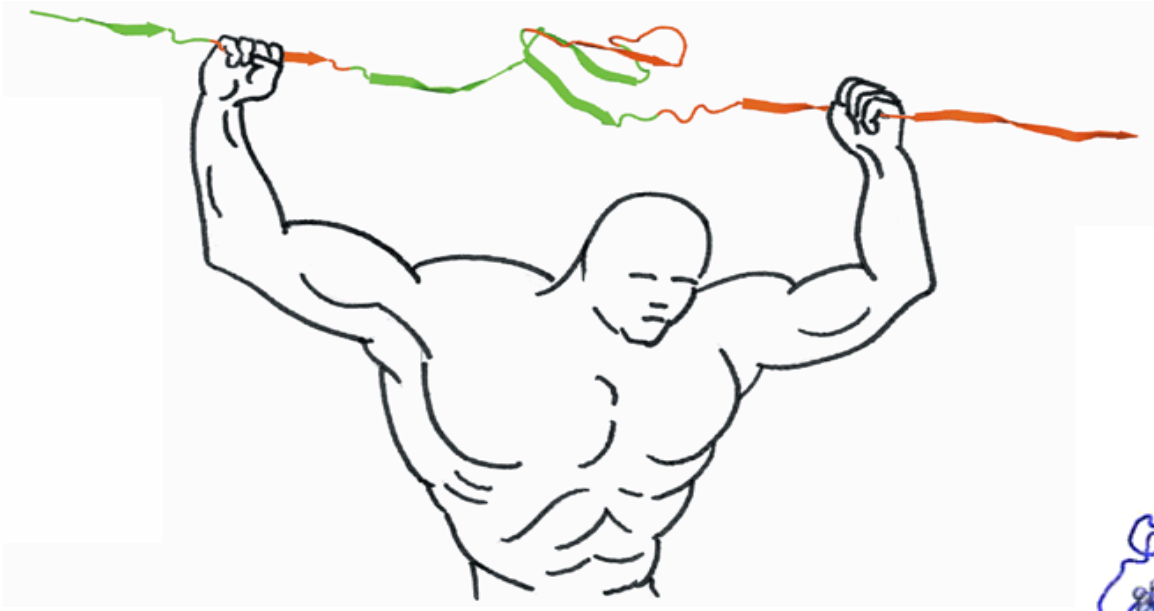
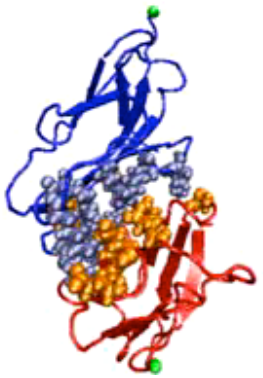
Mechanical Proteins





Stretching imunoglobulin and fibronectin domains of the muscle protein titin

Adhesion Proteins of the Immune System

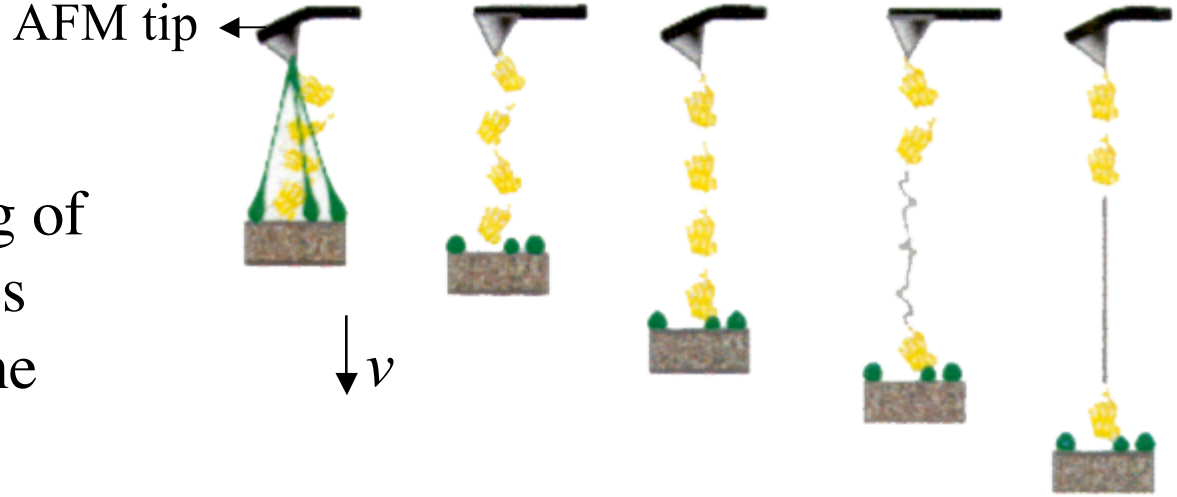
National Center for Research Resources

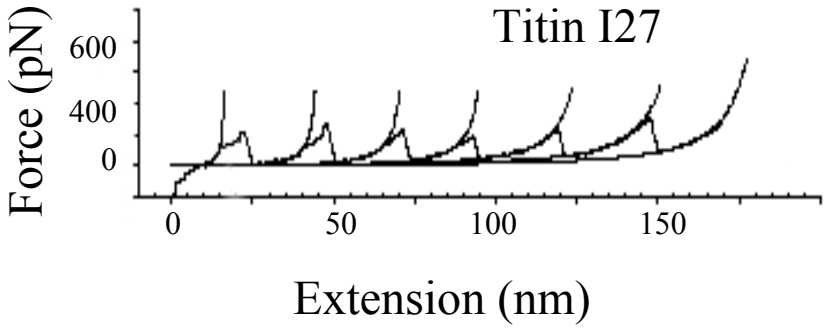
NIH Resource for Macromolecular Modeling and Bioinformatics
Theoretical Biophysics Group,
Beckman Institute, UIUC

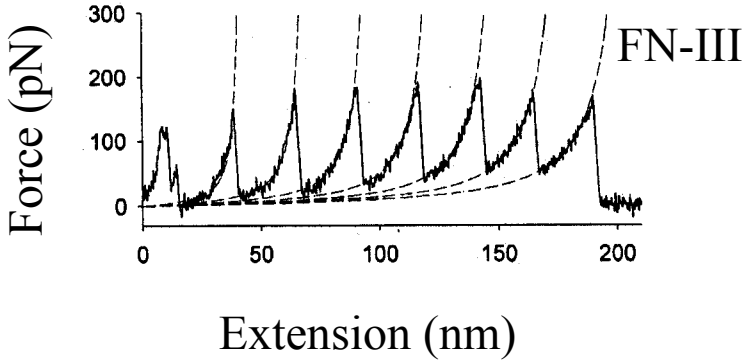
Immunoglobulin Domains

AFM Studies of Titin And FN-III Modules

- Forced unfolding of identical modules occurs one by one

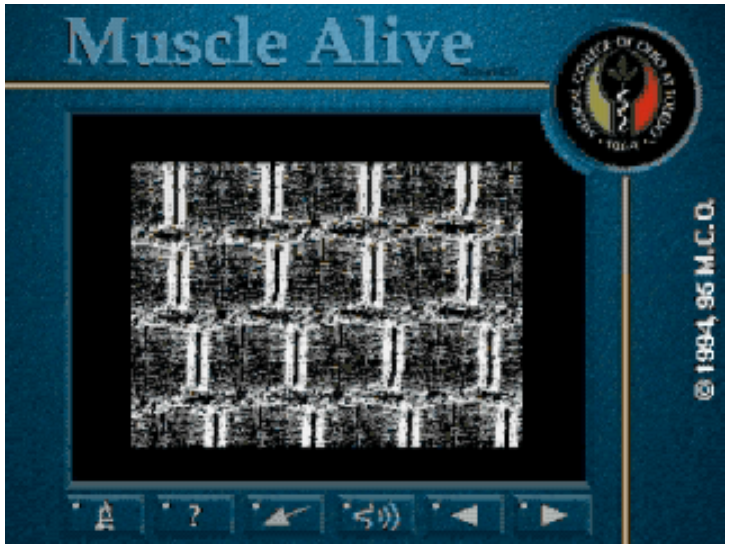


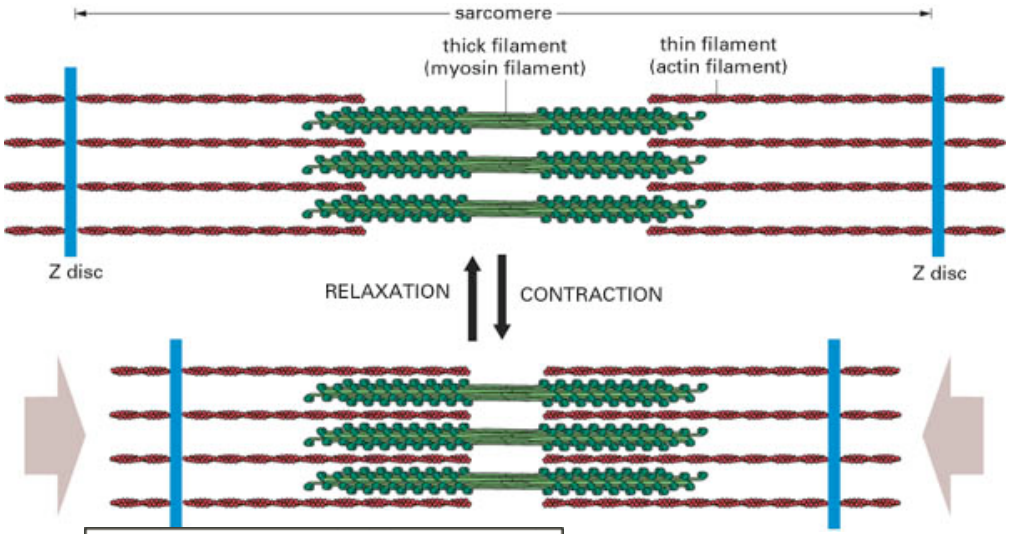


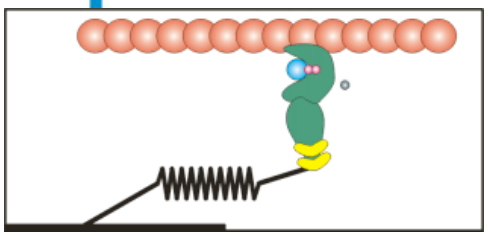


Reviewed in **Fisher** *et al*. *Nature Struct. Biol.* **7**:719-724 (2001)

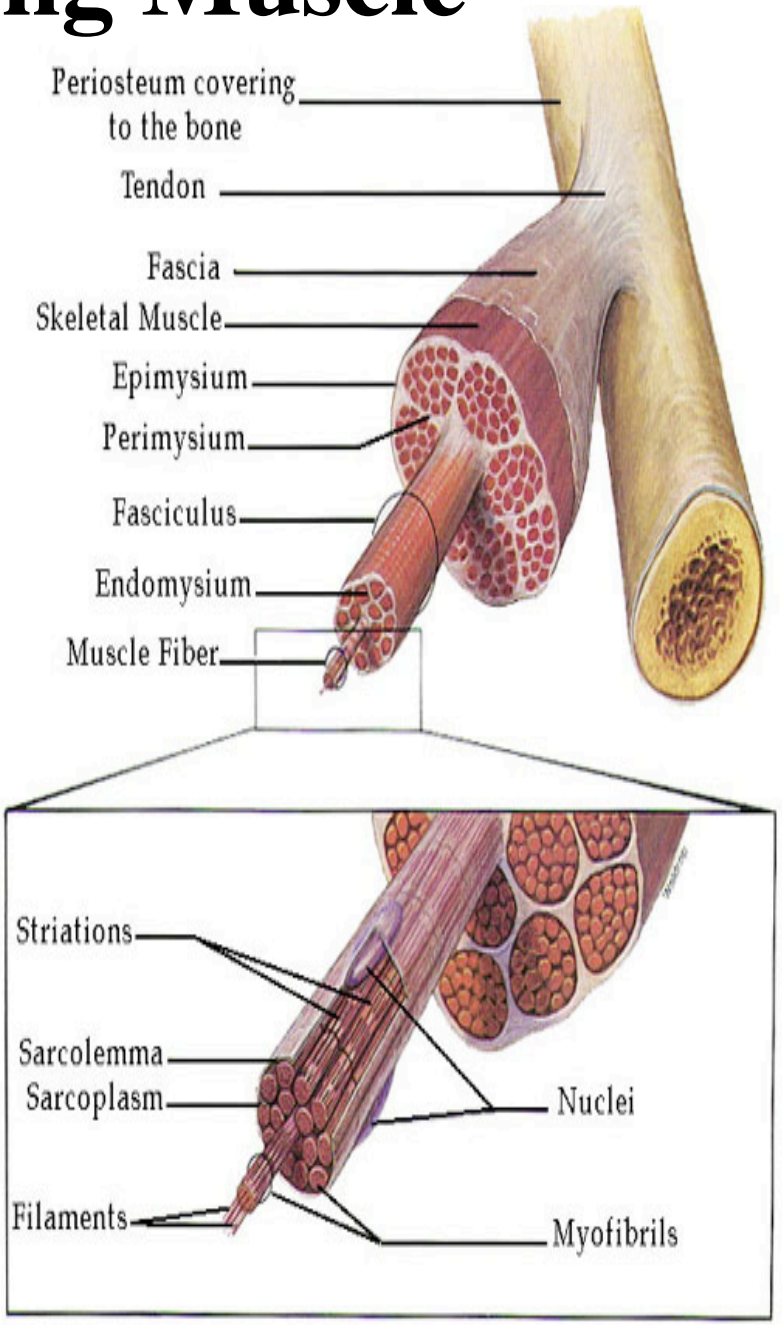
Contracting and Relaxing Muscle



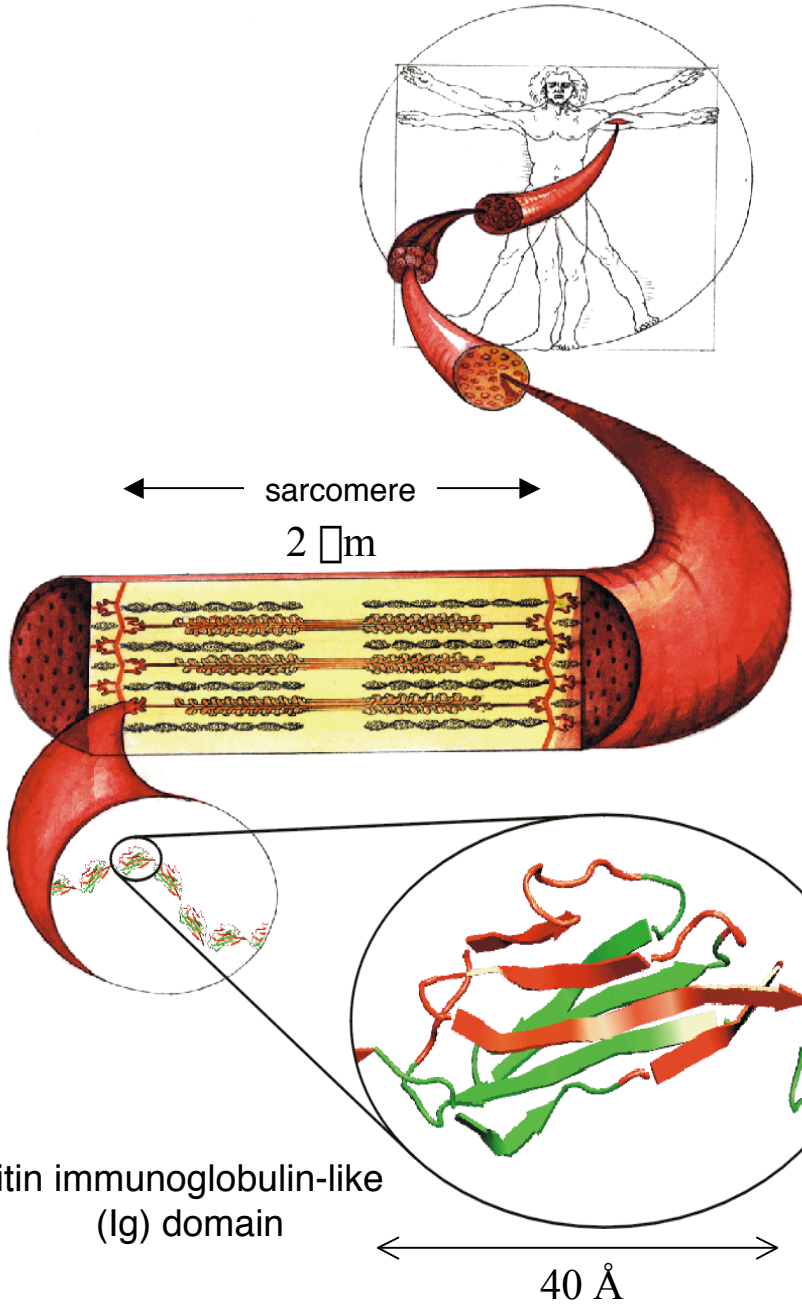


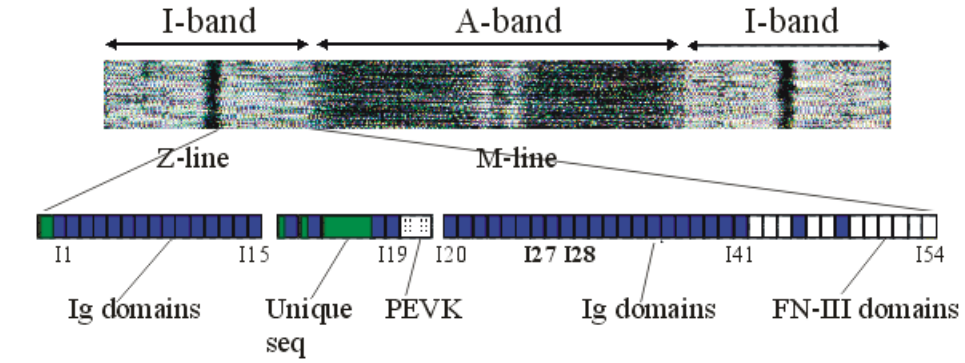


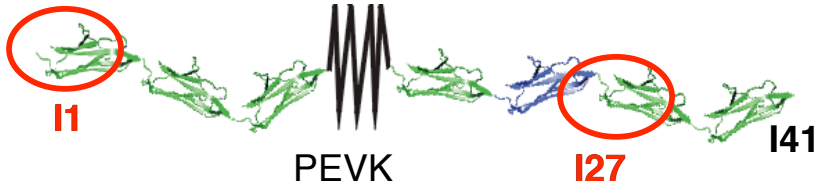
Needs ATP!



