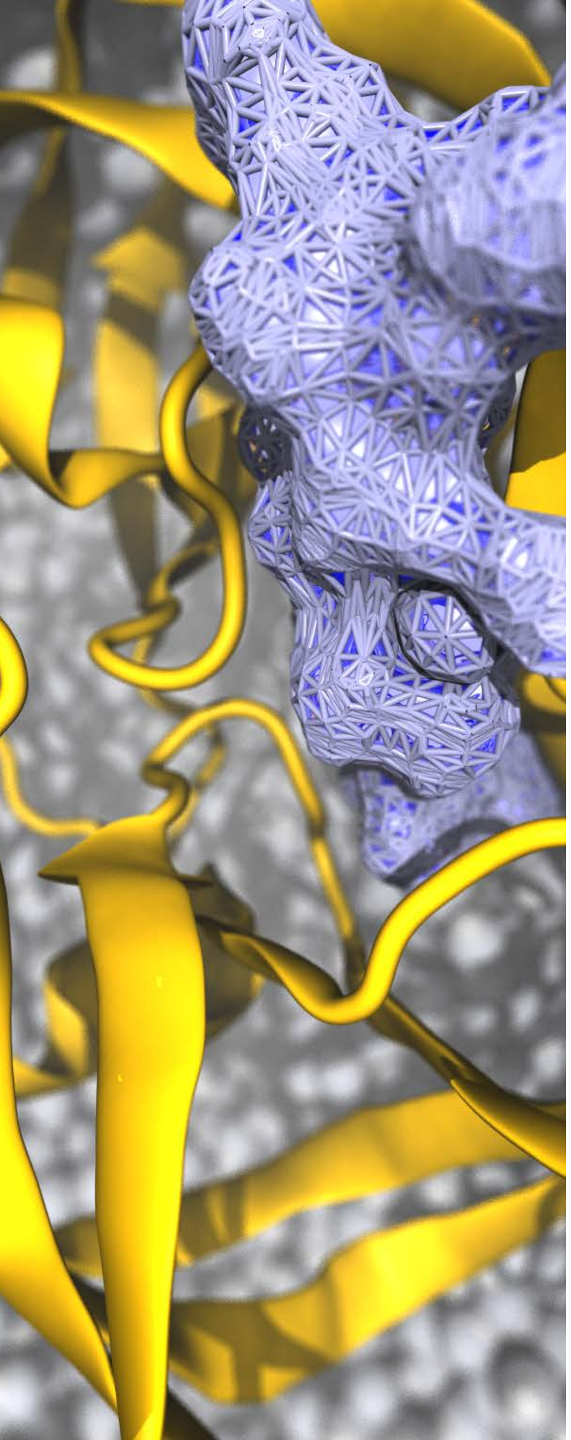

Probing Protein Mechanics with Molecular Dynamics Simulations and Single-Molecule Experiments

PRAC: The Computational Microscope

PI: Emad Tajkhorshid

Co-PIs: Rafael C. Bernardi, John E. Stone, and James C. Phillips

Rafael C. Bernardi

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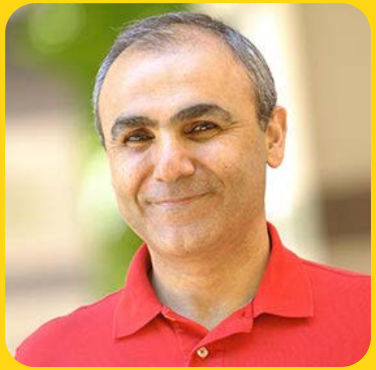
What are we doing in Illinois?



Prof. Emad Tajkhorshid

Prof. Zan Luthey-Schulten

Prof. Klaus Schulten

Development of NAMD & VMD:

- **Available for Free;**
- **One of the Most used Software in US Super Computer Centers;**

Over 700 publications;

Over 120k citations;

NIH Center for Macromolecular Modeling and Bioinformatics

NSF Center for the Physics of Living Cells

Probing Protein Mechanics with Molecular Dynamics Simulations and Single-Molecule Experiments

in silico (Steered Molecular Dynamics)

Prof. Klaus Schulten
Beckman Institute
University of Illinois

Prof. Zaida Luthey-Schulten
Department of Chemistry
University of Illinois

in vitro (AFM-based SMFS)

Prof. Hermann Gaub
LMU Munich, Germany

Prof. Michael Nash
University of Basel, Switzerland

1867
ILLINOIS
UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

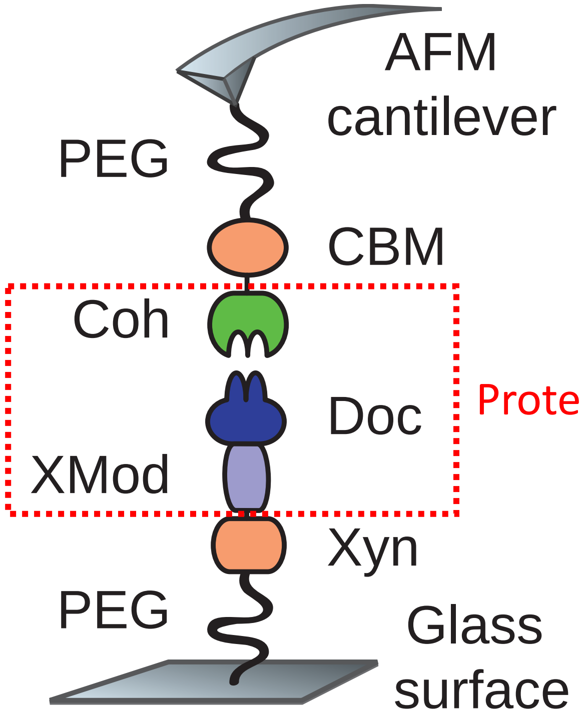
NIH Center for Macromolecular Modeling and Bioinformatics

NCSA Blue Waters Supercomputer

Combining *in silico* and *in vitro* Experiments

Unraveling Molecular Mechanisms of Extreme Mechanostability in Proteins

Experimental Setup
in vitro

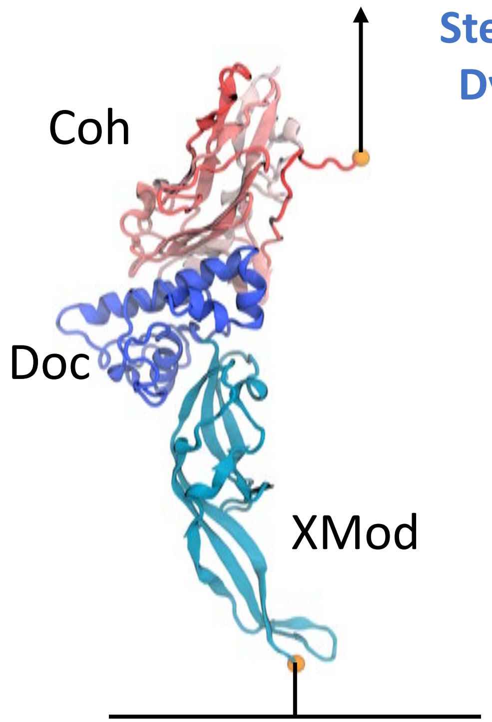


Single-Molecule Force Spectroscopy (SMFS)

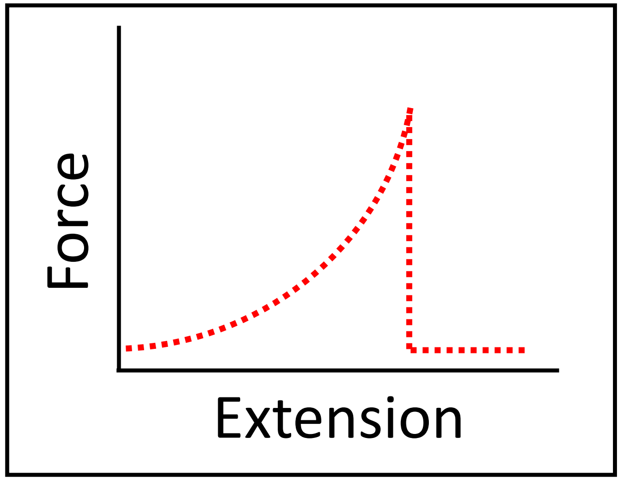
Atomic Force Microscope (AFM)

Protein Complex of Interest

Computational Setup
in silico

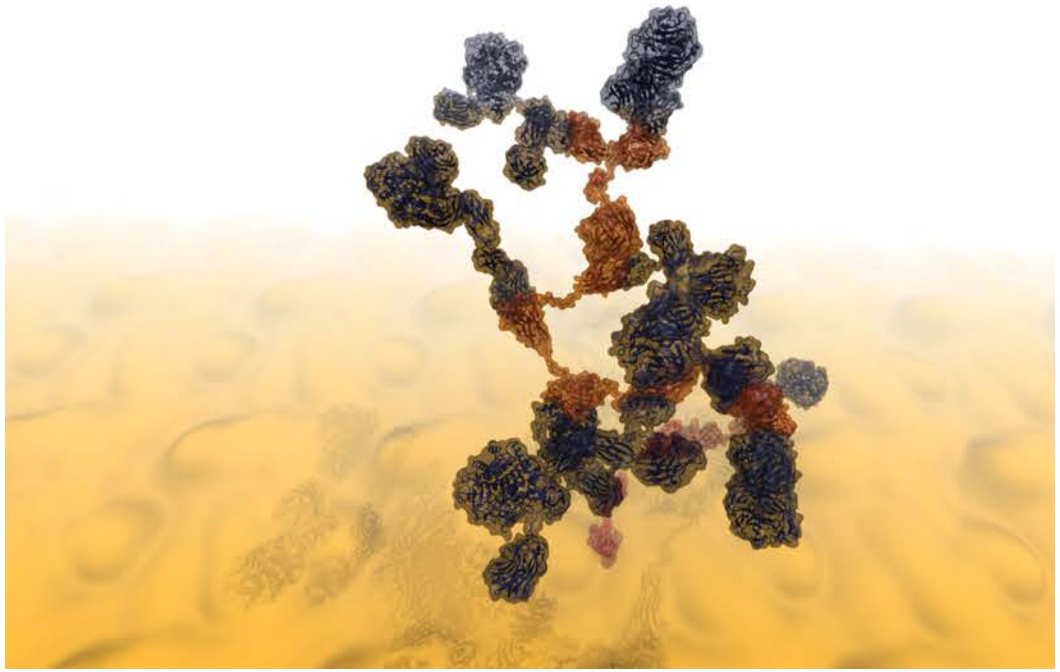


Steered Molecular Dynamics (SMD)



Extreme Mechanostability in Bacterial Proteins

Cellulosomes

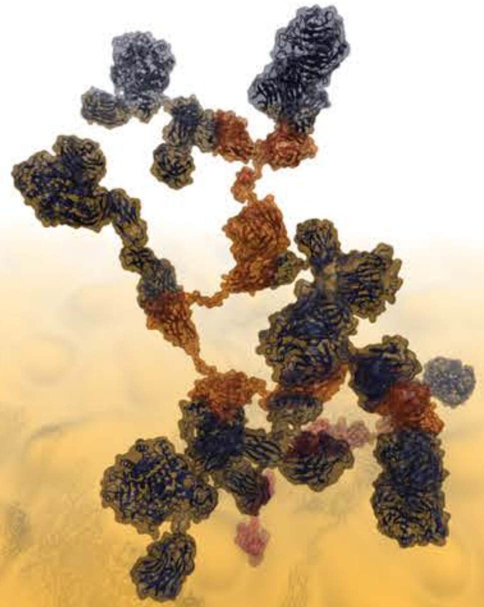


Adhesins

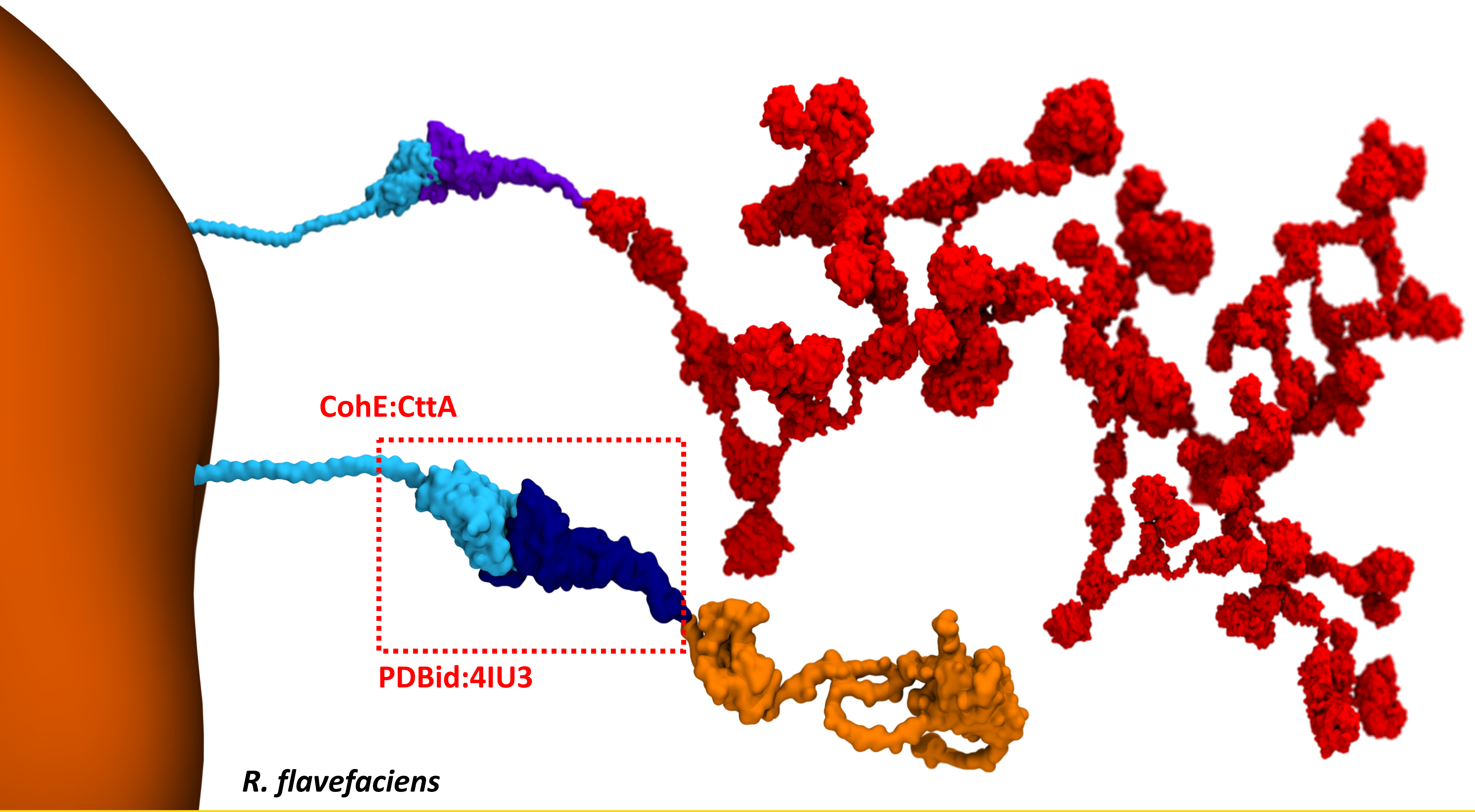
Cellulosomes are Used by Some Bacteria to Digest Plant Fiber

Cellulosomal organisms often live in a turbulent environment.

