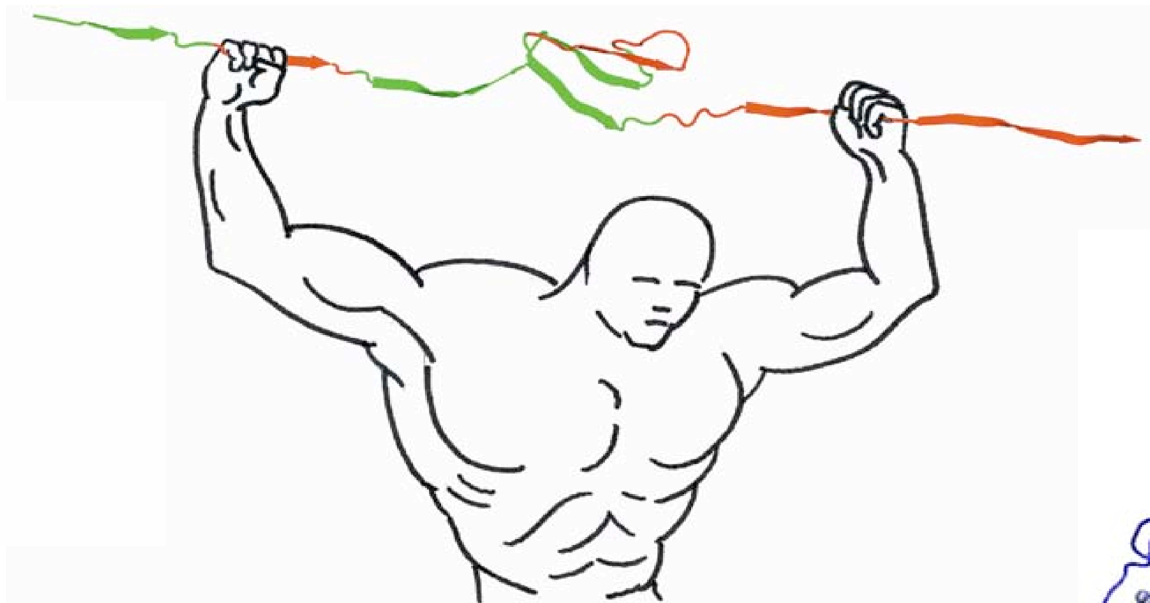
Mechanical Proteins



Stretching imunoglobulin and fibronectin domains of the muscle protein titin

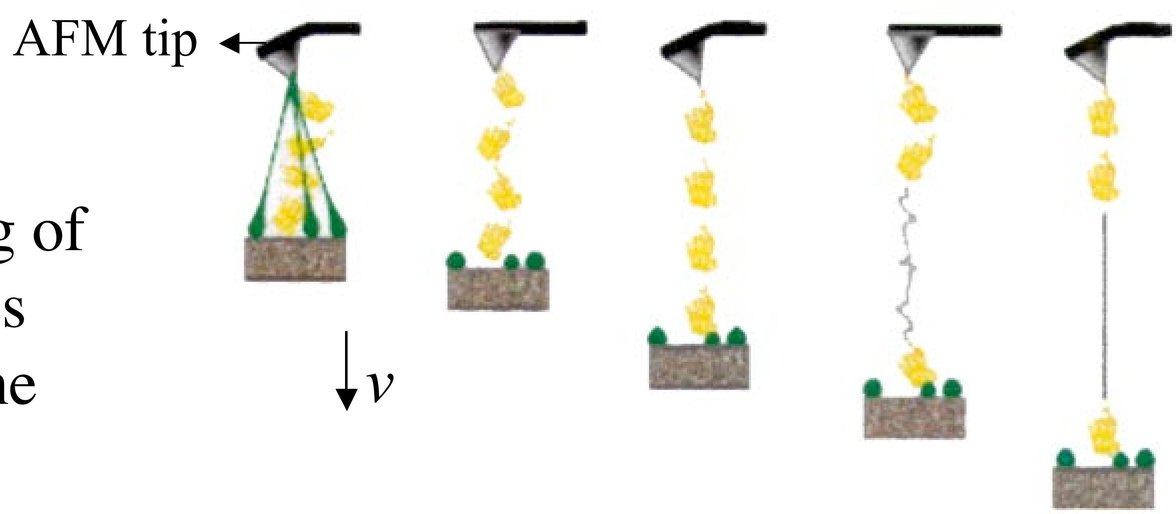
Adhesion Proteins of the Immune System

National Center for Research Resources

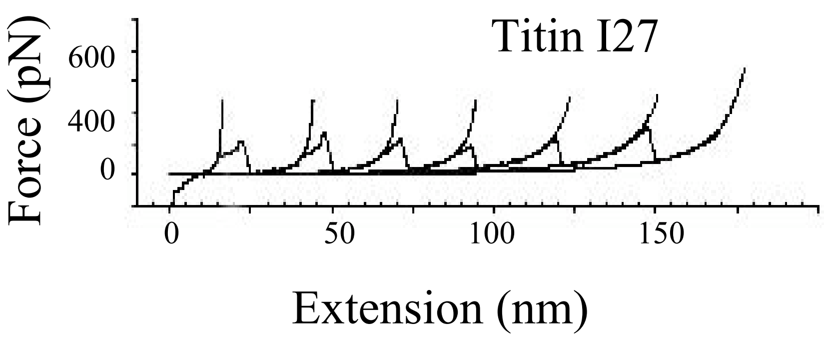
NIH Resource for Macromolecular Modeling and Bioinformatics
Theoretical Biophysics Group,
Beckman Institute, UIUC

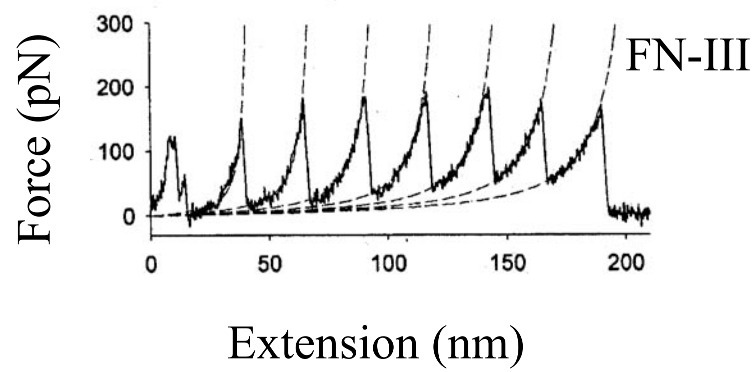
Immunoglobulin Domains

AFM Studies of Titin And FN-III Modules



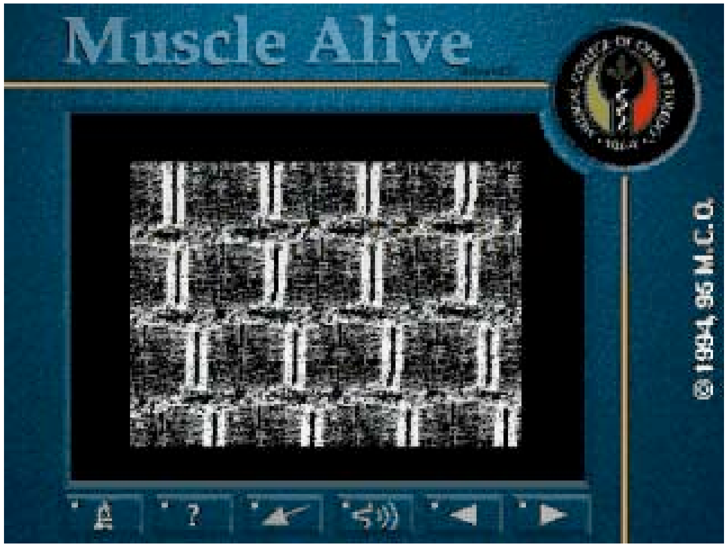
- Forced unfolding of identical modules occurs one by one

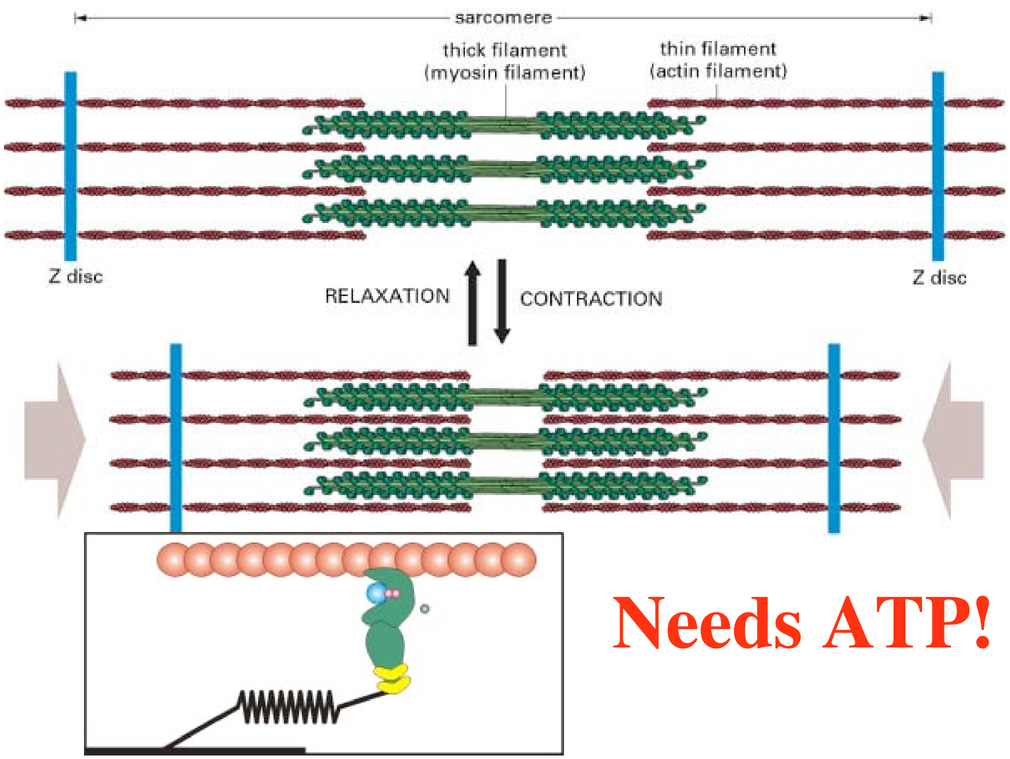




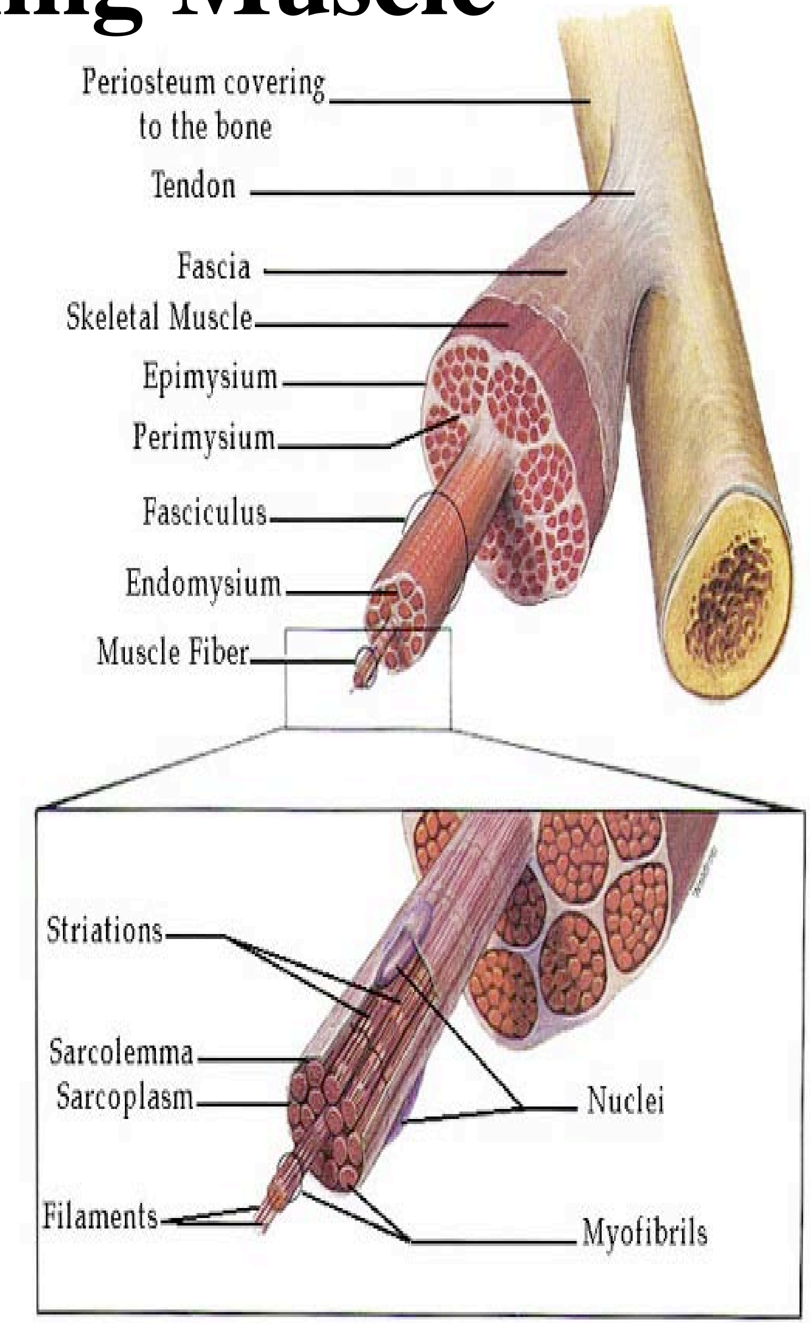
Reviewed in **Fisher** *et al*. *Nature Struct. Biol.* **7**:719-724 (2001)

Contracting and Relaxing Muscle

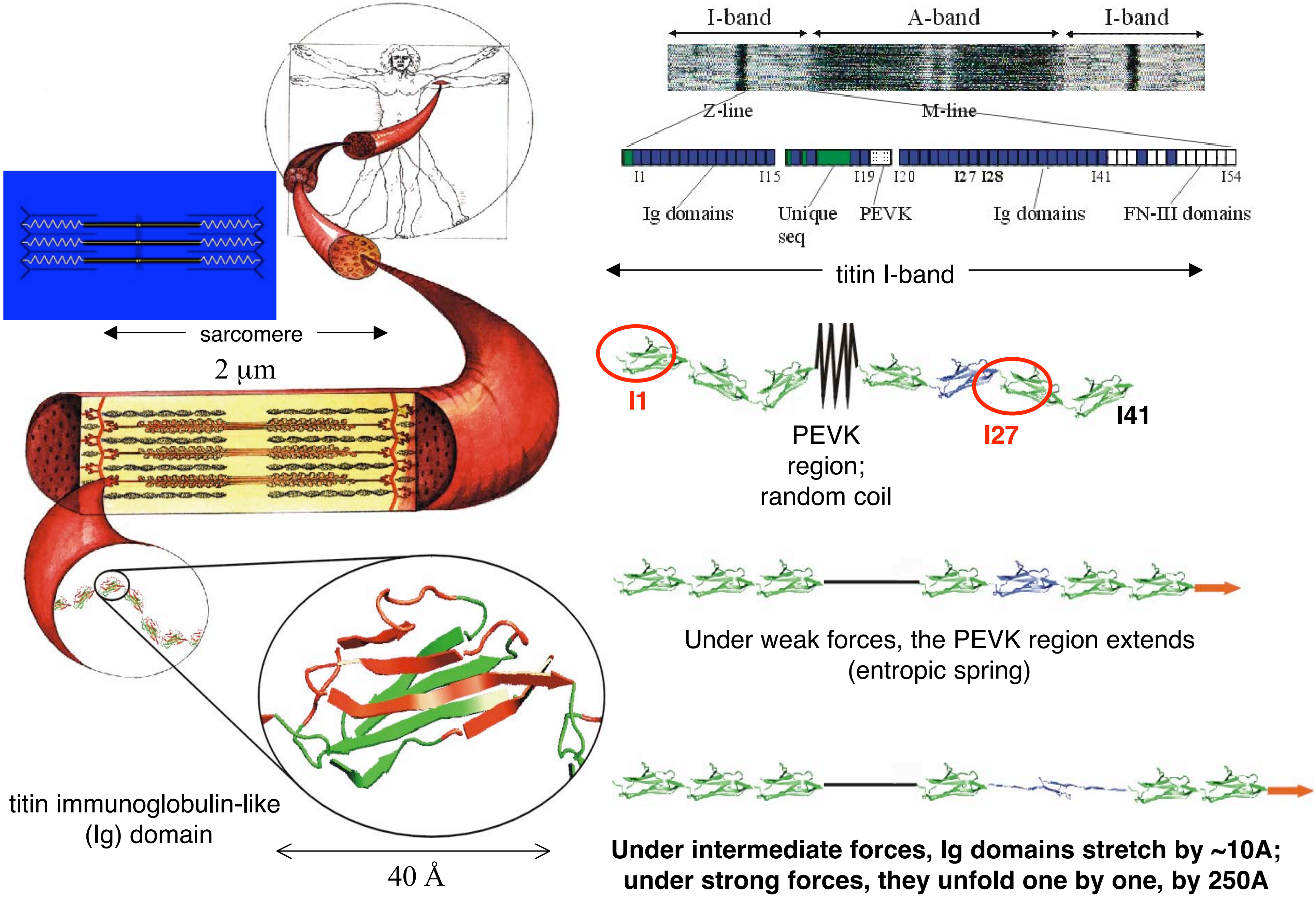




Needs ATP!



