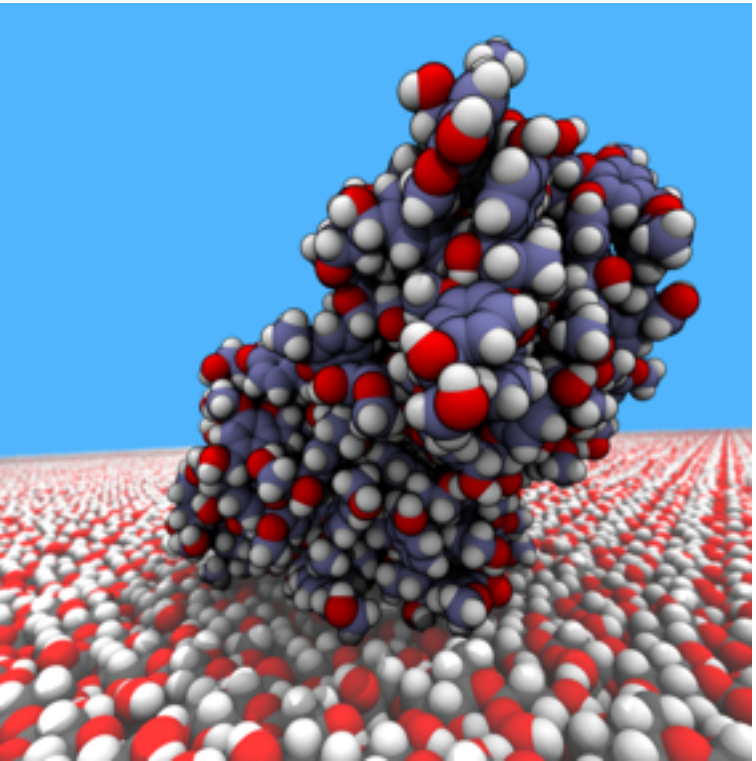

Molecular Interactions at the Cellulose-Lignin Interface Explored via Molecular Simulation

Josh Vermaas, Gregg Beckham, and Michael Crowley

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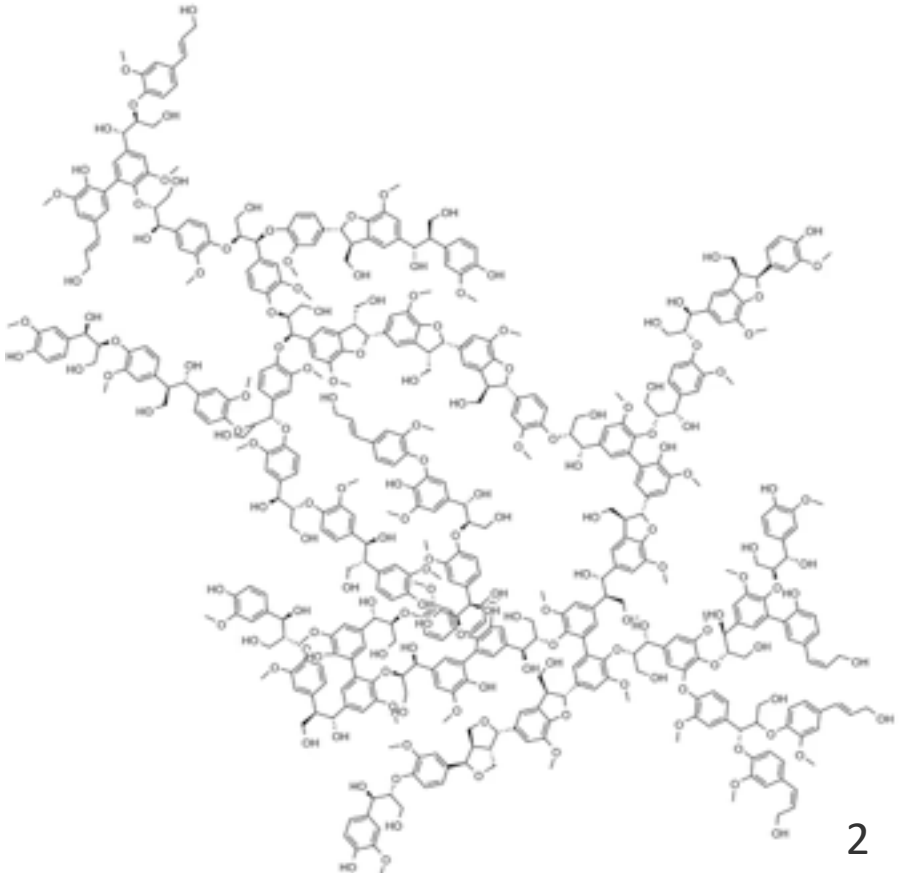
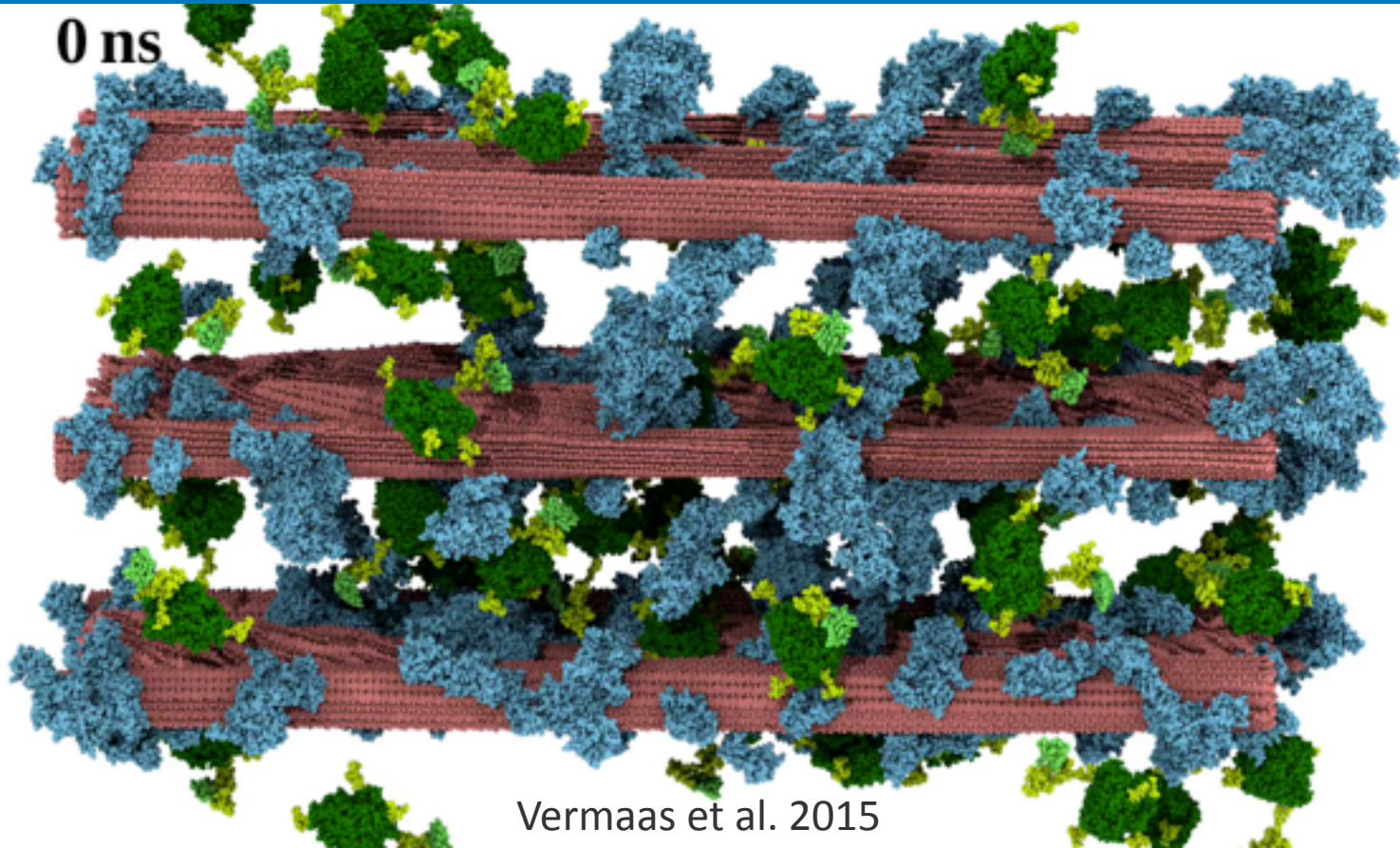
Lignin Introduction