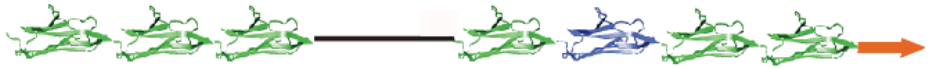
Titin the Longest Protein in the Human Genome







PEVK region; random coil



Under weak forces, the PEVK region extends (entropic spring)

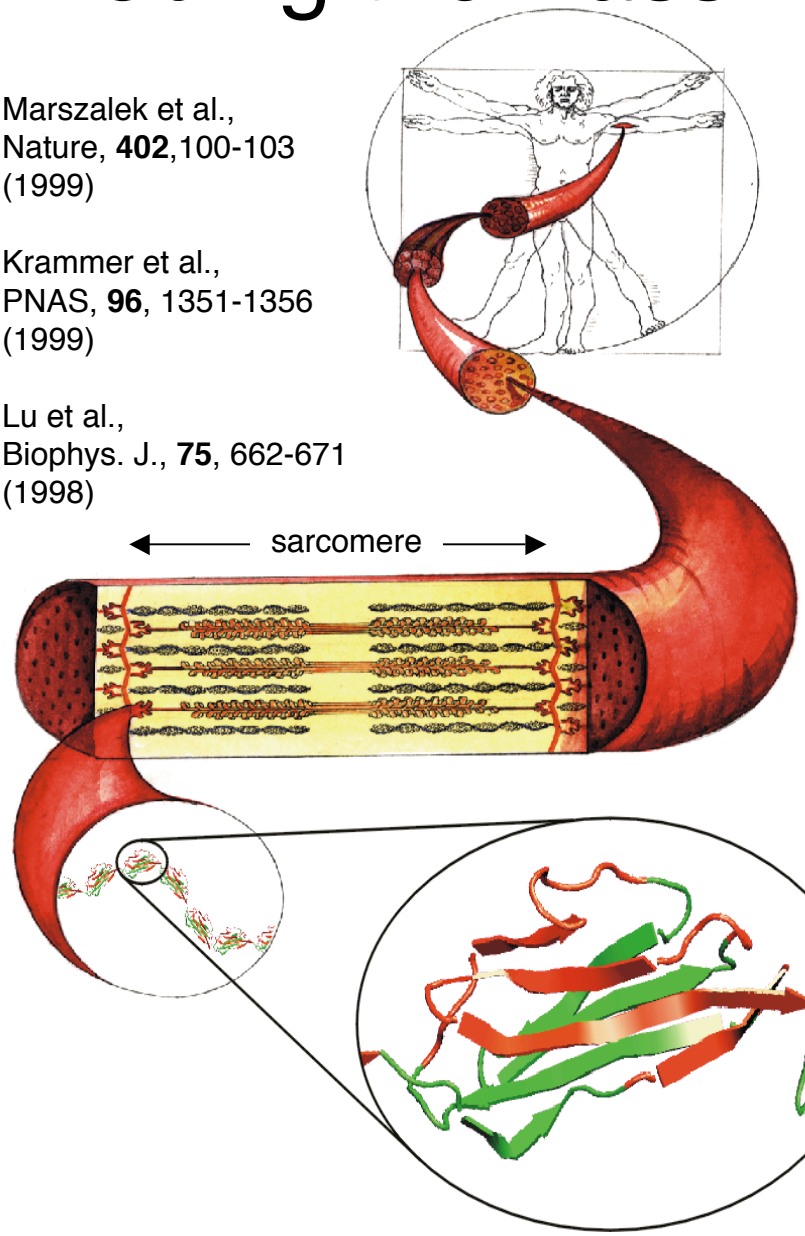
Under intermediate forces, Ig domains stretch by ~10A; under strong forces, they unfold one by one, by 250A

Probing the Passive Elasticity of Muscle: I27

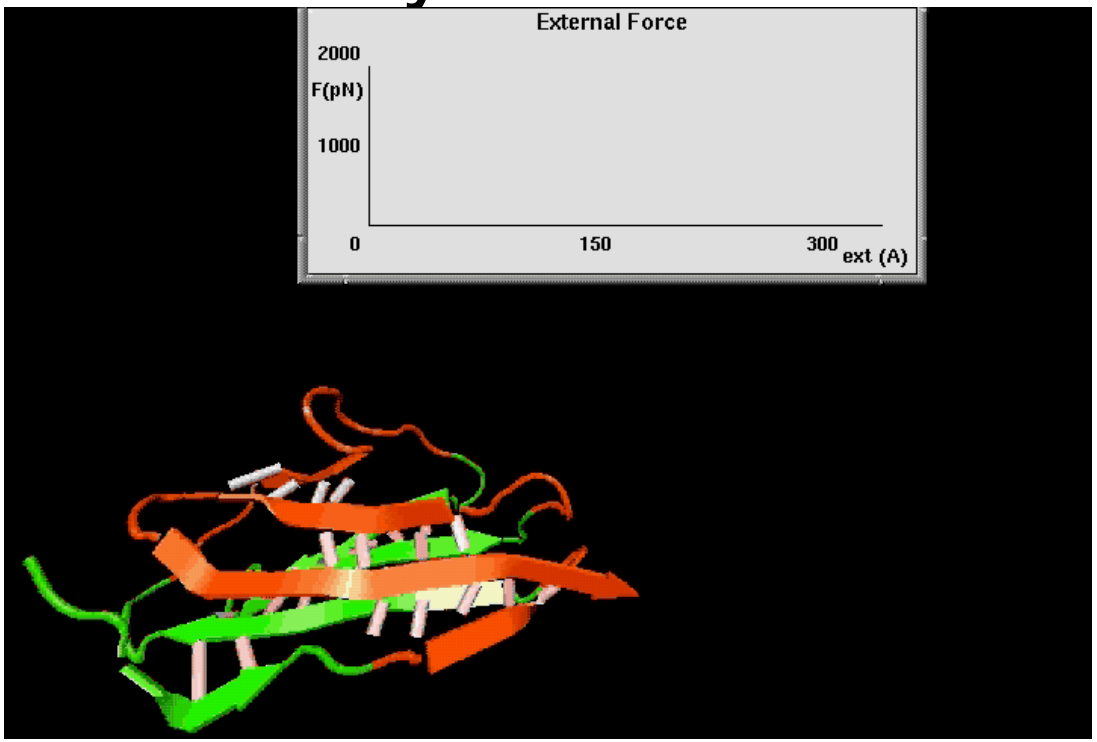
Marszalek et al.,
Nature, **402**,100-103
(1999)

Krammer et al.,
PNAS, **96**, 1351-1356
(1999)

Lu et al.,
Biophys. J., **75**, 662-671
(1998)



titin immunoglobulin-like (Ig) domain



Computational Stretching of Ig: Trajectory Animation

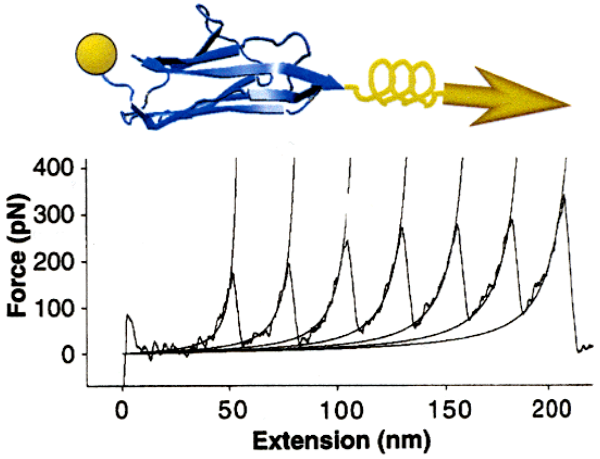
Gaub et al.,
Fernandez et al.

Hui Lu, Barry Israelewitz

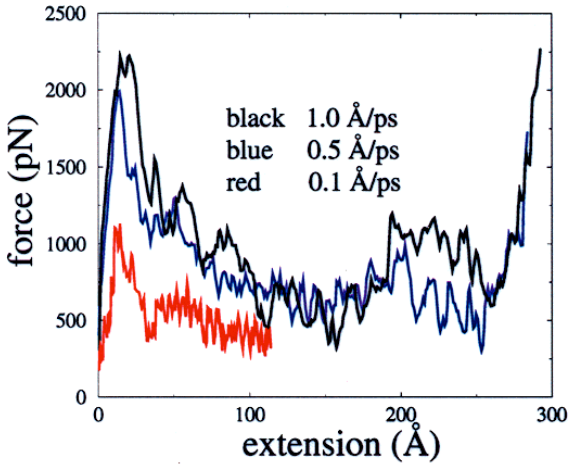
Quantitative Comparison

Bridging the gap between SMD and AFM experiments

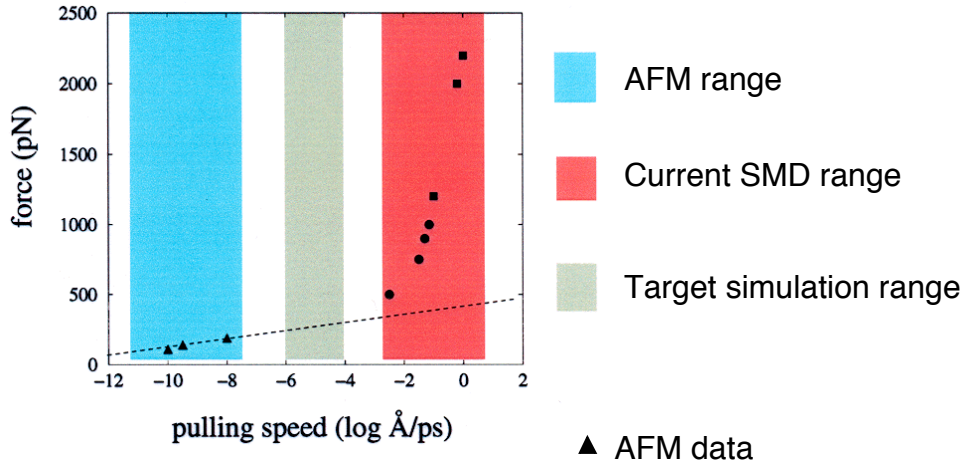
Steered Molecular Dynamics (SMD)



Force-extension curve



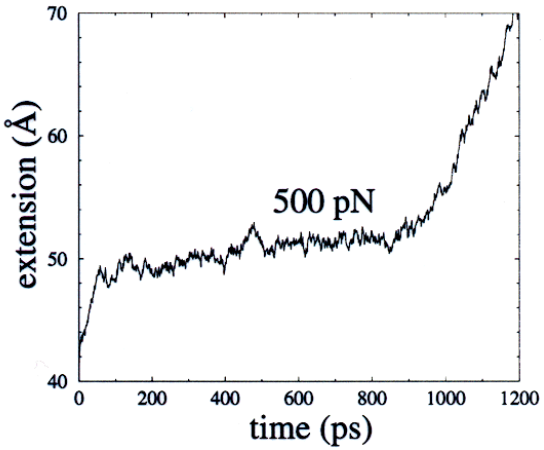
Force-pulling velocity relationship



■ ● SMD data

▲ AFM data

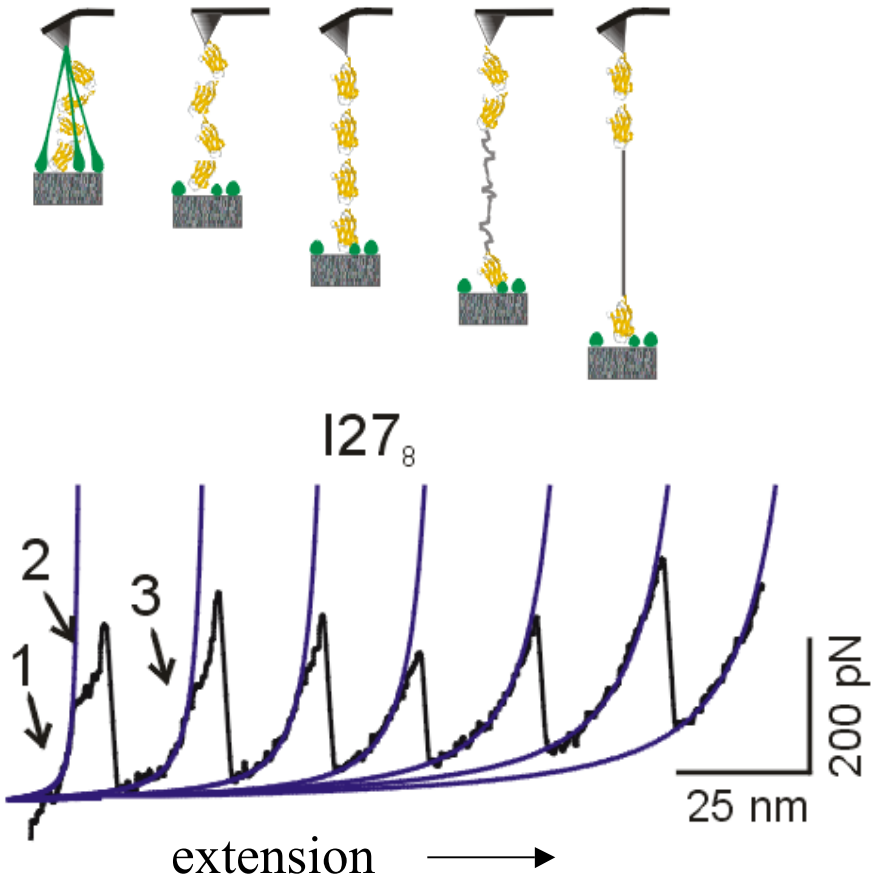
------ Extrapolation of AFM data



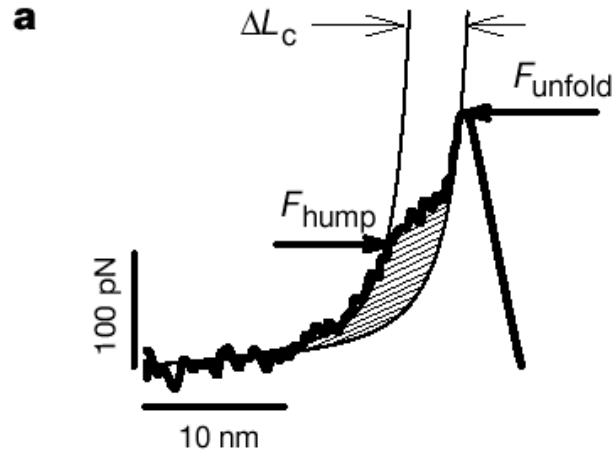
Extension curve

Hui Lu, Barry Isralewitz

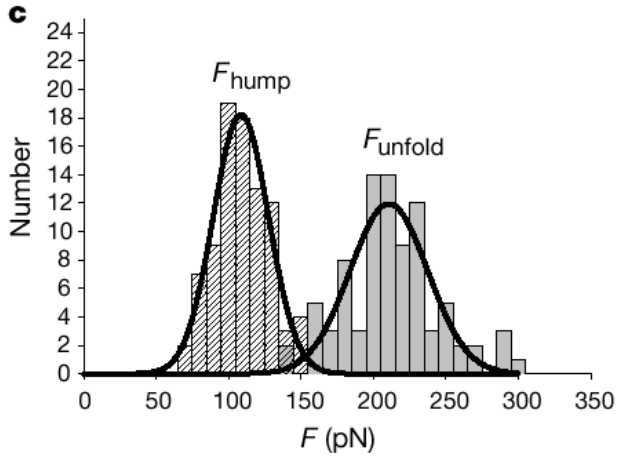
Stretching modular proteins – Detailed View



Schematic view and typical
Extension vs. force plot

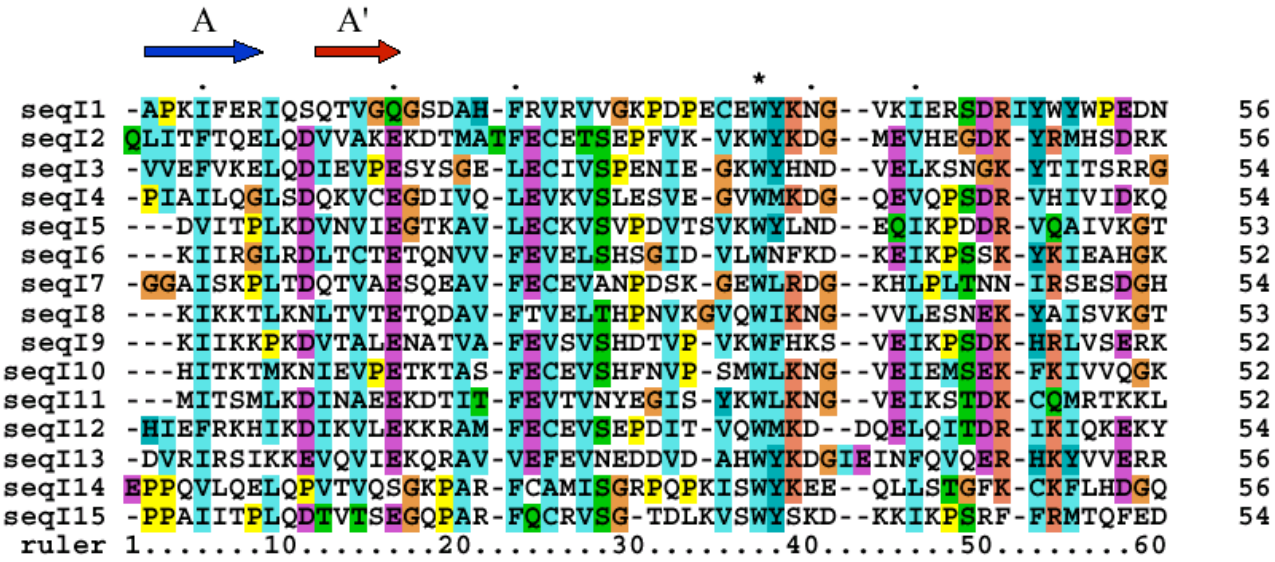


Extension occurs in two steps



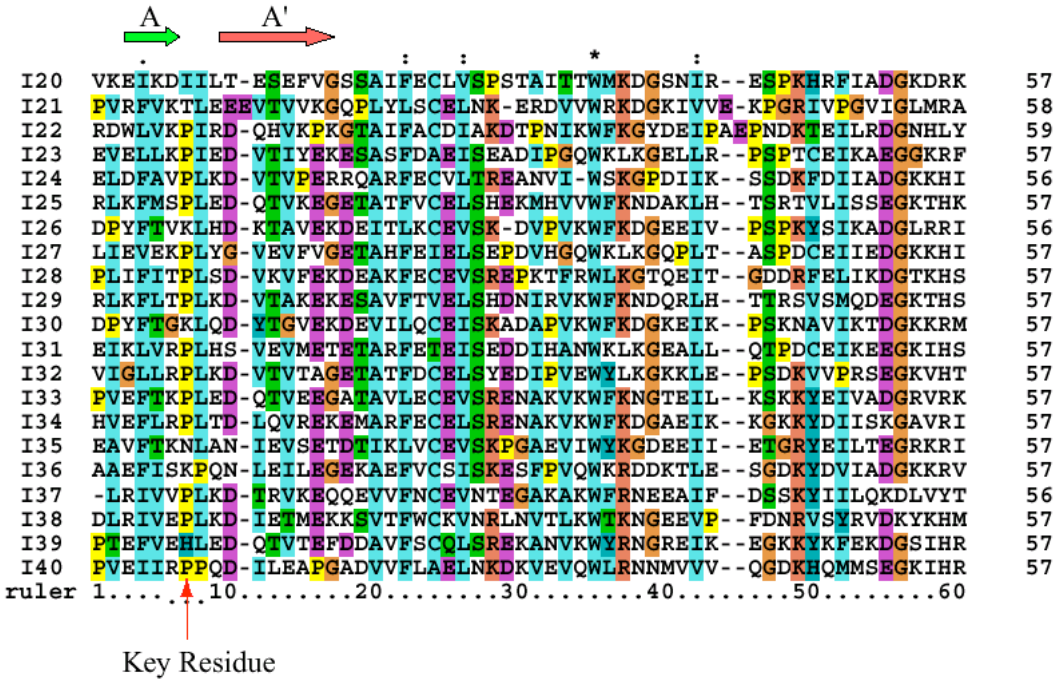
Distribution of measured forces
For step 1 and step 2

