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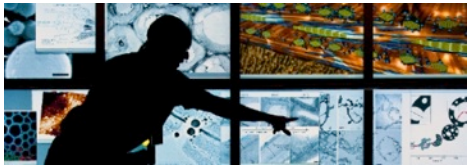
Understanding Free and Complexed Enzyme Mechanisms and Factors Contributing to Cell Wall Recalcitrance



Michael G. Resch
SIMB SBFC
Clearwater, FL
4/29/2014

NREL/PR-2700-60927
NREL is a national laboratory of the U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, operated by the Alliance for Sustainable Energy, LLC.

Acknowledgements

Funding

Office of Science

BESC BioEnergy Science Center

Biomass Technologies Office (BETO)

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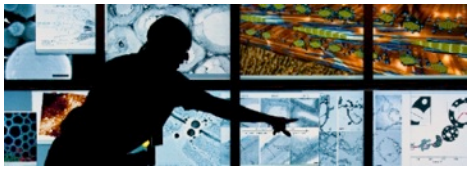

Task Leaders
Gregg Beckham
Michael Himmel
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TEM and SEM Imaging
Bryon Donohoe
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Intern
Jennifer Nill

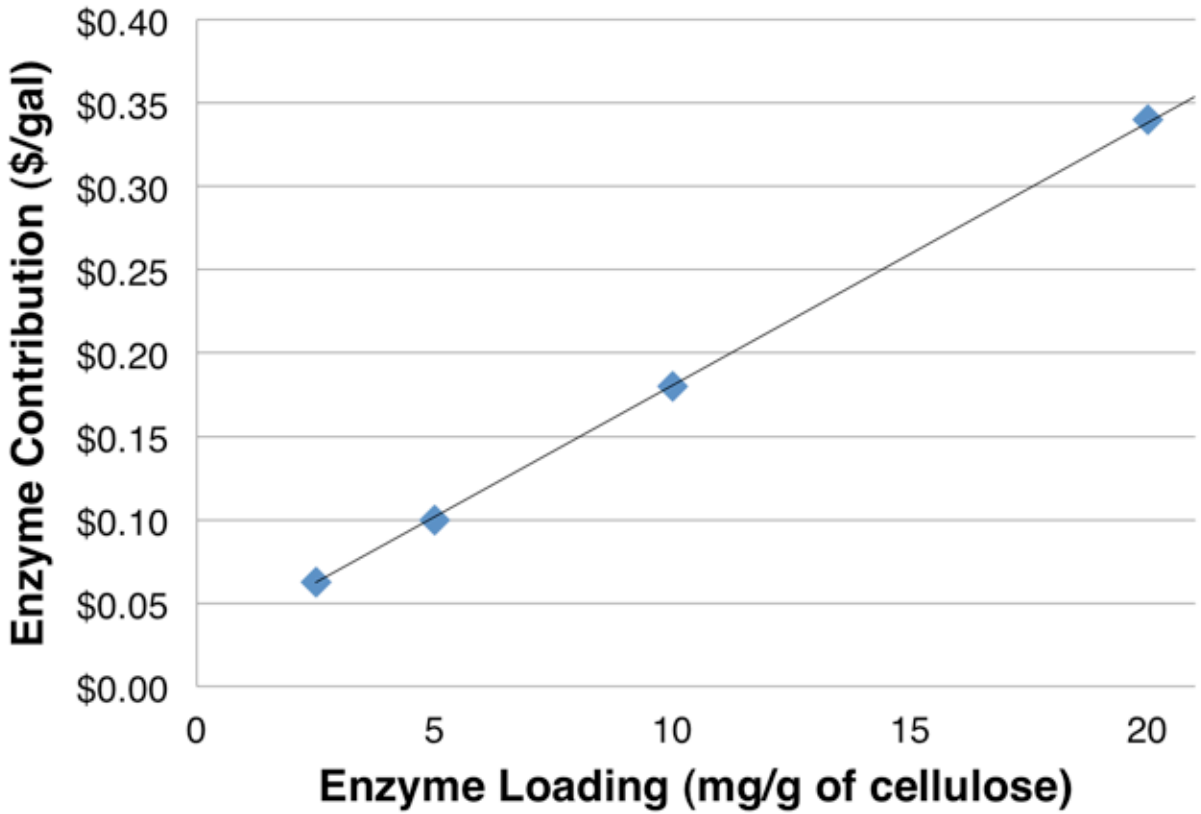
Clean Fractionation
Rui Katahira
Kellene McKinney

Cellulose Allomorphs and XRD
Ashutosh Mittal

BAT Team

Techno Economic Analysis
Mary Biddy

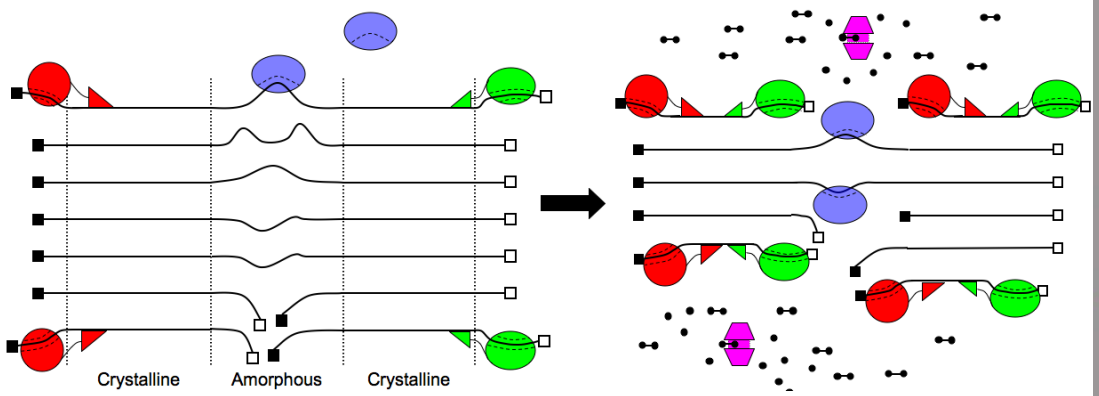
Economic Impact of Reducing Enzyme Loading



