Simulation of the Molecular Machinery For Second Generation Biofuel Production

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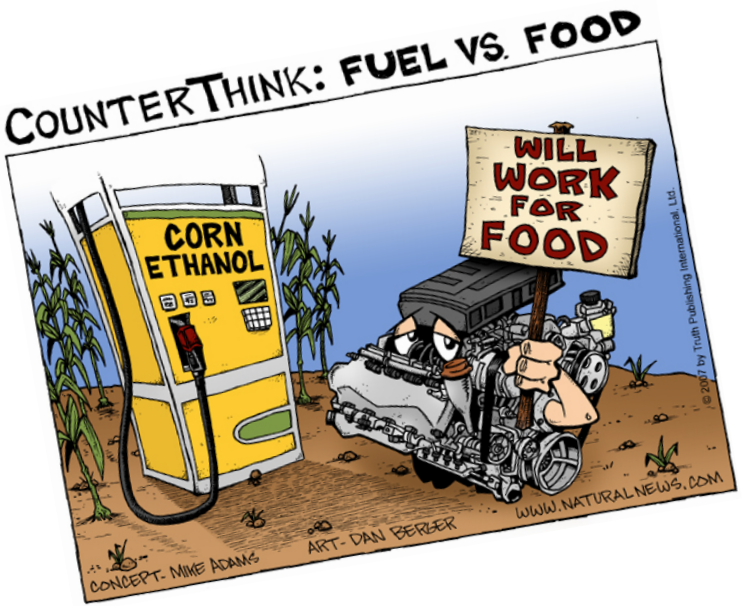
First-Generation Biofuel Production



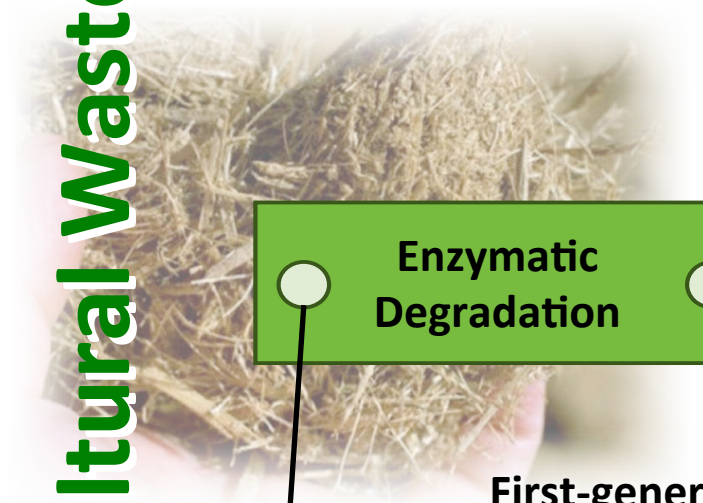
- **USA:** Largest Producer. **(CORN)**
- Cars can run on blends of up to 10% ethanol.
- E85 available.

- **Brazil:** Largest Biofuel Program. **(SUGARCANE)**
- No pure gasoline available (E18-E25).
- 97% of the cars sold in 2011 are flexfuel (E18 – E100).

- **Other Countries:** Sweden and France have E85 available.

Second-generation Biofuels

Agricultural Waste



Enzymatic Degradation → Carbohydrates Monomers → Yeast Fermentation → Ethanol Biofuel

First-generation biofuels

Uses food sources: corn starch, sugar cane, ... (carbohydrates monomers).

Second-generation biofuels

Uses agricultural waste: bagasse, corncob, woodchip, …

Microbes evolved the strategy, the main point is to reduce costs.

"Most bacteria release the cellulolytic enzymes to the environment and try to capture the enzymatic product, however some of them build large enzymatic complexes that are up to 50 times more efficient"

Simulations guiding Experiments

"Cellulosomes were discovered more than 30 years ago and due to the size and characteristics of this complex there is almost no clue why they are so efficient"

Prof. Isaac Cann

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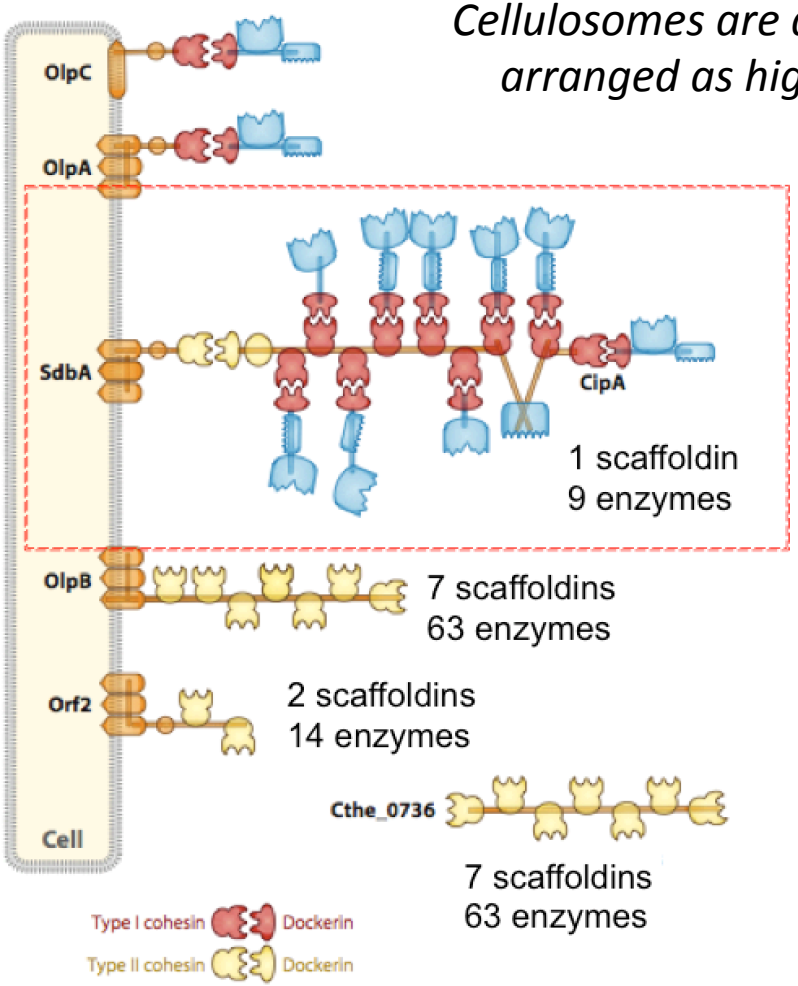
Why Blue Waters?