- Lignin is an aromatic heteropolymer that makes up between 15-40% of the dry weight of terrestrial plants
- Largest source of renewable aromatics
- Many potential industrial uses
- Implicated as a significant driver of cell wall recalcitrance

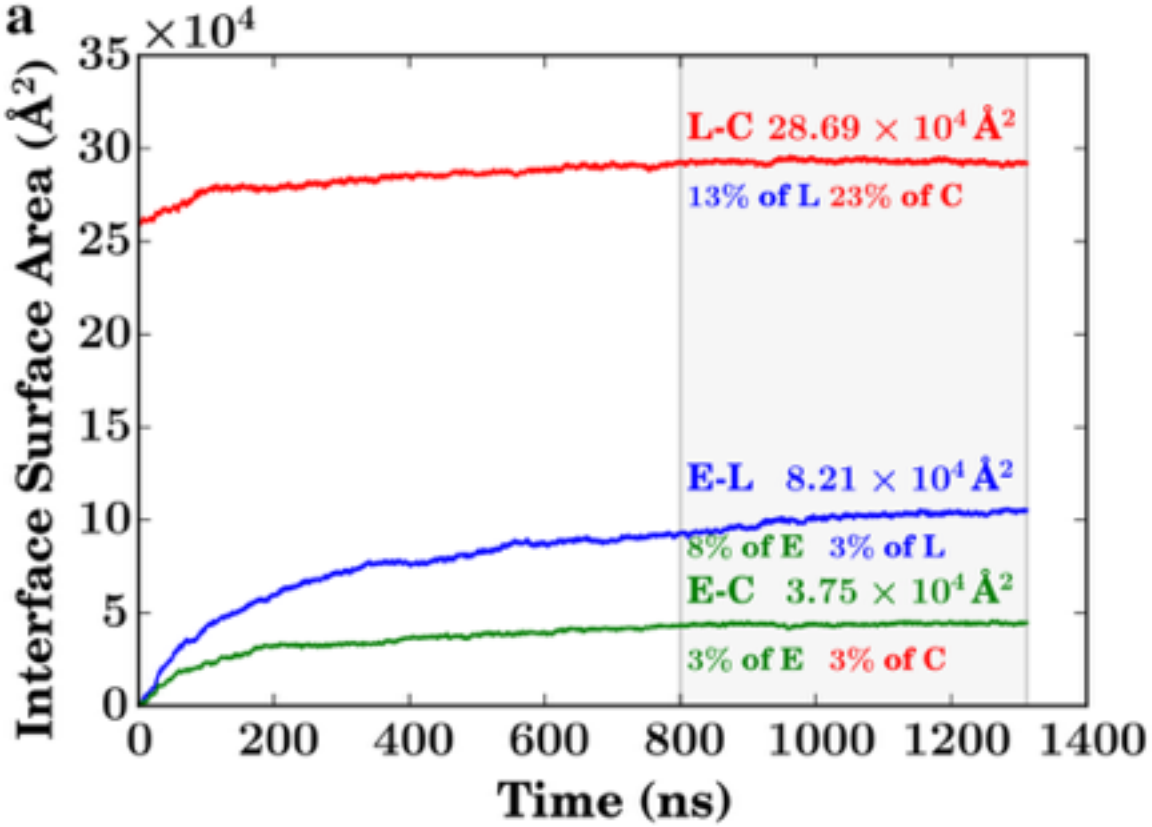


Lignocellulose Simulation



Vermaas et al. 2015

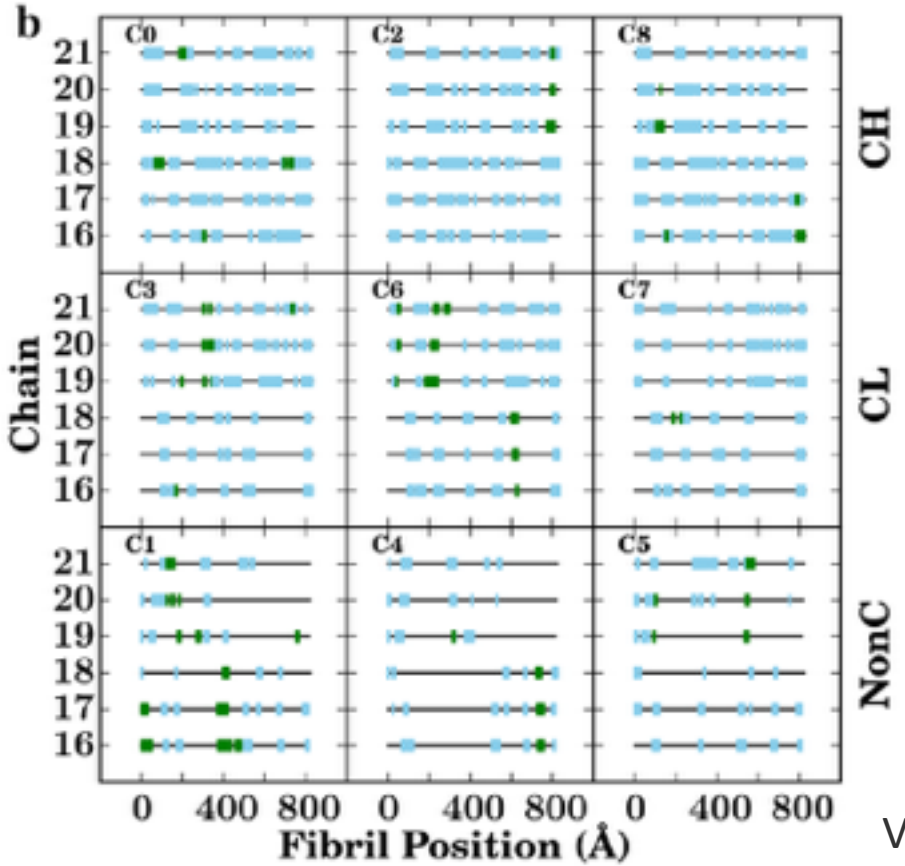
Lignin Binds to Cellulose…



Vermaas et al. 2015

- Lignin covers roughly a quarter of the cellulose surface
- Does it coat the surface evenly?
- Does lignin chemistry matter?