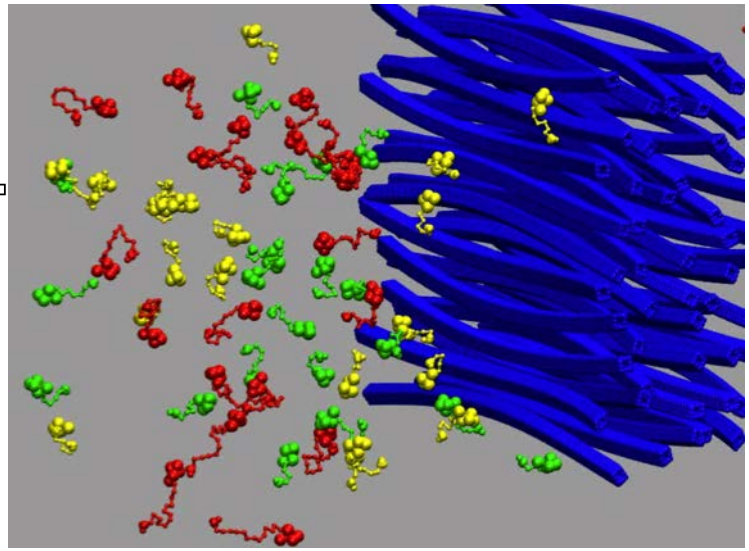
Humbird *et al*. 2011

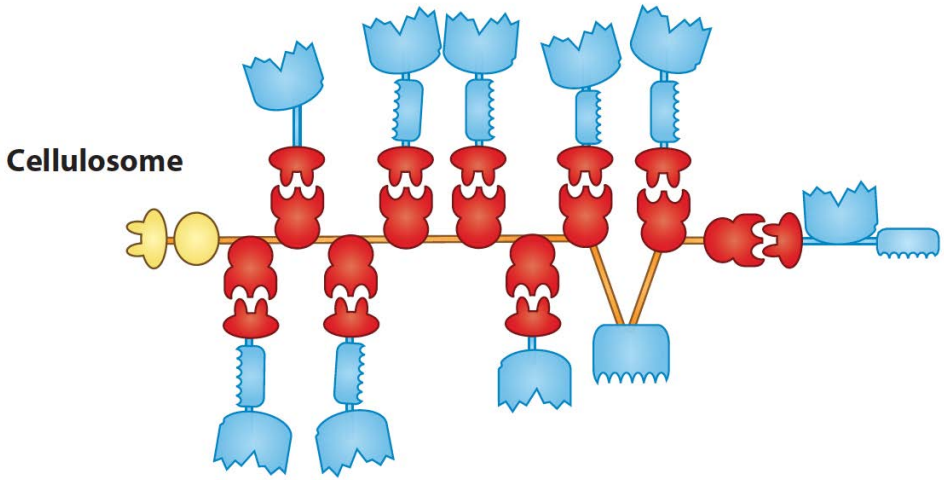
“Free” vs. Complexed Enzymes

“free” cellulases

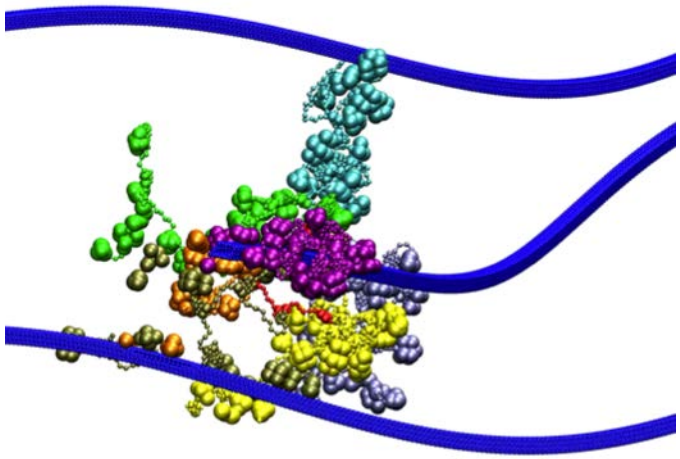


Lynd *et al. MMR* 2002



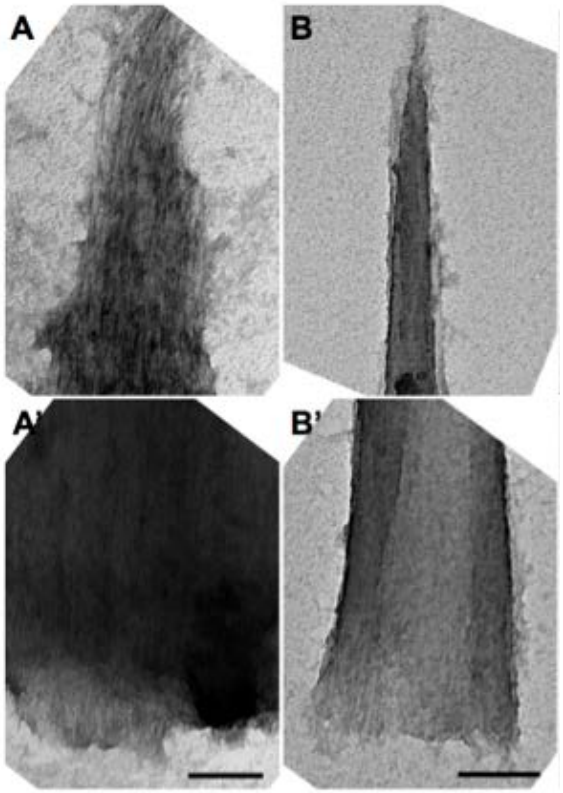


Gilbert and Fontes, *Ann. Rev. Biochem*. 2010

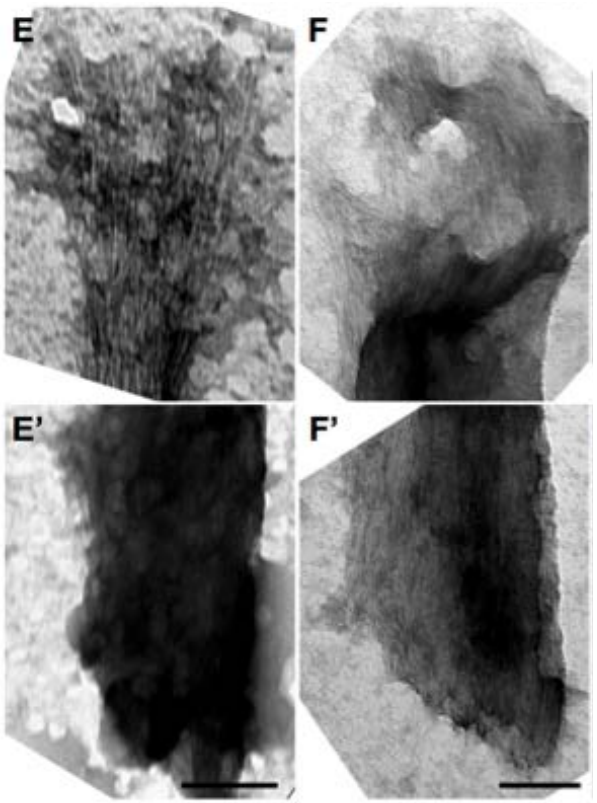


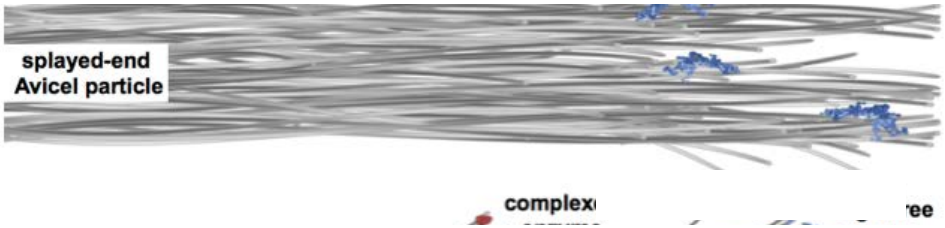
Different physical mechanisms of denconstruction

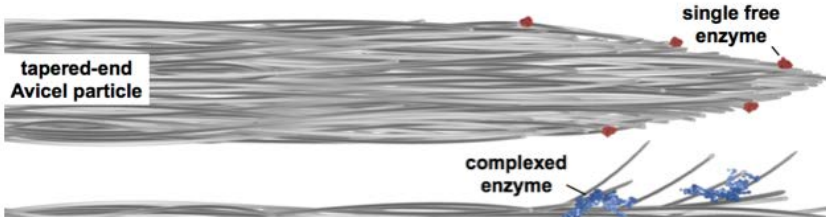
"Free" Cellulases



Cellulosomes



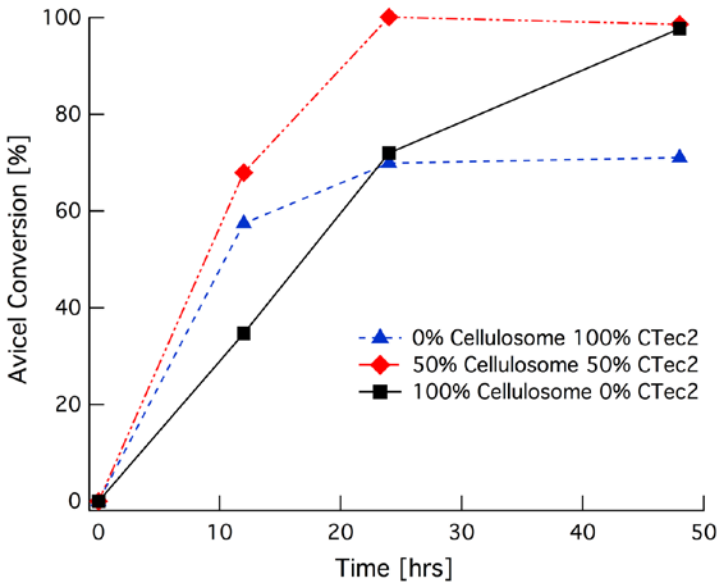


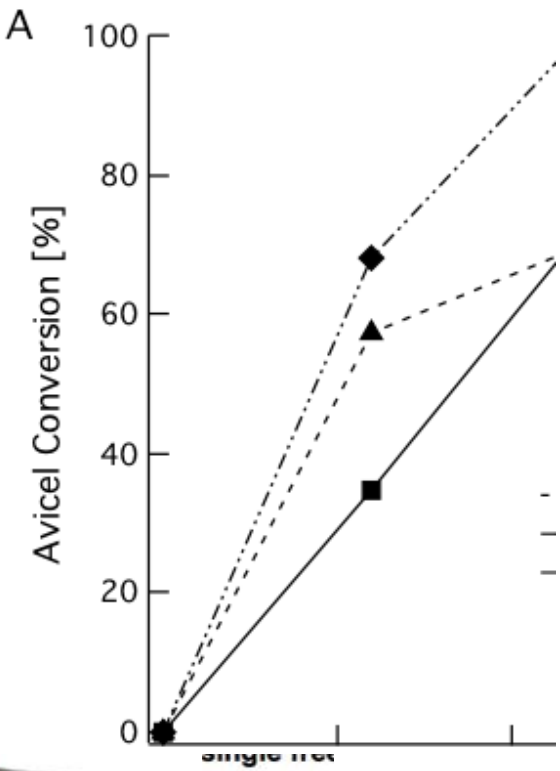


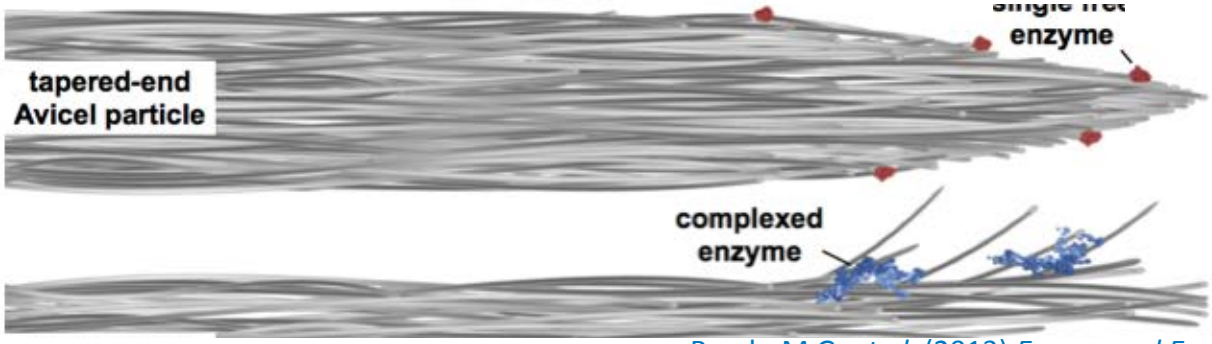
Resch, M.G. *et al.* (2013) *Energy and Environmental Science*

Do Free and complexed enzymes degrade cellulose synergistically?

