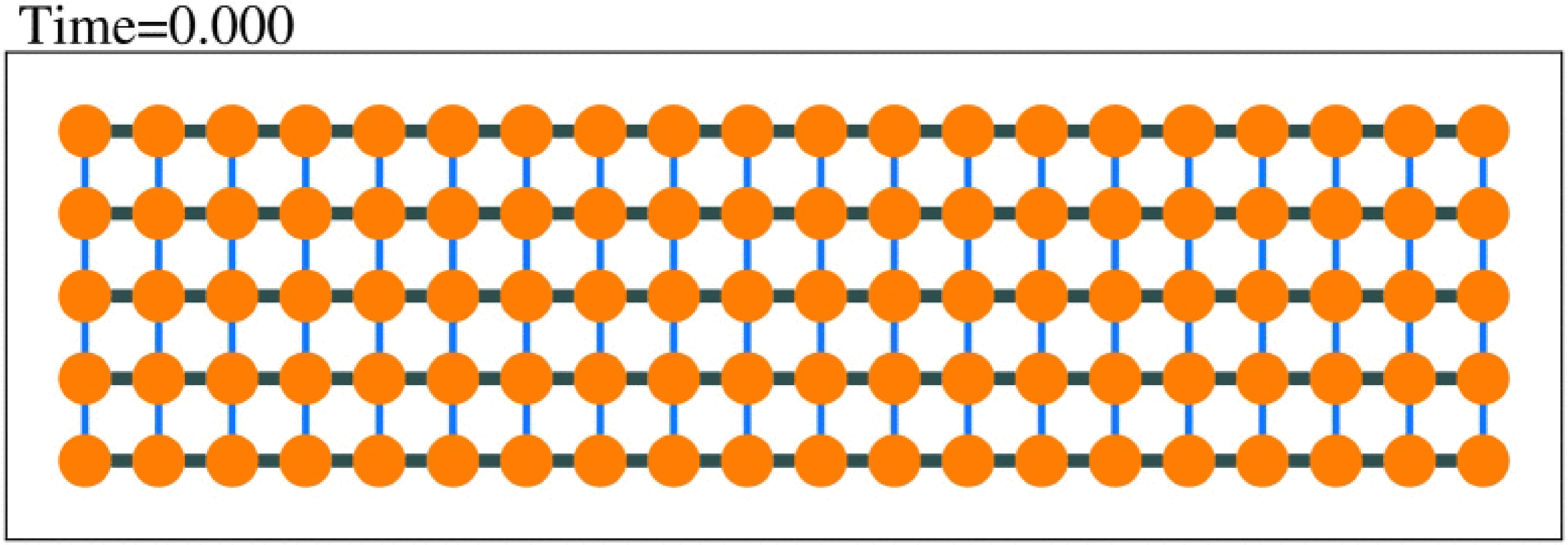
Time=10.662

Oligomers entering into solution

Asztalos, A., Daniels, M., Sethi, A., Shen, T., Langan, P., Redondo, A., and Gnanakaran, S., *A coarse-grained model for synergistic action of multiple enzymes on cellulose*, Biotechnology for Biofuels,. 2012 Aug