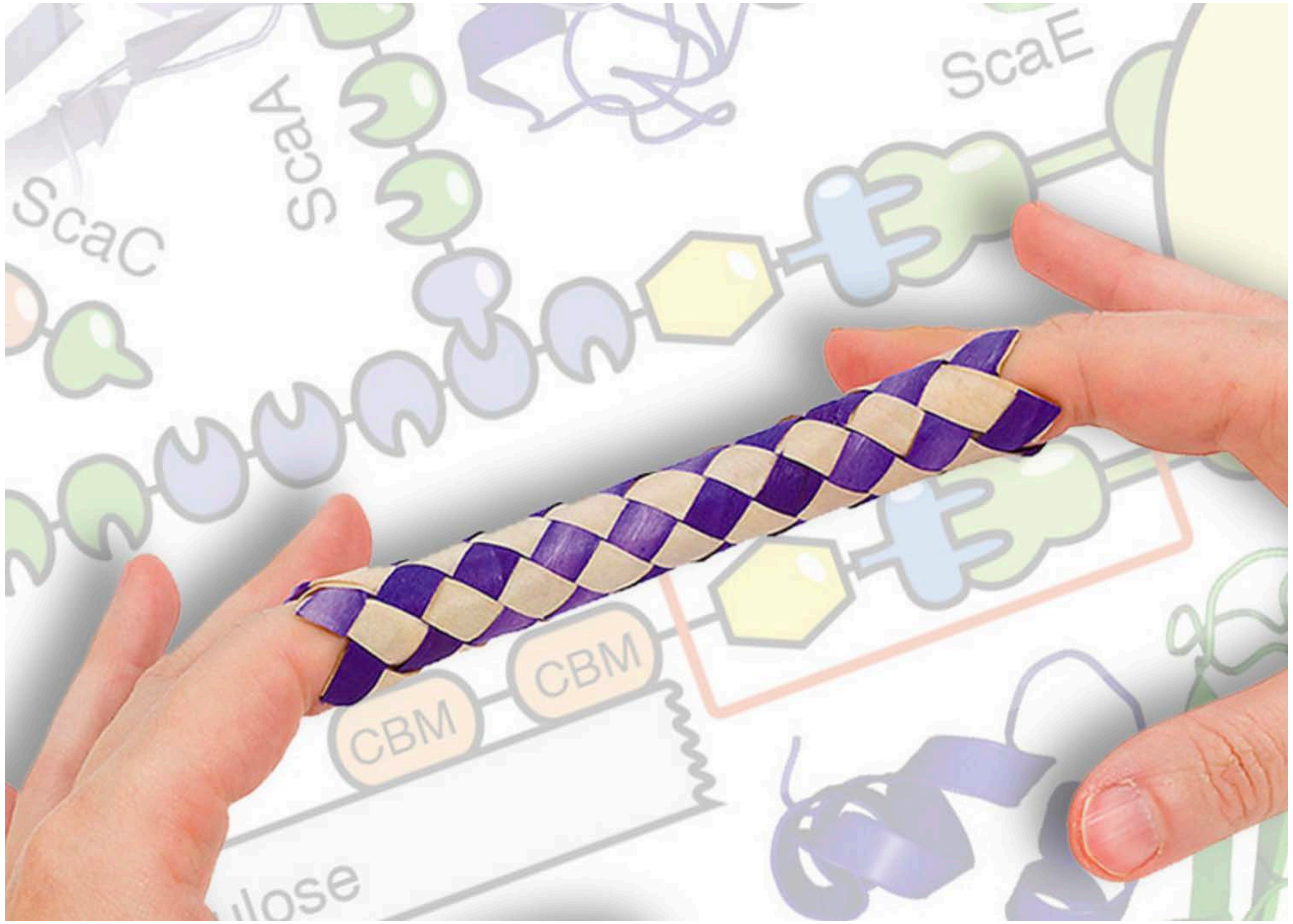
How Mechanically Stable are Cellulosomes?



RC Bernardi, et. al. **Enhanced sampling techniques in molecular dynamics simulations of biological systems.** BBA, 2015


CohE:CttA
PDBid:4IU3
R. flavefaciens

Strongest Non-Covalent Bond Ever Found



Molecular Finger Trap Puzzle

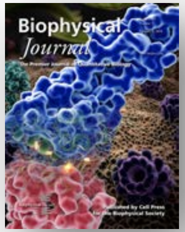
K_D = 20 nM

About the same as a typical antibody–antigen

Rupture Under Force = 600-750 pN

Antibody-antigen rupture at only ~60 pN

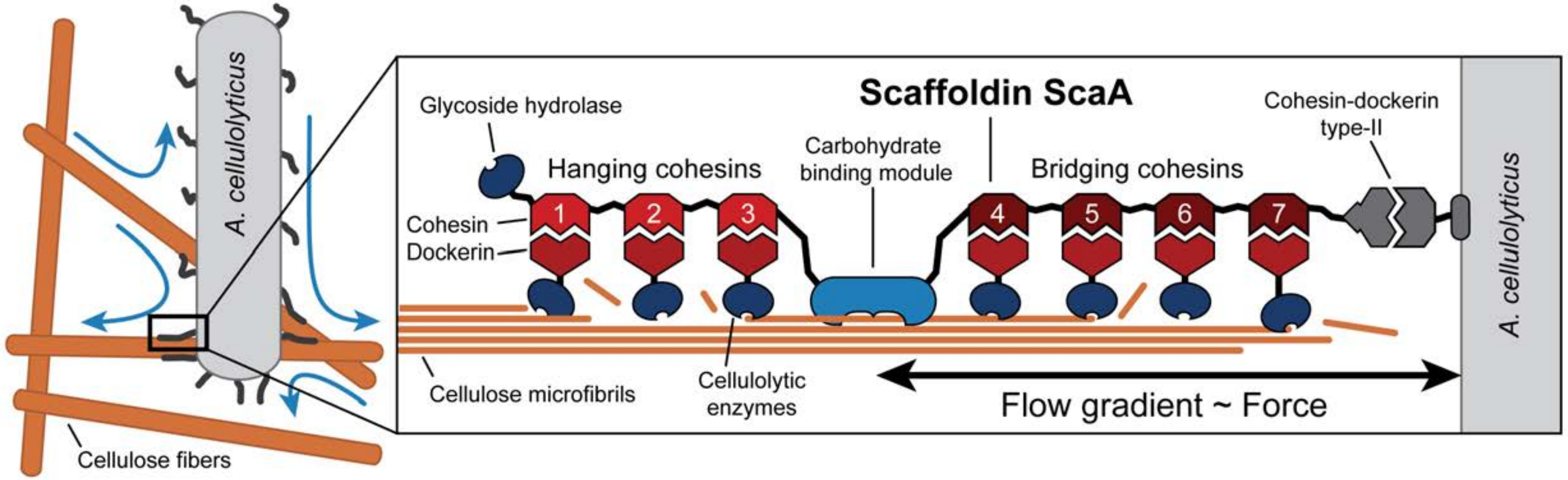
About half the rupture force of a covalent gold-thiol bond



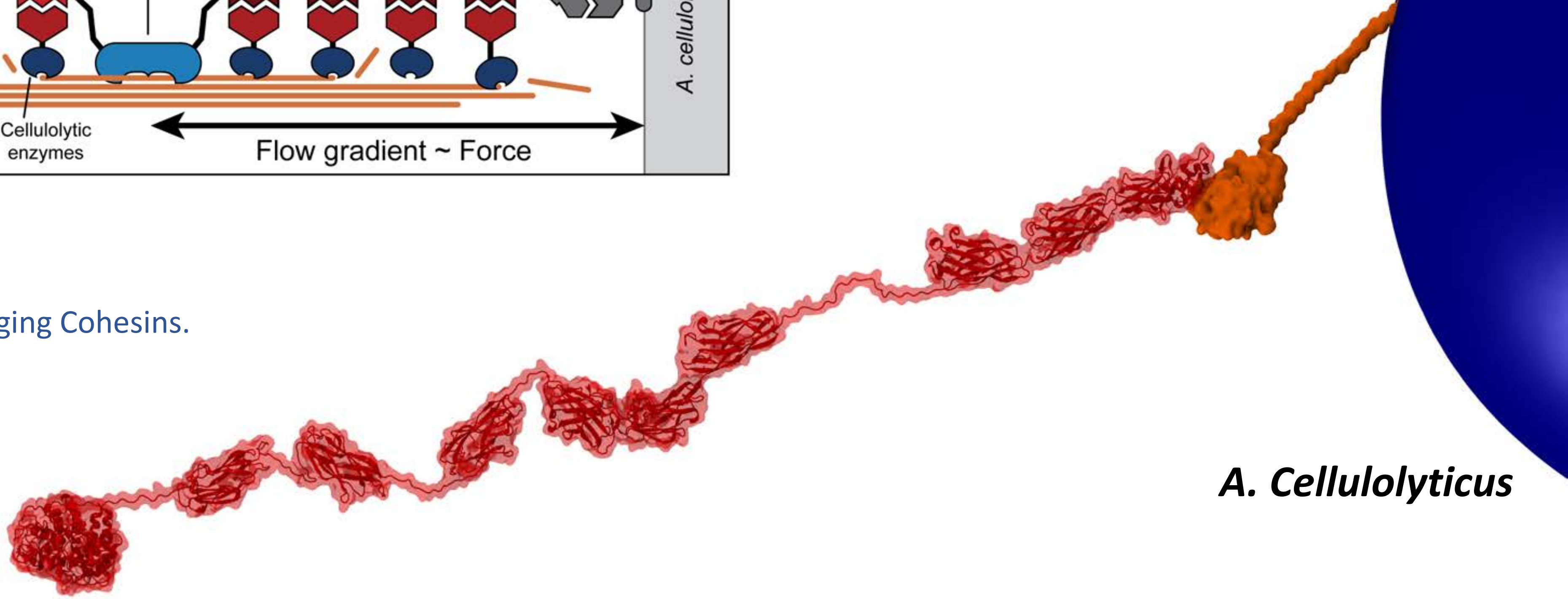
C Schoeler, KH Malinowska, RC Bernardi, et. al. **Ultrastable cellulosome-adhesion complex tightens under load.** Nature Communications, 2014
C Schoeler, RC Bernardi, et. al. **Mapping mechanical force propagation through biomolecular complexes.** Nano Letters, 2015
M Scheurer, P Rodenkirch, M Siggel, RC Bernardi, et. al. **PyContact: Rapid, customizable, and visual analysis of noncovalent interactions in MD simulations.** Biophysical Journal, 2018

Can we use simulations to engineer modified cellulosomal proteins?

Are the cohesins in a scaffold different?