… Particularly Hydrophobic Cellulose Faces



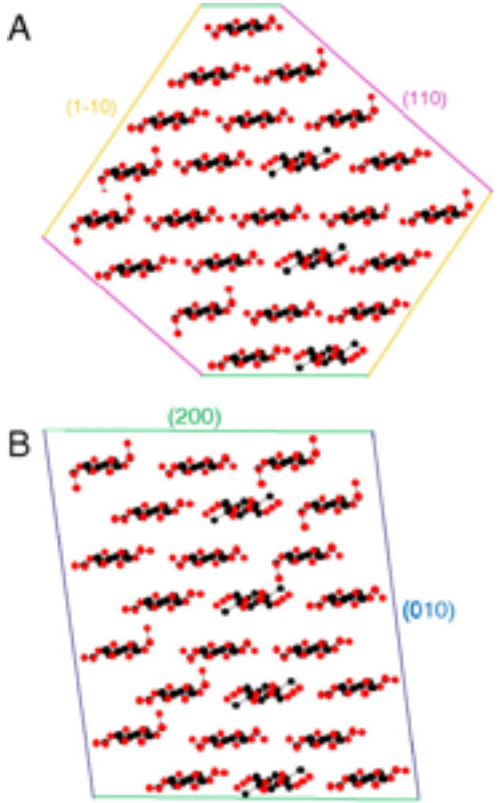
	A_T (10^4 Å^2)	A_E (10^4 Å^2)	A_L (10^4 Å^2)	A_E/A_T	A_L/A_T
CH	***6.51***	***0.18***	***3.02***	***0.03***	***0.46***
	6.74	*0.15*	*1.33*	*0.02*	*0.20*
CL	***6.51***	***0.23***	***2.11***	***0.04***	***0.32***
	6.74	*0.24*	*0.97*	*0.04*	*0.14*
NonC	***6.57***	***0.30***	***1.16***	***0.05***	***0.18***
	7.76	*0.36*	*1.17*	*0.05*	*0.15*

- Prefers hydrophobic faces
- Blocks cellulase binding
- Can we quantify the thermodynamic preference between faces?

Vermaas et al. 2015

The Many Faces of Cellulose

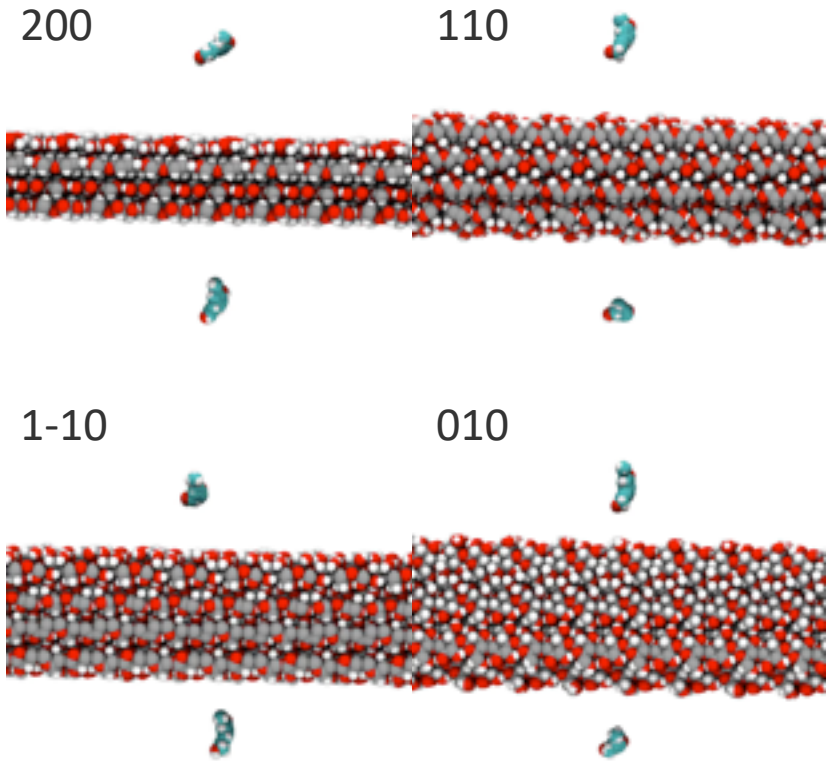
- Only the 200 face is hydrophobic
- Other primary faces are hydrophilic
- Normal cellulose twists
- Infinite cellulose does not



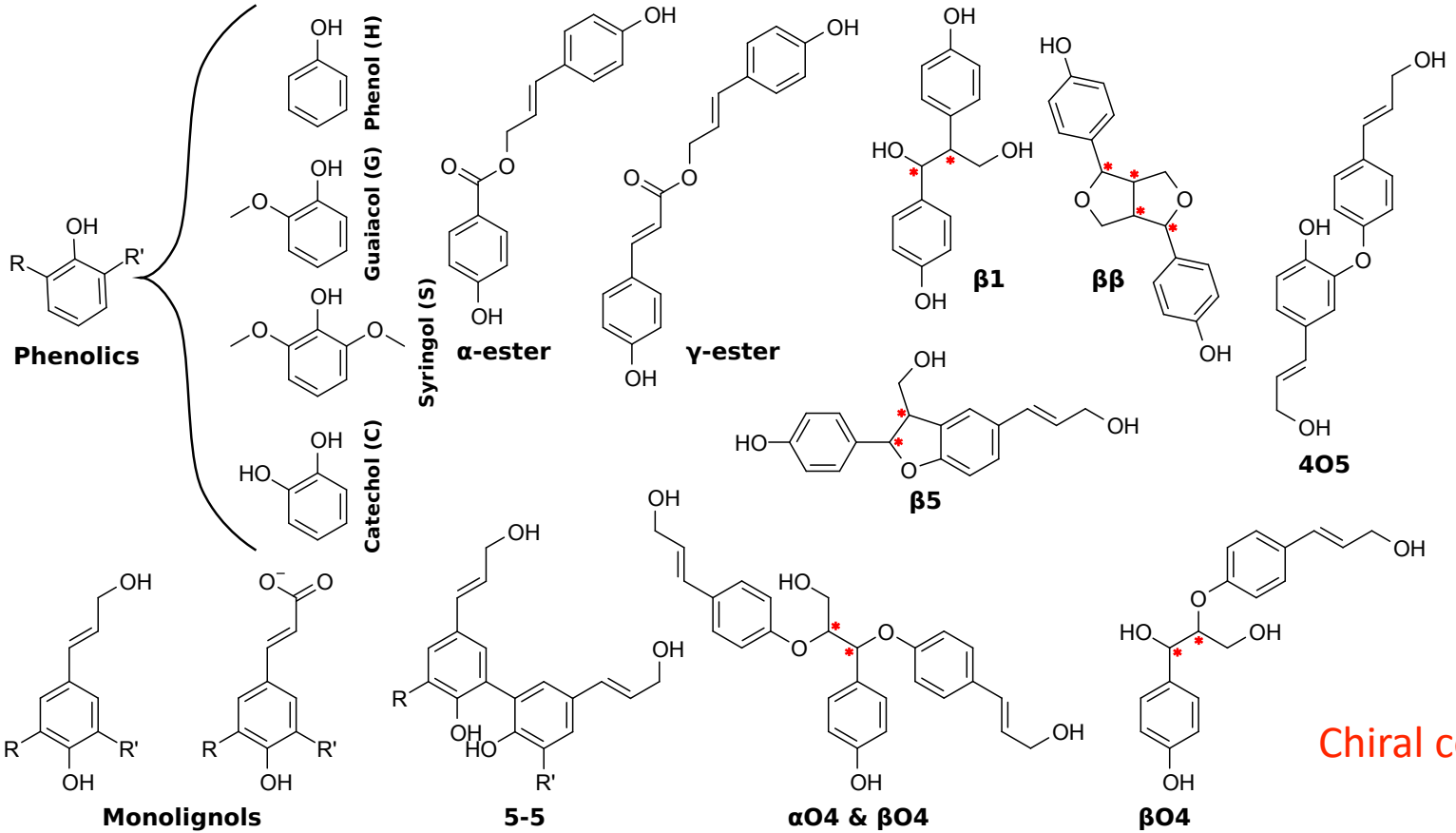
Fernandes et al. 2015

The Next Experiment

- Construct “infinite” sheets of cellulose with different faces exposed to solution
- Place lignin derived compounds in solution
- Simulate for 200 ns
- Determine binding free energies of compounds to individual faces



