Computational Biophysics Approaches to Mechanosensing

Rafael C. Bernardi

Department of Physics at Auburn University
NIH Center for Macromolecular Modeling and Bioinformatics
compbiophysics.auburn.edu
rcbernardi@auburn.edu
@rafaelcbernardi

MCB-2143787
CAREER: In Silico Single-Molecule Force Spectroscopy

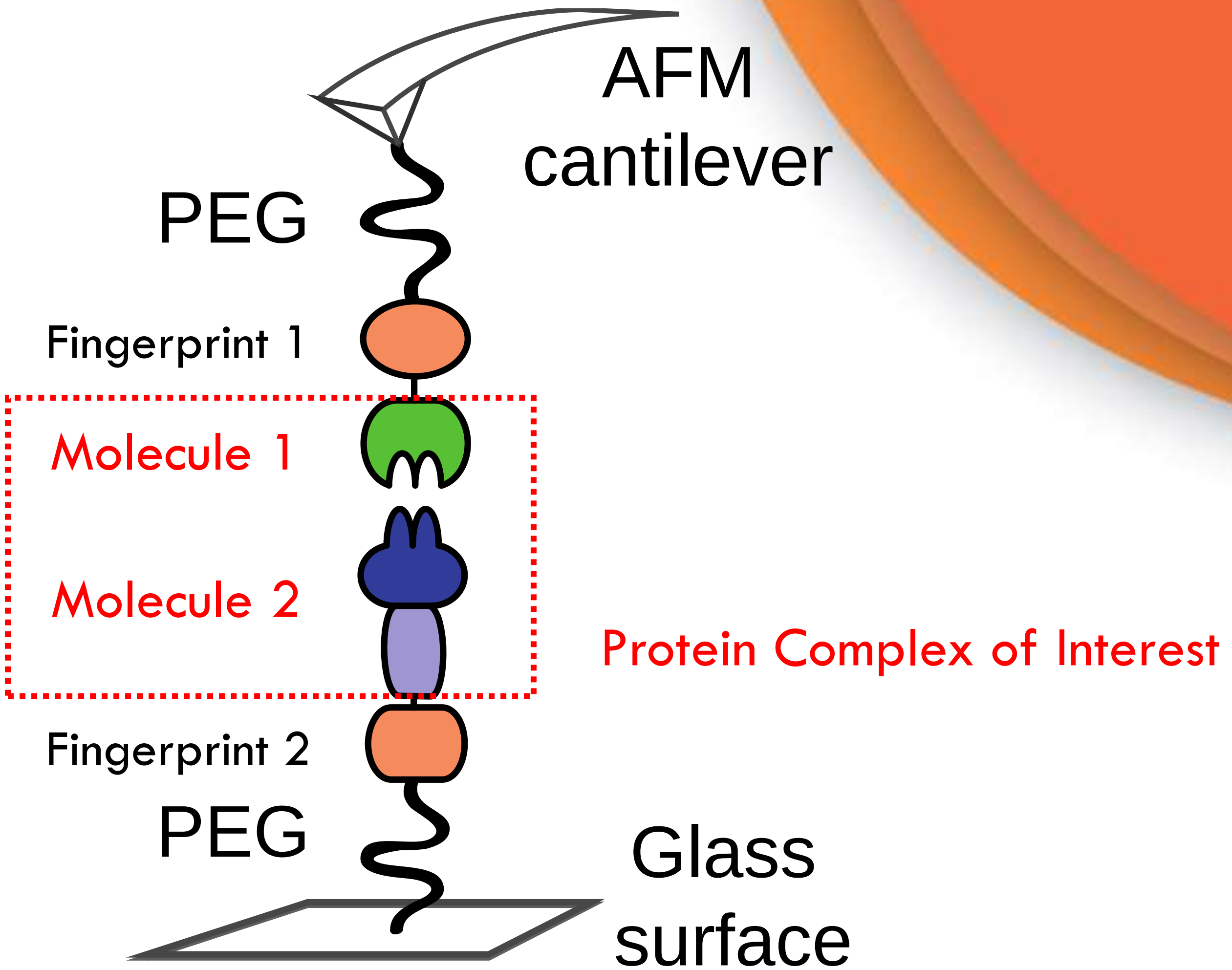
R24 GM-145965
Resource for Macromolecular Modeling and Visualization

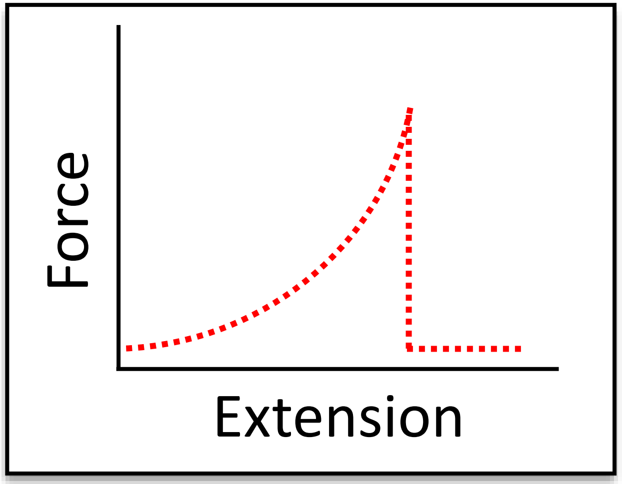
Single Molecule Force Spectroscopy

- Atomic Force Microscopy
- Optical Tweezers
- Magnetic Tweezers
- Centrifugal Force Microcopy
- …

Hermann Gaub
(LMU)

Atomic Force Microscope (AFM)



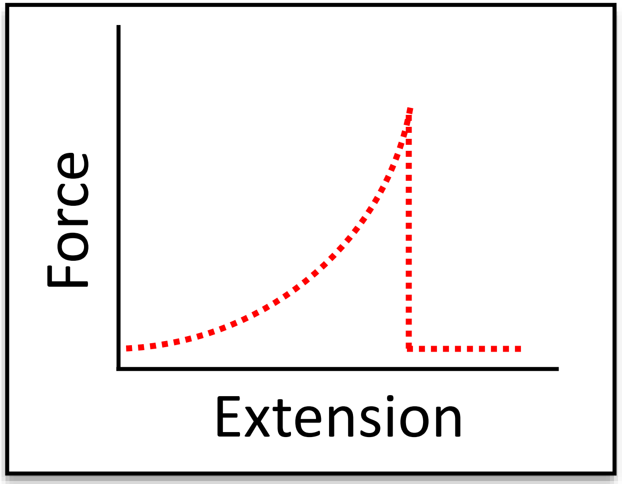


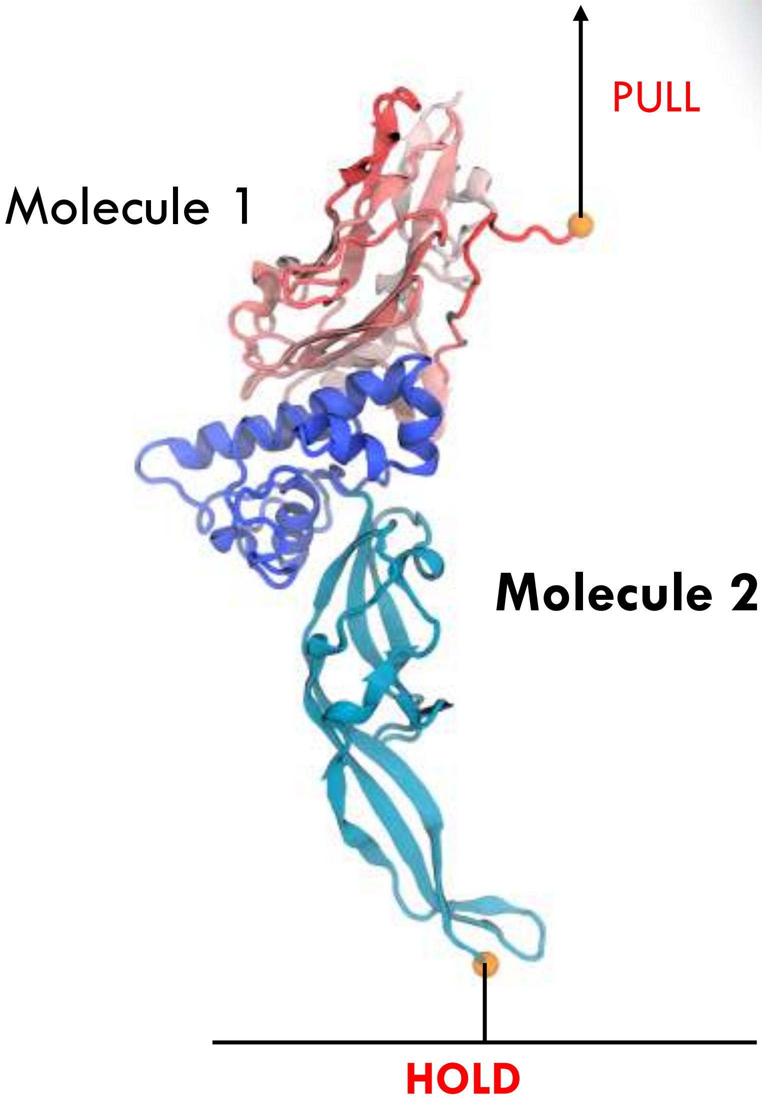
EL Florin, et. al.; Science, 1994
GU Lee, et. al.; Langmuir, 1994

Steered Molecular Dynamics Simulations

- Molecular Dynamics Simulations
- Pulling with a spring (Hooke's Law):

$$F = -k \cdot \Delta x$$





Helmut Grubmüller
MPI

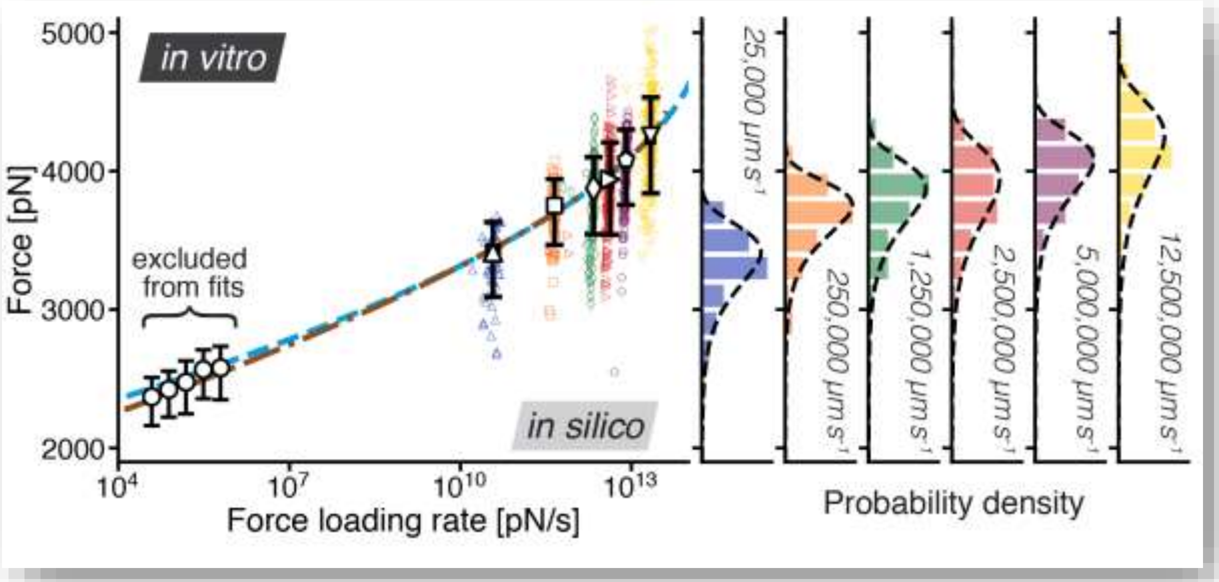
Klaus Schulten
UIUC

H Grubmüller, et. al.; Science, 1996
S Izrailev, et. al.; Biophysical Jounal, 1997

In Silico Single Molecule Force Spectroscopy

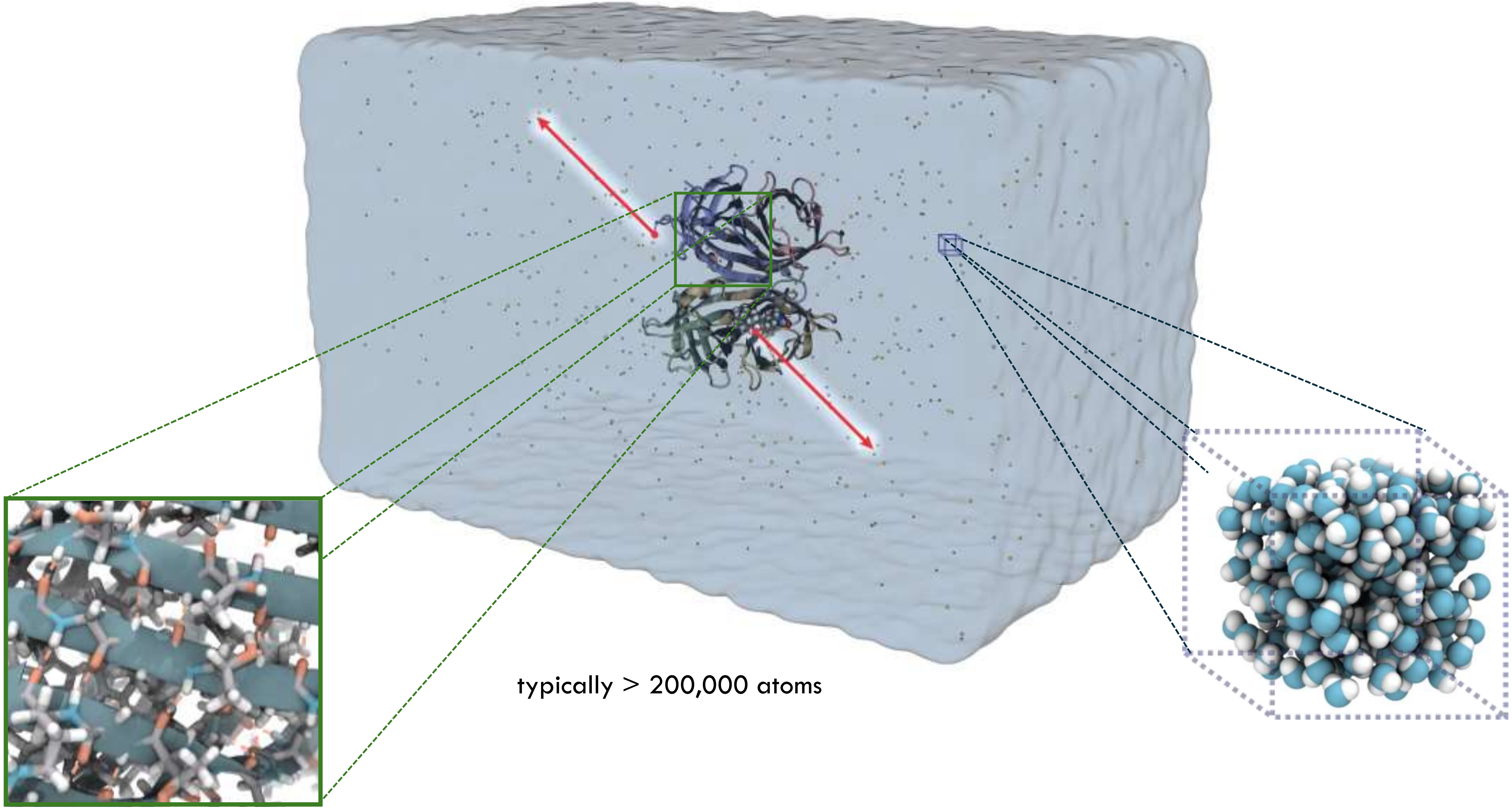
MCB-2143787
CAREER: In Silico Single-Molecule Force Spectroscopy

- Pulling and anchoring points mimic experiments.
- Thousands of simulation replicas.
- Dozens to hundreds of microseconds of all-atom SMD.
- Dozens of terabytes of trajectory data.
- Dynamic Network Analysis.
- Dimensionality reduction tools.
- AI tools for mutation prediction.



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

Molecular Dynamics Simulations



typically > 200,000 atoms

Molecular Dynamics Simulations Software

National Institutes of Health

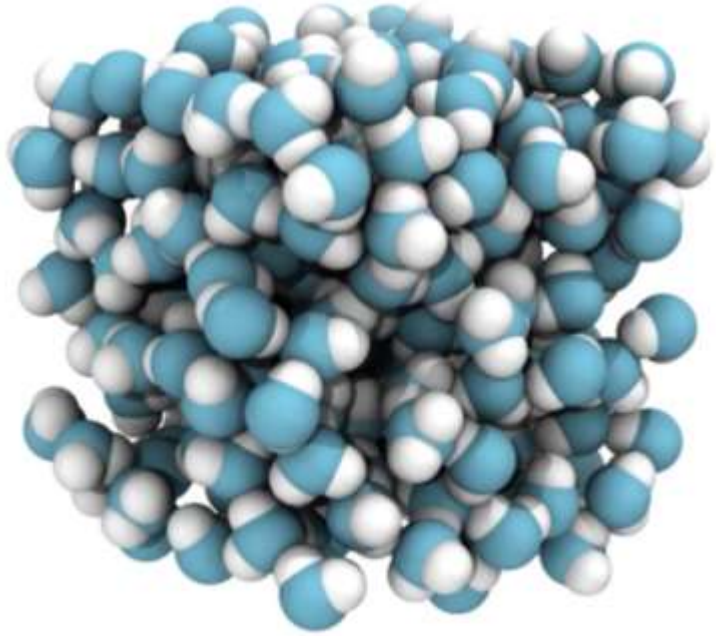
R24 GM-145965
Resource for Macromolecular Modeling and Visualization

NAMD
Scalable Molecular Dynamics

VMD
Visual Molecular Dynamics

QwikMD
Gateway for Easy Simulation

300,000 registered users;



M Spivak, …, RC Bernardi, E Tajkhorshid; **VMD as a platform for interactive small molecule preparation and visualization in quantum and classical simulations.** JCIM, 2023
JC Phillips, …, RC Bernardi, et. al.; **Scalable molecular dynamics on CPU and GPU architectures with NAMD.** The Journal of Chemical Physics, 2020
MCR Melo*, RC Bernardi*, et. al.; **NAMD goes quantum: An integrative suite for hybrid simulations.** Nature Methods, 2018
JV Ribeiro*, RC Bernardi*, et. al.; **QwikMD: Integrative Molecular Dynamics Toolkit for Novices and Experts.** Scientific Reports, 2016
W Humphrey, et. al.; Journal of Molecular Graphics, 1996

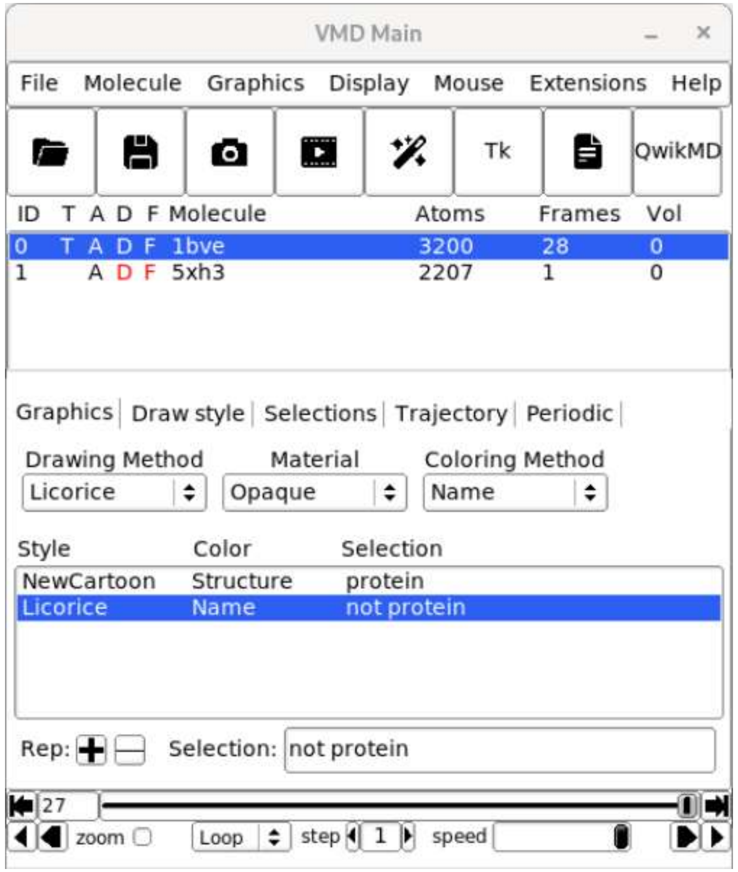
Molecular Dynamics Simulations Software

R24 GM-145965
Resource for Macromolecular Modeling and Visualization

VMD 2.0-alpha:

Release December 2024

New Interface



ID	T	A	D	F	Molecule	Atoms	Frames	Vol
0	T	A	D	F	1bve	3200	28	0
1		A	D	F	5xh3	2207	1	0