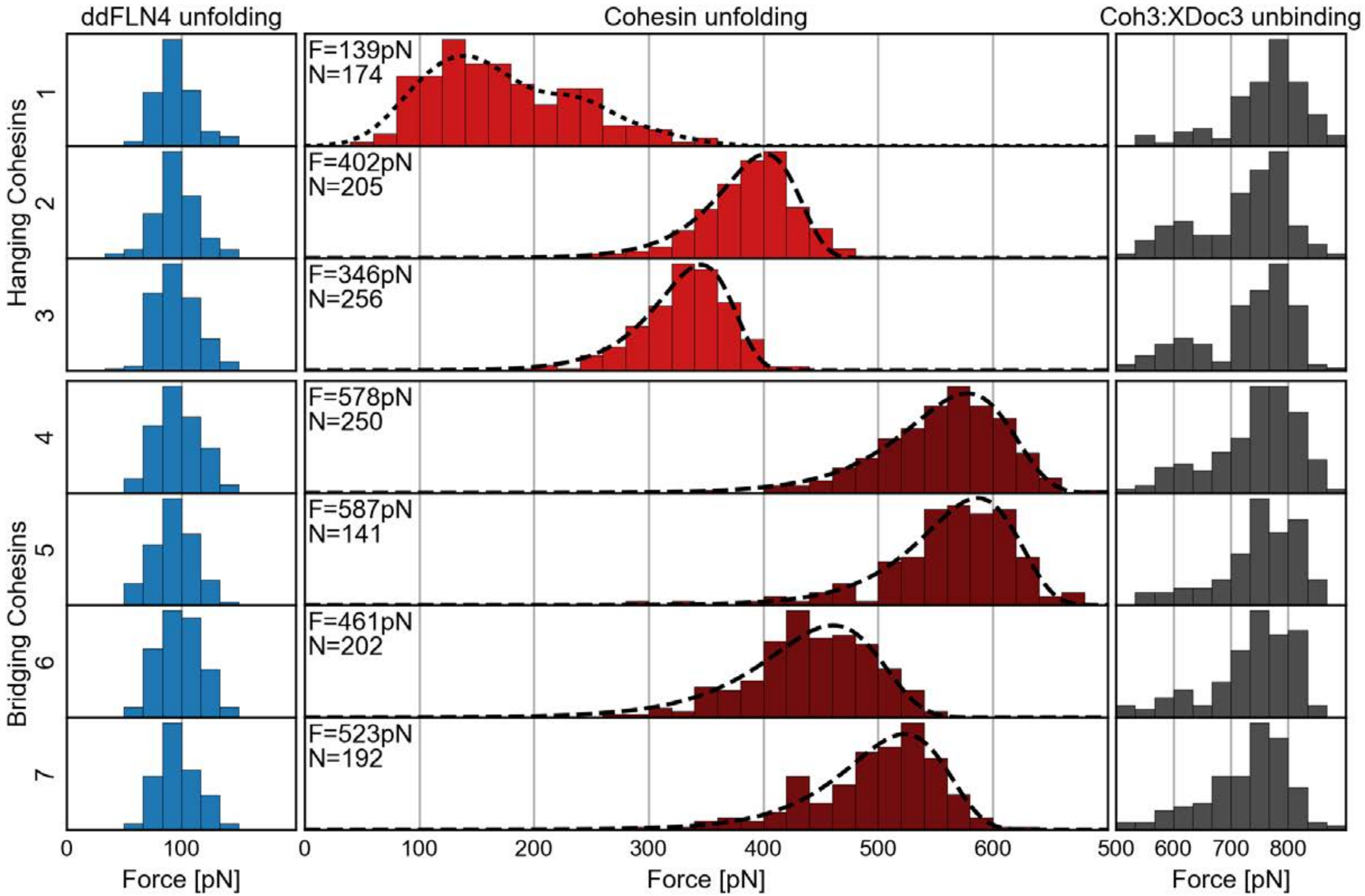


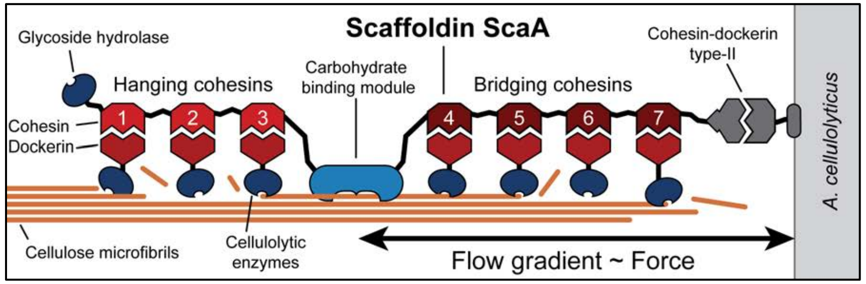
Bridging Cohesins are Stronger than Hanging Cohesins.
Proposed by *Valbuena, et. al. PNAS 2009*



T Verdorfer, RC Bernardi, et. al. **Combining in Vitro and in Silico Single-Molecule Force Spectroscopy to Characterize and Tune Cellulosomal Scaffoldin Mechanics.** JACS, 2017

Unfolding Cohesins: Are them different regarding their force resilience?

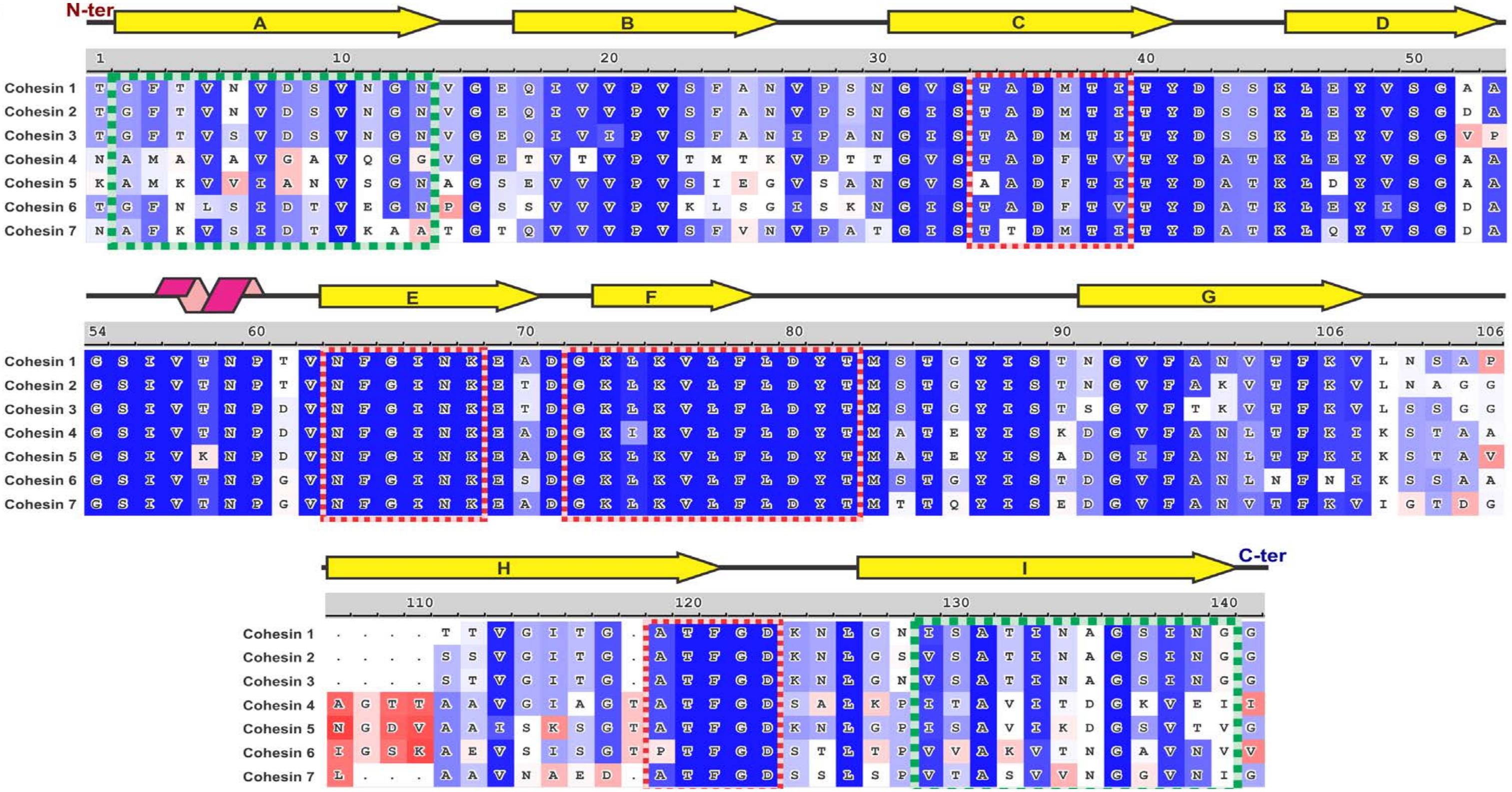




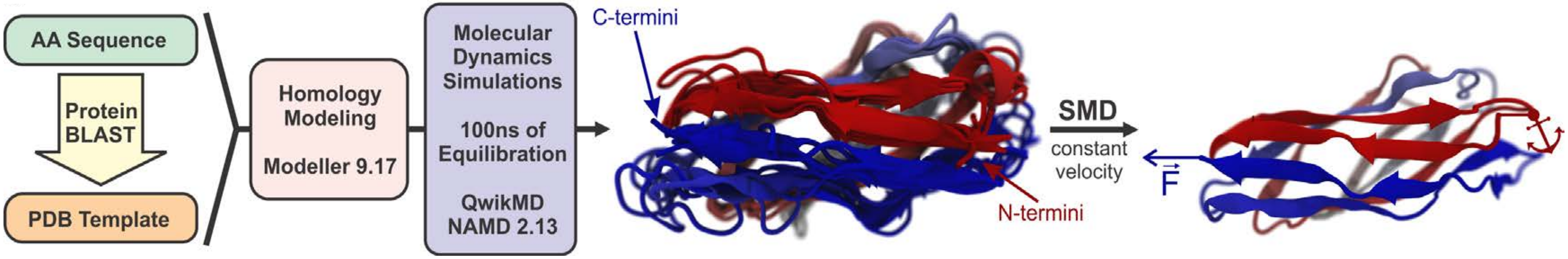
EXPERIMENTAL RESULTS

T Verdorfer, RC Bernardi, et. al. **Combining in Vitro and in Silico Single-Molecule Force Spectroscopy to Characterize and Tune Cellulosomal Scaffoldin Mechanics.** JACS, 2017

Very high Sequence Similarity



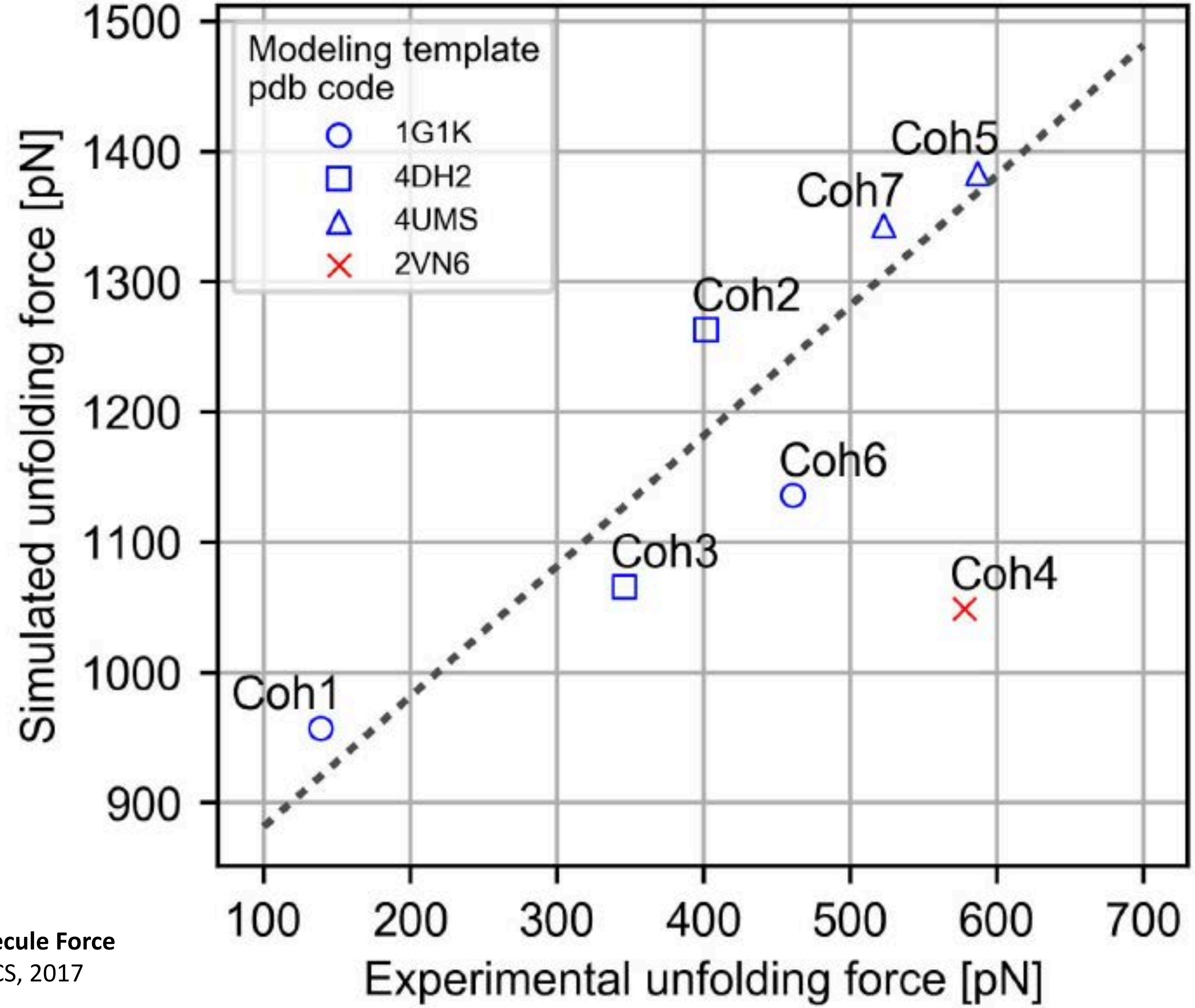
Modeling the Cohesins



T Verdorfer, RC Bernardi, et. al. **Combining in Vitro and in Silico Single-Molecule Force Spectroscopy to Characterize and Tune Cellulosomal Scaffoldin Mechanics.** JACS, 2017