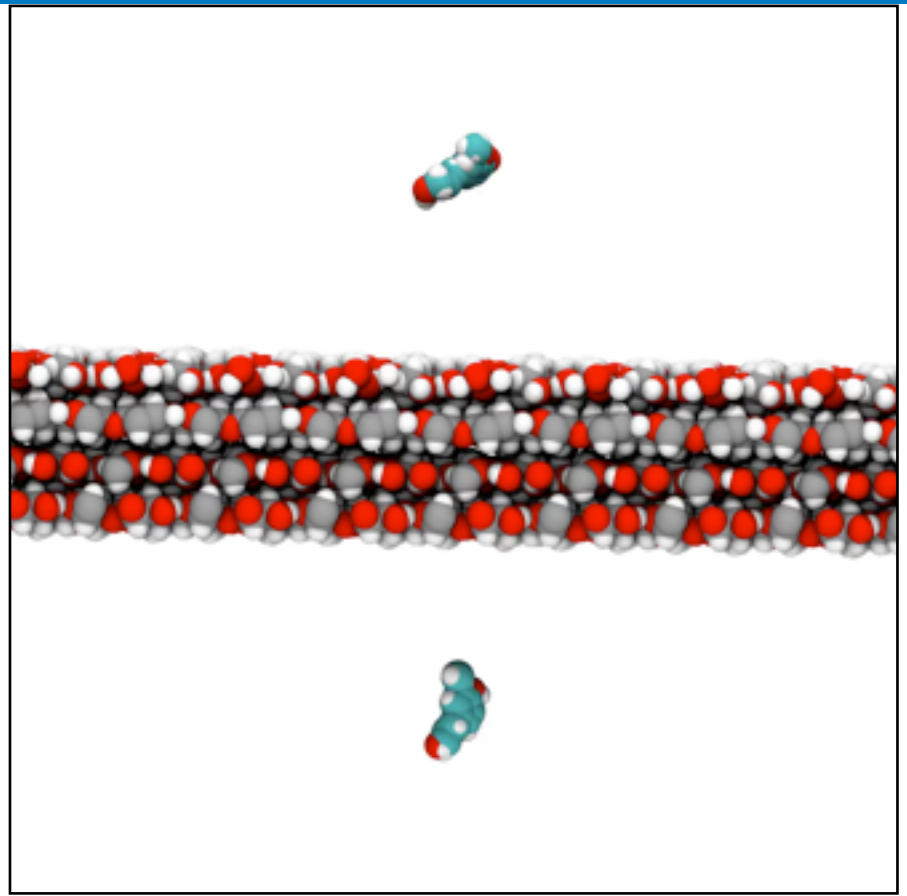
What Compounds?



Potential Binding Metrics for Free Energy Determination

$$\Delta G_{binding} = -RT \ln \frac{P_{bound}}{P_{unbound}}$$

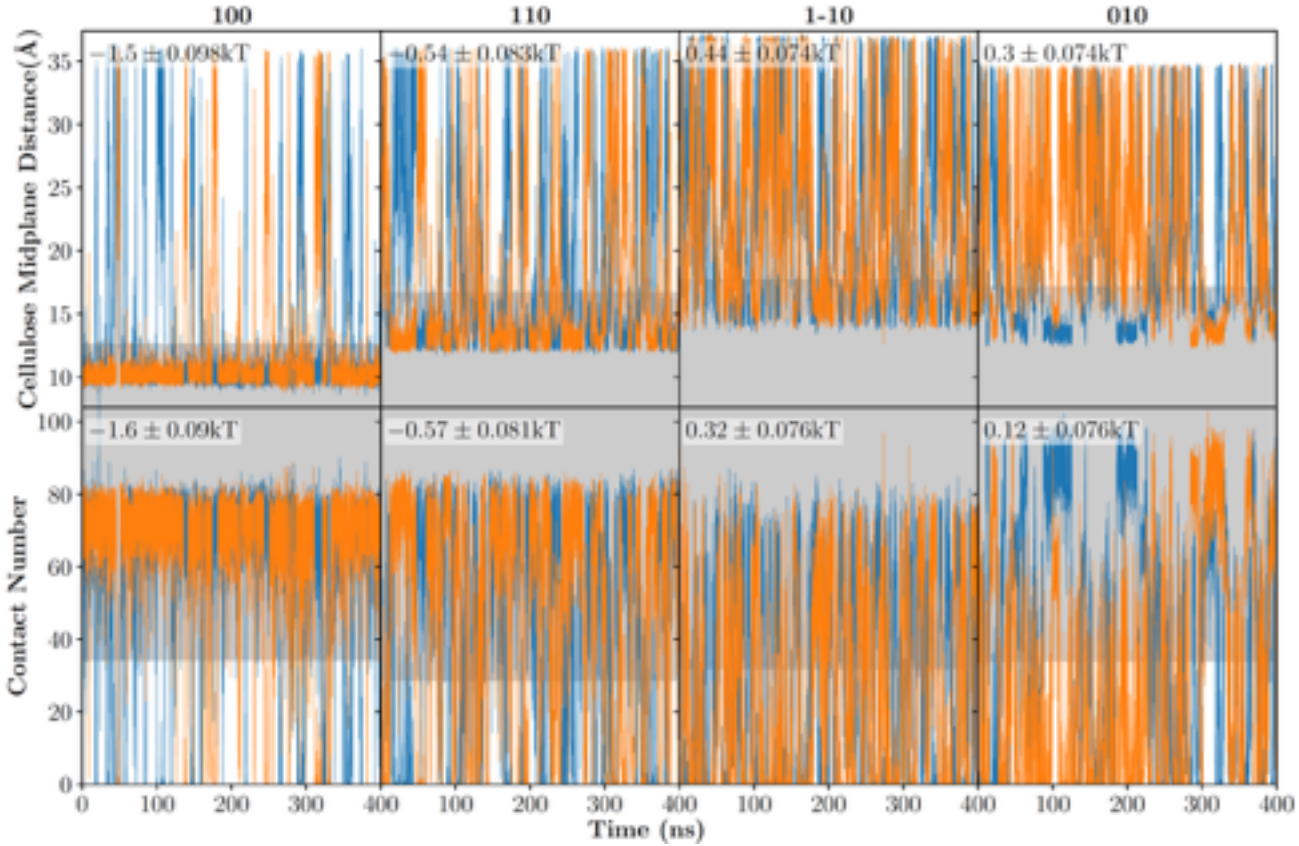
- Free energy is related to probability
- Trajectories can be used to determine relative probabilities
- How do we define a bound vs unbound state?



