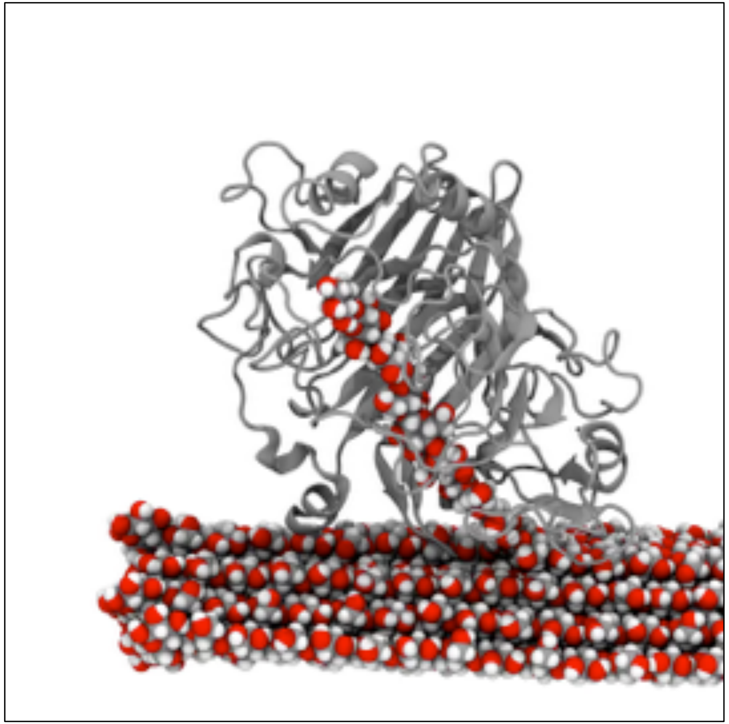

Dissociation mechanism of processive cellulases explored through molecular simulation

Josh Vermaas, Riin Kont, Gregg Beckham,
Michael Crowley, Mikael Gudmundsson,
Mats Sandgren, Jerry Ståhlberg,
Priit Väljamäe, and Brandon Knott

Fall 2019 ACS COMP Division
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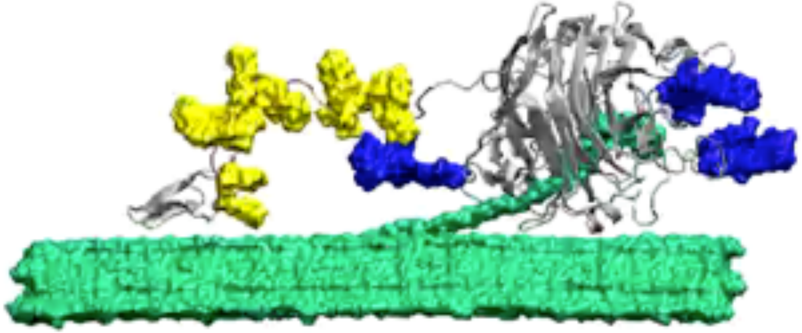
NREL/PR-2700-74304

