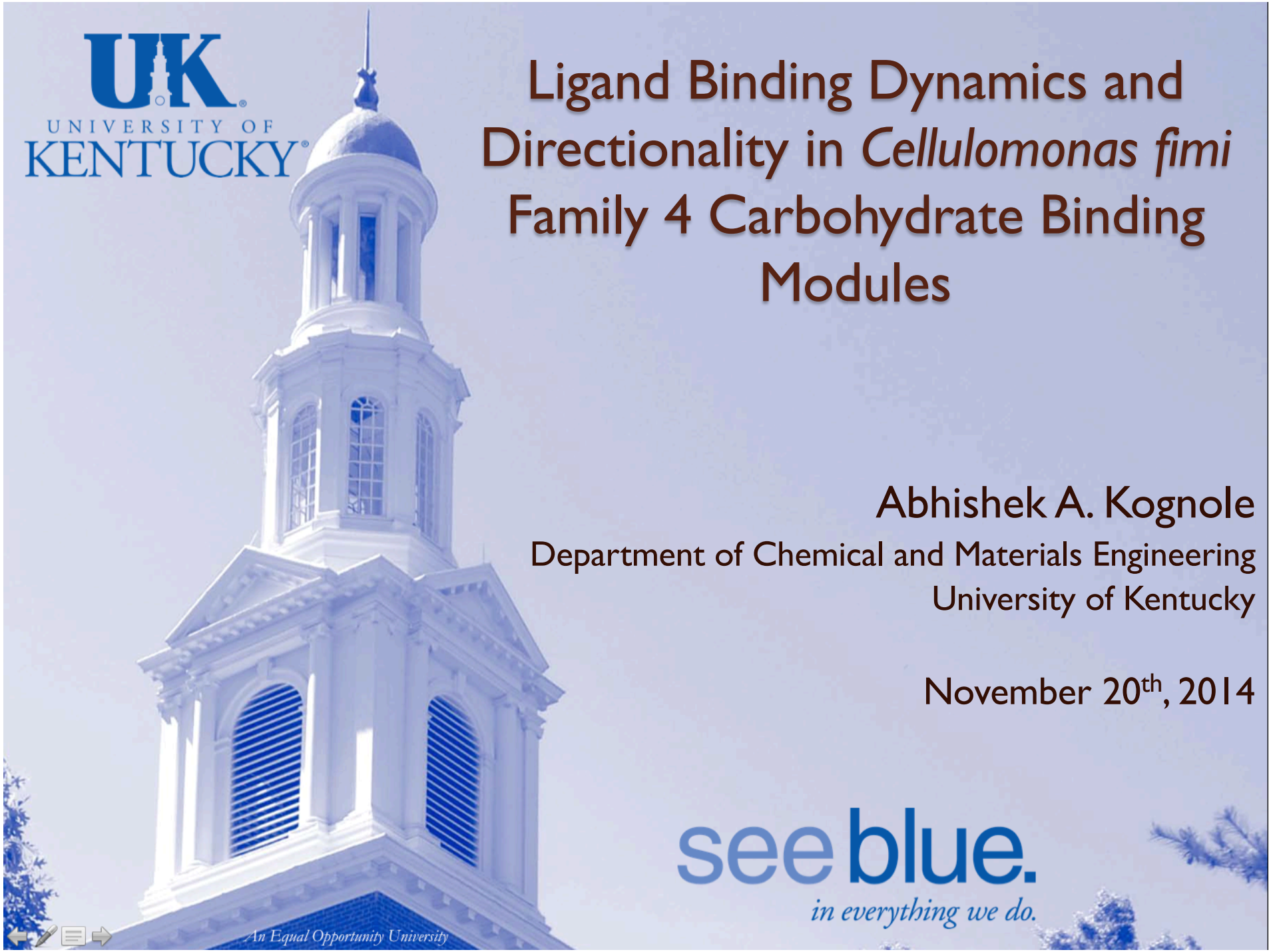


Ligand Binding Dynamics and Directionality in *Cellulomonas fimi* Family 4 Carbohydrate Binding Modules

Abhishek A. Kognole
Department of Chemical and Materials Engineering
University of Kentucky

November 20th, 2014

Carbohydrate Binding Modules (CBMs)