Distance and Contact Metrics, Compared

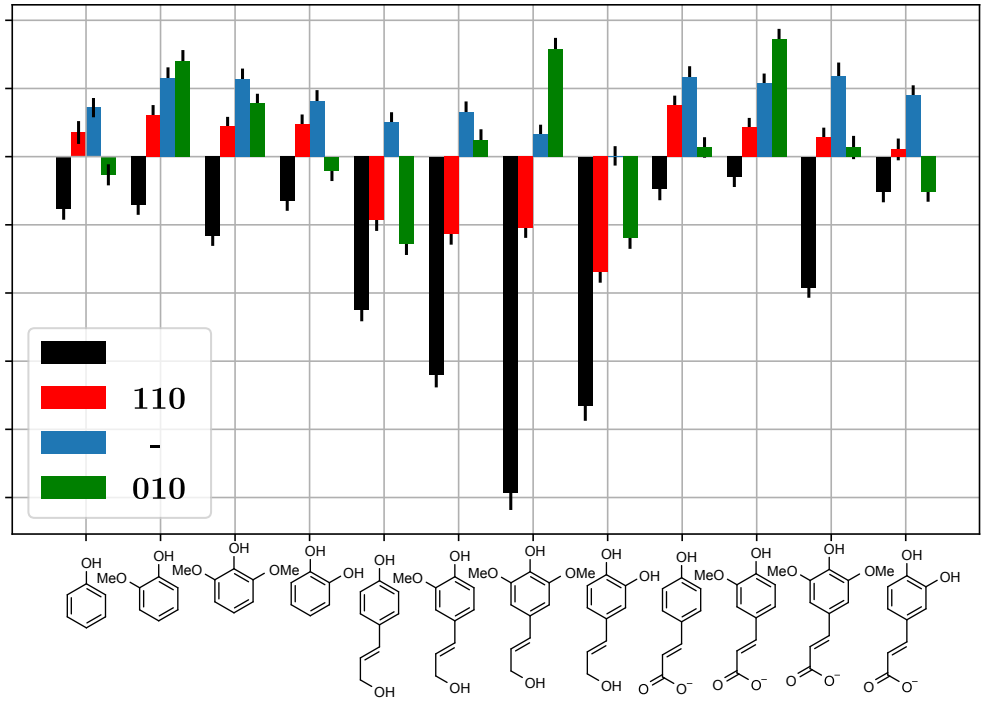
- Distance
  - Lignin Center of mass to cellulose center of mass
  - Very easy to explain
- Contact number
  - Pairwise contacts between cellulose and lignin
  - Is not biased by lignin shape

$$C = \sum_{i,j \in pairs} \frac{1 - \left(|x_i - x_j| / 12\text{Å}\right)^6}{1 - \left(|x_i - x_j| / 12\text{Å}\right)^{24}}$$



Cellulose Binding for Monomers

- Hydrophobic 200 face predominant for binding
- Ferulate prefers solution
  - Fits well with its physiological role
- 110 face better for binding than the other hydrophilic faces

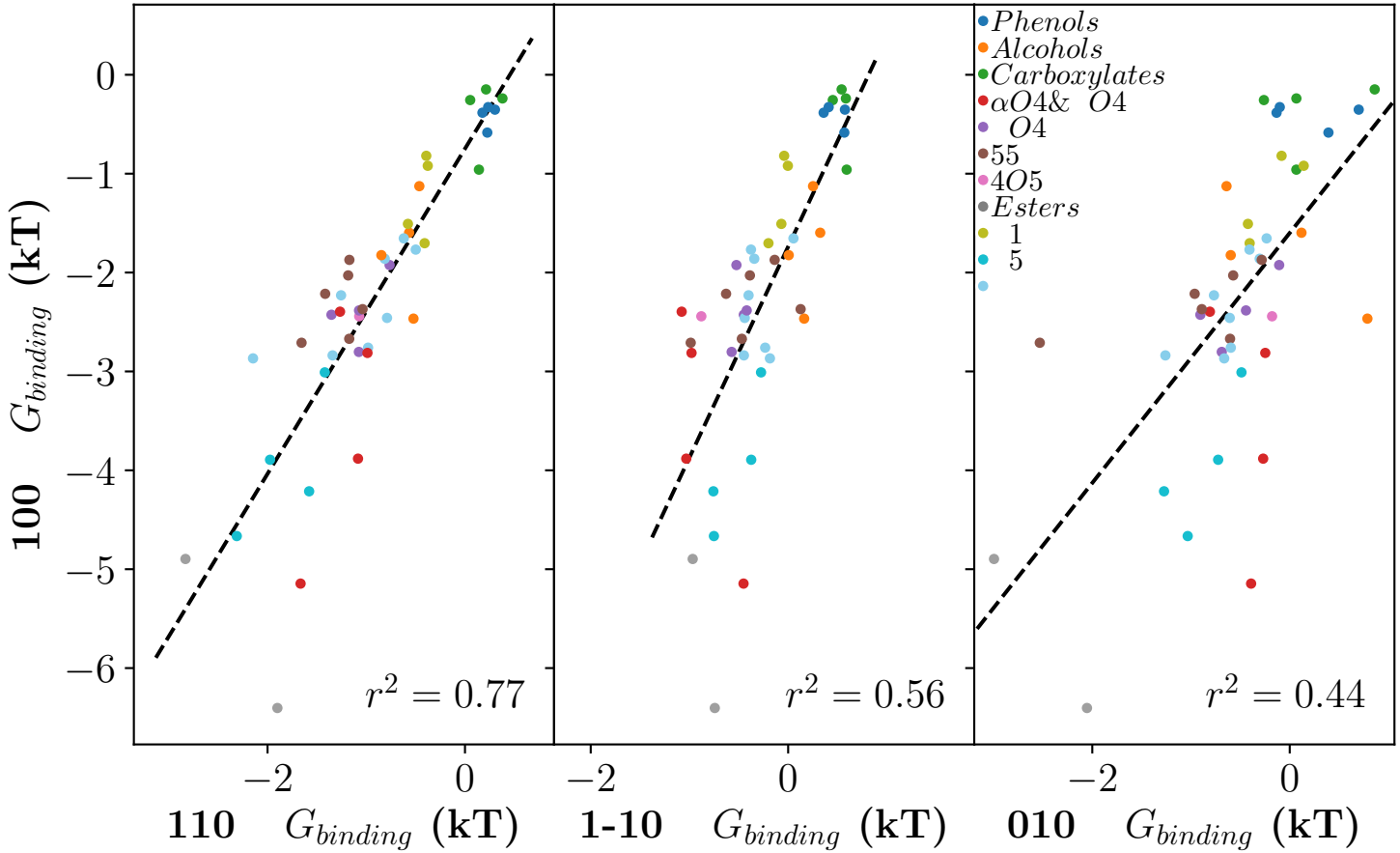


Multimeric Binding

- 200>>110>010>1-10
- Planar lignin molecules like esters demonstrate particular affinity to the 200 face
- Comparisons between stereoisomers suggest longer simulation may be beneficial