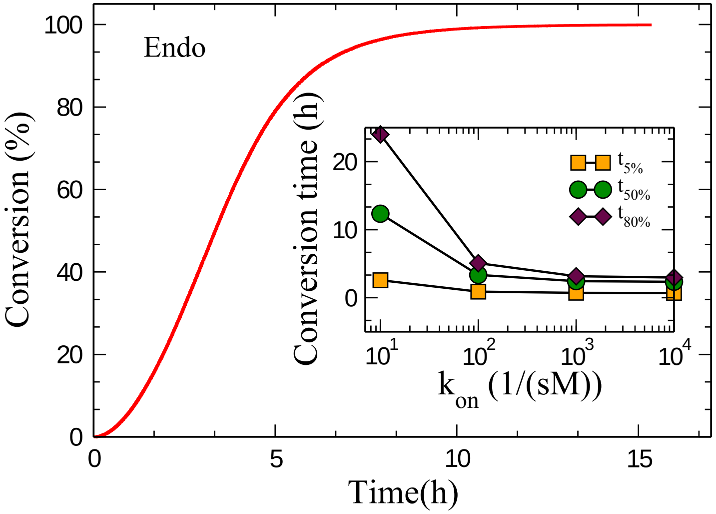
Current Studies – Enzymatic Degradation



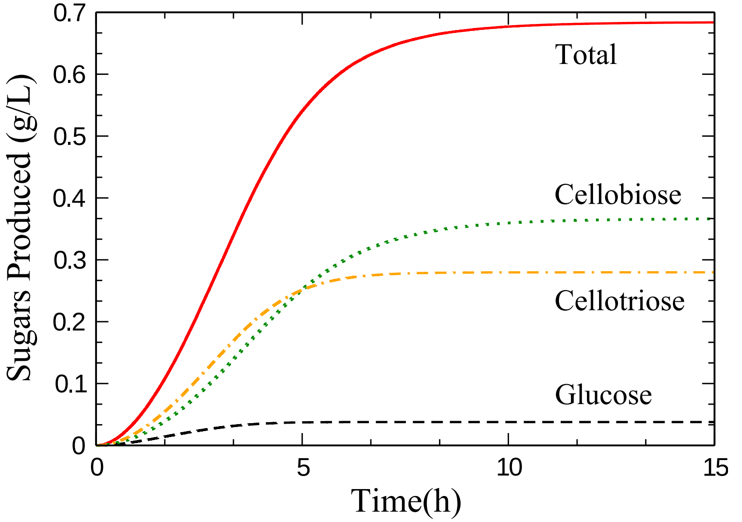


Results: Endo-enzymes

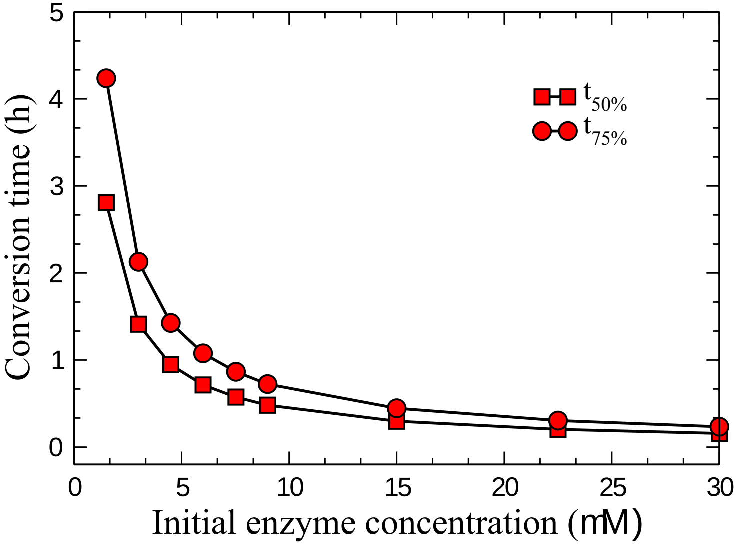
Time course of hydrolysis



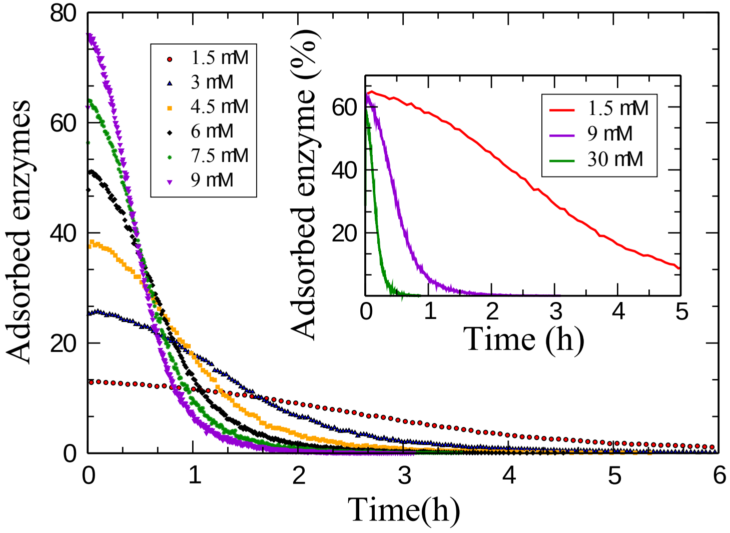
Distribution of oligomers



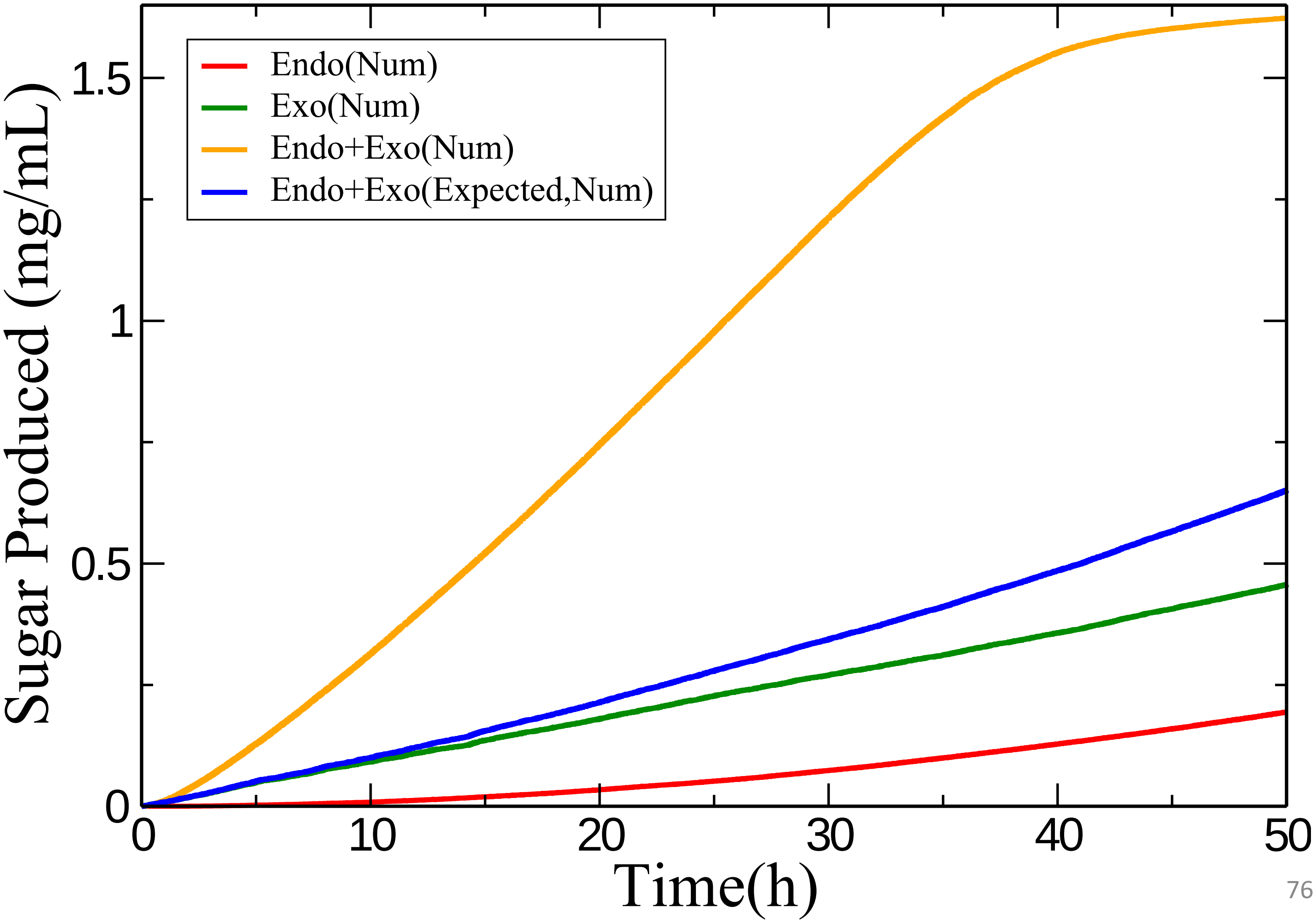
Enzyme loading



Enzyme adsorption



Results: Cellulase Cocktails



Summary

- Simple mechanistic kinetic model finds
  - Sliding rate of CBH1 is faster in Cellulose III
  - Binding rate of CBH1 is lower in Cellulose III

- Stochastic model that capture substrate properties during hydroylsis and effect of enzyme cocktail has been developed
  - possible 3d extension of the model