Titin the Longest Protein in the Human Genome

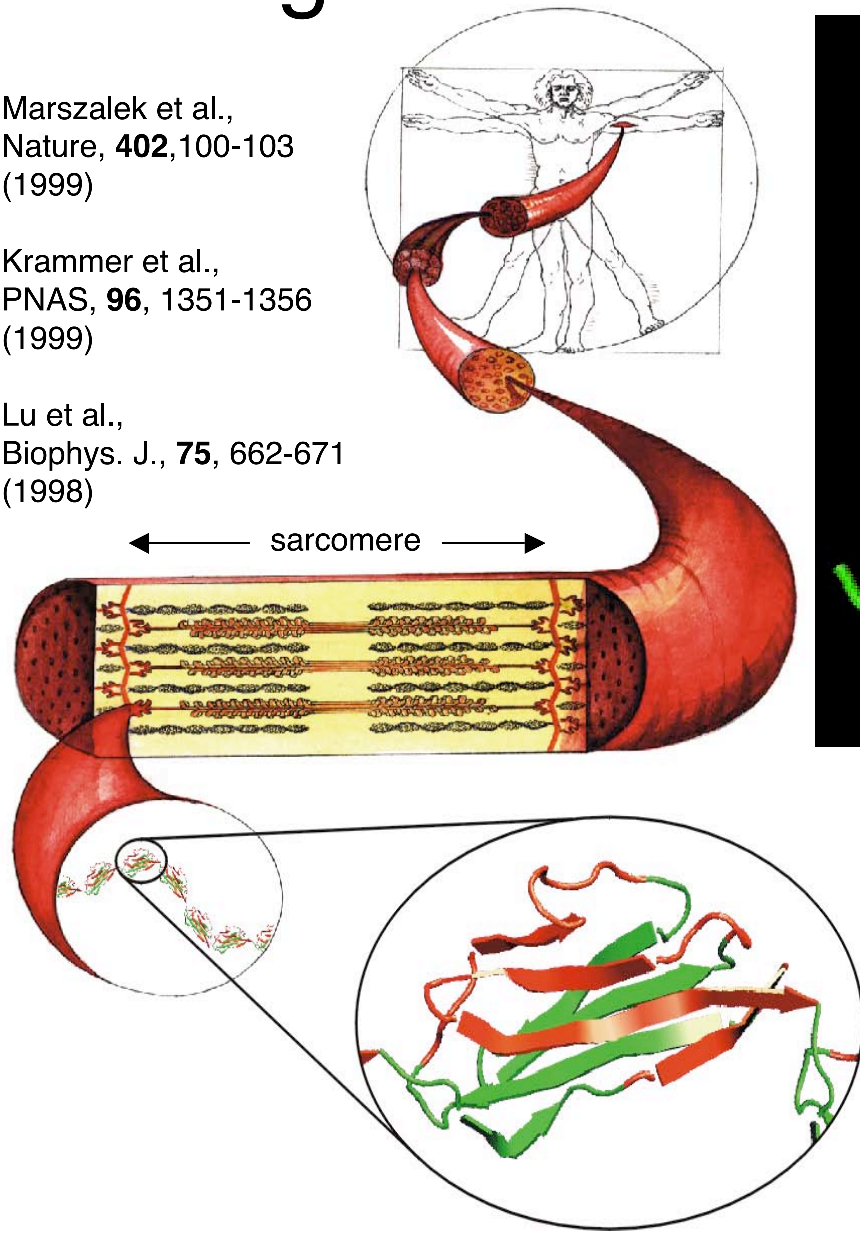


Probing the Passive Elasticity of Muscle: I27

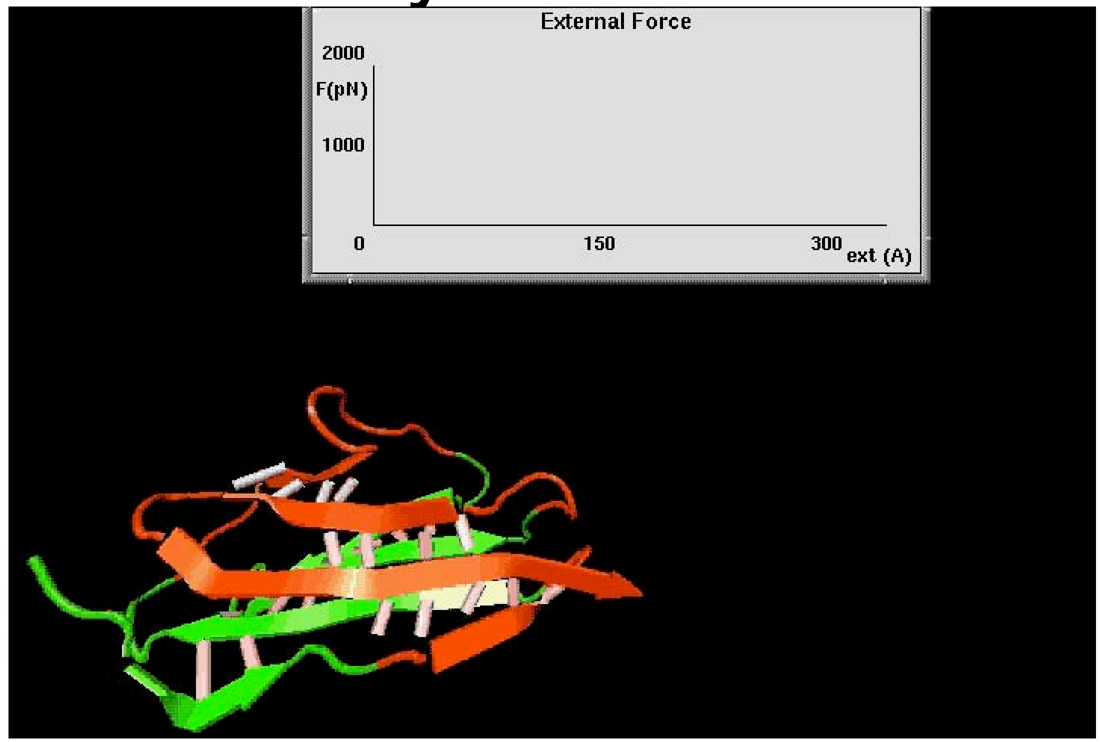
Marszalek et al.,
Nature, **402**,100-103
(1999)

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

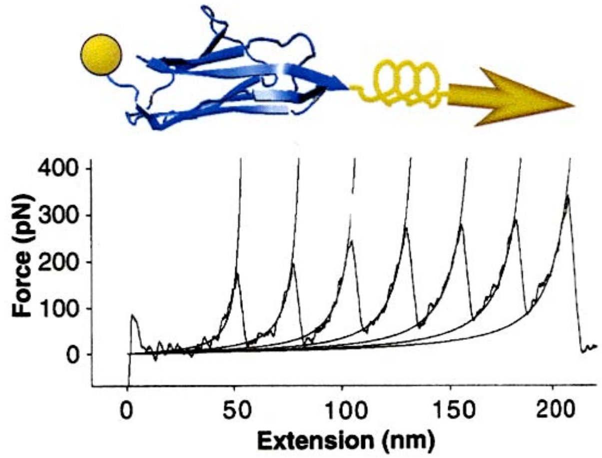
Gaub et al.,
Fernandez et al.

Hui Lu, Barry Isralewitz

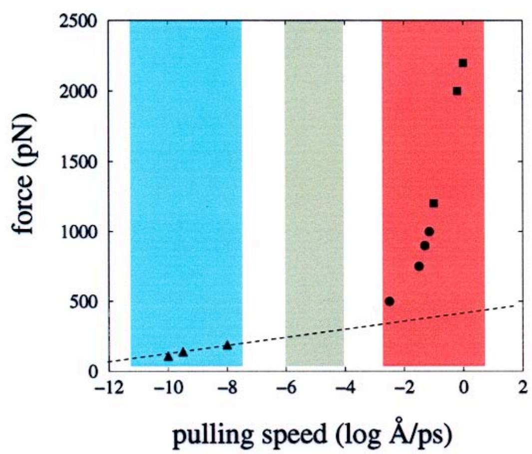
Quantitative Comparison

Bridging the gap between SMD and AFM experiments

Steered Molecular Dynamics (SMD)



Force-pulling velocity relationship



AFM range

Current SMD range

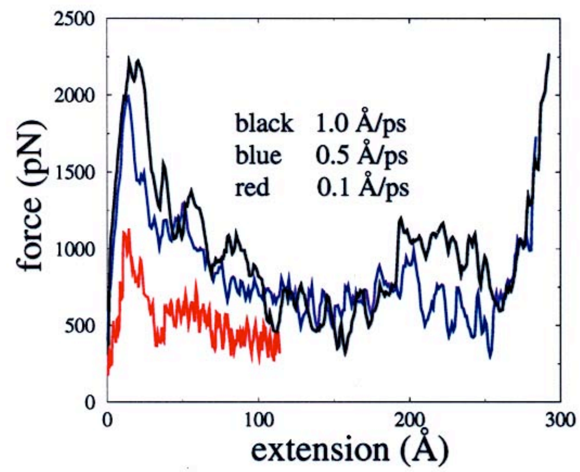
Target simulation range

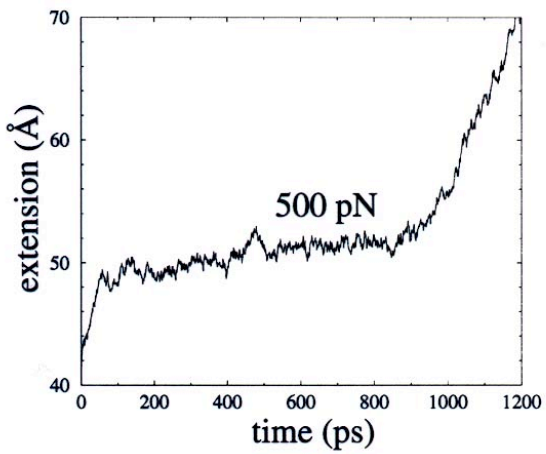
■ ● SMD data

▲ AFM data

------ Extrapolation of AFM data

Force-extension curve

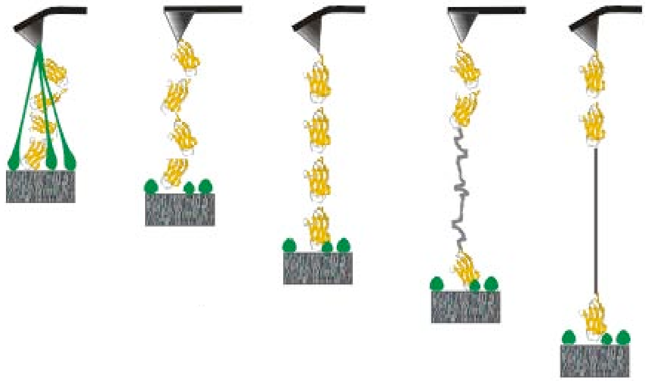


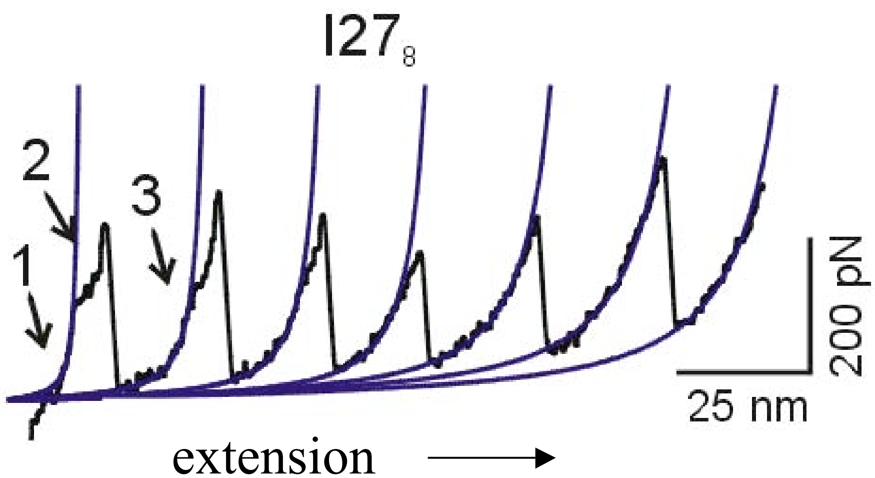


Extension curve

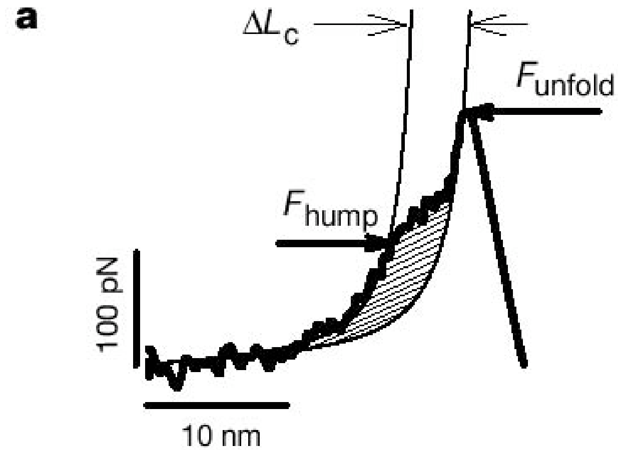
Hui Lu, Barry Isralewitz

Stretching modular proteins – Detailed View

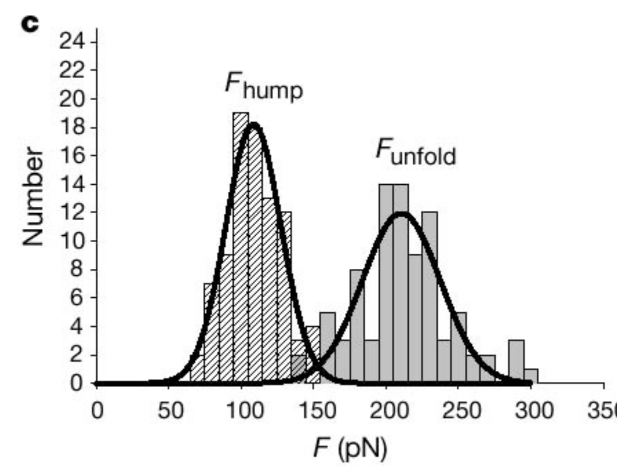




Schematic view and typical
Extension vs. force plot



Extension occurs in two steps



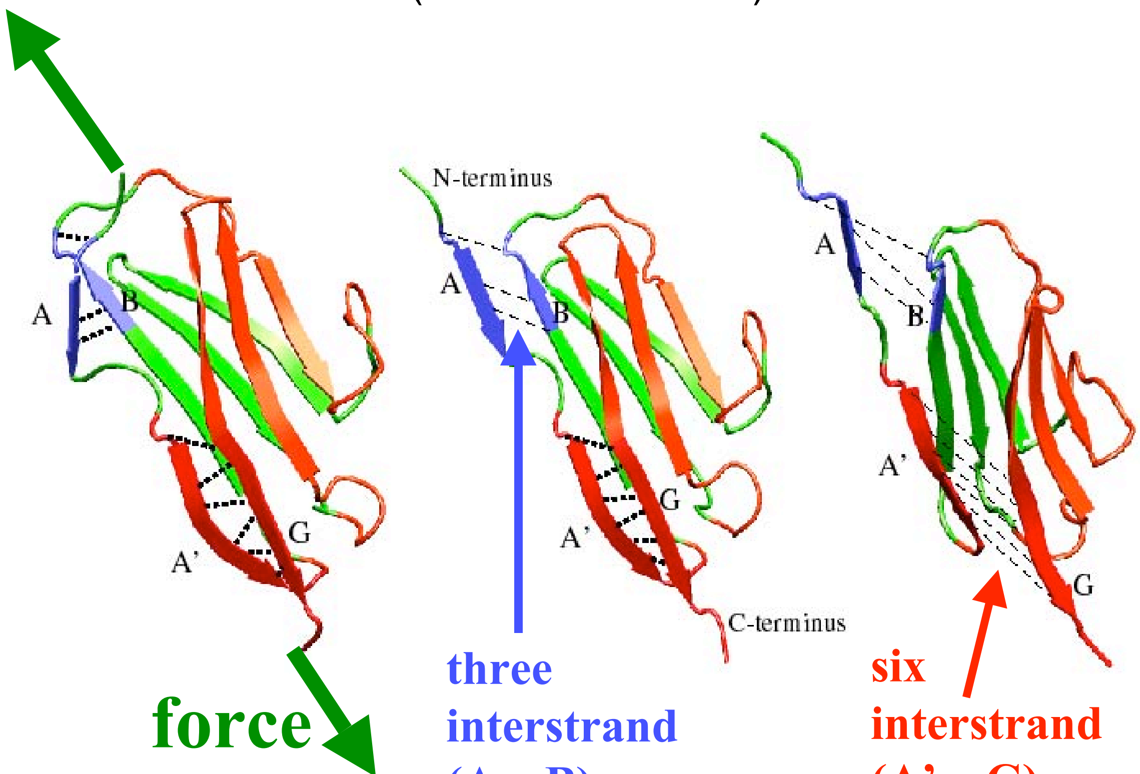
Distribution of measured forces
For step 1 and step 2