Energy

Biofuels

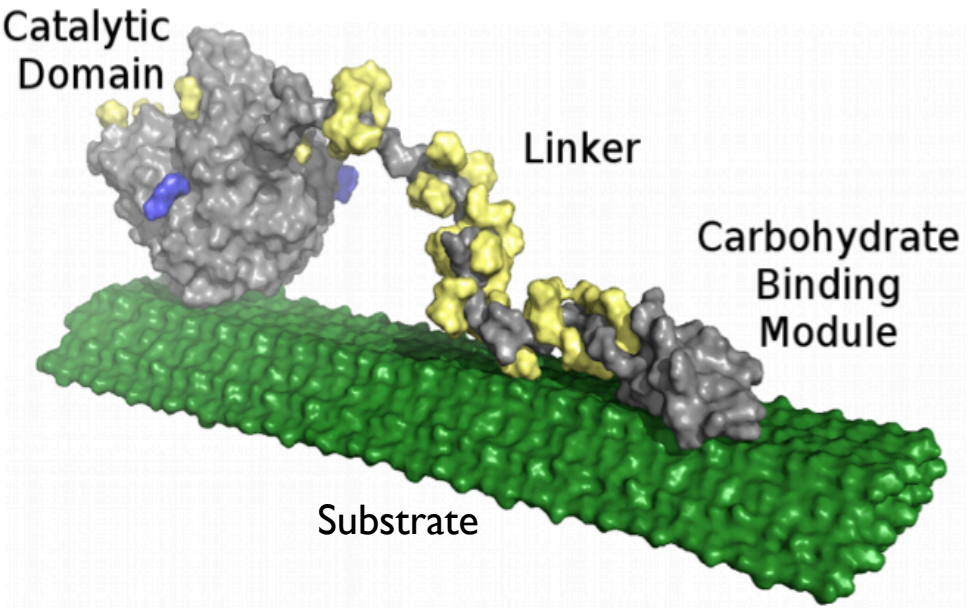
Biomass - Plant Cell Wall

Cellulose

Enzymatic Hydrolysis

Multi-modular Glycoside Hydrolases

Carbohydrate Binding Modules



Functions of CBM:

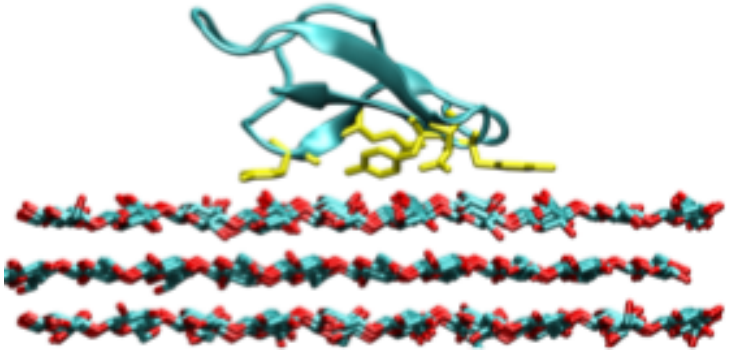
1. Maintain proximity to substrate
2. Target specific regions
3. Disrupt surface crystallinity

Boraston et al., *Biochem. J.*, 2004

Different Substrates – Different Types

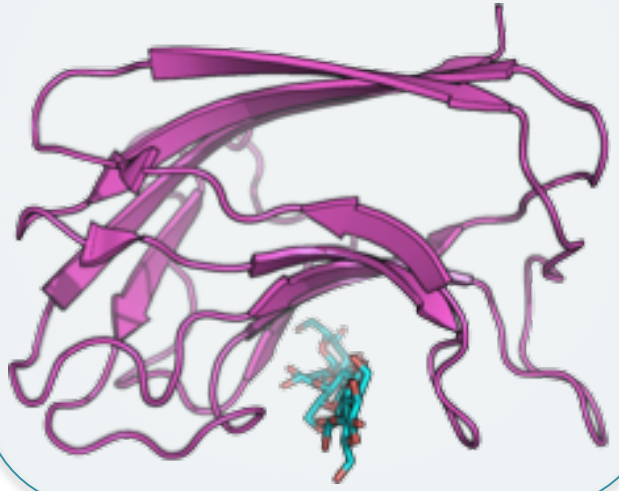
Type A

(crystalline polysaccharides)



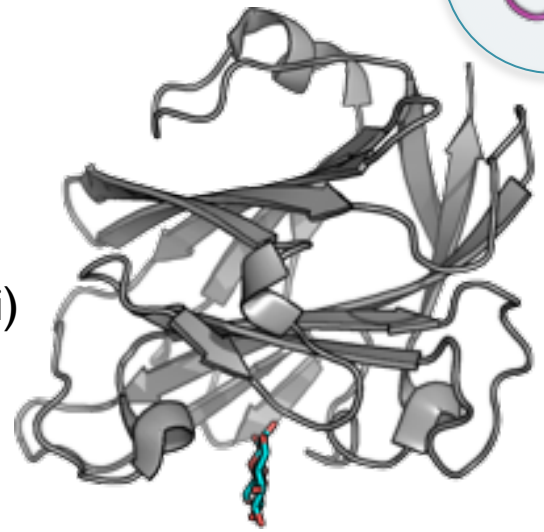
Type B

(internal glycan chains)



Type C

(glycan chain termini)