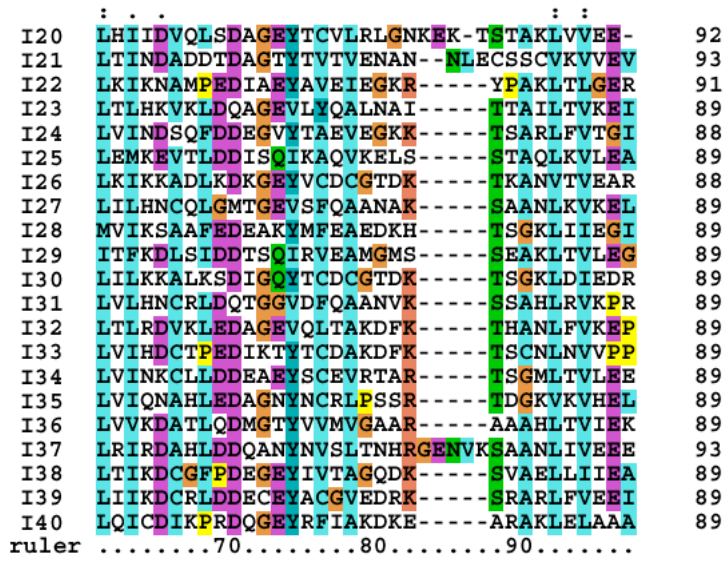
Sequence aligment of Ig modules from the proximal Ig region



I1

Sequence aligment of Ig modules from the distal Ig region



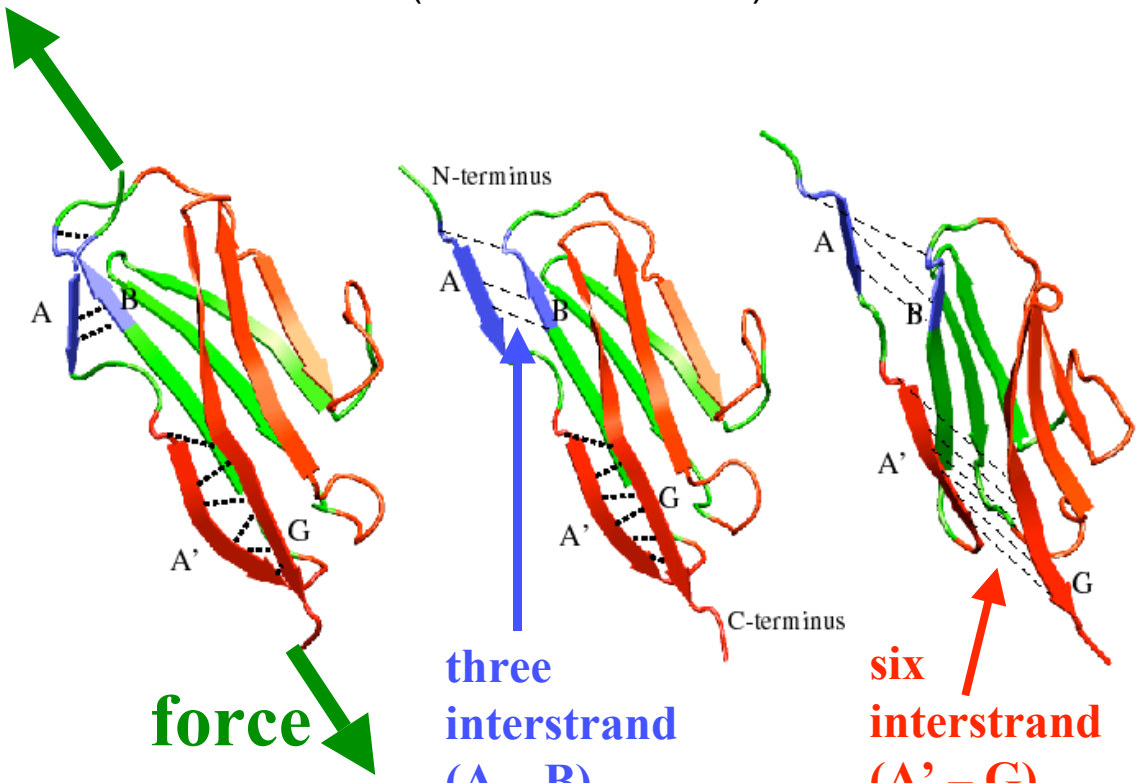


Two Force Bearing Components

I. Native structure
(2 A of extension)

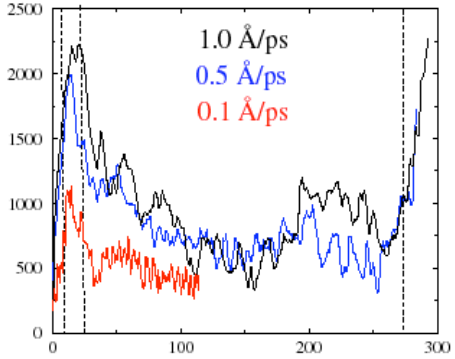
II. Mechanical unfolding intermediate
(10 A of extension)

III. Unfolded state
(250 A of extension)

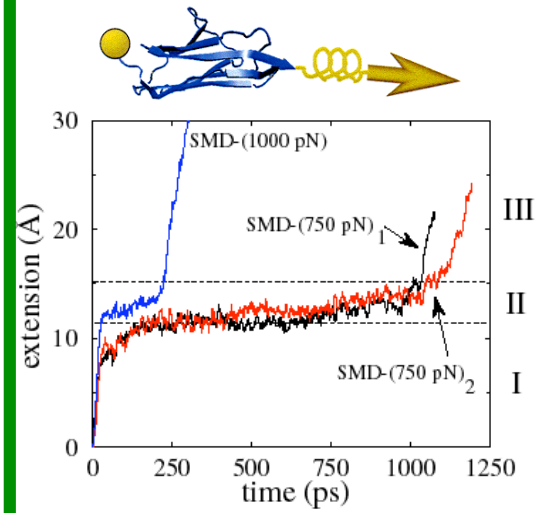


Hui Lu, Barry Israelewitz

Two types of SMD simulations



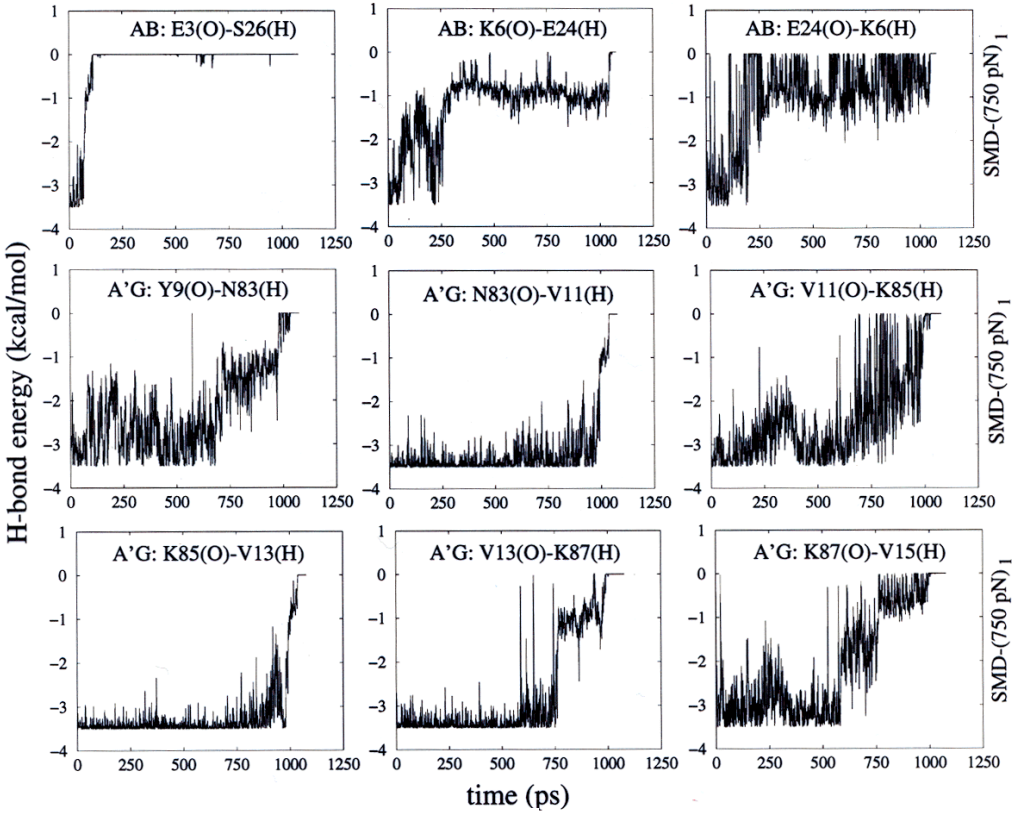
Constant velocity: Lu et al, Biophys J., 72, 1568 (1007)

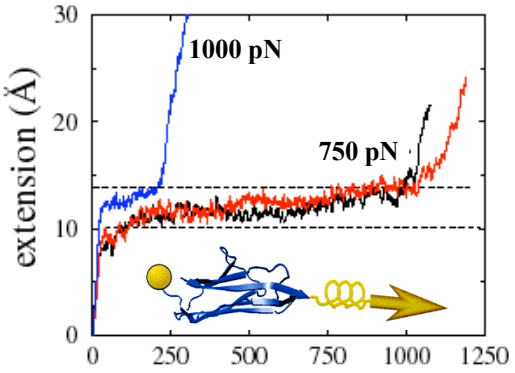


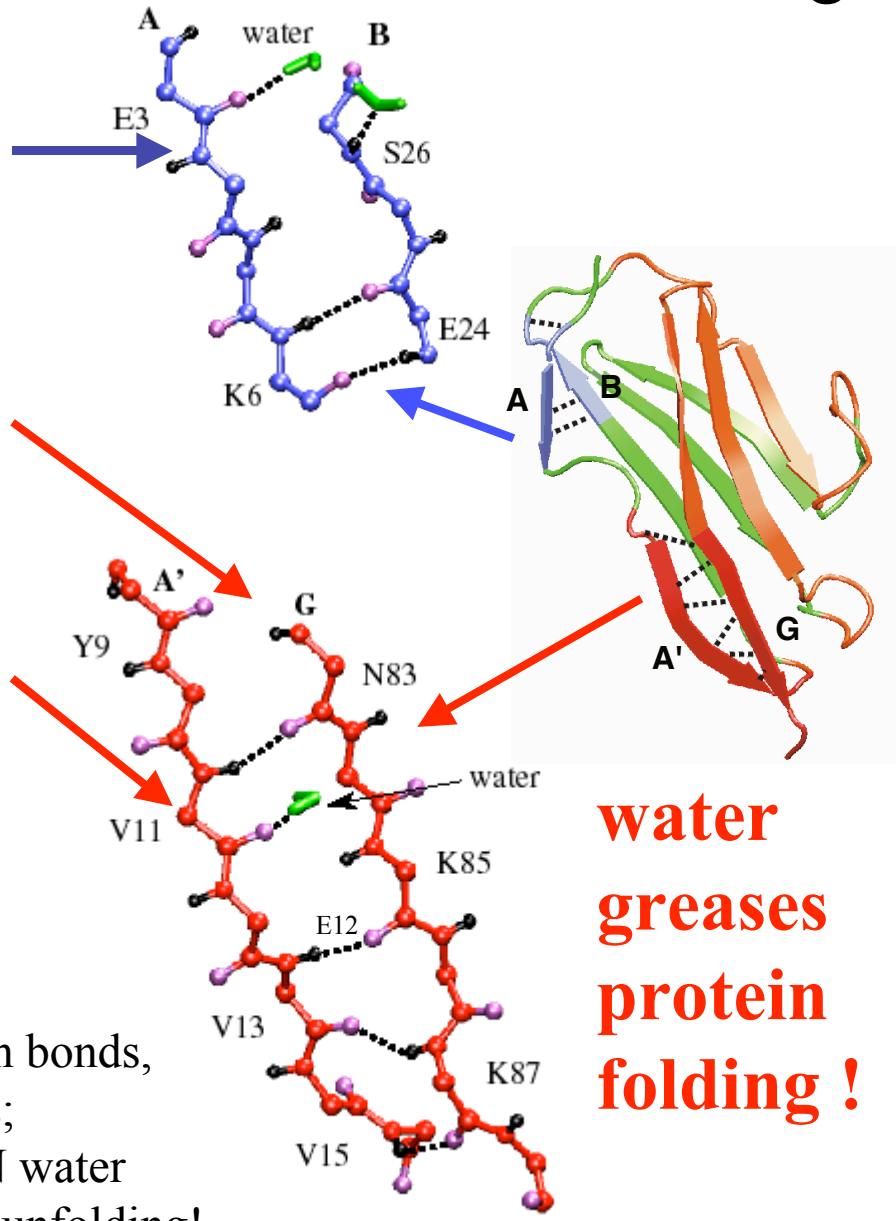
Constant force: Lu and Schulten Chem. Phys., 247 (1999) 141.

***Reviewed in* Israelewitz** *et al. Curr. Opinion Struct. Biol.* **11**:224-230 (2001)

Water-Backbone Interactions Control Unfolding







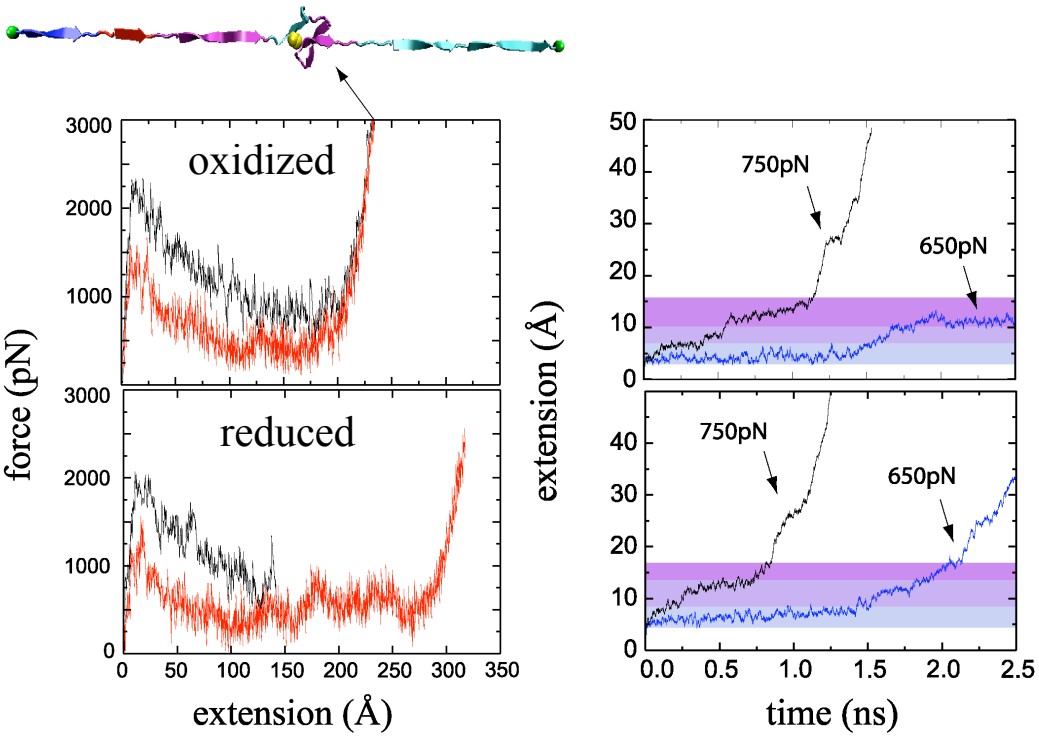
During stretching,
water molecules
attack repeatedly
interstrand hydrogen bonds,
about 100 times / ns;
for forces of 750 pN water
fluctuation controls unfolding!