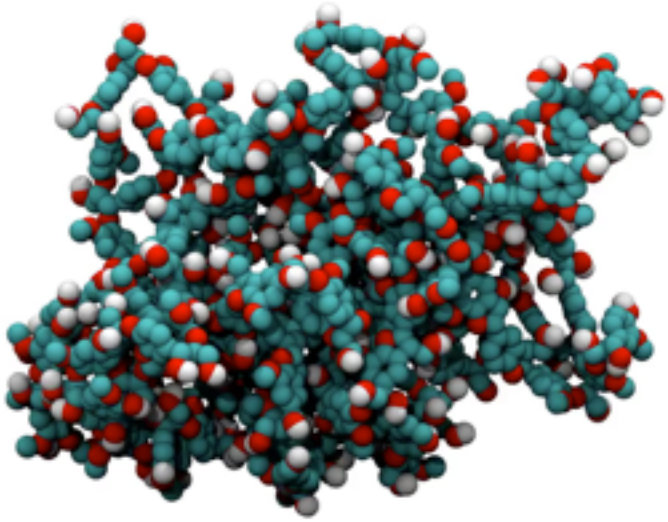
Molecule		Cellulose Face Binding Energy (kT)			
Linkage	Feature	200	110	1-10	010
βO4αO4	RR	-5.42 ± 1.00	-1.51 ± 0.11	-1.02 ± 0.09	-0.18 ± 0.08
	RS	-3.48 ± 0.24	-1.42 ± 0.09	-0.81 ± 0.07	-0.44 ± 0.07
	SR	-5.30 ± 1.00	-1.21 ± 0.09	-0.33 ± 0.06	-0.25 ± 0.07
	SS	-2.71 ± 0.16	-1.24 ± 0.09	-1.10 ± 0.08	-1.14 ± 0.08
βO4	RR	-2.44 ± 0.14	-1.36 ± 0.09	-0.46 ± 0.07	-1.05 ± 0.08
	RS	-2.16 ± 0.13	-0.69 ± 0.08	-0.67 ± 0.08	-0.18 ± 0.08
	SR	-2.53 ± 0.17	-0.91 ± 0.09	-0.83 ± 0.09	-0.07 ± 0.08
	SS	-2.83 ± 0.15	-0.47 ± 0.08	-0.71 ± 0.08	-0.80 ± 0.08
55	CC	-2.76 ± 0.14	-1.56 ± 0.10	-1.01 ± 0.07	-1.76 ± 0.11
	CG	-2.07 ± 0.11	-1.23 ± 0.10	-0.47 ± 0.07	0.10 ± 0.08
	CP	-2.45 ± 0.13	-1.70 ± 0.09	-0.88 ± 0.08	-1.02 ± 0.09
	GC	-3.10 ± 0.18	-1.22 ± 0.10	-0.95 ± 0.08	0.04 ± 0.07
	GG	-2.33 ± 0.13	-1.20 ± 0.09	-0.07 ± 0.07	-0.40 ± 0.07
	GP	-2.67 ± 0.15	-0.86 ± 0.07	-0.38 ± 0.08	-0.50 ± 0.09
	PC	-1.60 ± 0.09	-1.21 ± 0.08	-0.93 ± 0.08	-0.33 ± 0.09
	PG	-3.00 ± 0.17	-1.87 ± 0.10	0.05 ± 0.08	-0.92 ± 0.08
	PP	-2.16 ± 0.12	-1.21 ± 0.08	-0.57 ± 0.06	-0.78 ± 0.08
4O5		-2.70 ± 0.15	-1.23 ± 0.09	-1.09 ± 0.10	-0.75 ± 0.08
Ester	α	-7.85 ± 1.00	-2.65 ± 0.14	-0.66 ± 0.09	-2.11 ± 0.11
	γ	-5.51 ± 1.00	-2.36 ± 0.13	-0.72 ± 0.08	-2.36 ± 0.15
β1	RR	-0.55 ± 0.08	-0.29 ± 0.06	-0.20 ± 0.07	-0.01 ± 0.07
	RS	-0.86 ± 0.07	-0.71 ± 0.07	-0.54 ± 0.07	-0.78 ± 0.08
	SR	-1.66 ± 0.10	-0.59 ± 0.08	-0.40 ± 0.08	-0.45 ± 0.07
	SS	-0.88 ± 0.08	-0.43 ± 0.07	-0.24 ± 0.07	0.01 ± 0.08
β5	RR	-5.73 ± 1.00	-2.03 ± 0.11	-0.84 ± 0.09	-1.51 ± 0.09
	RS	-4.24 ± 0.35	-2.47 ± 0.15	-0.50 ± 0.08	-0.50 ± 0.07
	SR	-2.74 ± 0.18	-1.28 ± 0.09	-0.38 ± 0.08	-0.16 ± 0.08
	SS	-3.65 ± 0.24	-1.91 ± 0.11	-1.06 ± 0.08	-1.18 ± 0.10
$\beta\beta$	RRRR	-1.96 ± 0.11	-0.68 ± 0.08	-0.55 ± 0.08	-0.51 ± 0.07
	RRRS	-1.72 ± 0.10	-0.60 ± 0.07	-0.41 ± 0.07	-0.45 ± 0.08
	RRSR	-2.35 ± 0.14	-0.84 ± 0.09	-0.37 ± 0.08	-0.60 ± 0.08
	RRSS	-2.68 ± 0.14	-1.09 ± 0.08	-0.63 ± 0.08	-1.14 ± 0.09
	RSRR	-1.76 ± 0.10	-0.52 ± 0.07	-0.37 ± 0.07	-0.03 ± 0.07
	RSRS	-2.64 ± 0.16	-1.75 ± 0.09	-0.31 ± 0.07	-0.68 ± 0.07
	RSSR	-3.32 ± 0.22	-0.85 ± 0.09	-0.62 ± 0.07	-1.63 ± 0.10
	RSSS	-2.51 ± 0.17	-1.56 ± 0.10	-0.62 ± 0.07	-0.50 ± 0.07

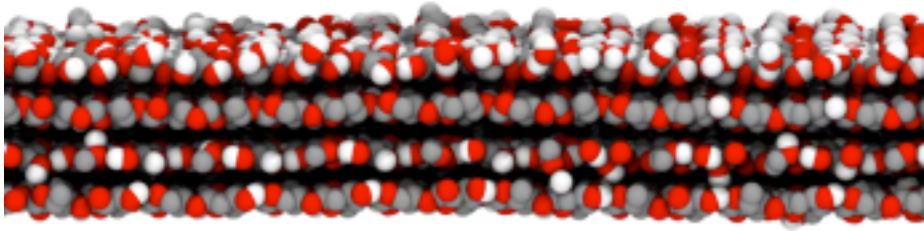
Cellulose Face-Dependent Binding Relationships



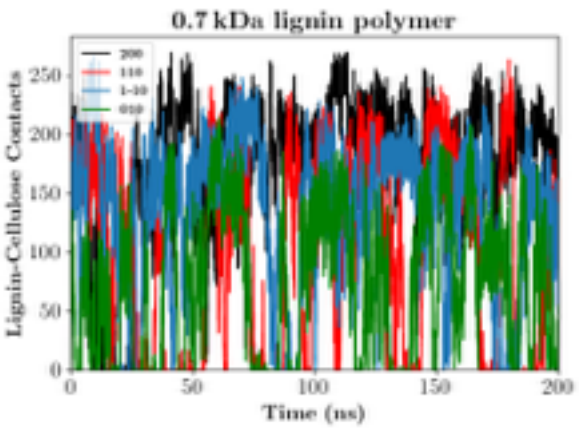
How Does Binding Scale with Polymer Size?

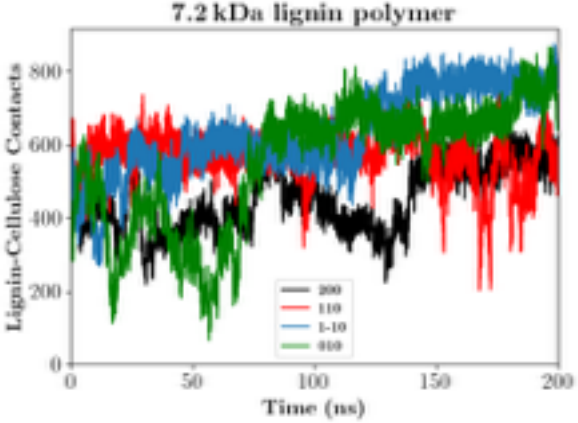
- Monomers and dimers are not perfect models for real lignin
- Draw from established lignin polymer libraries
- Determine lignin binding affinity