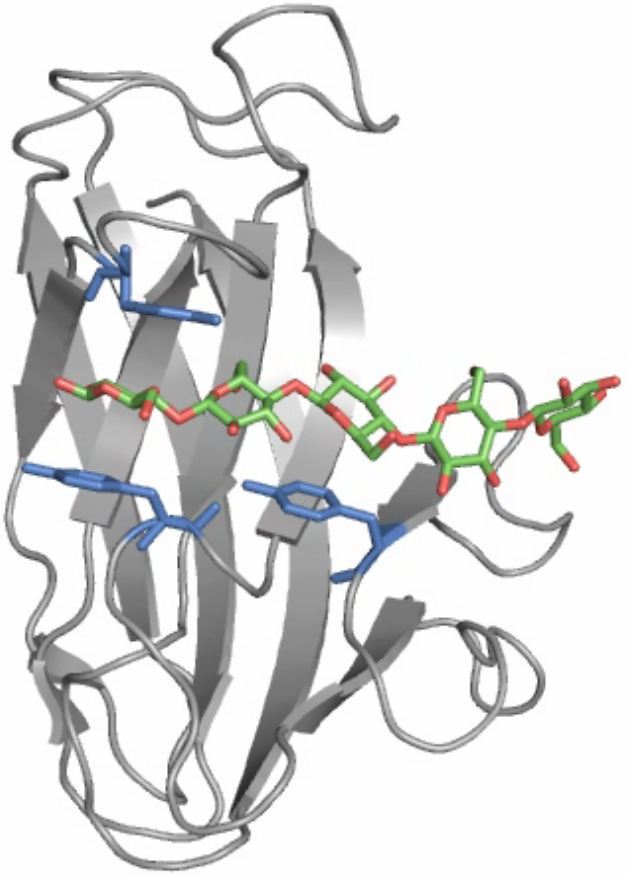
Boraston et al., *Biochem. J.*, 2004
Gilbert et al., *Curr. Opin. Struct. Biol.*, 2013

CBMs of *Cellulomonas fimi* β-1,4 glucanase C

*Cf*CBM4-1

Family 4 (Type B) CBM

- First N-terminal CBM
- β- sandwich fold
- Deep binding groove
- Specific to β-1,4 - glucan
- Enthalpically driven binding
- Prevalent hydrogen bonding

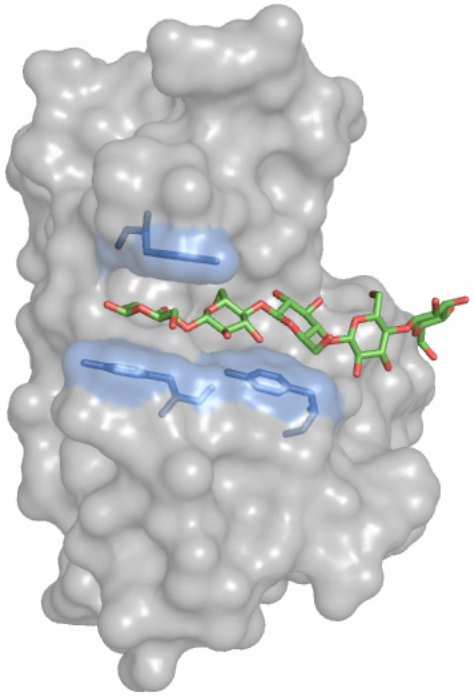


Boraston et al., *J. Mol. Bio.* 2002

CBMs of *Cellulomonas fimi* β-1,4 glucanase C

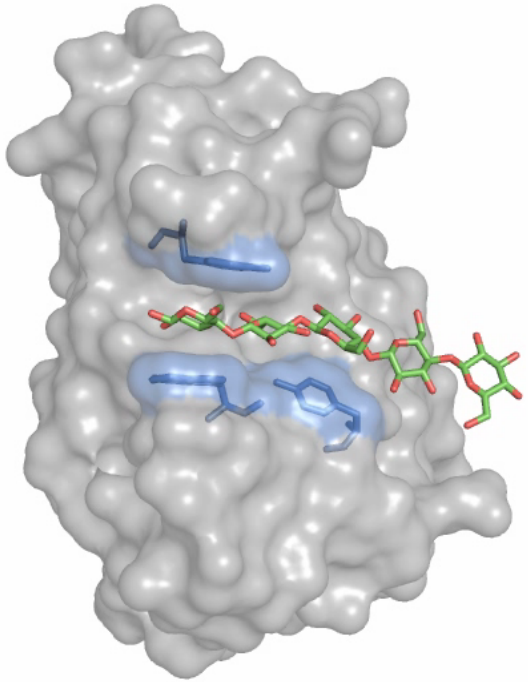
*Cf*CBM4-1 (CBD_{N1})

PDB ID : 1GU3



*Cf*CBM4-2 (CBD_{N2})