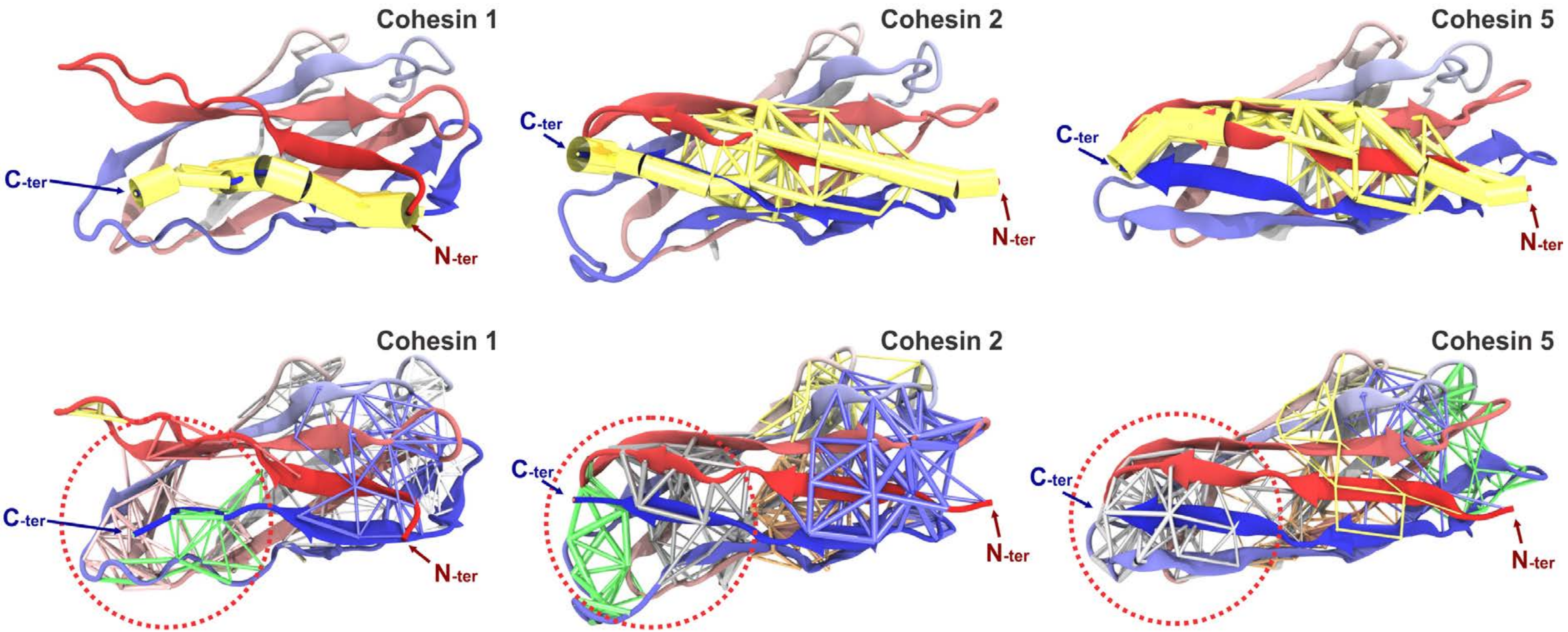
Simulations vs Experiments

Simulations and Experiments agree extremely well, except for Cohesin 4.



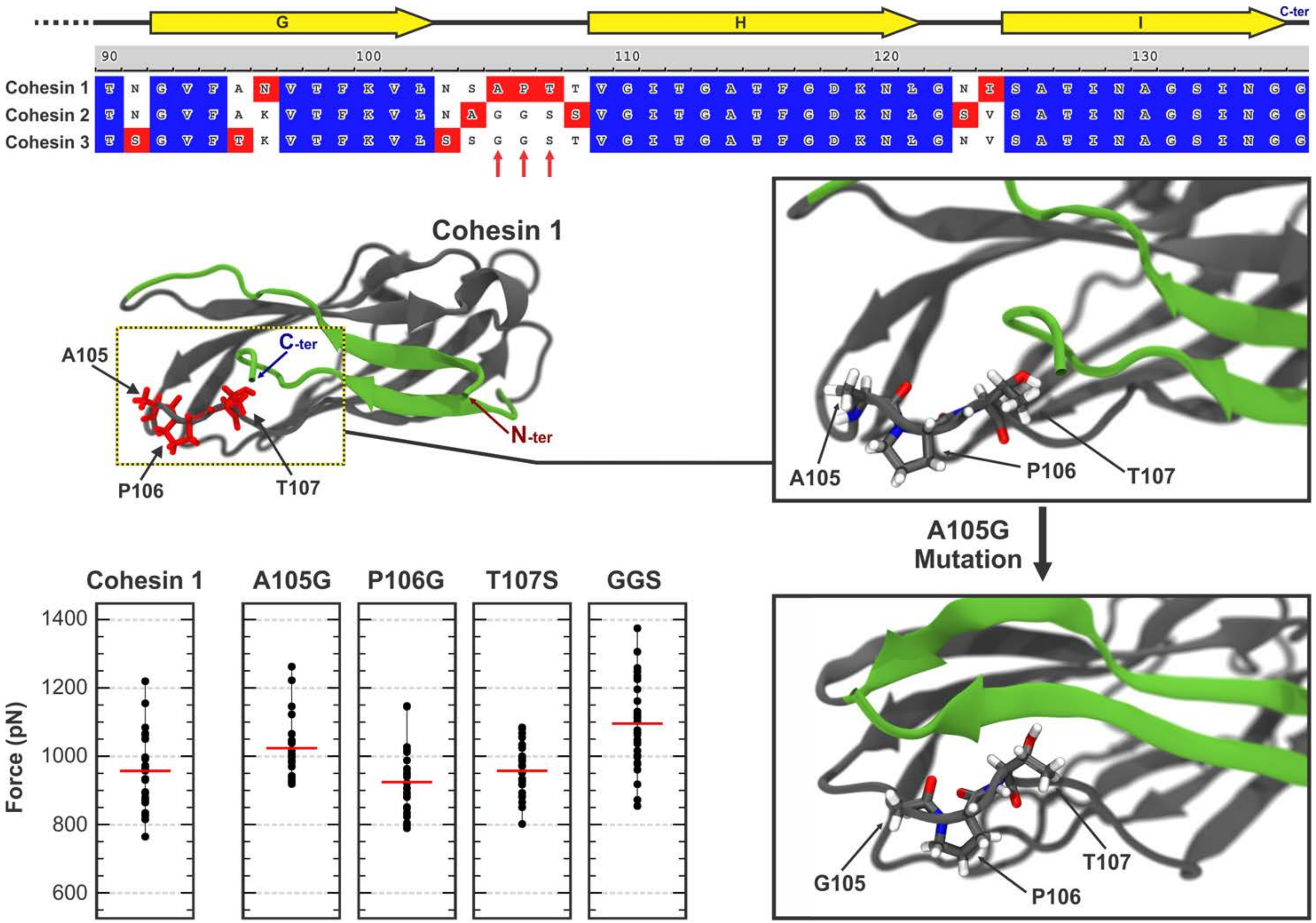
T Verdorfer, RC Bernardi, et. al. **Combining in Vitro and in Silico Single-Molecule Force Spectroscopy to Characterize and Tune Cellulosomal Scaffoldin Mechanics.** JACS, 2017

Why are Cohesins Different in Force Resilience?



T Verdorfer, RC Bernardi, et. al. **Combining in Vitro and in Silico Single-Molecule Force Spectroscopy to Characterize and Tune Cellulosomal Scaffoldin Mechanics.** JACS, 2017

Engineering new Cohesins



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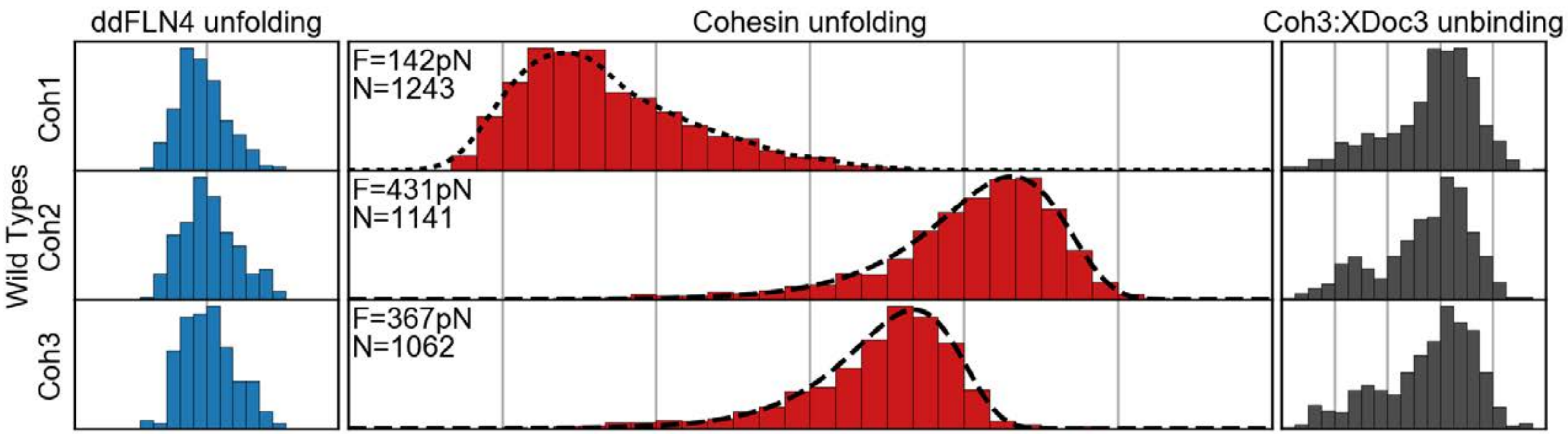
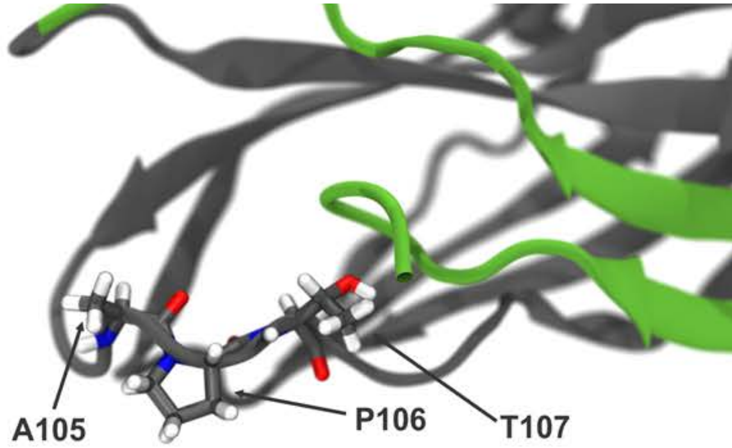
Engineering new Cohesins