Drawing Method: Licorice
Material: Opaque
Coloring Method: Name

Style	Color	Selection
NewCartoon	Structure	protein
Licorice	Name	not protein

Selection: not protein

27

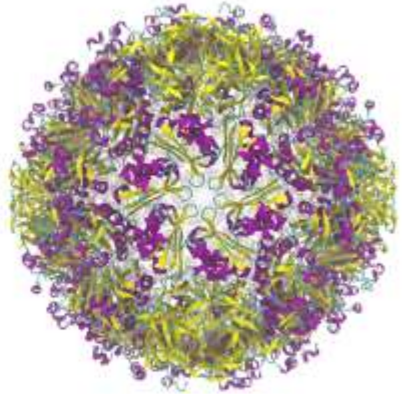
1

NAMD 3.0:

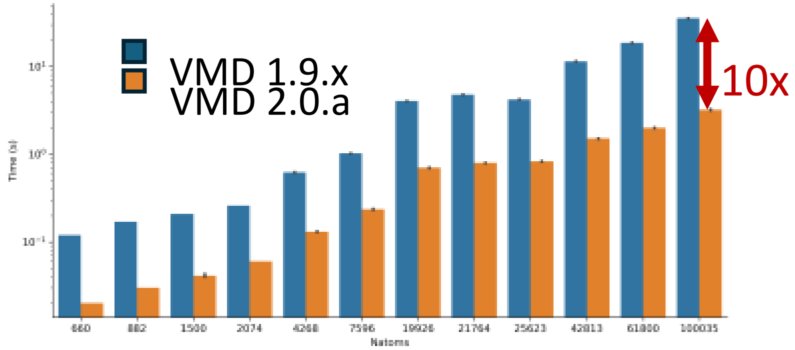
Released June 2024

- GPU-resident
- Support for Multi-GPU
- Full capability of NAMD 2

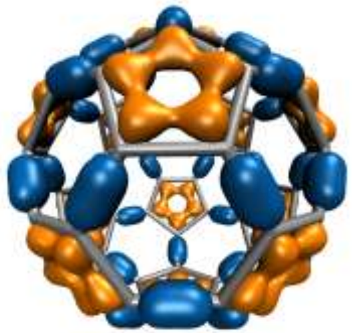
Secondary Structure Representation / Live Rendering



Surface Representation

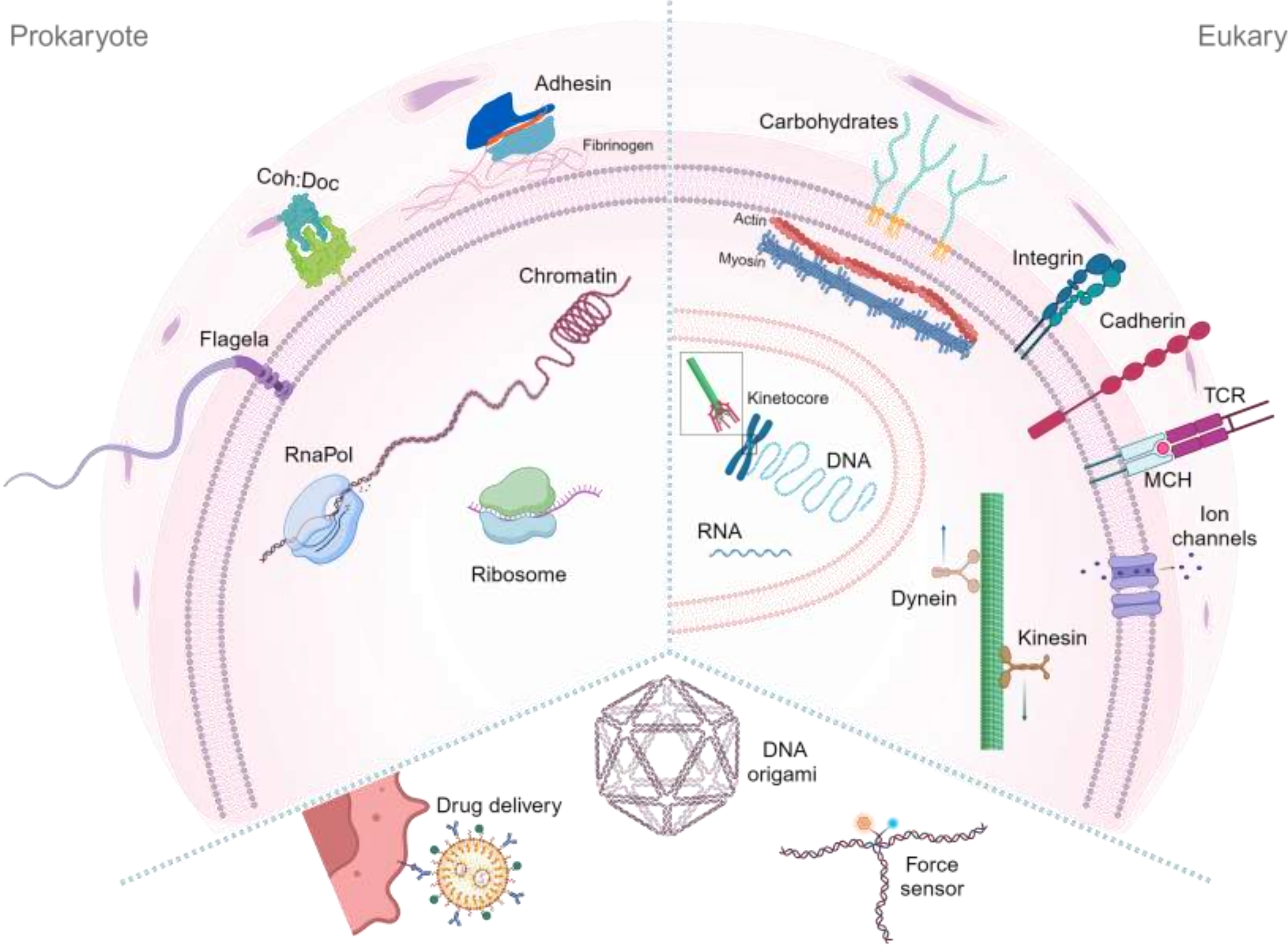


QM Visualization



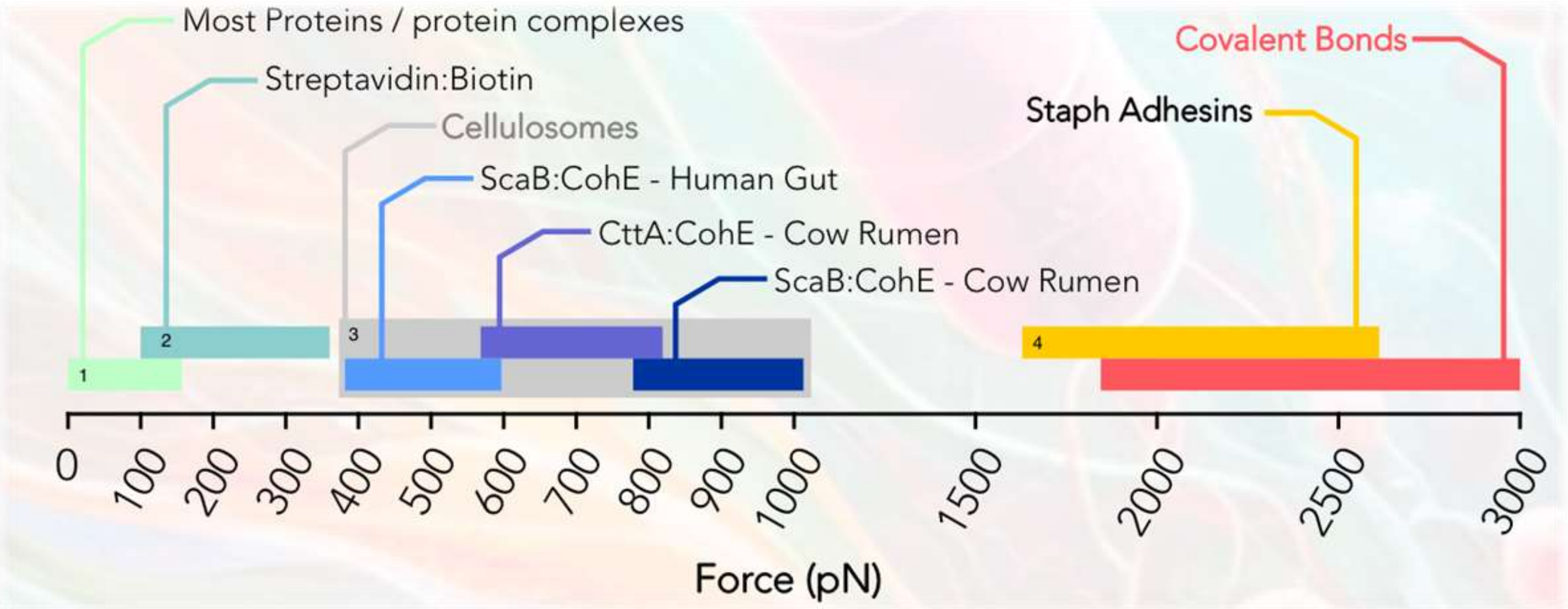
Rafael C. Bernardi
rcbernardi@auburn.edu

Mechanostability and Mechanoactive Biomolecules



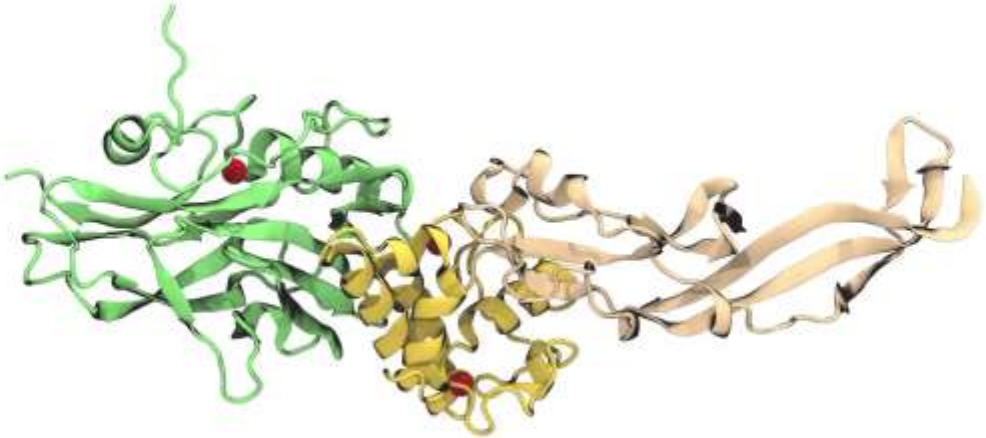
PSFC Gomes, MCR Melo, RSL Rosa, BL Riberio, RC Bernardi; **in preparation**

Forces in Biology



PSFC Gomes, MCR Melo, RSL Rosa, BL Riberio, RC Bernardi; **in preparation**

Molecular Mechanisms of Mechanostability



Catch-Bond Mechanism
Ultra-mechanostable Protein Complex
Cohesin:Dockerin

Nat. Comm., 2014 & 2020; Nano Letters, 2015; JACS 2017 & 2019

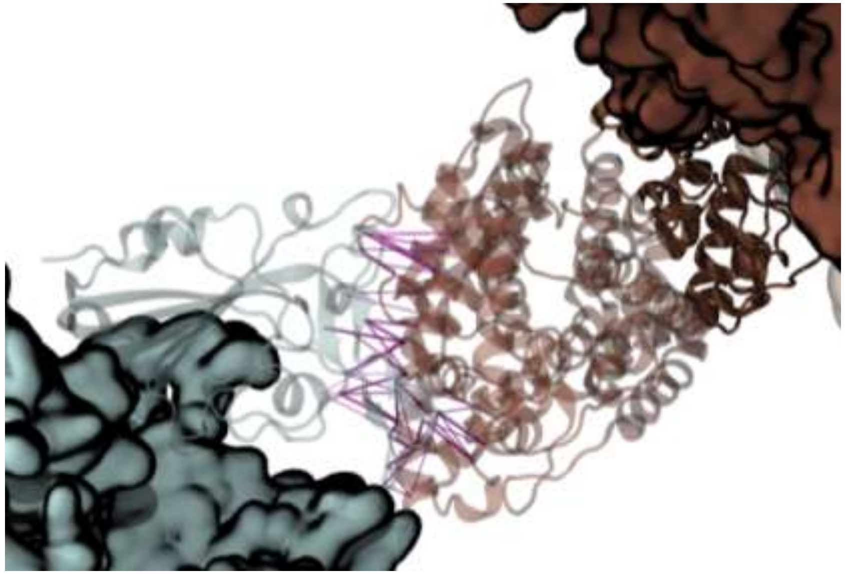
Hermann Gaub
(LMU)

Michael Nash
(U. Basel / ETH)

Hermann Gaub
(LMU)

Jan Lipfert
(Utrecht U.)

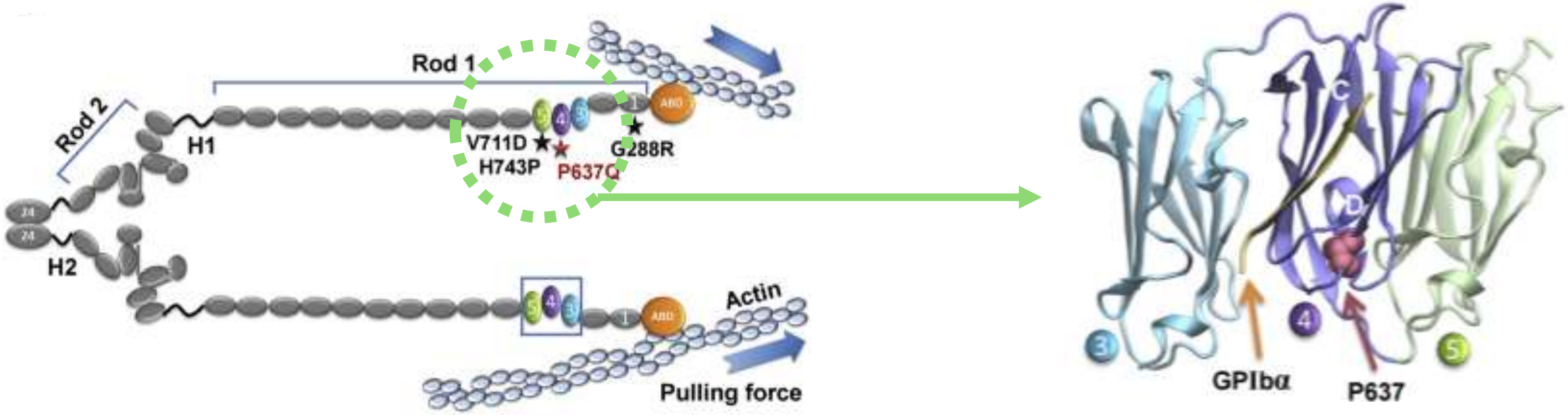
David Alsteens
(CU Louvain)



Evolution of Mechanostability
Coronavirus SARS-CoV-2

PNAS, 2022; Nature Nanotechnology, 2024;
Nature Communications, 2024

Molecular Mechanisms of Mechanostability

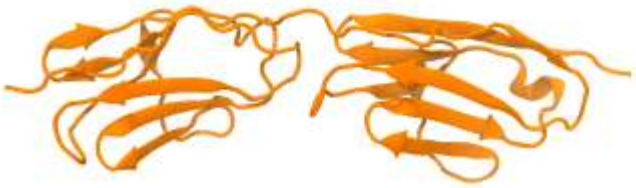


Ulla Pentikäinen
(U. Turku0

Mutations in Filamins that are associated with genetic diseases

Scientific Reports, 2017; Structure, 2019

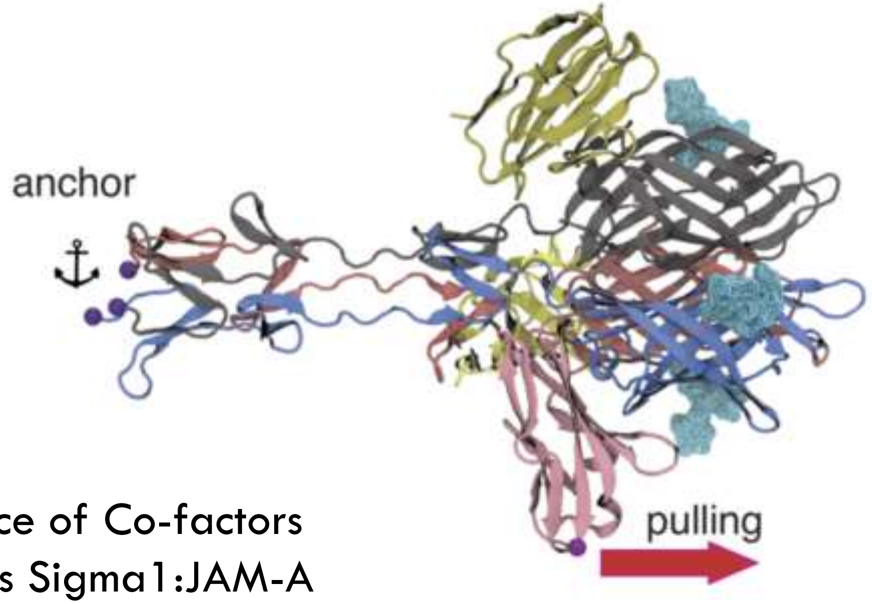
Ionel Popa
(U. Wisconsin)



Mutations in Titin that are associated with genetic diseases

In preparation

David Alsteens
(CU Louvain)

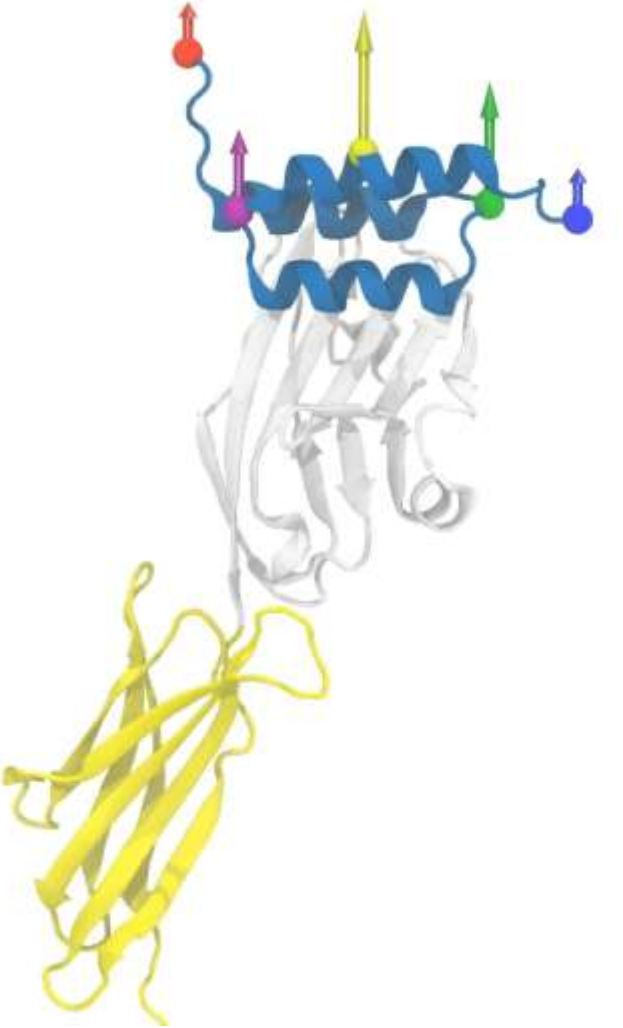


Influence of Co-factors
Reovirus Sigma1:JAM-A

PNAS, 2023

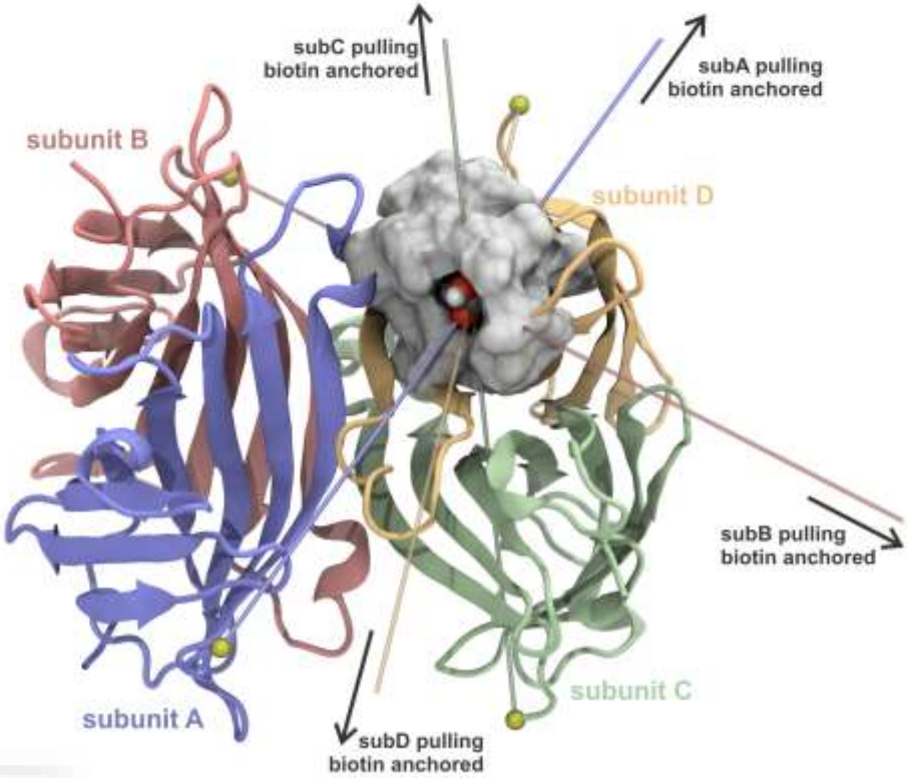
Mechanical Anisotropy

Michael Nash
(U. Basel / ETH)