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)



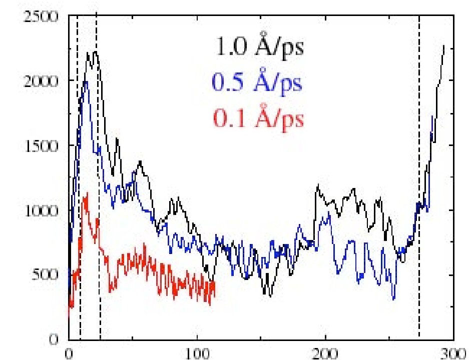
force

three interstrand (A – B) hydrogen bonds

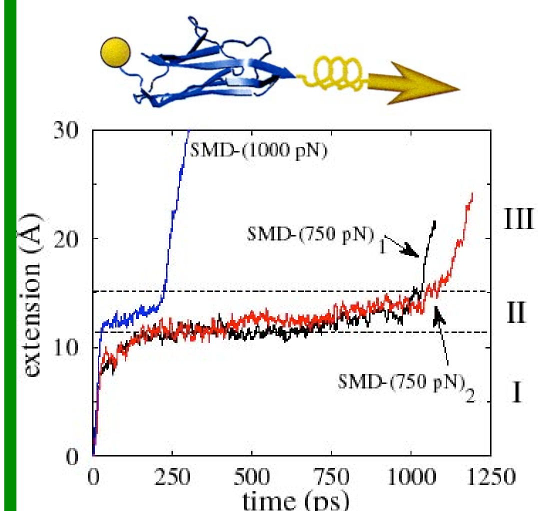
six interstrand (A' – G) hydrogen bonds

Hui Lu, Barry Israelewitz

Two types of SMD simulations



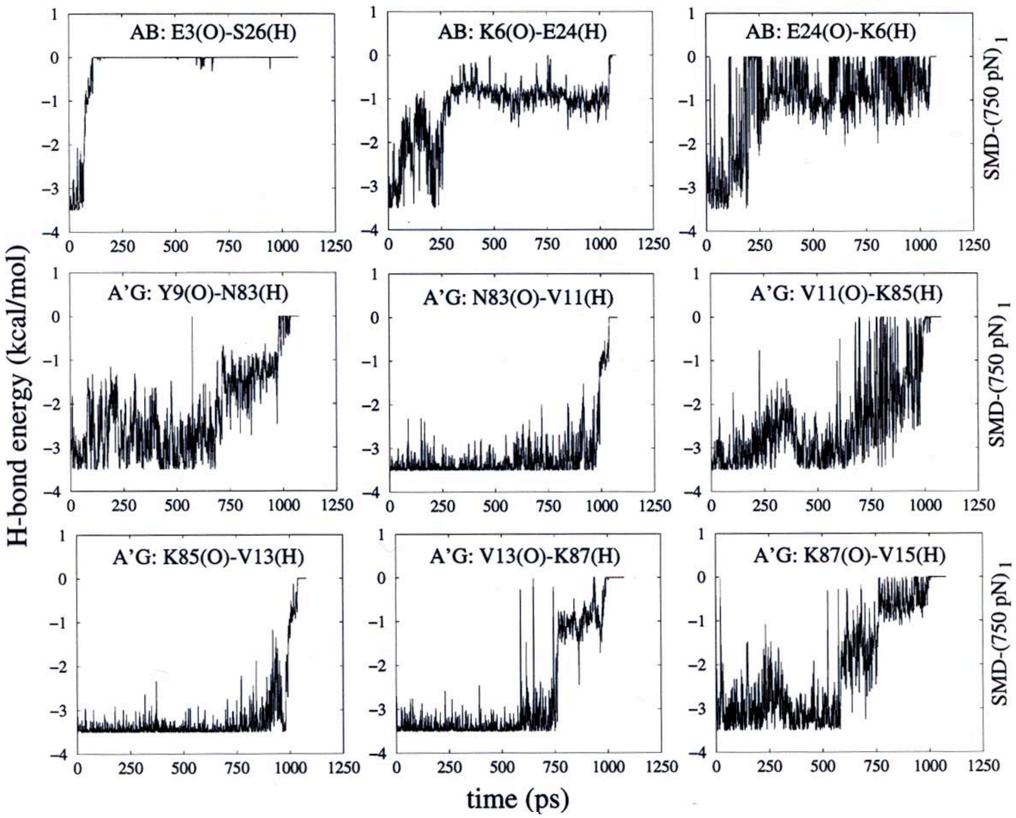
Constant velocity: Lu et al, Biophys J., 72, 1568 (1007)

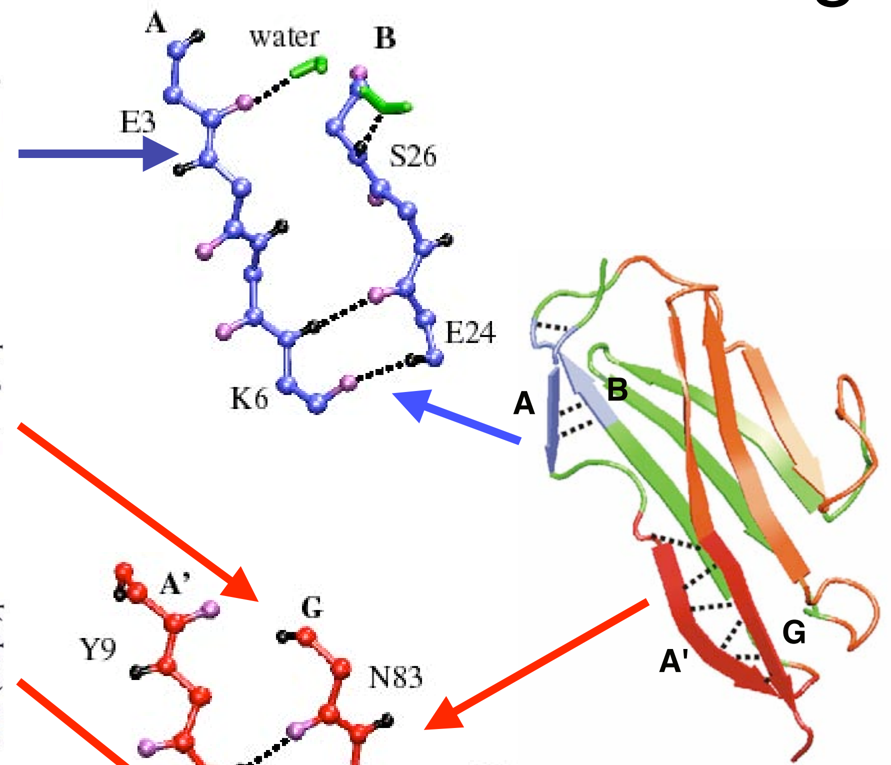


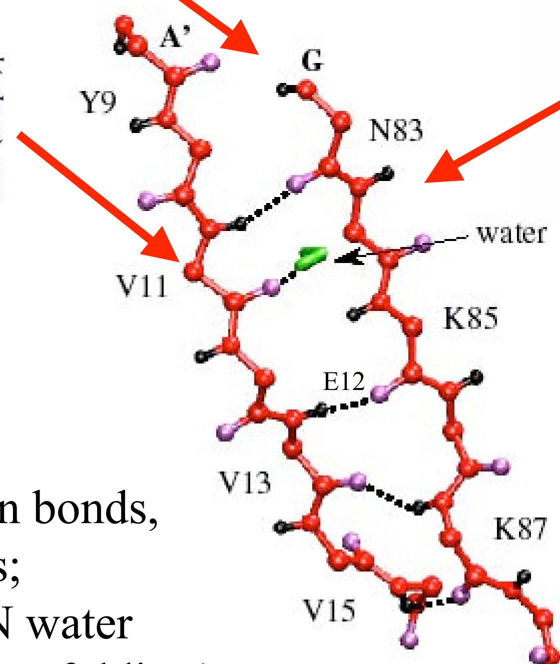
Constant force: Lu and Schulten Chem. Phys., 247 (1999) 141.

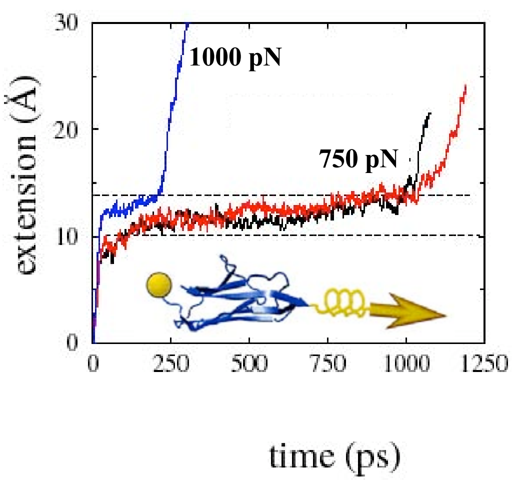
***Reviewed in* Israelewitz *et al. Curr. Opinion Struct. Biol.* 11:224-230 (2001)**

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!

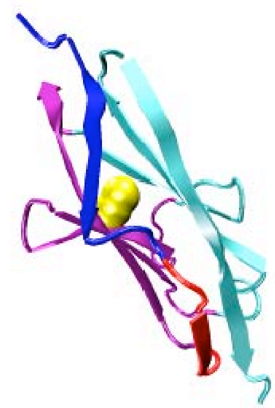
water greases protein folding !

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

Sequence aligment of Ig modules from the proximal Ig region

```
             A          A'
       .          .      .             *  .     .
 seqI1 -APKIFERIQSQTVGQGSDAH-FRVRVVGKPDPECEWYKNG--VKIERSDRIYWYWPEDN     56
 seqI2 QLITFTQELQDVVAKEKDTMATFECETSEPFVK-VKWYKDG--MEVHEGDK-YRMHSDRK     56
 seqI3 -VVEFVKELQDIEVPESYSGE-LECIVSPENIE-GKWYHND--VELKSNGK-YTITSRRG     54
 seqI4 -PIAILQGLSDQKVCEGDIVQ-LEVKVSLESVE-GVWMKDG--QEVQPSDR-VHIVIDKQ     54
 seqI5 ---DVITPLKDVNVIEGTKAV-LECKVSVPDVTSVKWYLND--EQIKPDDR-VQAIVKGT     53
 seqI6 ---KIIRGLRDLTCTETQNVV-FEVELSHSGID-VLWNFKD--KEIKPSSK-YKIEAHGK     52
 seqI7 -GGAISKPLTDQTVAESQEAV-FECEVANPDSK-GEWLRDG--KHLPLTNN-IRSESDGH     54
 seqI8 ---KIKKTLKNLTVTETQDAV-FTVELTHPNVKGVQWIKNG--VVLESNEK-YAISVKGT     53
 seqI9 ---KIIKKPKDVTALENATVA-FEVSVSHDTVP-VKWFHKS--VEIKPSDK-HRLVSERK     52
seqI10 ---HITKTMKNIEVPETKTAS-FECEVSHFNVP-SMWLKNG--VEIEMSEK-FKIVVQGK     52
seqI11 ---MITSMLKDINAEEKDTIT-FEVTVNYEGIS-YKWLKNG--VEIKSTDK-CQMRTKKL     52
seqI12 -HIEFRKHIKDIKVLEKKRAM-FECEVSEPDIT-VQWMKD--DQELQITDR-IKIQKEKY     54
seqI13 -DVRIRSIKKEVQVIEKQRAV-VEFEVNEDDVD-AHWYKDGIEINFQVQER-HKYVVERR     56
seqI14 EPPQVLQELQPVTVQSGKPAR-FCAMISGRPQPKISWYKEE--QLLSTGFK-CKFLHDGQ     56
seqI15 -PPAIITPLQDTVTSEGQPAR-FQCRVSG-TDLKVSWYSKD--KKIKPSRF-FRMTQFED     54
 ruler 1.......10........20........30........40........50........60
```

```
          : :      *                    : :
 seqI1 VCELVIRDVTAEDSASIMVKAINIAGETSSHAFLLVQAK     95
 seqI2 VHFLSILTIDTSDAEDYSCVLVEDE-NVKTTAKLIVEGA     94
 seqI3 RQNLTVKDVTKEDQGEYSFVID----GKKTTCKLKMKPR     89
 seqI4 SHMLLIEDMTKEDAGNYSFTIP--ALGLSTSGRVSVYSV     91
 seqI5 KQRLVINRTHASDEGPYKLIVG----RVETNCNLSVEKI     88
 seqI6 IYKLTVLNMMKDDEGKYTFYAG----ENMTSGKLTVA--     85
 seqI7 KRRLIIAATKLDDIGEYTYKVA----TSKTSAKLKVEAV     89
 seqI8 IYSLRIKNCAIVDESVYGFRLG----RLGASARLHVETV     88
 seqI9 VHKLMLQNISPSDAGEYTAVVG----QLECKAKLFVETL     87
seqI10 LHQLIIMNTSTEDSAEYTFVCG----NDQVSATLTVTPI     87
seqI11 THSLNIRNVHFGDAADYTFVAG----KATSTATLYVEAR     87
seqI12 VHRLLIPSTRMSDAGKYTVVAG----GNVSTAKLFVEGR     89
seqI13 IHRMFISETRQSDAGEYTFVAG----RNRSSVTLYVNAP     91
seqI14 EYTLLLIEAFPEDAAVYTCEAKNDYGVATTSASLSVEVP     95
seqI15 TYQLEIAEAYPEDEGTYTFVANNAVGQVSSTANLSLEAP     93
 ruler .........70........80........90.........
```



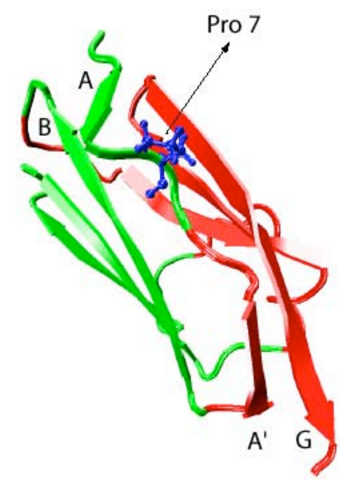
I1

Sequence aligment of Ig modules from the distal Ig region

```
            A          A'
            .                         :     :           *           :
I20    VKEIKDIILT-ESEFVGSSAIFECLVSPSTAITTWMKDGSNIR--ESPKHRFIADGKDRK     57
I21    PVRFVKTLEEEVTVVKGQPLYLSCELNK-ERDVVWRKDGKIVVE-KPGRIVPGVIGLMRA     58
I22    RDWLVKPIRD-QHVKPKGTAIFACDIAKDTPNIKWFKGYDEIPAEPNDKTEILRDGNHLY     59
I23    EVELLKPIED-VTIYEKESASFDAEISEADIPGQWKLKGELLR--PSPTCEIKAEGGKRF     57
I24    ELDFAVPLKD-VTVPERRQARFECVLTREANVI-WSKGPDIIK--SSDKFDIIADGKKHI     56
I25    RLKFMSPLED-QTVKEGETATFVCELSHEKMHVVWFKNDAKLH--TSRTVLISSEGKTHK     57
I26    DPYFTVKLHD-KTAVEKDEITLKCEVSK-DVPVKWFKDGEEIV--PSPKYSIKADGLRRI     56
I27    LIEVEKPLYG-VEVFVGETAHFEIELSEPDVHGQWKLKGQPLT--ASPDCEIIEDGKKHI     57
I28    PLIFITPLSD-VKVFEKDEAKFECEVSREPKTFRWLKGTQEIT--GDDRFELIKDGTKHS     57
I29    RLKFLTPLKD-VTAKEKESAVFTVELSHDNIRVKWFKNDQRLH--TTRSVSMQDEGKTHS     57
I30    DPYFTGKLQD-YTGVEKDEVILQCEISKADAPVKWFKDGKEIK--PSKNAVIKTDGKKRM     57
I31    EIKLVRPLHS-VEVMETETARFETEISEDDIHANWKLKGEALL--QTPDCEIKEEGKIHS     57
I32    VIGLLRPLKD-VTVTAGETATFDCELSYEDIPVEWYLKGKKLE--PSDKVVPRSEGKVHT     57
I33    PVEFTKPLED-QTVEEGATAVLECEVSRENAKVKWFKNGTEIL--KSKKYEIVADGRVRK     57
I34    HVEFLRPLTD-LQVREKEMARFECELSRENAKVKWFKDGAEIK--KGKKYDIISKGAVRI     57
I35    EAVFTKNLAN-IEVSETDTIKLVCEVSKPGAEVIWYKGDEEII--ETGRYEILTEGRKRI     57
I36    AAEFISKPQN-LEILEGEKAEFVCSISKESFPVQWKRDDKTLE--SGDKYDVIADGKKRV     57
I37    -LRIVVPLKD-TRVKEQQEVVFNCEVNTEGAKAKWFRNEEAIF--DSSKYIILQKDLVYT     56
I38    DLRIVEPLKD-IETMEKKSVTFWCKVNRLNVTLKWTKNGEEVP--FDNRVSYRVDKYKHM     57
I39    PTEFVEHLED-QTVTEFDDAVFSCQLSREKANVKWYRNGREIK--EGKKYKFEKDGSIHR     57
I40    PVEIIRPPQD-ILEAPGADVVFLAELNKDKVEVQWLRNNMVVV--QGDKHQMMSEGKIHR     57
ruler  1.........10........20........30........40........50........60
```