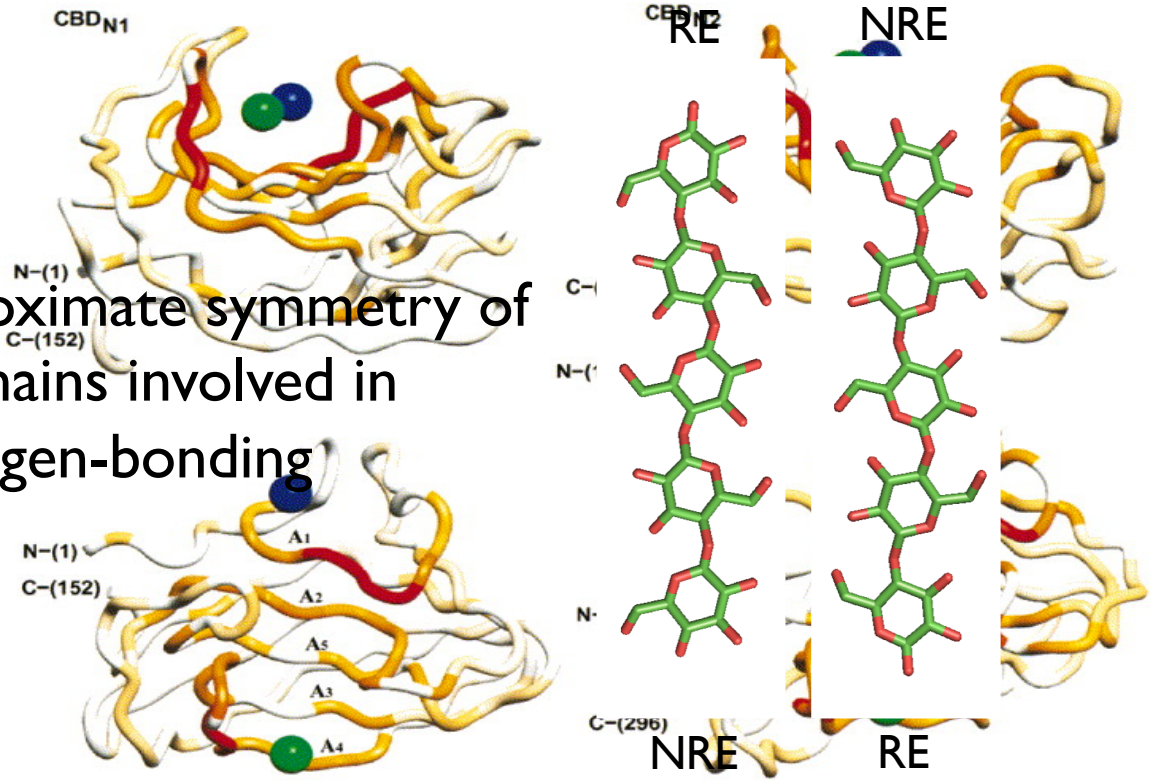
PDB ID : 1CX1



Reducing end (RE) specific or Non-reducing end (NRE) specific or Directionless binding?

Boraston et al., *J. Mol. Bio.* 2002 & Brun et. al., Biochem. 2000

Bi-directional Binding



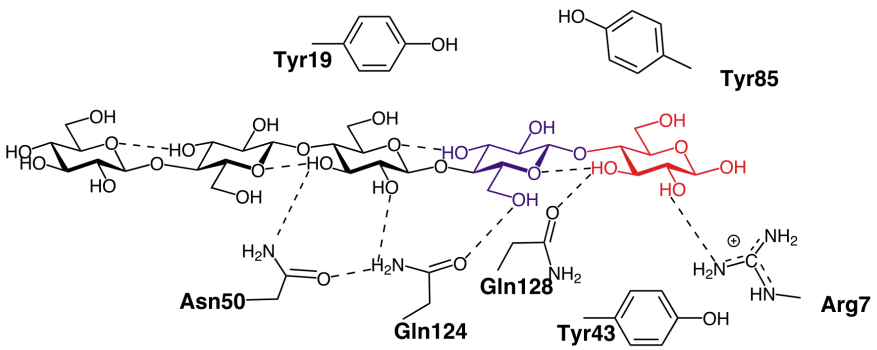
Approximate symmetry of sidechains involved in hydrogen-bonding

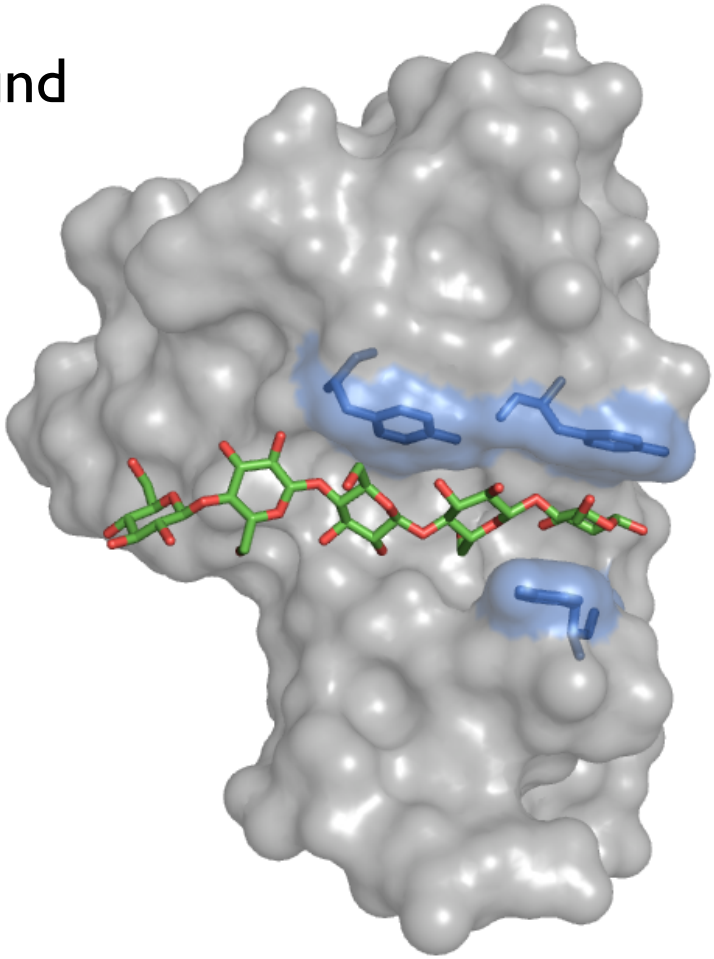
- Both *Cf*CBM4's bind 2,2,6,6,-tetramethylpiperidine-1-oxyl-4-yl (TEMPO) spin-labeled cellotriose and cellotetraose
- Associate in either orientation across beta sheets