Epitope Mapping
Mechano-anisotropy
Affibody:PD-L1

JACS, 2024; ACS Nano 2024



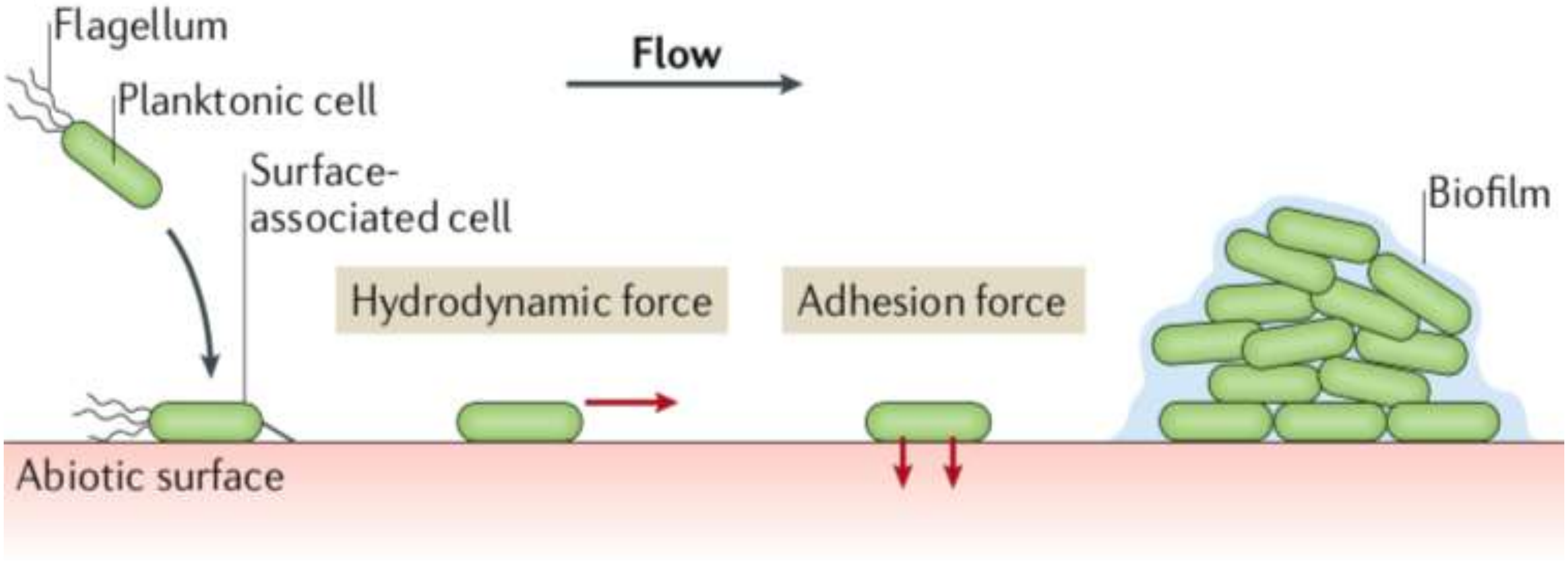
Hermann Gaub
(LMU)

Mechano-anisotropy
Strepavidin:Biotin

Nano Letters, 2019; Science Adv. 2020

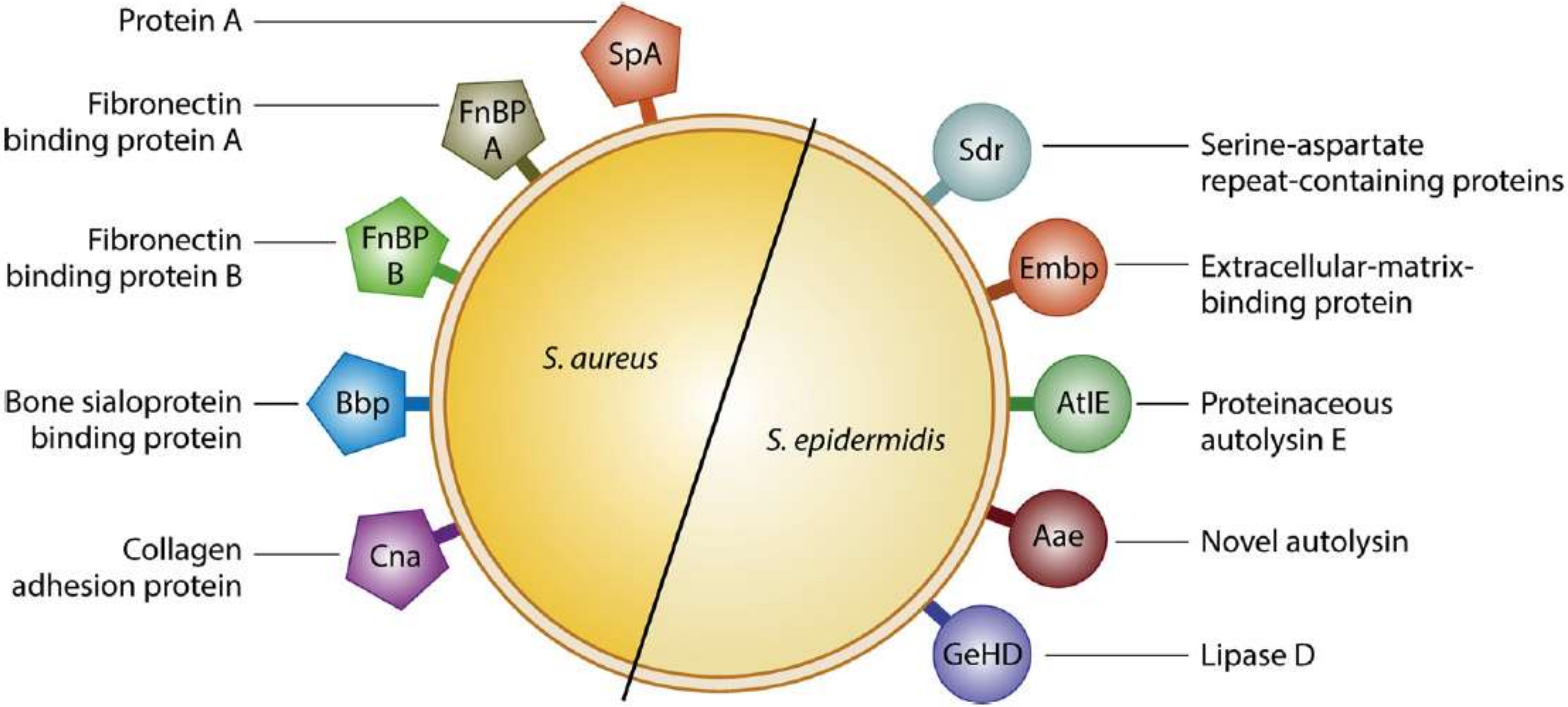
But why are adhesins so different and so interesting?

Staphylococci Biofilm Formation



YF Dufrêne & A Persat; Nature Reviews Microbiology, 2020

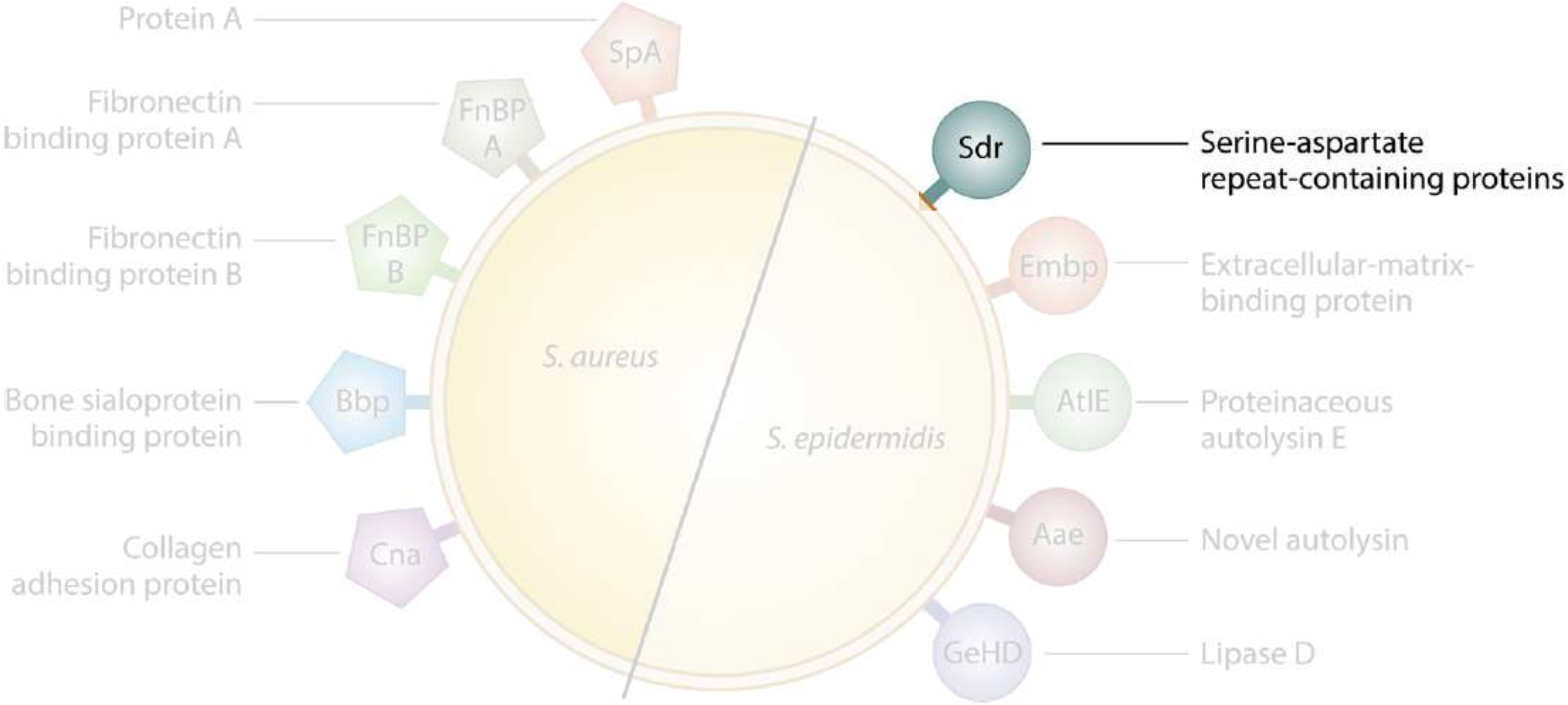
MSCRAMMs
Microbial Surface Components Recognizing Adhesive Matrix Molecules



Human targets include Fibrinogen (Fg, all chains), Fibronectin (Fn), Keratin, Collagen, Elastin, Complement Factor H, …

Adapted from: Kavanagh, et. al.; Clinical Microbiology Reviews, 2018

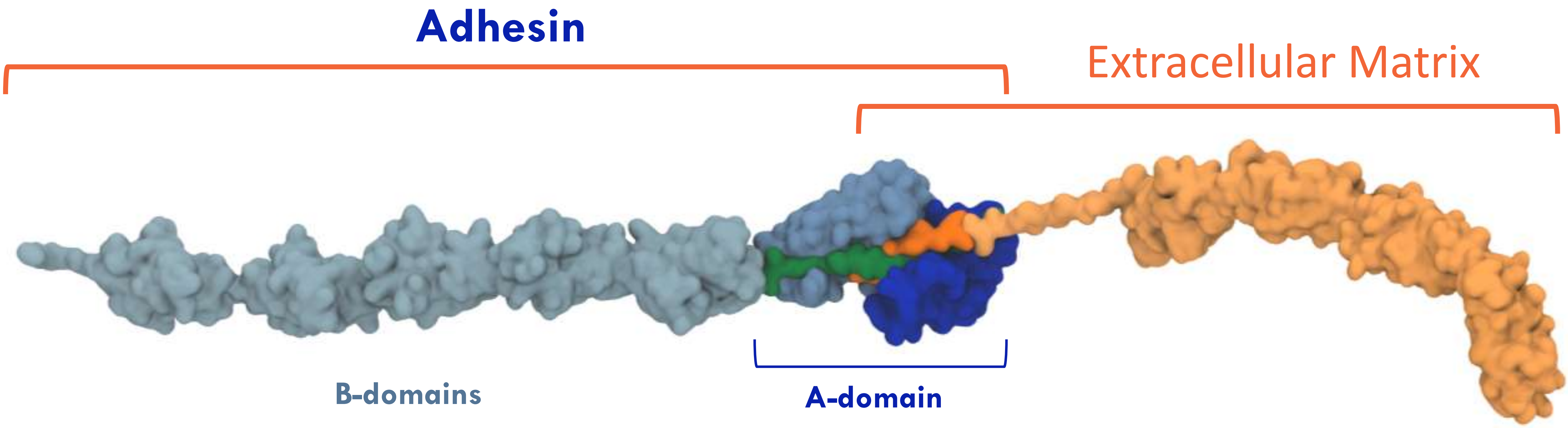
MSCRAMMs
Microbial Surface Components Recognizing Adhesive Matrix Molecules



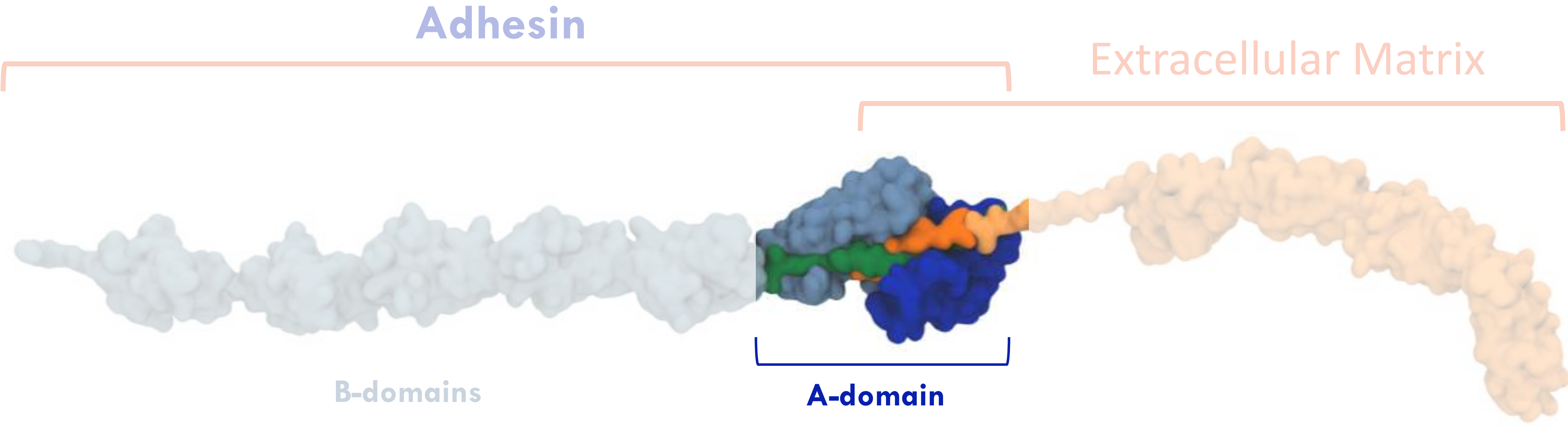
Fgβ / DSG-1

Adapted from: Kavanagh, et. al.; Clinical Microbiology Reviews, 2018

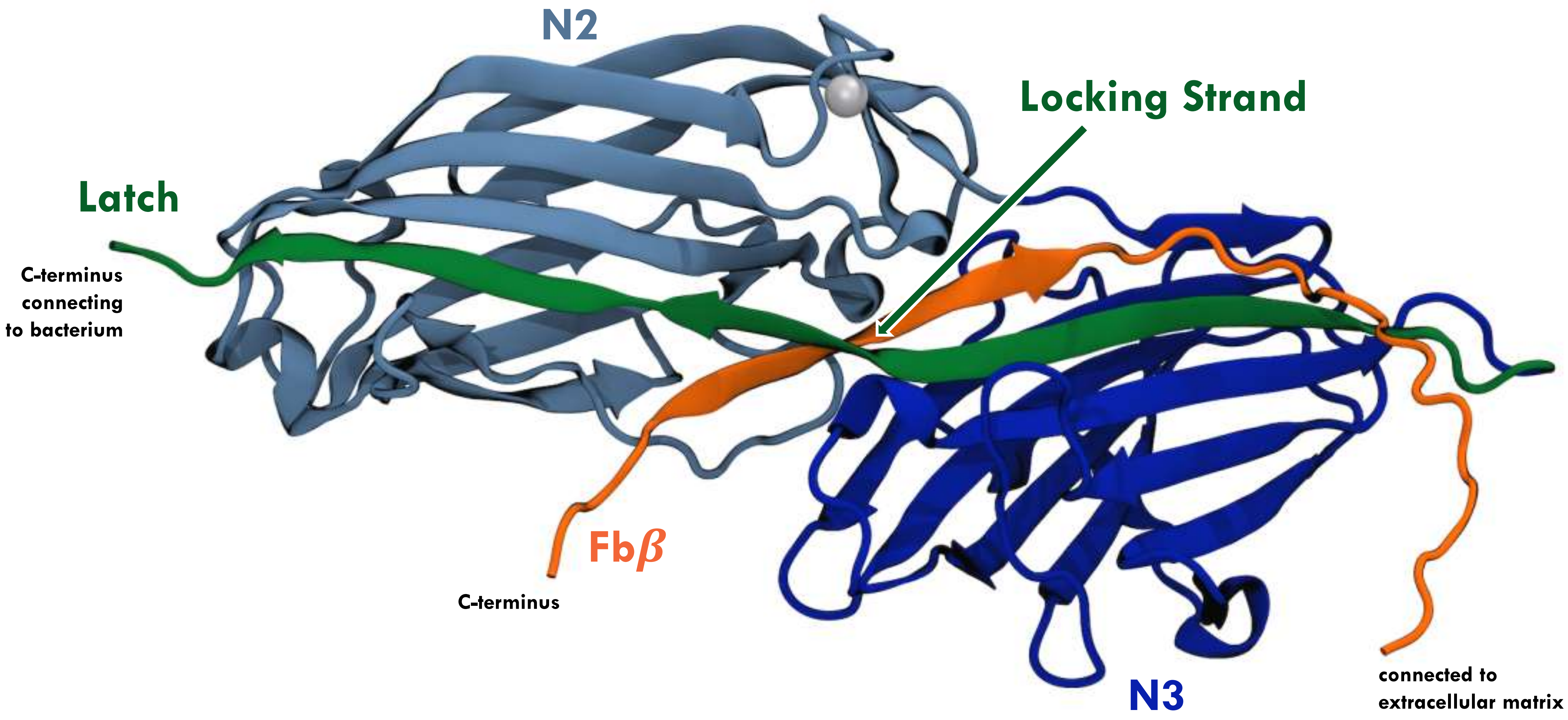
Adhesins


Bacteria
Human
Adhesin
Extracellular Matrix
B-domains
A-domain

Adhesins



The "Dock, Lock, and Latch" (DLL) Mechanism



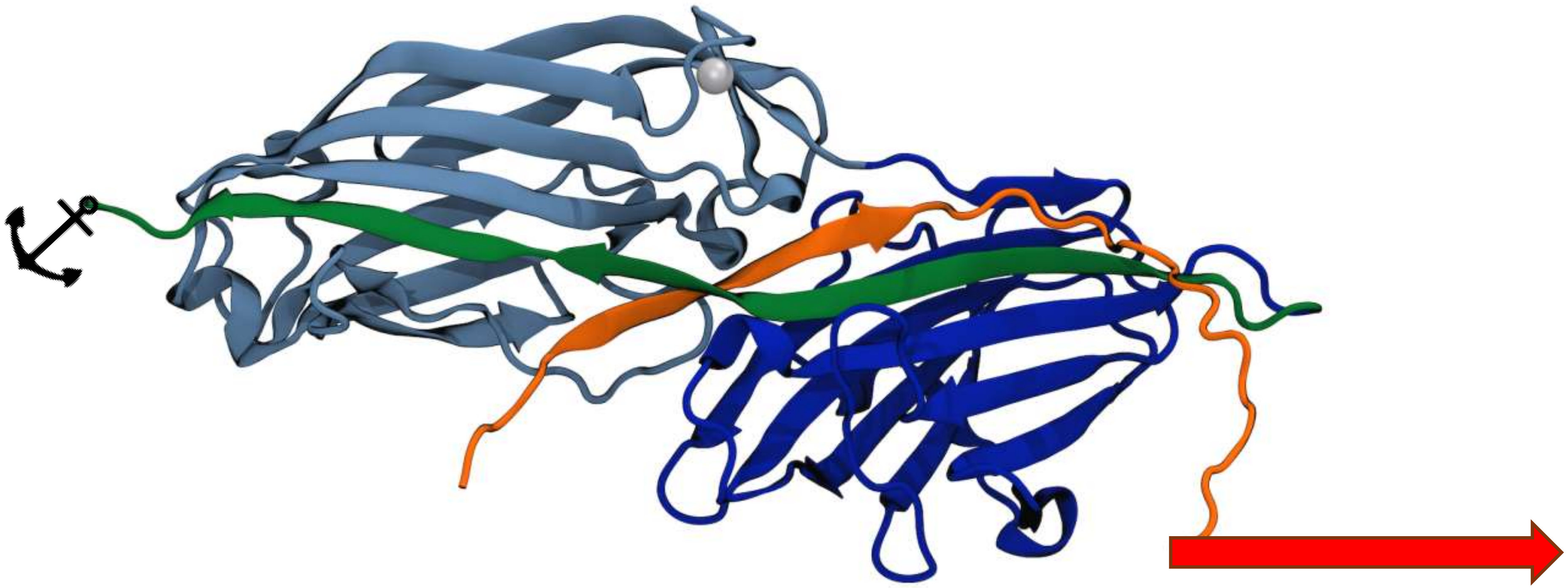
The "Dock, Lock, and Latch" (DLL) Mechanism