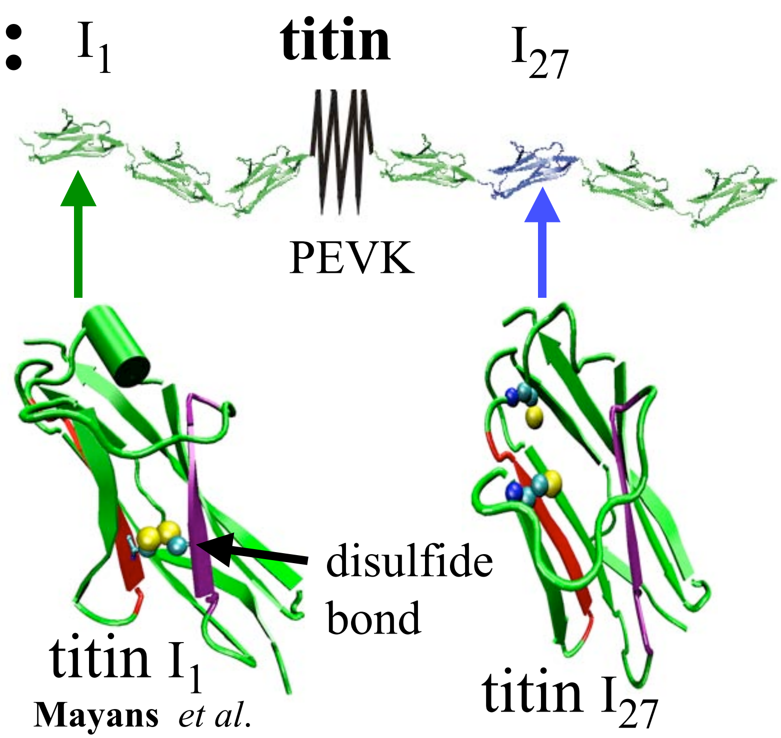
Key Residue

```
       :  .  .                          :  :
I20    LHIIDVQLSDAGEYTCVLRLGNKEK-TSTAKLVVEE-     92
I21    LTINDADDTDAGTYTVTVENAN--NLECSSCVKVVEV     93
I22    LKIKNAMPEDIAEYAVEIEGKR-----YPAKLTLGER     91
I23    LTLHKVKLDQAGEVLYQALNAI-----TTAILTVKEI     89
I24    LVINDSQFDDEGVYTAEVEGKK-----TSARLFVTGI     88
I25    LEMKEVTLDDISQIKAQVKELS-----STAQLKVLEA     89
I26    LKIKKADLKDKGEYVCDCGTDK-----TKANVTVEAR     88
I27    LILHNCQLGMTGEVSFQAANAK-----SAANLKVKEL     89
I28    MVIKSAAFEDEAKYMFEAEDKH-----TSGKLIIEGI     89
I29    ITFKDLSIDDTSQIRVEAMGMS-----SEAKLTVLEG     89
I30    LILKKALKSDIGQYTCDCGTDK-----TSGKLDIEDR     89
I31    LVLHNCRLDQTGGVDFQAANVK-----SSAHLRVKPR     89
I32    LTLRDVKLEDAGEVQLTAKDFK-----THANLFVKEP     89
I33    LVIHDCTPEDIKTYTCDAKDFK-----TSCNLNVVPP     89
I34    LVINKCLLDDEAEYSCEVRTAR-----TSGMLTVLEE     89
I35    LVIQNAHLEDAGNYNCRLPSSR-----TDGKVKVHEL     89
I36    LVVKDATLQDMGTYVVMVGAAR-----AAAHLTVIEK     89
I37    LRIRDAHLDDQANYNVSLTNHRGENVKSAANLIVEEE     93
I38    LTIKDCGFPDEGEYIVTAGQDK-----SVAELLIIEA     89
I39    LIIKDCRLDDECEYACGVEDRK-----SRARLFVEEI     89
I40    LQICDIKPRDQGEYRFIAKDKE-----ARAKLELAAA     89
ruler  ........70........80........90.......
```

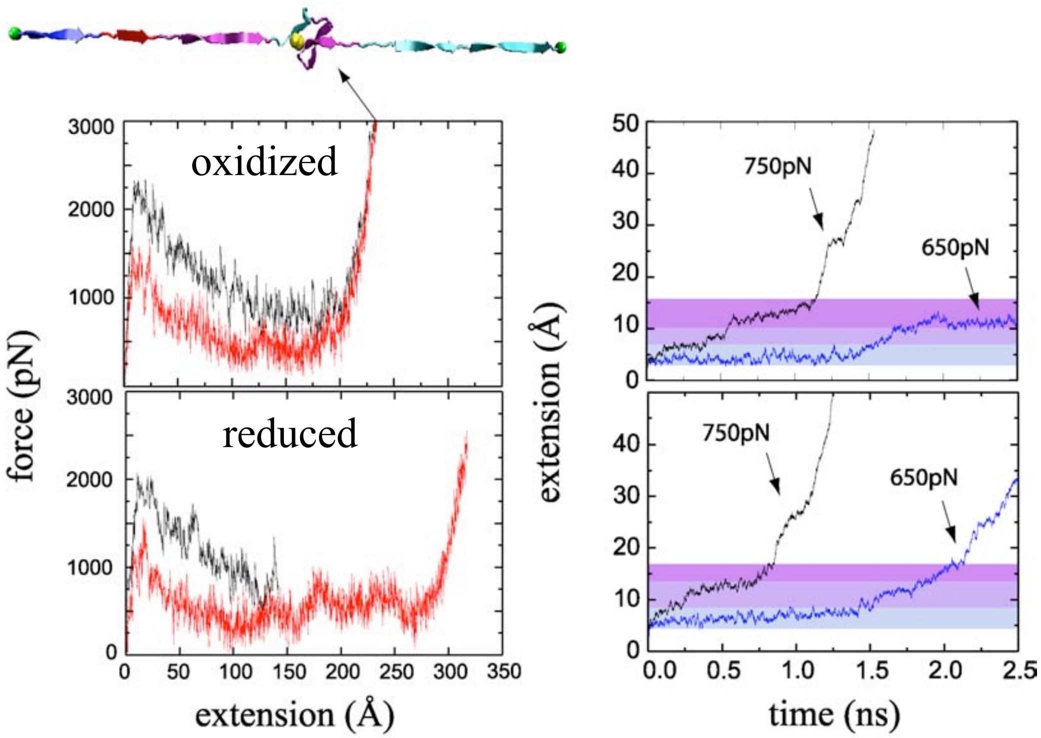


Titin Domain Heterogenity:

Comparing Oxidized and Reduced I1

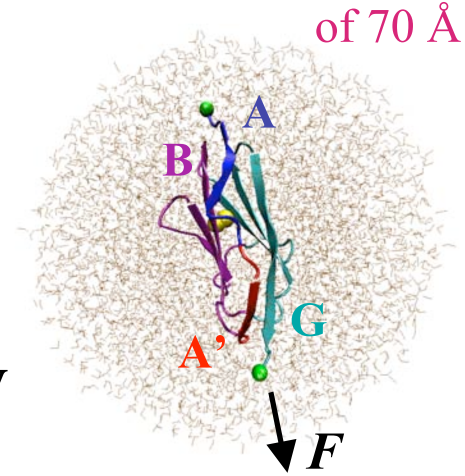


Mayans *et al.*
Structure **9**:331-340 (2001)



Stretching I_1 with constant velocity

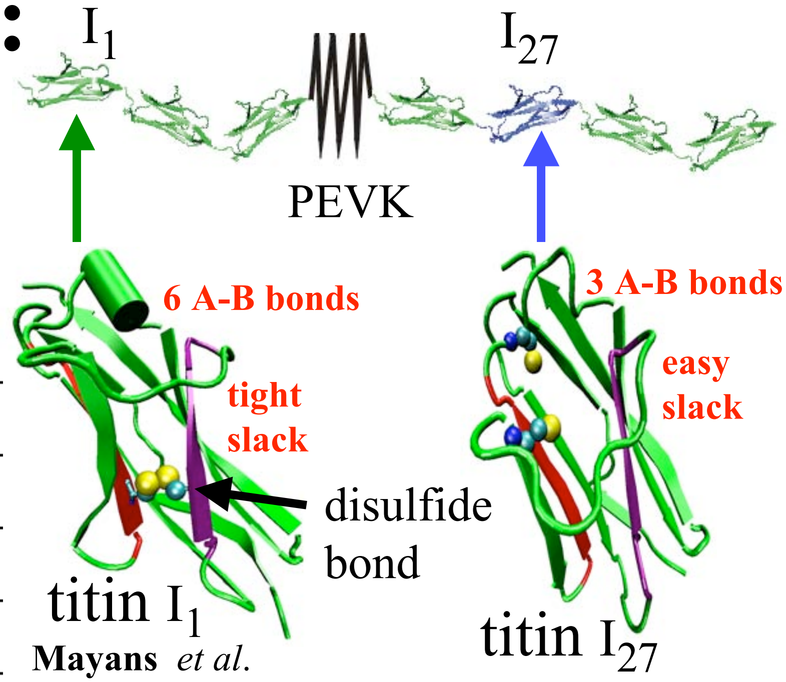
I_1 in 70 Å water sphere of 70 Å (18,000 atoms)



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

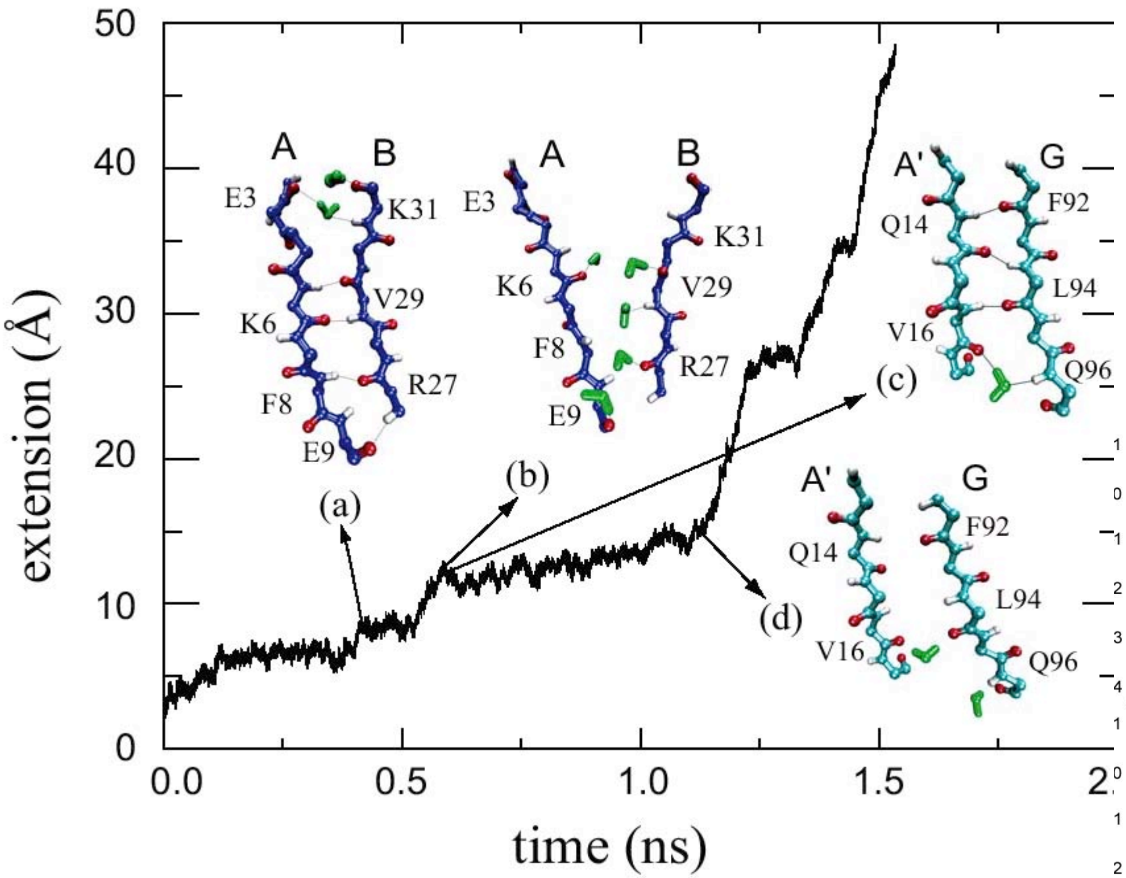
Mu Gao

Titin Domain Heterogenity:

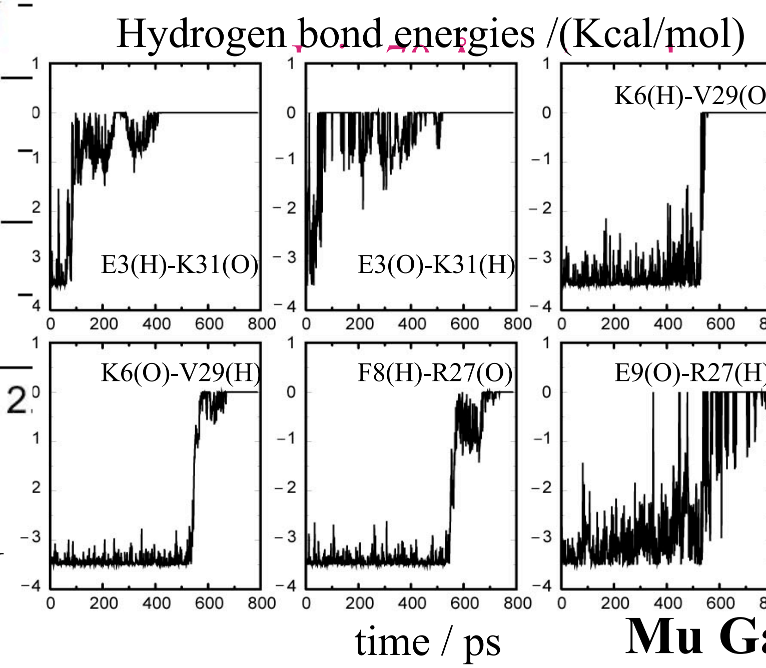


Mayans *et al.*
Structure **9**:331-340 (2001)

pre-stretching slack tight in I1



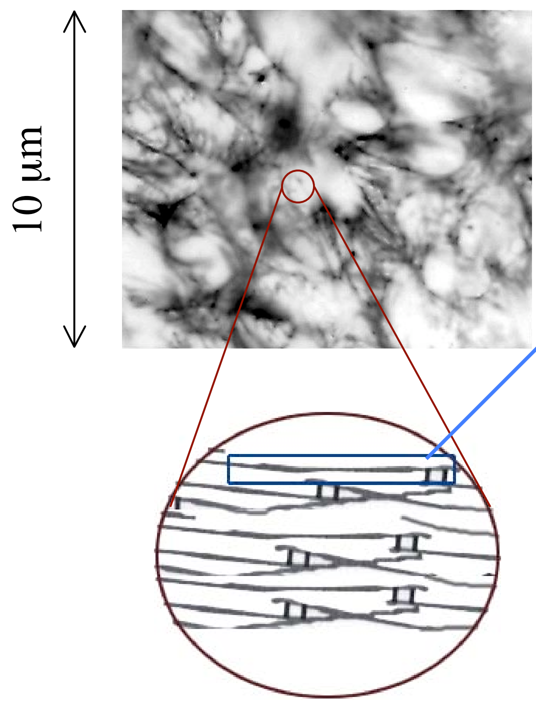
Stretching with constant force of 750 pN