Avicel conversion at 10 mg/g







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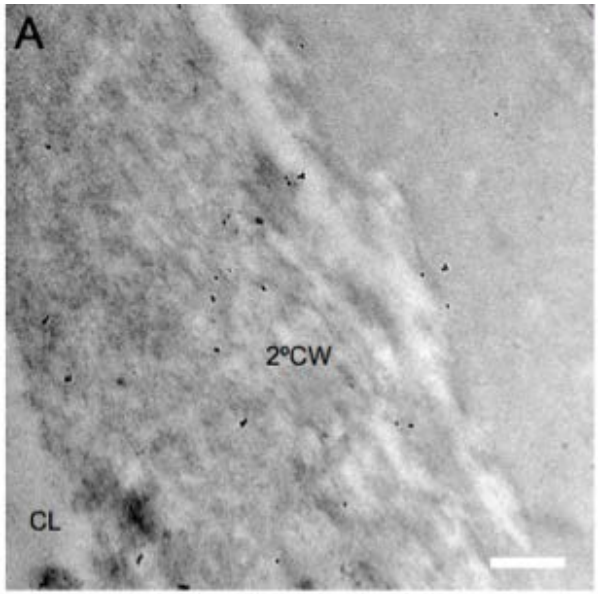
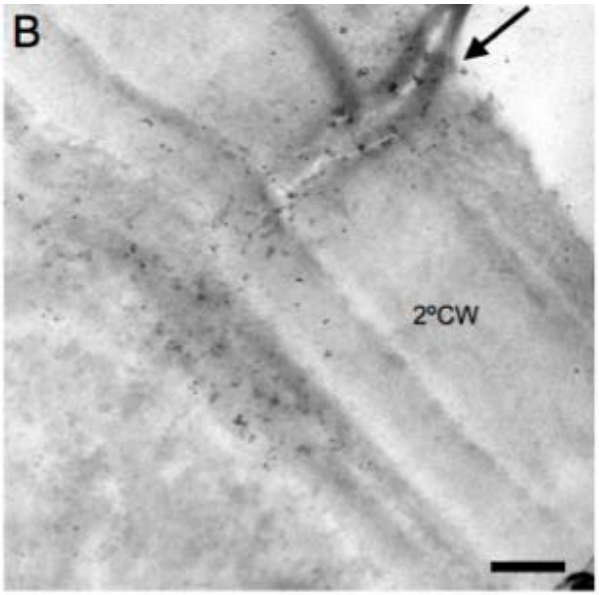
Enzyme Accessibility is Different

"Free" Enzymes



- Cellulases are evenly distributed
- Cellulosomes are near the cell walls

Cellulosomes



Possible factors limiting cellulosome activity on DA pretreated biomass

- Accessibility
- Low k_{off} compared to free cellulases
- Binding to lignin

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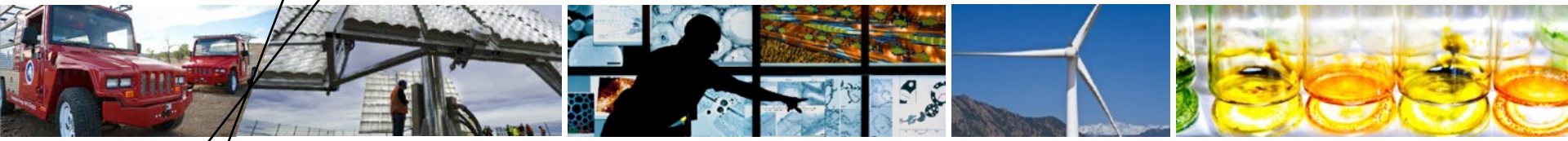
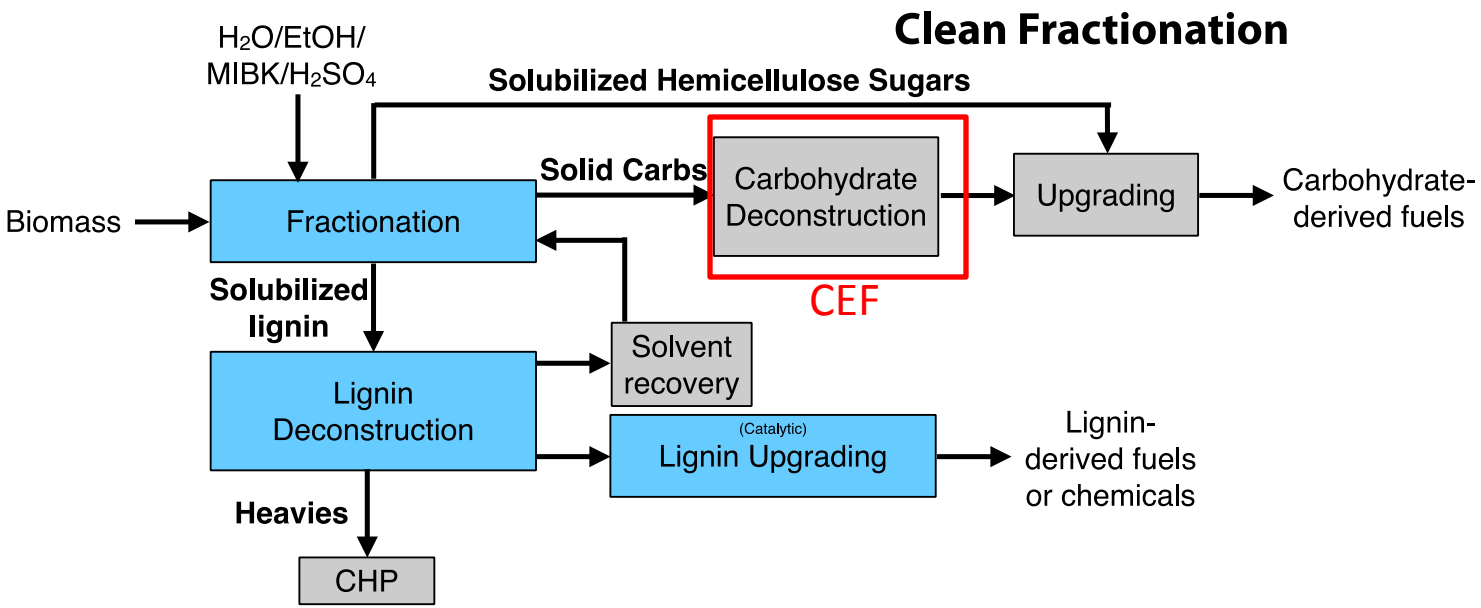


Clean Fractionation Pretreatment (Organosolve)

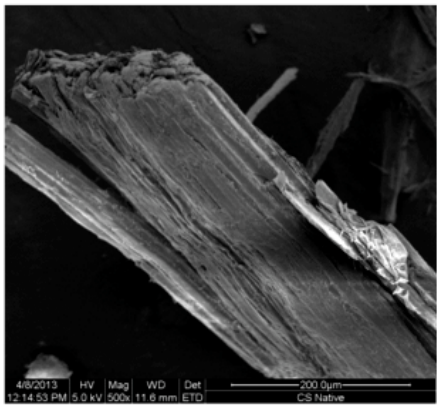


CEF Chemical Composition

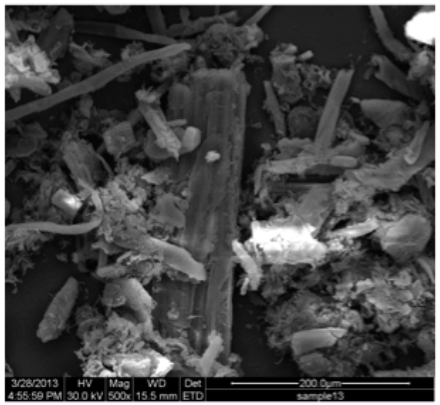
Solvent	Temp (°C)	Acid (mol/L)	Lignin (%)	Glucan (%)	Xylan (%)
MIBK/**Acetone**/H2O	140	0.025	10.63	63.31	17.47
MIBK/**Acetone**/H2O	140	0.05	3.67	82.28	6.56
MIBK/**Acetone**/H2O	140	0.1	3.33	87.19	3.08

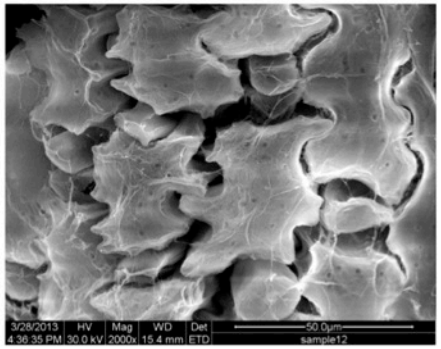
How does Clean Fractionation affect biomass morphology?