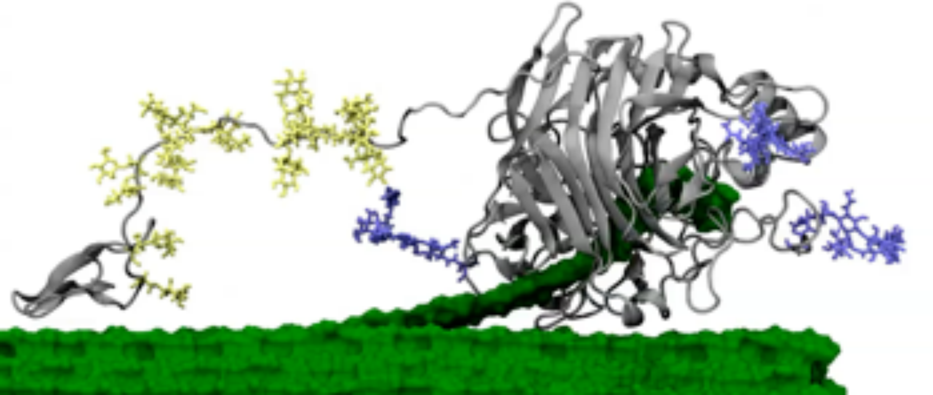
Computational Characterization of Cel7A

Binding/Association

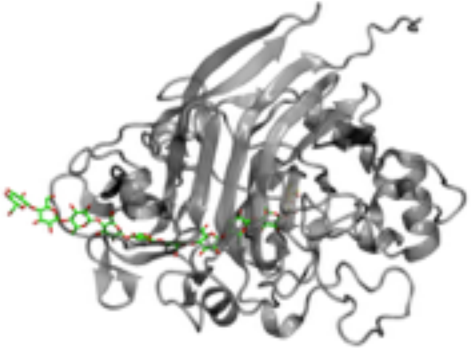


Payne et. al. PNAS 2011

Amore et. al. PNAS 2017

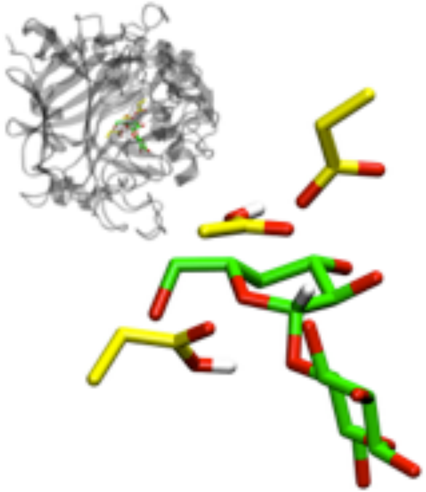


Processivity



Knott et. al. JACS 2014

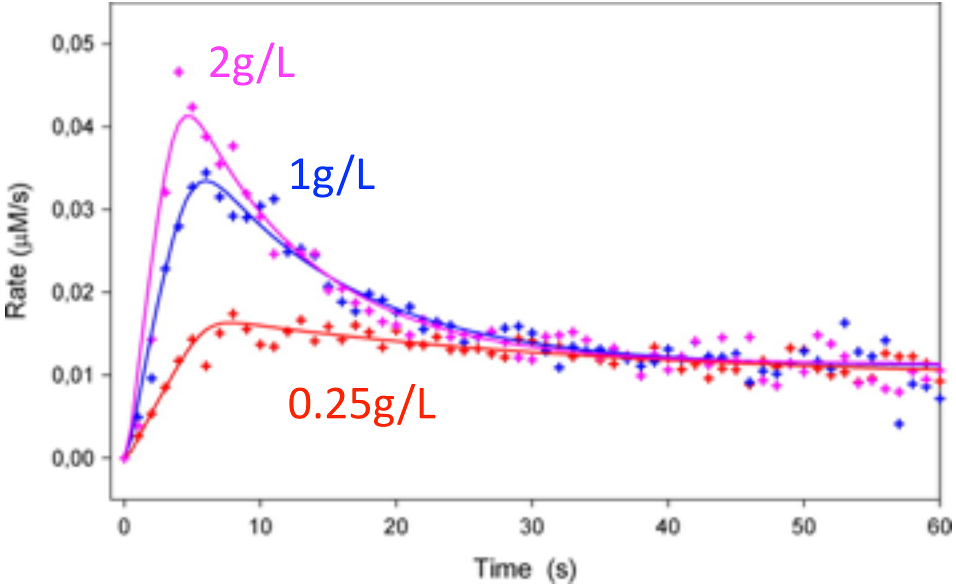
Catalysis



Knott et. al. JACS 2013

Cel7A is Dissociation Limited

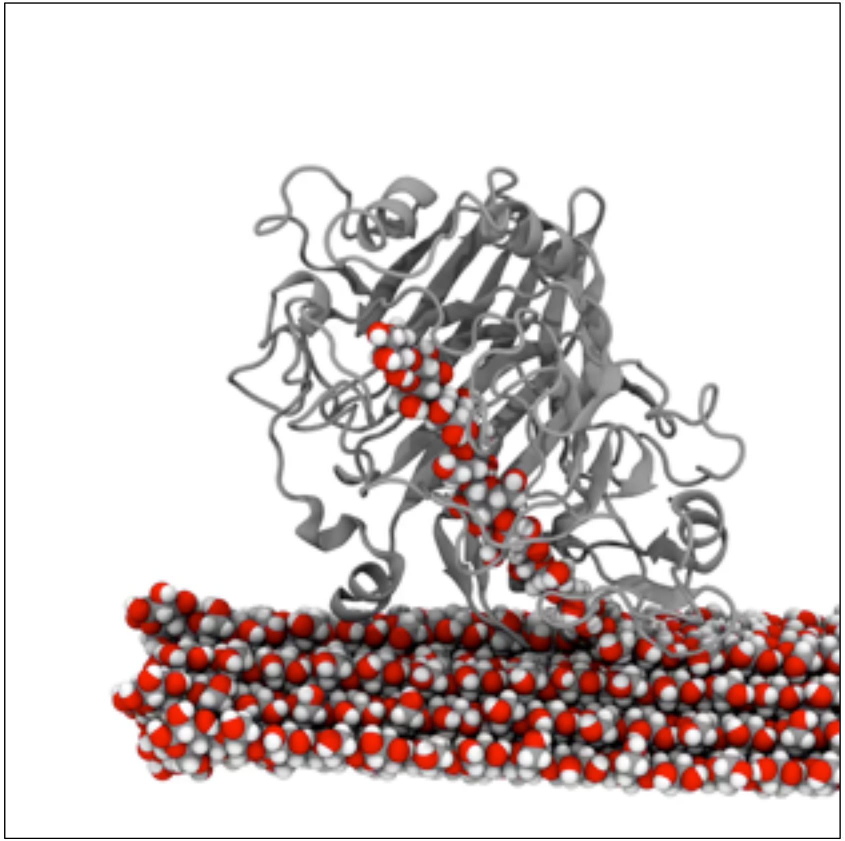
- Cel7A used extensively to hydrolyze cellulose within an industrial context
- With an excess of substrate, the enzyme is limited by its substrate dissociation
- How does the enzyme typically dissociate?
- What interactions could be altered to improve dissociation?



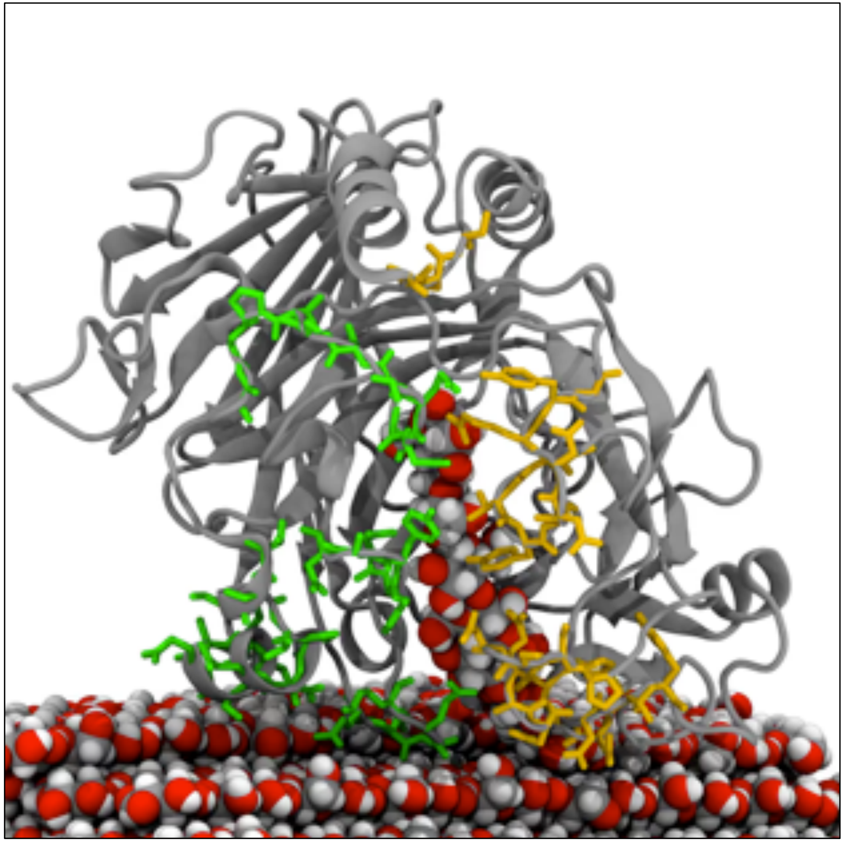
Amorphous cellulose conversion rate at fixed cellulase concentration

Cruys-Bagger et. al. JBC 2012

Two Potential Mechanisms



Dethreading



Clamshell

Mechanism Evaluation Metrics

- Dissociation rates have been determined experimentally
- Use simulation to compute kinetics along both pathways
- Compare results (including with new mutants!)