From single enzymes to multi-enzymatic complexes

Cellulosomes

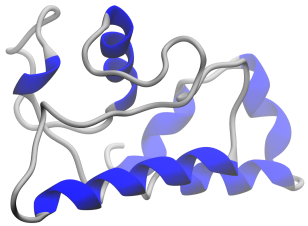
Cellulosomes are a large consortium of enzymes arranged as highly efficient nanomachines.



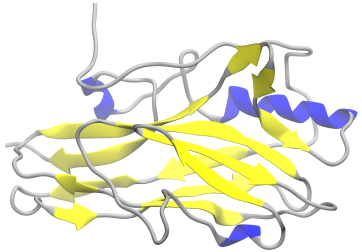
C. thermocellum Cellulosomes

- *Clostridium thermocellum cellulosome consists of **up to 63 different enzymes***
- *Ruminococcus flavefaciens cellulosome consists of **more than 200 different enzymes***

Building Blocks:



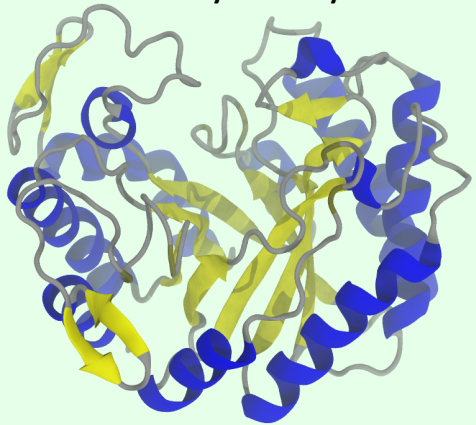
Dockerins



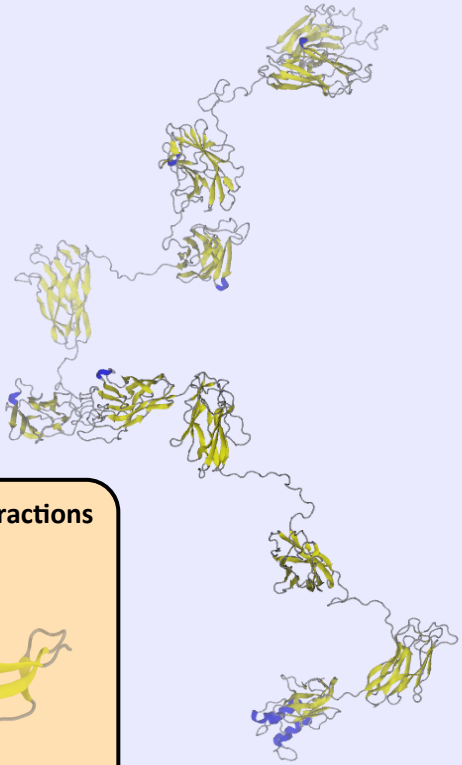