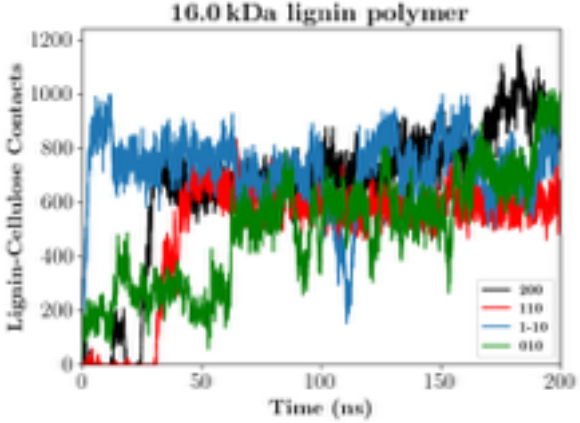


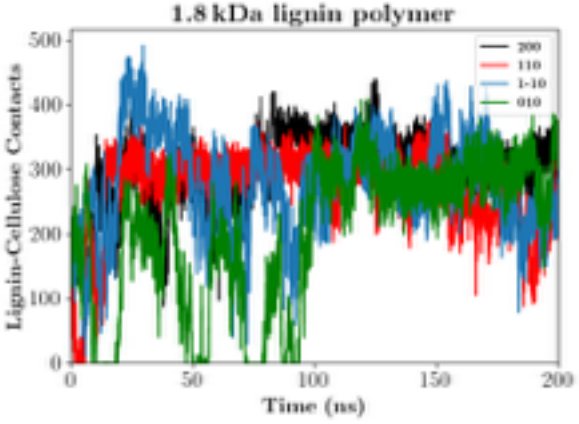


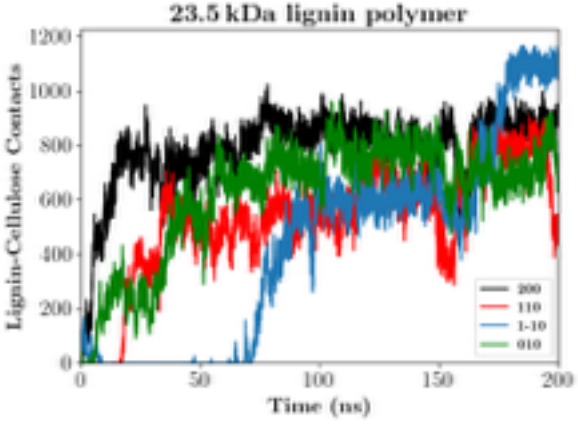
Unbiased Lignin Contacts to Cellulose

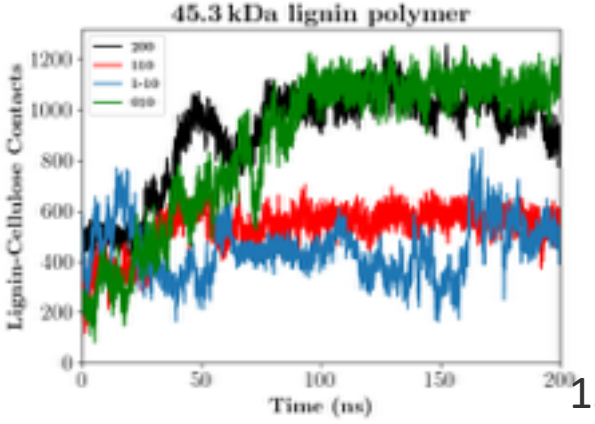






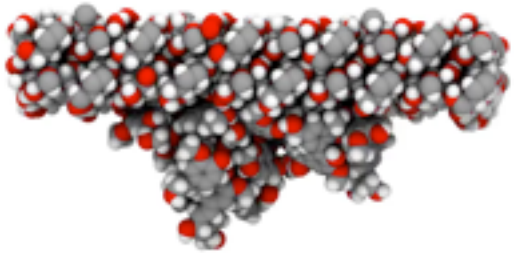




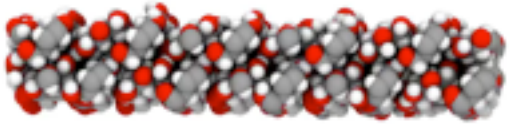


Technical Challenges

- Binding is spontaneous and irreversible on typical MD timescales for larger lignin
- Use replica exchange umbrella sampling on a contact-number reaction coordinate to estimate binding free energy



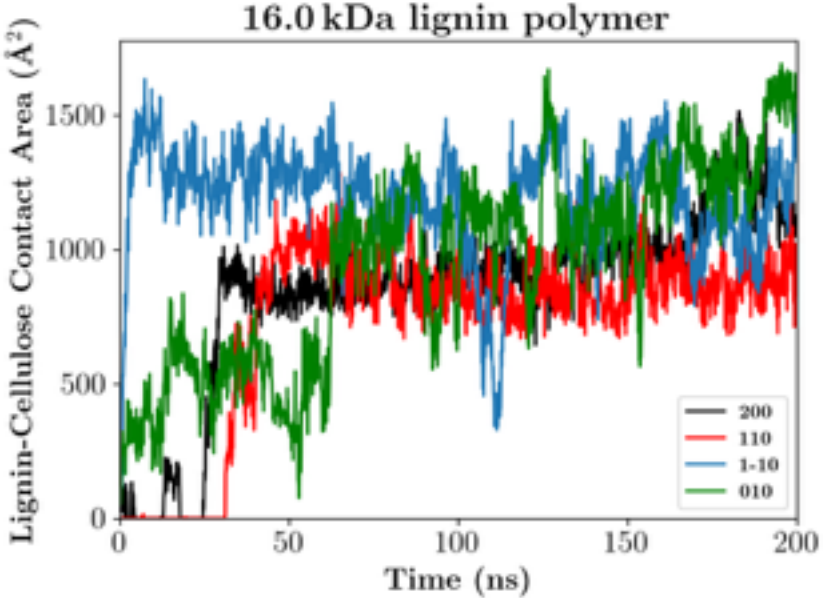
$$C = \sum_{i,j \in pairs} \frac{1 - \left(|x_i - x_j| / 12\text{Å}\right)^6}{1 - \left(|x_i - x_j| / 12\text{Å}\right)^{24}}$$

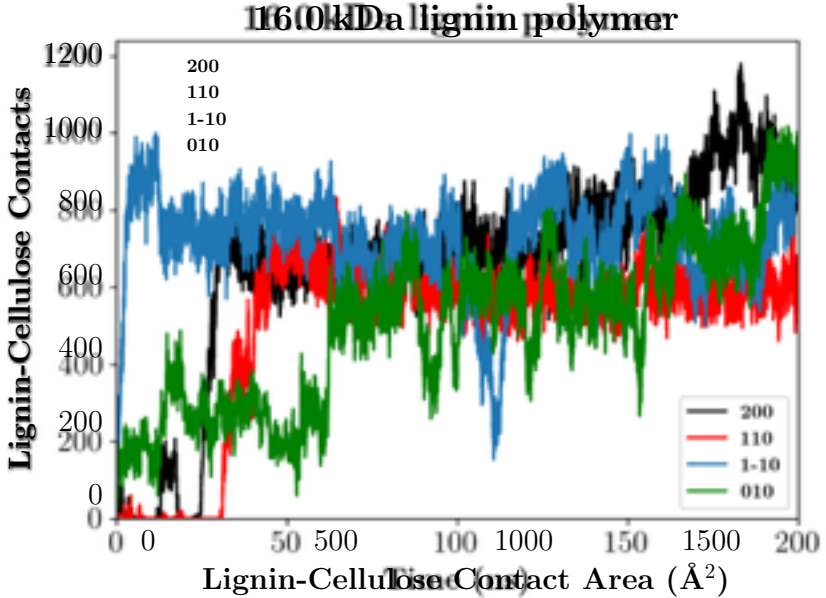


Surface Area Comparison

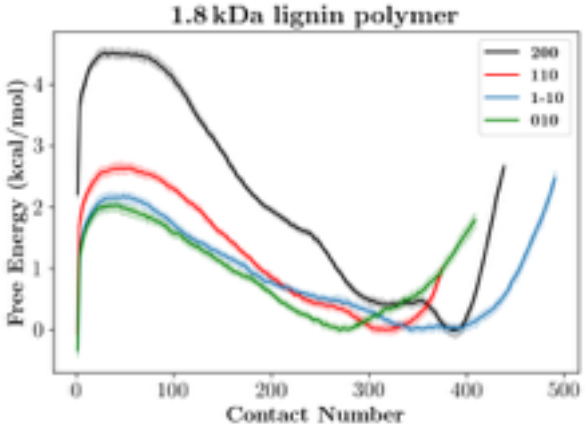
- Contact number as defined is a strong proxy for contact surface area between lignin and cellulose