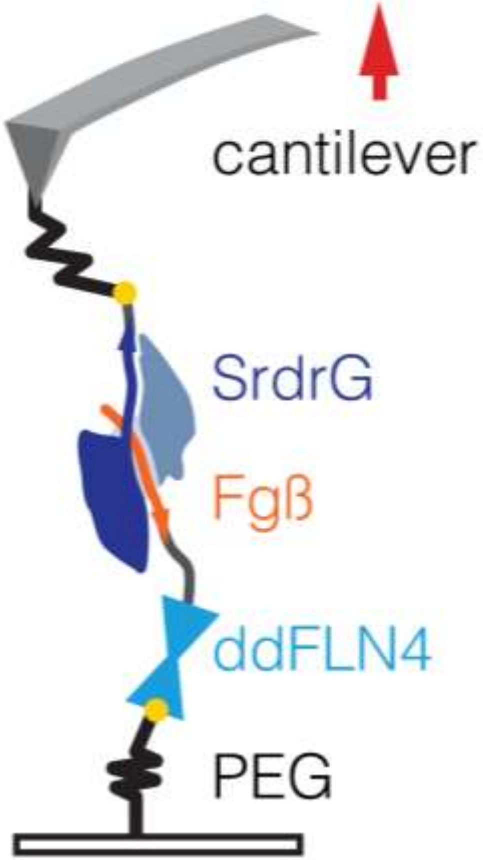
Rafael C. Bernardi
rcbernardi@auburn.edu

Unbinding Mechanics

- AFM-based SMFS

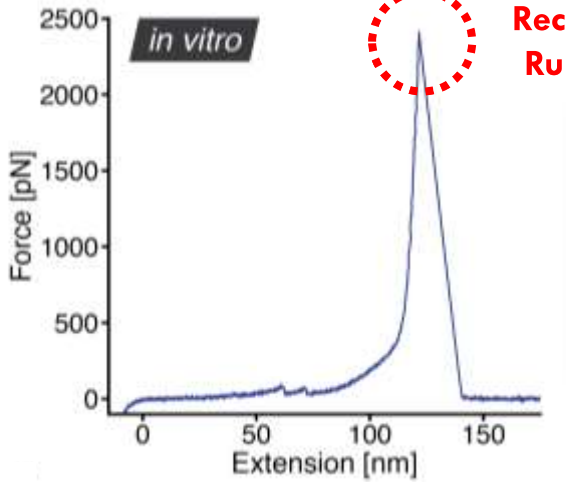
- SMD-based SMFS





The Hyperstrong Bond

- AFM-based SMFS

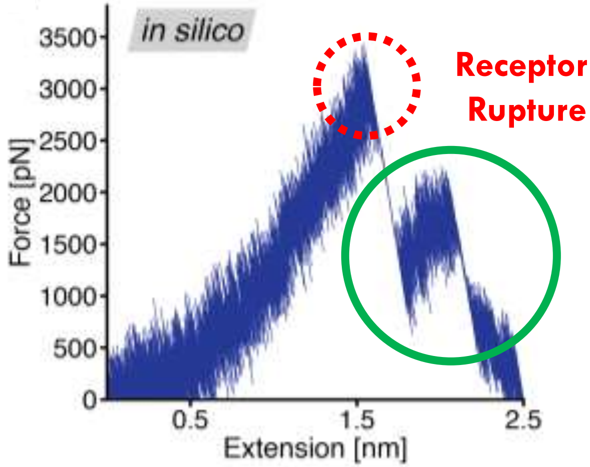


Receptor:Ligand Rupture Event

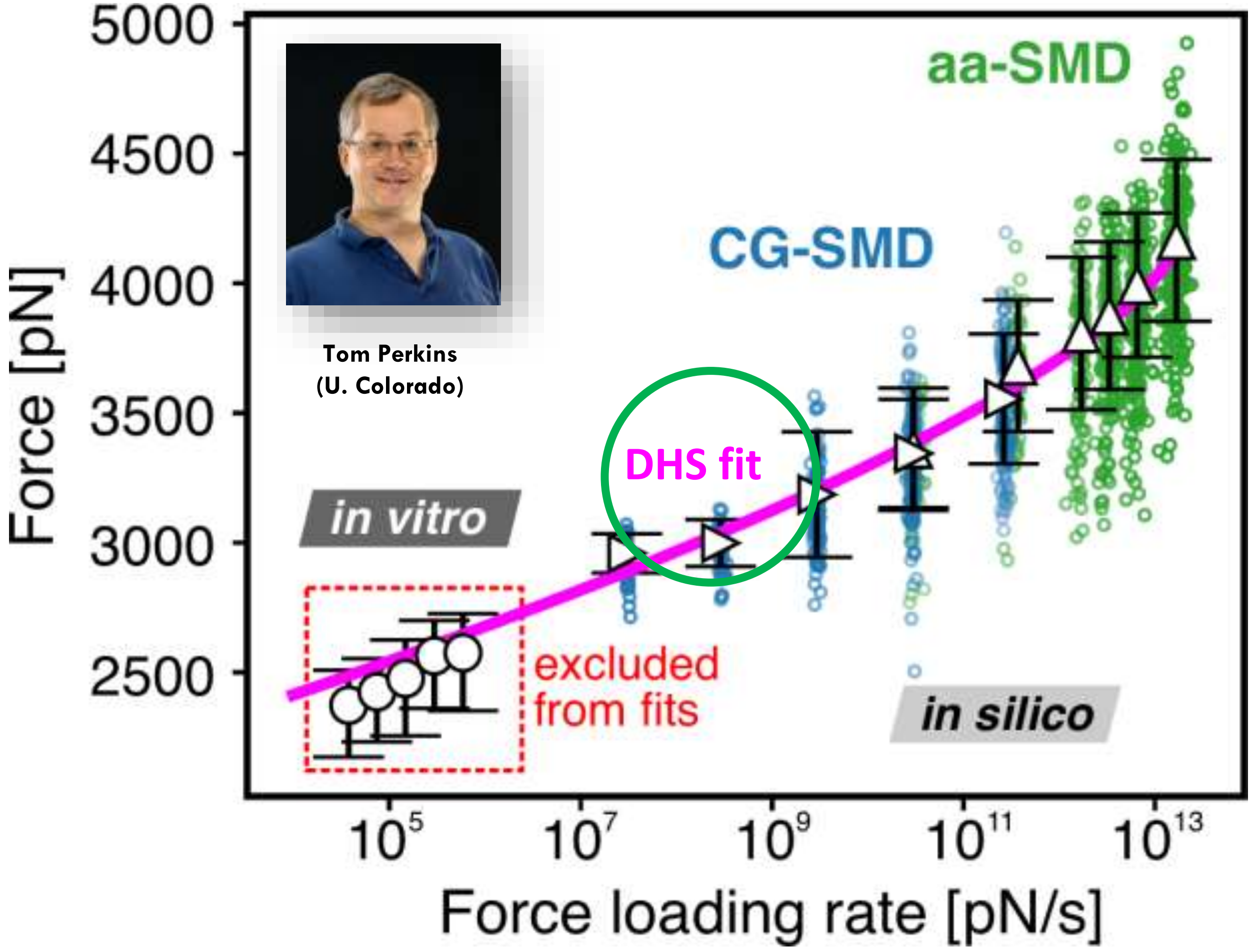
Hermann Gaub
(LMU)

Lukas Milles
(LMU/MPI)

- SMD-based SMFS



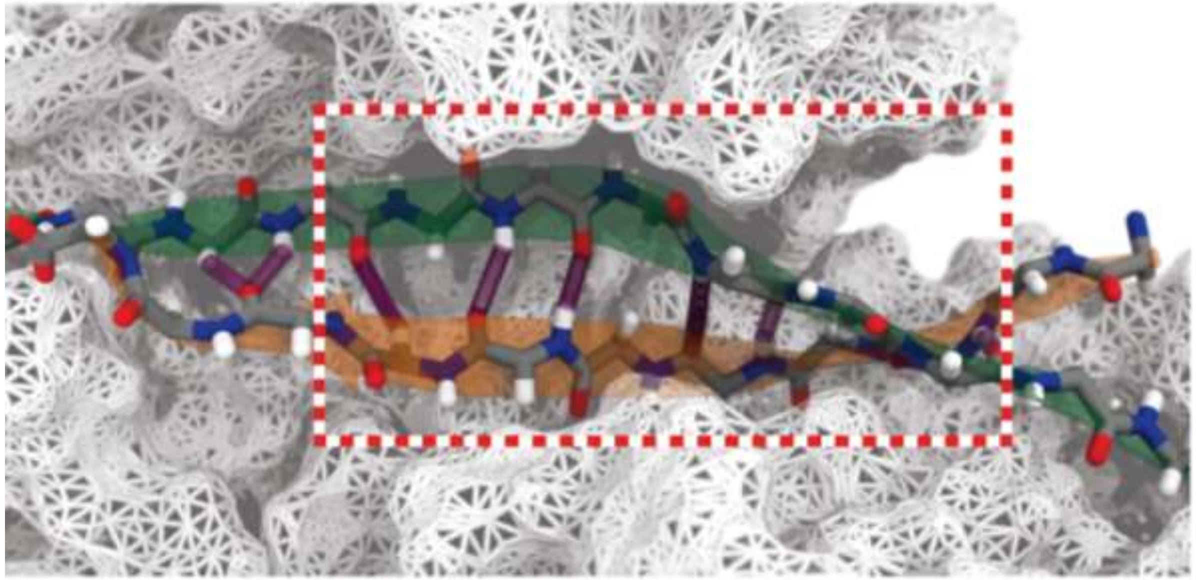
Receptor:Ligand Rupture Event

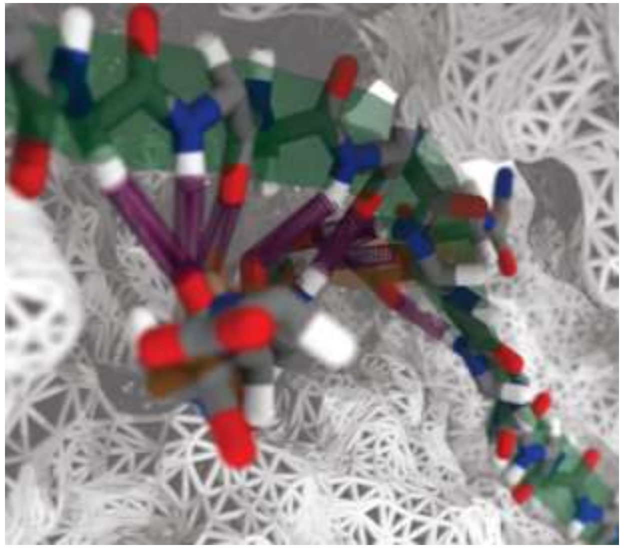


Tom Perkins
(U. Colorado)

LF Milles, K Schulten, HE Gaub, RC Bernardi; **Molecular mechanism of extreme mechanostability in a pathogen adhesin.** Science, 2018
DEB Gomes, …, RC Bernardi; **Bridging the gap between in vitro and in silico single-molecule force spectroscopy.** bioRxiv, 2022

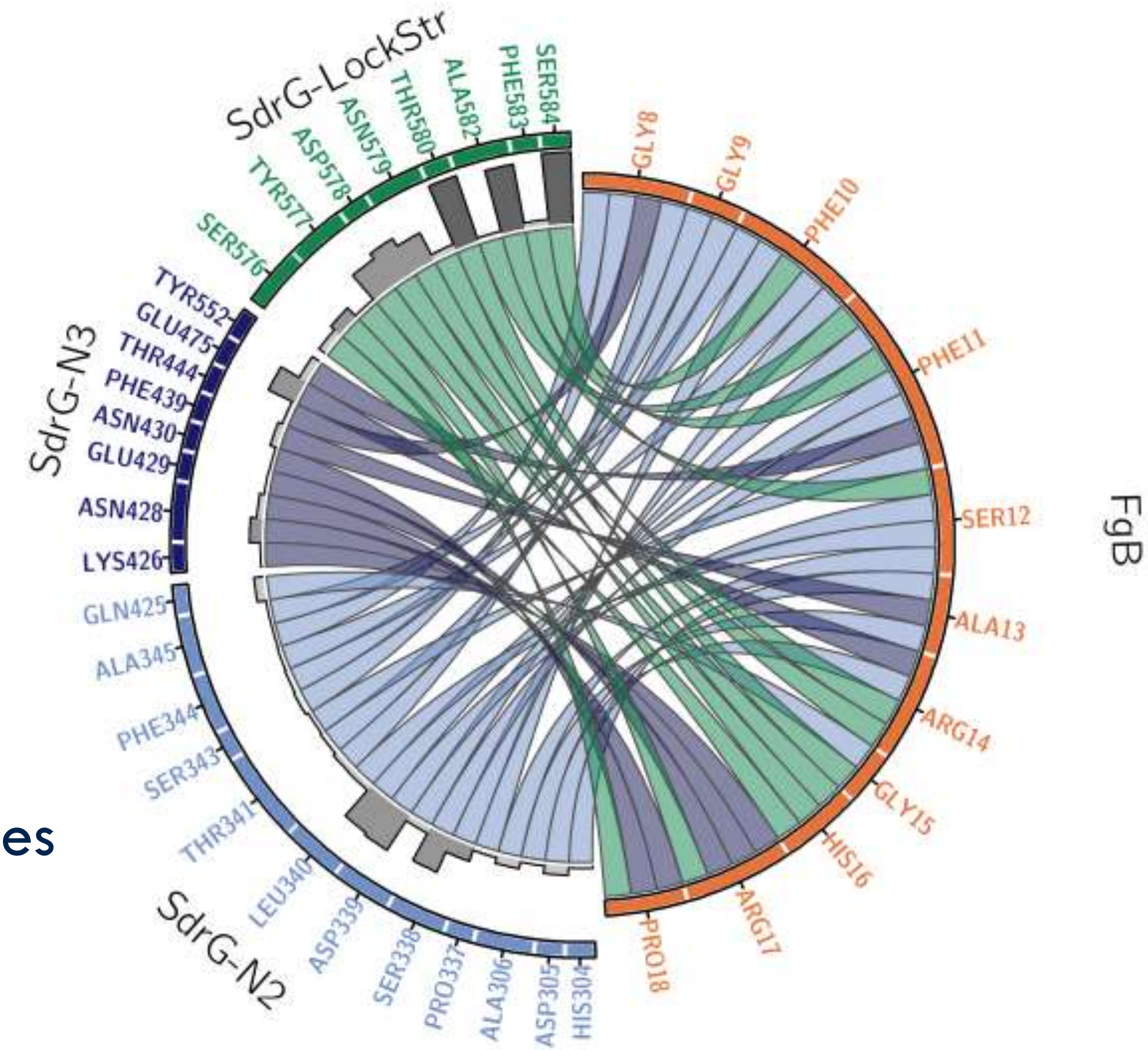
Resilience to mechanical loads is due to a network of hydrogen bonds.





A bond with a twist!

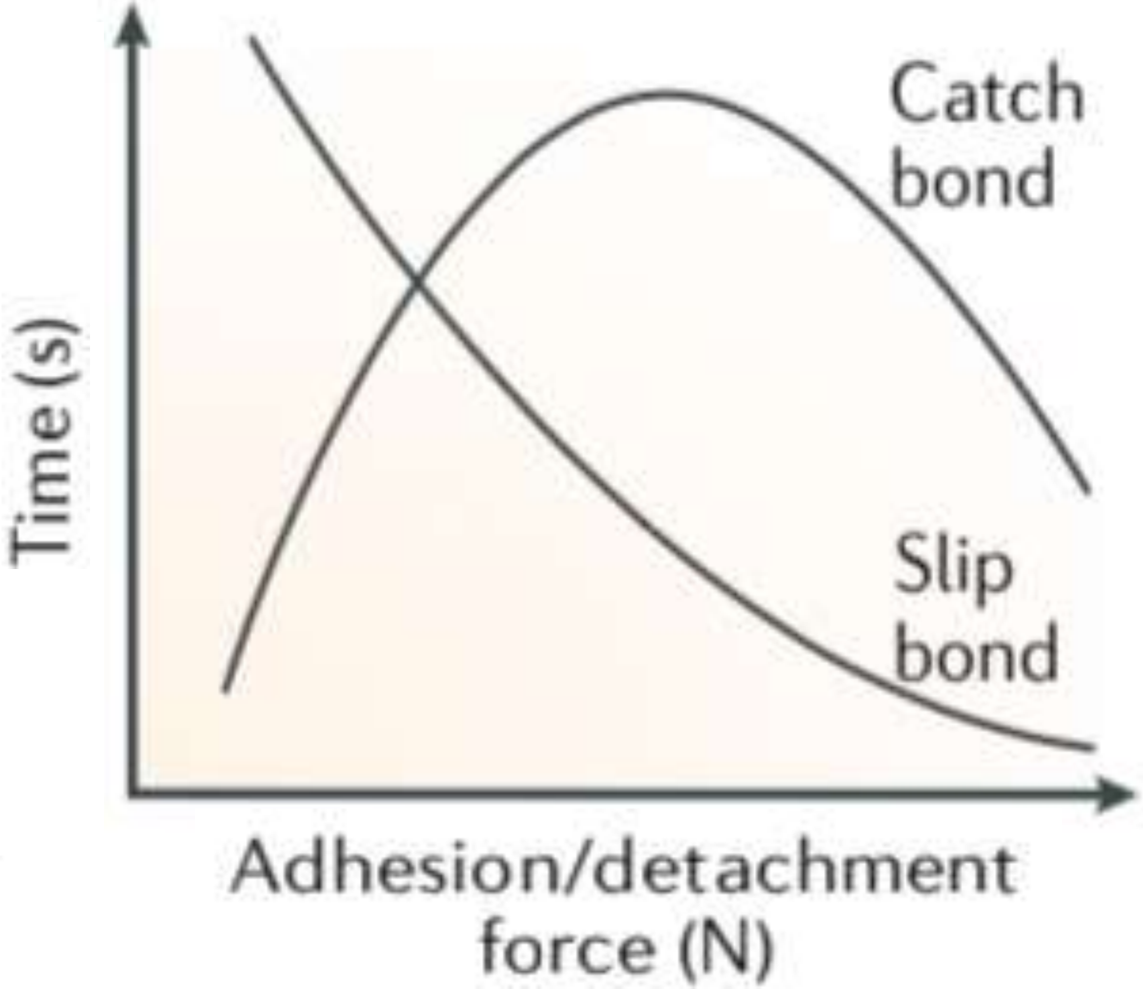
The corkscrew shape makes the complex hyperstable



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

Is this a hyperstable catch-bond?