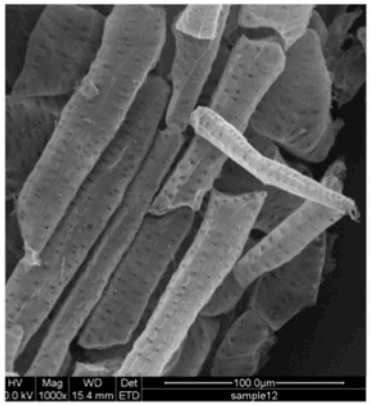
Native Vascular bundle



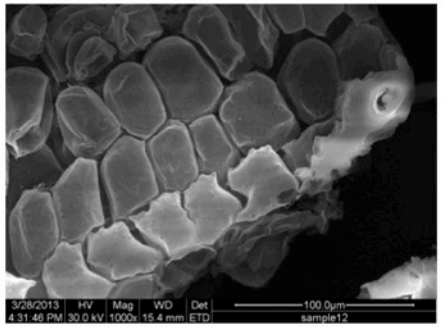
Dilute acid-treated

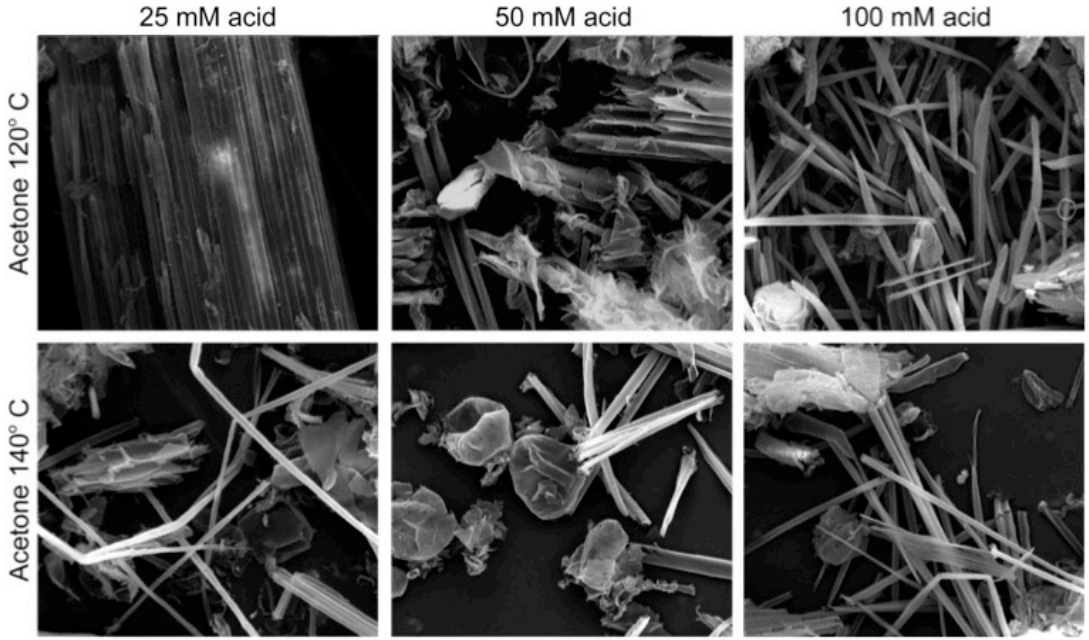






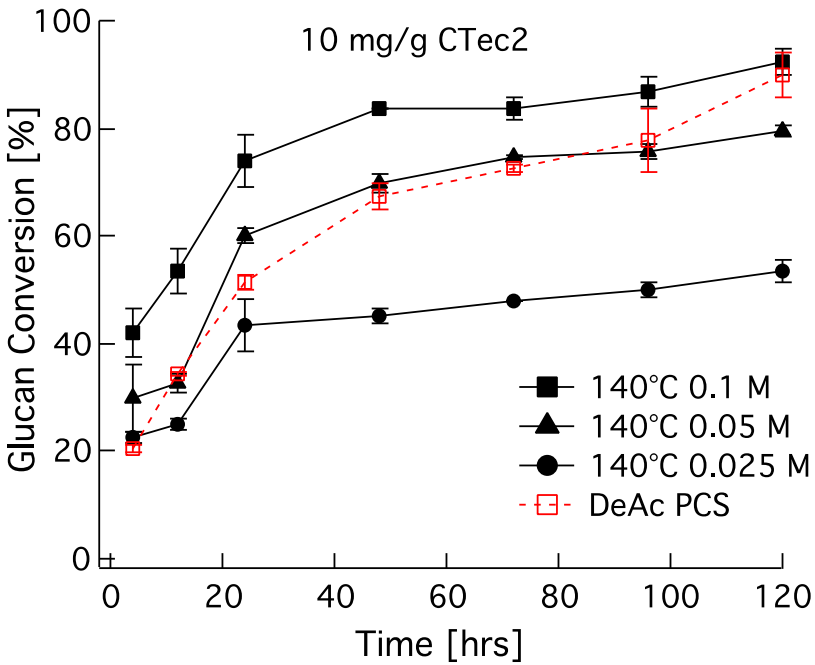
140° C 0.1M

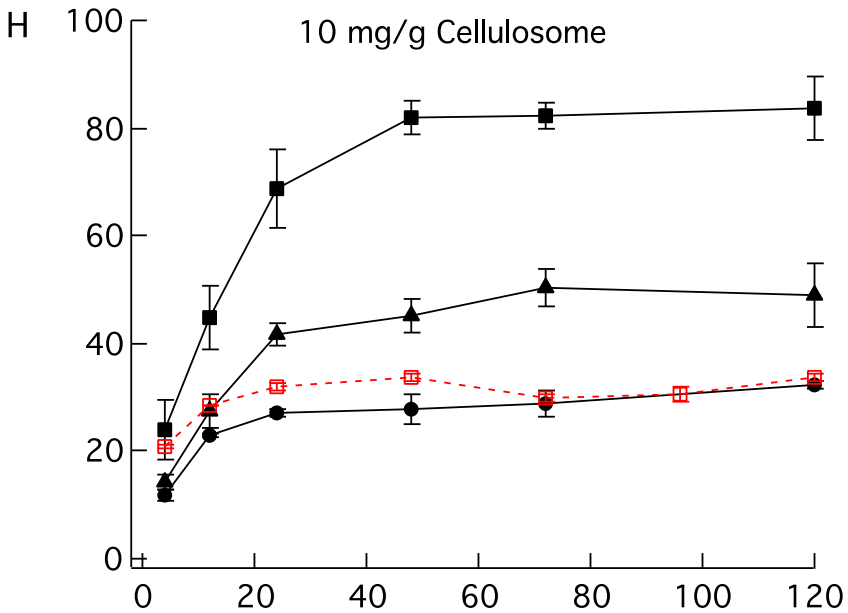




Katahira, R. *et al* (in review)

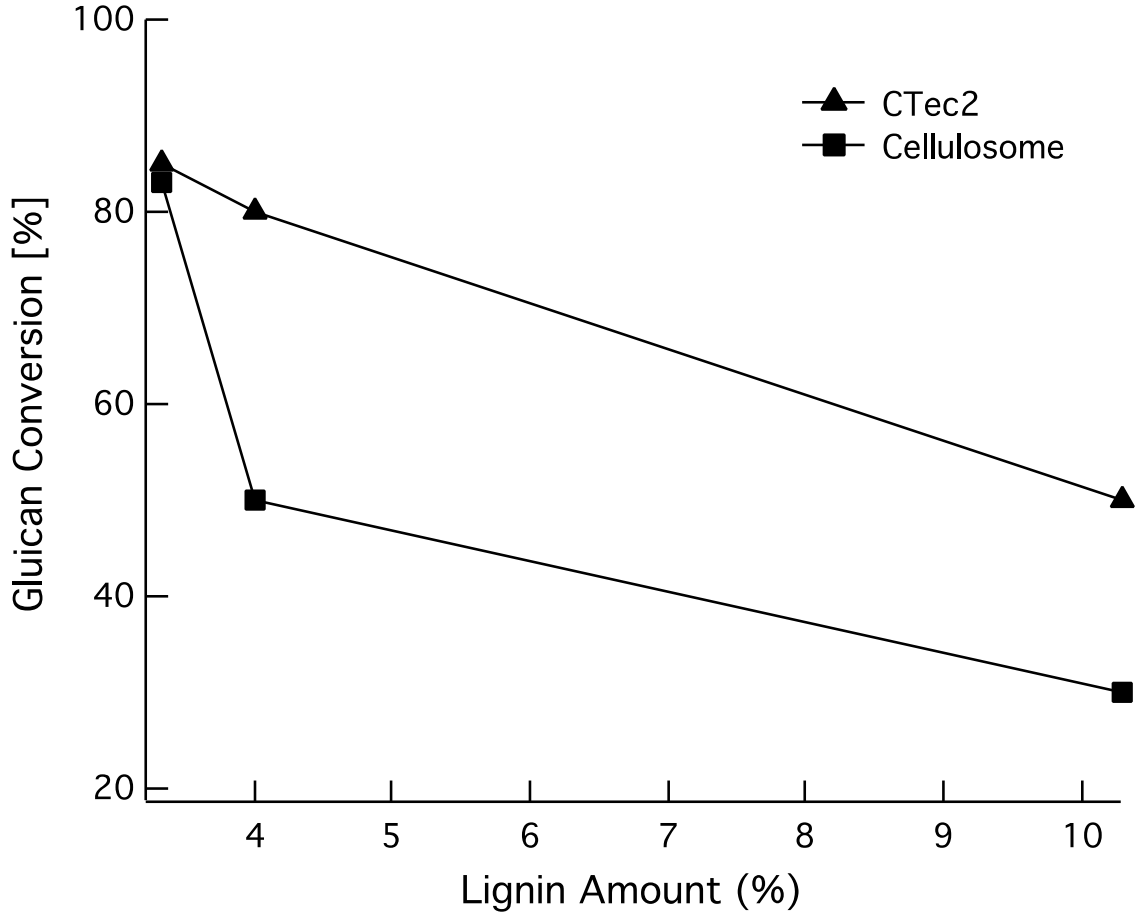
How does Clean Fractionation affect enzyme saccharification?





Resch, M. *et al* (in review)

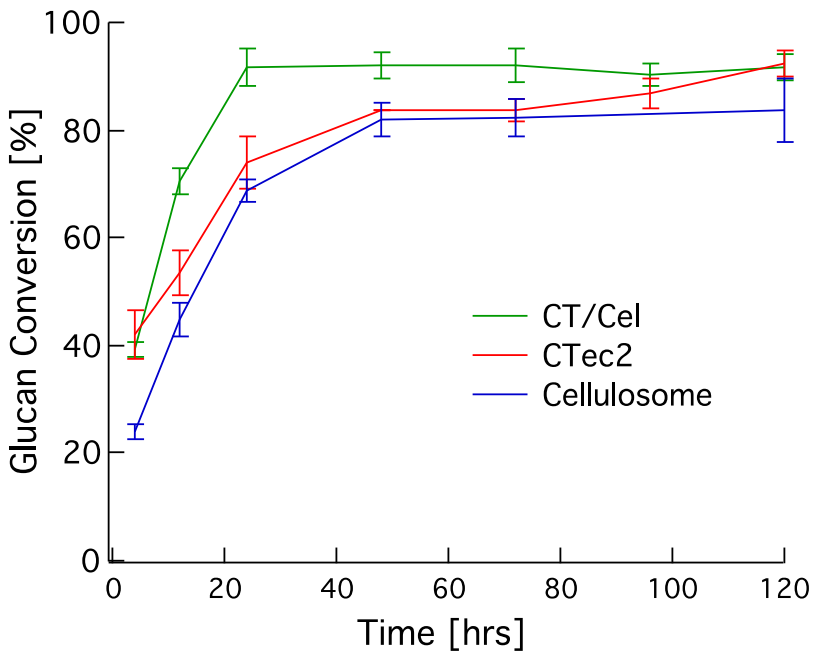
Affect of PT Severity on Enzymatic Saccharification