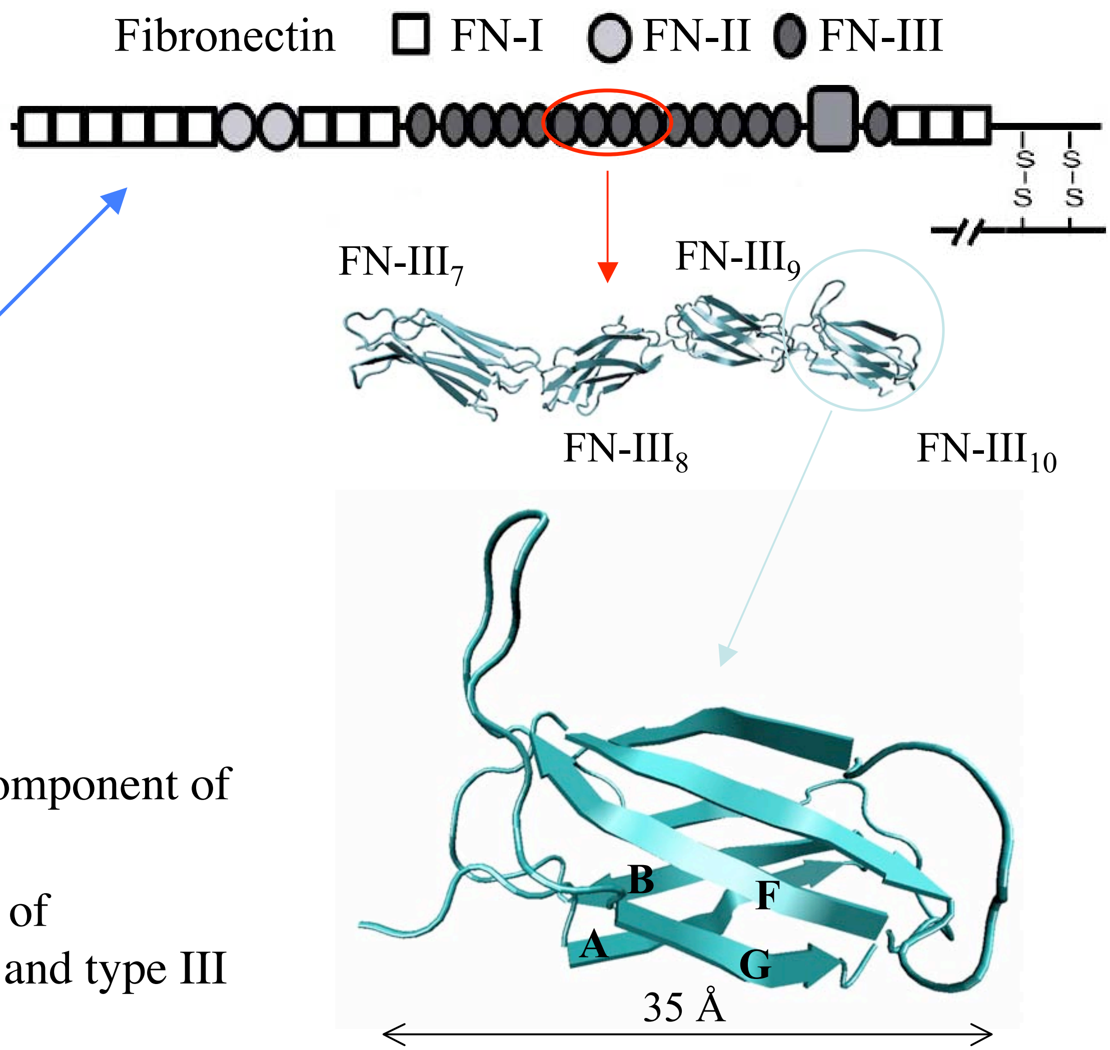


Mu Gao

Fibronectins

Architecture and Function of Fibronectin Modules

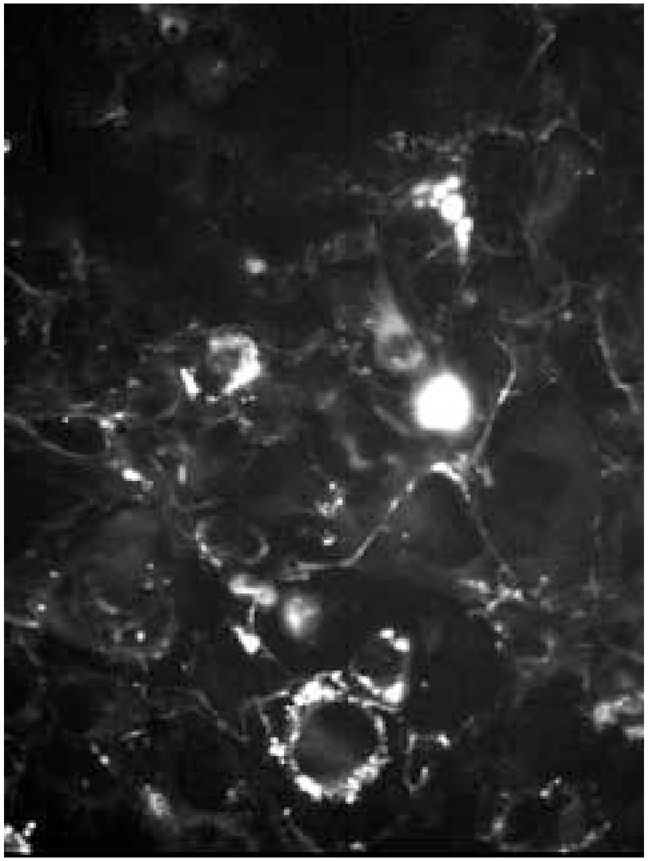




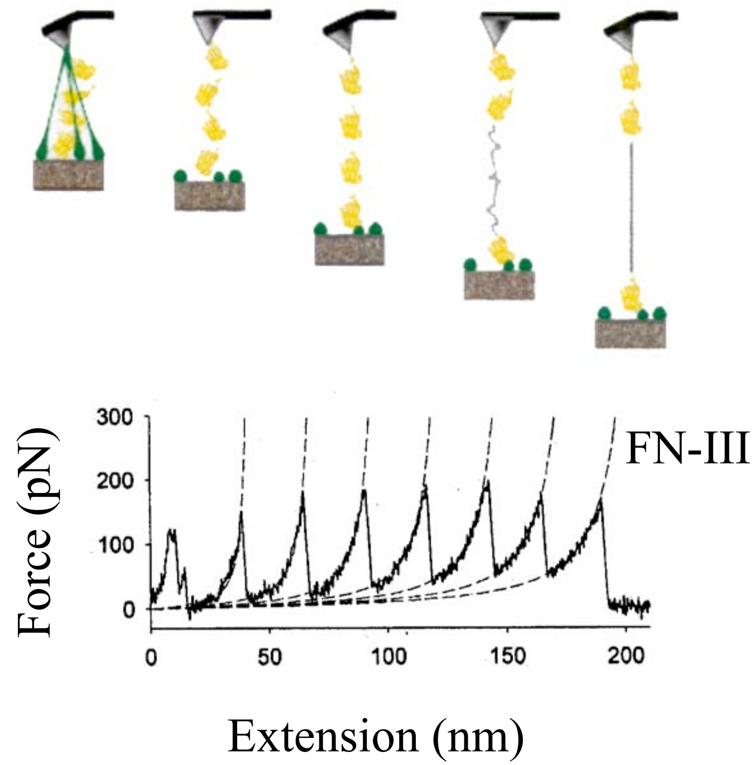
- Fibronectin is a major component of extracellular matrix
- It consists of three types of modules: type I, type II, and type III
- Highly extensible

Andre Krammer V. Vogel, U. Wash.

Fibronectin Matrix in Living Cell Culture



100 μm



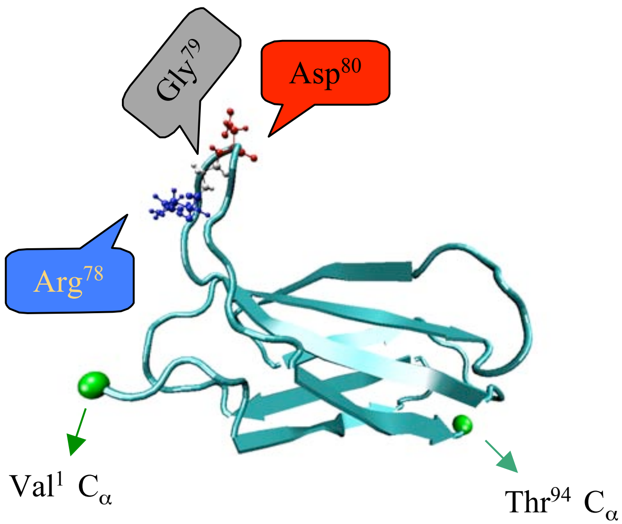
Atomic force microscopy observations

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

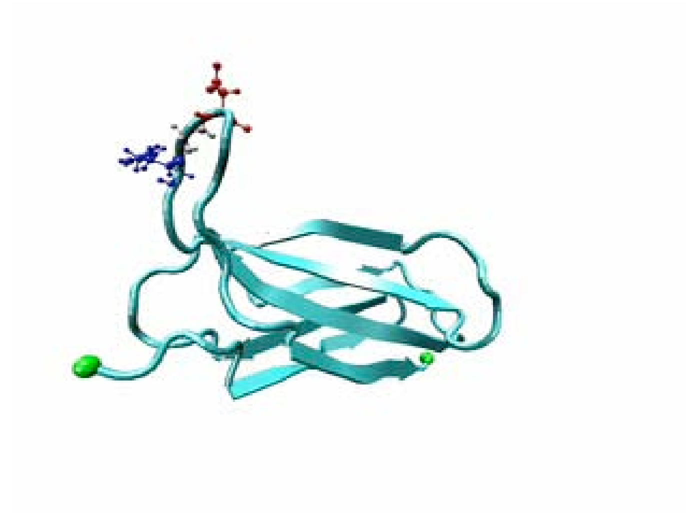
Andre Krammer V. Vogel, U. Wash.

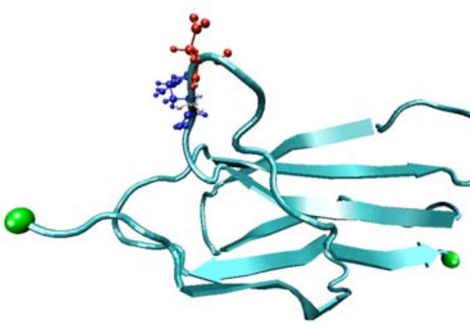
RGD Loop of FnIII$_{10}$

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



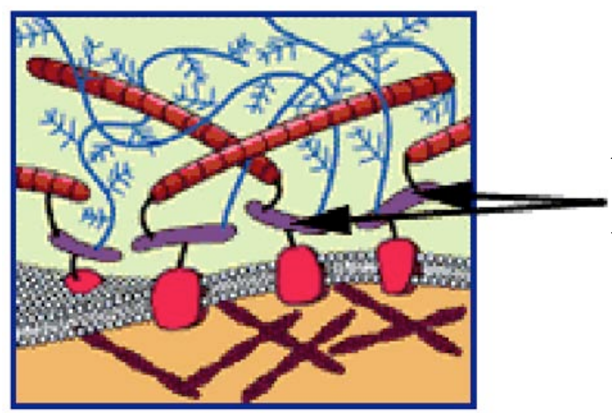
Native FnIII$_{10}$





→ $\boldsymbol{F}$ = 500 pN

Extension of 13 Å



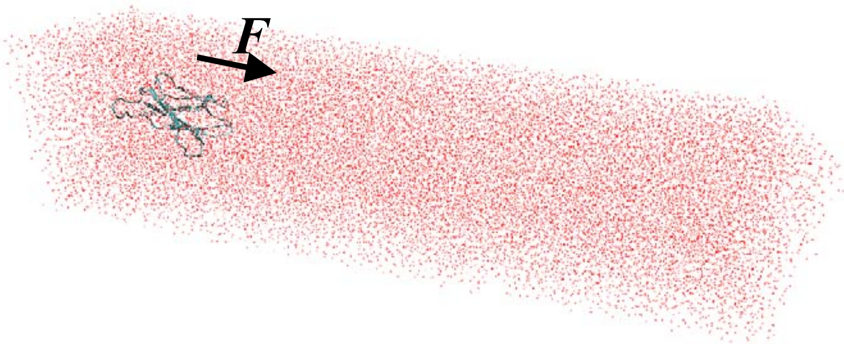
fibronectin binding with RGD loop to integrin

integrin interacting with extracellular matrix

Andre Krammer V. Vogel, U. Wash.

http://www.ks.uiuc.edu

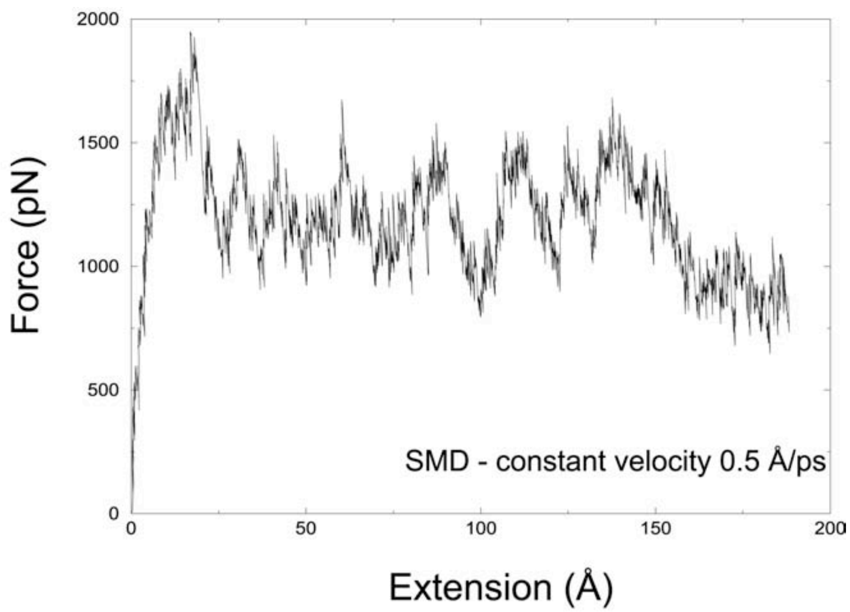
Probing Unfolding Intermediates in FN-III_{10}

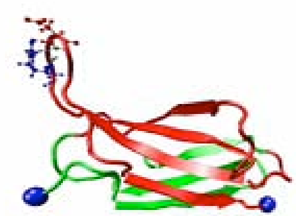


$FnIII_{10}$ module solvated in a water box $55 \times 60 \times 367$ Å^3 (**126,000 atoms)**

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.**

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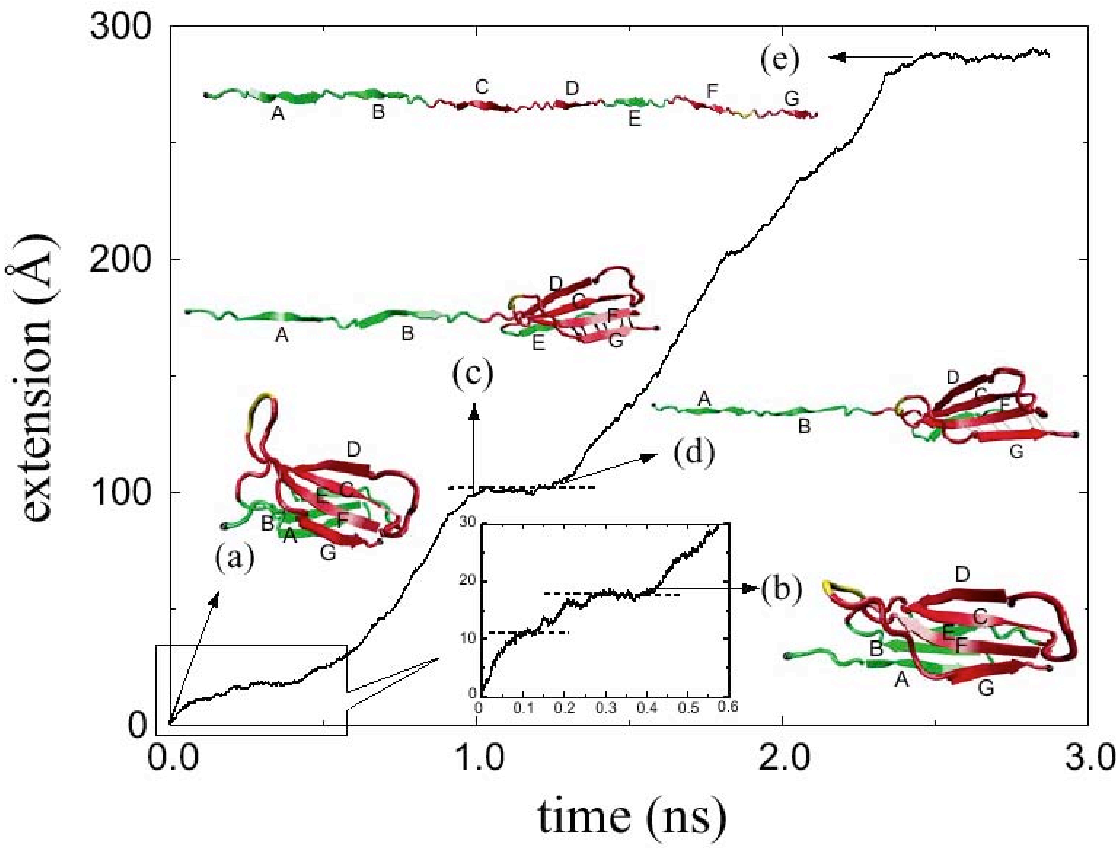
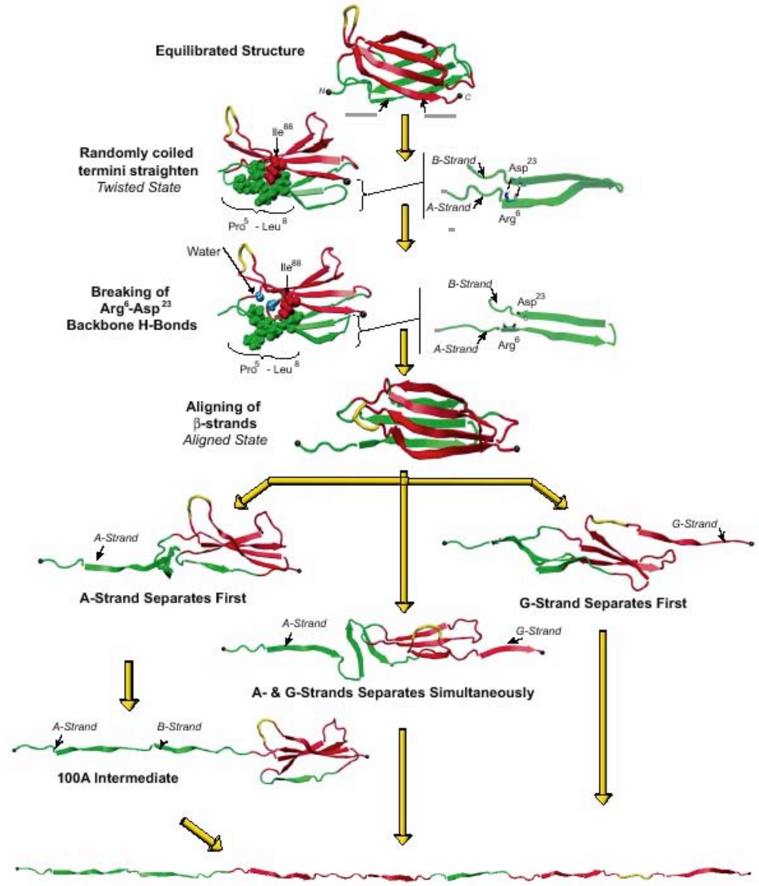