A Hyperstable Catch-Bond



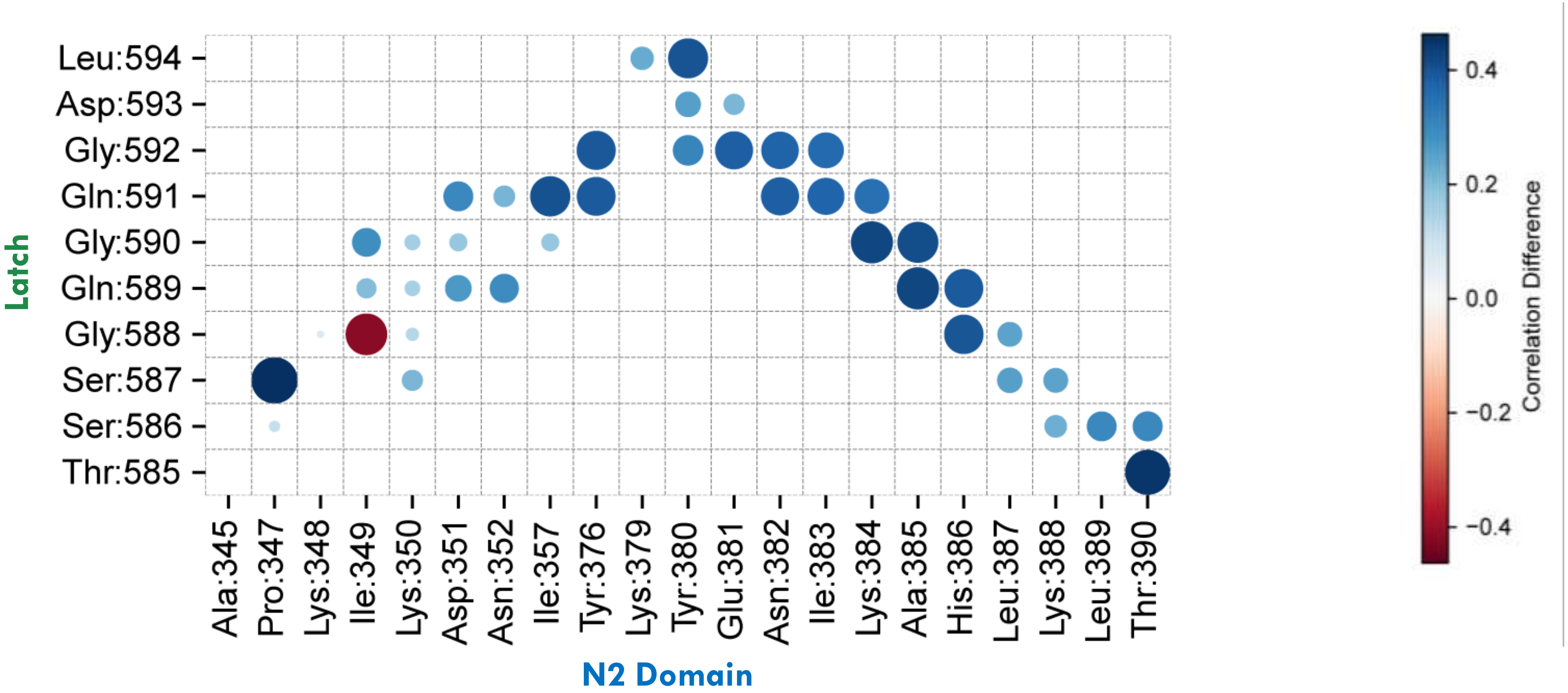
Adapted from: YF Dufrêne & A Persat; Nature Reviews Microbiology, 2020

Molecular Finger Trap Puzzle

Common Affinity
High Mechanostability

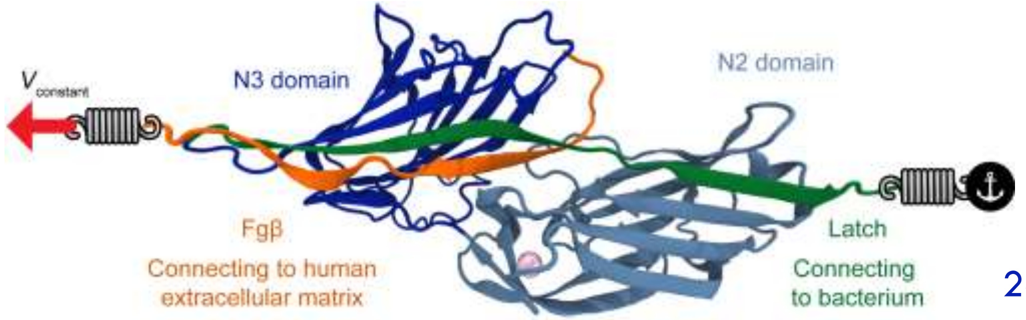
Network Edges as Intermolecular Interactions Descriptors

Marcelo CR Melo
(now at Colorado State)

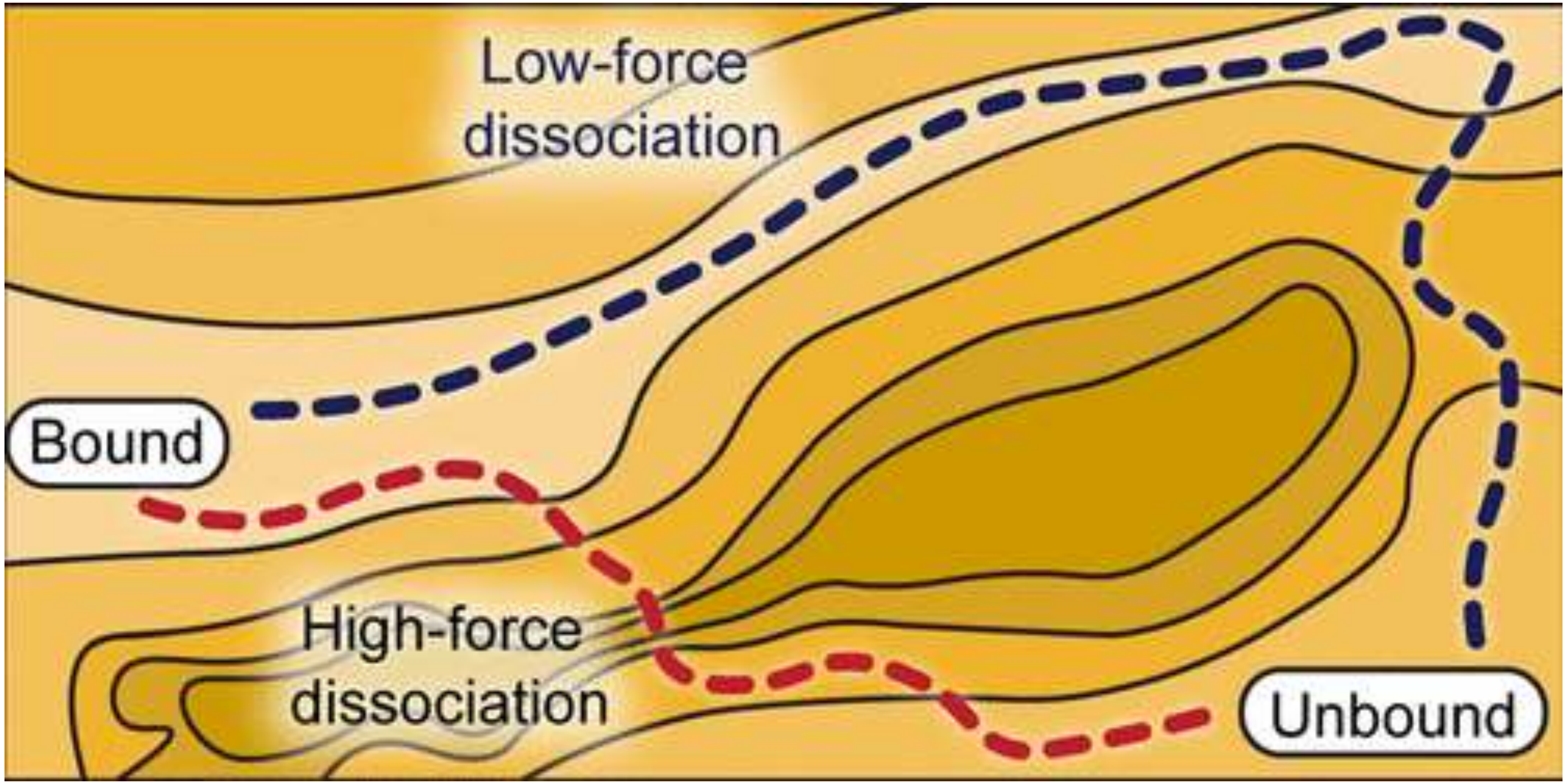


BLUE = Stronger correlation in the AFM-like pulling
RED = Stronger correlation in the MT-like force clamping

MCR Melo, …, RC Bernardi; **Molecular origins of force-dependent protein complex stabilization during bacterial infections.** JACS, 2023



Catch-Bond Mechanism offers a Different Unbinding Pathway

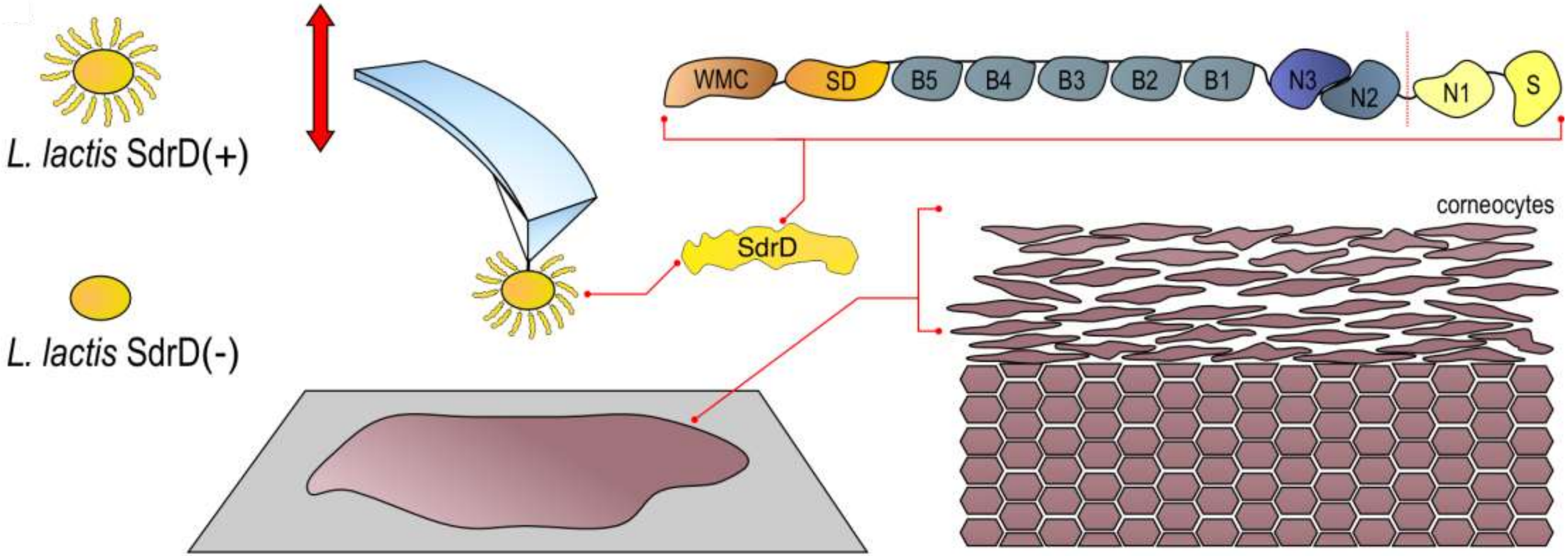


MCR Melo, …, RC Bernardi; **Molecular origins of force-dependent protein complex stabilization during bacterial infections.** JACS, 2023

How these adhesins interact with cells?

Rafael C. Bernardi
rcbernardi@auburn.edu

SdrD Interaction with Corneocytes

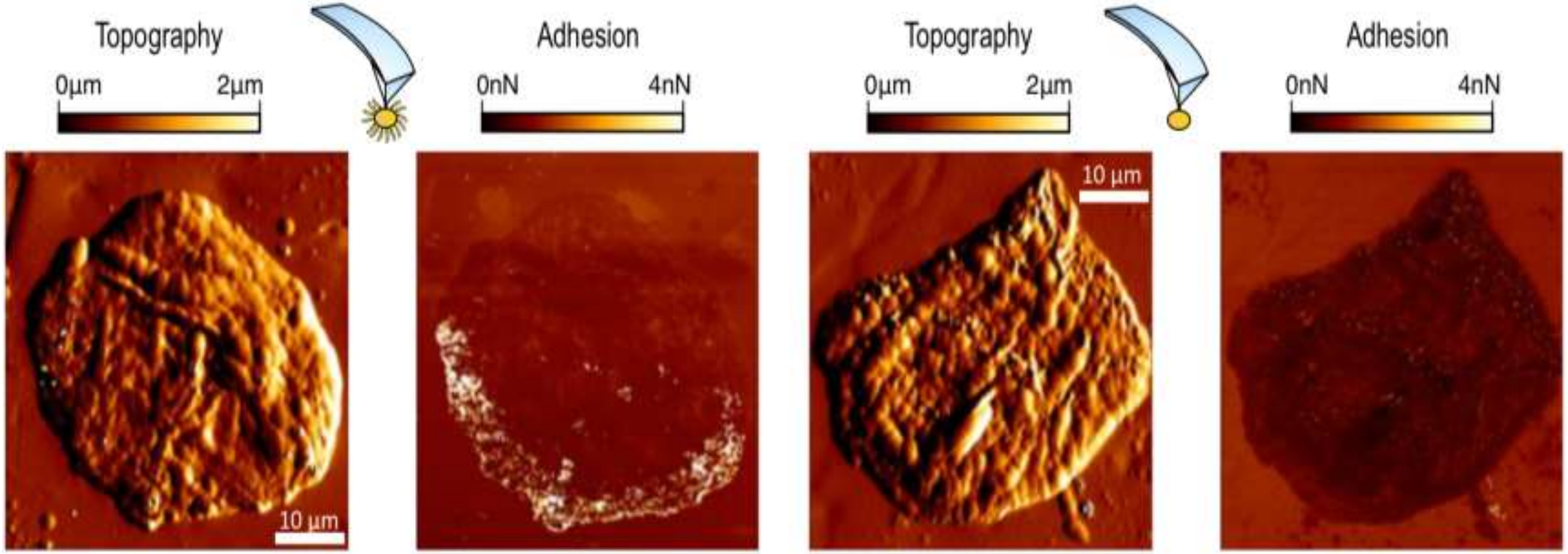


Yves Dufrêne
(CU Louvain)

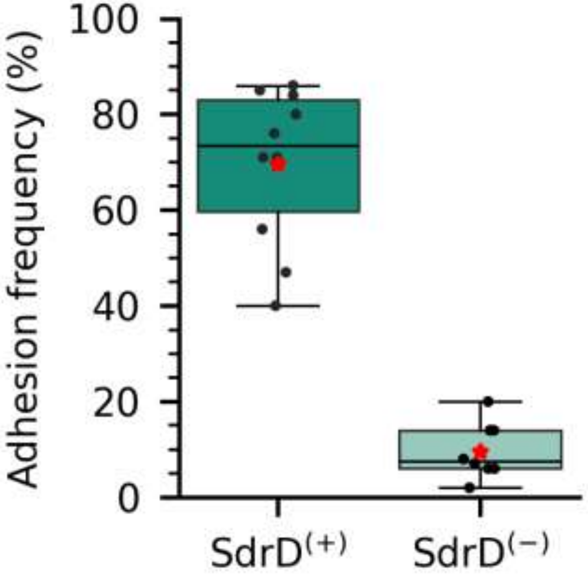
Rafael C. Bernardi
rcbernardi@auburn.edu

C Chantraine*, PSFC Gomes*, …, RC Bernardi#, YF Dufrêne#. **Under review**

SdrD Interaction with Corneocytes are at Cellular Junction Points



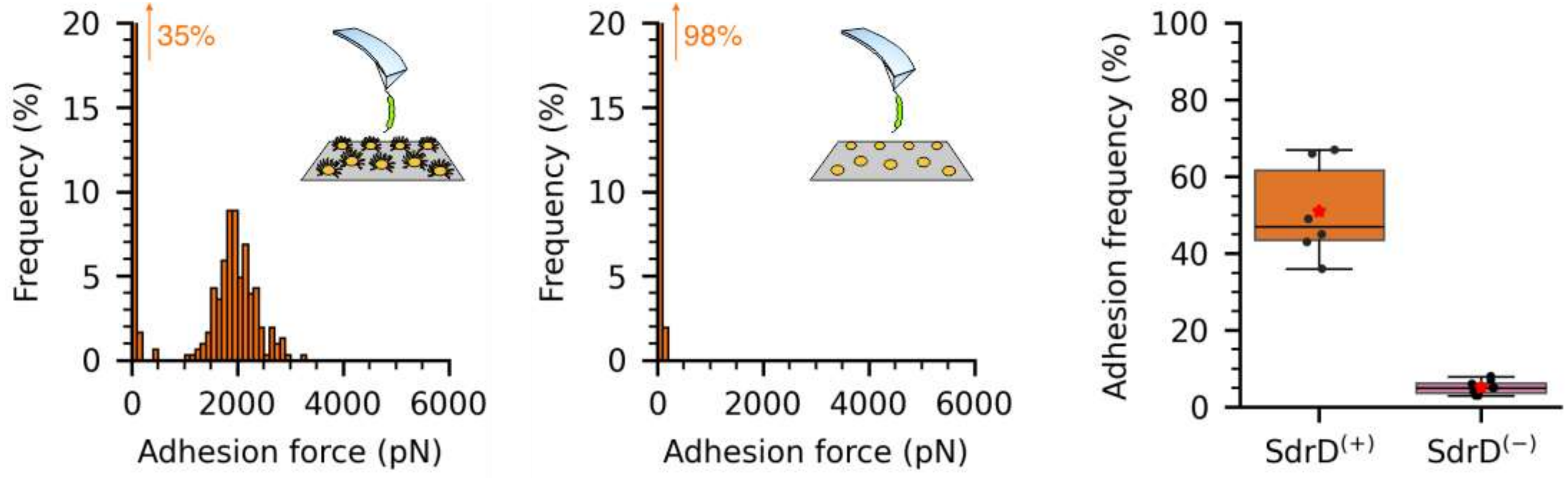
SrdD mediates *Staphylococcus Aureus* binding to Corneocytes

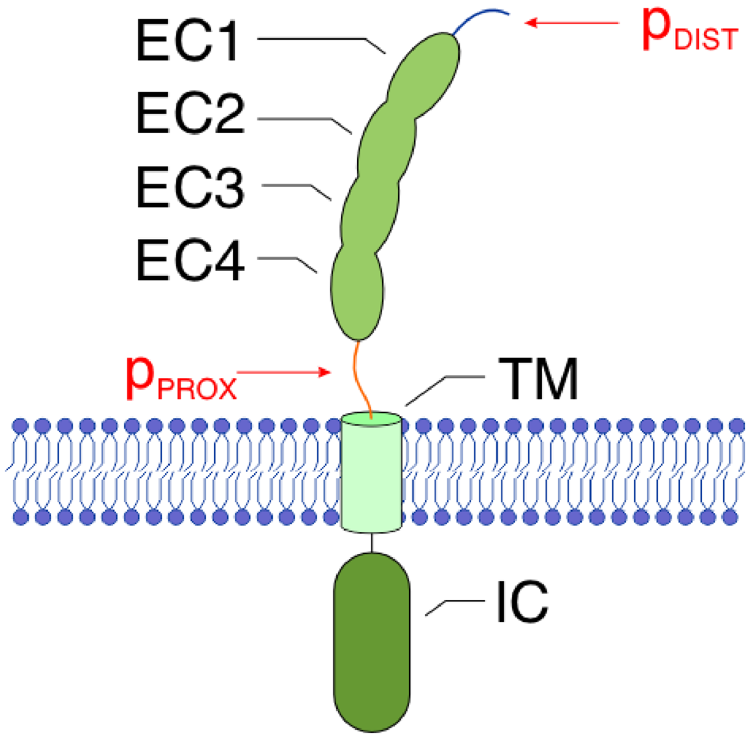


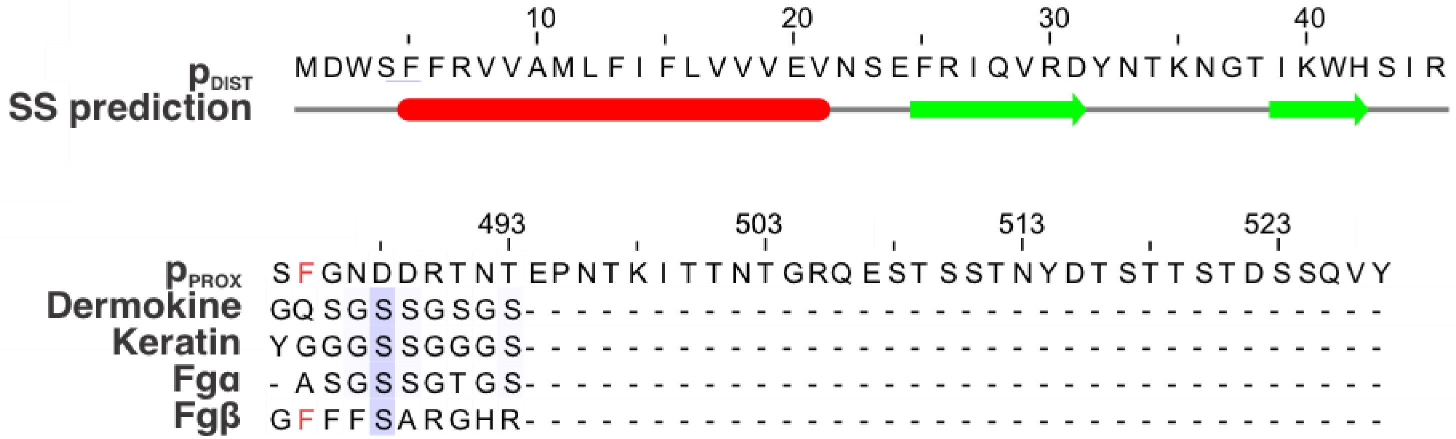
C Chantraine*, PSFC Gomes*, …, RC Bernardi#, YF Dufrêne#. **Under review**