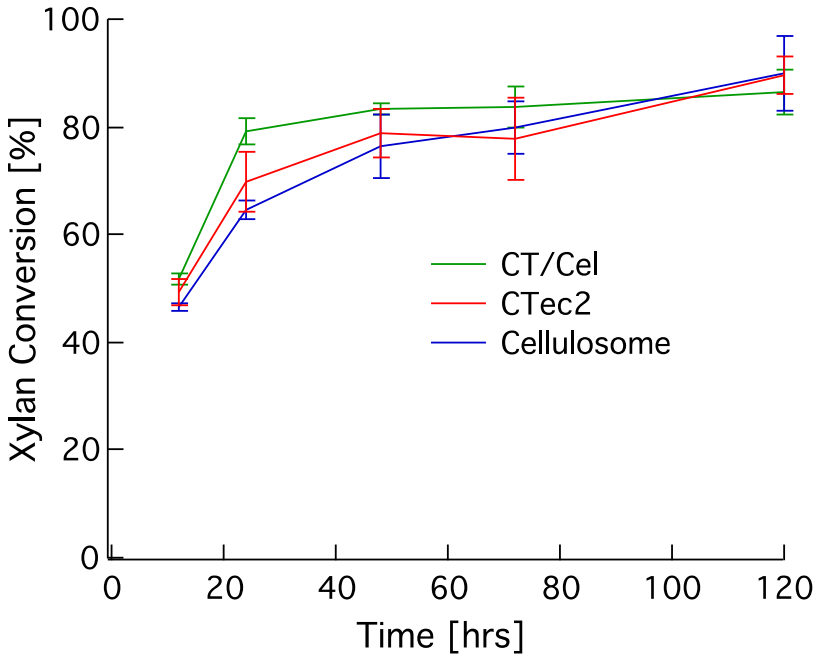


10 mg/g enzyme loading,
72 hours

Resch, M. *et al* (in review)

Synergistic Combination of CTec2 and Cellulosomes



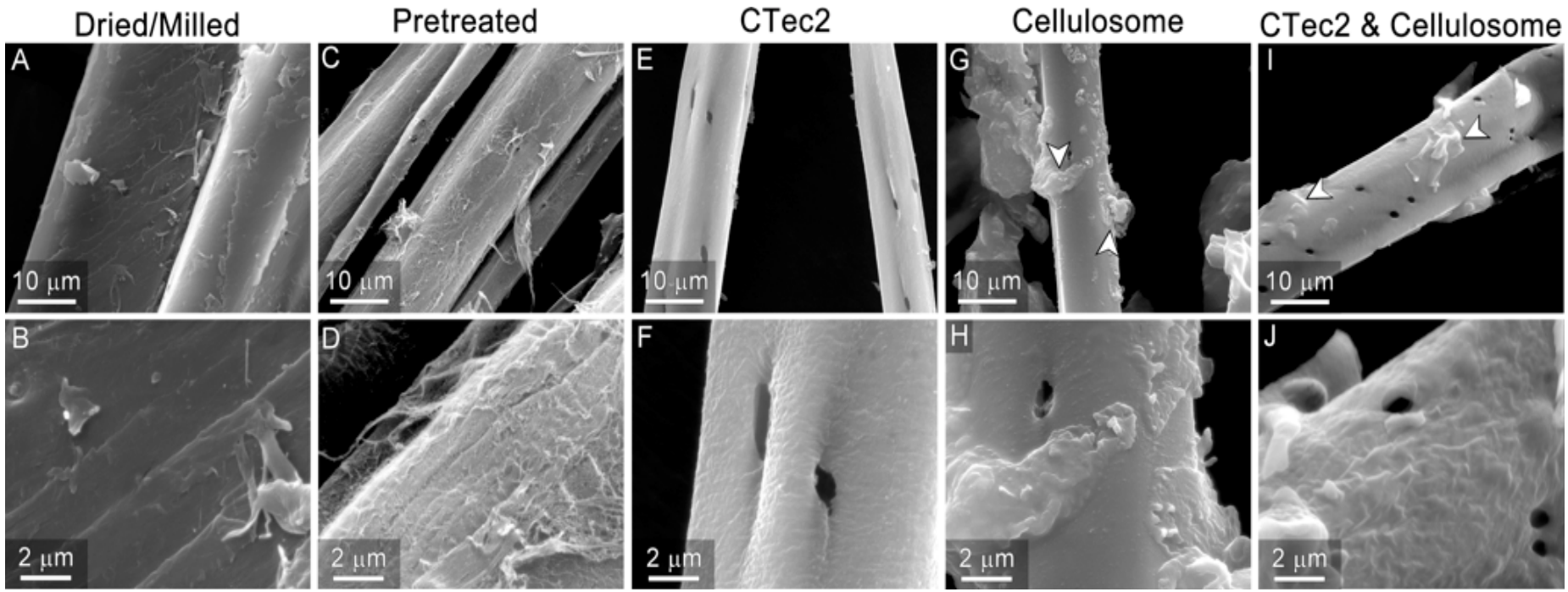


Resch, M. *et al* (in review)

Technoeconomic Results from CF Work

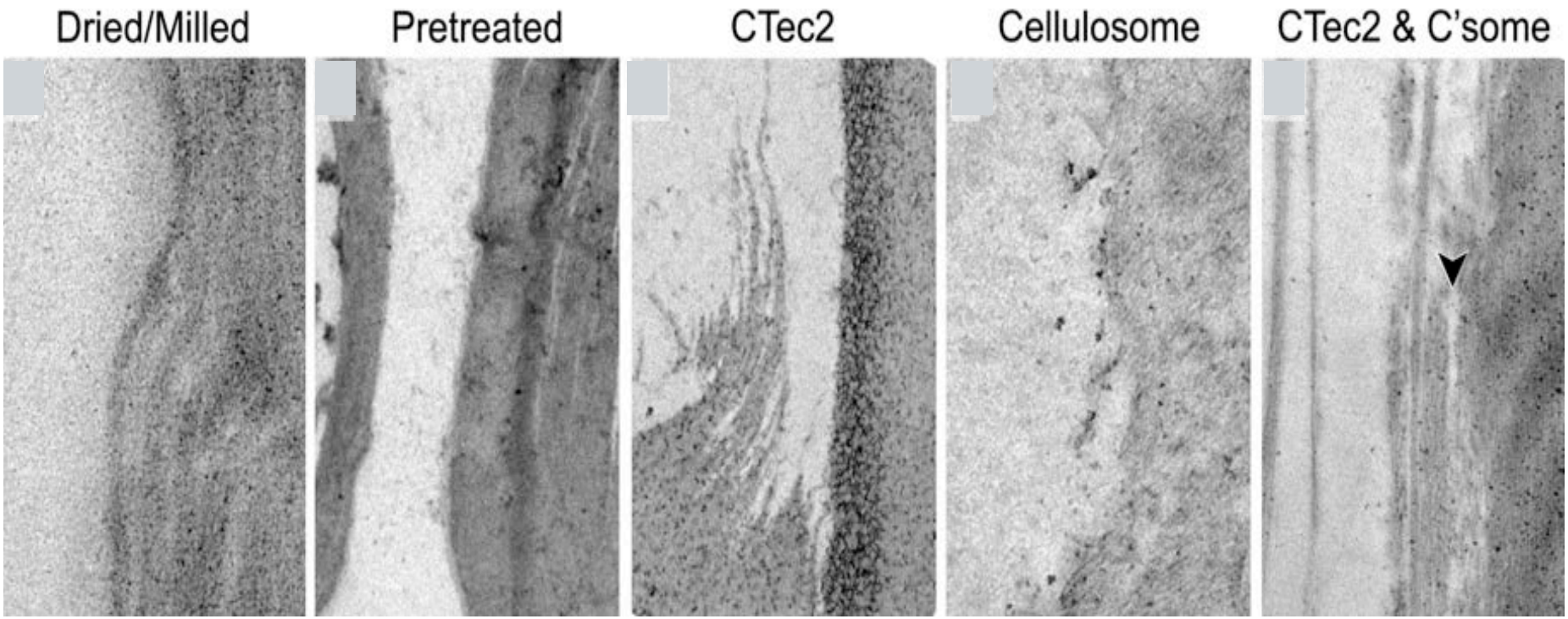
Mary Biddy (NREL)

SEM of Digested Cellulose Enriched Fraction



Resch, M. *et al* (in review)