Johnson et. al., J. Mol. Bio. 1999

Crystallographic Evidence of Binding

- *Cf*CBM4-1 – Cellopentaose bound complex captured in crystal structure (1GU3)

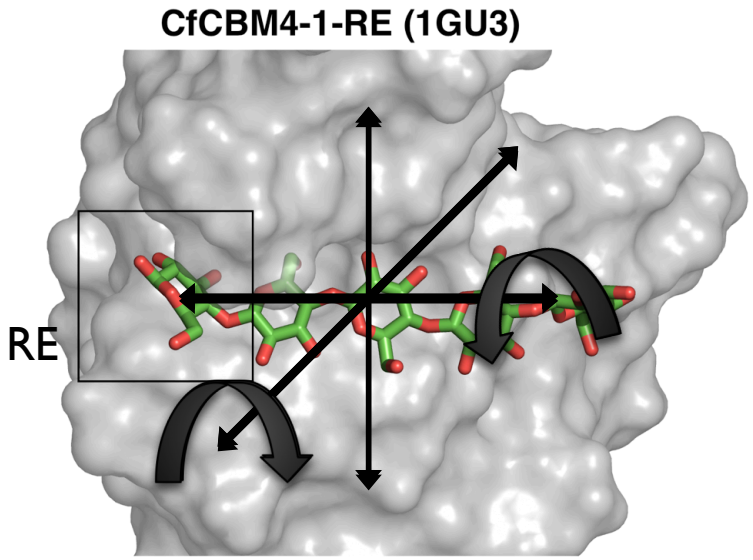




- Only hydrophilic edge of sugar pointing inward toward binding groove

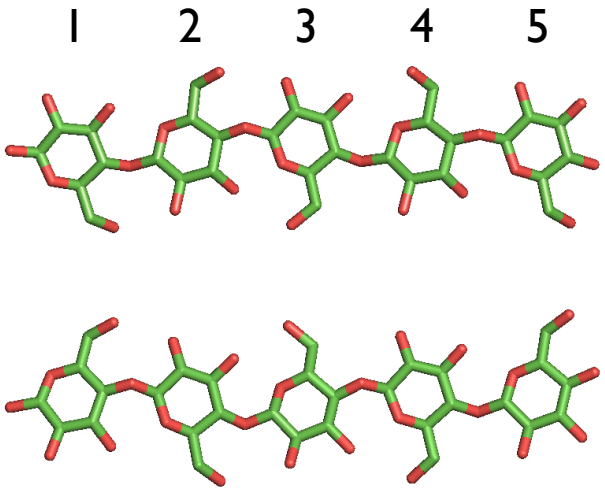
Boraston et al., *J. Mol. Bio.* 2002

Examining Binding with MD Simulation



Stability of Ligand in Binding Groove

- Placing the sidechains specifically involved in hydrogen bonding interactions on opposite edge of the binding groove results in unfavorable protein-ligand interactions



*Cf*CBM4-1-RE

*Cf*CBM4-1-RE '

Ligand Binding Free Energy Calculation

Free Energy Perturbation
with Replica Exchange Molecular Dynamics
(FEP/λ-REMD)

$$\text{CfCBM4-1} * \text{Cellopentaose}_{(solv)} \xrightarrow{\Delta G_1} \text{CfCBM4-1}_{(solv)} + \text{Cellopentaose}_{(vac)}$$