Specific stretching and unfolding pathway for constant force (500 pN) stretching: scenario with A rupturing first

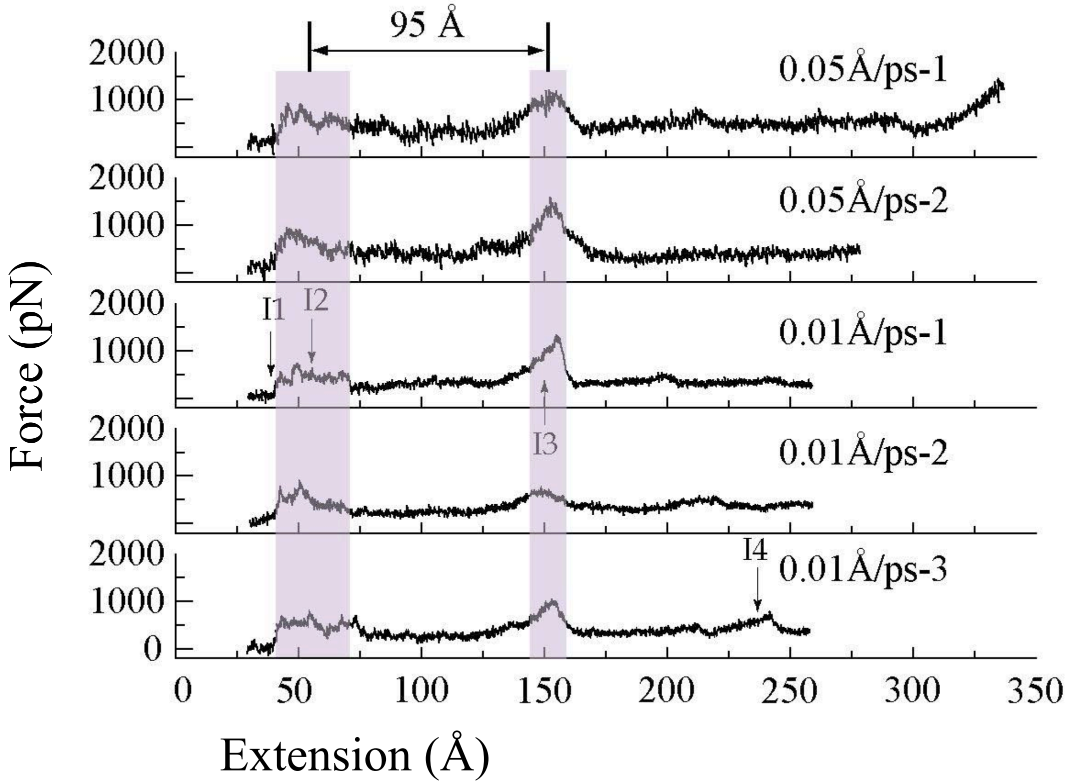


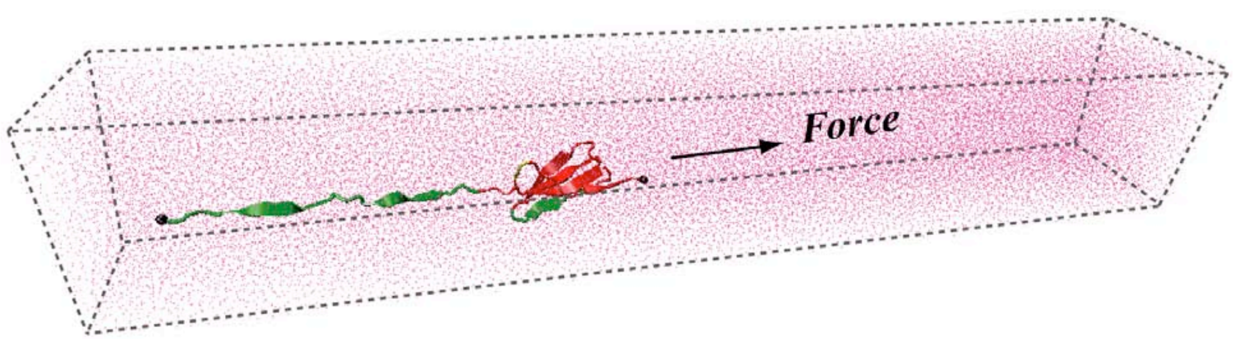
Complete stretching and unfolding pathway: after straightening and partial A-B separation, A, , or A+G or G rupture first

Stretching FN-III$_1$: Pronounced Intermediate

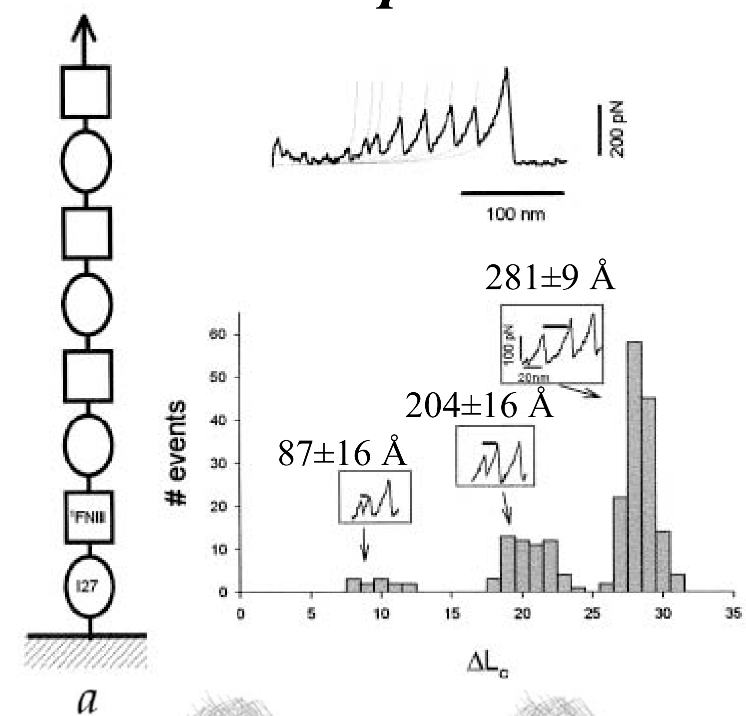
M. Gao, D. Craig, O. Lequin, I. D. Campbell, V. Vogel, and K. Schulten. **Structure and functional significance of mechanically unfolded fibronectin type III1 intermediates.** *Proc. Natl. Acad. Sci. USA,* 100:14784–14789, 2003.

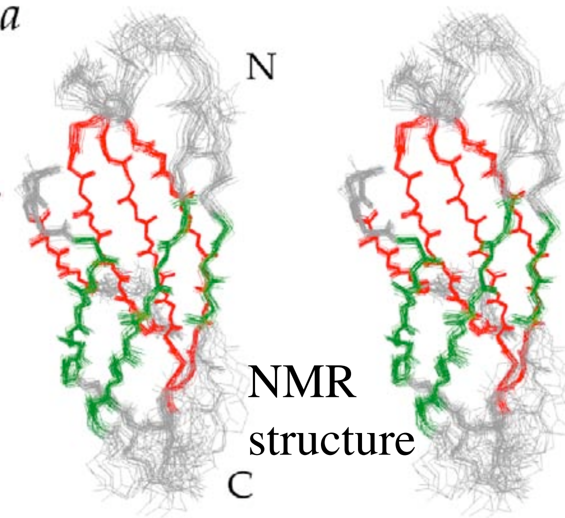
SMD simulations (150,000 atoms)





AFM experiments





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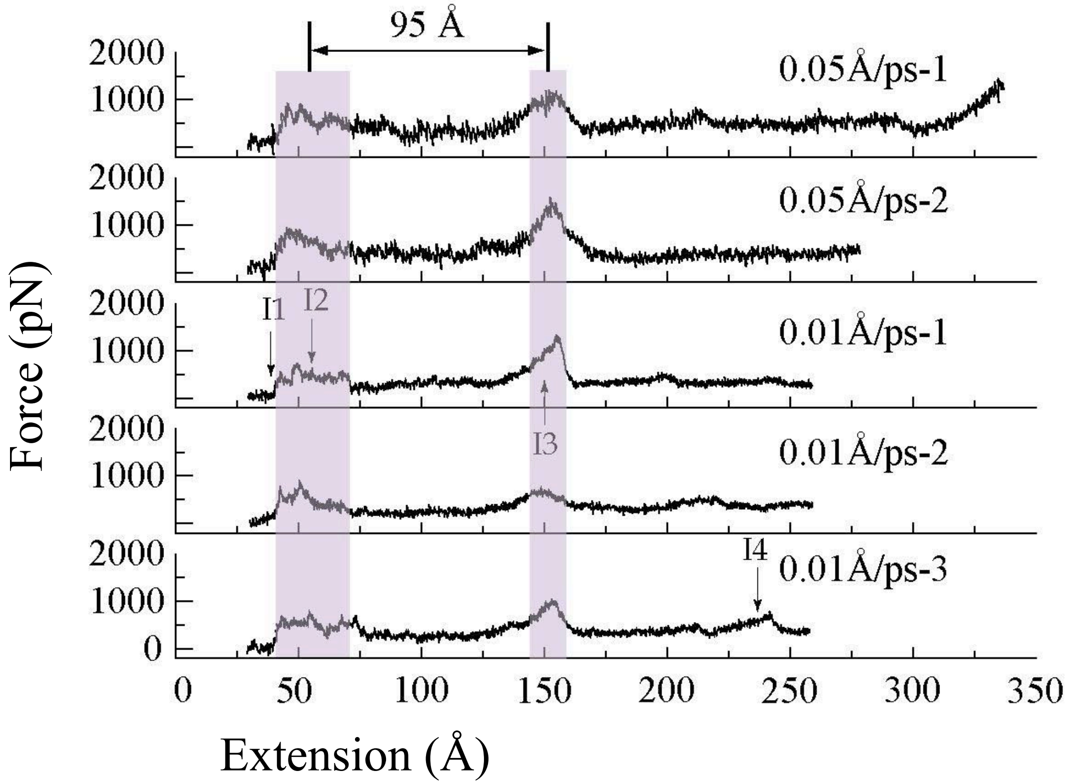

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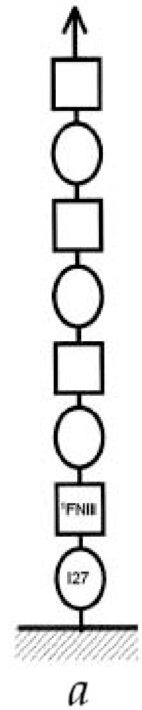

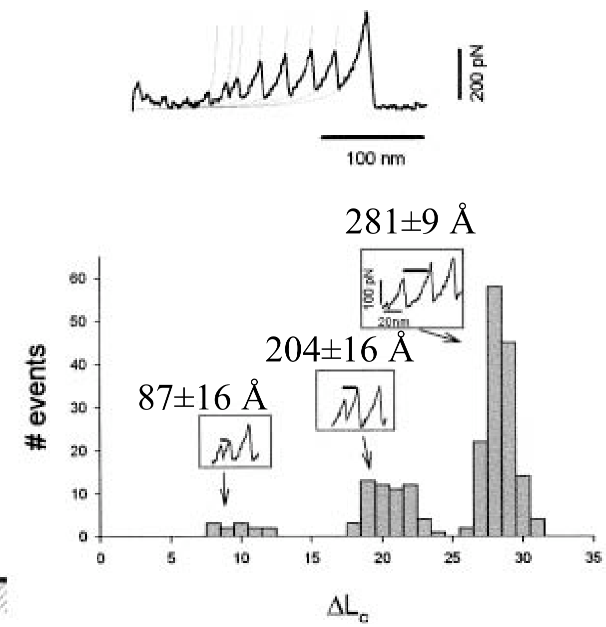

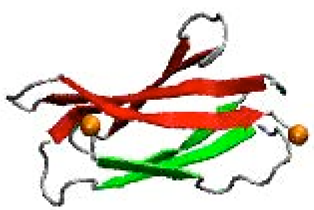

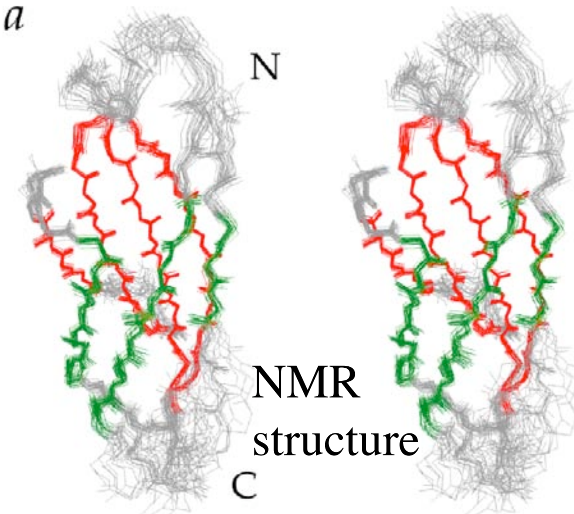

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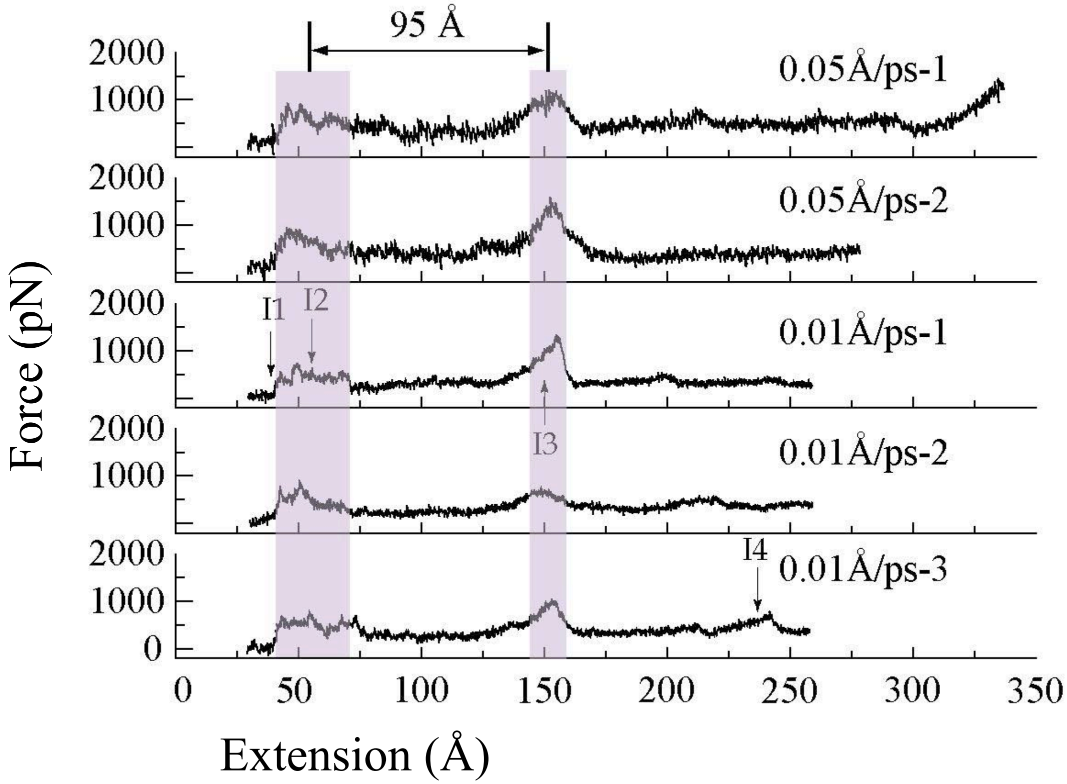
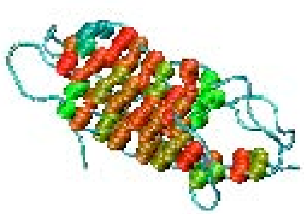
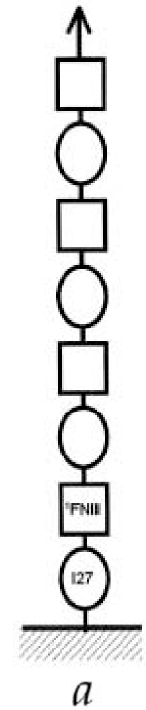
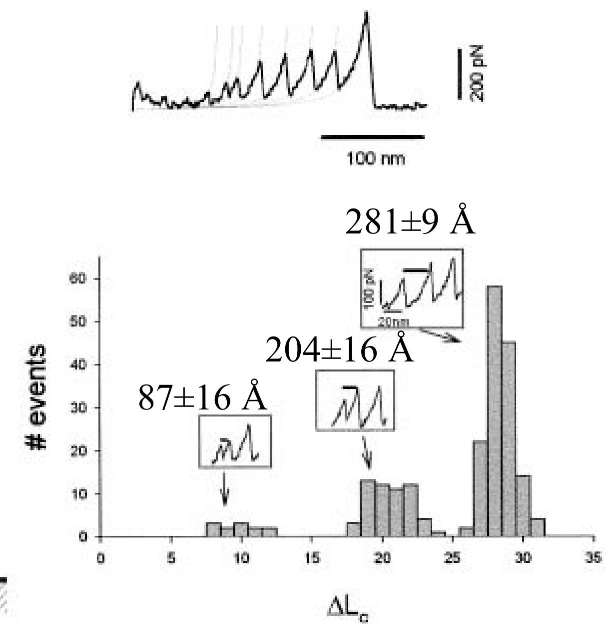
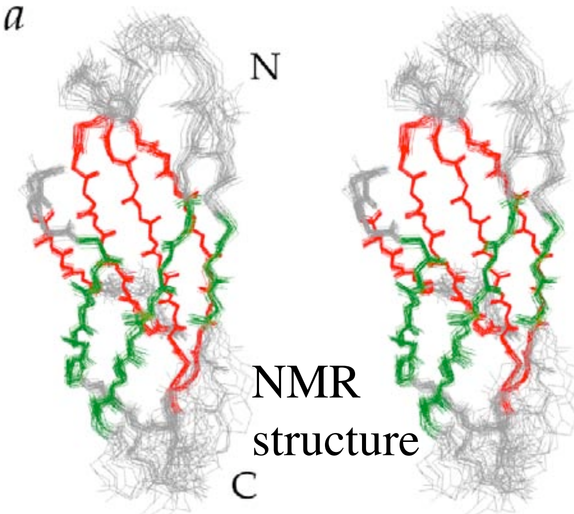