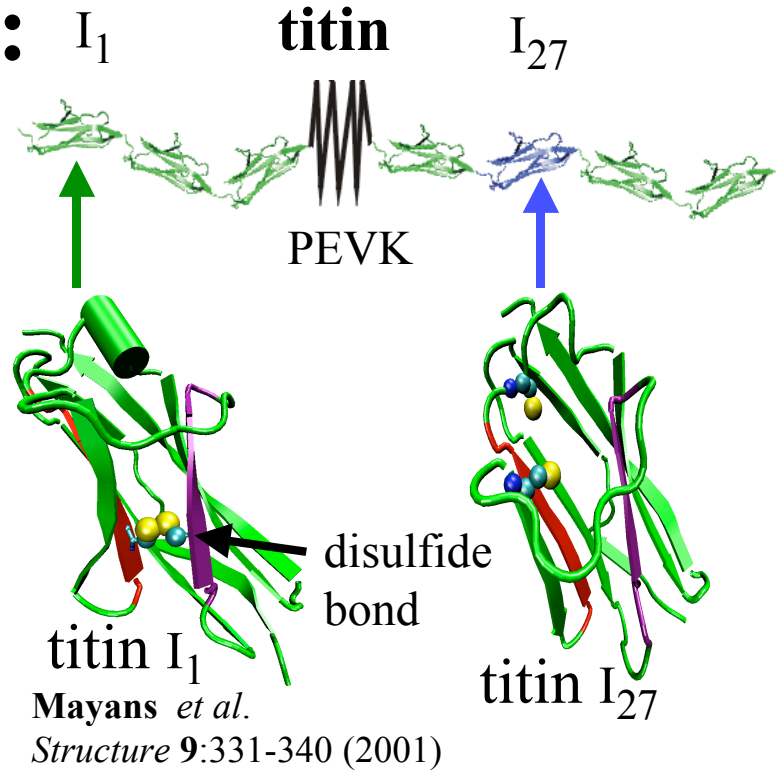
water greases protein folding !

Lu and Schulten, Biophys J.79: 51-65 (2000)

Titin Domain Heterogenity:

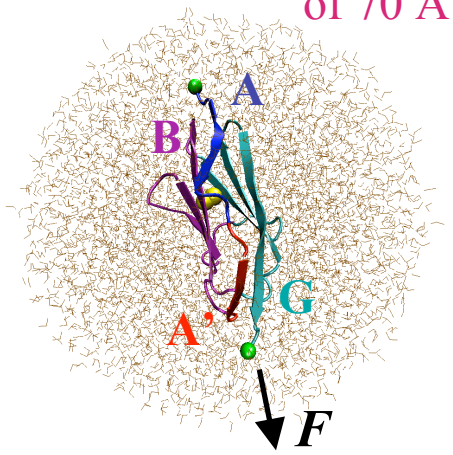
Comparing Oxidized and Reduced I1





Mayans *et al.*
Structure **9**:331-340 (2001)

I_1 in 70 Å water sphere of 70 Å (18,000 atoms)



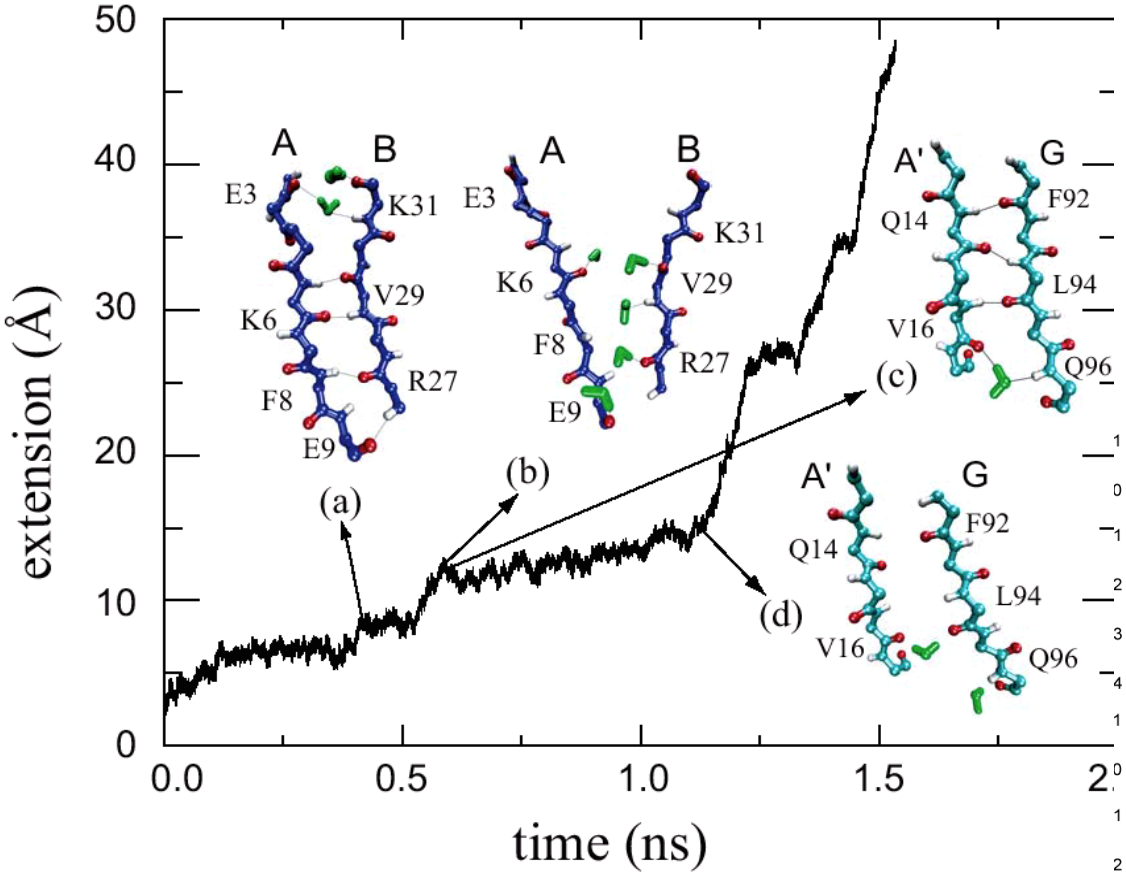
Use of program NAMD on 32 Processor (1.1 GHz Athlon) Linux cluster: 1 day/ns

Stretching I_1 with constant velocity

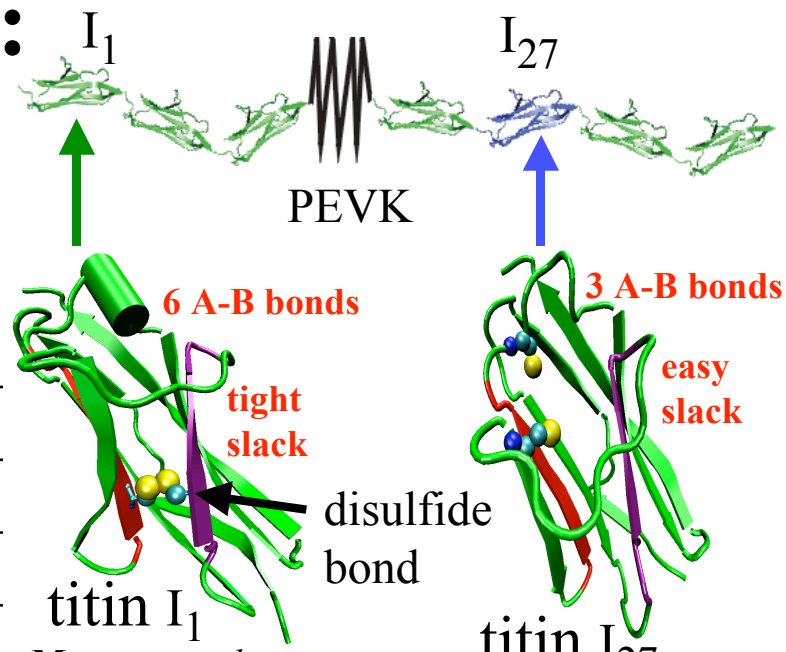
Mu Gao

Titin Domain Heterogenity:

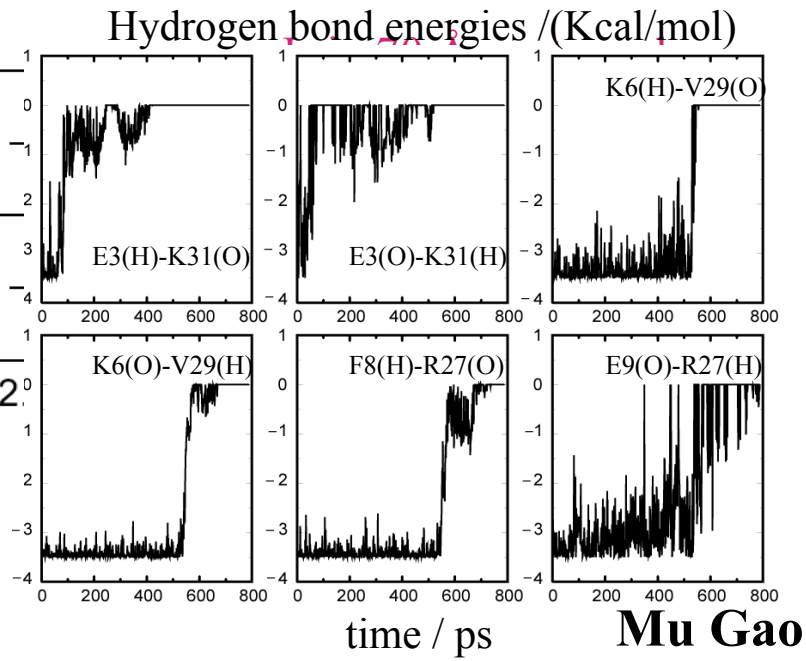
pre-stretching slack tight in I1



Stretching with constant force of 750 pN



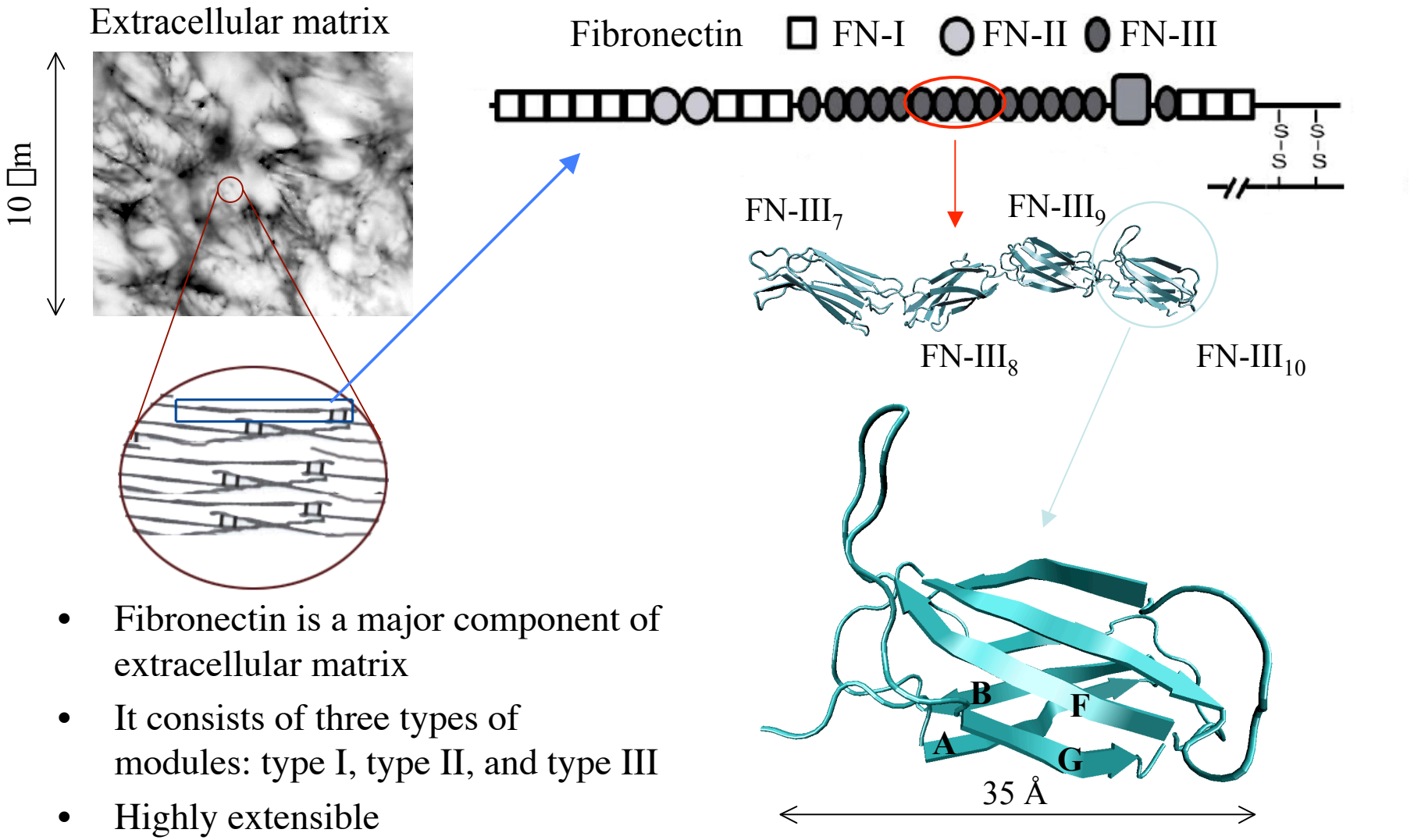
Mayans *et al.*
Structure **9**:331-340 (2001)



Mu Gao

Fibronectins

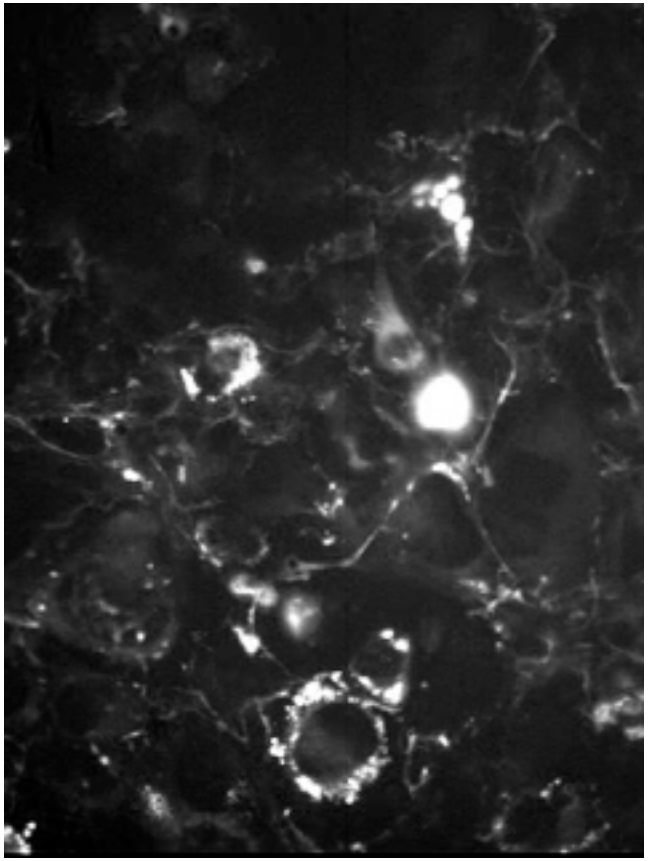
Architecture and Function of Fibronectin Modules



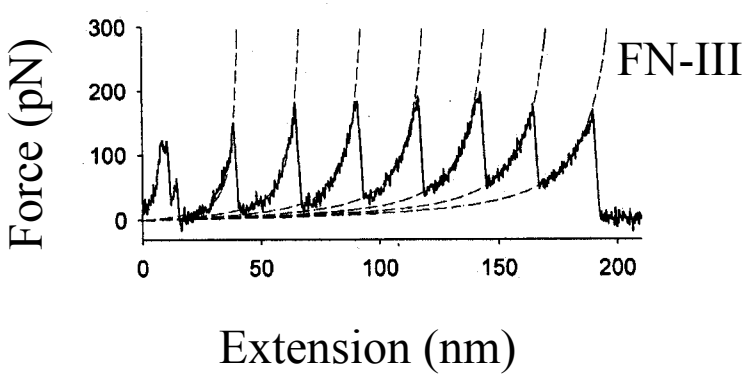
- Fibronectin is a major component of extracellular matrix
- It consists of three types of modules: type I, type II, and type III
- Highly extensible

Andre Krammer V. Vogel, U. Wash.

Fibronectin Matrix in Living Cell Culture



100 □m



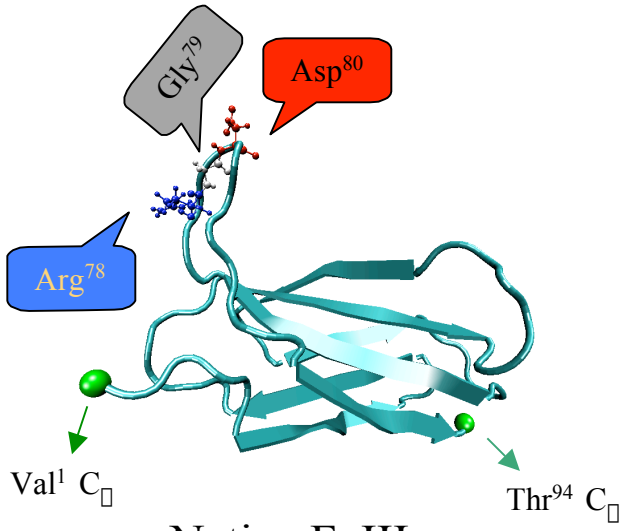
Atomic force microscopy observations

Ohashi *et al*. *Proc. Natl. Acad. Sci USA* **96**:2153-2158 (1999)

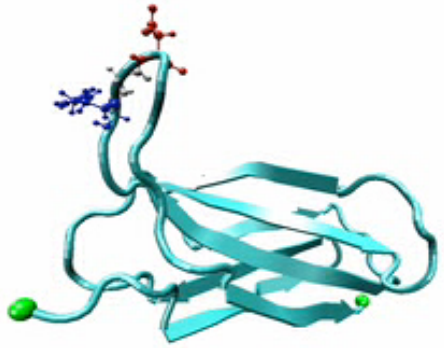
Andre Krammer V. Vogel, U. Wash.

