Studying Anaerobic Fungi For Use In Biofuel Applications

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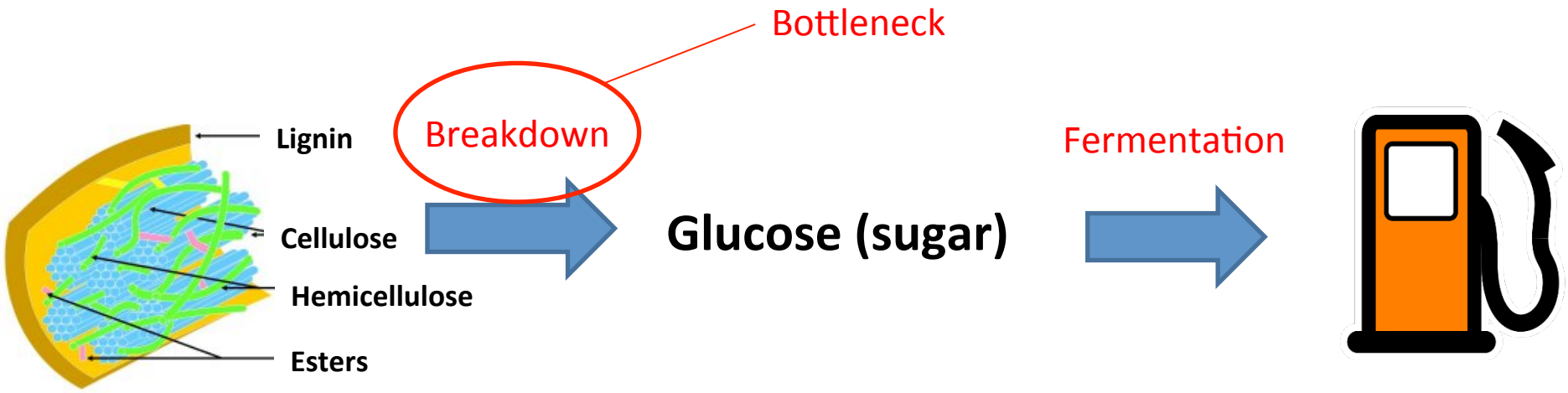
U.S. DEPARTMENT OF ENERGY

ICB

Image courtesy of: eandt.theiet.org

Anaerobic Fungi Naturally Break Down Cellulose in to Sugars

- Break down followed by fermentation leads to valuable products



Lignofuel.wordpress.com

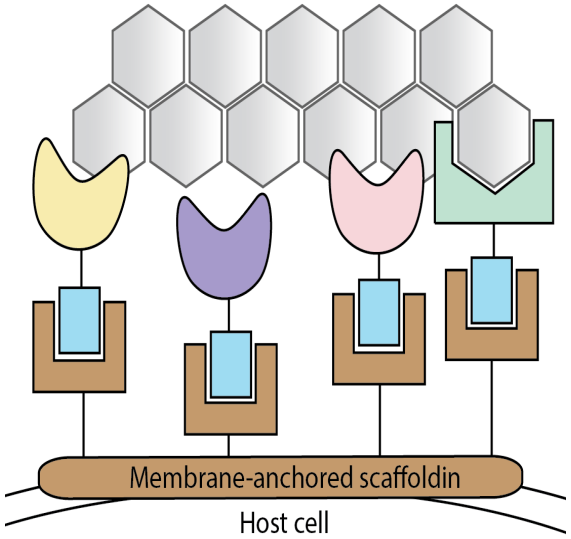
4vector.com

Why do we care?

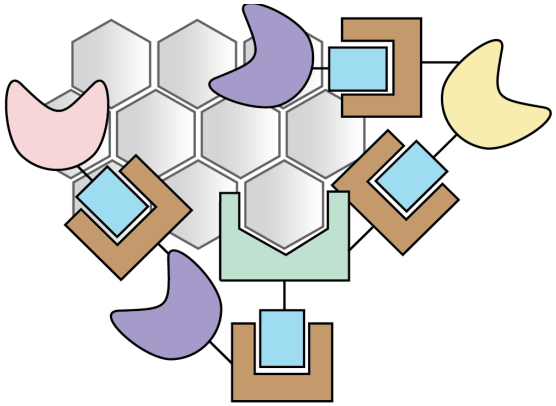
- Cellulosic biofuels are a promising renewable energy source
- Will help strengthen the economy, national security, and decrease environmental impacts of energy use