AFM experiments



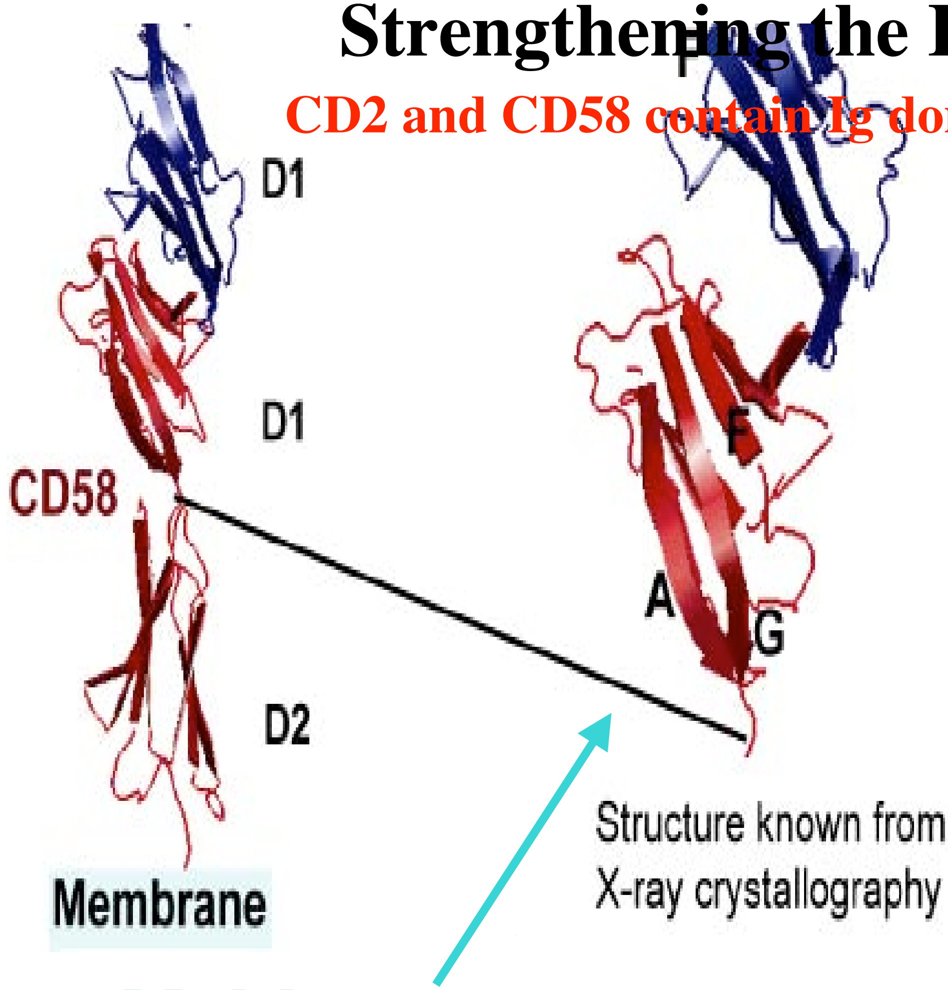




Adhesion Proteins of the Immune System

Strengthening the Immune System

CD2 and CD58 contain Ig domains

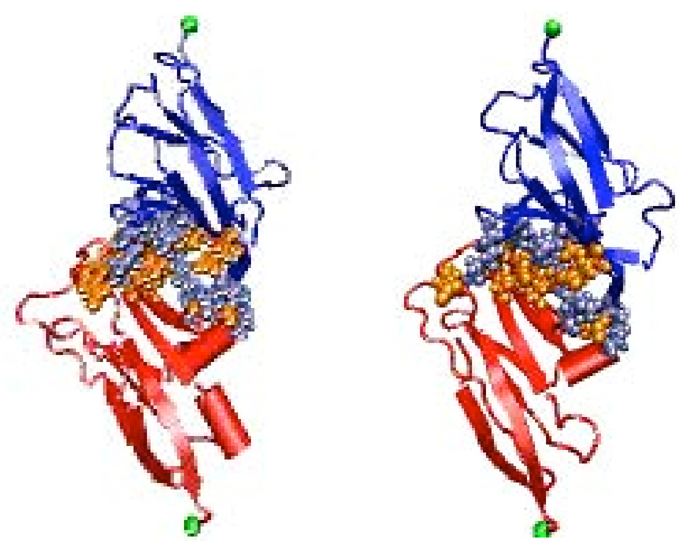


- Model Based on Crystal Structure: *Wang et al. (1999) Cell, **97**, 791*
- **90,000 atoms**
- NAMD simulation on Linux cluster 120 ps so far
- 1 – 0.05 Å / ps pulling velocity

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

0.05 Å / ps

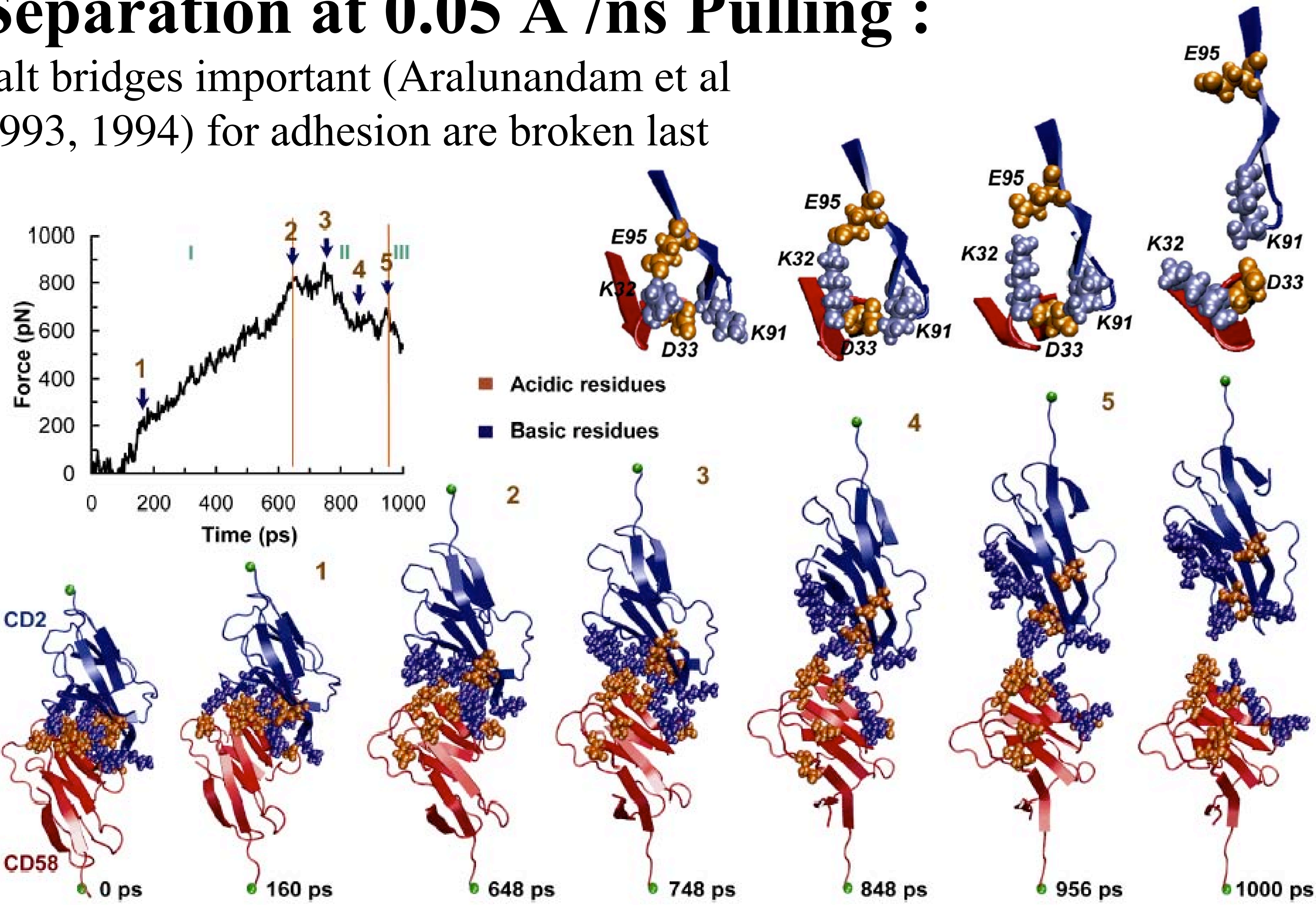
slow



1 Å / ps

Separation at 0.05 Å /ns Pulling :

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

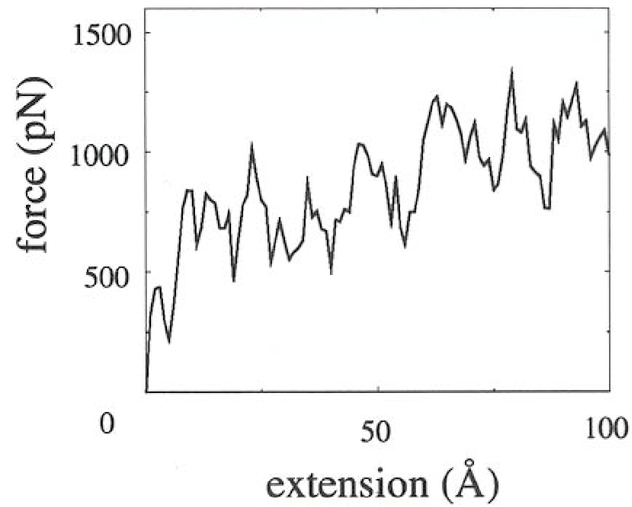


Marcos Bayas, D. Leckband, K. Schulten, Biophys. J. 84:2223-2233, 2003

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

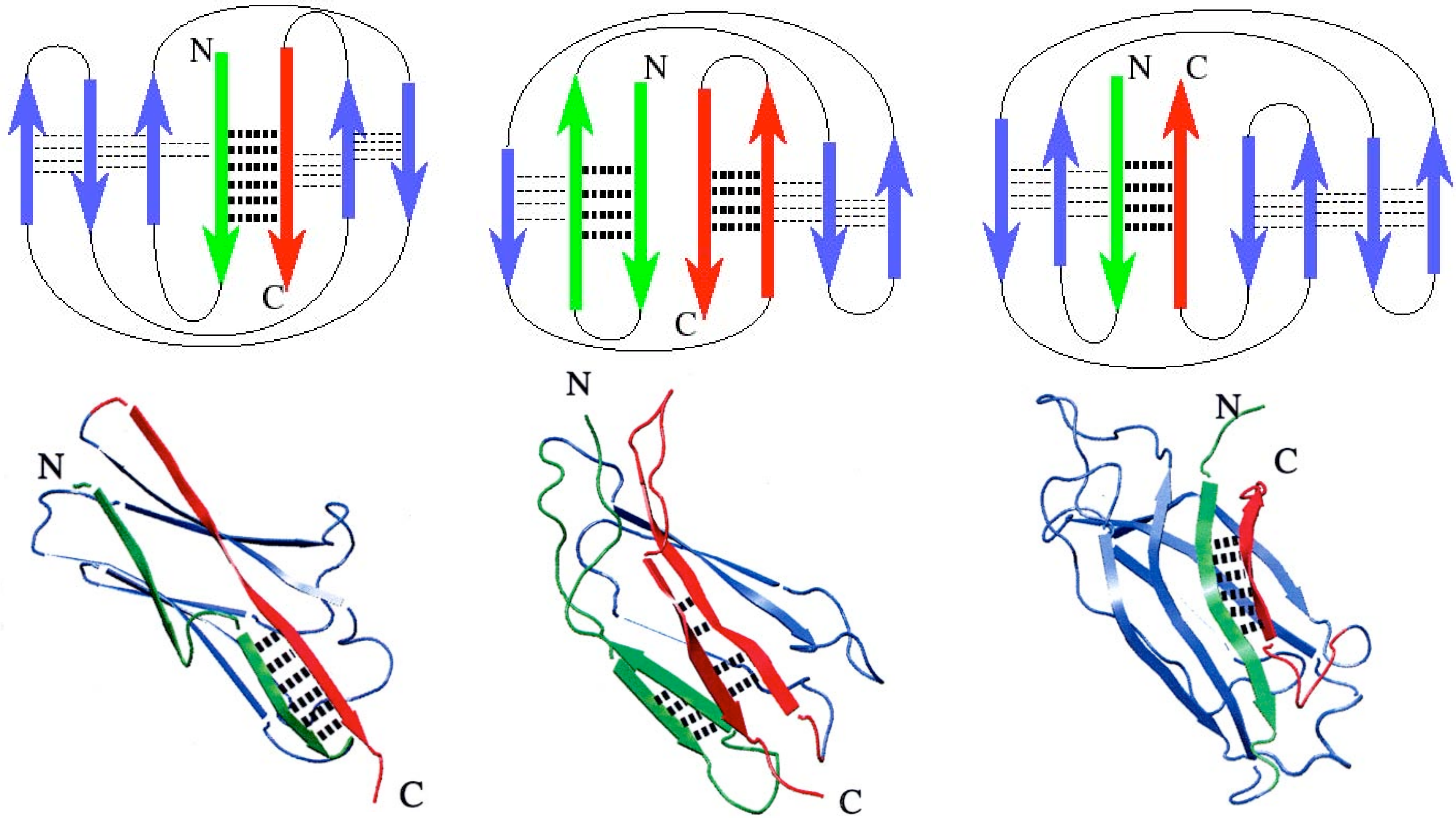
Lu and Schulten, Proteins, 35, 453-463 (1999)

Classification of β Sandwich Domains

Class I a:
strong resistance

Class I b:
less resistance

Class I c:
little resistance

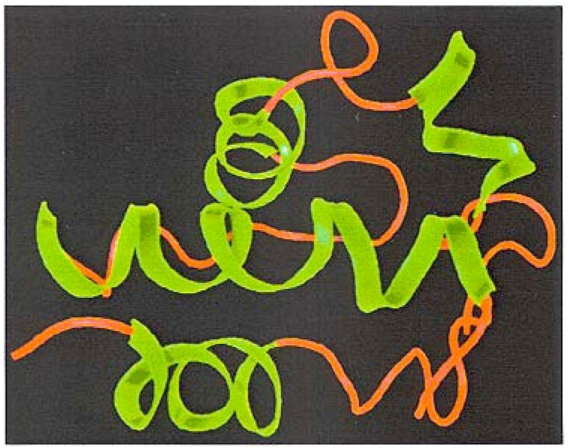


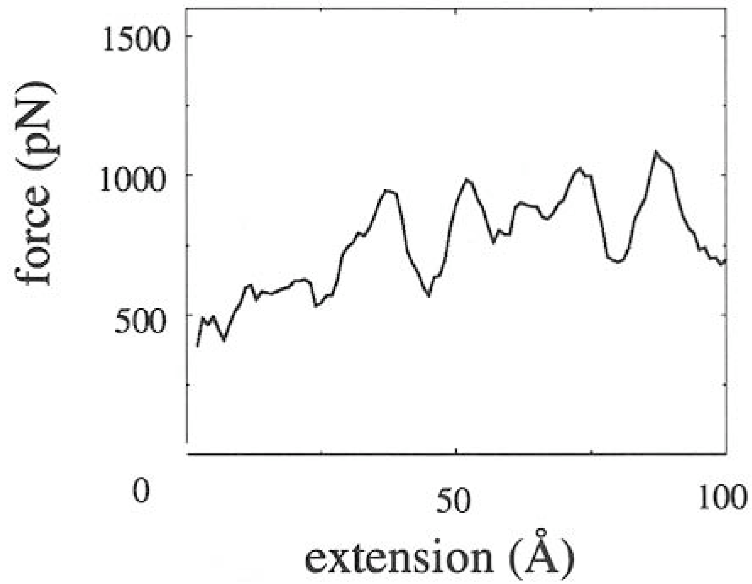
Lu and Schulten, Proteins, 35, 453-463 (1999)