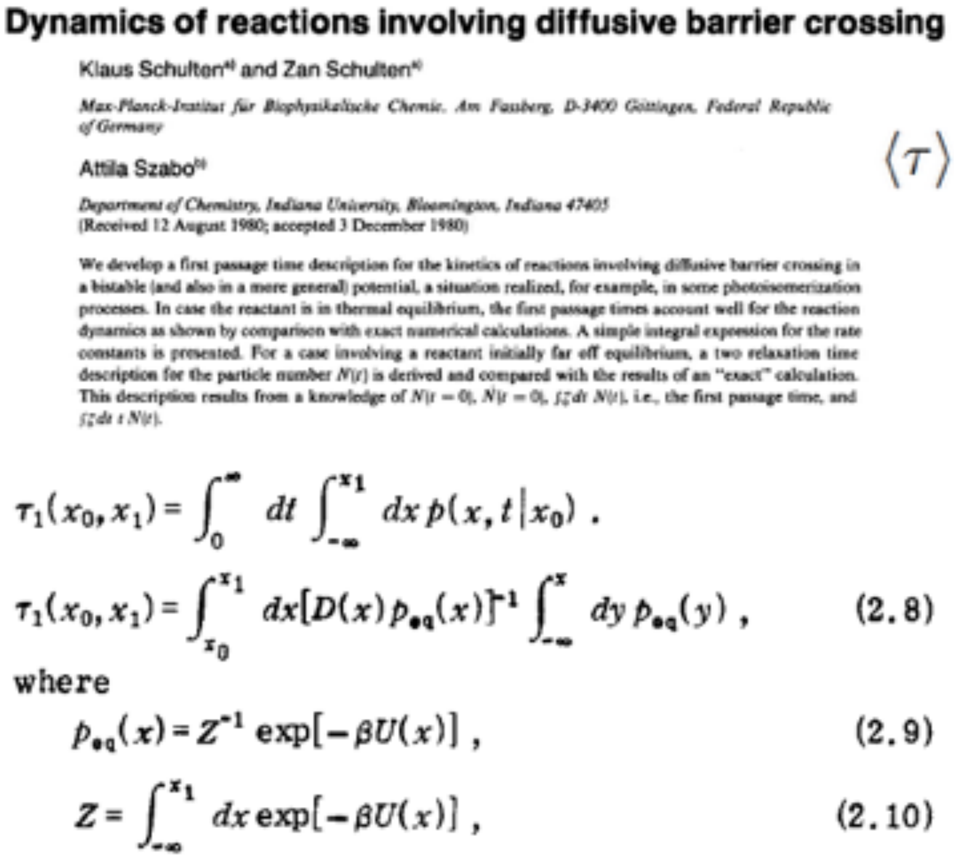
Measurement Technique	k_{off} (s^{-1})
AFM[a]	0.2
Biochemical Assay[b]	0.01
Biochemical Assay[c]	0.0032

[a] Nakamura et. al. JACS 2014

[b] Cruys-Bagger et. al. JBC 2012

[c] Kurašin and Väljamäe, JBC 2011

Computing Kinetic Parameters



Dynamics of reactions involving diffusive barrier crossing

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(Received 12 August 1980; accepted 3 December 1980)

We develop a first passage time description for the kinetics of reactions involving diffusive barrier crossing in a bistable (and also in a more general) potential, a situation realized, for example, in some photoisomerization processes. In case the reactant is in thermal equilibrium, the first passage times account well for the reaction dynamics as shown by comparison with exact numerical calculations. A simple integral expression for the rate constants is presented. For a case involving a reactant initially far off equilibrium, a two relaxation time description for the particle number $N(t)$ is derived and compared with the results of an "exact" calculation. This description results from a knowledge of $N(t=0)$, $\dot{N}(t=0)$, $\int_0^\infty dt\, N(t)$, i.e., the first passage time, and $\int_0^\infty dt\, t\, N(t)$.

$$\tau_1(x_0, x_1) = \int_0^\infty dt \int_{-\infty}^{x_1} dx\, p(x, t | x_0) \,.$$

$$\tau_1(x_0, x_1) = \int_{x_0}^{x_1} dx [D(x) p_{eq}(x)]^{-1} \int_{-\infty}^{x} dy\, p_{eq}(y) \,, \qquad (2.8)$$

where

$$p_{eq}(x) = Z^{-1} \exp[-\beta U(x)] \,, \qquad (2.9)$$

$$Z = \int_{-\infty}^{x_1} dx \exp[-\beta U(x)] \,, \qquad (2.10)$$

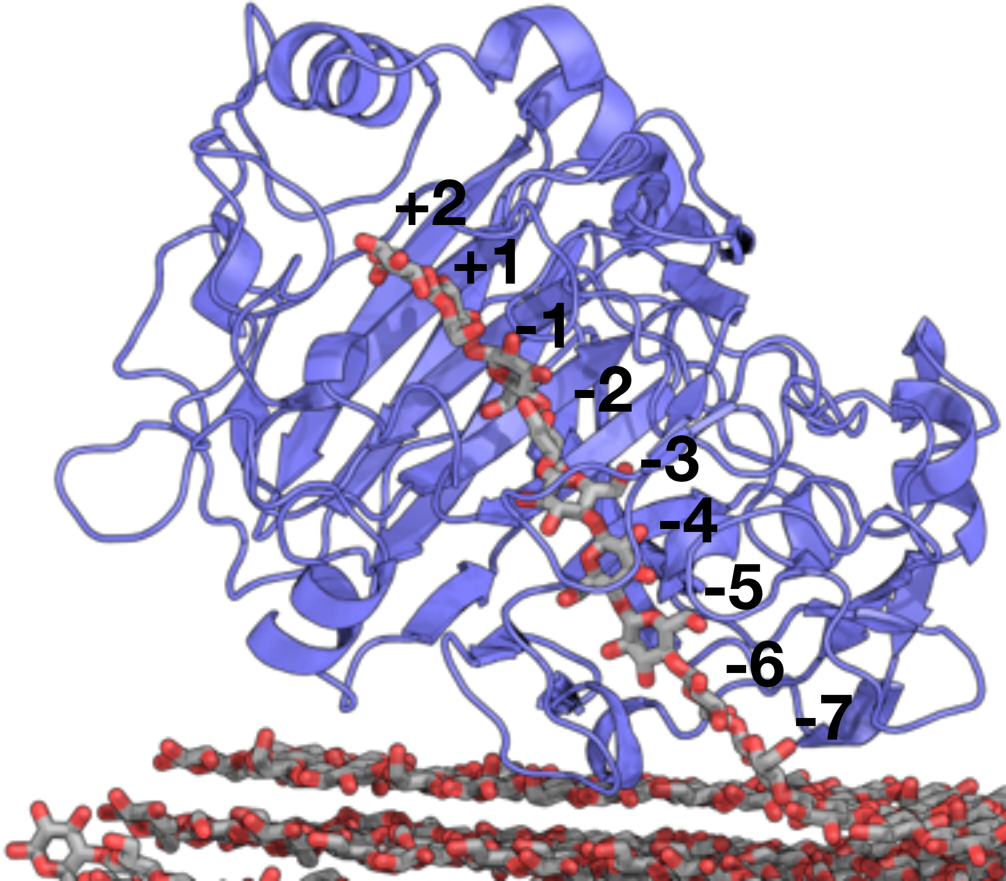
$$\langle \tau \rangle = \int_{x_i}^{x_f} dx' \frac{e^{W(x')\beta}}{D(x')} \int_{x_i}^{x'} dx'' e^{-W(x'')\beta}$$