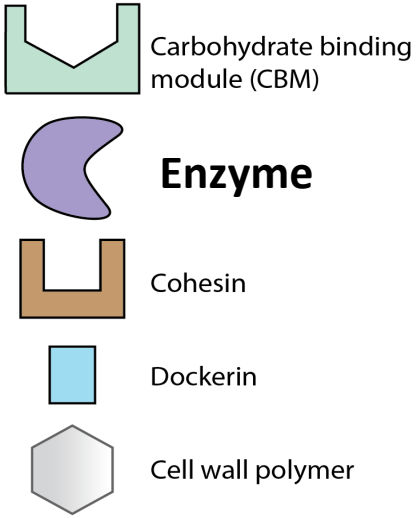
Competing Models for Enzyme Complexes

Model A: Enzymes are anchored



Model B: Enzymes run freely





Courtesy of: Charles Haitjema

Known:

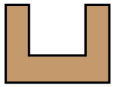
- Enzymes assemble in to large complexes
- Identity of Dockerin domains

Unknown:

- Cohesin binding partners for dockerin domains
- Which model most closely resembles how the enzymes behave

Research Goals

Short Term

Identify Cohesin domains 

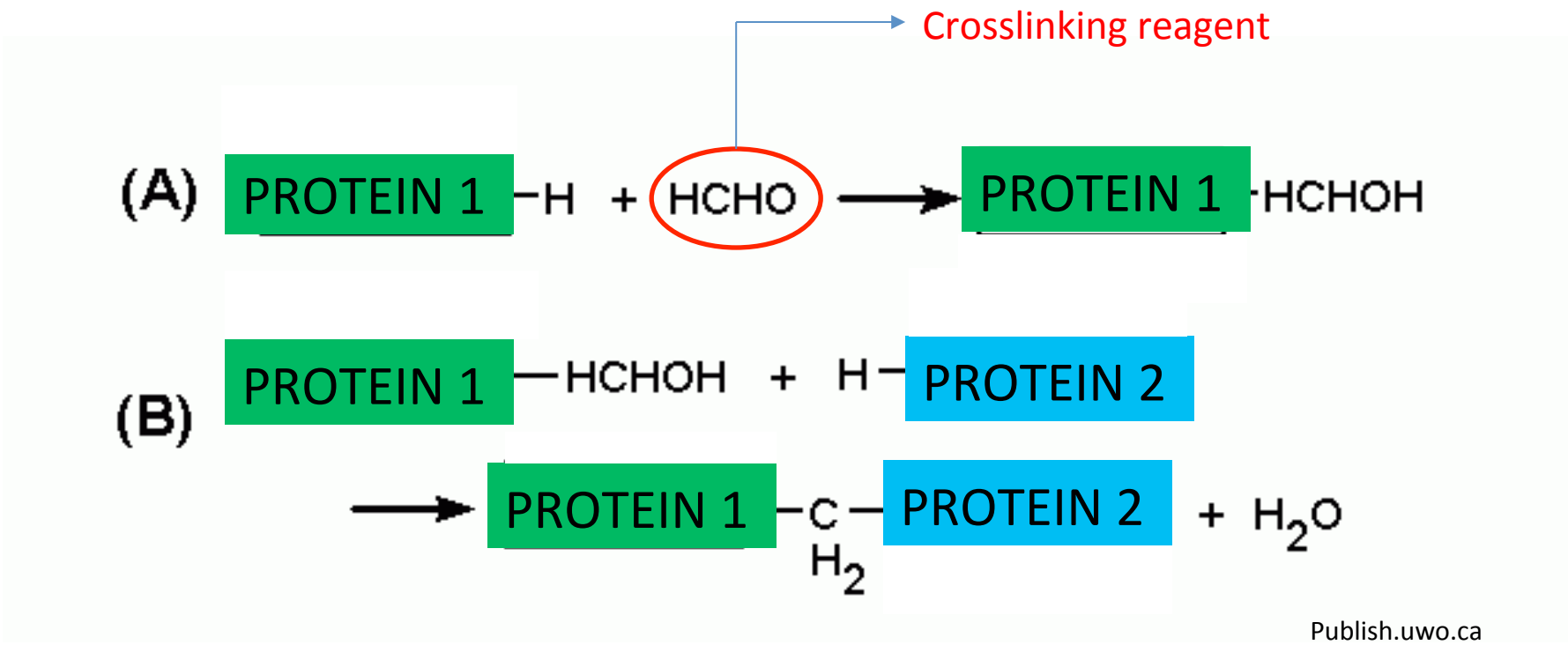
Identify which model most closely matches how the enzymes actually behave