SdrD:DSG-1 Interaction

DSG-1 as the SdrD target in Corneocytes



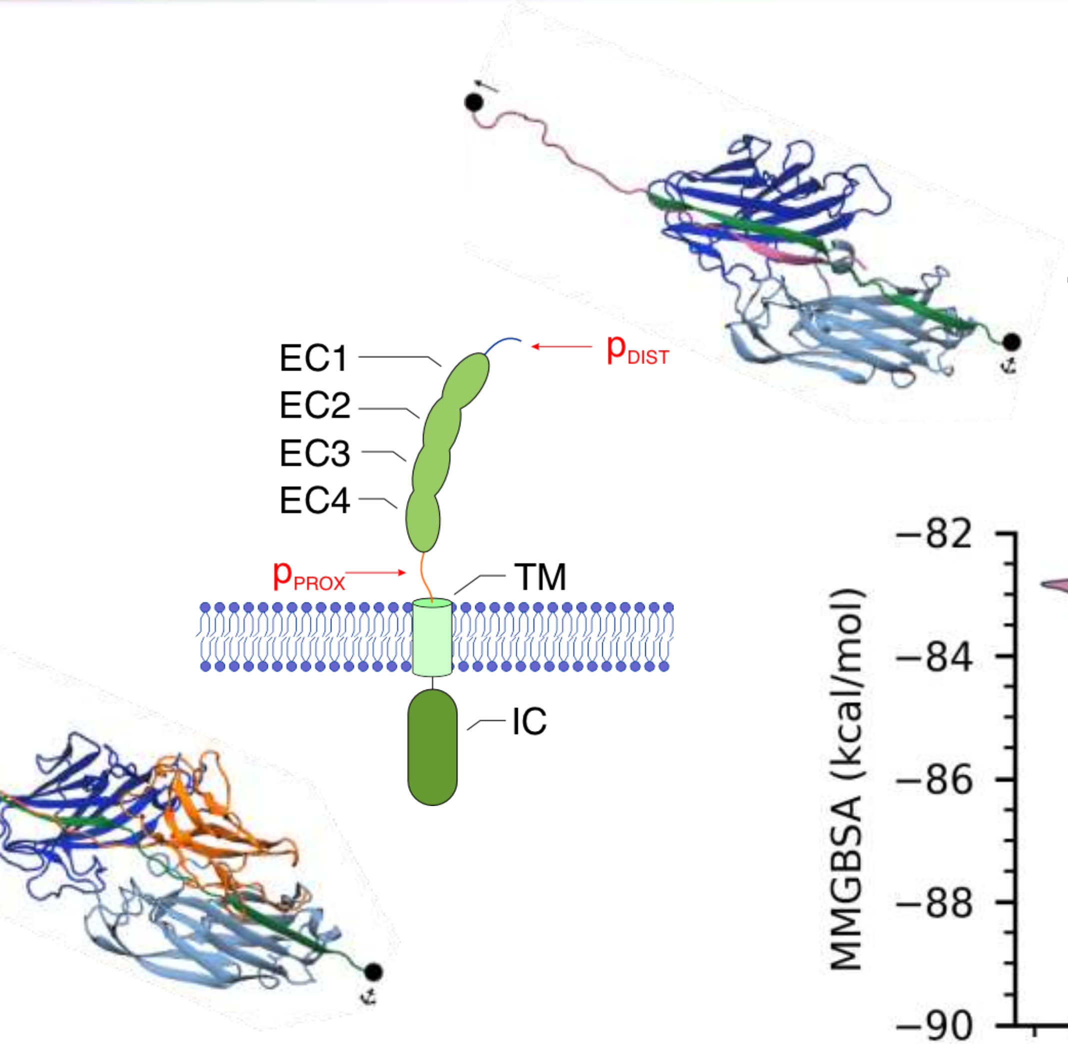




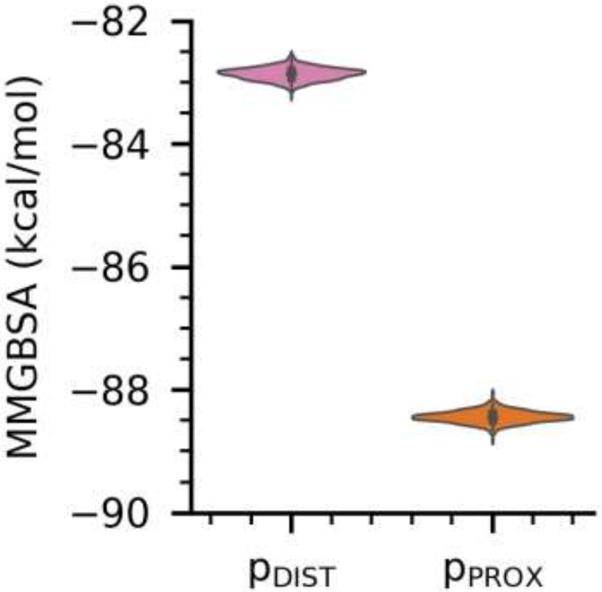
C Chantraine*, PSFC Gomes*, …, RC Bernardi#, YF Dufrêne#. **Under review**

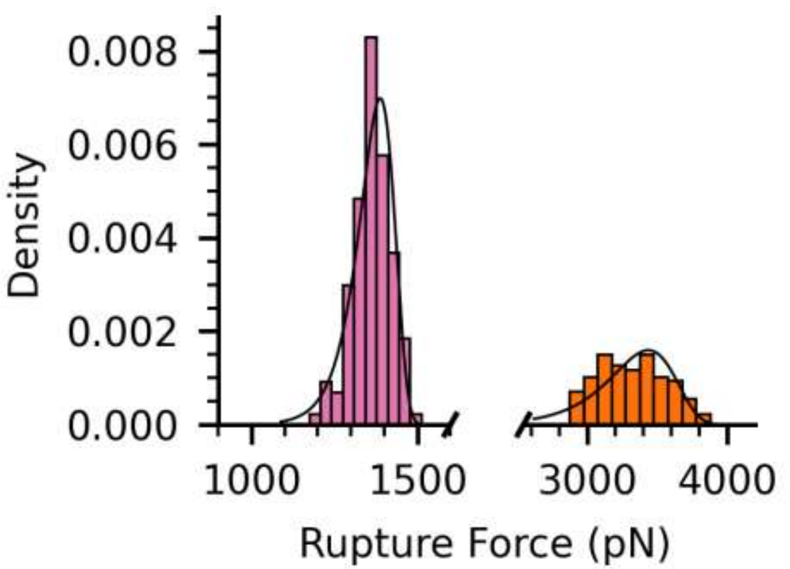
SdrD:DSG-1 Interaction

Priscila SFC Gomes



The proximal peptide is probably the target of SdrD

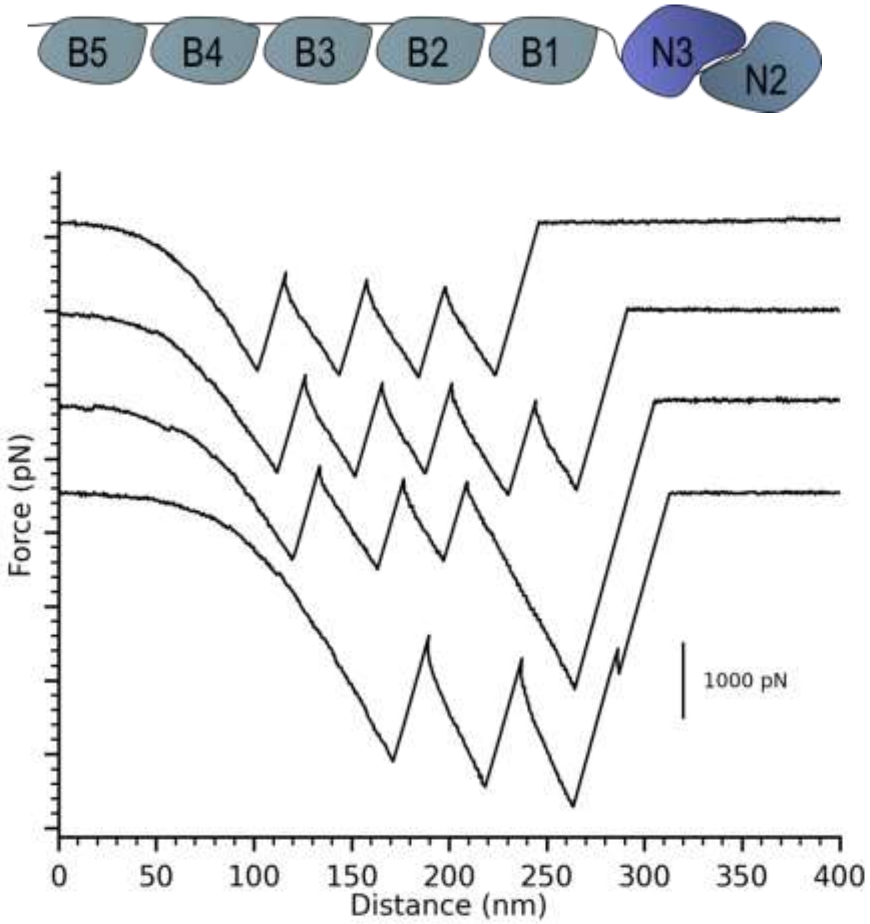


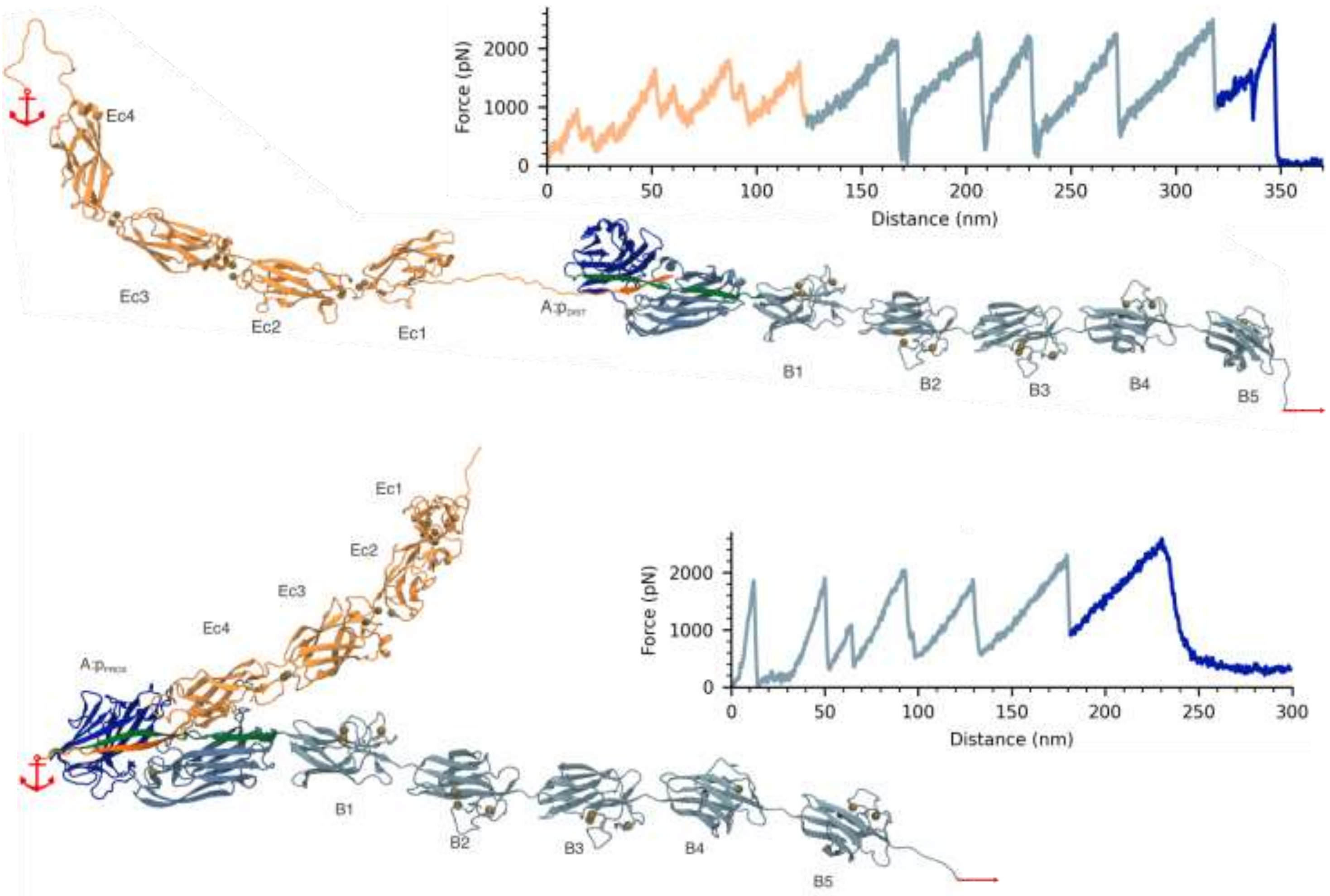


Rafael C. Bernardi
rcbernardi@auburn.edu

C Chantraine*, PSFC Gomes*, …, RC Bernardi#, YF Dufrêne#. **Under review**

B-domains Unfolding Pattern



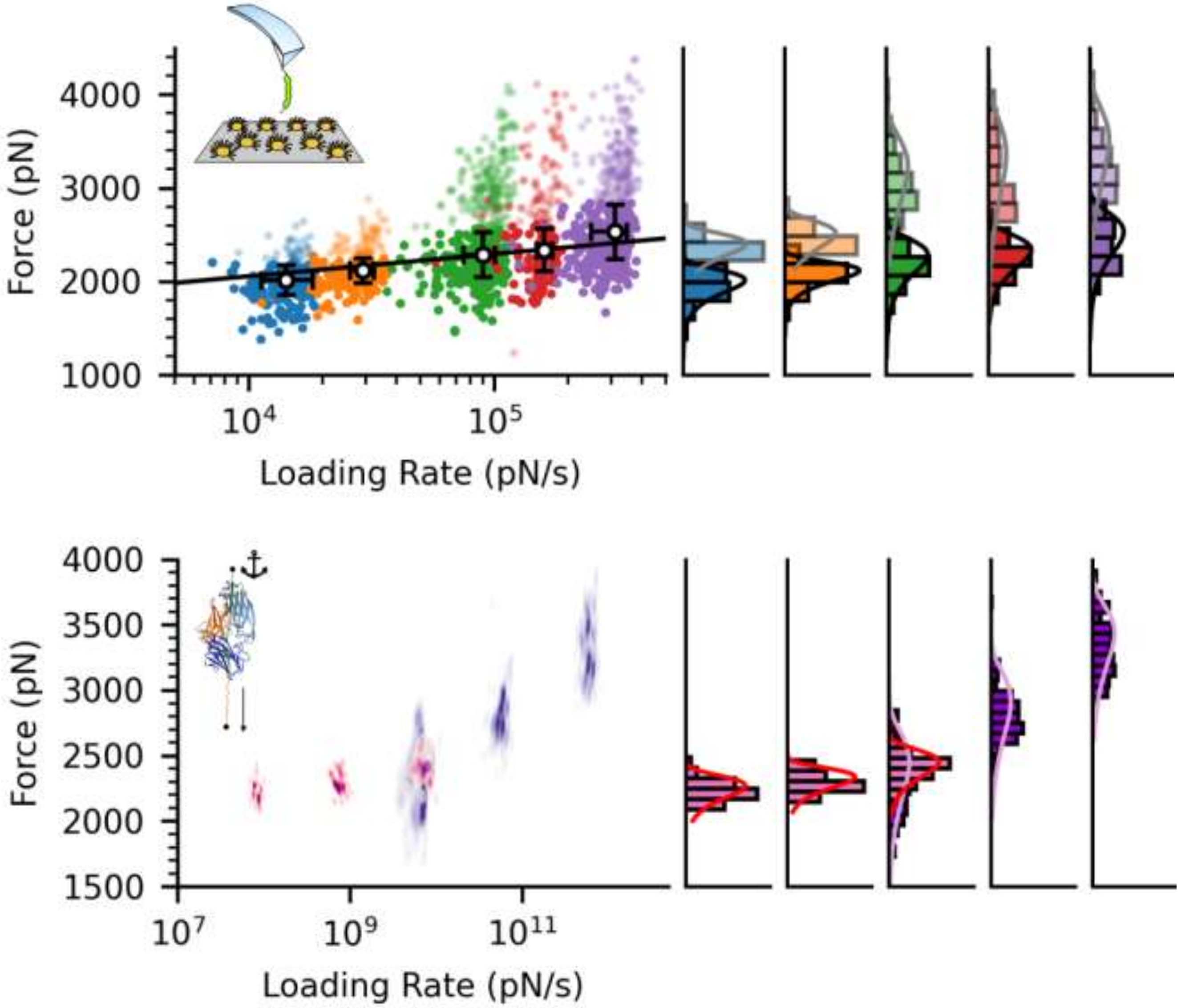


The proximal peptide is probably the target of SdrD

Rafael C. Bernardi
rcbernardi@auburn.edu

C Chantraine*, PSFC Gomes*, …, RC Bernardi[#], YF Dufrêne[#]. **Under review**

Single Molecule Force Spectroscopy *in vitro* and *in silico*



Simulation Details:

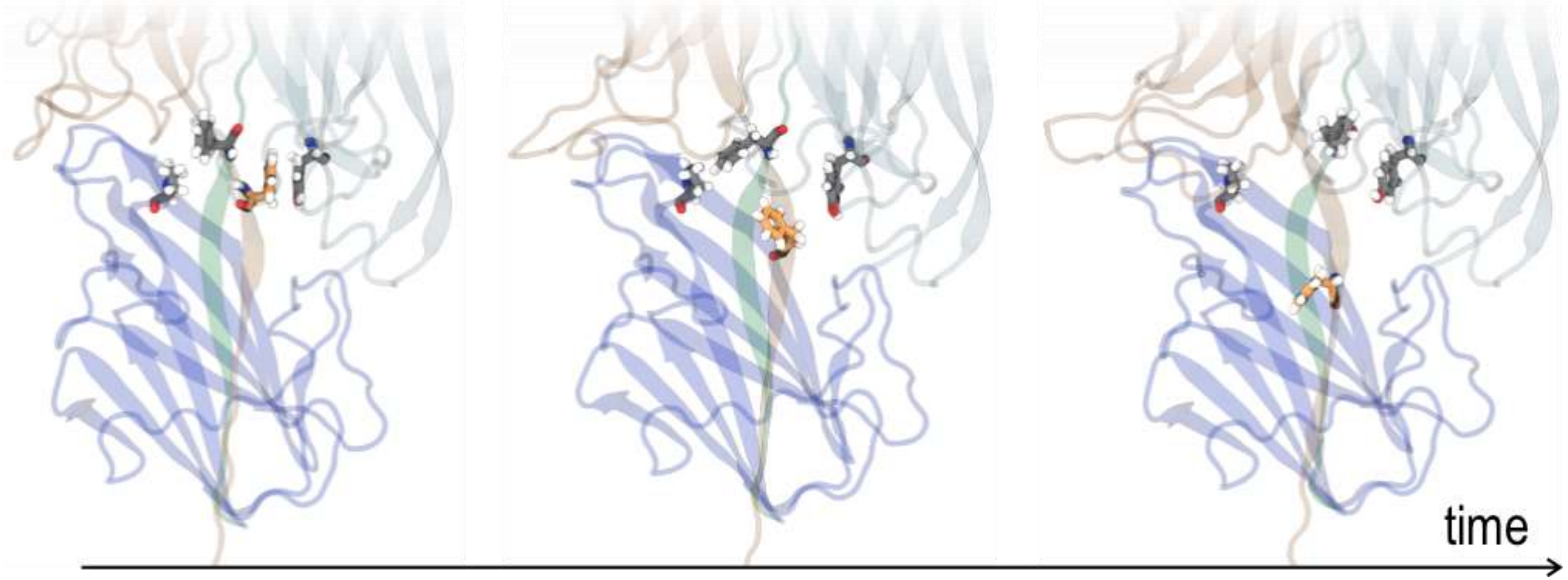
- **System Size:** ~200,000 atoms
- **Simulation Time:**
  - 107 μs all-atom MD (aa-MD)
  - 760 μs coarse-grained MD (CG-MD) - Gromacs
- **NAMD 3.0:**
  - CHARMM 36 force field
  - 4 fs timestep
  - 12 Å cutoff for long-range interactions + PME

Performance:

- **Lab DGX-A100 Cluster:**
  - 265 ns/day per GPU (2.1 μs/day)
- **NCSA Delta (4 A-100 GPUs):**
  - 271 ns/day per GPU (1.1 μs/day total)
- **NCSA Delta AI (GH200 Nodes with 4 GPUs):**
  - 381 ns/day per GPU (1.5 μs/day total)