TEM of Digested Cellulose Enriched Fraction



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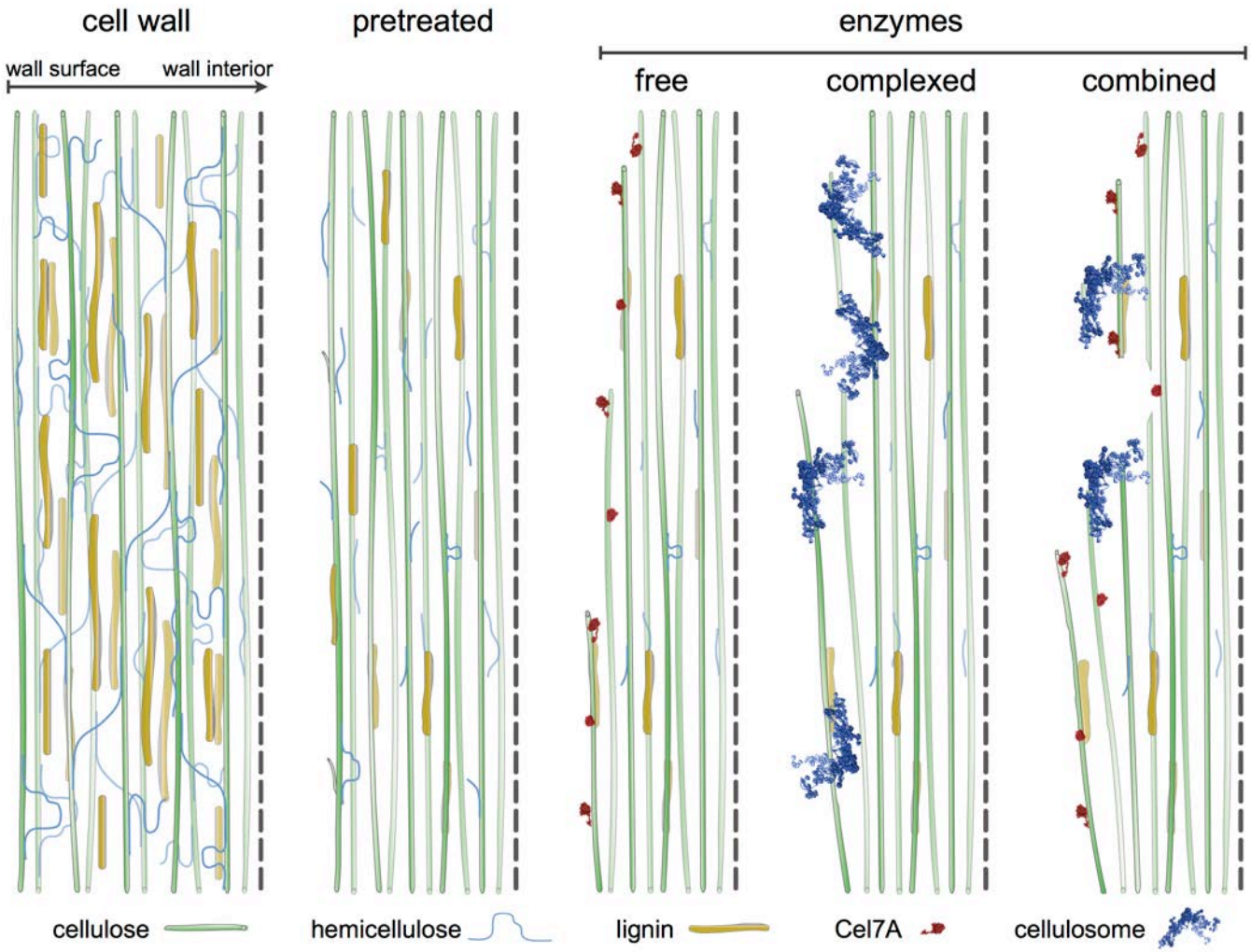
Model of Enzymatic Deconstruction Mechanisms



Ciesielski, P., Resch, M. *et al* (in review)

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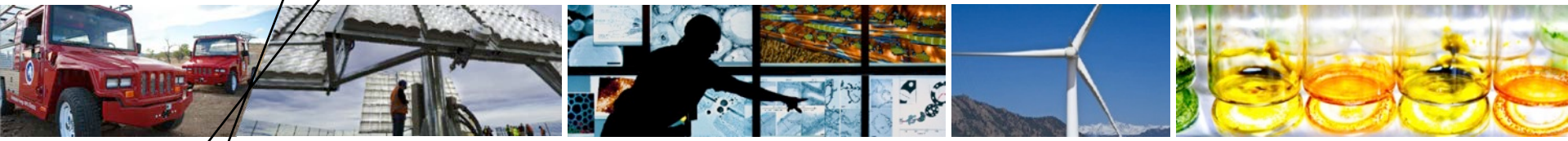
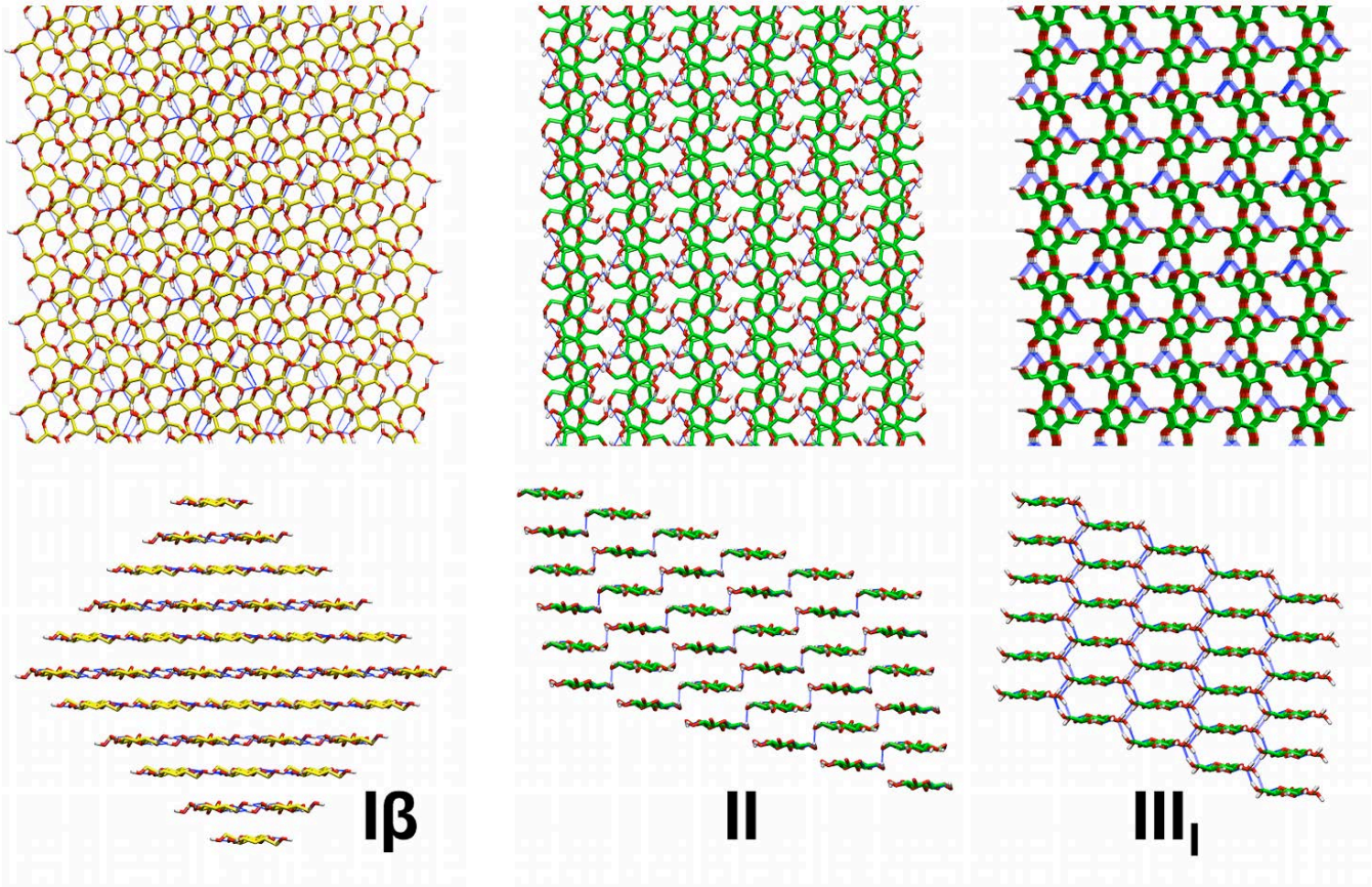


Cellulose Allomorphs

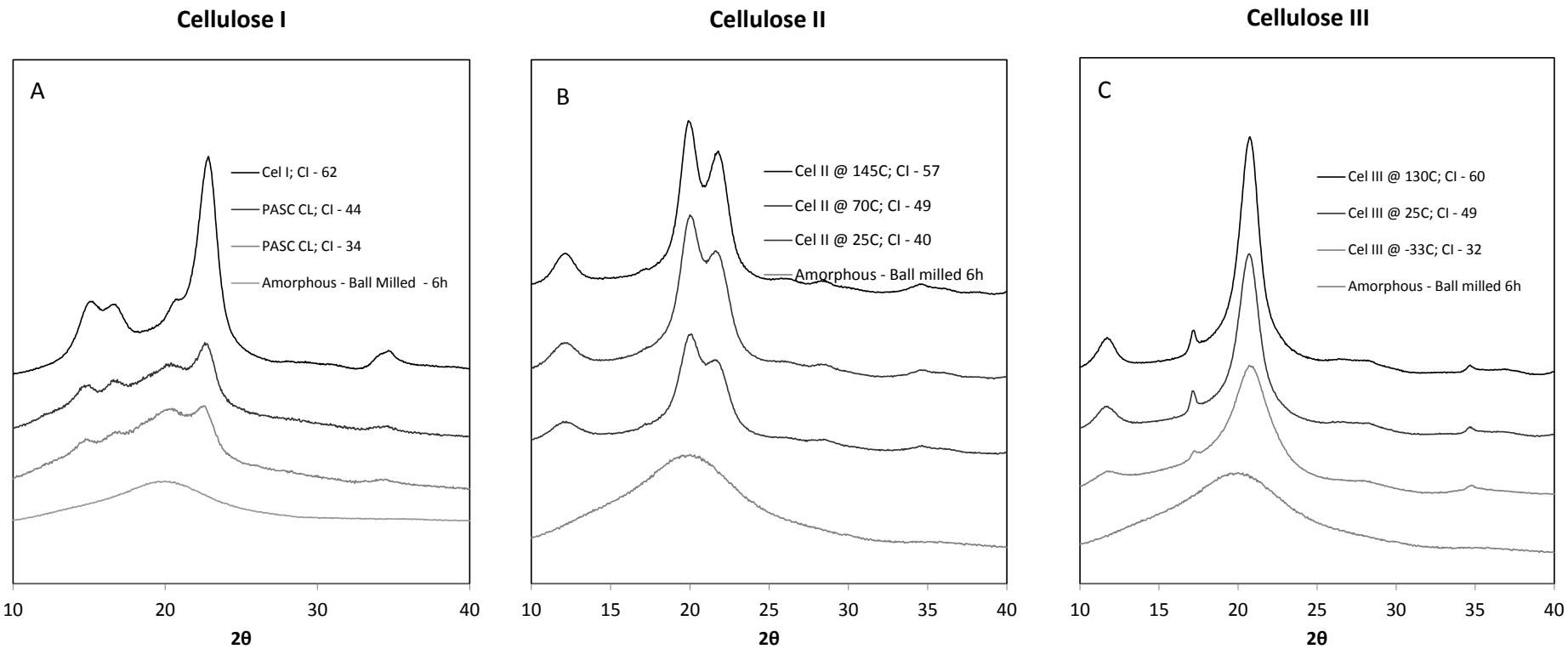
Allomorph EH Study Objectives

- ✓ **Isolate cellulose allomorphs with identical crystallinity.**
- ✓ **How do complexed, free and a combination perform on cellulose I, II and III?**
- ✓ **Does the crystallinity change over the course of digestion?**
- ? **How does cellulose type effect the hydrolysis mechanism with free, complexed and combination?**