Mean first passage time is just related to free energy profile and diffusivity

$$\langle \tau \rangle = \int_0^\infty k t e^{-kt} dt = \frac{1}{k}$$

Definition of mean first passage time directly relates to rate constants

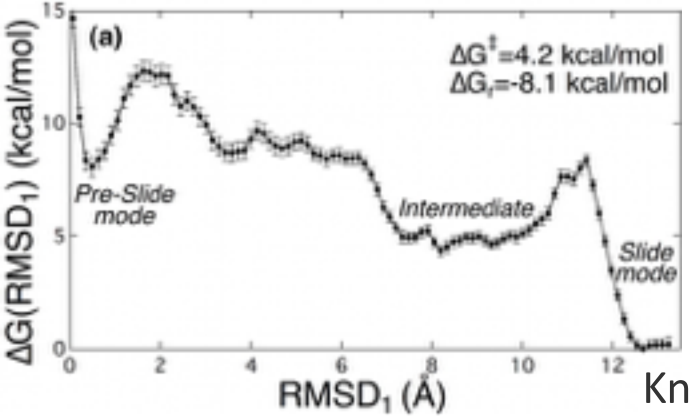
Reaction Coordinate for the Dethreading Mechanism



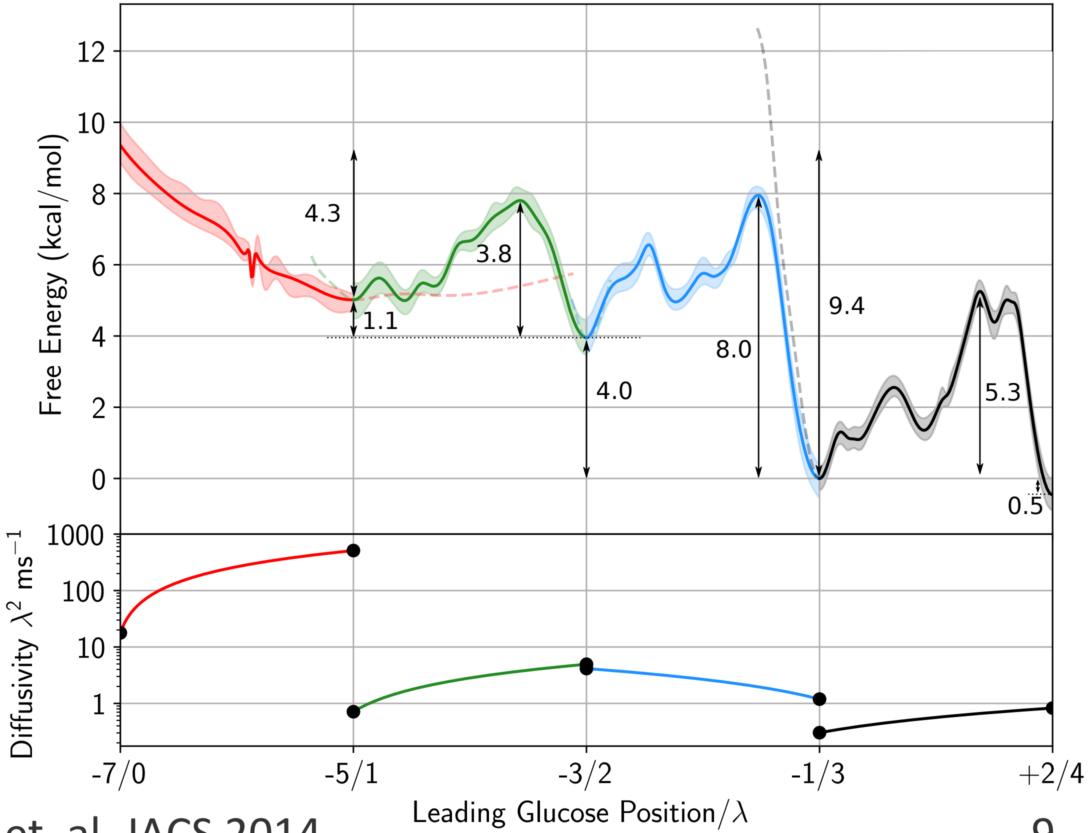
- Define contacts between individual glucose units and the surrounding enzyme and cellulose fibril for different loading states
- Interpolate between loading states (C_A-C_B)
- Use umbrella sampling to determine the free energy and diffusion coefficients

Dethreading Free Energy Results

- Moving from -1 state to -3 state is rate-limiting
- Movement from -1 to +2 state consistent with previous simulation