Cohesins

Studying Cellulosome Components

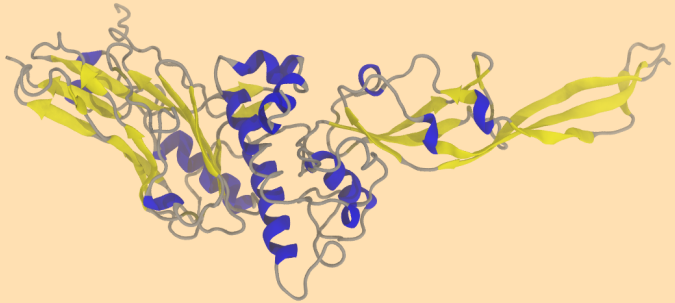
Glycoside Hydrolases Enzymes



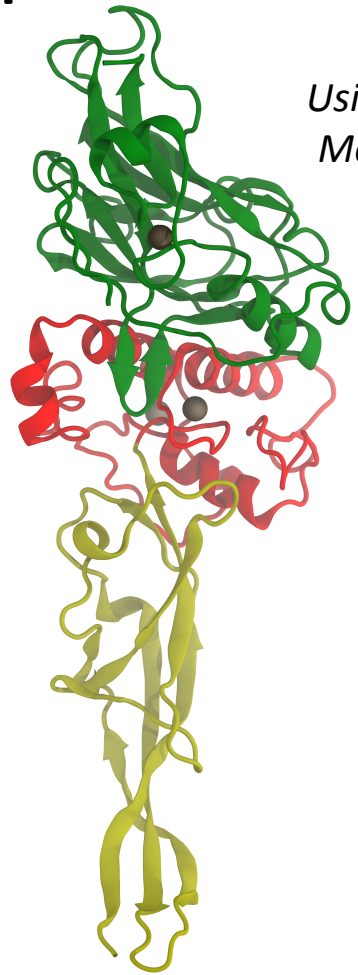
Cellulosome Scaffolding



Dockerin – Cohesin Interactions



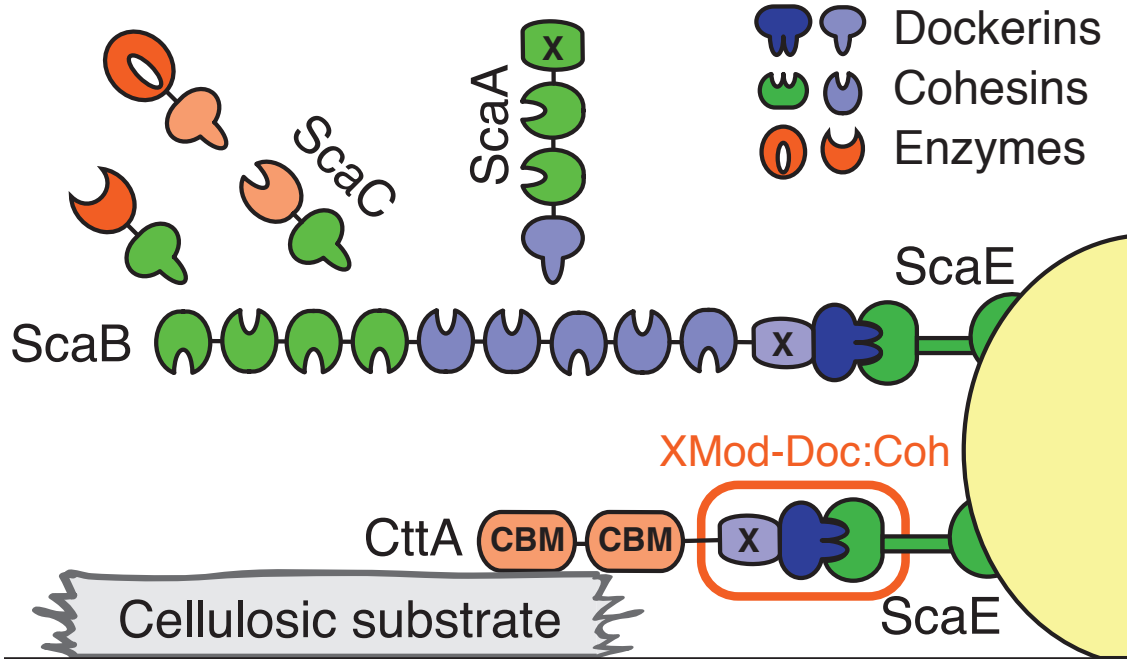
Type III Cohesin-Dockerin



Using Atomic Force Microscopy and Steered Molecular Dynamics we are characterizing Cohesin-Dockerin interactions.

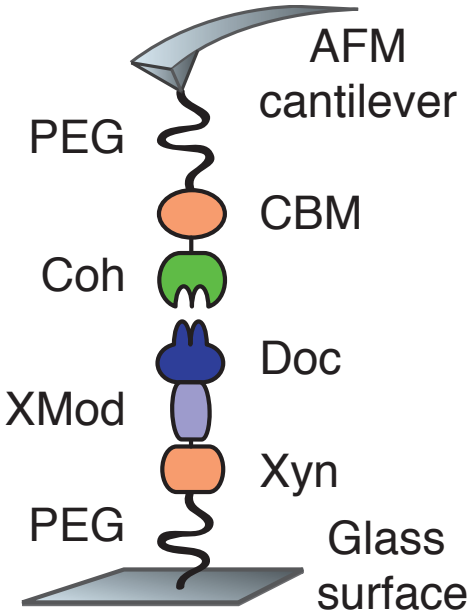


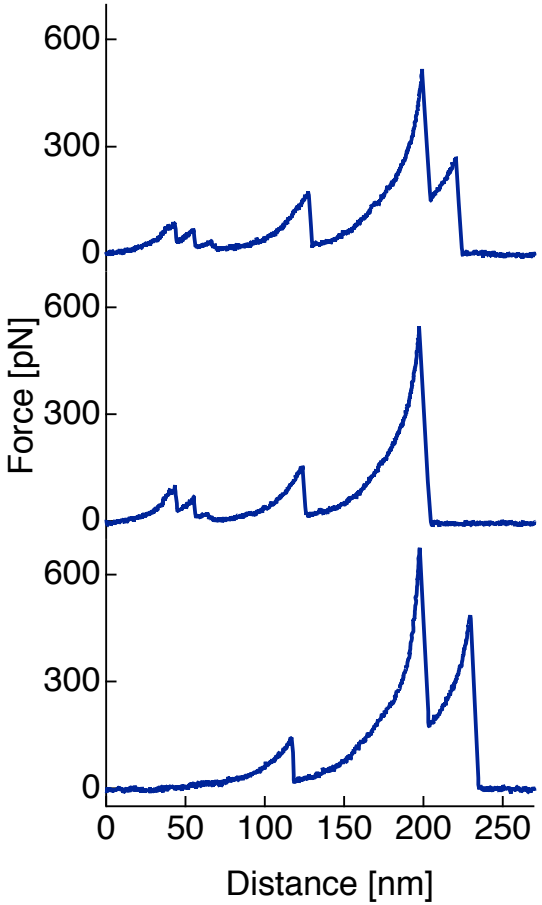
Collaboration with: Prof. Ed Bayer &
Prof. Hermann Gaub & Dr. Michael Nash