Intermediate

Gain a complete understanding of the enzymes that mediate the breakdown of biomass

Long Term

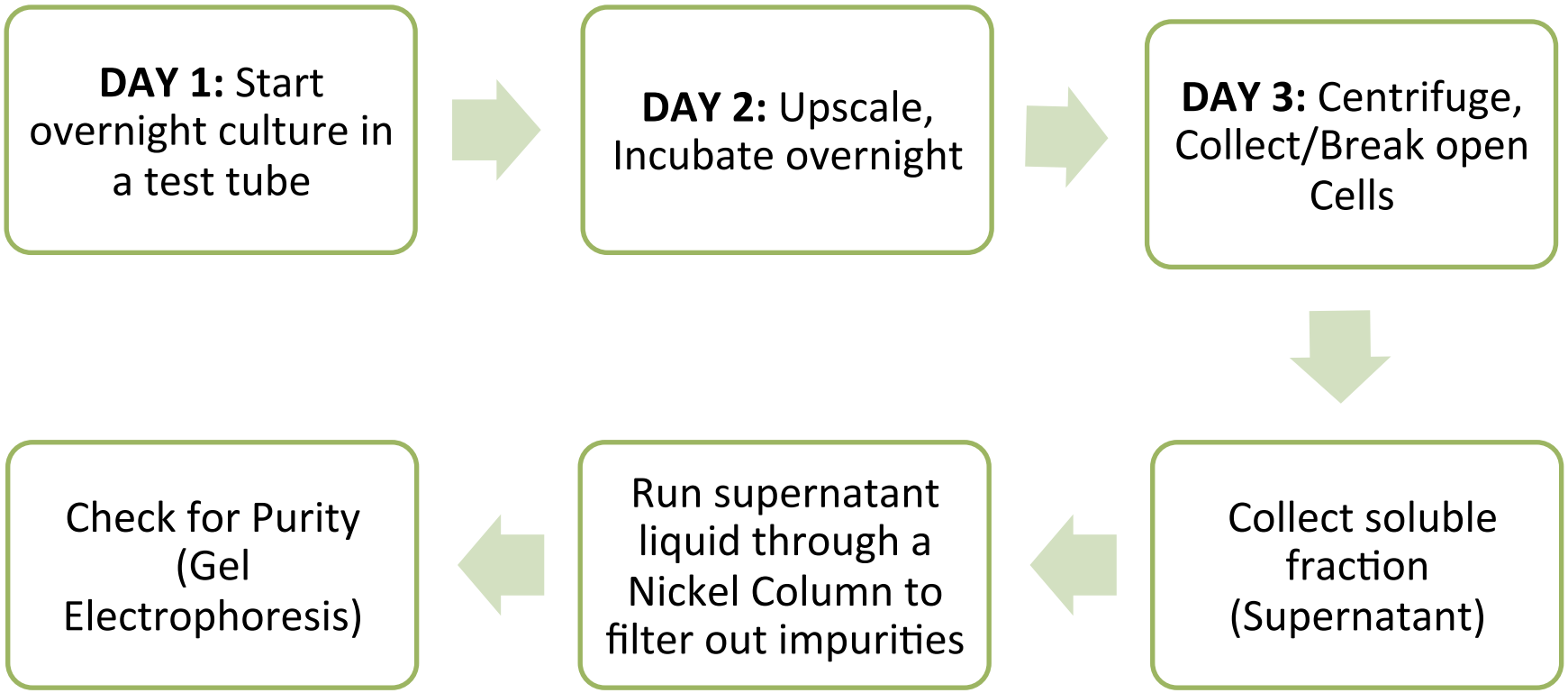
Crosslinking Experiments Are Used to Observe How Proteins Interact