Crystal Structure Models



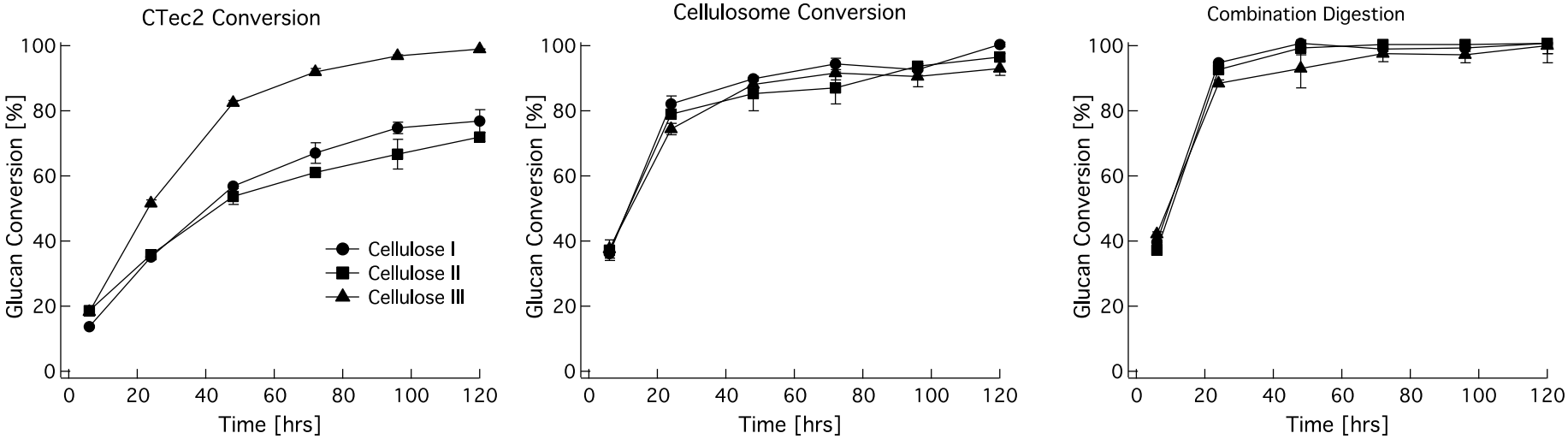
Cotton linters Allomorphs - XRD



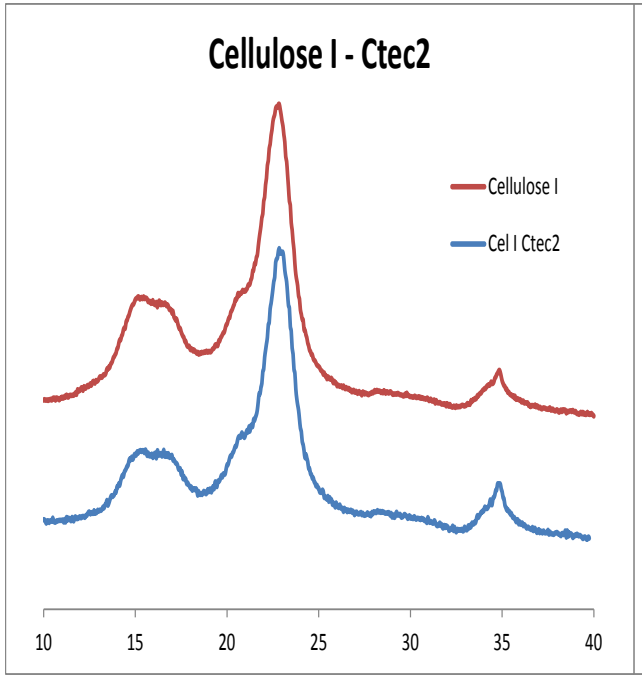
Allomorph	Crystallinity Index (CrI)		
Cellulose I	62	44	34
Cellulose II	57	49	40
Cellulose III	60	49	32

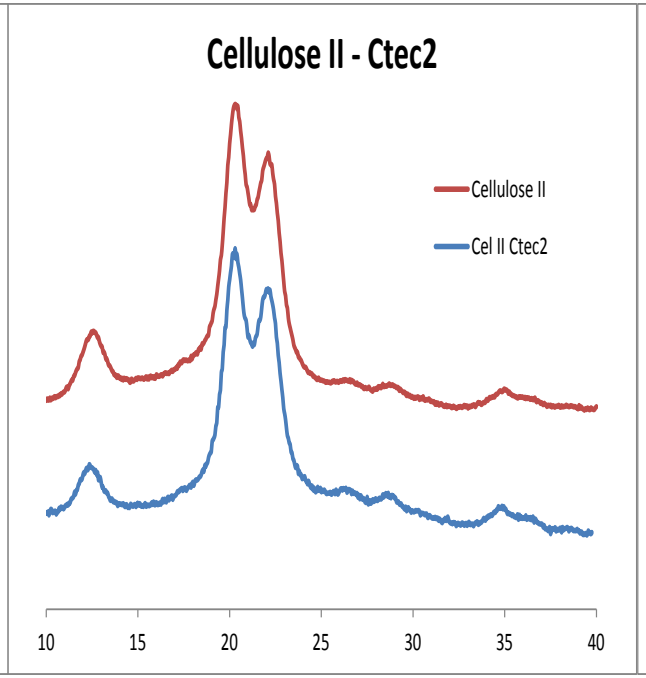
Ashutosh Mittal

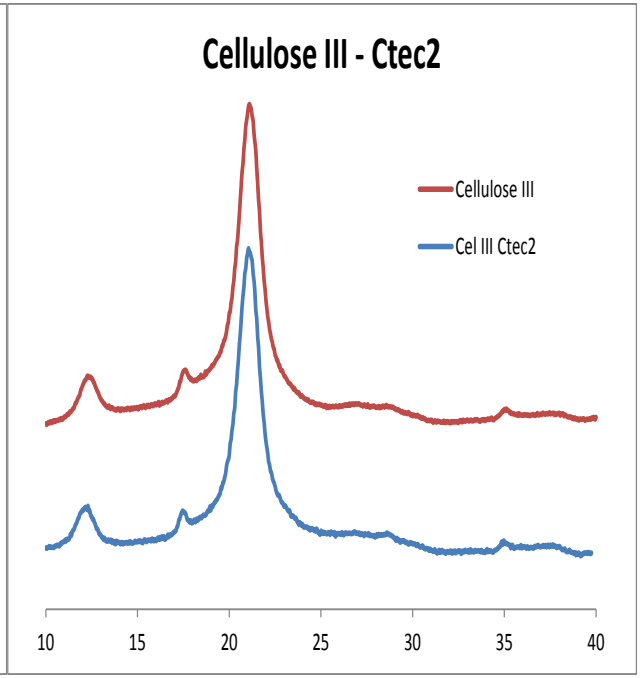
Enzymatic Hydrolysis of Cellulose Allomorphs



XRD – 50% EH with Ctec2

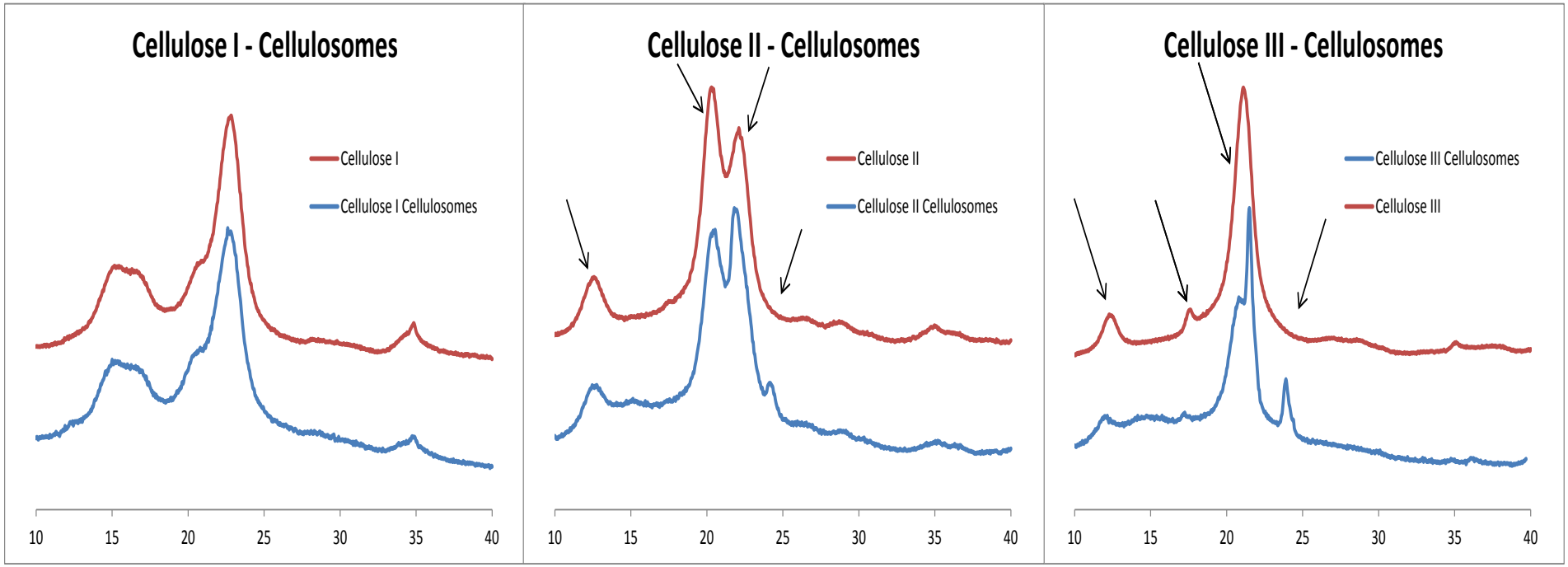






Ashutosh Mittal

XRD – 50% EH with Cellulosomes



XRD of cellulose II and III allomorphs change after 50% digestion with Cellulosomes