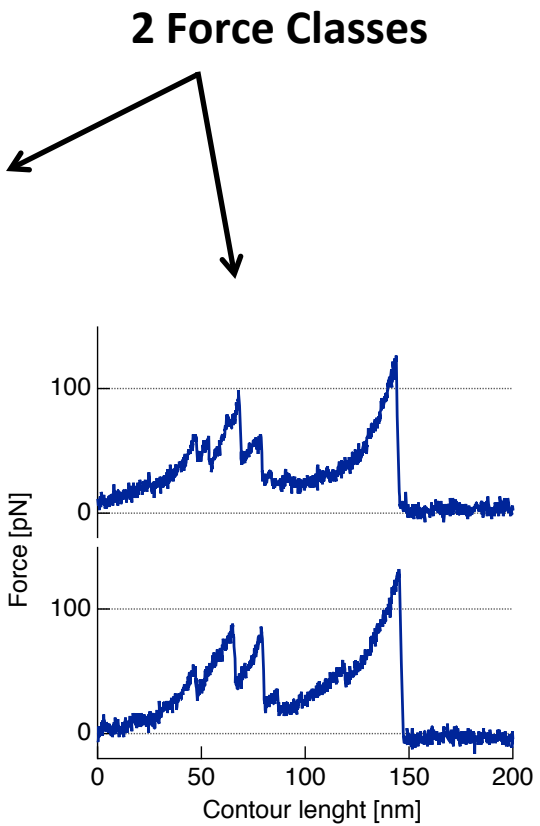


R. flavefaciens Cellulosomes

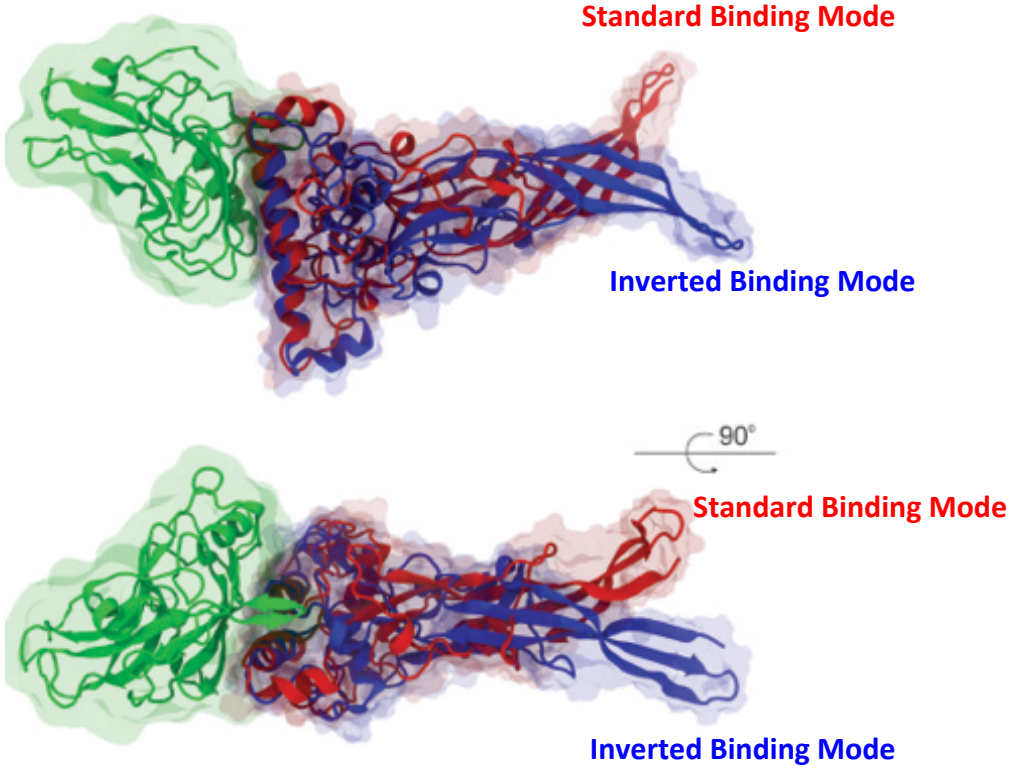
Single Molecule Experiments:

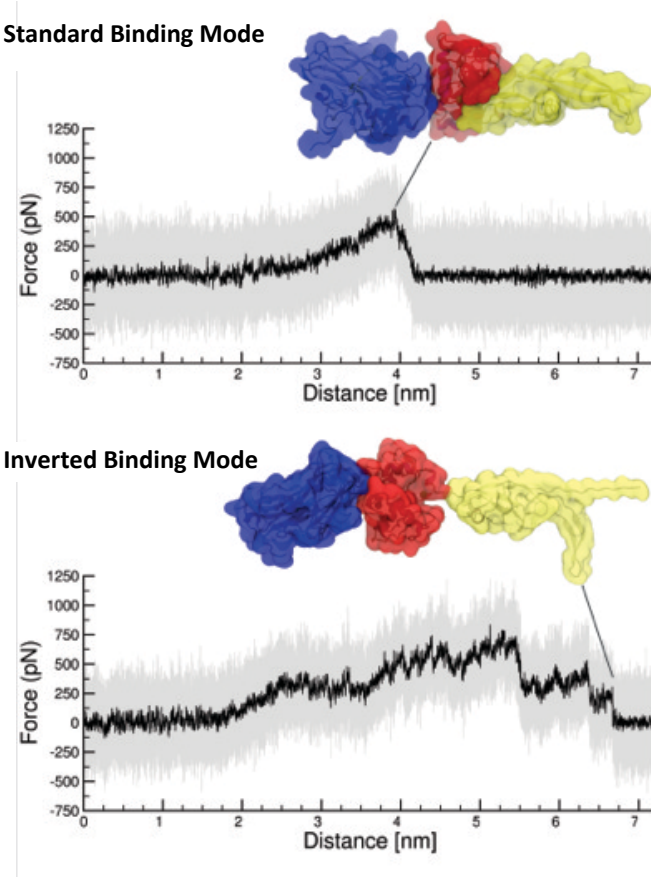




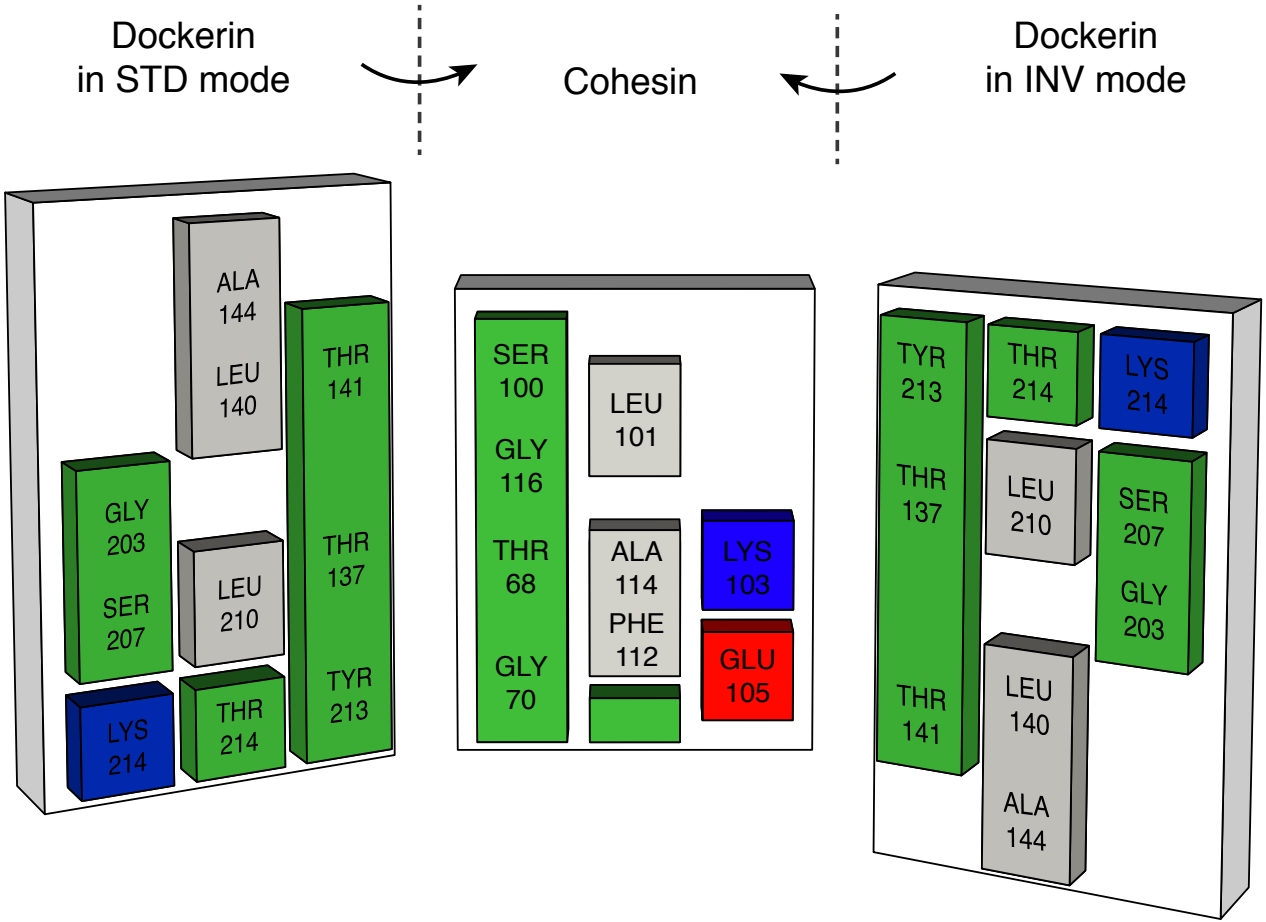


Inverted Binding Mode – Low Force Class?