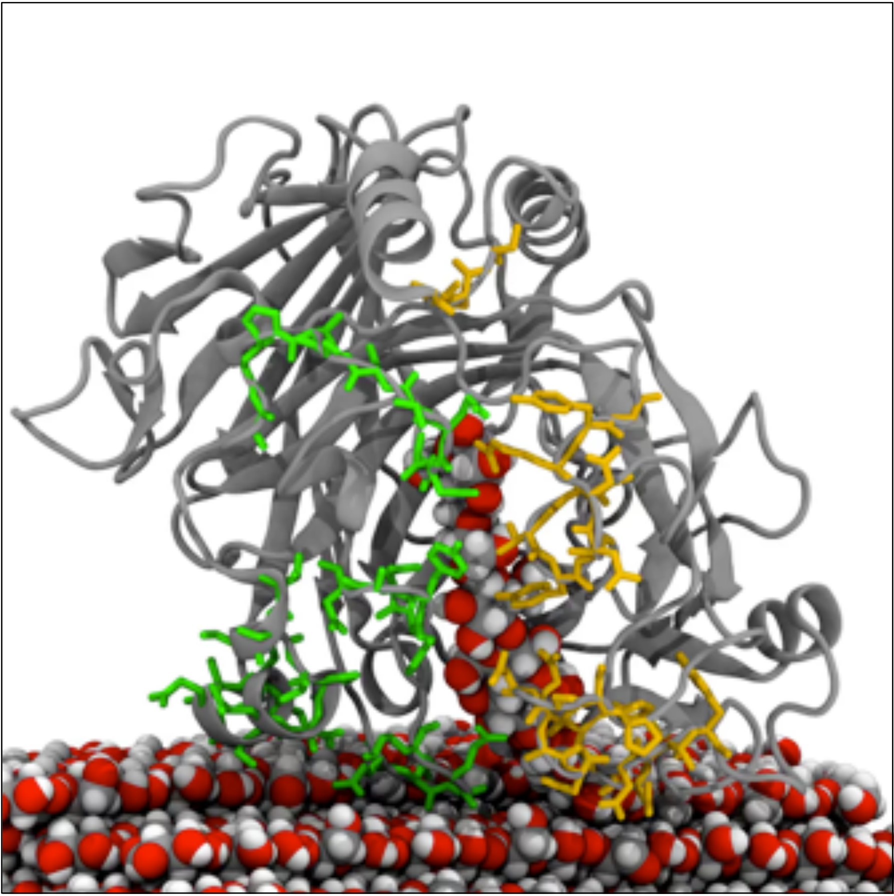


Knott et. al. JACS 2014

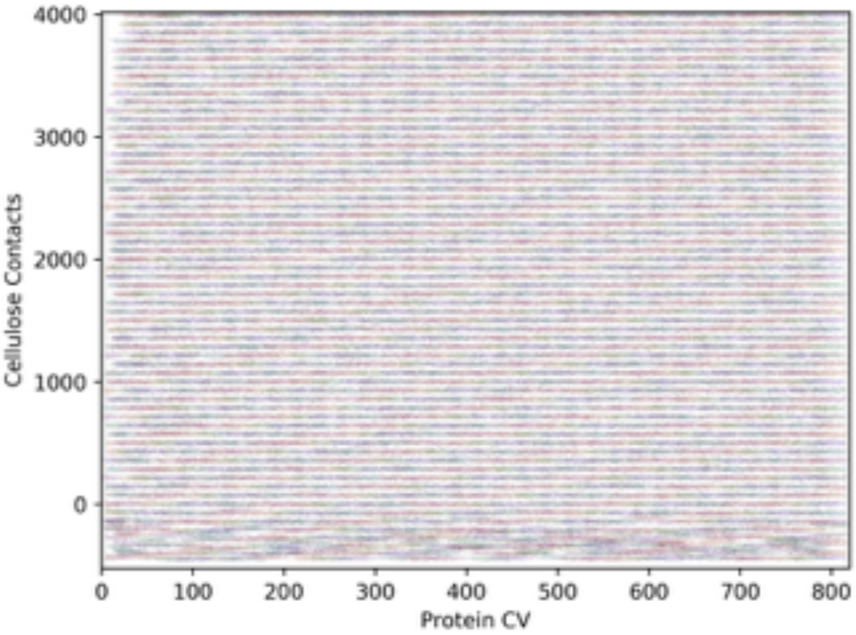


Dimensionality Problem

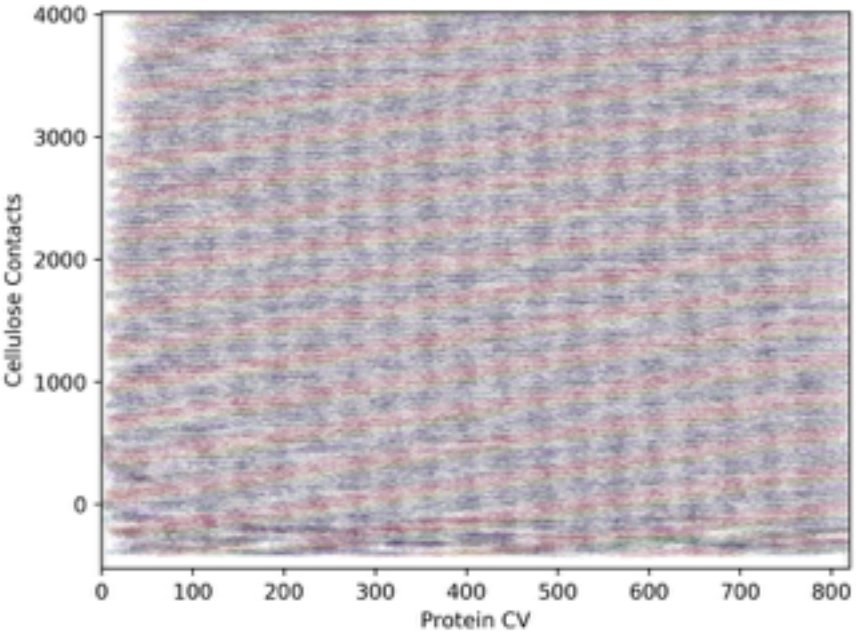
- Dethreading is fundamentally one-dimensional
- Clamshell mechanism is two-dimensional
  - Enzyme loop contacts
  - Cellulose-enzyme contacts
- Early science allocation on Eagle used to probe this two-dimensional reaction coordinate