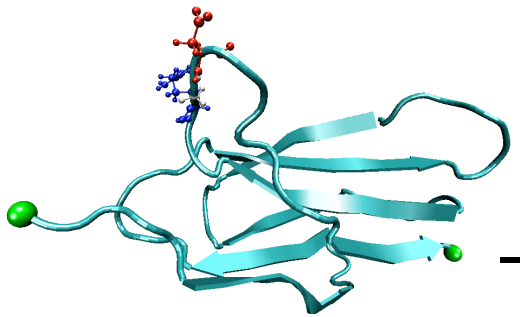
RGD Loop of $FnIII_{10}$

Krammer *et al.*
Proc. Natl. Acad. Sci USA
96:1351-1356 (1999)



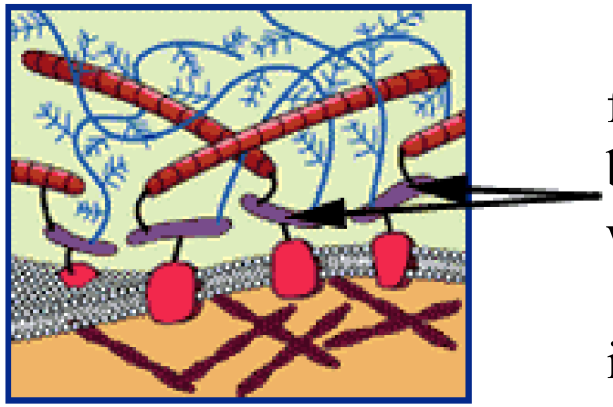
Native $FnIII_{10}$





→ ***F*** = 500 pN

Extension of 13 Å



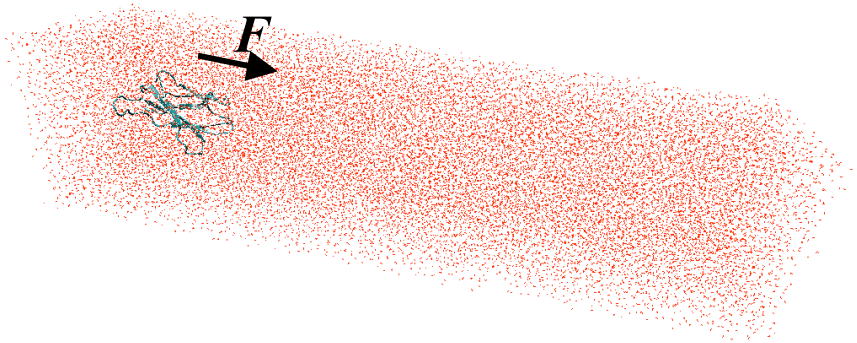
fibronectin binding with RGD loop to integrin

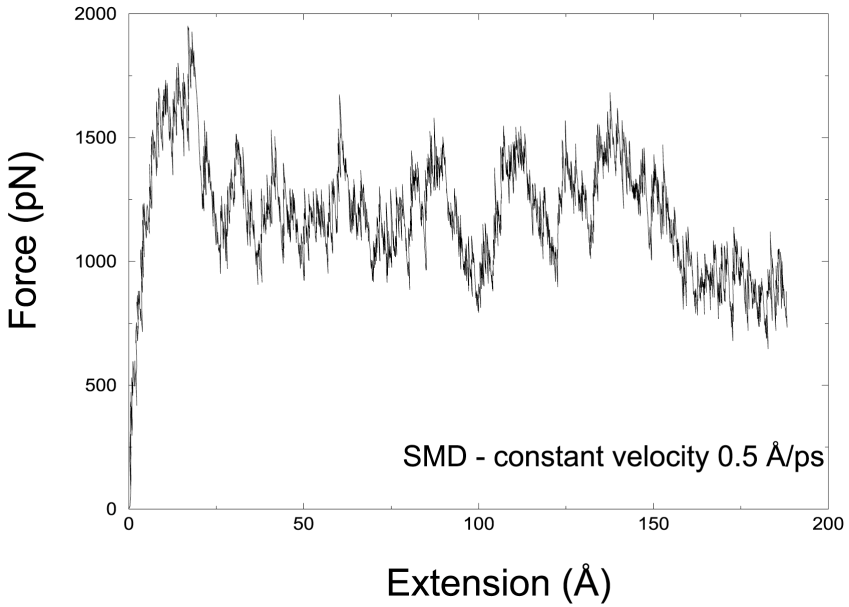
integrin interacting with extracellular matrix

Andre Krammer V. Vogel, U. Wash.

http://www.ks.uiuc.edu

Probing Unfolding Intermediates in FN-III$_{10}$





FnIII$_{10}$ module solvated in a water box 55×60×367 Å^3 **(126,000 atoms)**

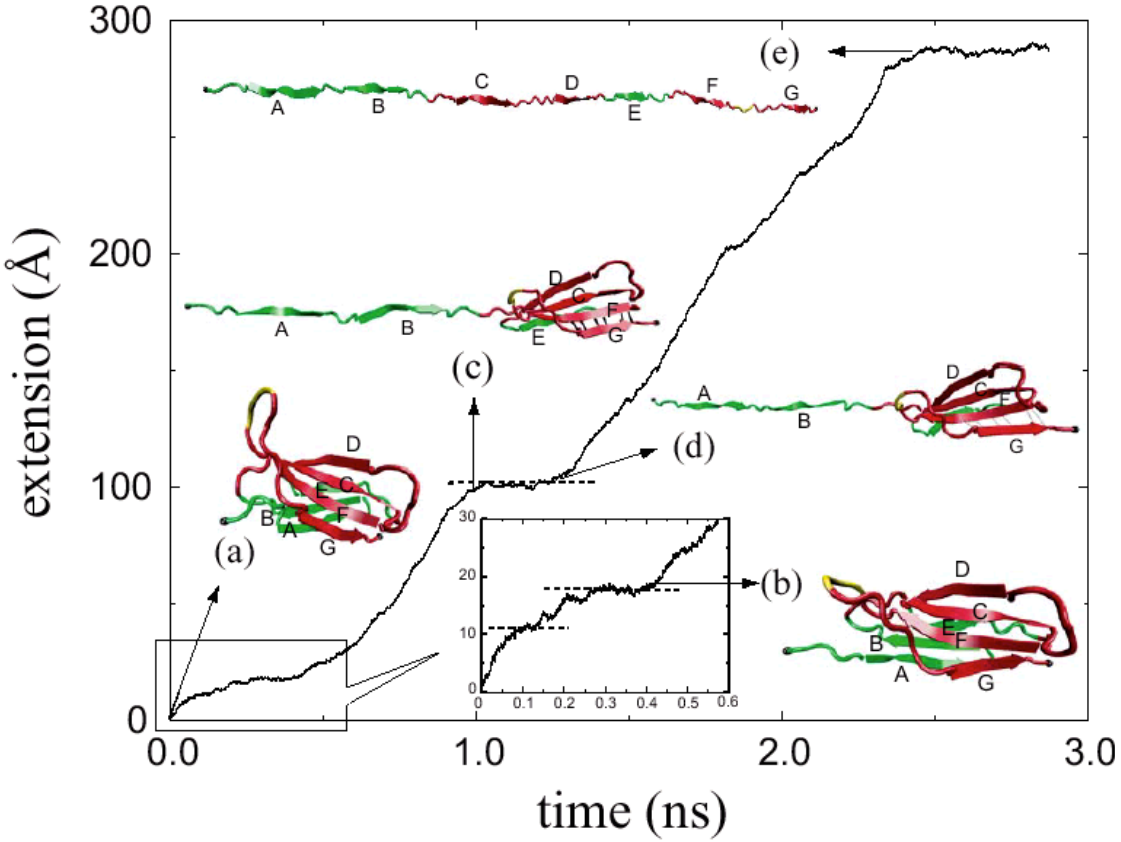
Steered Molecular Dynamics, periodic boundary conditions, NpT ensemble, Particle Mesh Edwald for full electrostatics

NAMD on Linux cluster of 32 Athlon 1.1GHz processors: **10 days/ns**

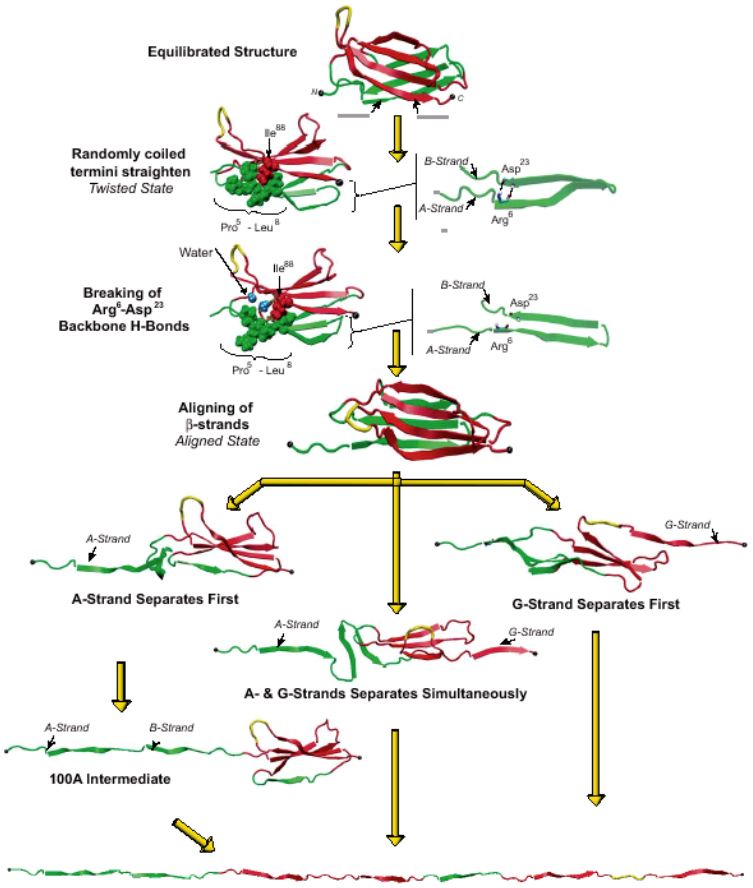
Mu Gao, U. Illinois

A. Krammer, D. Craig, V. Vogel, U. Wash.

Probing Unfolding Intermediates in FN-III$_{10}$



Specific stretching and unfolding pathway for constant force (500 pN) stretching: scenario with A rupturing first

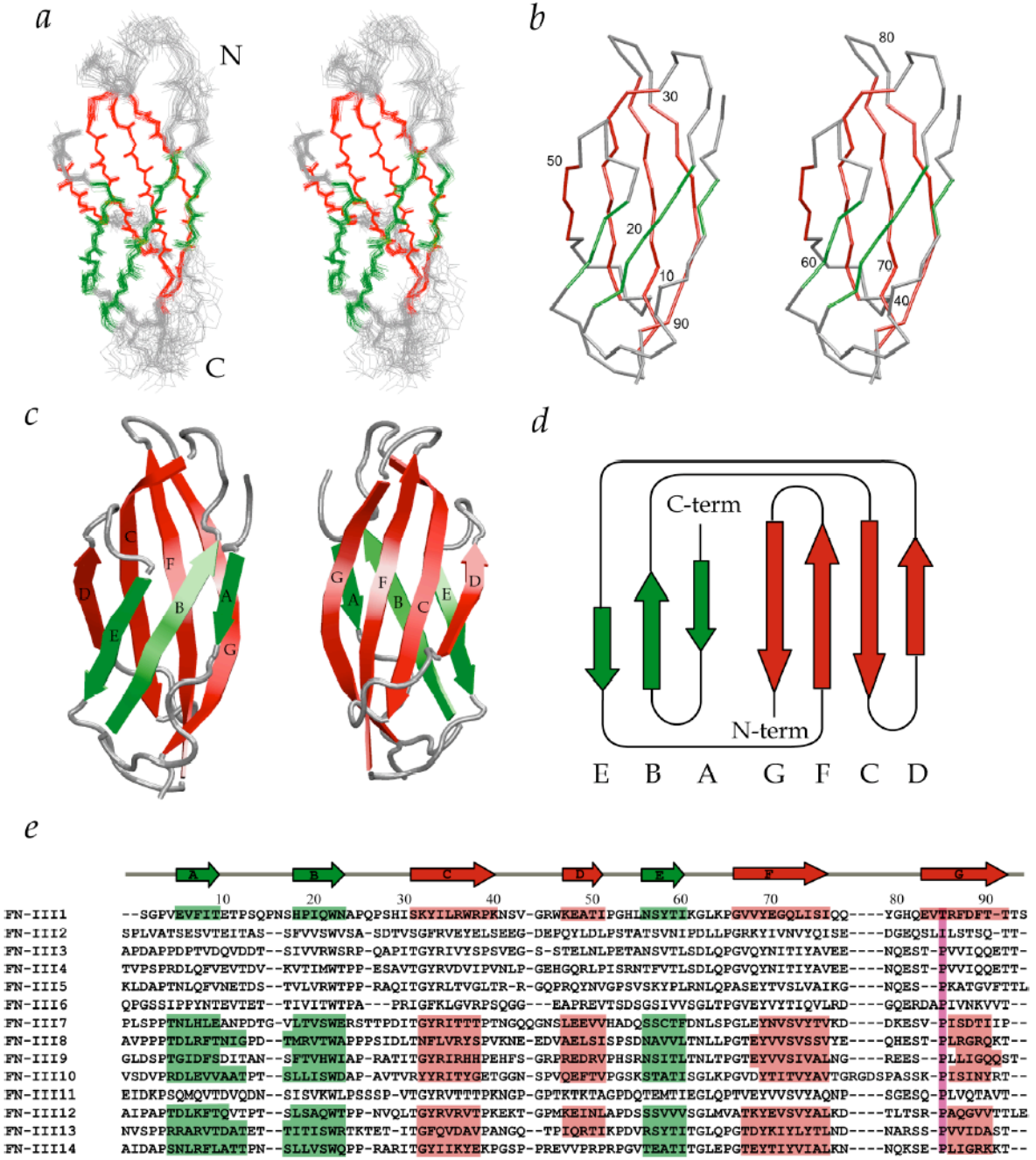


Complete stretching and unfolding pathway: after straightening and partial A-B separation, A, , or A+G or G rupture first