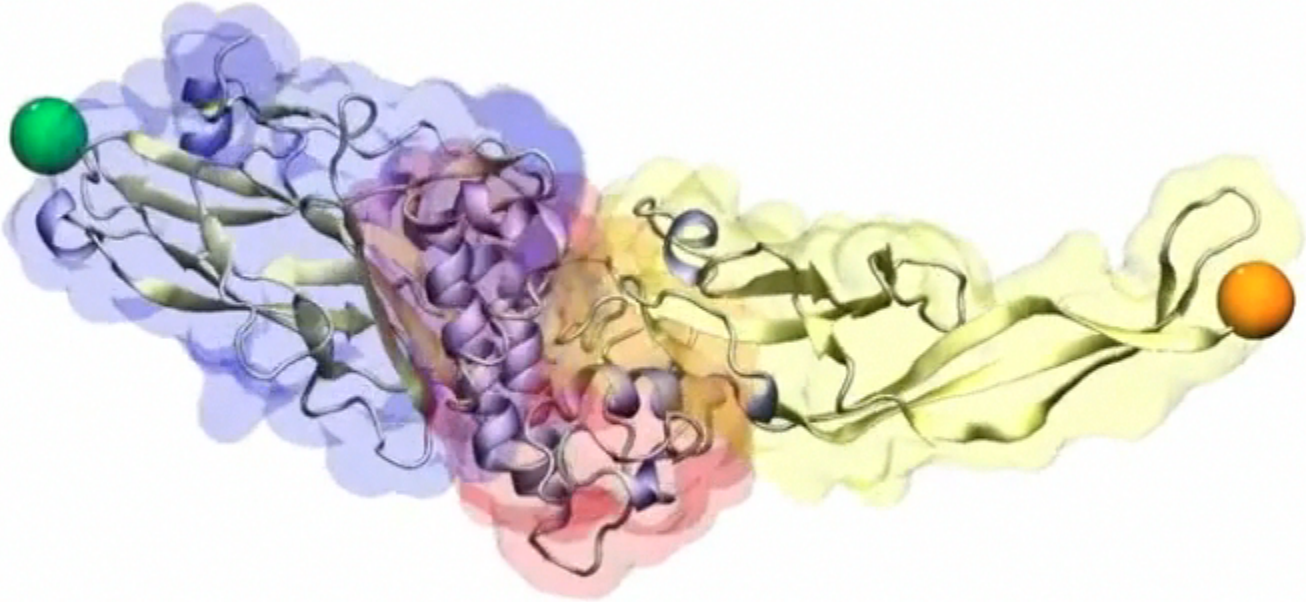
Why the Inverted Binding Mode is also stable?



White: Hydrophobic
Green: Hydrophilic
Blue: Positively charged
Red: Negatively charged

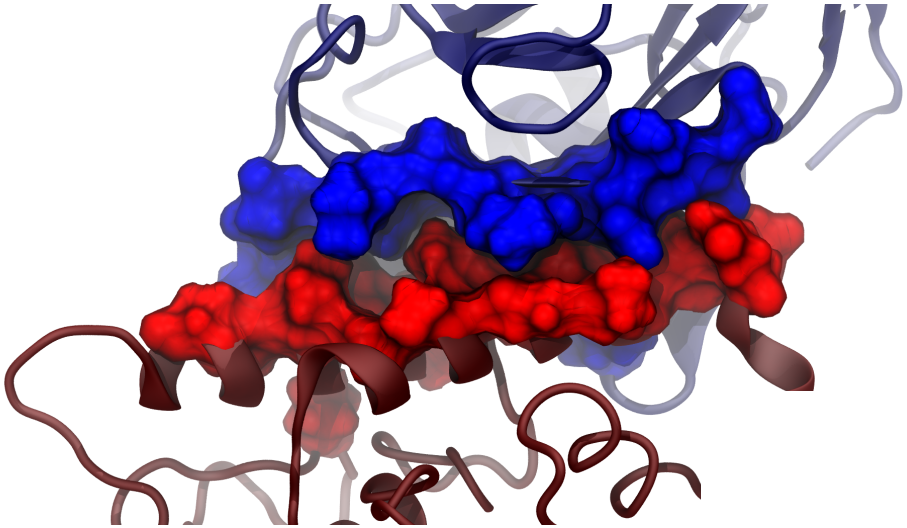
Cohesin-Dockerin Complex – SMD

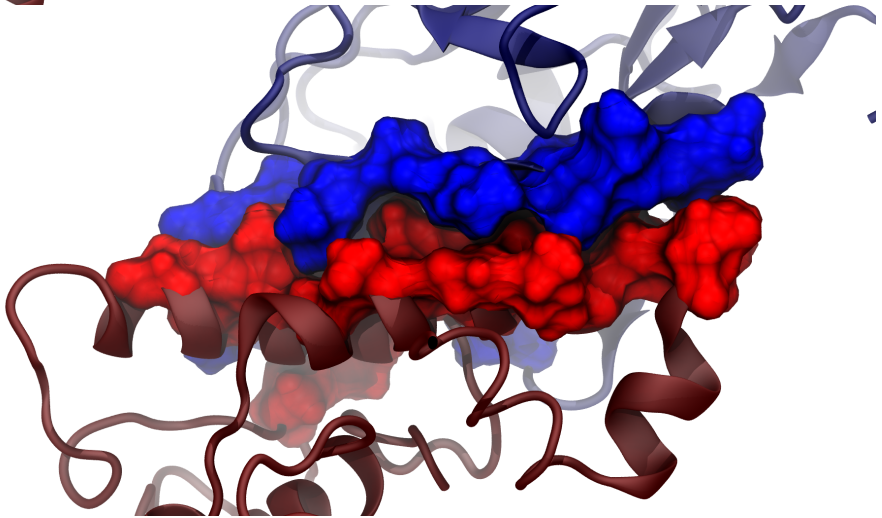
Pulling speeds: 0.25, 0.625 and 1.25 Å/ns