Publish.uwo.ca

Before crosslinking can be executed, pure protein is needed……

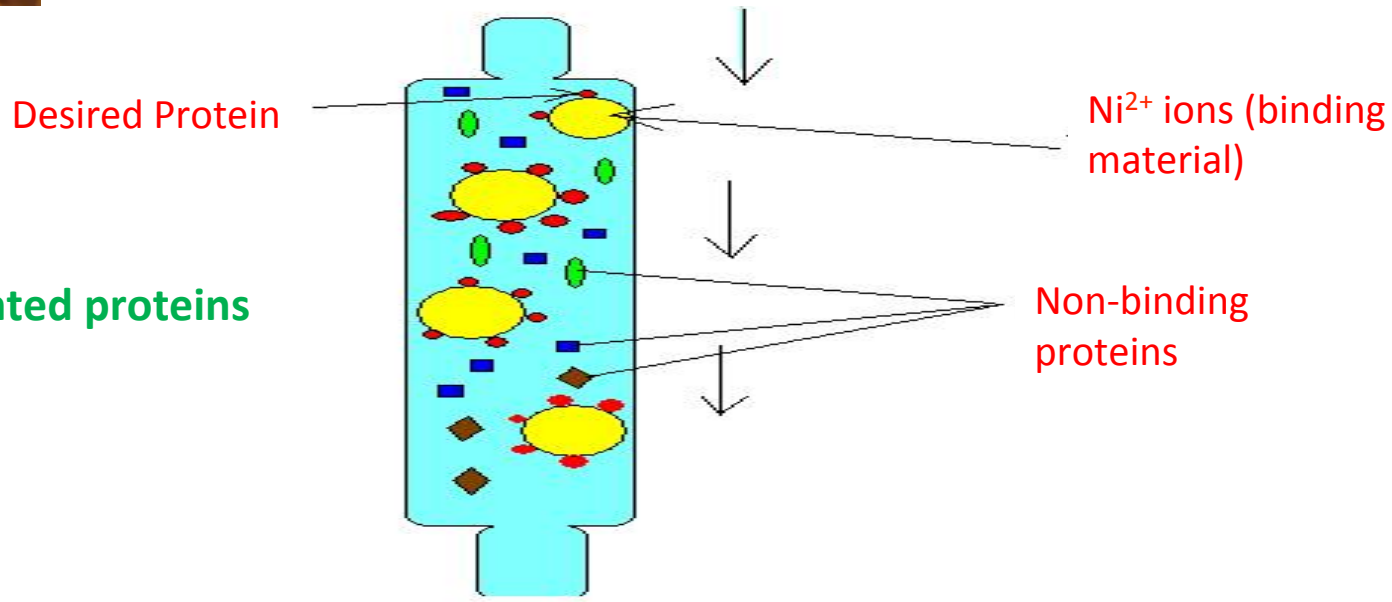
Protein Purification Procedure



Protein Purification Summary

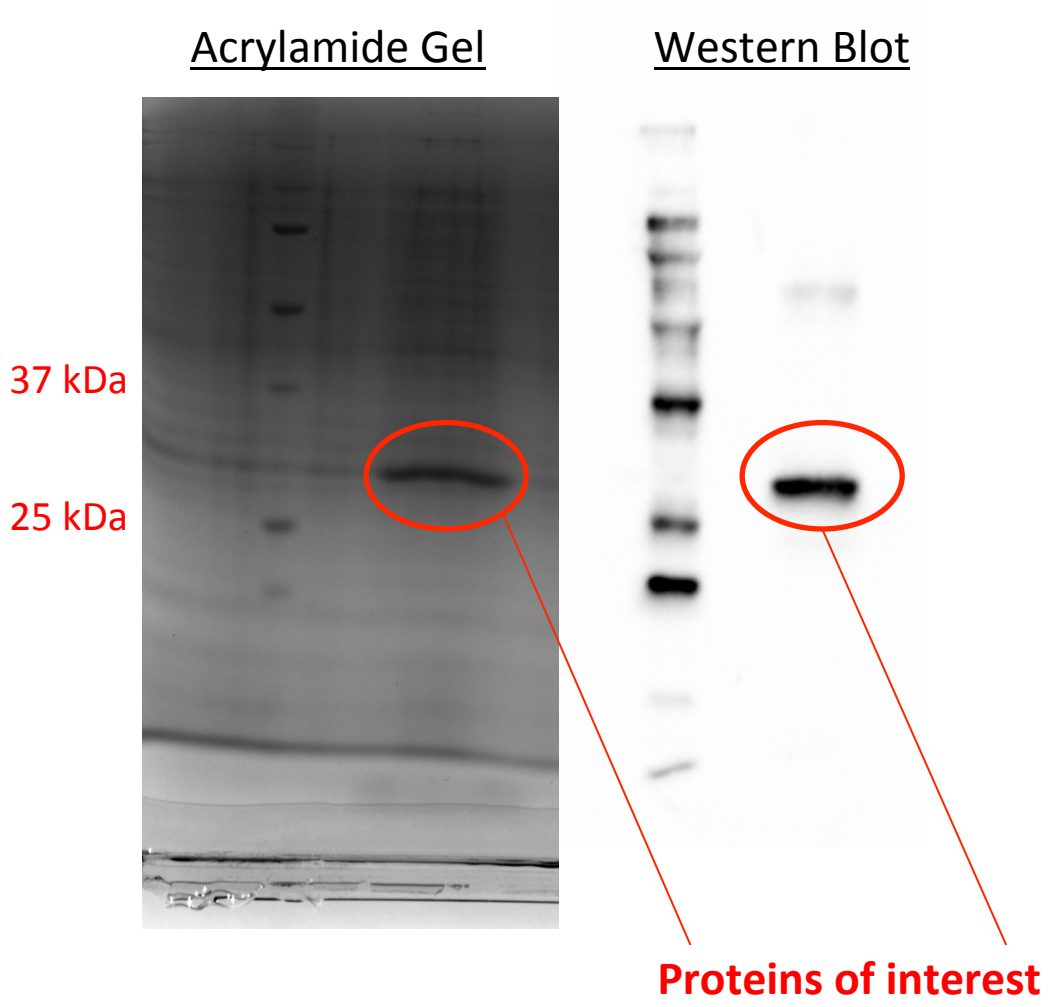
1) Start Culture

2) Break Open Cells



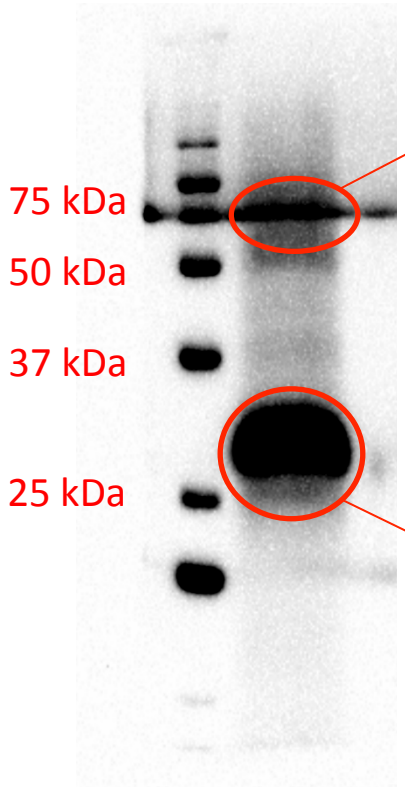
3) Filter out unwanted proteins

Results of Protein Purification



Successful purification of 13208 Dockerin protein (Finally!!). Ready to use in crosslinking experiments to identify binding partners.

Crosslinking Experiments Reveal A Binding Partner With Mass of ~75 kDa