Modeling Fibronectin type III_1

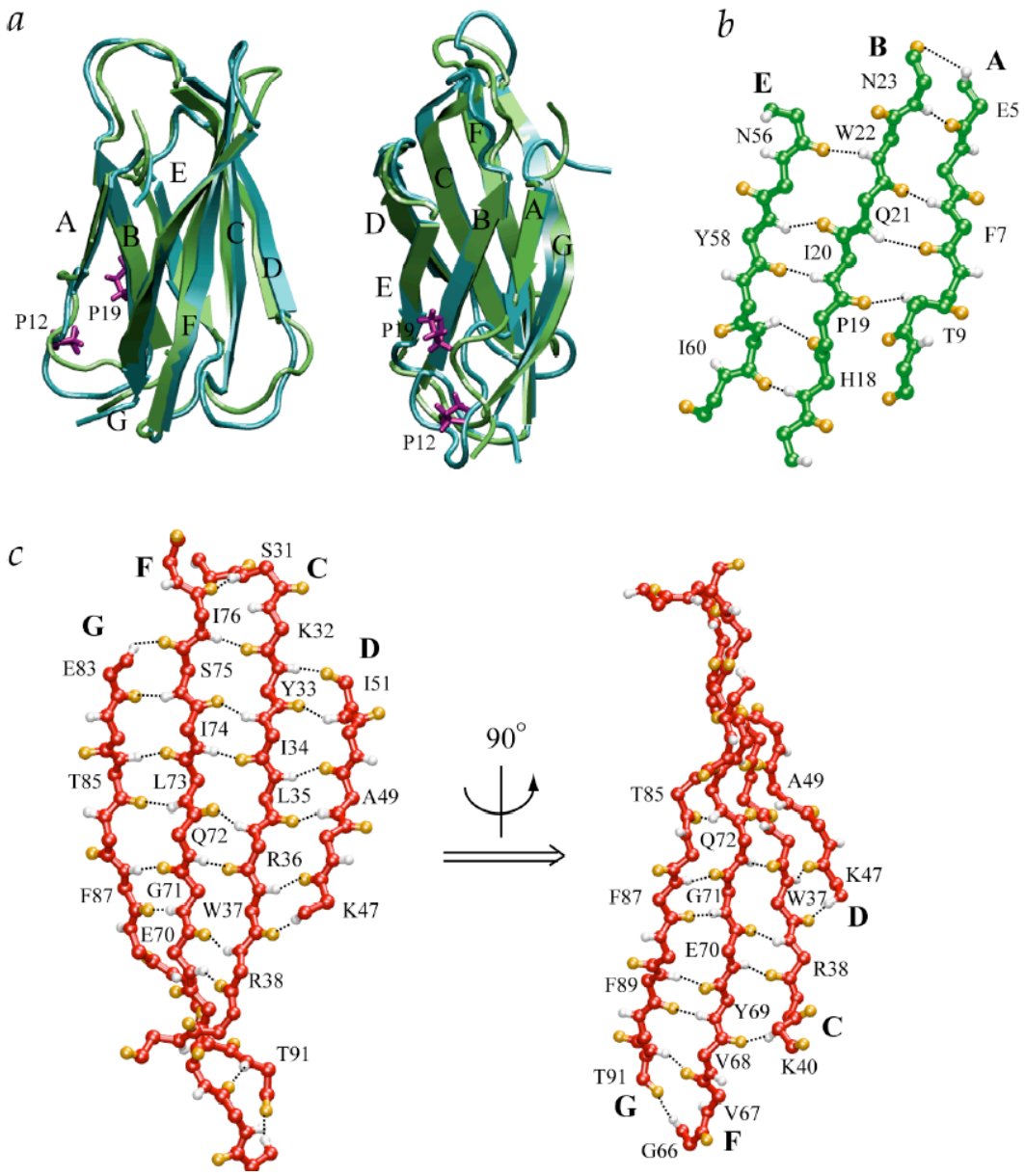
NMR Structure + SMD Modeling

Mu Gao
Collaboration with V. Vogel, David Craig, O. Lequin

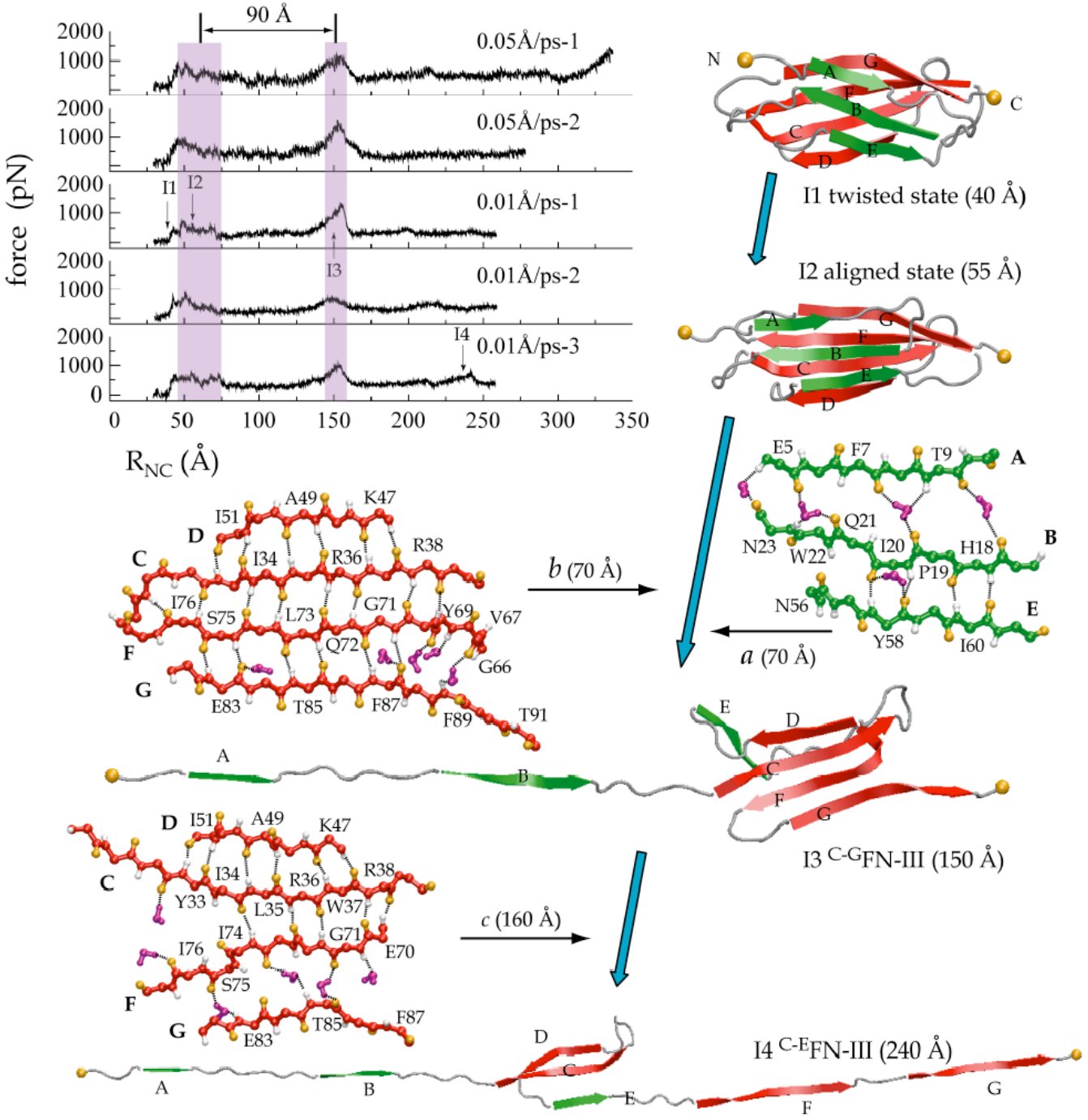


Architecture of beta-sheets

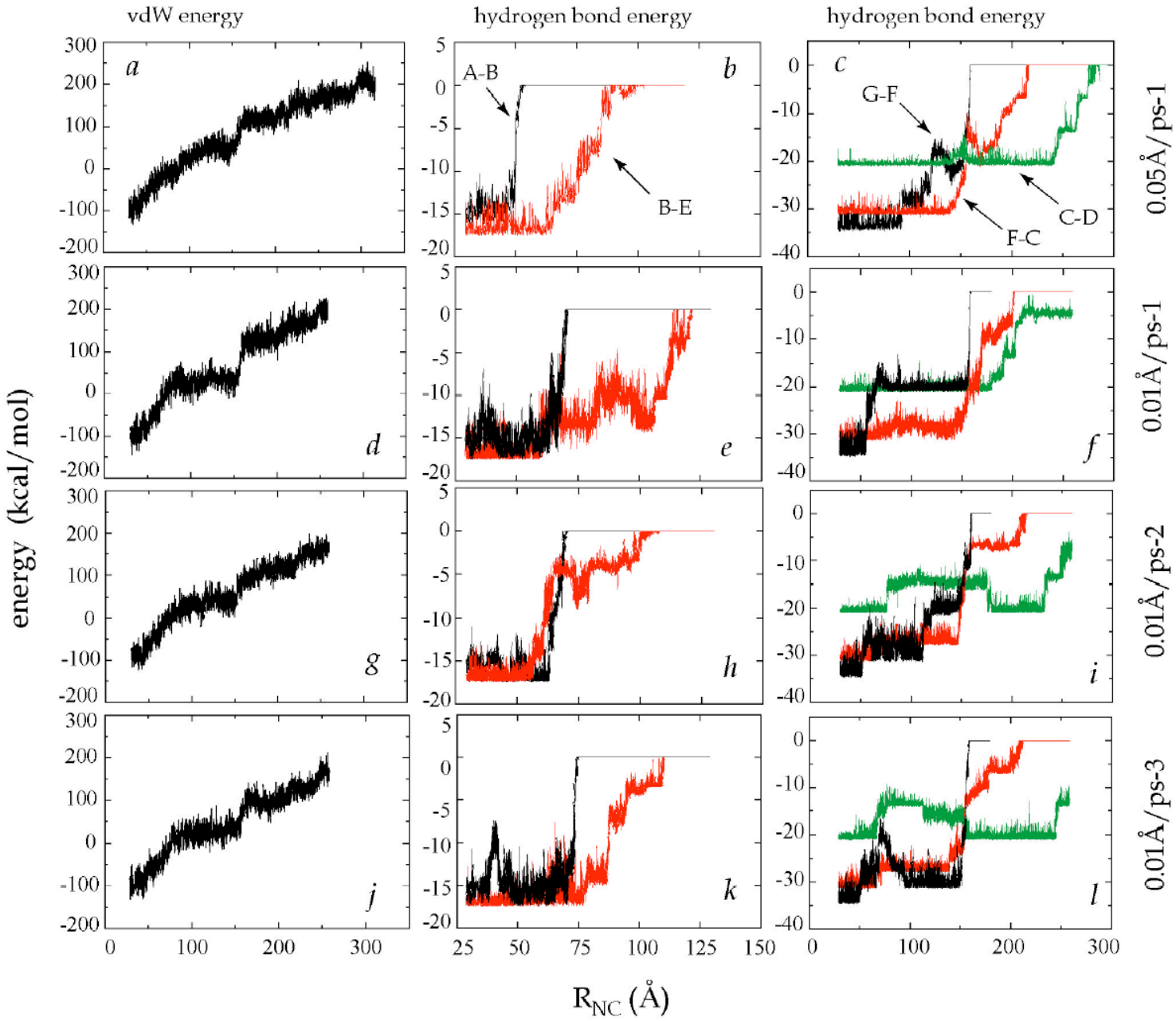
This protein features a particularly strong (red) beta-sheet



Constant velocity pulling stretches and breaks first the green beta-sheet, exhibits then a stable intermediate, before the red beta-sheet is stretched and broken



Energetics of FN-III-1 during stretching



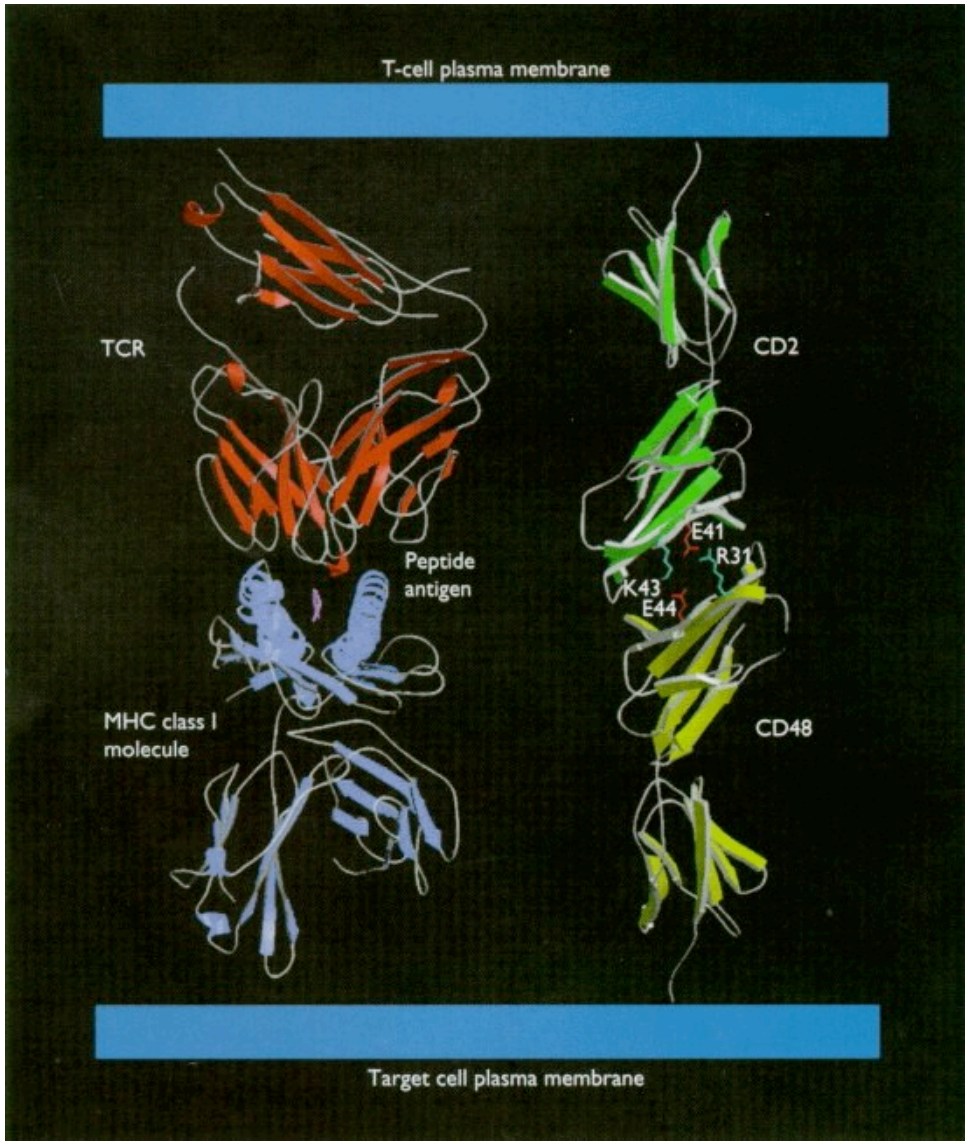
Adhesion Proteins of the Immune System

Strengthening the Immune System

M. Bayas and D. Leckband

Infections are battled best by the human immune system when there exists a memory from a previous disease or vaccination. The first step in using this line of defense is recognition: Cells of the immune system capture antigens, e.g., microbes in the respiratory tract, then mature in the lymph system, and finally present on their surface pieces of the antigen to T-cells that may recognize the antigen and become activated. The recognition of the antigen by T-cells is dramatically enhanced through surface receptors, CD2 and CD58, on the T-cell and the antigen presenting cell. The receptors stick out from their cell, adhere to one another, and conjoin the T-cell and antigen presenting cell long enough to enable recognition and activation. The molecular basis of this adhesion has been probed in a recent collaborative study with UIUC chemical engineer D. Leckband. Starting from the available crystallographic structure of the CD2-CD58 complex the researchers carried out 90,000 and 100,000 atom simulations using NAMD and pulled the complex apart in steered molecular dynamics simulations. An analysis of the simulations with VMD revealed in atomic level detail how the human immune system is strengthened through elastic adhesion.

CD2-CD48 interaction enhances antigen recognition between t-lymphocyte cells and antigen presenting cells



CD2-CD-48: Cluster of differentiation (CD) antigen number for unique reactivity patterns with leukocytes.

TCR: t cell receptor .

MHC: major histocompatibility Complex.

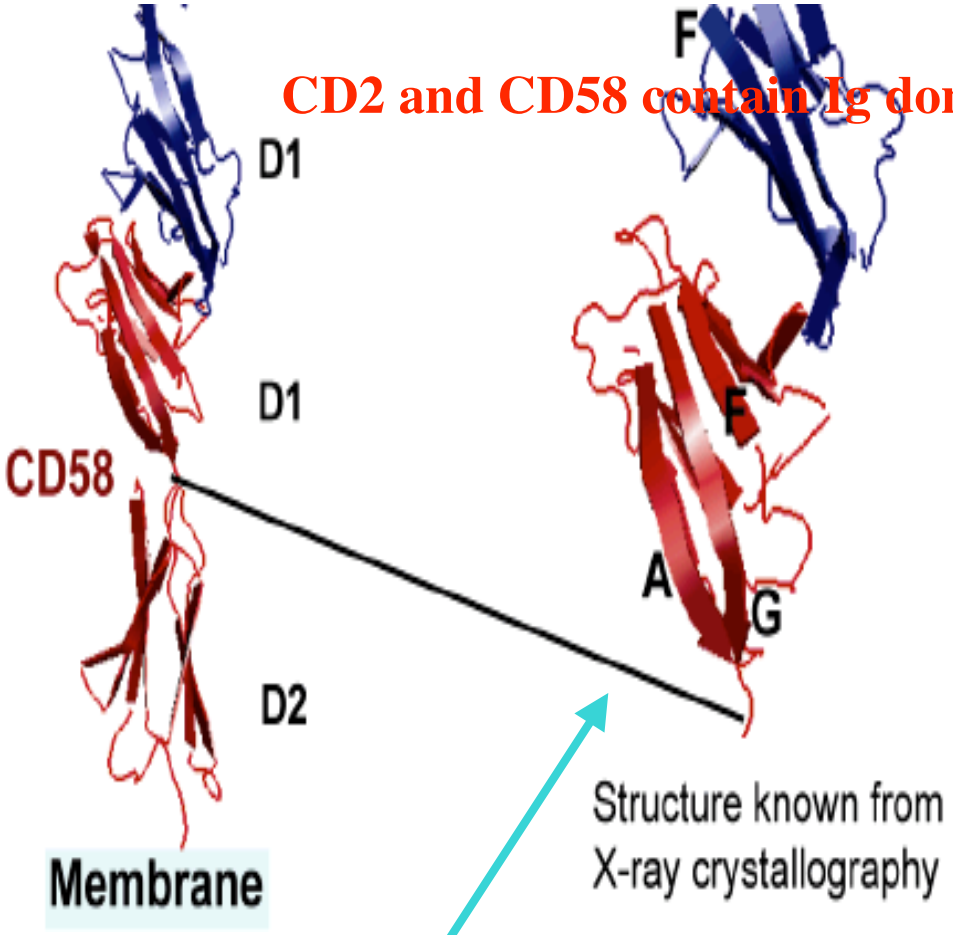
Thousands of CD's with low adhesion affinity assist cells in antigen recognition

CD2-CD48 salt bridges contribute to specificity, but are they also important for the tensile strength of interaction? Yes!

How do Ig domains of CD2-CD48 contribute to adhesion elasticity: through unfolding and/or deformation?
Through unfolding, even though Ig domains do not contain disulfide bonds!

Marcos Bayas, D. Leckband, K. Schulten, Biophys. J. 84:2223-2233, 2003

CD2 and CD58 contain Ig domains



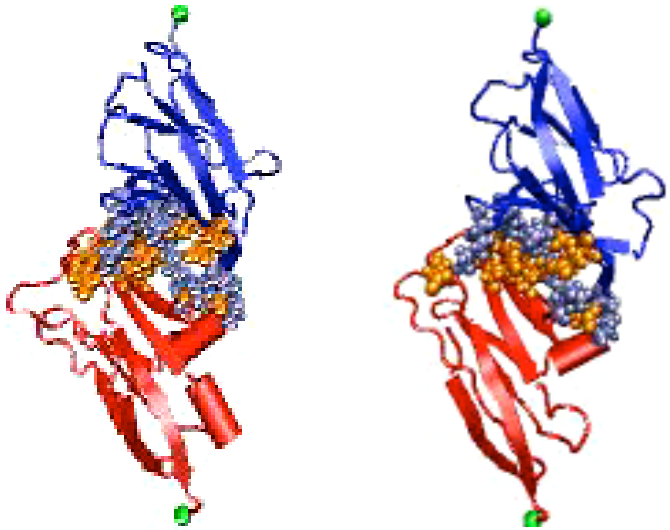
0.05 Å / ps

slow

- **Model** Based on Crystal Structure:
  *Wang et al. (1999) Cell, **97**, 791*
- **90,000 atoms**
- NAMD simulation on Linux cluster
  120 ps so far
- 1 – 0.05 Å / ps pulling velocity

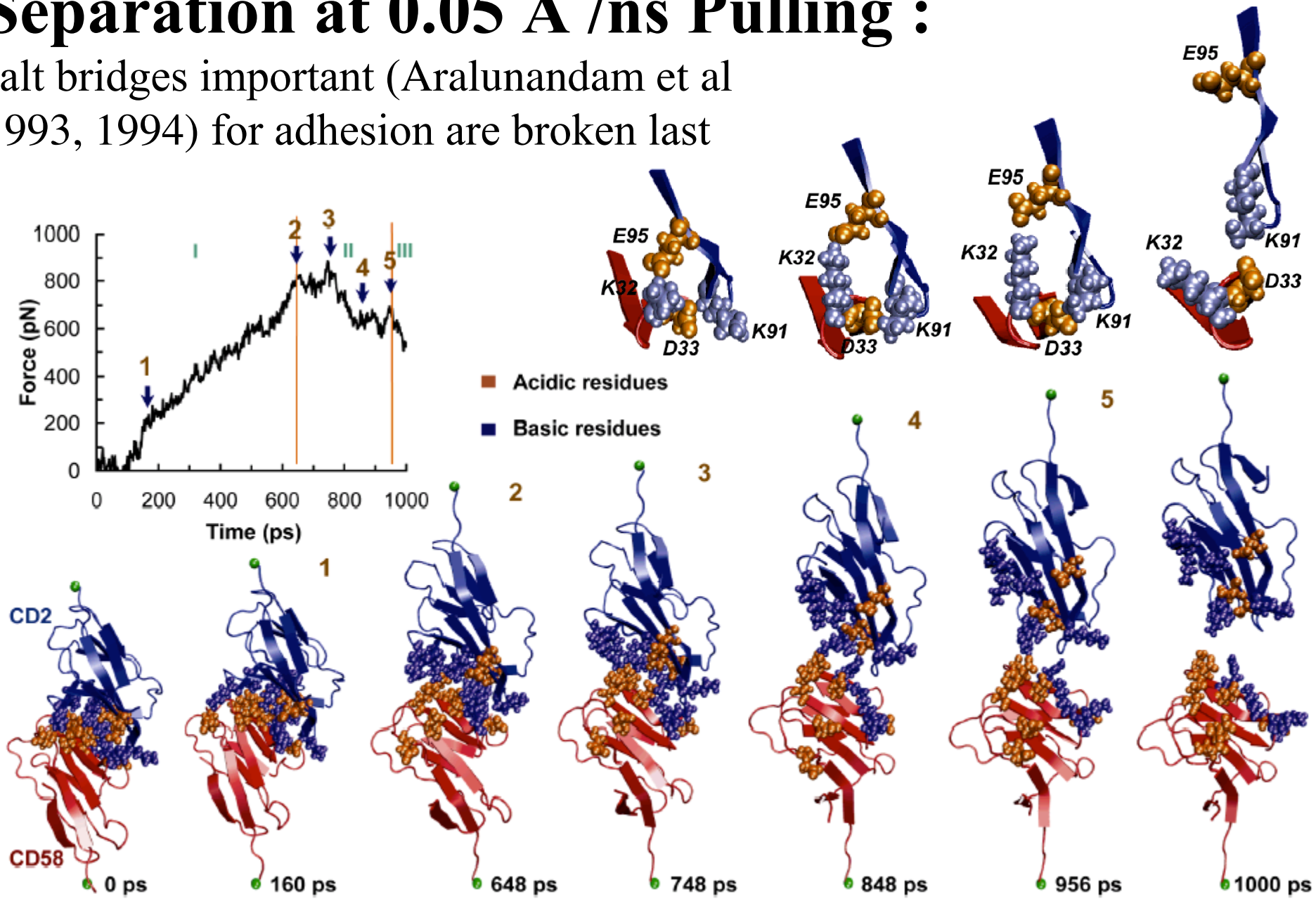
Marcos Bayas, D. Leckband, K. Schulten, submitted