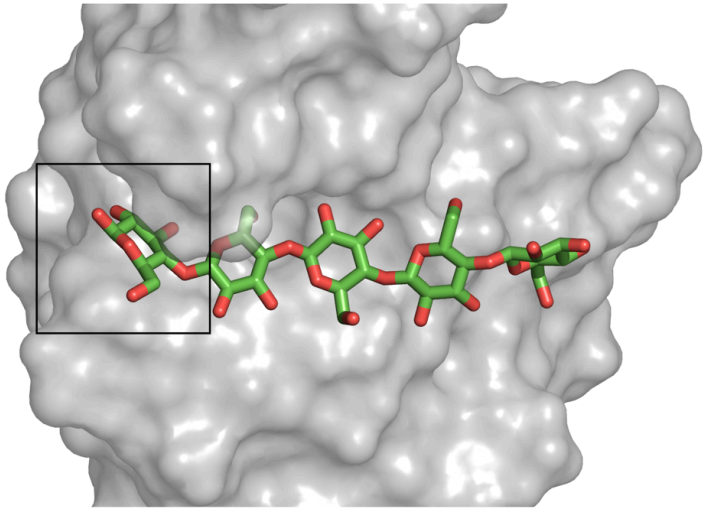
$$\text{Cellopentaose}_{(solv)} \xrightarrow{\Delta G_2} \text{Cellopentaose}_{(vac)}$$

$$\text{CfCBM4-1}_{(solv)} + \text{Cellopentaose}_{(solv)} \xrightarrow{\Delta G_b} \text{CfCBM4-1} * \text{Cellopentaose}_{(solv)}$$

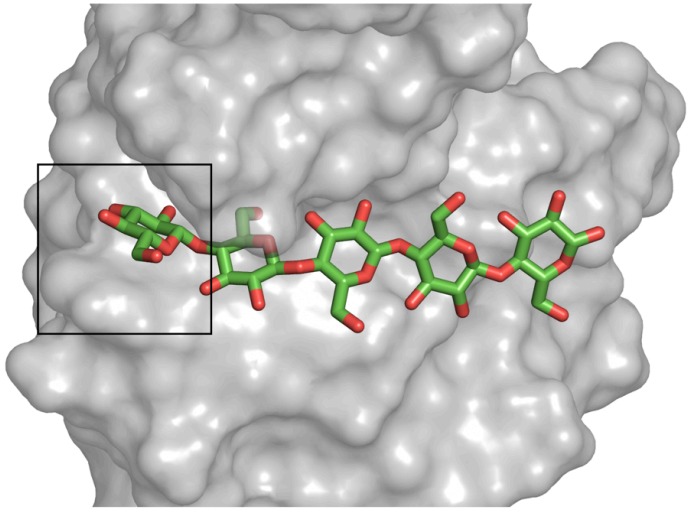
$$\Delta G_b = \Delta G_2 - \Delta G_1$$

Thermodynamic Preference for Direction?

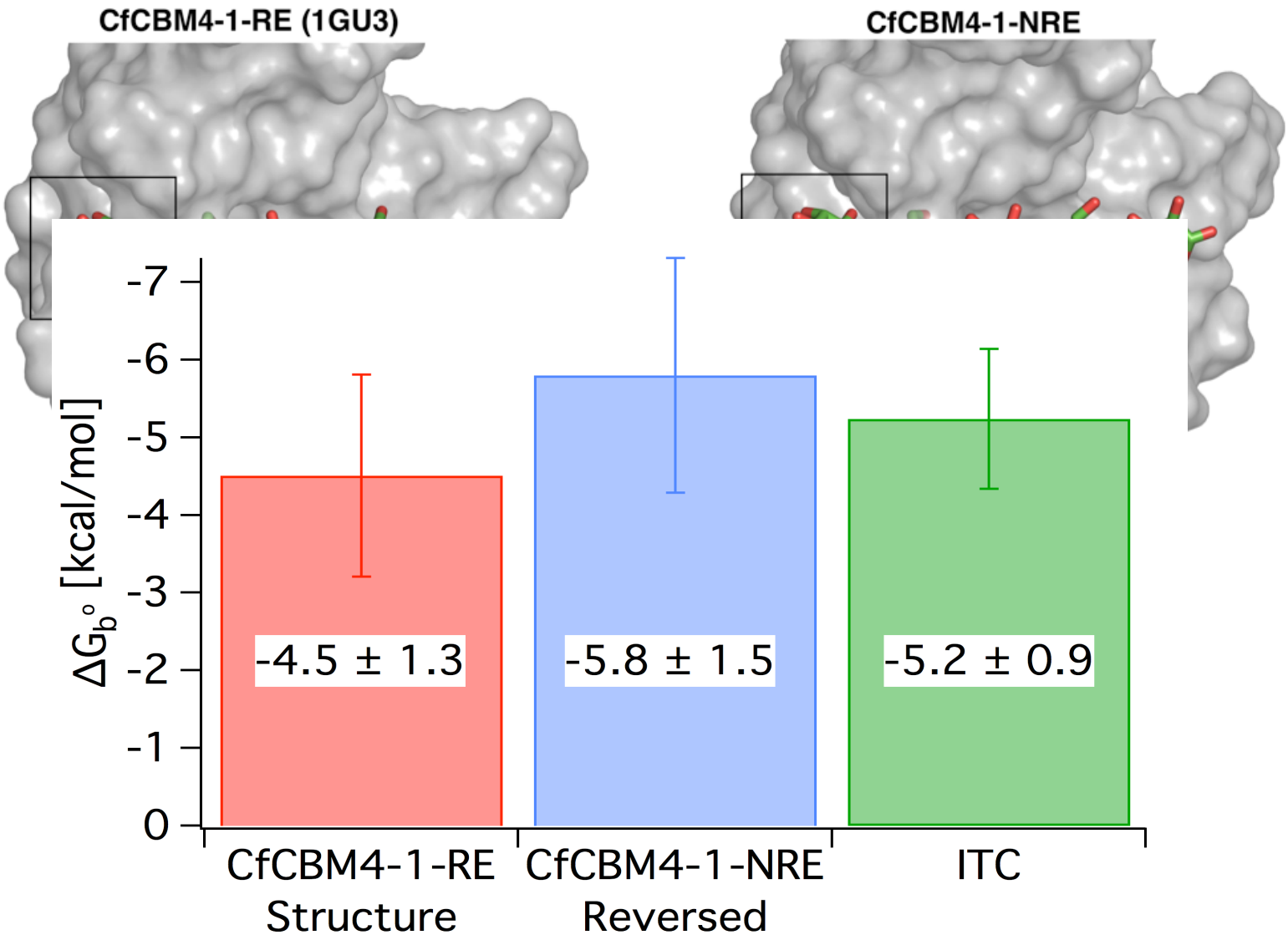
CfCBM4-1-RE (1GU3)