Clamshell Umbrella Configuration



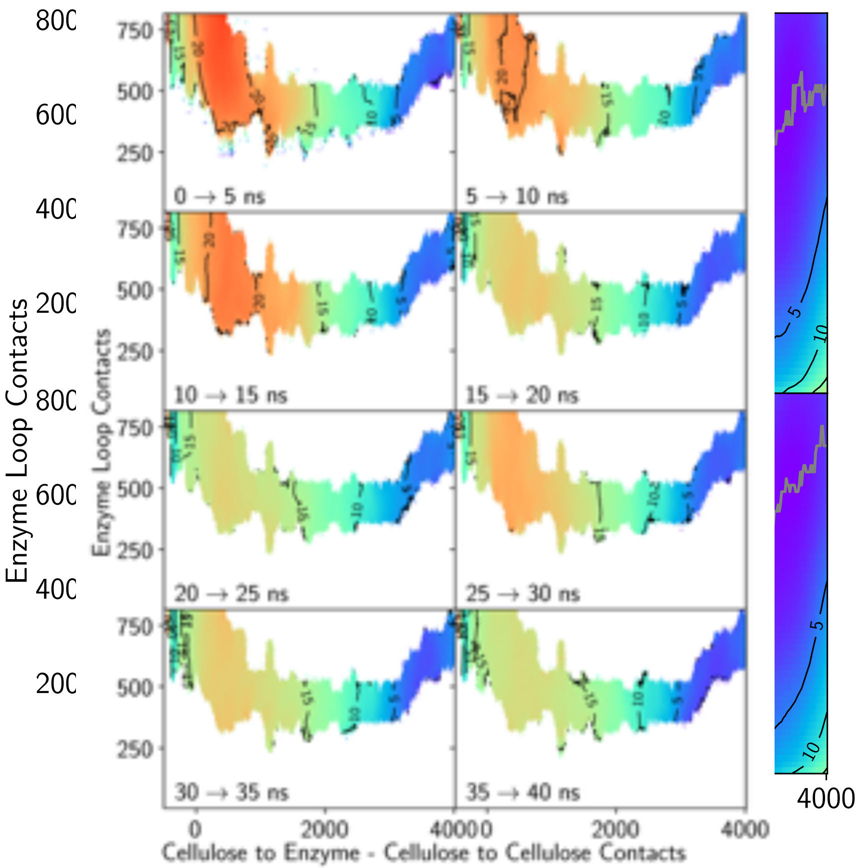
64x33 initial guess



20x105 refinement

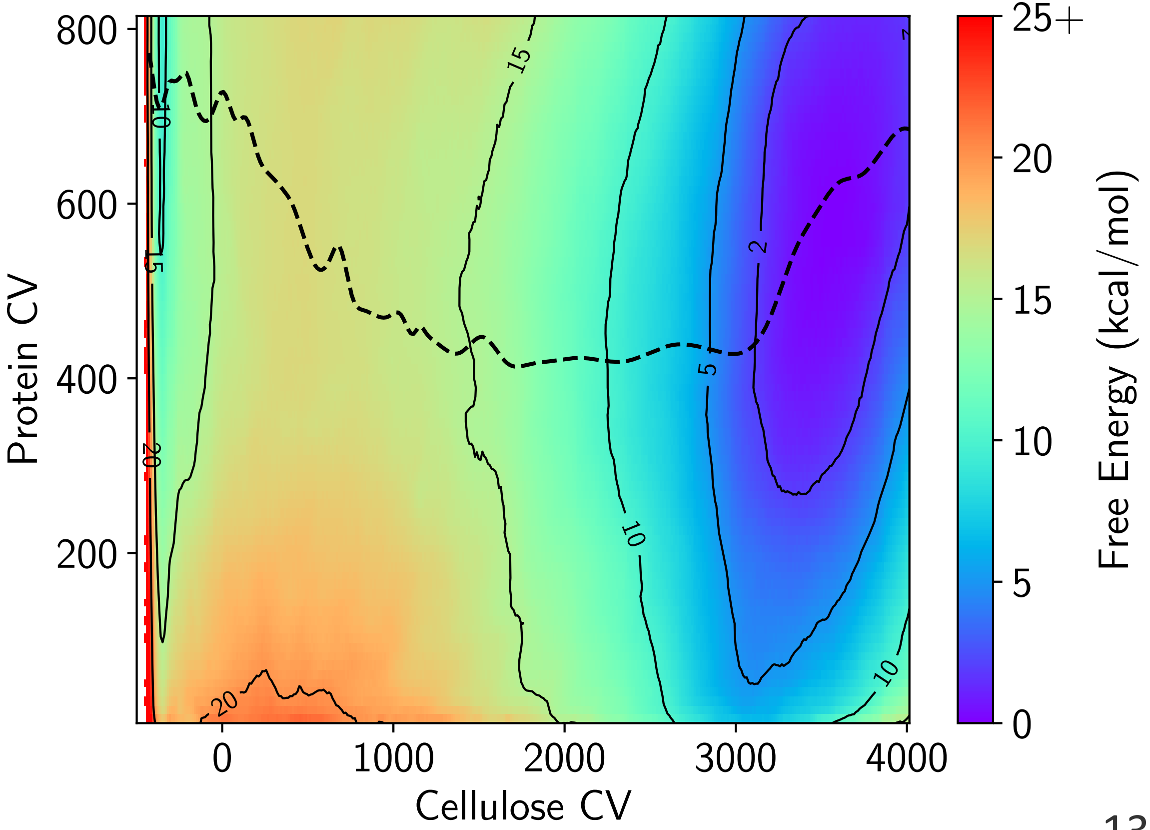
Slow Convergence and a Remedy

- Subsets of the total sampling indicate relatively slow convergence
- Expected given the fast SMD pulls to populate the windows
- Extended sampling over the predominant path increases sampling efficiency significantly



Clamshell Free Energy Surface

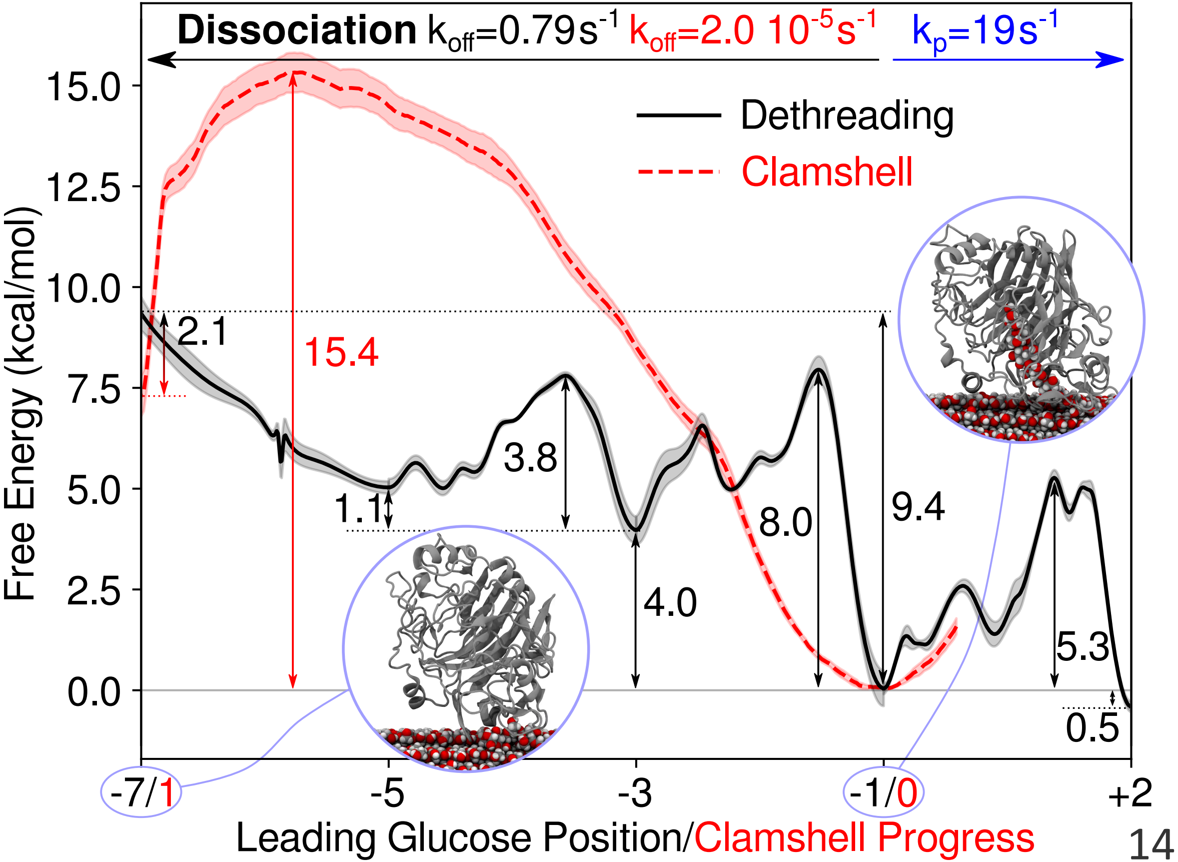
- Opening Cel7A loops is low energy
- The large barrier occurs at intermediate states where many interactions must be broken simultaneously