Extreme High Force

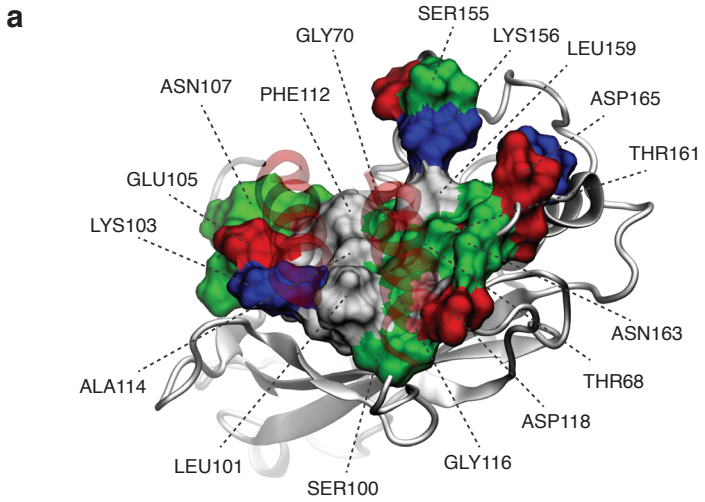
Catch Bond?

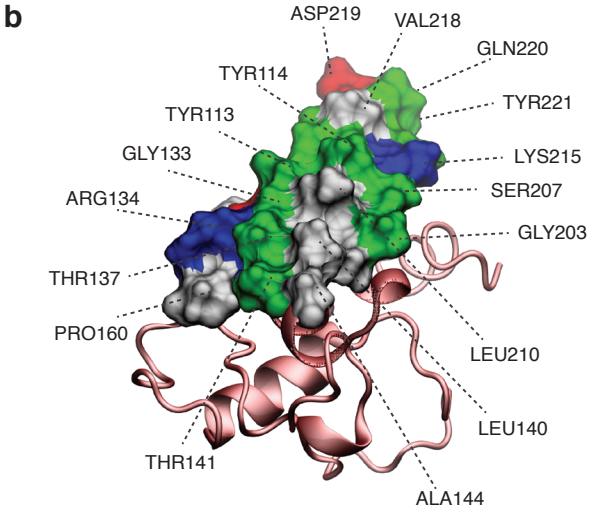


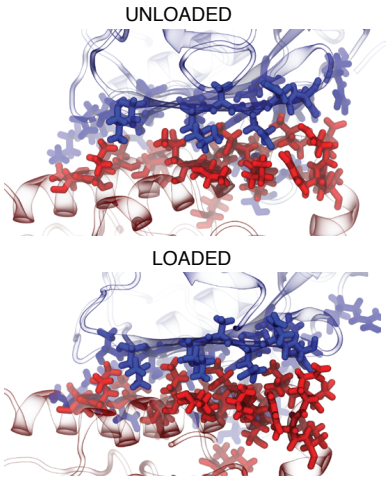


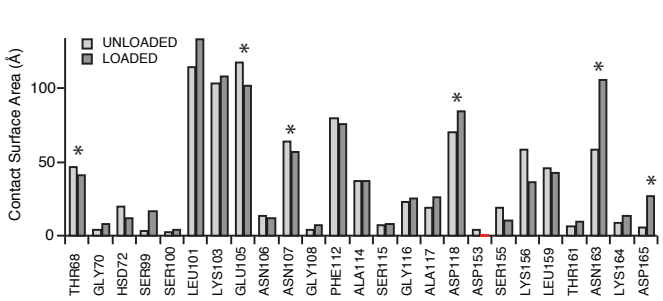
Dockerin – Cohesin Interactions

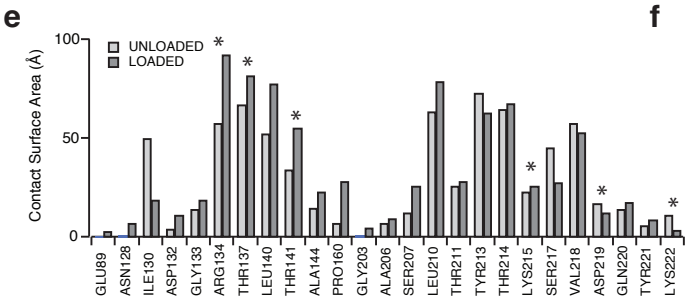
Contact Surface

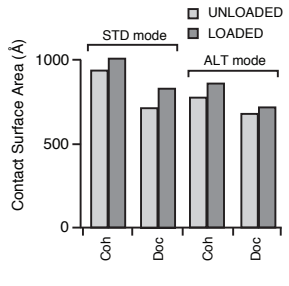








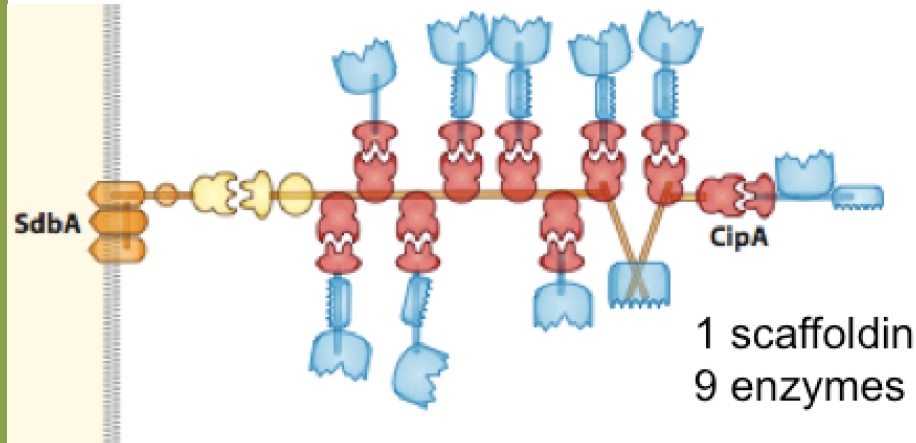




Building Atomistic Cellulosome Model

Building a Cellulosome Model

- SdbA: SHL domain, Unknown domain, Type II cohesin
- CipA: Type II Dockerin-XModule, 7 Type I cohesin, CBM, 2 Type I cohesin
- 5 Enzymatic Domains: (1is9, 1daq), (2cn3, 1ohz), (3c7e, 1gmm, 1gmm, 1daq), (3k4z, 1r15, 3pdd, 3zqx, 1daq), (3zm8, 1daq)