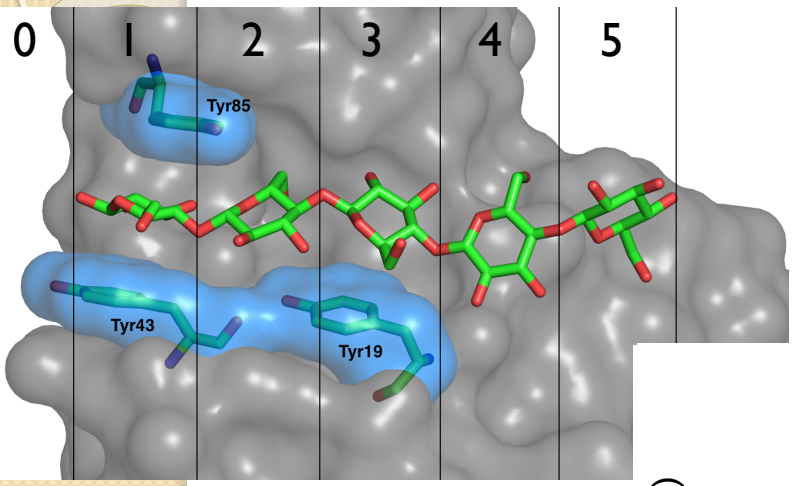
CfCBM4-1-NRE



Thermodynamic Preference for Direction?



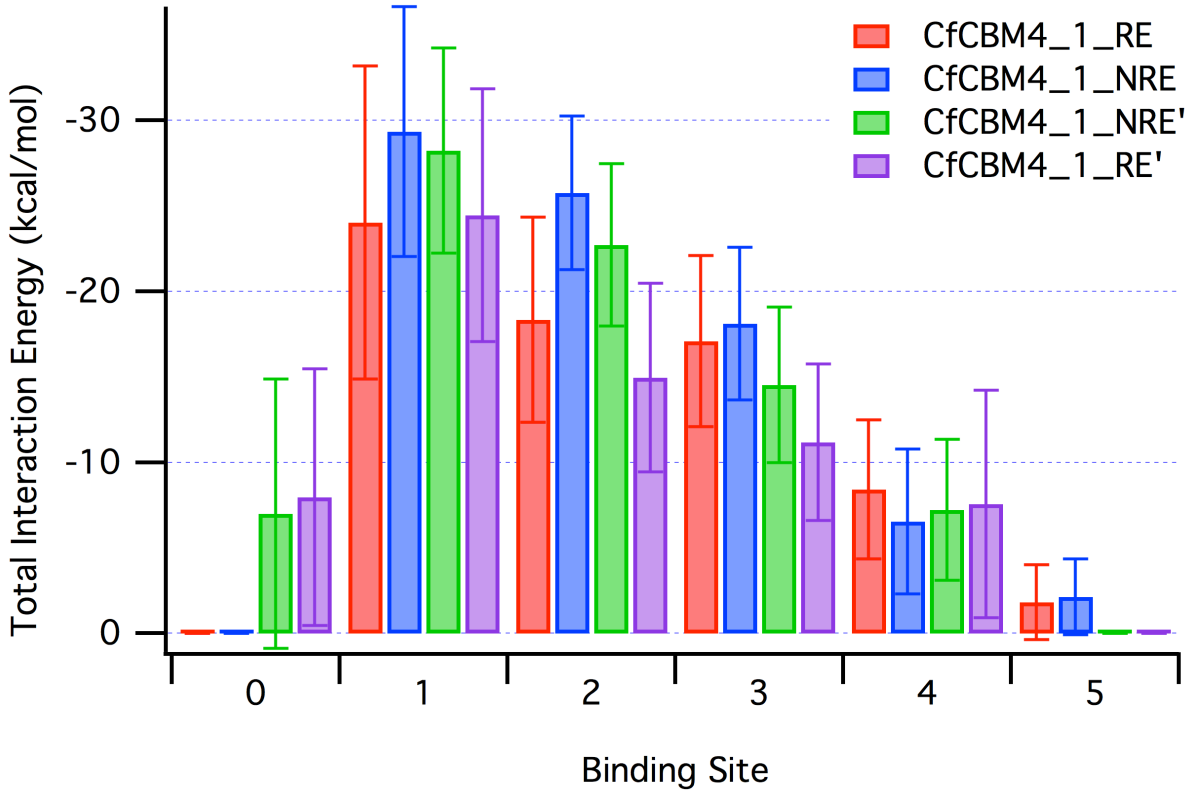
Ligand Binding Dynamics Supports Bi-directional Binding