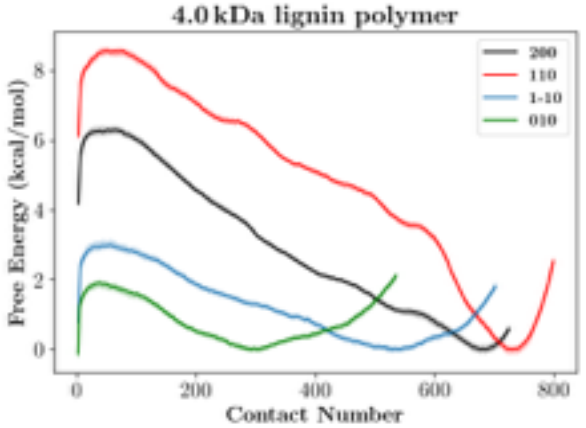
$$C = \sum_{i,j \in pairs} \frac{1 - \left(|x_i - x_j| / 12\text{Å}\right)^6}{1 - \left(|x_i - x_j| / 12\text{Å}\right)^{24}}$$

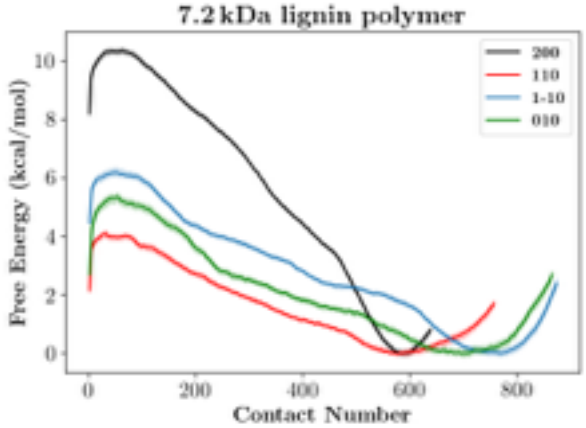


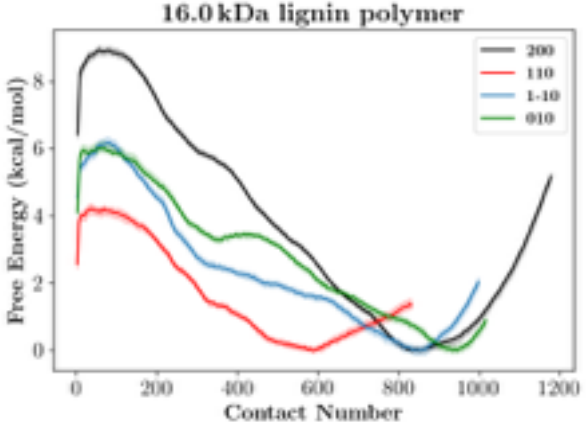


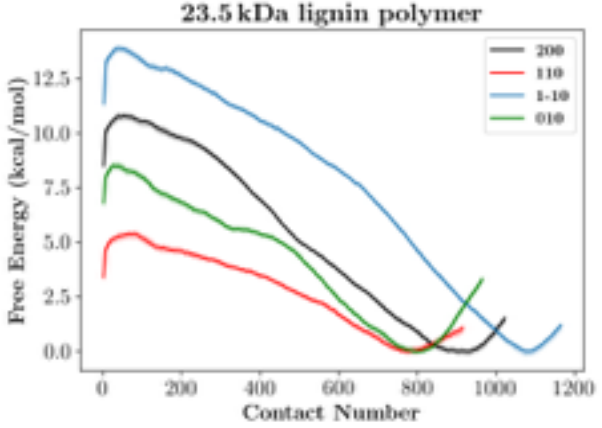
Binding Free Energy Profiles

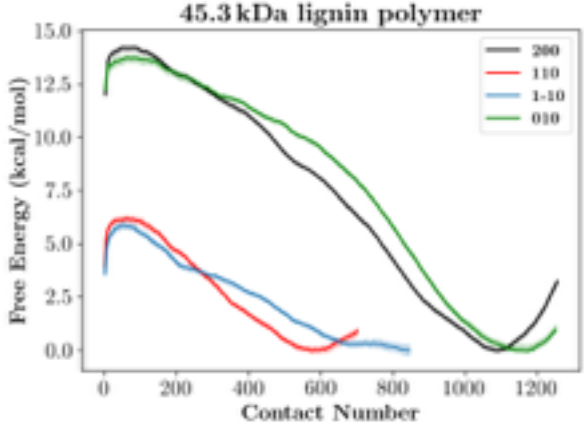




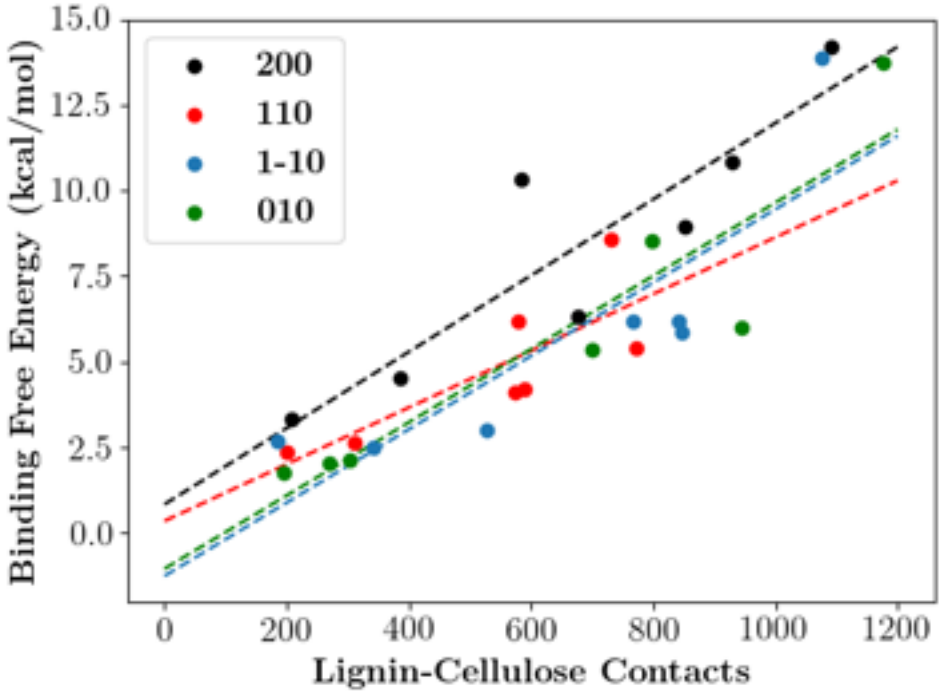








Size Dependence of Lignin-Cellulose Interactions



- Binding free energy grows directly with the number of contacts present at the free energy minimum
- Slopes are consistent across faces

Cellulose Face	
200	0.0111
110	0.0083
1-10	0.0107
0 10	0.0107

Summary

- Binding strength quantification can inform lignin modification
- Lignin related compounds prefer to bind to hydrophobic cellulose faces by about a factor of 2
  - May be related to the “flatness” of the 200 face, which fits well with planar aromatic rings, creating more nonspecific interaction sites
- Lignin contact number/contact area is more important than polymer size in determining binding strength
  - Lignin agglomeration is not considered, but would likely additively increase binding strength

Gregg Beckham

Michael Crowley

Questions?

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