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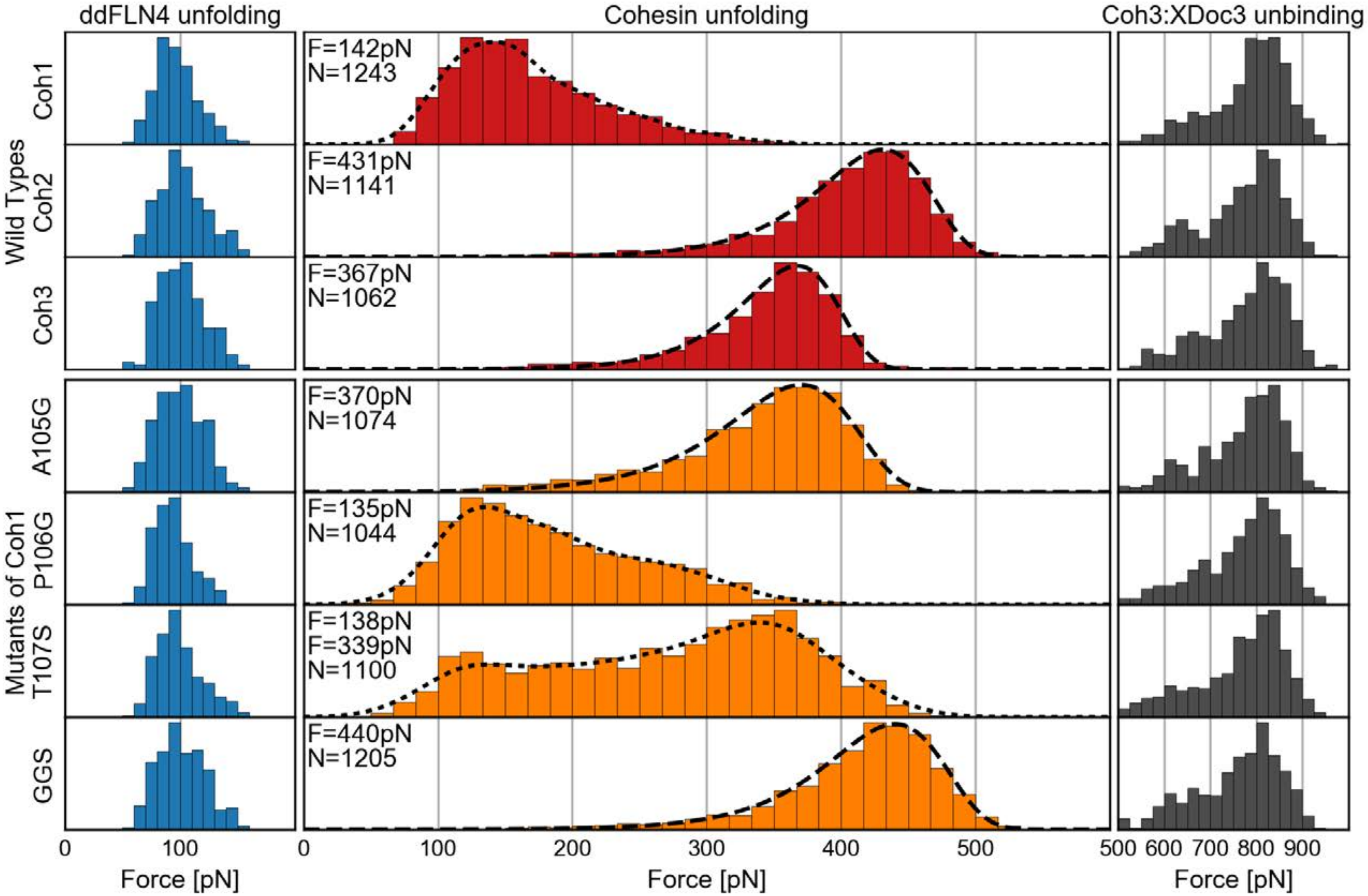
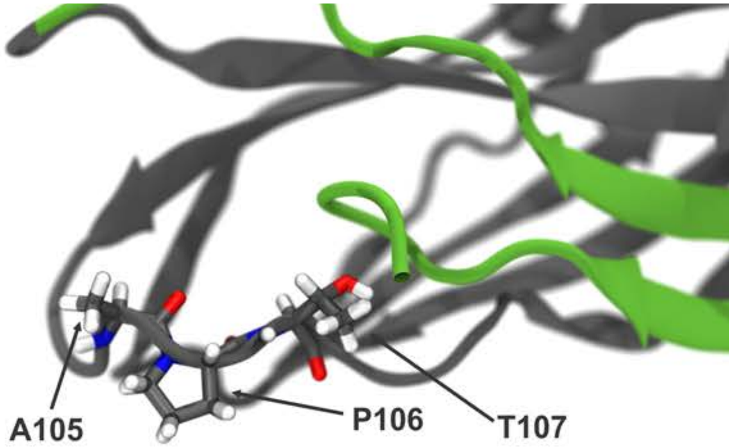
Engineering new Cohesins





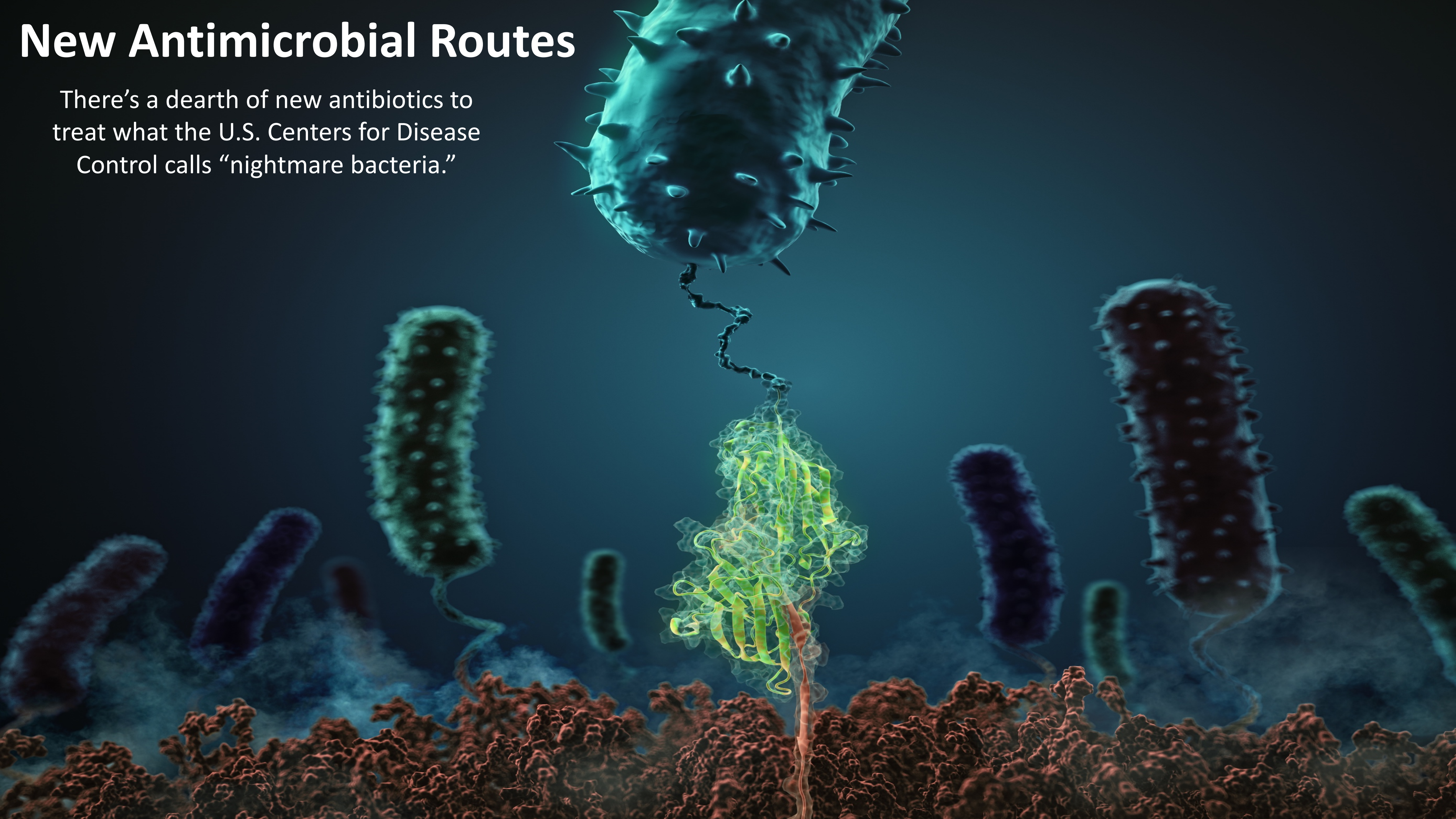
T Verdorfer, RC Bernardi, et. al. **Combining in Vitro and in Silico Single-Molecule Force Spectroscopy to Characterize and Tune Cellulosomal Scaffoldin Mechanics.** JACS, 2017

?
...FKVLNSAPTTVGI...
...FKVLNSGPTTVGI...
...FKVLNSSPTTVGI...
...FKVLNSPPTTVGI...
...FKVLNSRPTTVGI...
SMD
H
...FKVLNS NH CH C O PTTVGI...
CH3
...FKVLNS NH CH C O PTTVGI...

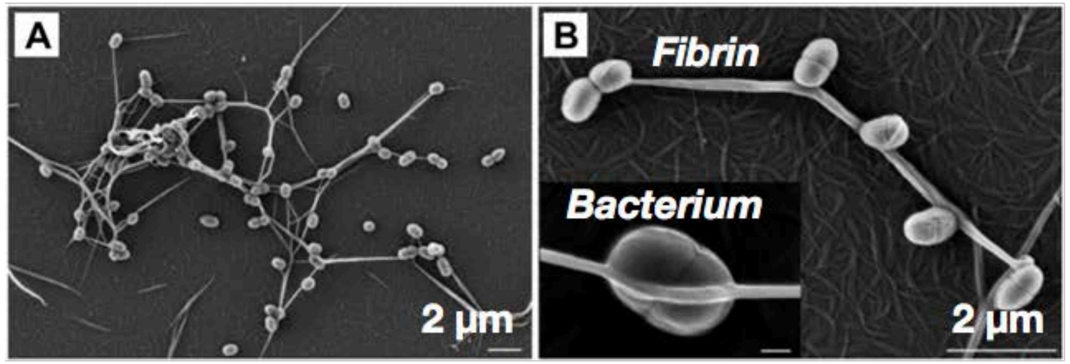
Are there other Bacterial proteins taking advantage of mechanically strong interactions?

New Antimicrobial Routes

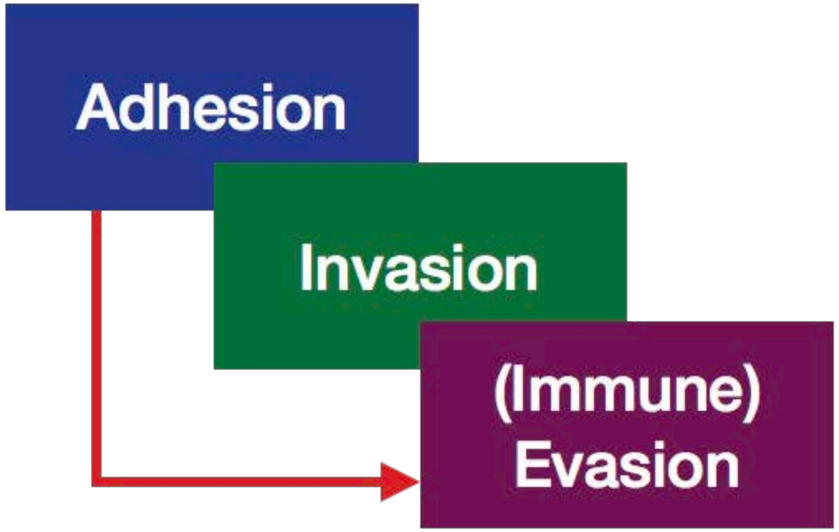
There's a dearth of new antibiotics to treat what the U.S. Centers for Disease Control calls "nightmare bacteria."