Hydrogen bonding is approximately the same in each binding site regardless of orientation

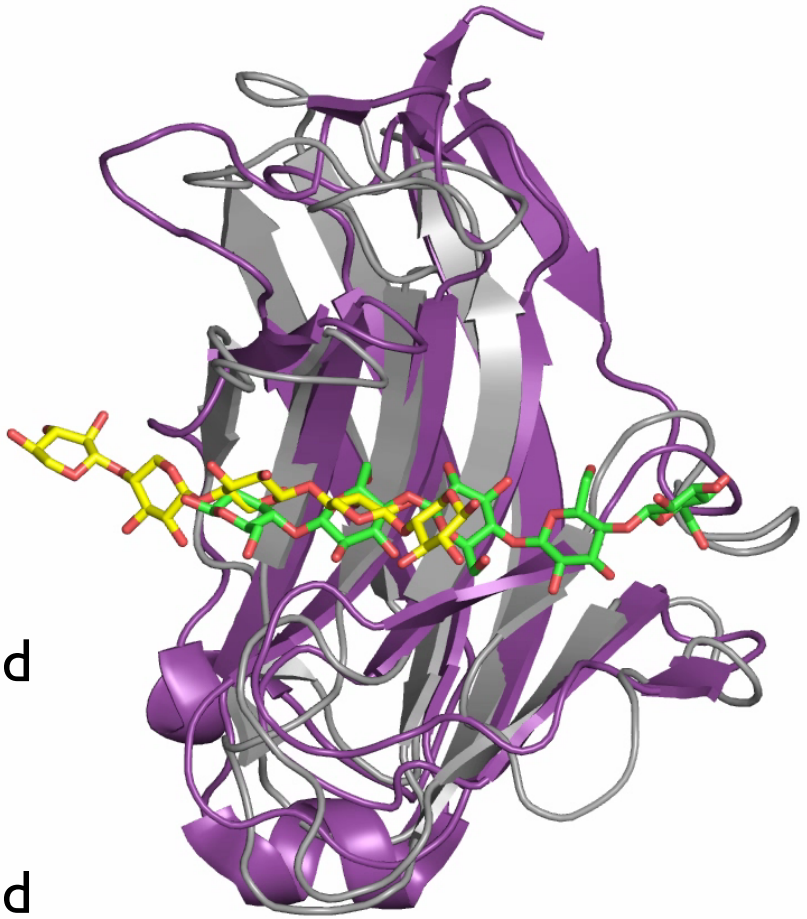
Interaction energy is approximately the same in each binding site regardless of orientation

Higher Root Mean Square Fluctuations (RMSF) for unfavorable ligand orientations



General to β-sandwich CBMs?

- 29 of the 71 CBM families demonstrate the β-sandwich fold
- 10 of these 29 families have glycan bound structures available (34 structures in total)
  - 22 structures observe the ligand in the same direction as the 1GU3 structure
  - 12 structures observe the ligand in the opposite direction of the 1GU3 structure



Purple : *Pc*CBM15 (1GNY)

Conclusions & Future Work

- Modeling and simulation techniques have enabled us to build unique systems and study the various aspects of protein-carbohydrate recognition process.
- Calculated binding free energies suggest that *Cf*CBM4-1 does not have significant preference for direction in binding to cellopentaose.
- Favorable ligand orientations in binding site are defined by hydrogen bonding interactions and otherwise result in unfavorable interactions.
- These findings will contribute in our ongoing investigations of molecular-level mechanisms of carbohydrate recognition in Type B CBMs.

Acknowledgements

Funding

Division of Chemistry
Award # 1404849

Computational Resources

CCS
Center for Computational Sciences

XSEDE
Extreme Science and Engineering Discovery Environment

Principal Investigator :
Dr. Christina M. Payne

Suvamay Jana

Extra slides…

Computational Approach

1. Docking of ligand in the binding cleft and setup the protein-ligand complex.
2. Solvation and energy minimization of protein-ligand-solvent system.
3. Heating and equilibration of the system in NPT ensemble.
4. Production run of Molecular Dynamic simulation for 250 ns in NVT ensemble.
5. Binding free energy calculation by FEP/λ-REMD.
6. Analysis of the trajectories and comparison of ΔG..

Examining Binding with MD Simulation