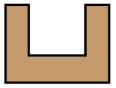


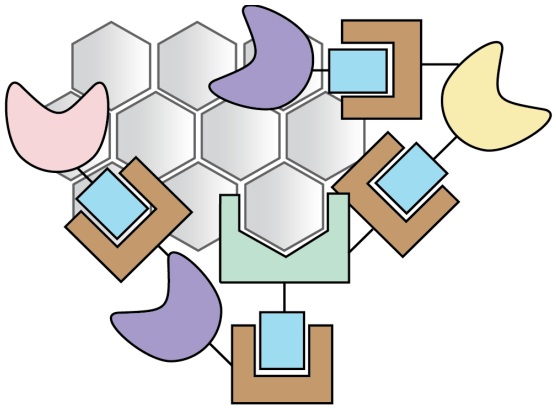
Crosslinked Protein

Dockerin Protein

Further analysis is needed to determine molecular structure of the binding partner

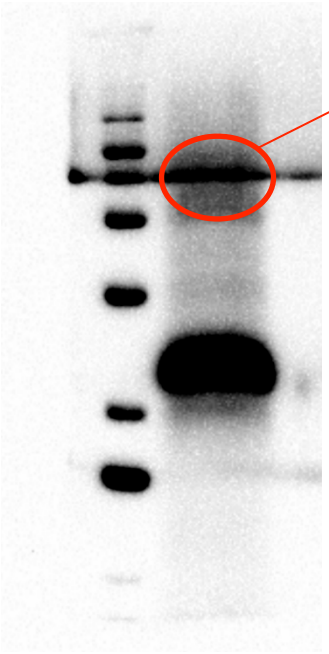
Summary

Cohesin binding partners  for Dockerin domains  have been identified by use of a chemical crosslinker



Future Work

Fully characterize Anaerobic gut fungi enzyme complex



? Further analyze binding partners fungal proteins using mass spectrometry

Use this understanding to create synthetic enzymes to breakdown biomass