Collaboration with: Prof. Ed Bayer & Prof. Hermann Gaub & Dr. Michael Nash

Cellulosomes

Generalized Simulated Annealing – GSAFold

GSAFold NAMD Plugin – Allows *ab initio* structure prediction

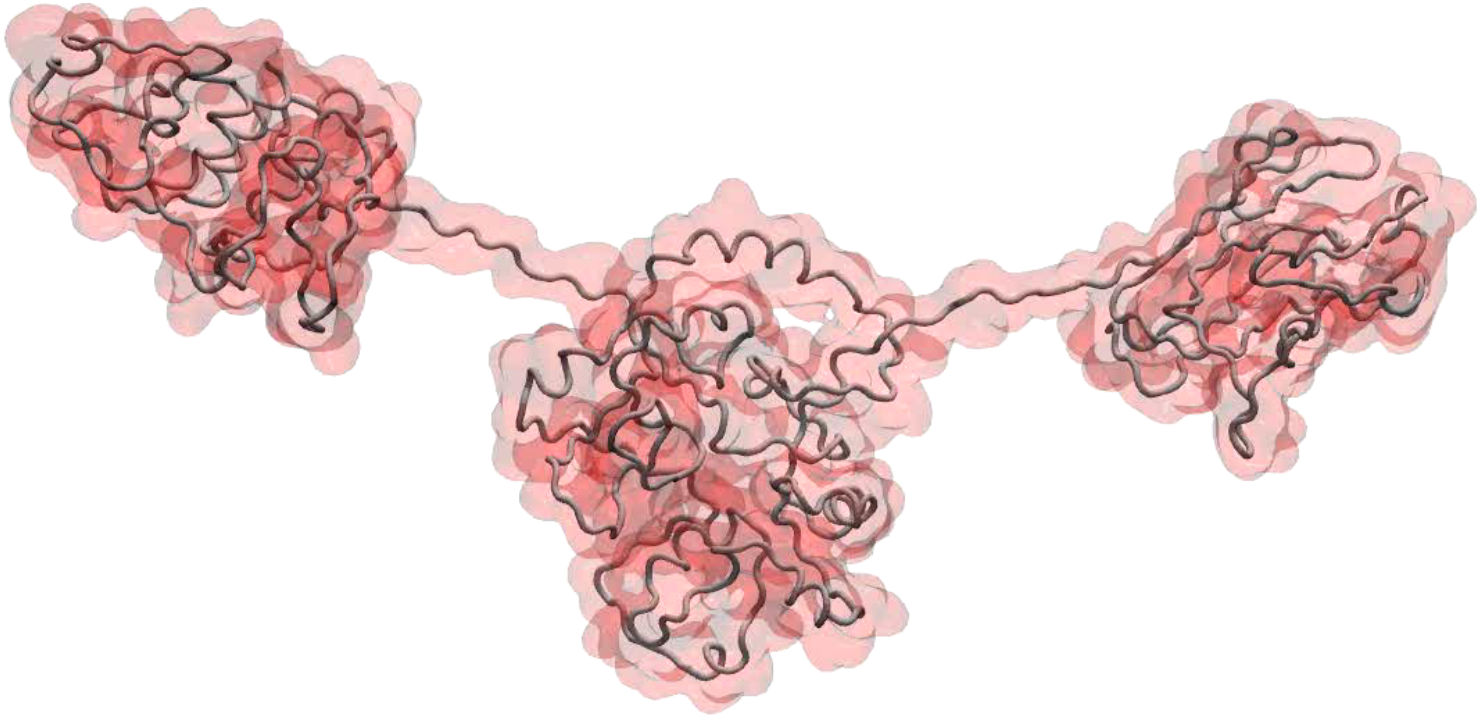
New implementation on Blue Waters to allow conformational search

- Amino acid residues connecting Type I Cohesin domains are disordered proteins and a random conformation with lower energy can be found using stochastic searching algorithms such as the GSA.

- GSAFold coupled to NAMD searches low-energy conformations to be used as starting points for the molecular dynamics studies.

Collaboration with: Marcelo Melo

Cellulosomes



Acknowledgments

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Thank You!!!