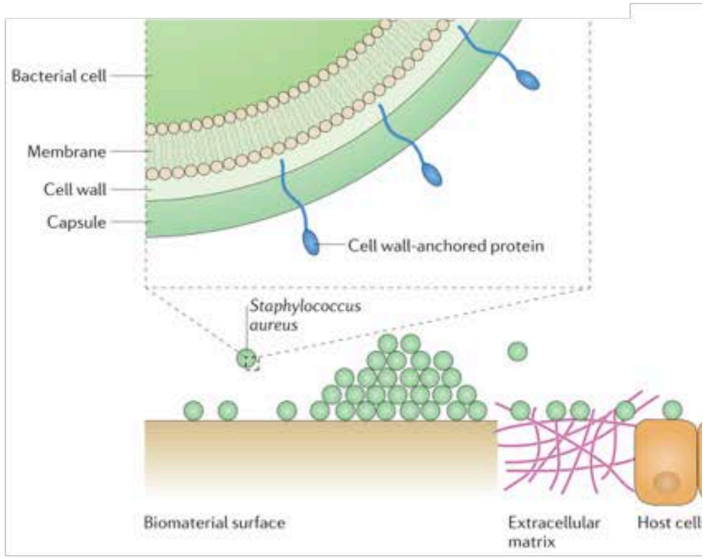


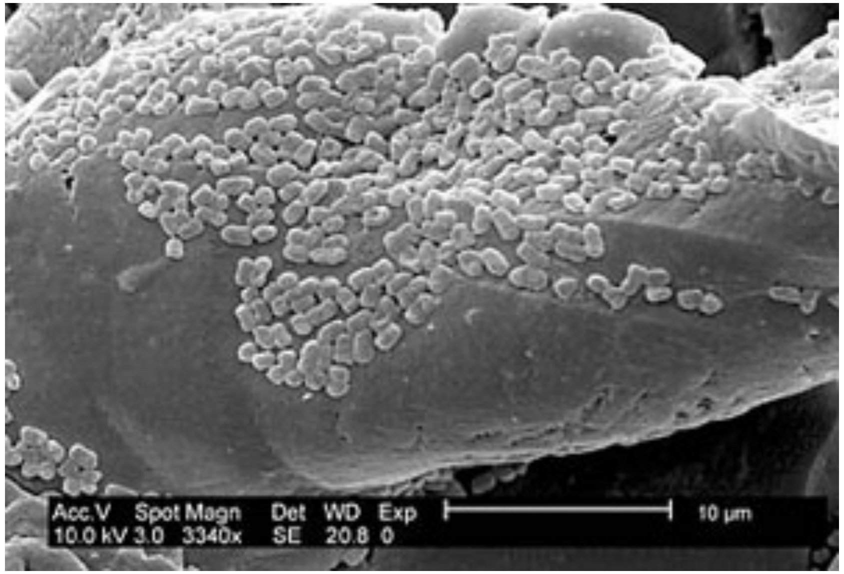
Adhesion by Pathogenic Bacteria



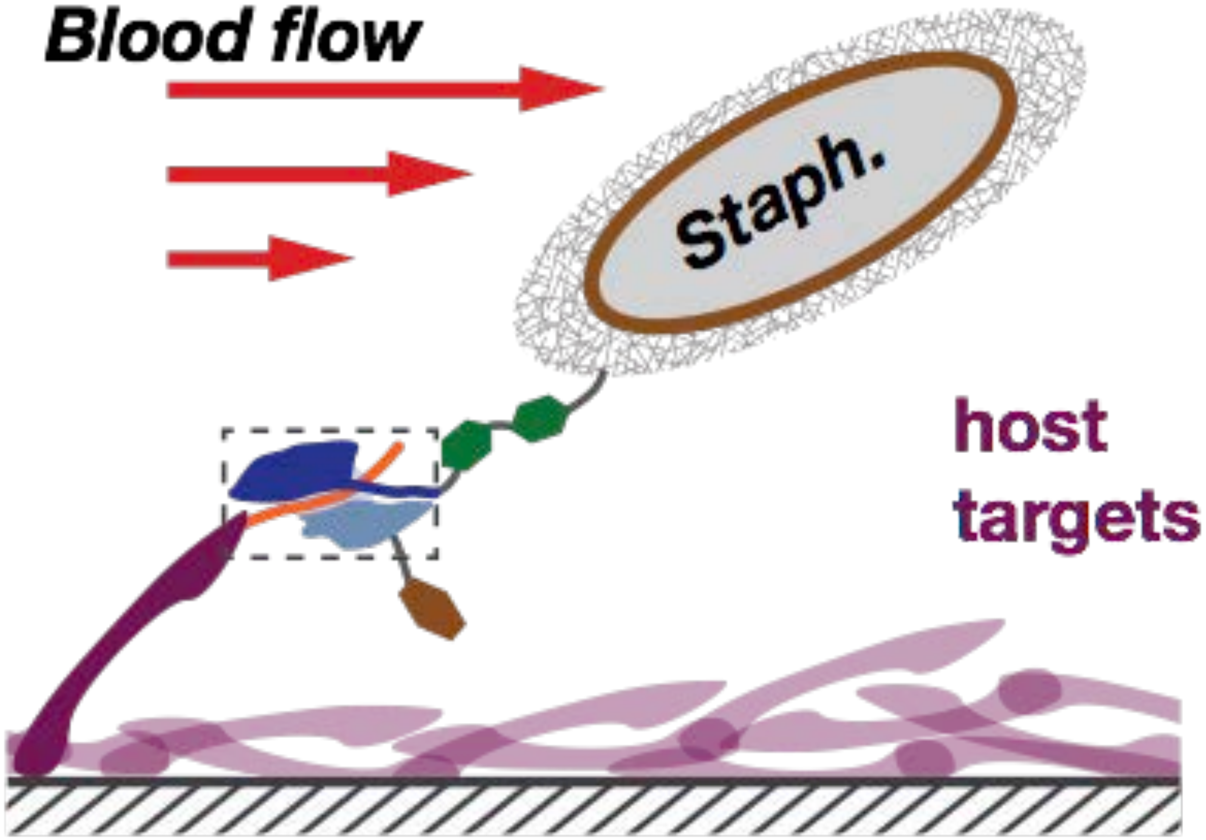
L. Lactis expressing a thioester adhesin (Sfbl-A40), a covalent **"chemical harpoon"**







Adhesion Mechanism - *Staph Infections*

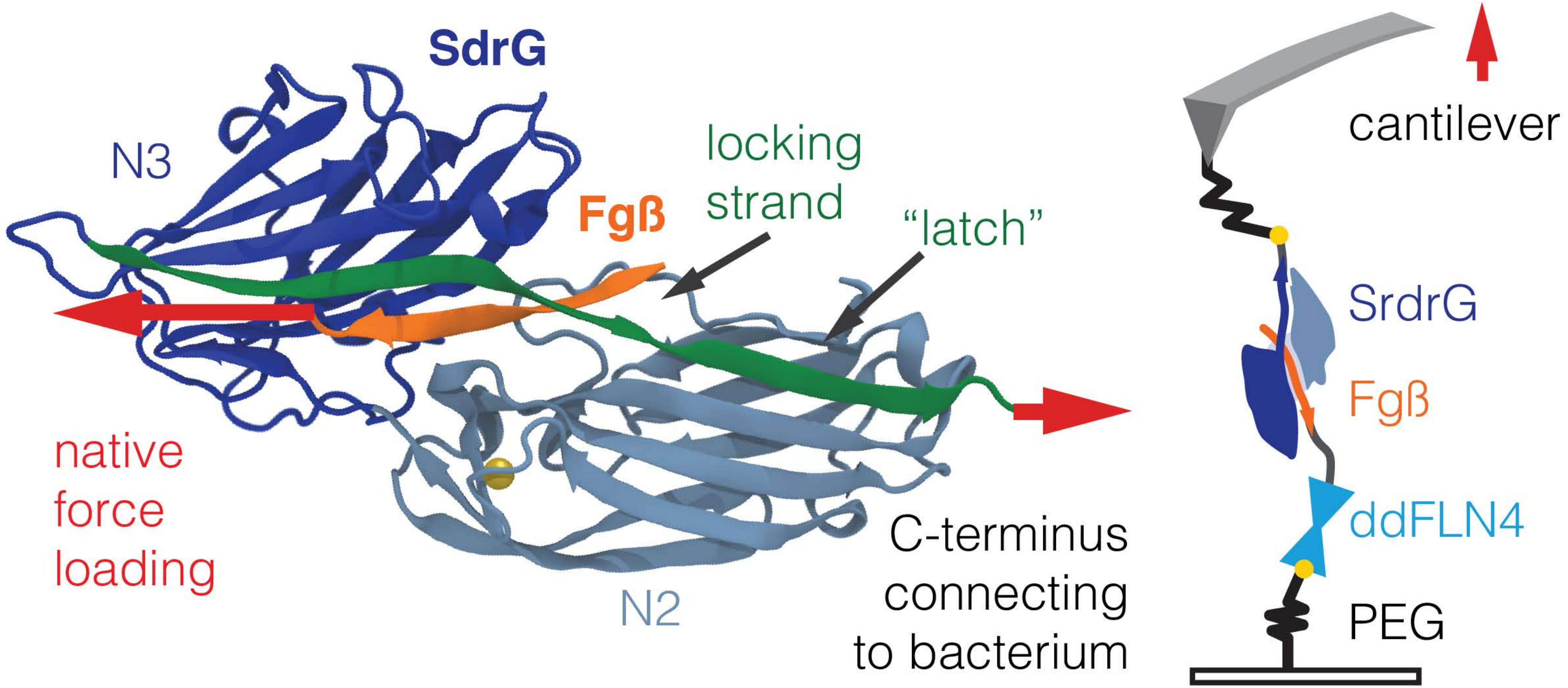


MSCRAMMs

Microbial Surface Components Recognizing Adhesive Matrix Molecules

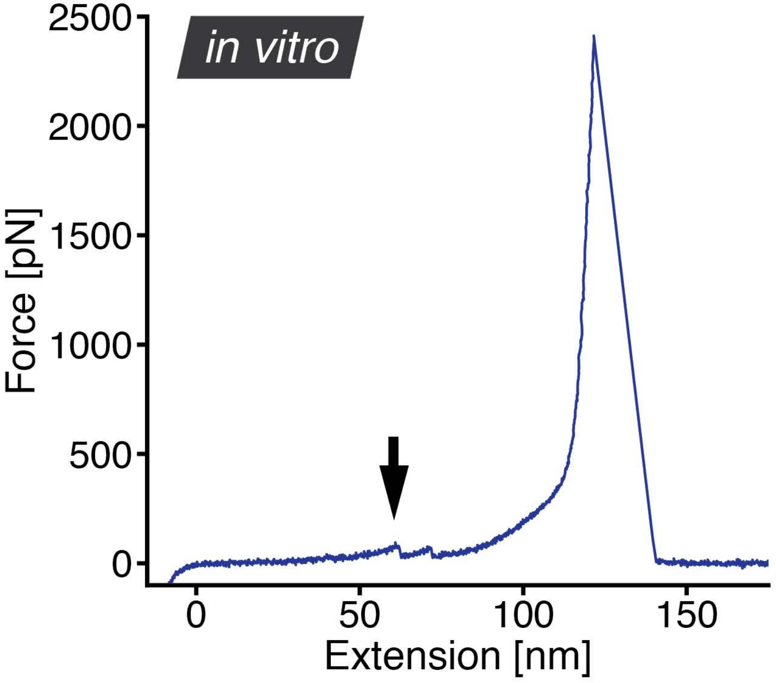
Targets include Fibrinogen (Fg, all chains), Fibronectin (Fn), Keratin, Collagen, Elastin, *Complement* Factor H

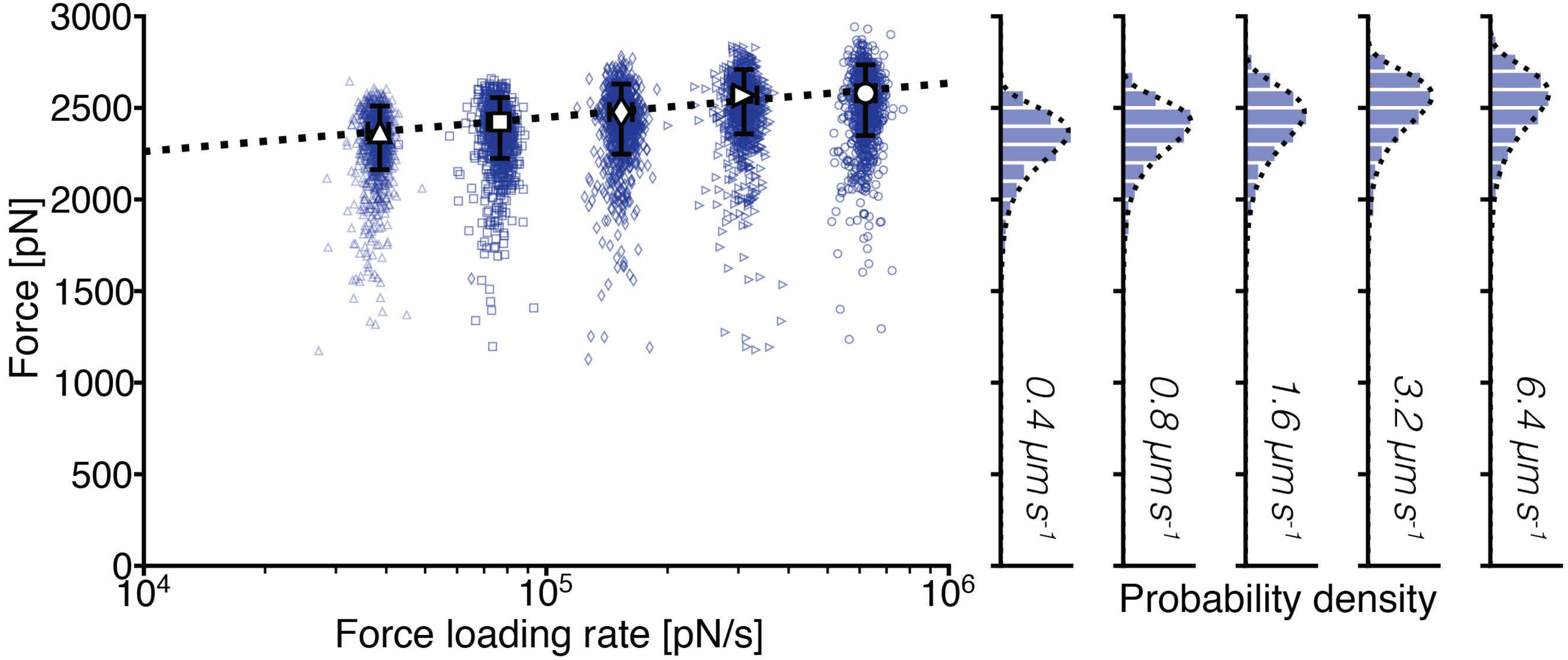
Experimental Setup *in silico* and *in vitro*



LF Milles, K Schulten, HE Gaub, RC Bernardi. **Molecular mechanism of extreme mechanostability in a pathogen adhesin.** Science, 2018