Dethreading Consistent with Experiment

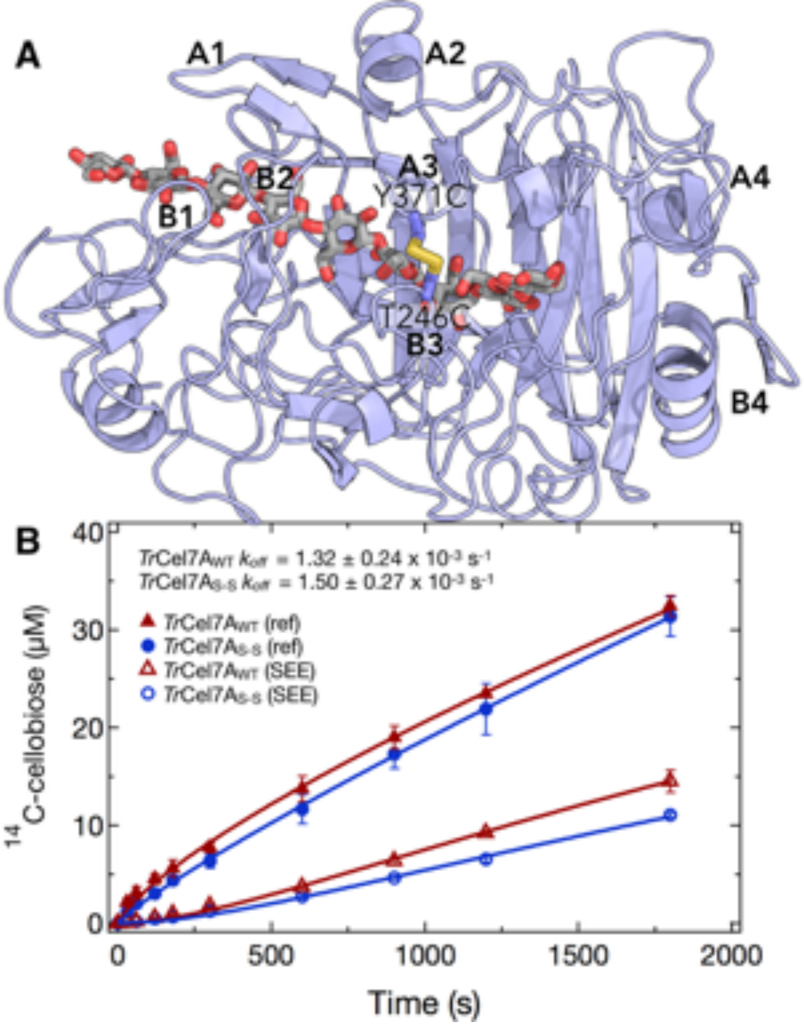
- Dethreading kinetics close to what is seen in AFM

Measurement Technique	k_{off} (s^{-1})
AFM[a]	0.2
Biochemical Assay[b]	0.01
Biochemical Assay[c]	0.0032



Mechanism Confirmation

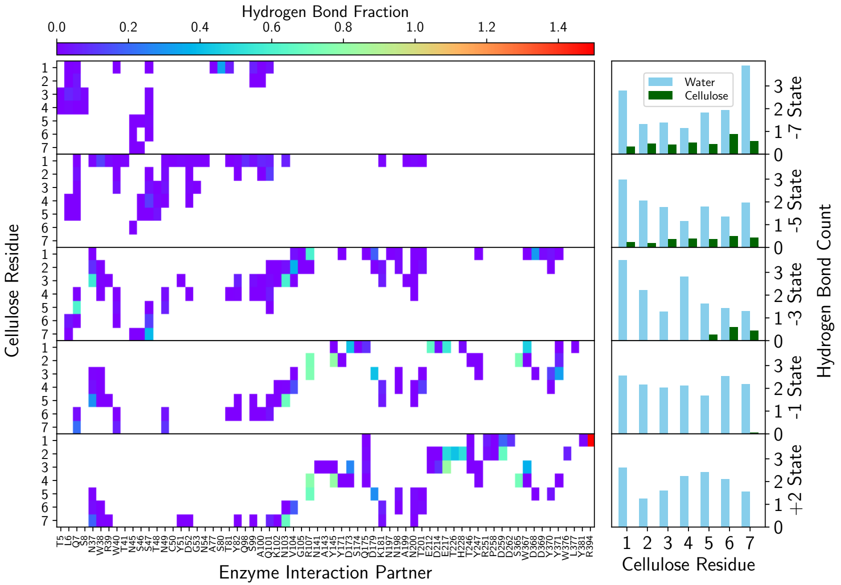
- Experimental collaborators in Sweden and Estonia can measure the dissociation rate for a cross linked enzyme variant
- Dissociation rates are unperturbed by the crosslinking, suggestive of the dethreading mechanism predominating