Increasing Spatial & Temporal Scales

Optimal Cellulase Cocktails for Efficient Degradation of Cellulose

Biotechnol. Biofuels. 2012,5:55

Plasticity of Hydrogen Bond Network in Cellulose

Biophys. J. 2009, 96:3032

Bulk Properties of Cellulose

Research in Progress

Transformation of Cellulose I to III

***J. Am. Chem. Soc.* 2011** 133, 11163

J. Phys. Chem. B. 2011 115: 9782

Amorphous Cellulose

J. Am. Chem. Soc. 2009 131: 14786

Stacking & Hydrogen Bonding in Cellulose

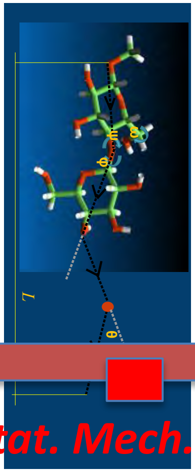
J. Phys. Chem. A. 2011 115: 14191

***Cellulose*. 2011**. 18, 191

***Acta Crystal. D*. 2010**. 66:1184

Stereochemistry of glucose

Manuscript in Preparation



Quantum Mechanical

Classical MD Simulations

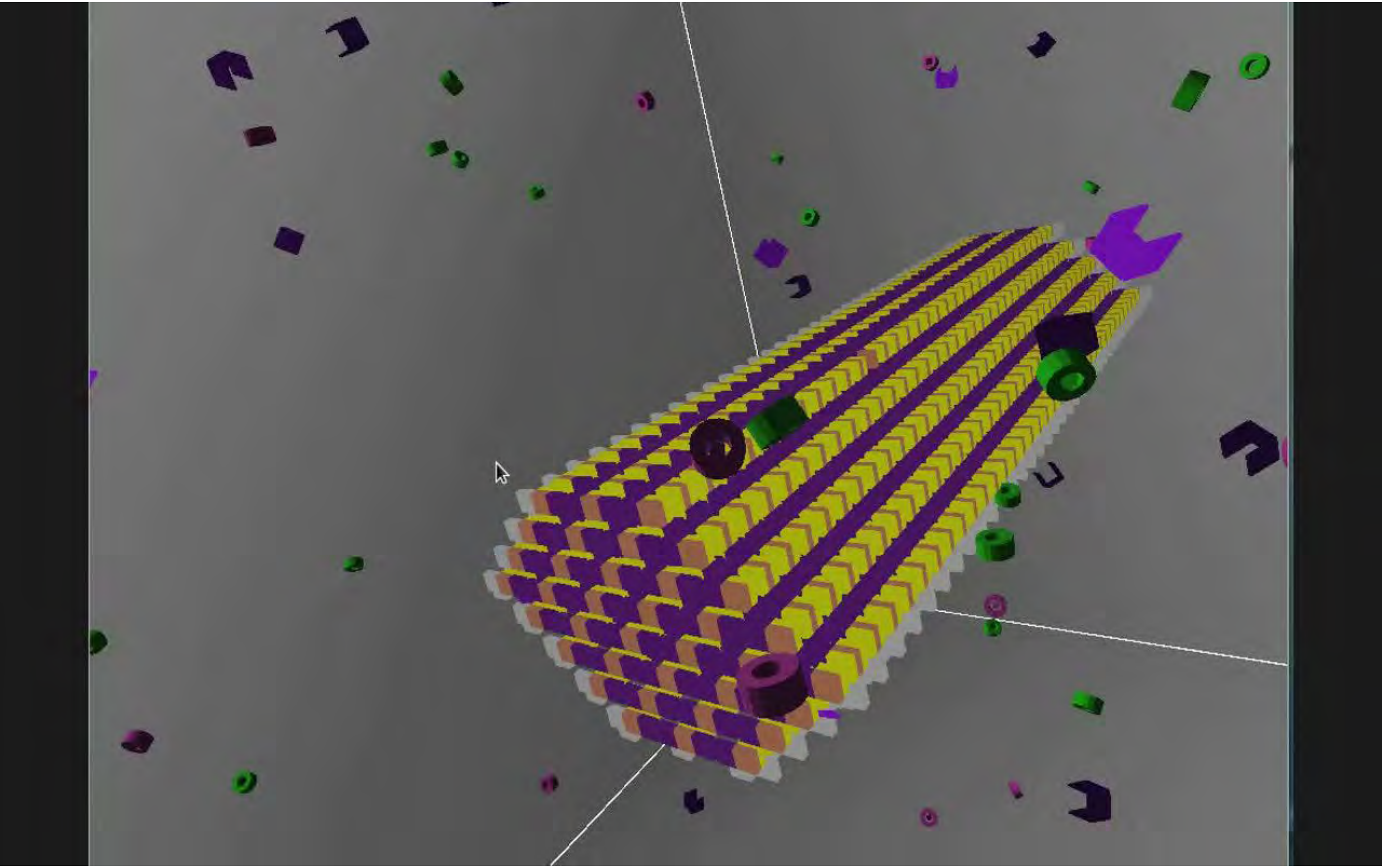
Coarse-grained Simulations

Stat. Mech. Models