The Hyperstable SdrG:Fgβ interaction

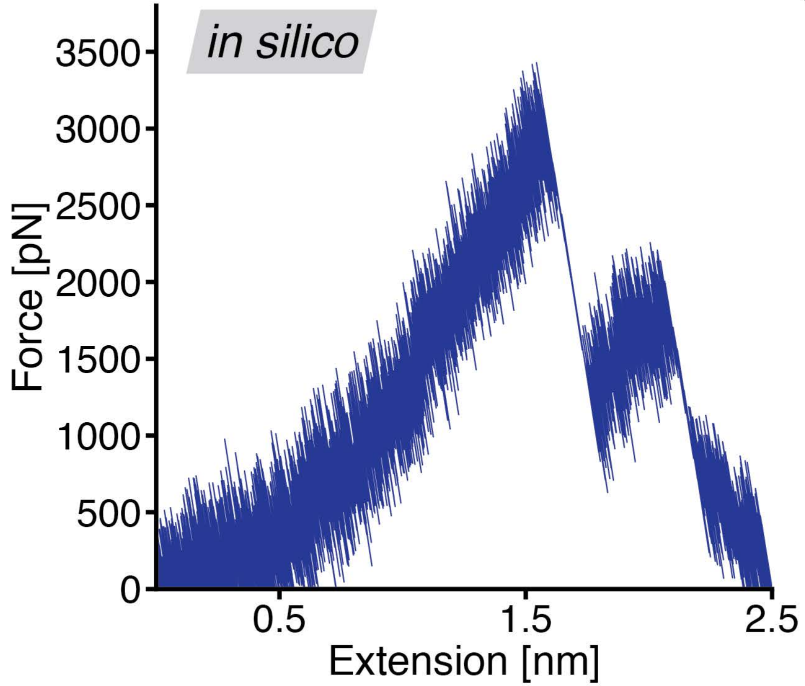


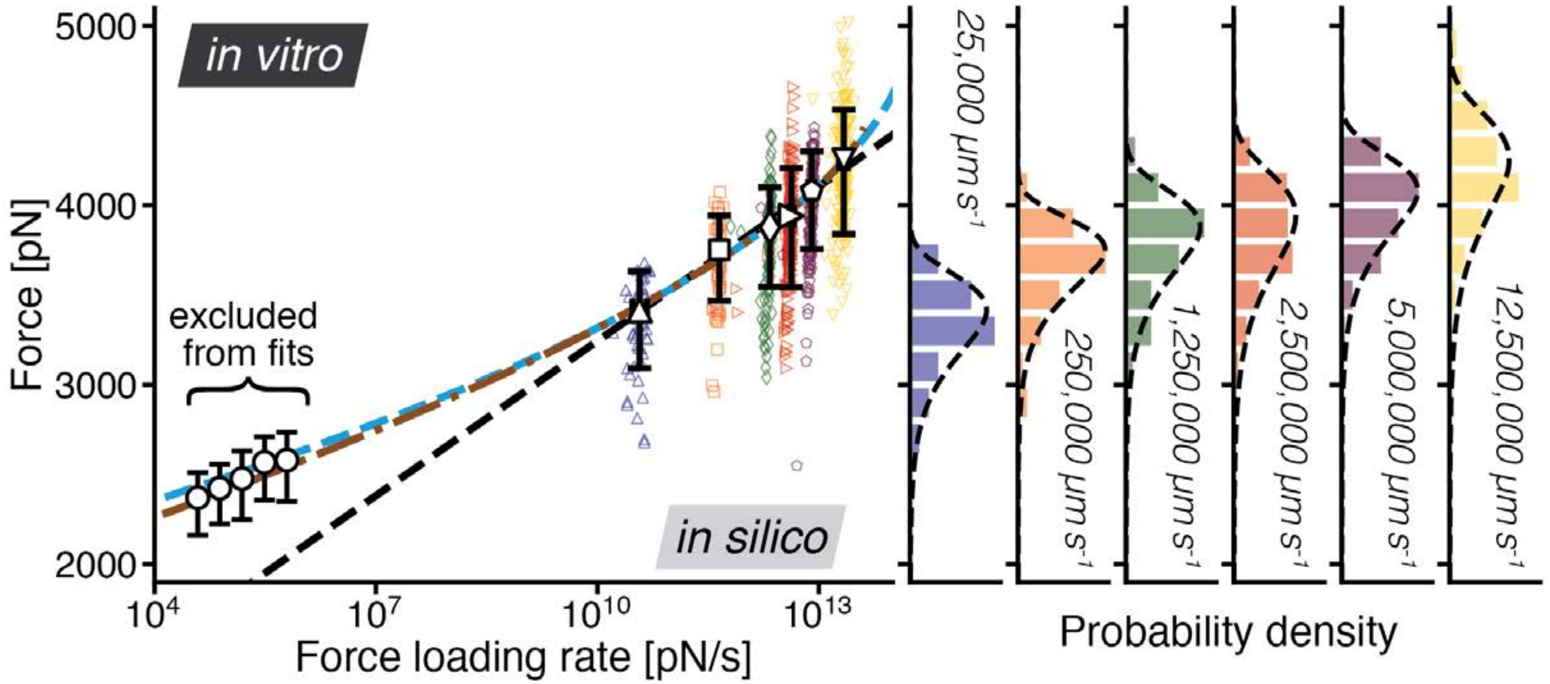


LF Milles, K Schulten, HE Gaub, RC Bernardi. **Molecular mechanism of extreme mechanostability in a pathogen adhesin.** Science, 2018

Bringing Molecular Dynamics to the same Statistical Standards of Single Molecule Force Spectroscopy

Over 2400 Steered Molecular Dynamics Simulations

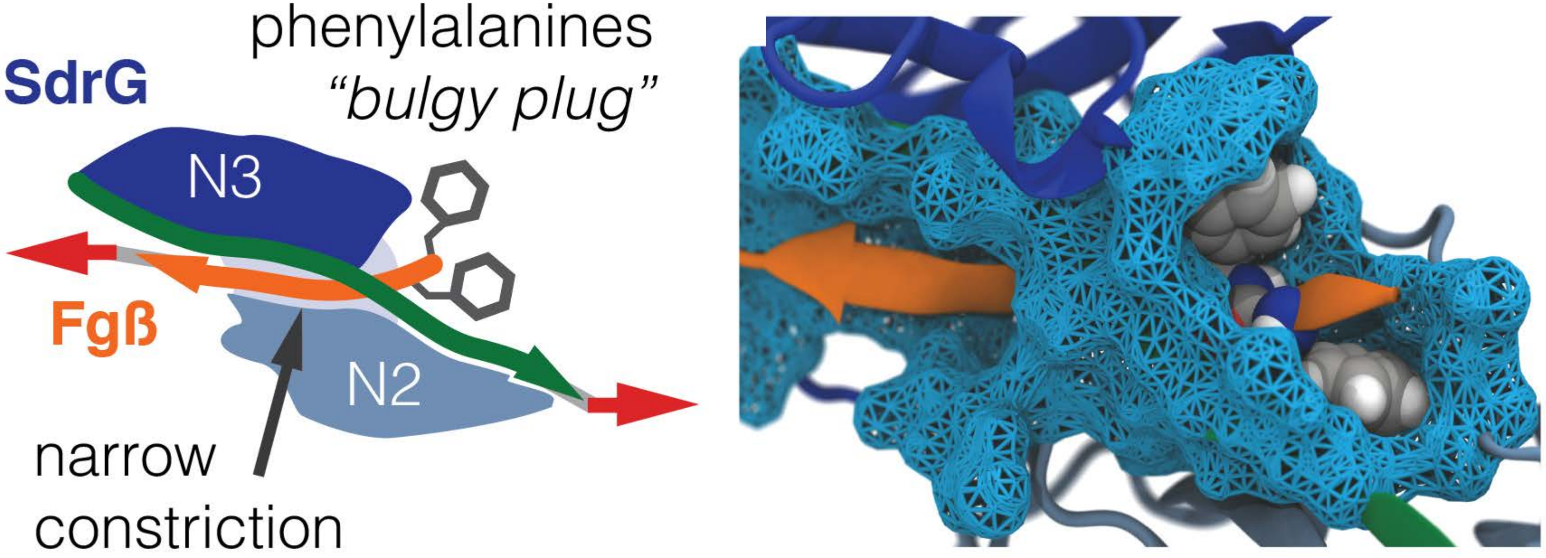




LF Milles, K Schulten, HE Gaub, RC Bernardi. **Molecular mechanism of extreme mechanostability in a pathogen adhesin.** Science, 2018

The Mechanism of the Hyperstable SdrG:Fgβ interaction

The Hyperstable SdrG:Fgβ interaction



complex strength:

		in vitro		*in silico*	
Fgß WT	G–FFSARG...	**100%**	2204 pN	**100%**	3362 pN
FgßF3	GFFFSARG...	101%	2236 pN	104%	3486 pN
FgßF1	G–AFSARG...	97%	2148 pN	92%	3082 pN
FgßF0	G–AASARG...	91%	1999 pN	82%	2758 pN

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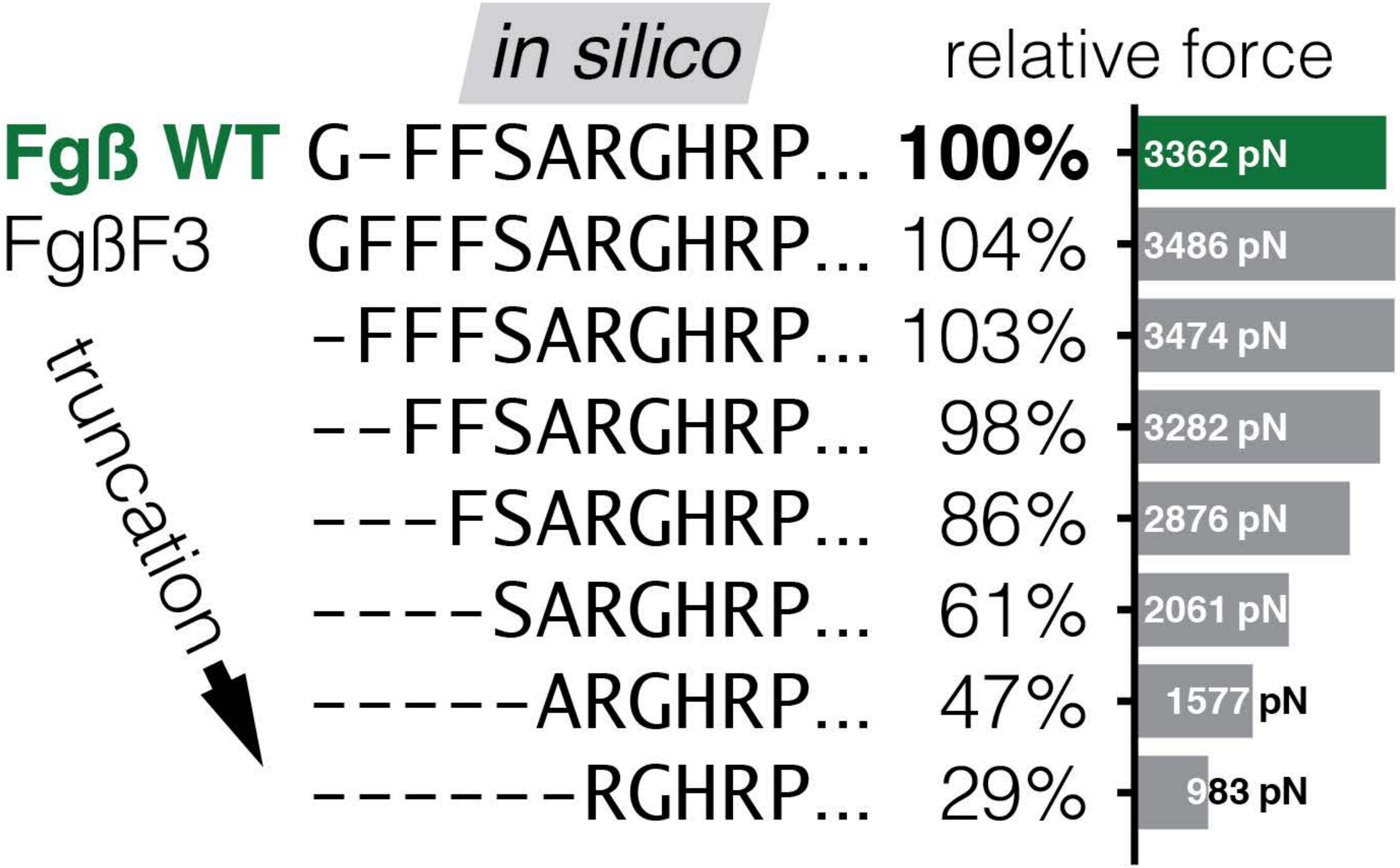
The Hyperstable SdrG:Fgβ interaction



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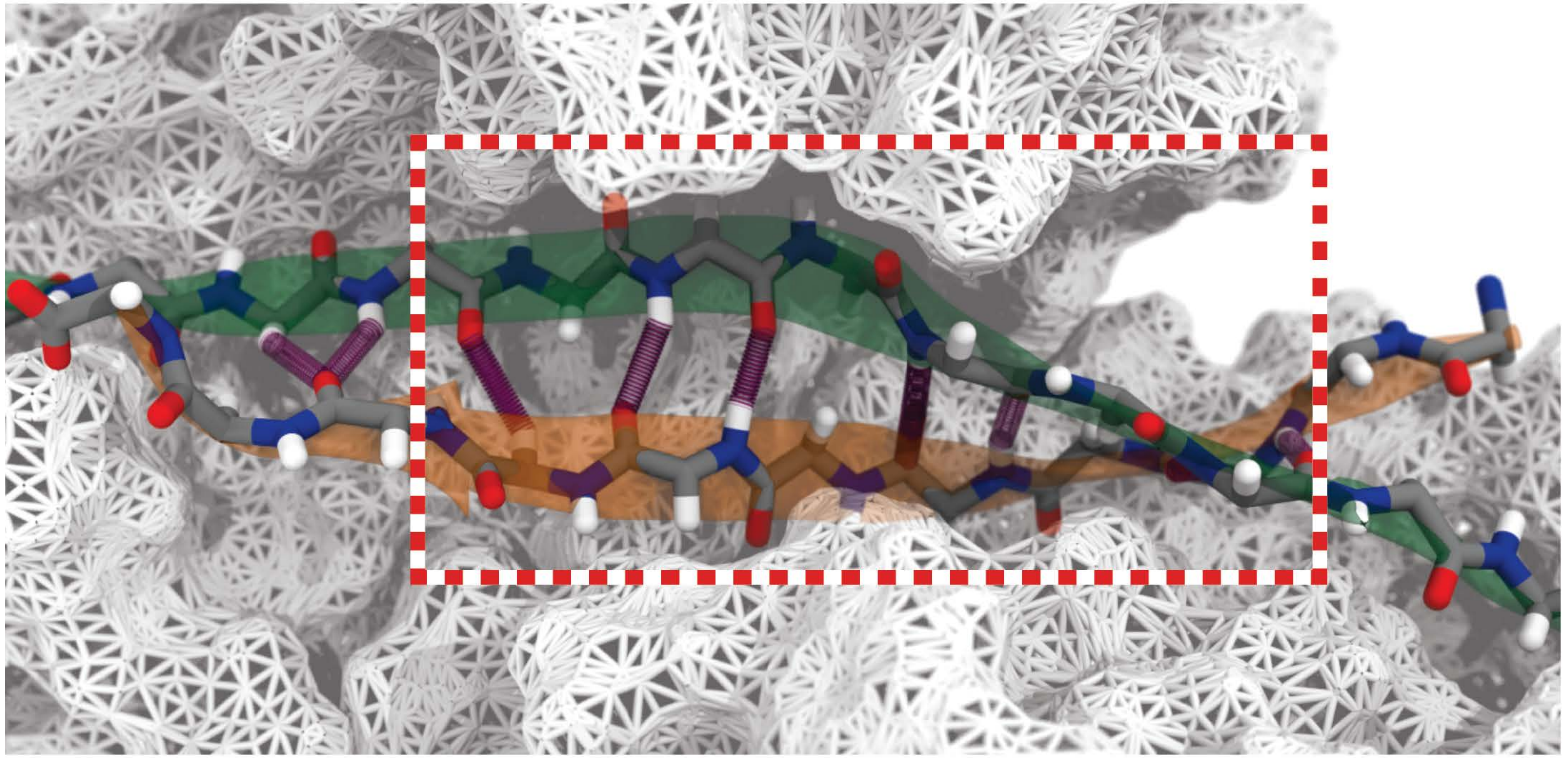