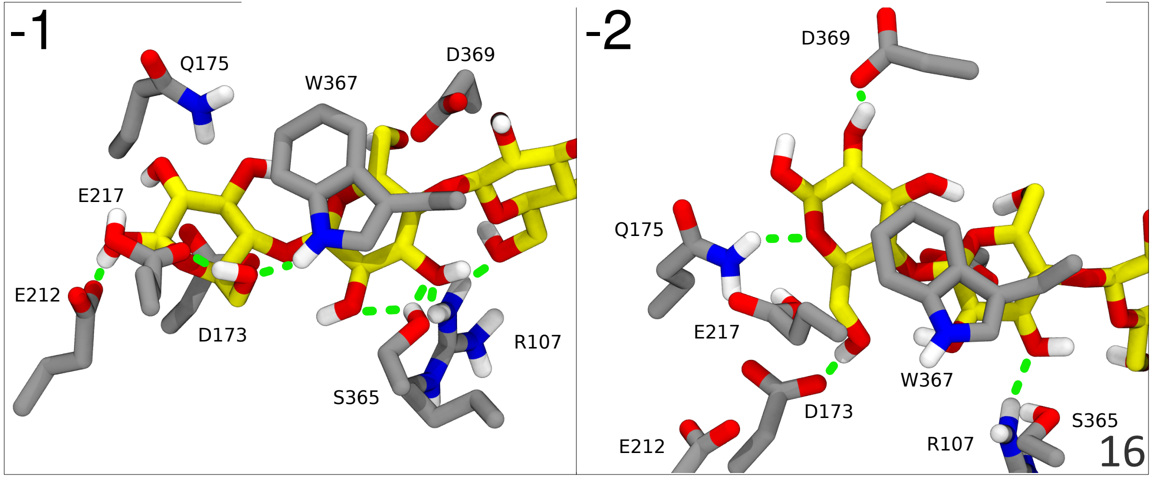


Courtesy of Riin Kont and Priit Väljamäe

What could we mutate?

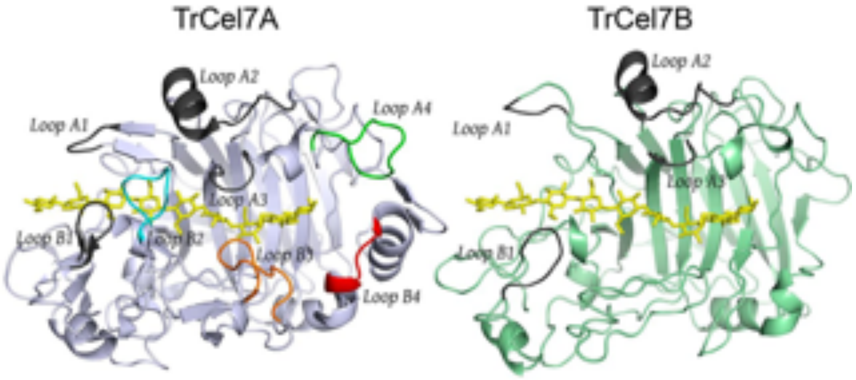
- Given the barrier to dethreading from the -1 to -2 state, what can be changed?
- Strongest interactions to -1 state are catalytic residues
- W367 may be one of the better options



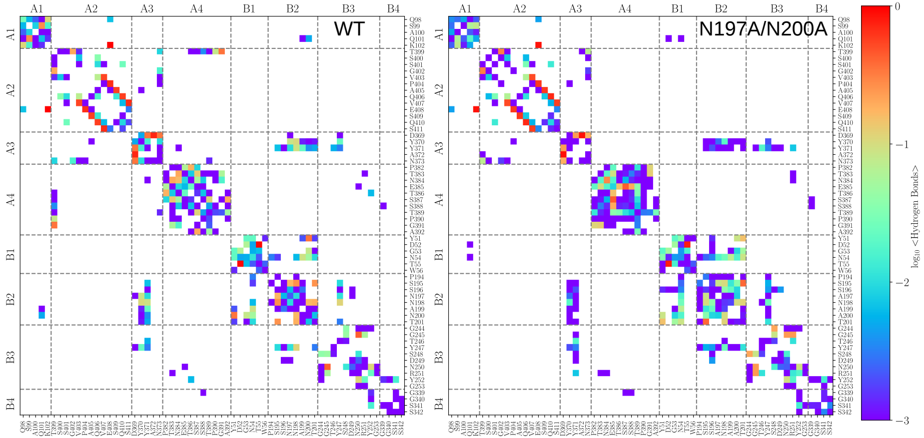


Mechanism Caveat

- Particular cellulases are indicative of the clamshell mechanism
- May be related to the continued closure of the loops, as connections between the two halves of the shell are relatively few and far between

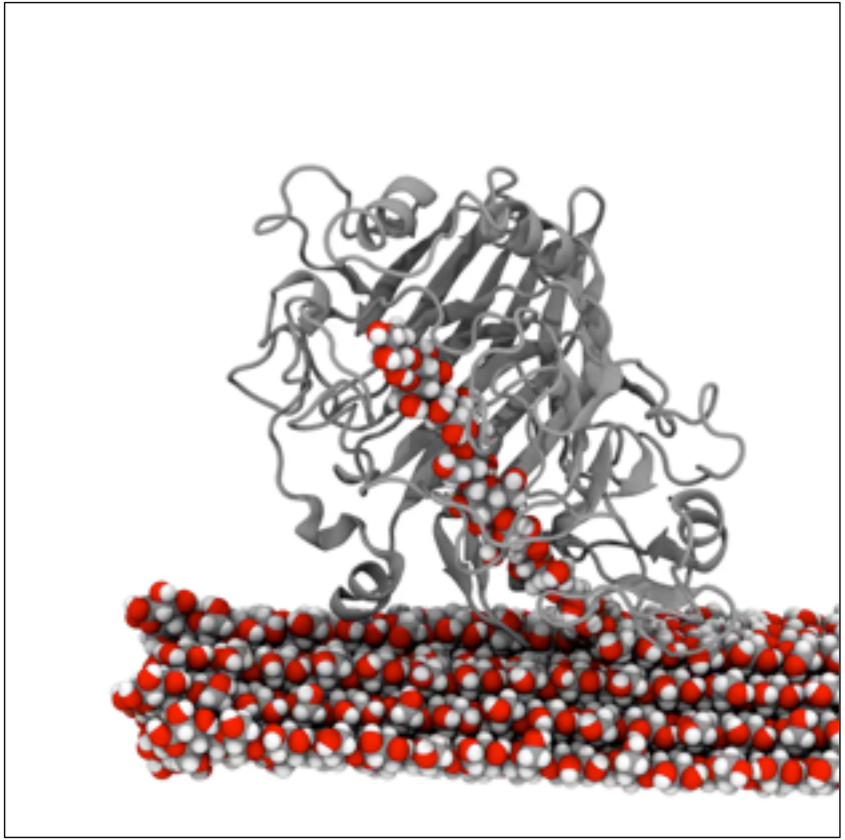


Schiano-di-Cola et. al. JBC 2019



Cel7A Summary

- For the wild type, the dethreading mechanism is clearly preferred
  - Matches experimental dissociation rates
  - Robust to mutagenesis experiments
- Clamshell-type mechanisms may depend on the loops simply not being present, as they are in some endoglucanases



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Mats Sandgren

Jerry Ståhlberg

Priit Väljamäe

Questions?

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