C Chantraine*, PSFC Gomes*, …, RC Bernardi#, YF Dufrêne#. **Under review**

SdrD:DSG-1 vs SdrG:Fgβ

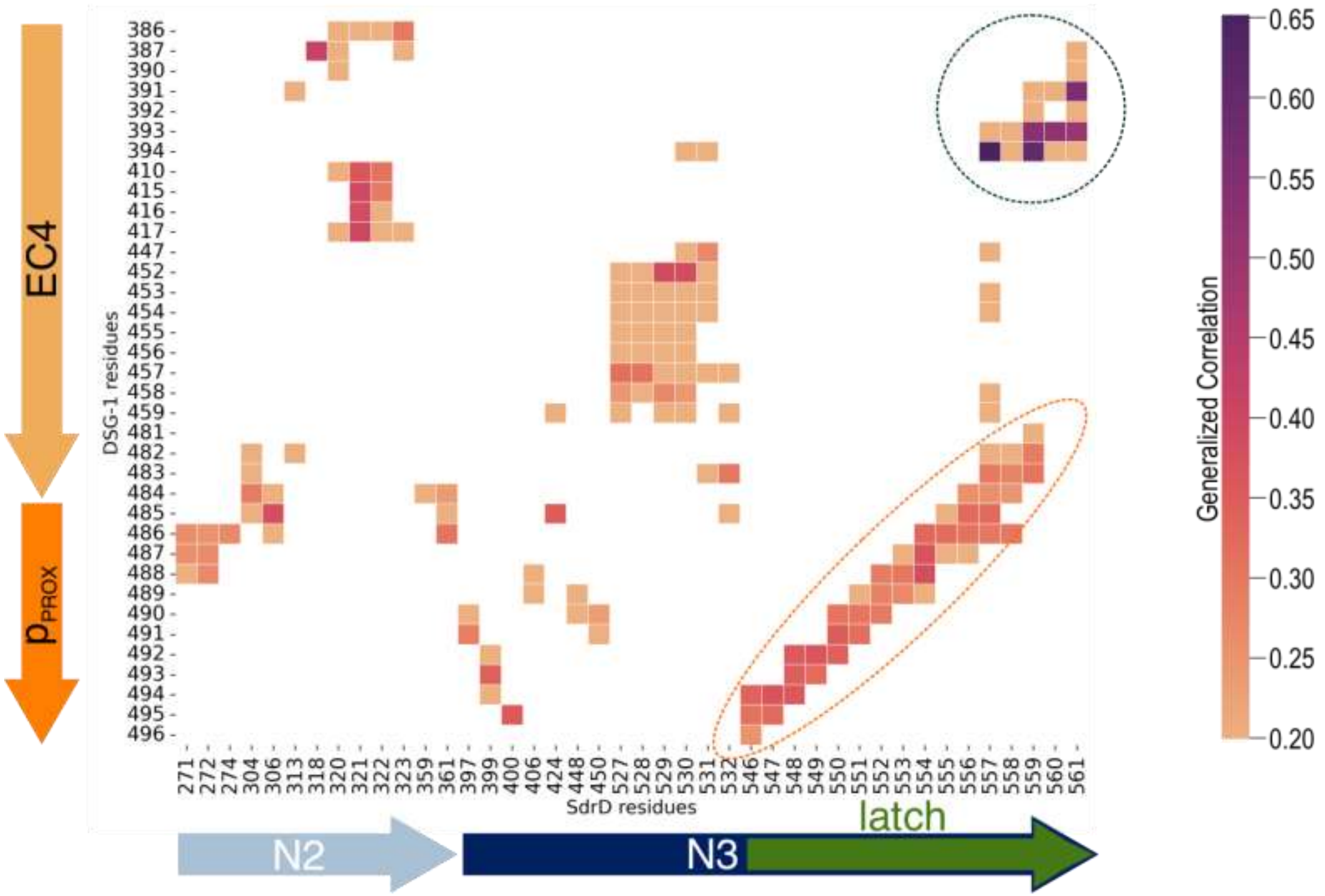


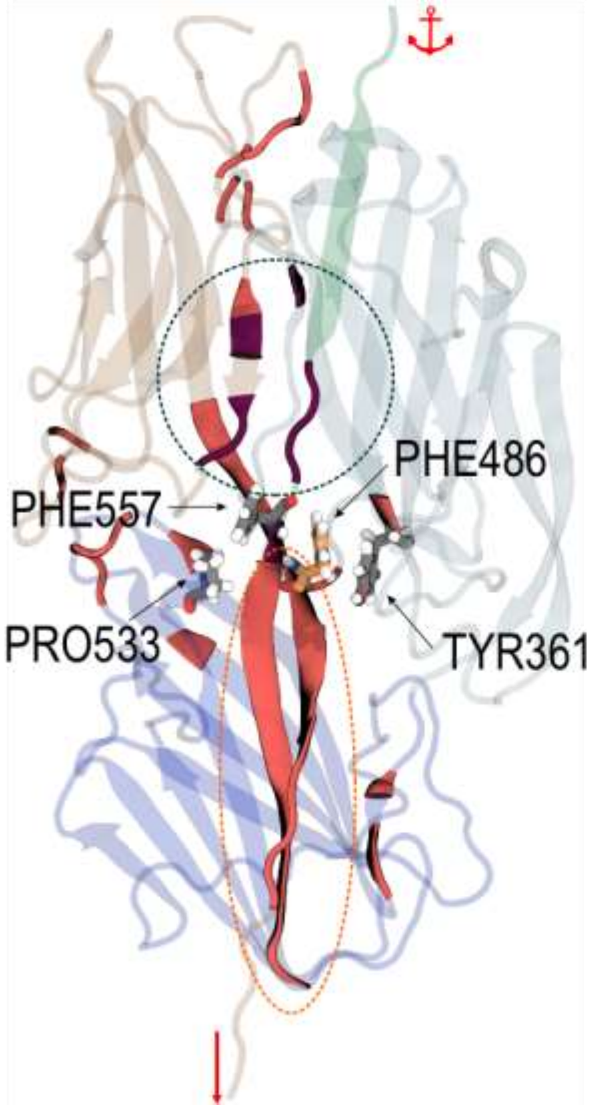
π-stacking at the junction residues is a key distinguishing feature of the SdrD:DSG-1 complex when compared to the SdrG:Fgβ complex.

Rafael C. Bernardi
rcbernardi@auburn.edu

C Chantraine*, PSFC Gomes*, …, RC Bernardi#, YF Dufrêne#. **Under review**

Network Analysis of the Hyper Mechanostable SdrD:DSG-1 Complex

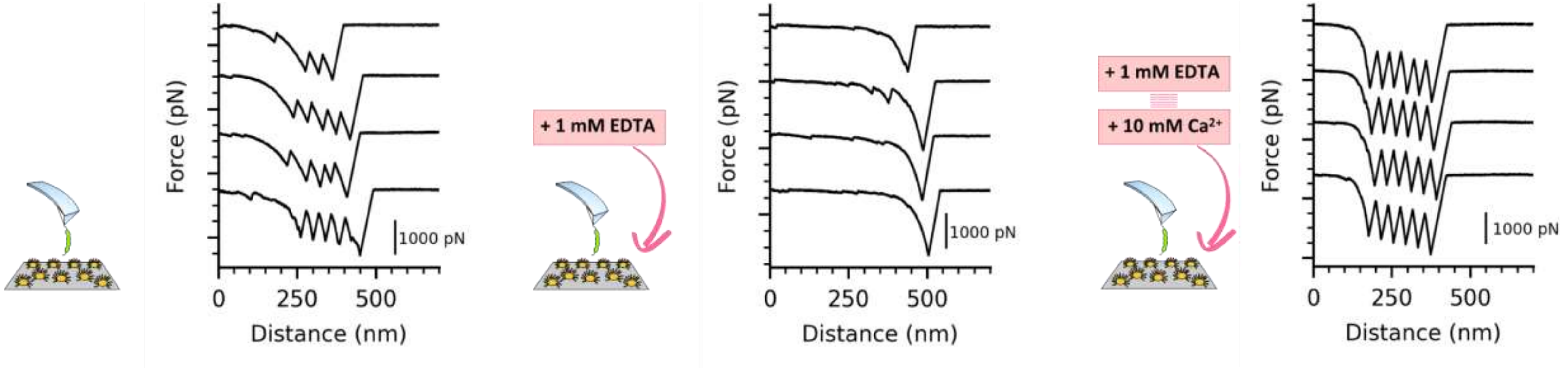


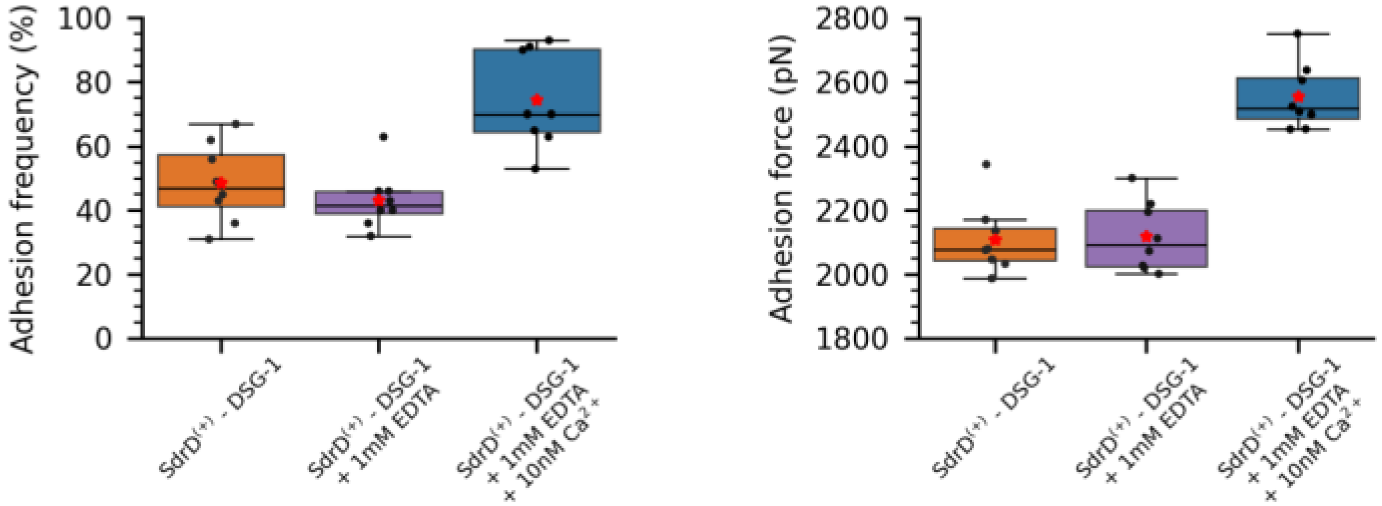


Rafael C. Bernardi
rcbernardi@auburn.edu

C Chantraine*, PSFC Gomes*, …, RC Bernardi#, YF Dufrêne#. **Under review**

Calcium Regulation of SdrD:DSG-1 Complex

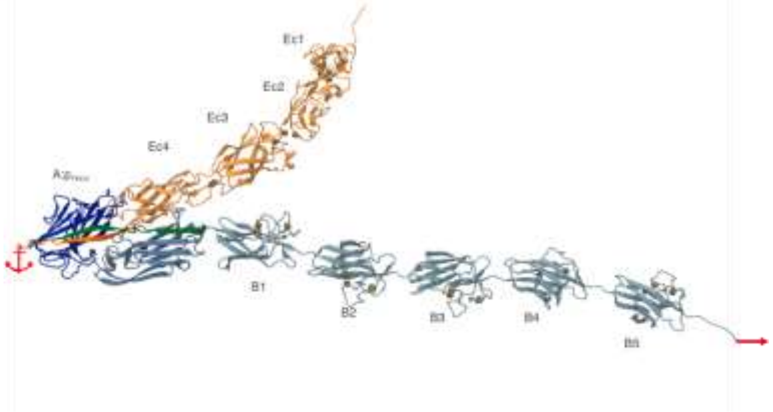


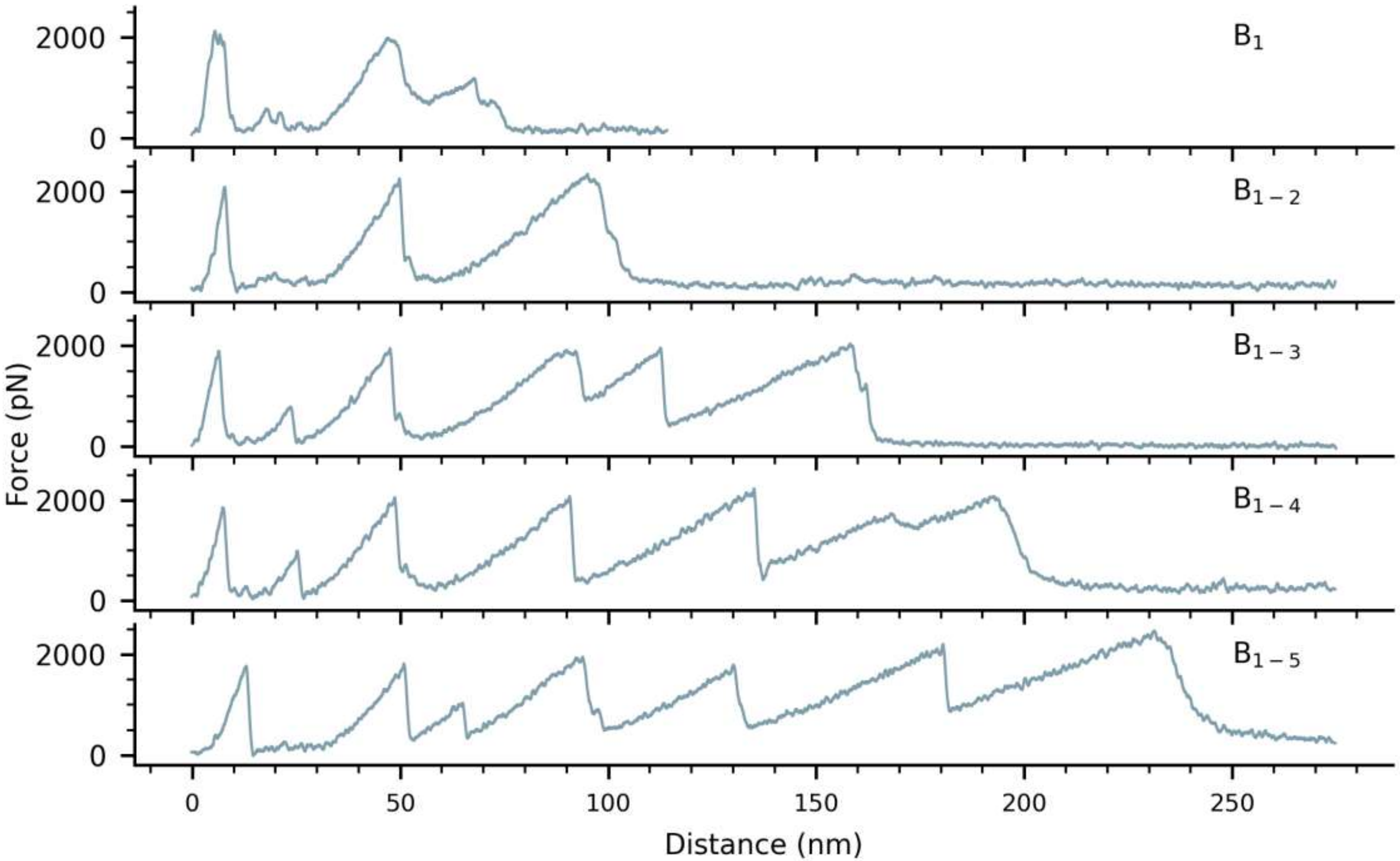


Calcium stabilizes the complex, making this the most mechanically robust protein-protein interaction observed to date.

C Chantraine*, PSFC Gomes*, …, RC Bernardi#, YF Dufrêne#. **Under review**

Is the number of B-domains Influencing the Mechanostability of the A-domain?

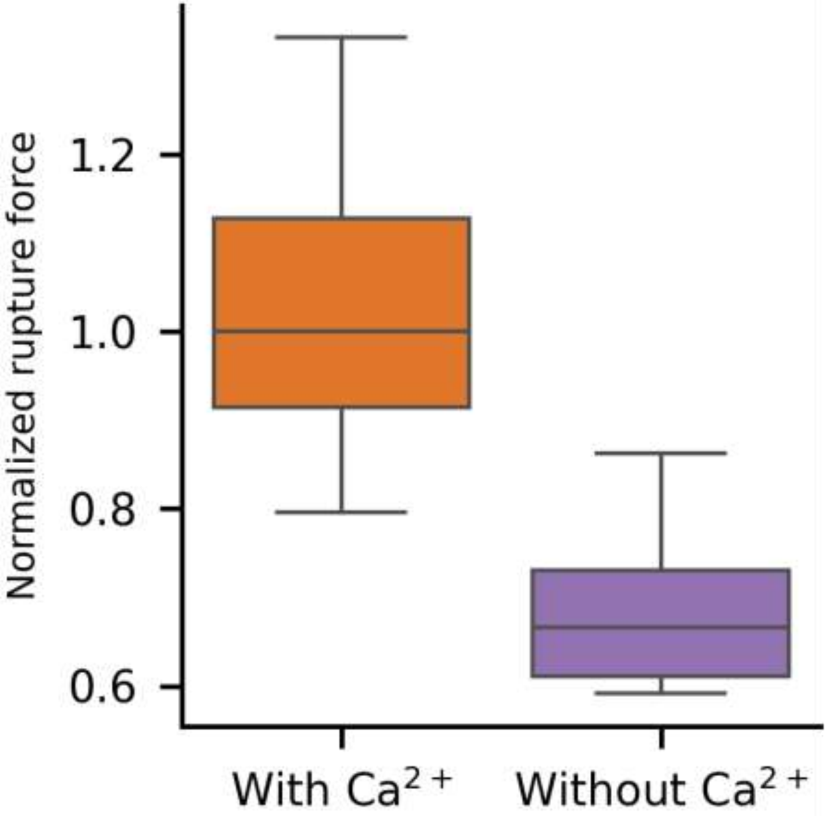


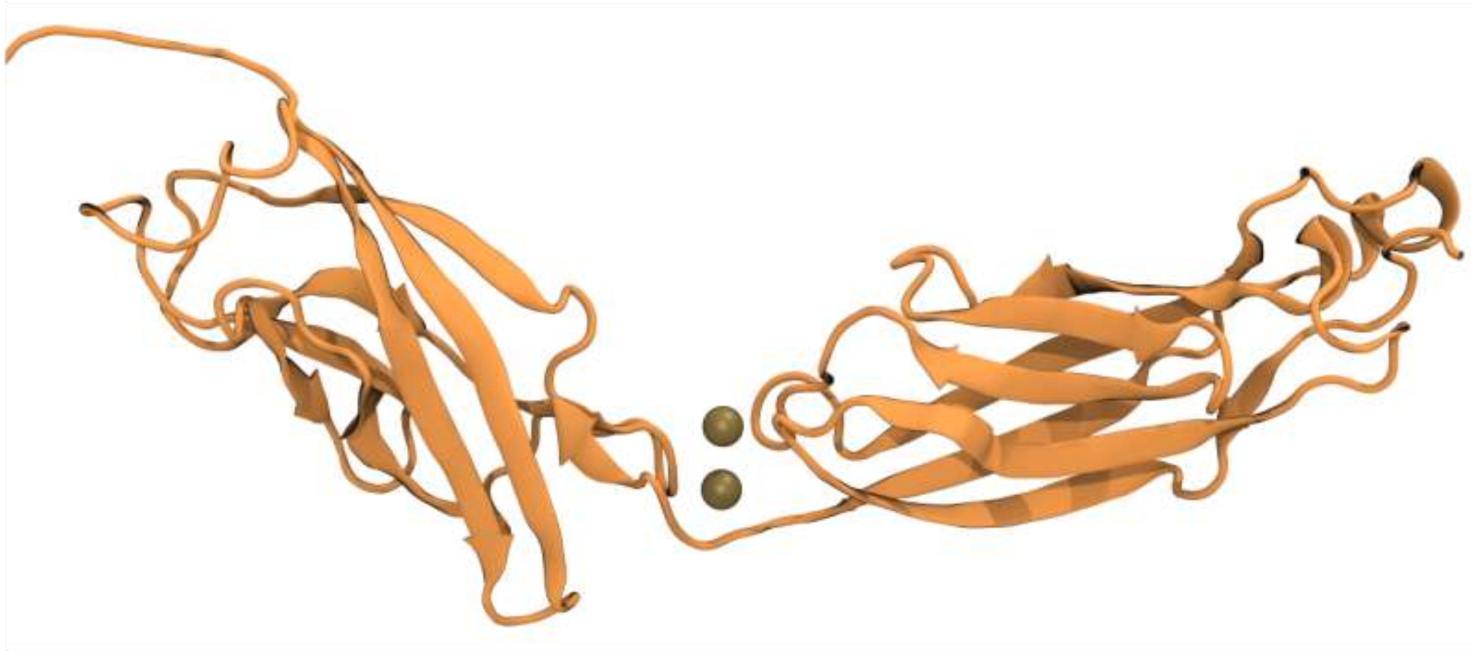


The B-domains have no direct impact on the A-domain.