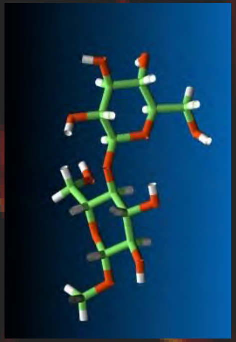
Rule-based Models

Multi-resolution Theoretical Approaches

Enzymatic Digestion Game



Bullet Physics – heavily used by the game developers

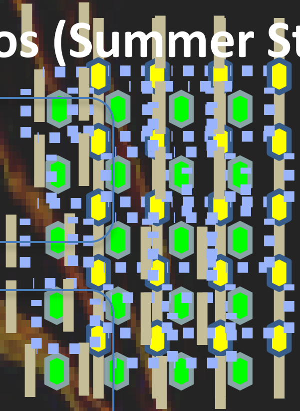


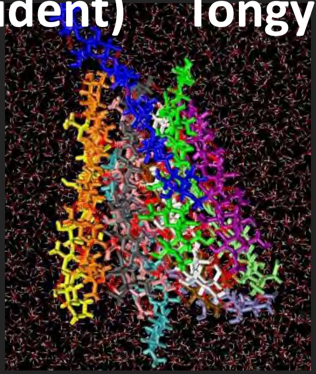
Giovanni Bellesia
Partha Ramakrishnan
Anurag Sethi
Jianhui Tian

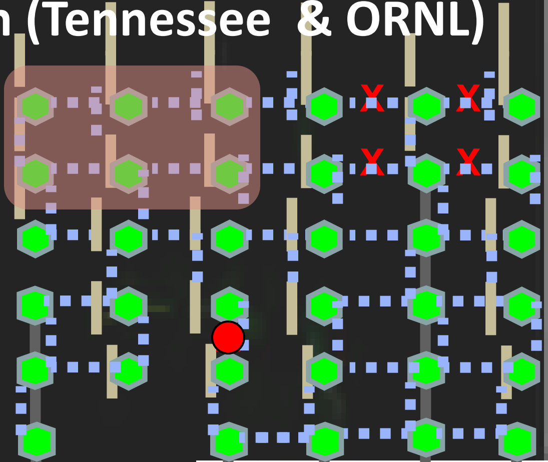
Bruce Dale
ShiShir Chundenwat
(GLBRC)

Andrea Asztalos (Summer Student)

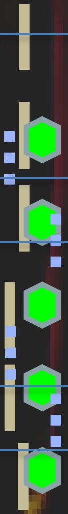
Tongye Shen (Tennessee & ORNL)







Paul Langan

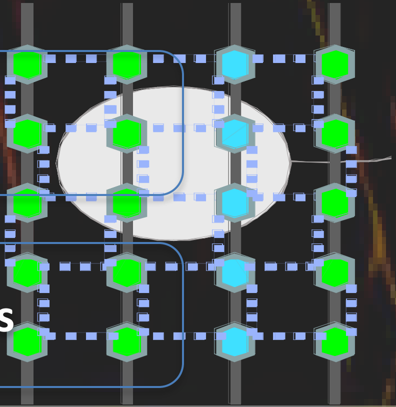


Tony Redondo

Peter Goodwin

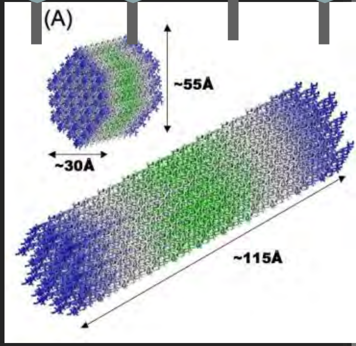
Cliff Unkefer

Marcus Daniels



Linda Broadbelt
Heather Mayes
(Northwestern)

Tom Elder
(USDA)



Alfred French
Glenn Johnson
(USDA)

Cesar Lopez
Siewert Marrink
(Groningen)