1 Å / ps



Separation at 0.05 Å /ns Pulling :

salt bridges important (Aralunandam et al 1993, 1994) for adhesion are broken last

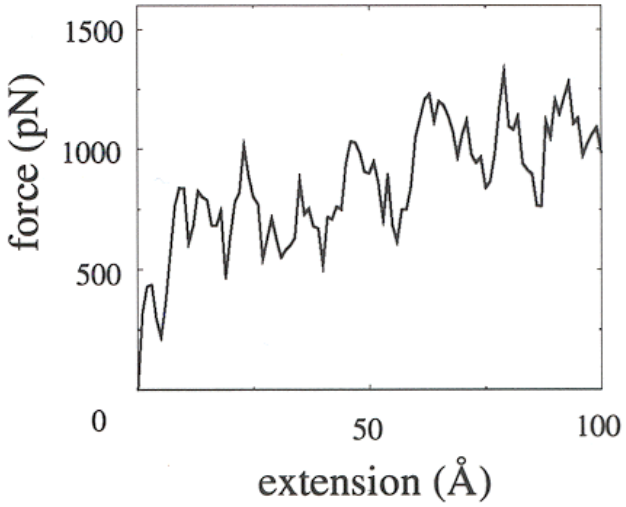


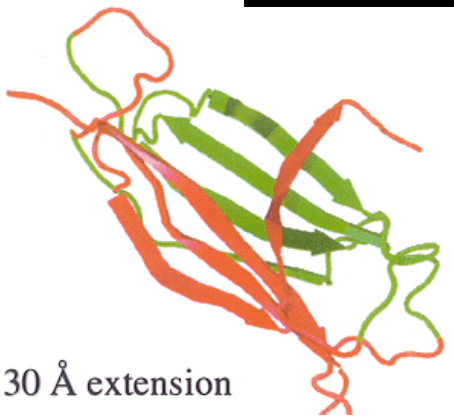
Marcos Bayas, D. Leckband, K. Schulten, Biophys. J. 84:2223-2233, 2003

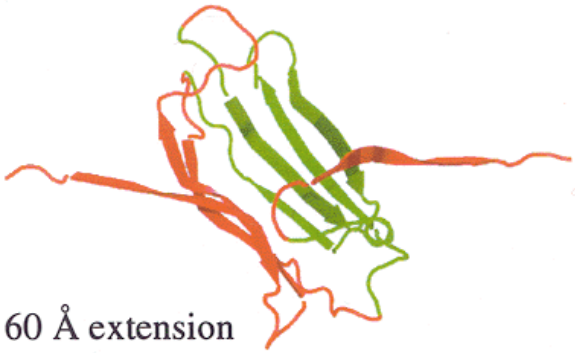
Behavior of Non-Mechanical Proteins

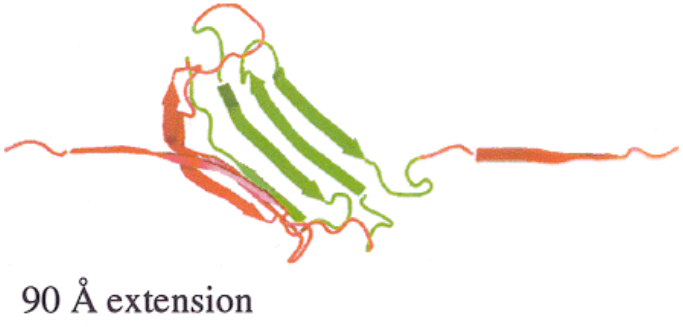
Force-induced Unfolding of Other Domains

C2 domain of synaptotagmin I (all sheet protein)







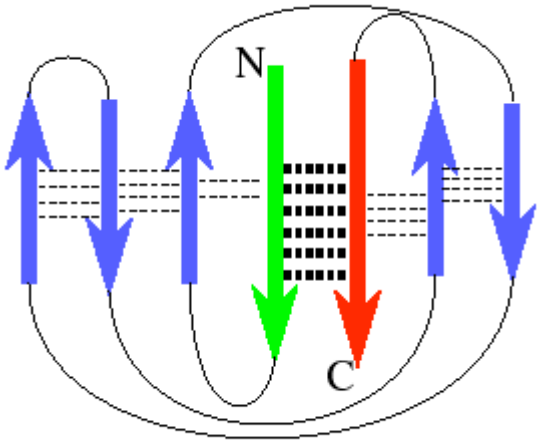


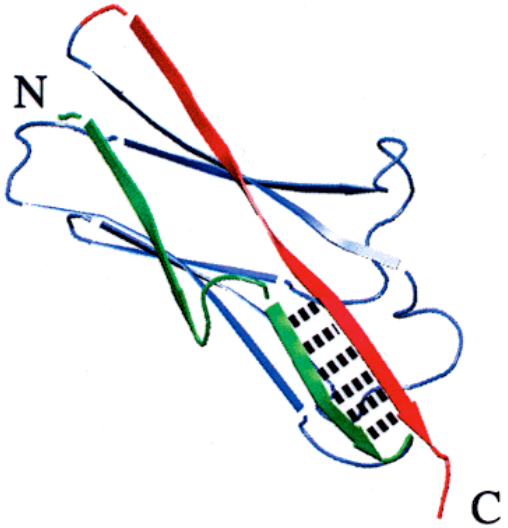
- no initial force peak
- much less resistance to external forces than Ig and FnIII
- during unfolding hydrogen bonds not required to be broken in clusters

Lu and Schulten, Proteins, 35, 453-463 (1999)

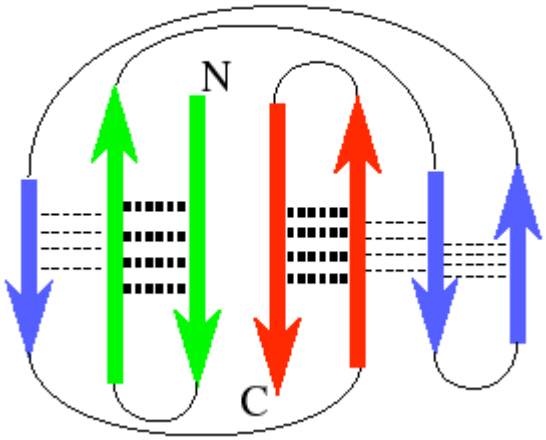
Classification of Sandwich Domains

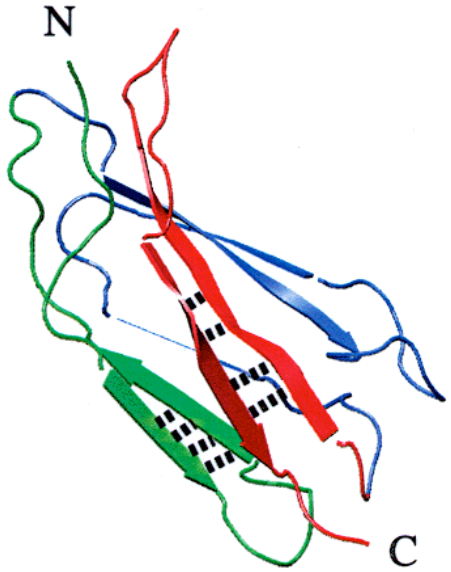
Class I a:
strong resistance



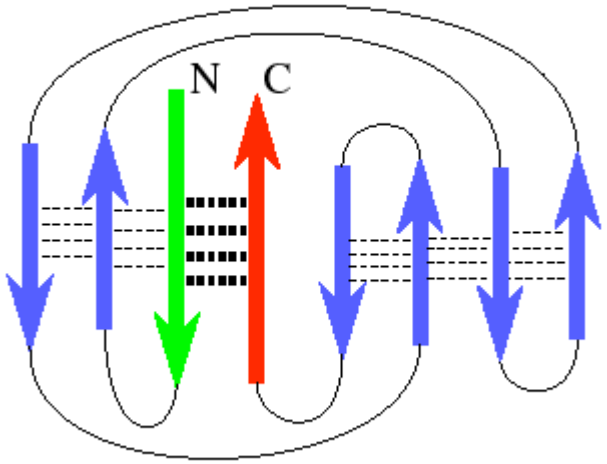


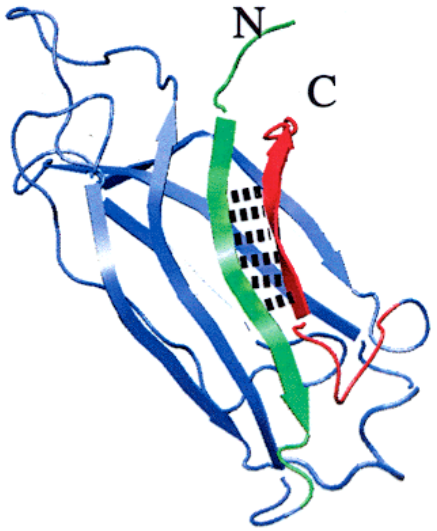
Class I b:
less resistance





Class I c:
little resistance



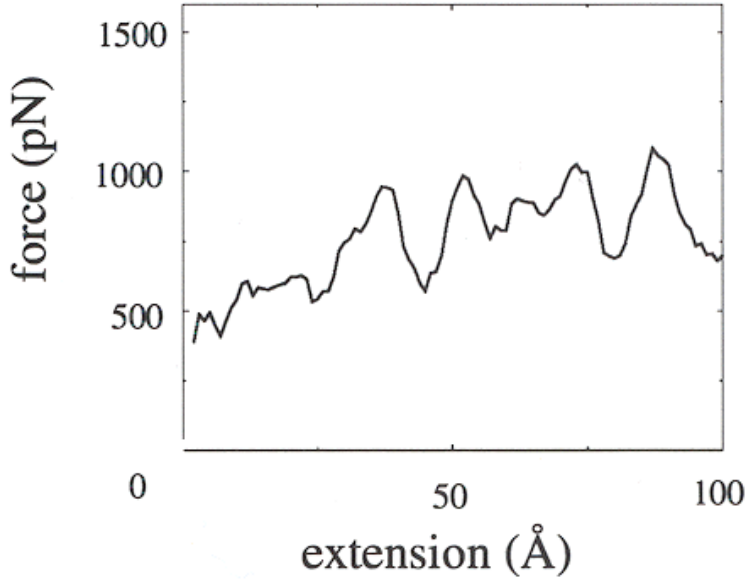


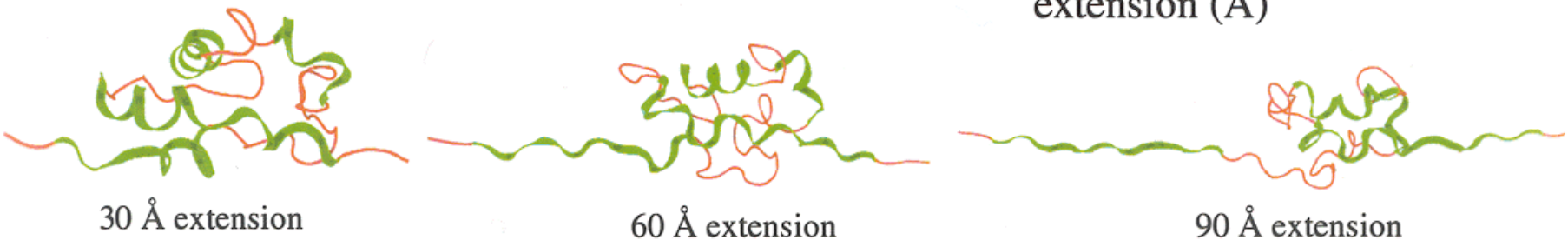
Lu and Schulten, Proteins, 35, 453-463 (1999)

NIH Resource for Macromolecular Modeling and Bioinformatics
Theoretical Biophysics Group, Beckman Institute, UIUC

Force-induced unfolding of alpha-helical protein

Cytochrome C6 (all helix protein)

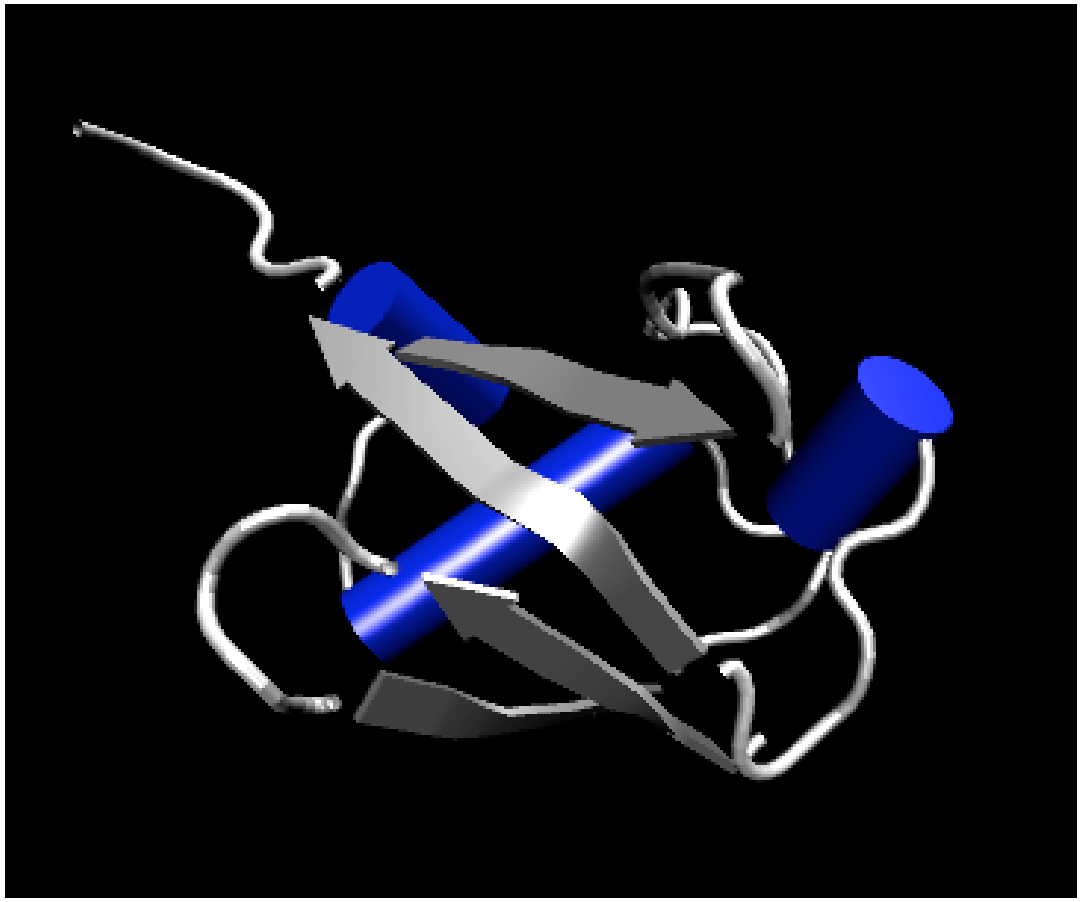




- no initial force peak
- much less resistance to external forces than Ig and FnIII
- during unfolding hydrogen bonds not required to be broken in clusters