C Chantraine*, PSFC Gomes*, …, RC Bernardi#, YF Dufrêne#. **Under review**

Calcium Regulation of SdrD:DSG-1 Complex



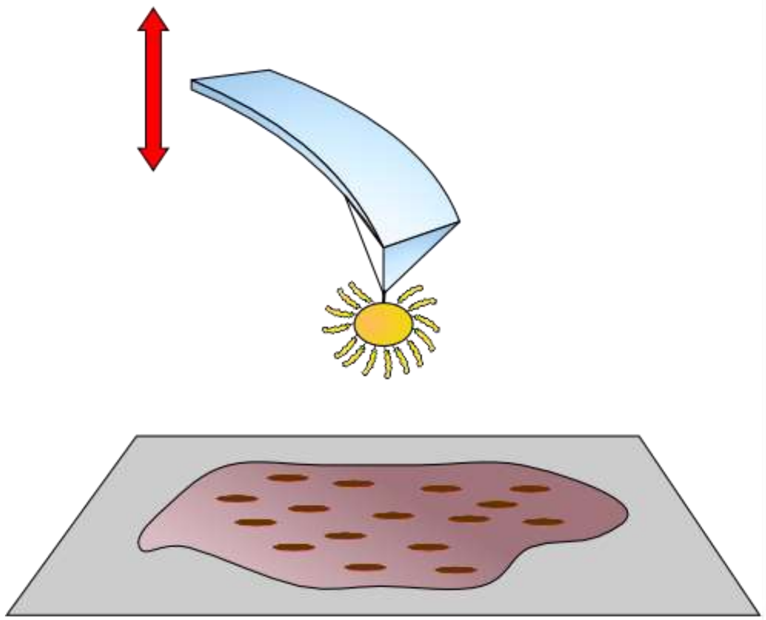


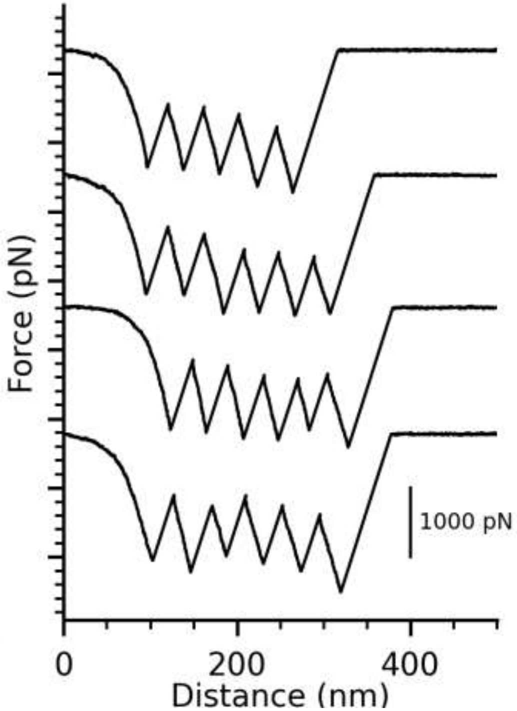
Rafael C. Bernardi
rcbernardi@auburn.edu

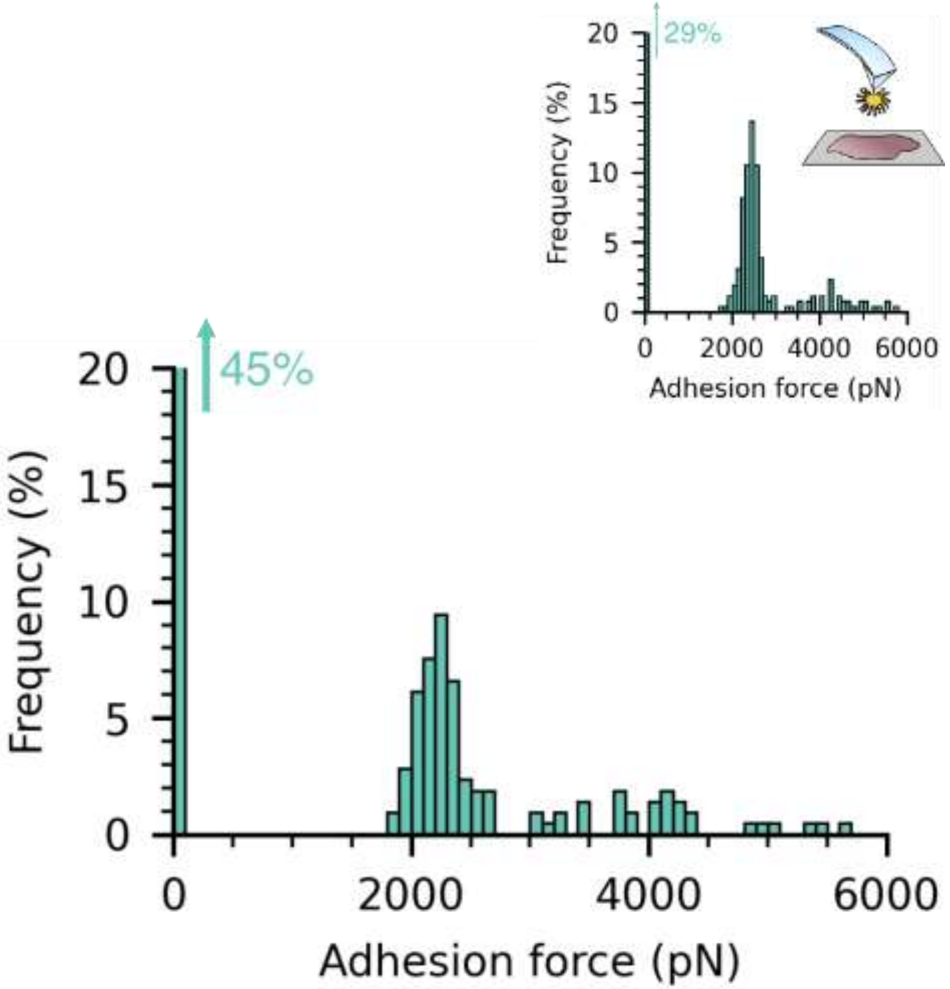
C Chantraine*, PSFC Gomes*, …, RC Bernardi#, YF Dufrêne#. **Under review**

How does calcium affect infections?

SdrD:(Atopic Dermatitis – AD cells)



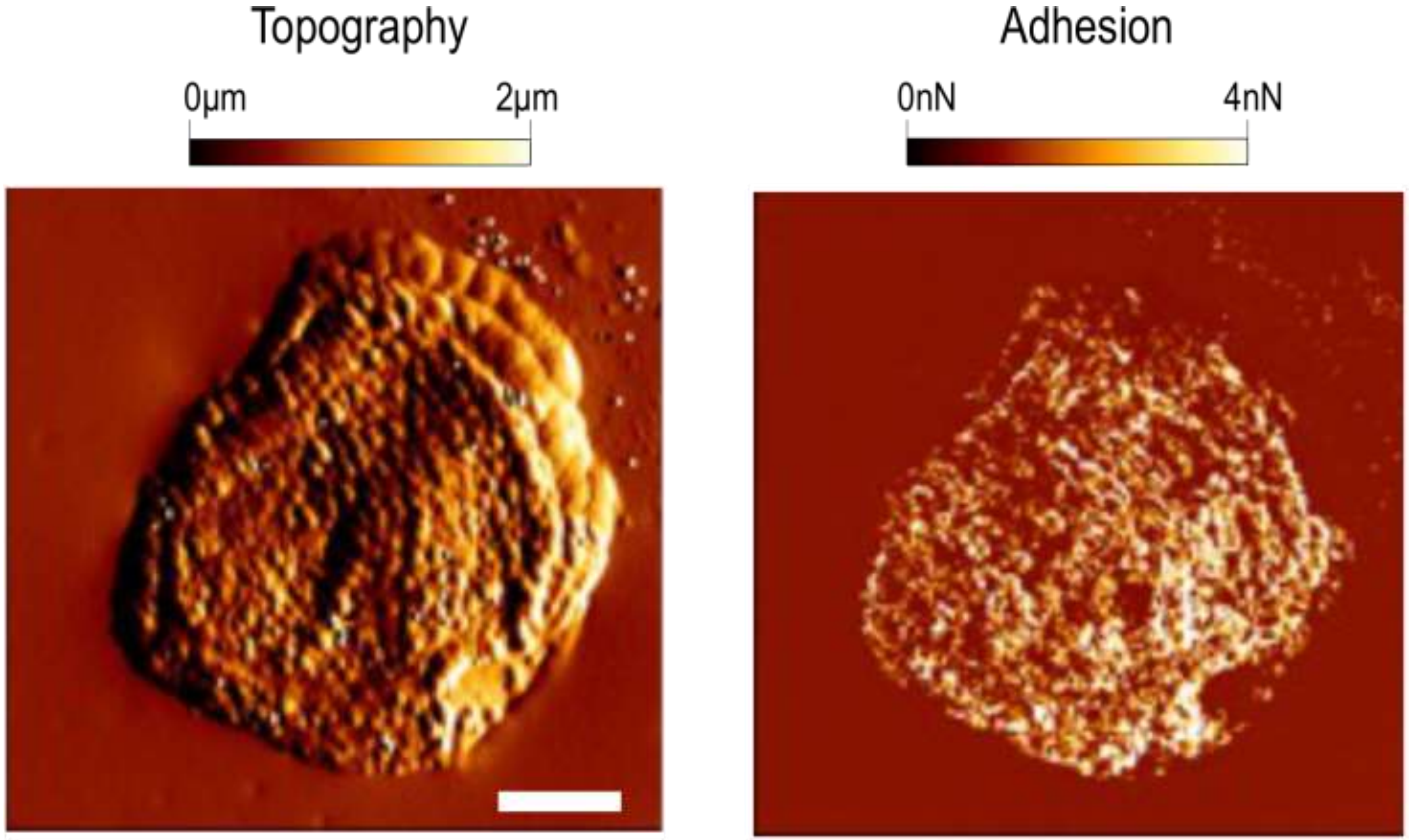




SdrD binds to AD cells with the same strength as it does to healthy cells.

C Chantraine*, PSFC Gomes*, …, RC Bernardi#, YF Dufrêne#. **Under review**

AD-cells are More Susceptible to SdrD Binding



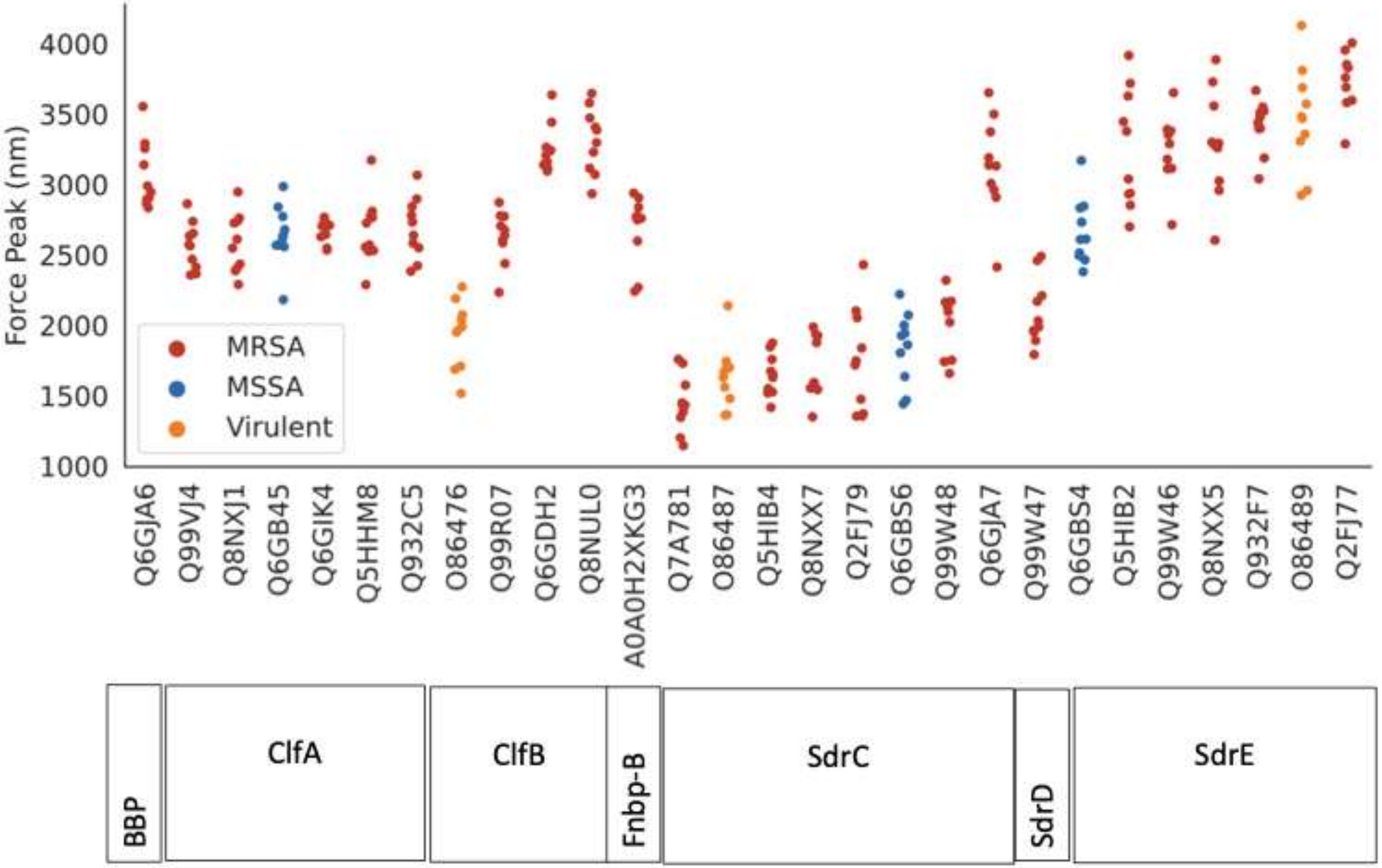
SCFS experiments with AD corneocytes show increased adhesion frequencies at higher rupture forces, likely due to the abnormal, diffuse distribution of DSG-1 on the cell surface

C Chantraine*, PSFC Gomes*, …, RC Bernardi#, YF Dufrêne#. **Under review**

Evolution of Mechanostability

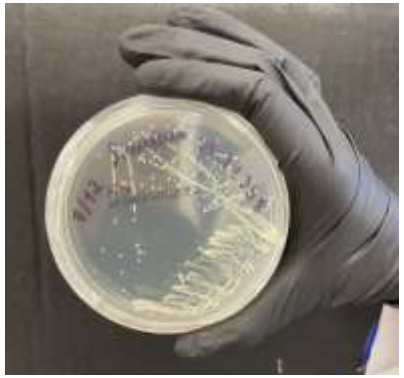
How Adhesin’s Bonds Became Hyperstable

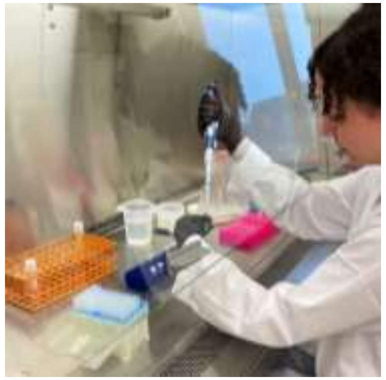
Priscila SFC Gomes



PSFC Gomes, …, RC Bernardi; **Protein structure prediction in the era of AI: challenges and limitations when applying to in-silico force spectroscopy.** Frontiers in Bioinformatics, 2022

How Adhesin's Bonds Became Hyperstable





Historical strains 19th century:

S. aureus subspecies aureus Rosenbach 1884

- DNA extraction using E.Z.N.A. Bacterial DNA kit (OMEGA Bio-Tek)

Sequencing

Michael Nash
(U. Basel / ETH)

UniProt

Isolates with MSSA and MRSA phenotypes:

- Strain NCTC 8325 (early 60s)
- Strain N315 (1982)
- Strain Mu50 (1997)

Protein sequences:

- 3D model construction
- All-atom MD simulations
- Force Resilience distribution

PSFC Gomes, ..., RC Bernardi. **in preparation**

Summary

- Introduction to *in silico* SMFS
- Many biological systems respond to force in surprising, unexpected ways.
- Advanced software and exascale computing are revolutionizing our understanding of mechano-active proteins.
- Bacteria exploit hyperstable non-covalent bonds to adhere to host cells.
- Calcium plays a key role in regulating these adhesive interactions.
- Dynamic network analysis provides valuable insights into protein-protein interactions.
- Evolutionary pressures are driving changes in pathogen adhesion properties.

62nd Hands-On Workshop on Computational Biophysics

December 16 – 20, Auburn, AL

Explore cutting-edge computational biophysics techniques in this workshop, led by experts from the NIH Center. Topics include:

- Molecular Dynamics Simulations with NAMD 3.0
- Biomolecular Visualization and Analysis with VMD 2.0
- Using Supercomputer Resources with Cybershuttle

Learn more

Marcelo C. R. Melo

Colorado State University

Sudhakar Pamidighantam

Georgia Institute of Technology

Suresh Marru

Georgia Institute of Technology

Jérôme Henin

CNRS, France

David Hardy

University of Illinois at Urbana-Champaign

Giacomo Fiorin

National Institutes of Health

Rafael C. Bernardi

Auburn University

JC Gumbart

Georgia Institute of Technology

Diego E. B. Gomes

Auburn University

Emad Tajkhorshid

University of Illinois at Urbana-Champaign

Chris Chipot

CNRS, France

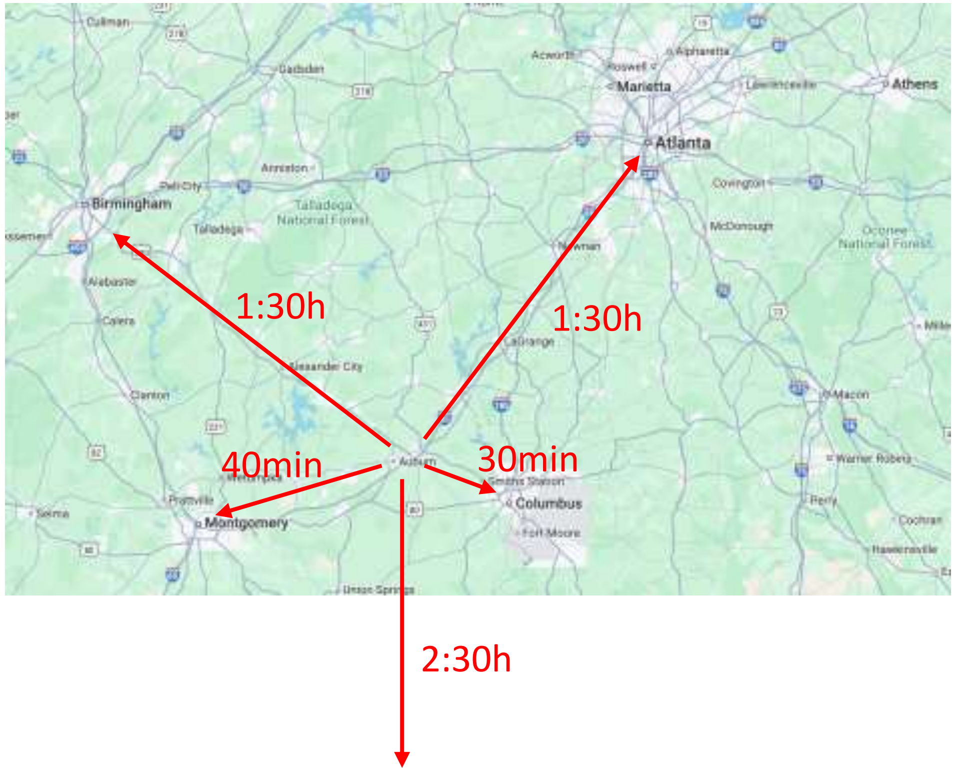
John Stone

NVIDIA

Join Auburn Physics

Engage in cutting-edge research in Atomic, Molecular, and Optical Physics; **Biophysics**; Condensed Matter Physics; Plasma Physics; Space Physics; and Physics Education Research.

Experience the newly built Leach Science Center with advanced labs, study spaces, and a rooftop astronomy terrace.



Why Choose Auburn University for Your PhD?

- R1: Top-Tier Research University
- Ranked #1 for Student Happiness
- Ranked among Top 15 Most Beautiful Campus in the U.S.
- 33,000+ Students in a Thriving Academic Community

Rafael C. Bernardi
rcbernardi@auburn.edu

Computational Biophysics Group @Auburn University

Dr. Diego EB Gomes
Dr. Marcelo CR Melo
Dr. Priscila SFC Gomes
Bruce Shukla
Bruno L Ribeiro
Maggie Pace
Meredith Forrester
Raisa SL Rosa
Toby Sizemore
Walter do Santos Filho

Collaborators

University of Illinois at Urbana-Champaign
Prof. Emad Tajkhorshid
Prof. Klaus Schulten
Prof. Zan Luthey-Schulten

Ludwig Maximilians University of Munich, Germany
Prof. Hermann Gaub

University of Basel & ETH Zurich, Switzerland
Prof. Michael Nash

Ultrech University, The Netherlands
Prof. Jan Lipfert

Weizmann Institute, Israel
Prof. Ed Bayer

Catholic University of Louvain, Belgium
Prof. David Alsteens
Prof. Yves Dufrene

University of Colorado
Tom Perkins

University of Wisconsin – Milwaukee
Ionel Popa

Funding

NSF

MCB-2143787
CAREER: In Silico Single-Molecule Force Spectroscopy

NIH
National Institutes of Health

R24 GM-145965
Resource for Macromolecular Modeling and Visualization

AUBURN UNIVERSITY
COLLEGE OF SCIENCES AND MATHEMATICS

May the Force be with you!