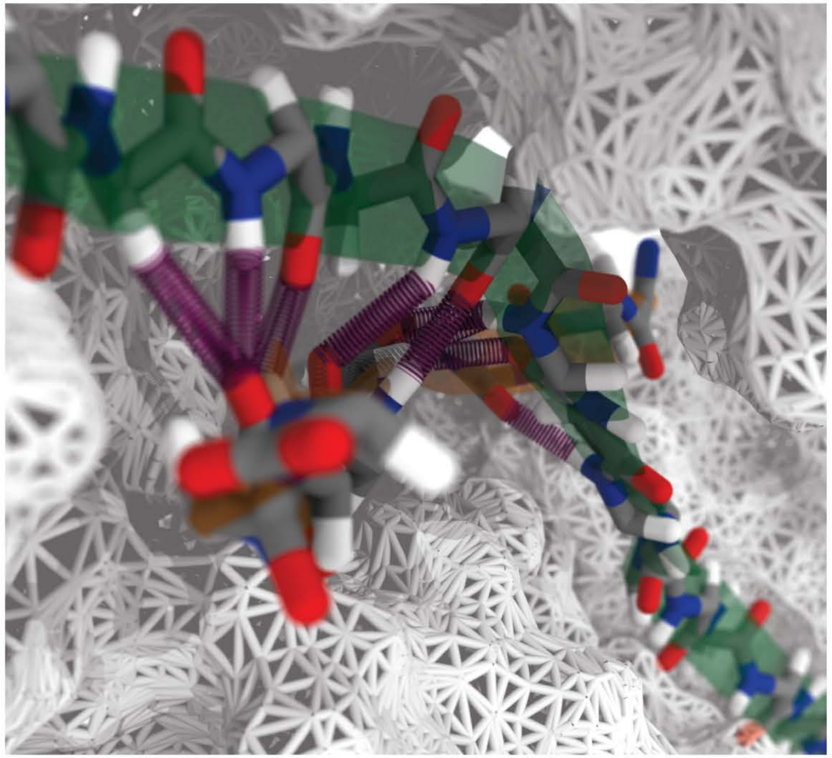

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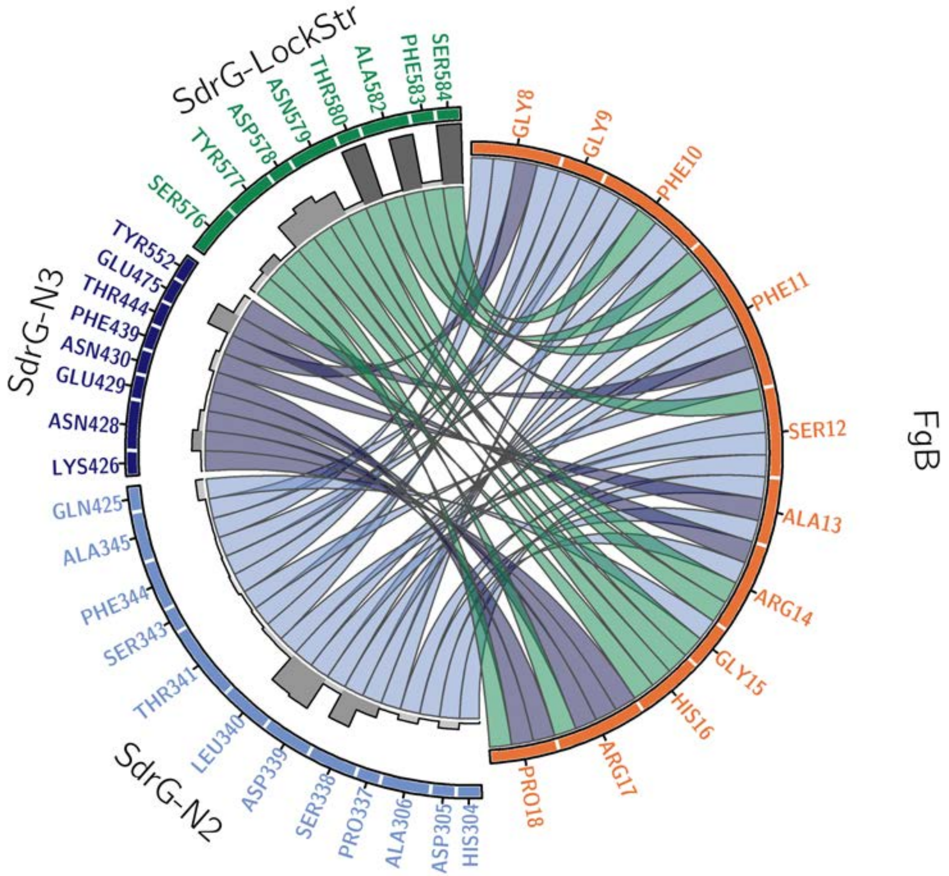
Sequence Independent?



Mapping Hydrogen Bond Prevalence

all-GLY	no-F	F	FF	FFF		FFF	FF	F	no-F	all-GLY
100%	68%	56%	61%	51%	8	0%	0%	0%	0%	0%
100%	66%	44%	36%	13%	9	45%	0%	0%	0%	0%
100%	100%	100%	100%	100%	10	100%	100%	0%	0%	0%
100%	100%	100%	100%	100%	11	97%	98%	100%	0%	0%
100%	100%	100%	100%	100%	12	100%	100%	100%	100%	0%
73%	27%	31%	29%	27%	13	26%	29%	32%	38%	0%
100%	100%	100%	100%	100%	14	71%	79%	83%	91%	0%
60%	39%	44%	45%	40%	15	0%	0%	0%	0%	0%
100%	100%	100%	100%	95%	16	33%	32%	29%	29%	0%
42%	3%	4%	6%	6%	17	58%	60%	63%	62%	0%
90%	3%	4%	4%	3%	18	59%	62%	58%	53%	0%

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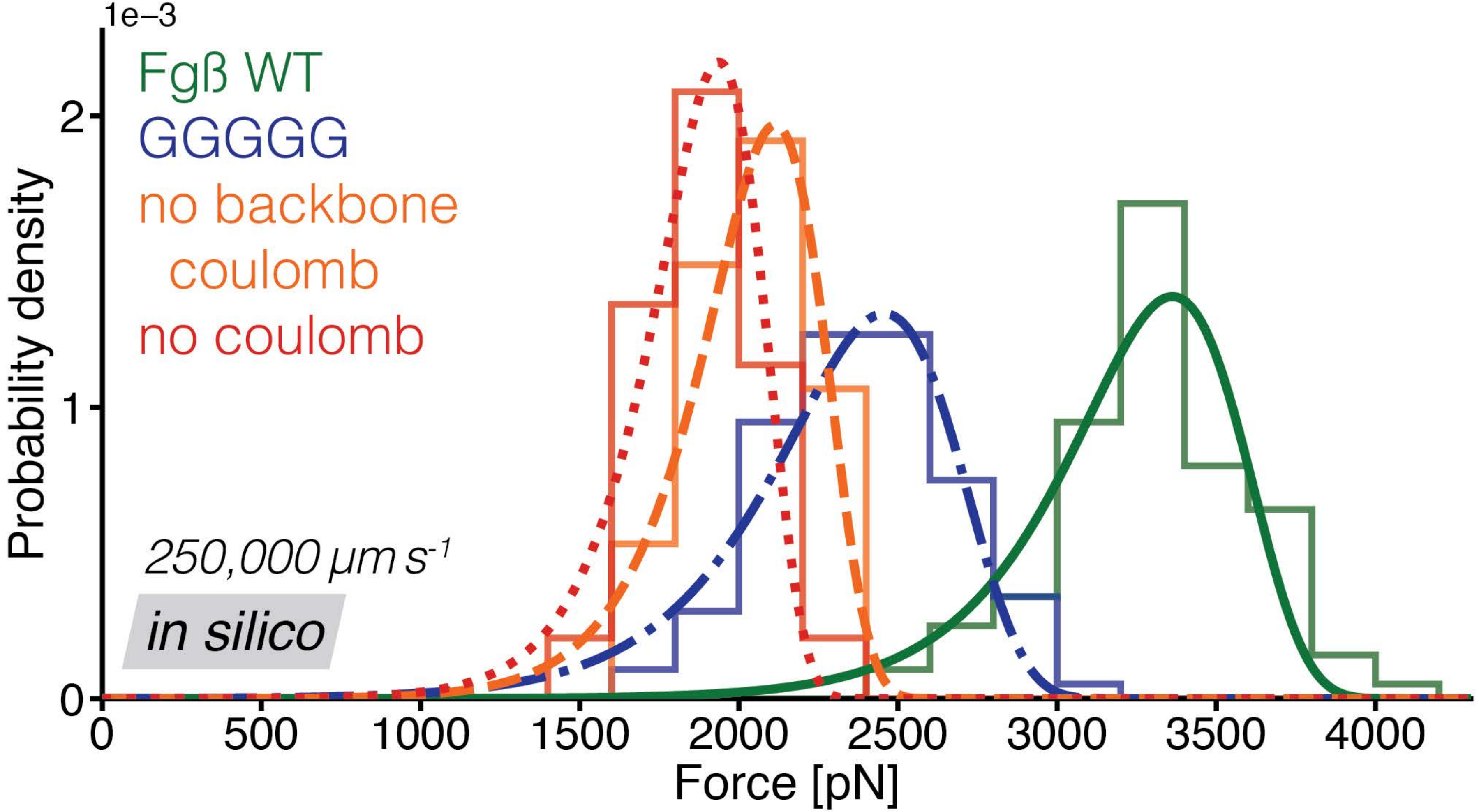
Sequence Independent?



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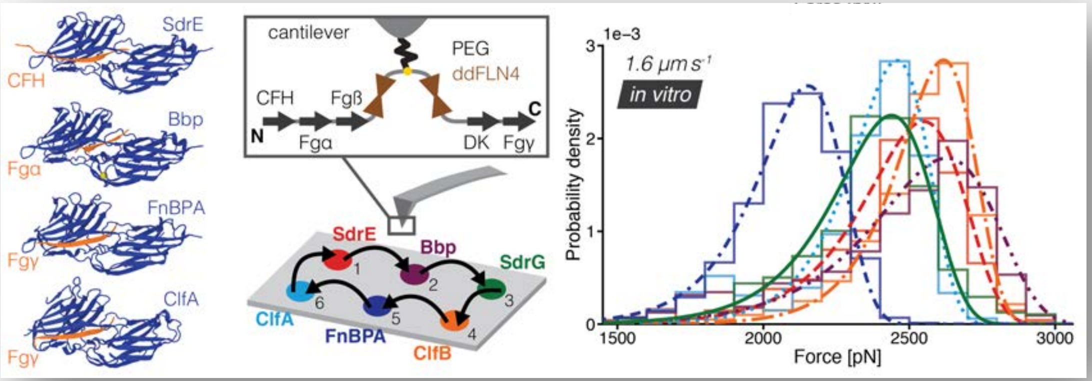
Sequence Independence

A Huge Evolutionary Advantage



LF Milles, K Schulten, HE Gaub, RC Bernardi. **Molecular mechanism of extreme mechanostability in a pathogen adhesin.** Science, 2018

Thank you all for your attention

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Dr. João Ribeiro
(QwikMD)
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John Stone
(VMD QM/MM, QwikMD)

Institute for Genomic Biology

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Ludwig Maximilians University of Munich, Germany

Prof. Hermann Gaub (AFM-based SMFS)

Dr. Constantin Schoeler (Cohesin:Dockerin)
Dr. Klara Malinowska (Cohesin:Dockerin)
Tobias Verdorfer (ScaA Cohesins)
Lukas Milles (Bacteria Adhesion)
Ellis Durner (ScaB Cohesin:Dockerin)

University of Basel & ETH Zurich, Switzerland

Prof. Michael Nash (Cellulosome Mechanics)

Weizmann Institute, Israel

Prof. Ed Bayer (Cellulosome Mechanics/Structure)

University of Heidelberg, Germany

Maximilian Scheurer (QM/MM)

Max Planck Institute – Mülheim, Germany

Prof. Frank Neese (QM/MM)
\

Federal University of Paraíba, Brazil

Prof. Gerd Rocha (QM/MM)

Computational Resources:

Funding and Support:

ENERGY BIOSCIENCES INSTITUTE
NATIONAL INSTITUTES OF HEALTH
NSF
U.S. DEPARTMENT OF ENERGY