- Change in the intensity of XRD from various crystal planes
- Emergence of a new peak at 2θ = 24°

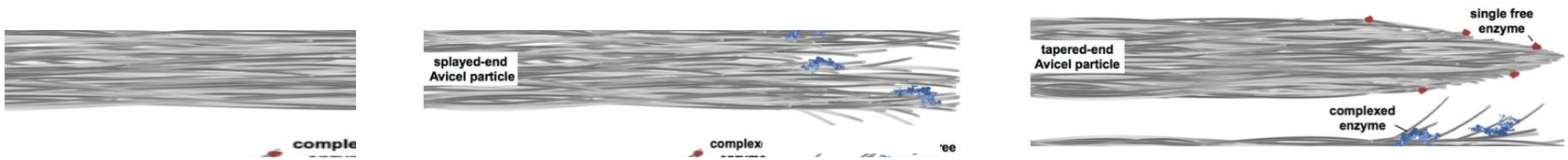
Do cellulosomes attack crystalline regions selectively?

Currently looking at the digestions of cellulose allomorphs with combination of Ctec2 and Cellulosomes

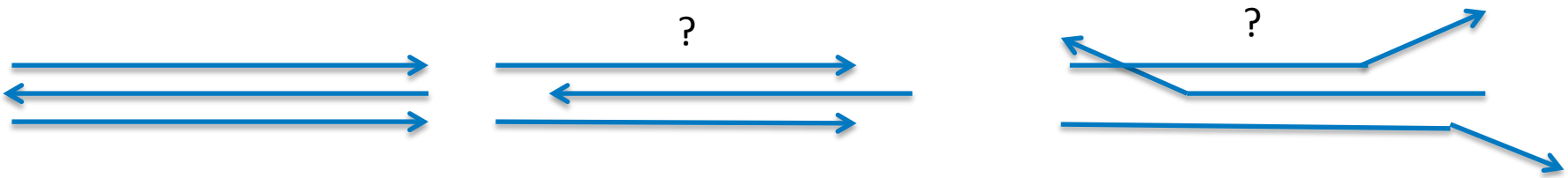
Free

Complexed

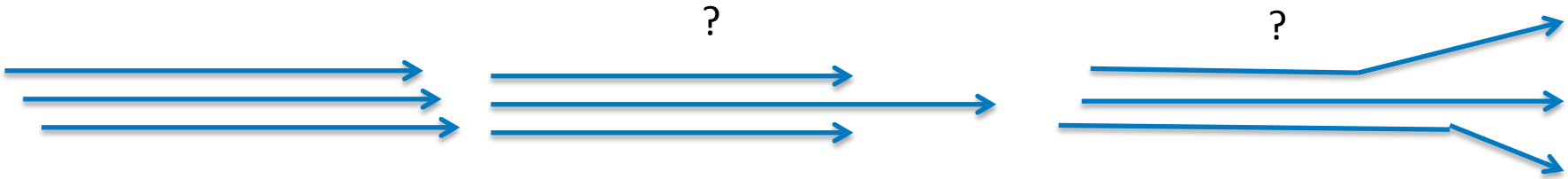
I



II



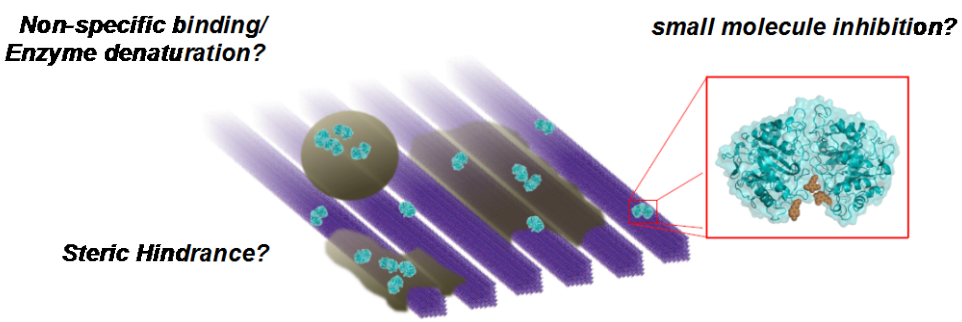
III

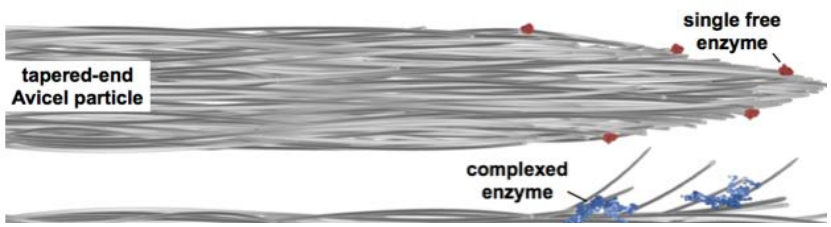


Conclusions

- "Free" enzymes and cellulosomes have distinct mechanisms in cellulose and biomass deconstruction
- "Free" enzymes and cellulosomes are synergistic
- Biomass composition and enzyme accessibility to cellulose ends affect cellulosomes more than "free" enzymes
- Combining properties of "free" and multi-domain enzymes in commercial enzyme cocktails could lead to enzyme cost reductions

Is Lignin More Inhibitory to Cellulosomes than Free enzymes?





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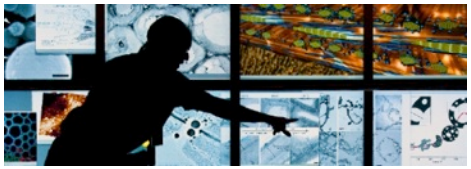

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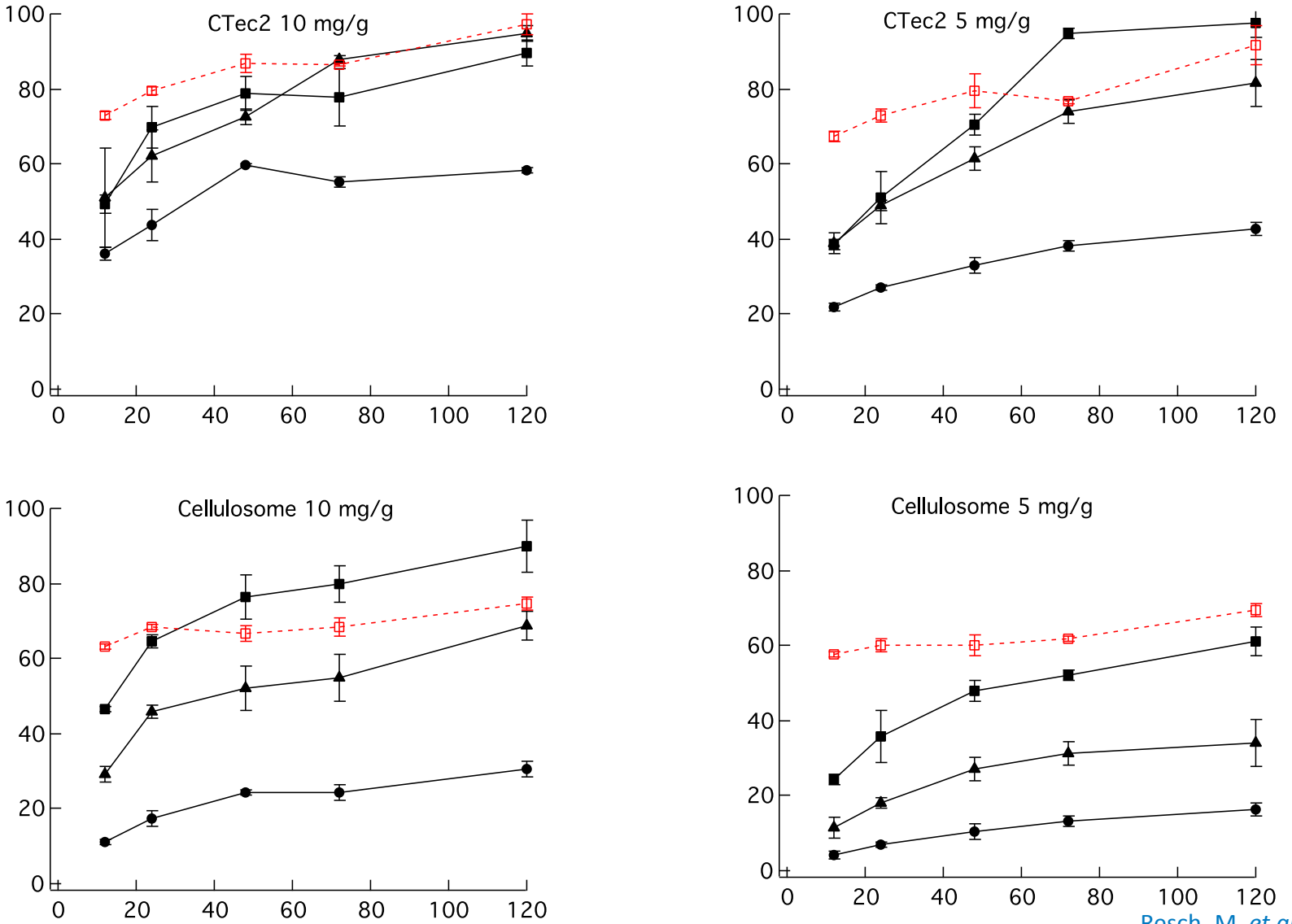
Clean Fractionation – Xylan Conversion



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