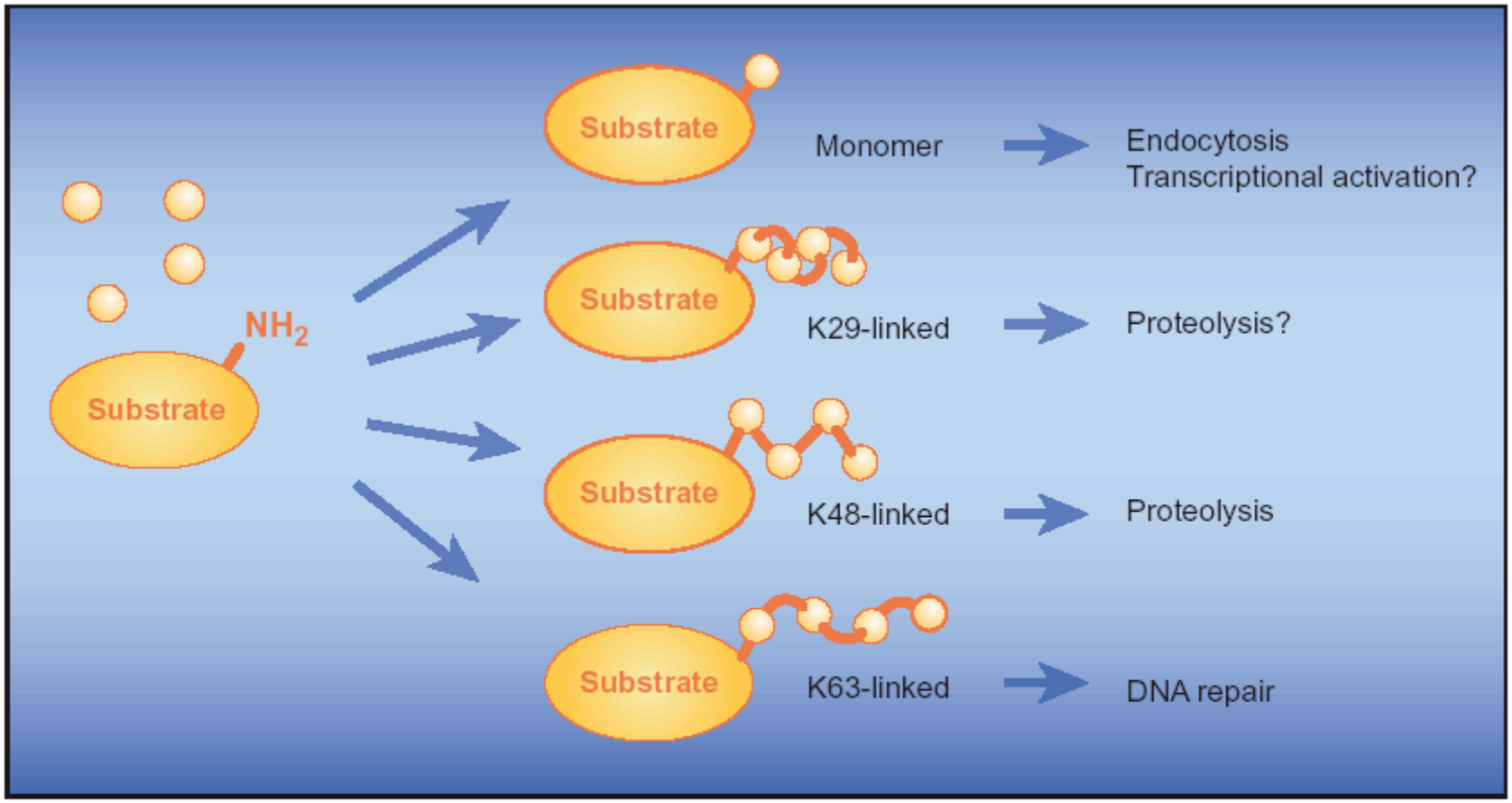
Lu and Schulten, Proteins, 35, 453-463 (1999)

Ubiquitin



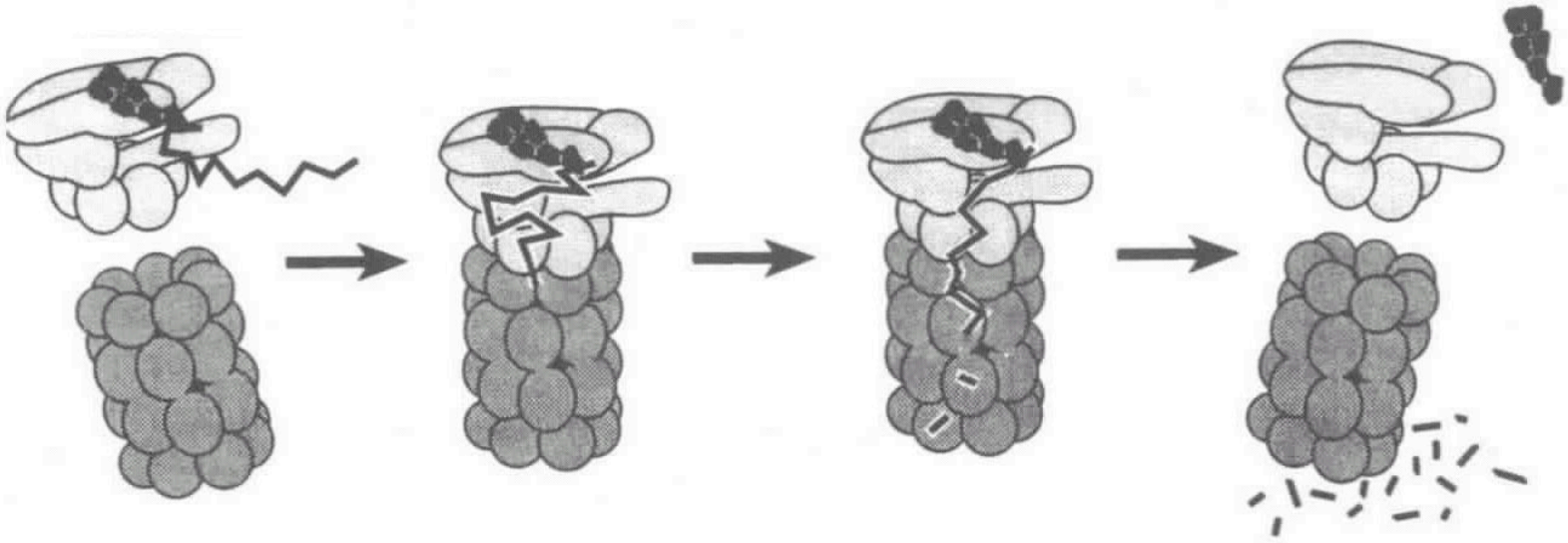
Fatemeh Araghi, Timothy Isgro, Marcos Sotomayor

Monoubiquitylation versus multi-ubiquitylation



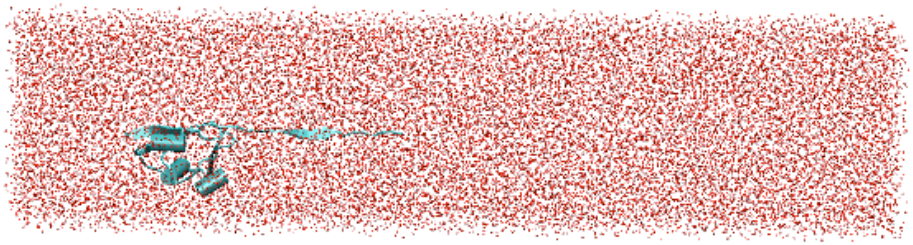
Multifaceted. Ubiquitin can attach to its various substrate proteins, either singly or in chains, and that in turn might determine what effect the ubiquitination has. (K29, K48, and K63 refer to the particular lysine amino acid used to link the ubiquitins to each other.)

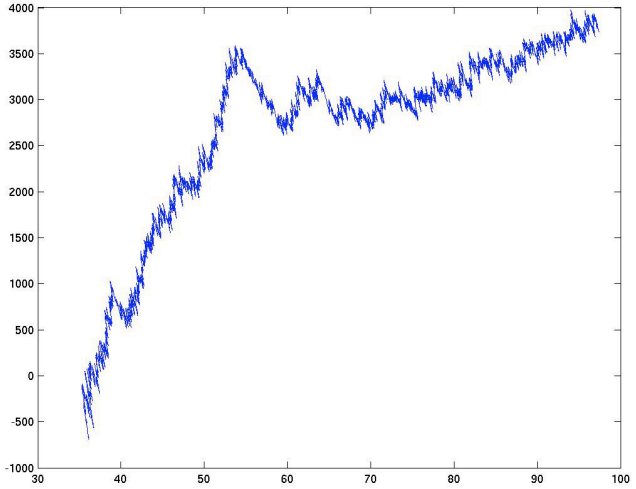
Structure-Function Relationship



Proteasome Degradation

First SMD Simulation

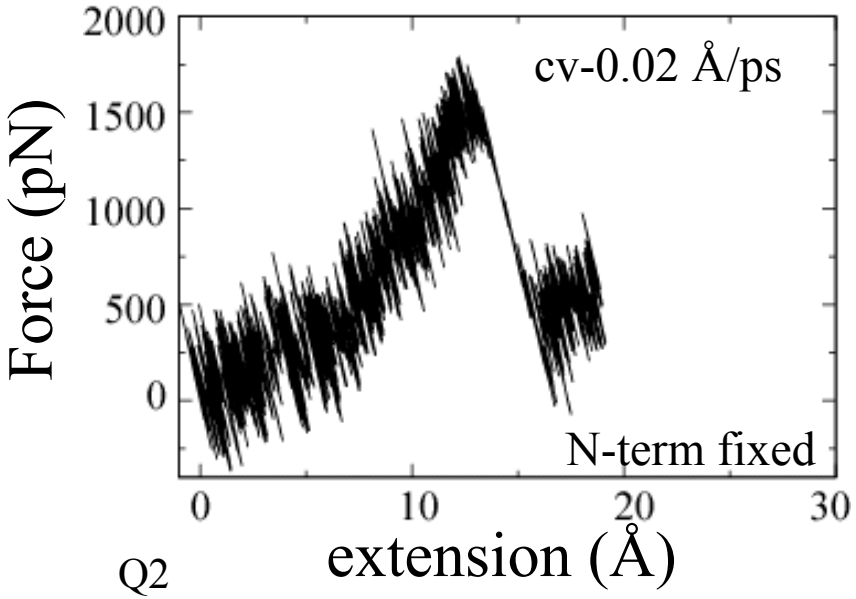


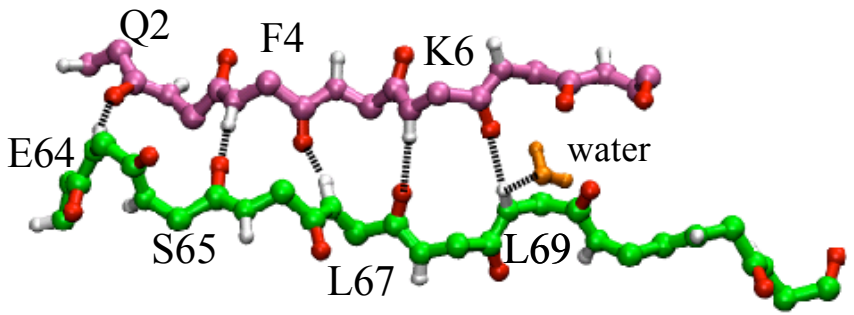


First peak when the first beta strand is stretched out

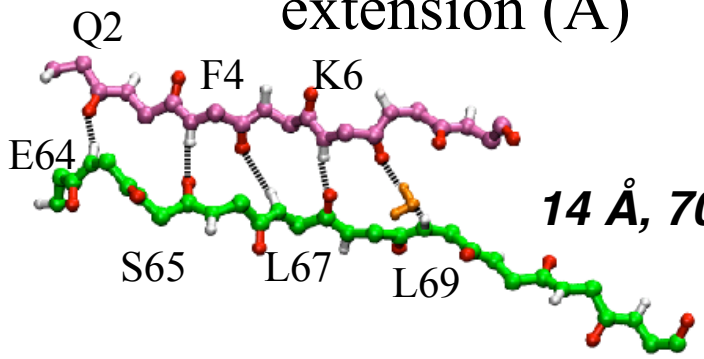
- SMD simulation, with constant velocity
- Box of water 70x240x70 A ~81K atoms
- smd velocity 0.4 A/ps
- smd spring constant 7 kcal/mol A^2

Ubiquitin Unfolding I

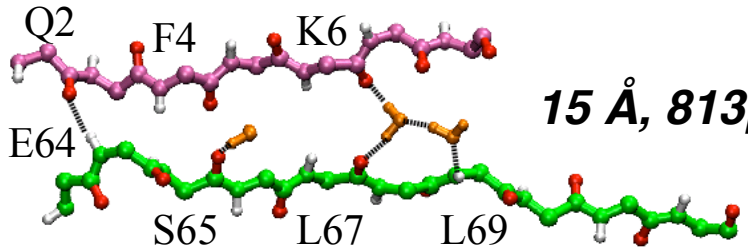




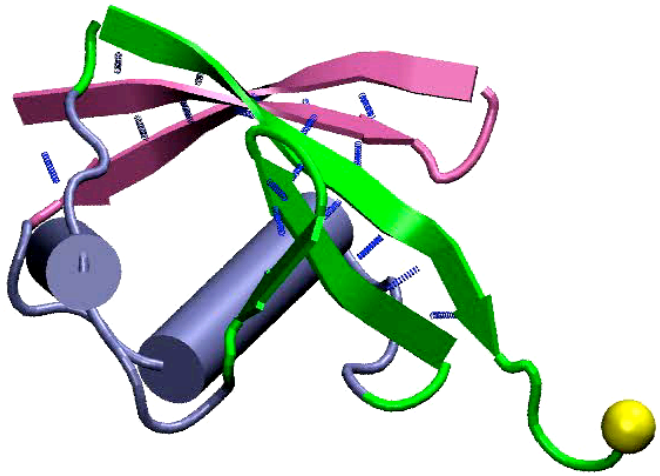
0 Å, 1 ps



14 Å, 700ps

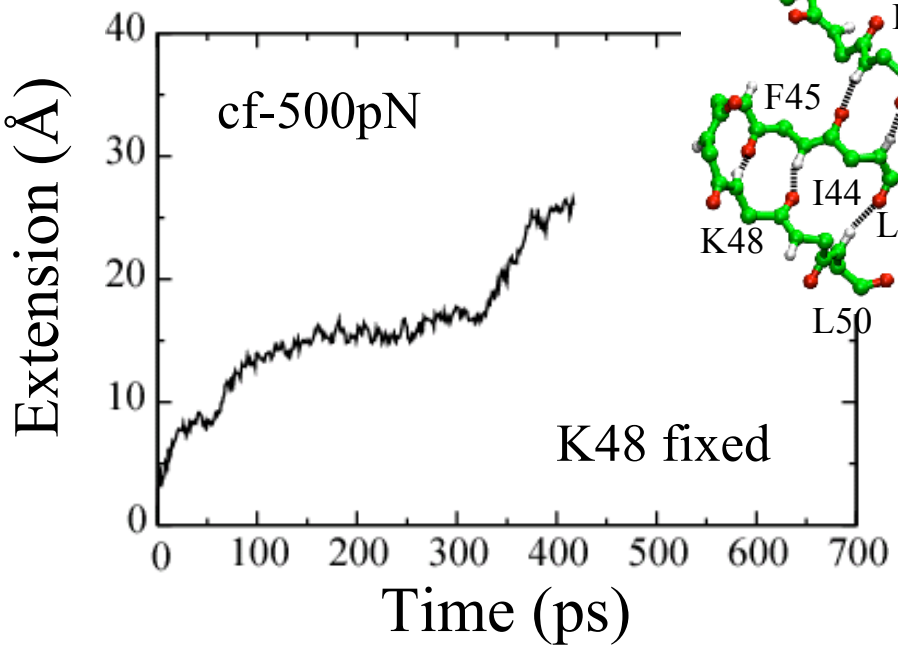


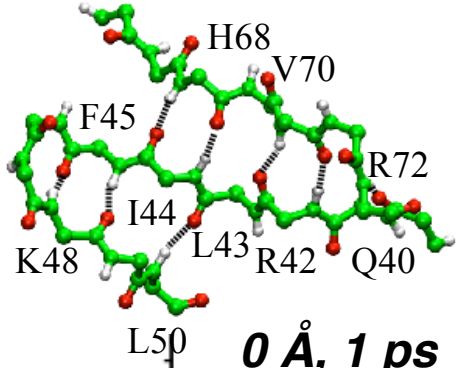
15 Å, 813ps



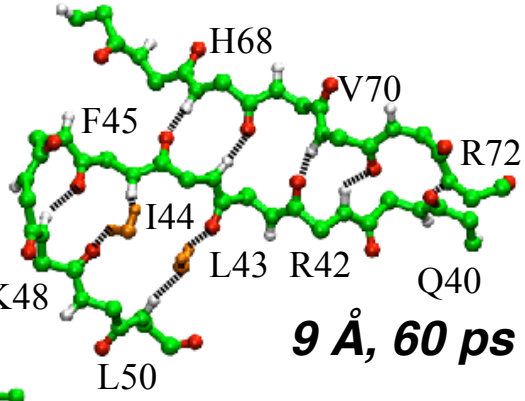
Mu Gao

Ubiquitin Unfolding II

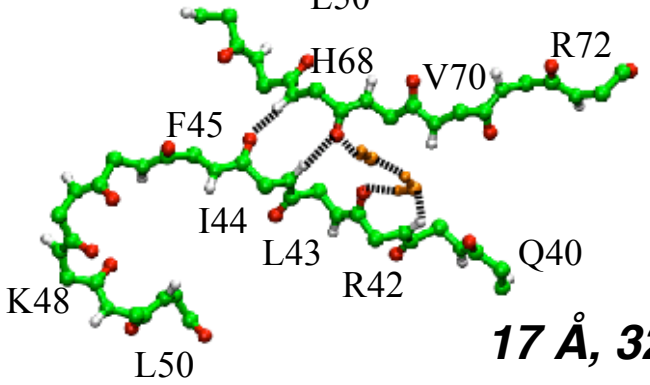




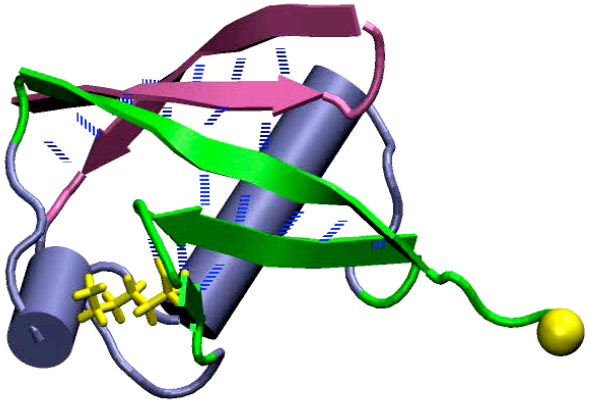
0 Å, 1 ps



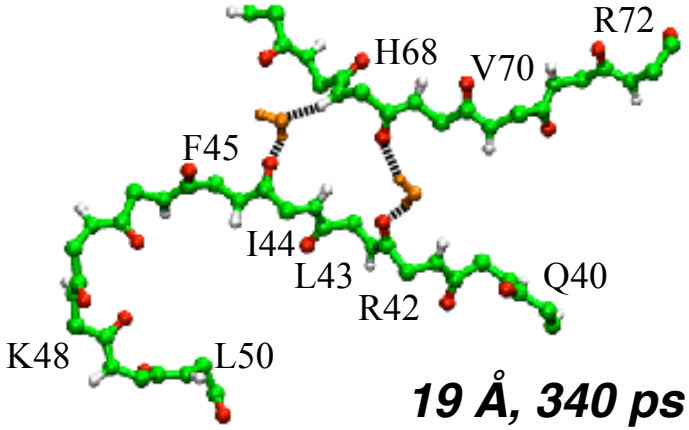
9 Å, 60 ps



17 Å, 320 ps



Mu Gao



19 Å, 340 ps

Pulling Dimer

- SMD (v=0.4 A/ps k=7 kcal/mol A^2) constant P
- Two monomers seperatre.