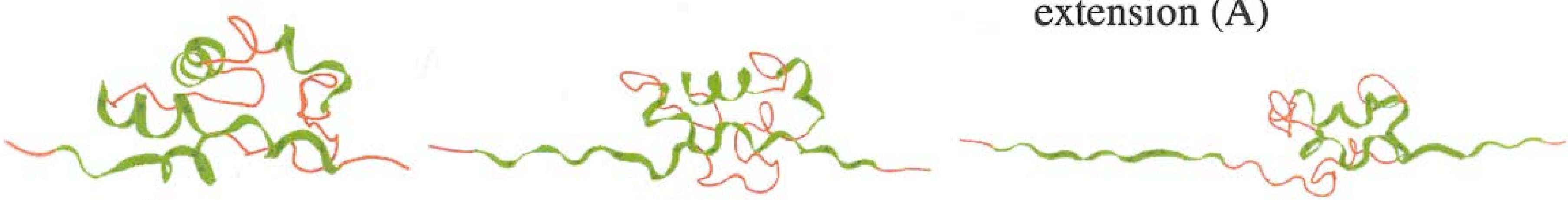
NIH Resource for Macromolecular Modeling and Bioinformatics
Theoretical Biophysics Group, Beckman Institute, UIUC

Force-induced unfolding of alpha-helical protein

Cytochrome C6 (all helix protein)





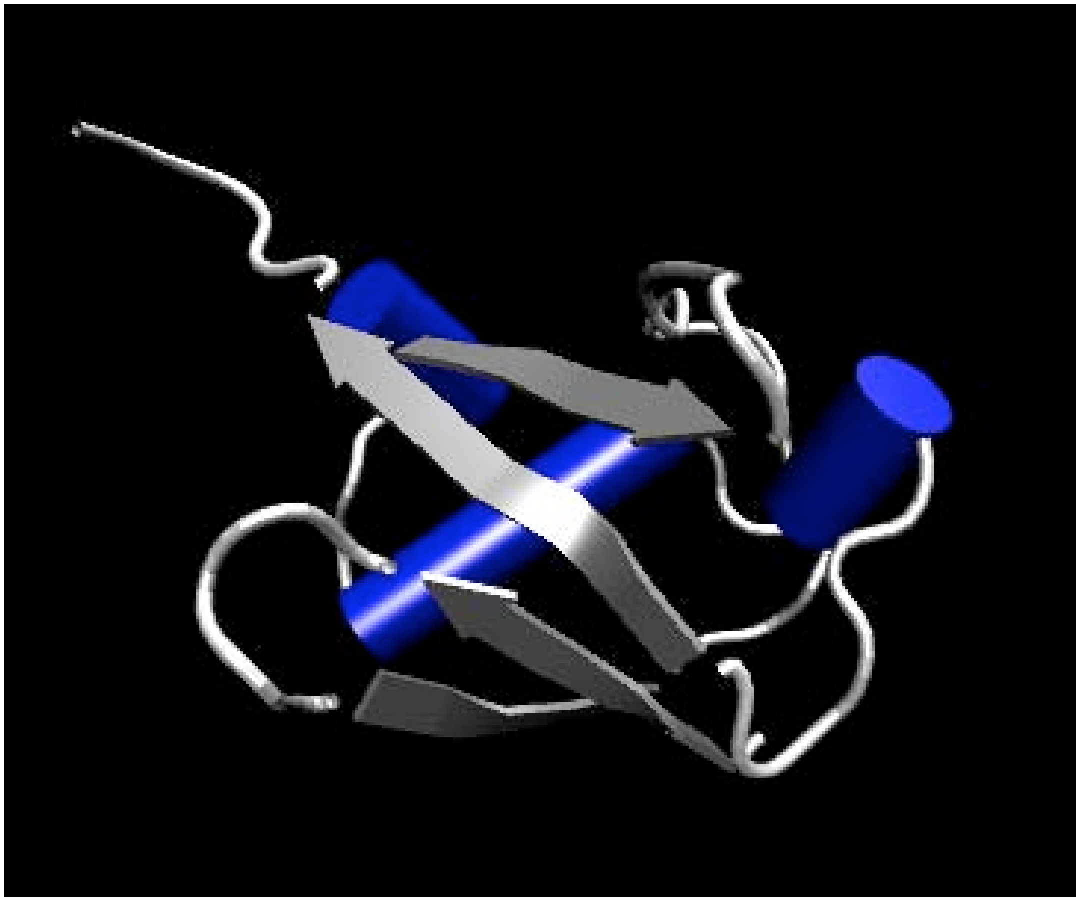


30 Å extension 60 Å extension 90 Å extension

- 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

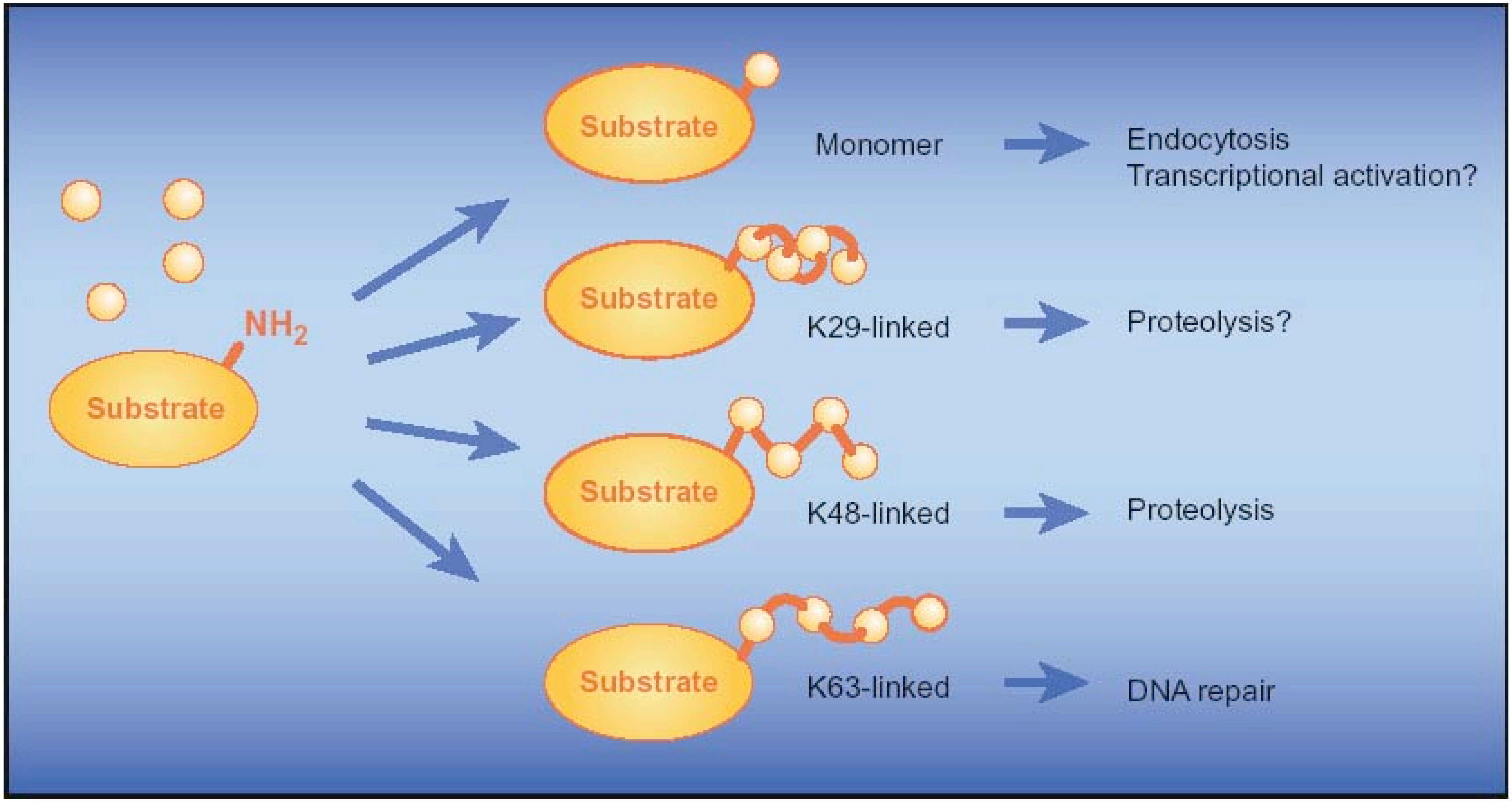
Lu and Schulten, Proteins, 35, 453-463 (1999)

Ubiquitin



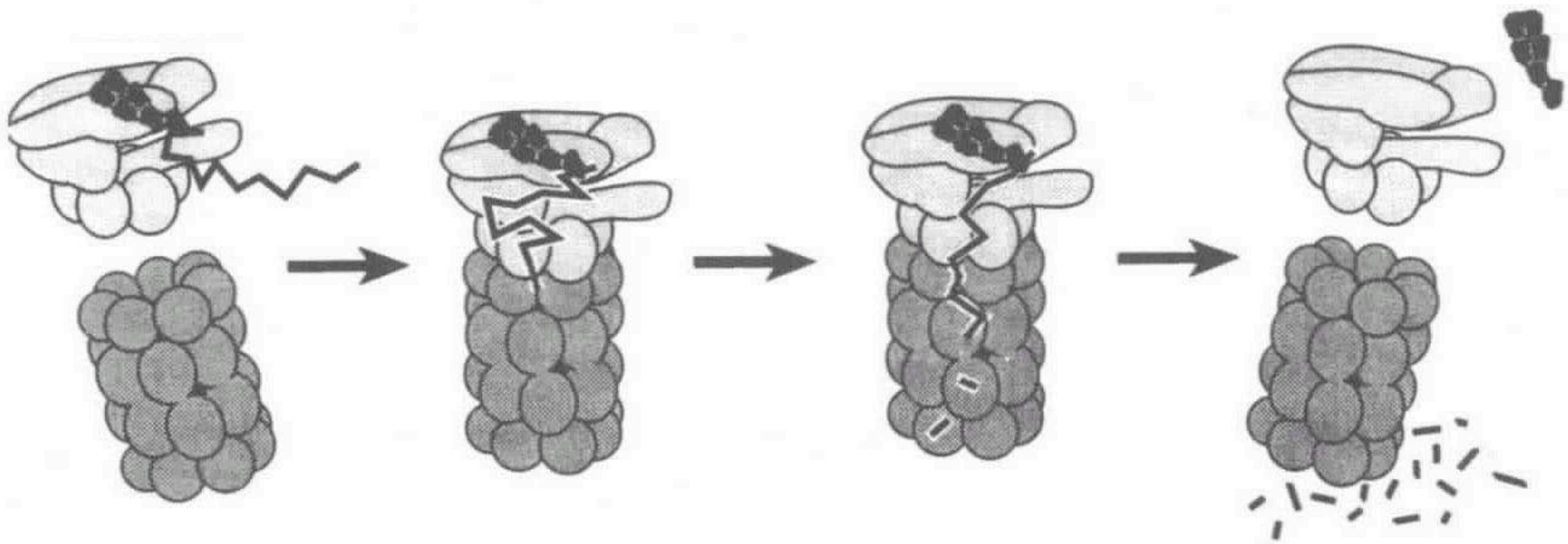
Fatemeh Araghi, Timothy Isgro, Marcos Sotomayor

Monoubiquitylation versus multi-ubiquitylation



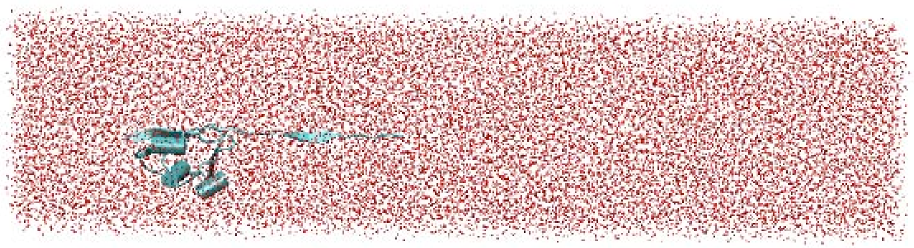
Multifaceted. Ubiquitin can attach to its various substrate proteins, either singly or in chains, and that in turn might determine what effect the ubiquitination has. (K29, K48, and K63 refer to the particular lysine amino acid used to link the ubiquitins to each other.)

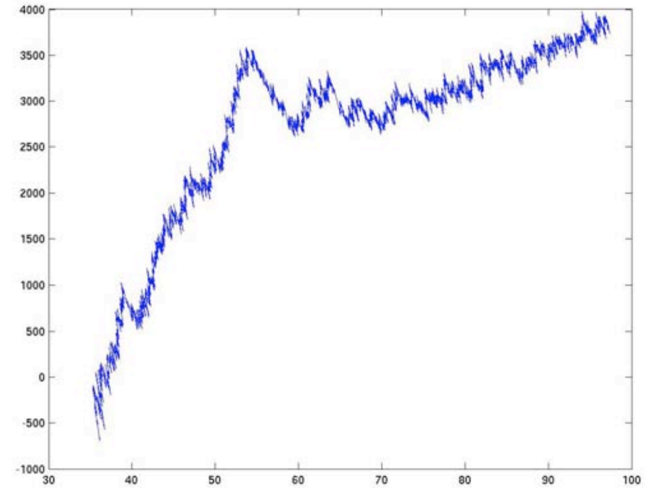
Structure-Function Relationship



Proteasome Degradation

First SMD Simulation

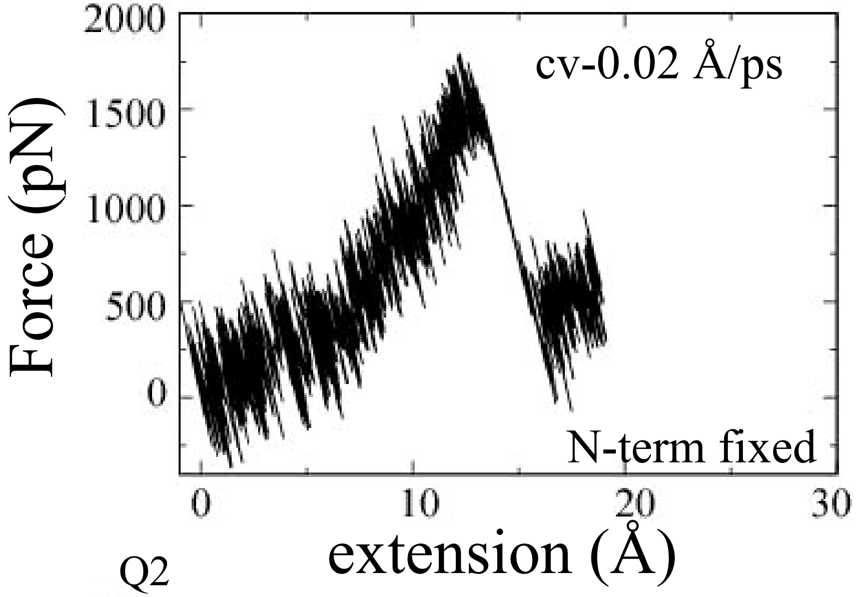


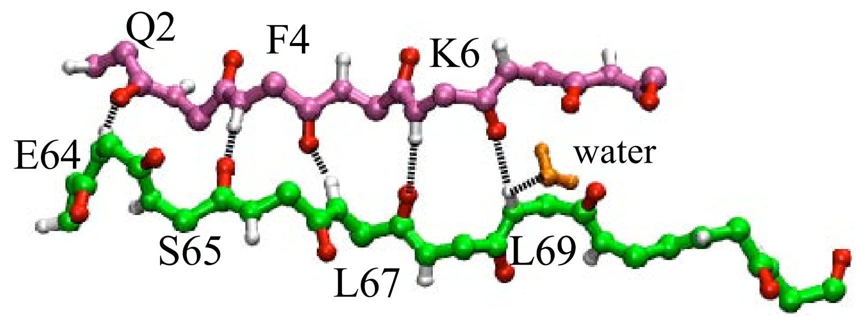


First peak when the first beta strand is stretched out

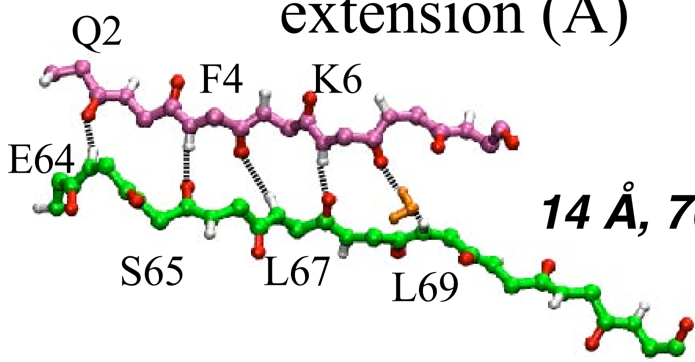
- SMD simulation, with constant velocity
- Box of water 70x240x70 A ~81K atoms
- smd velocity 0.4 A/ps
- smd spring constant 7 kcal/mol A^2

Ubiquitin Unfolding I

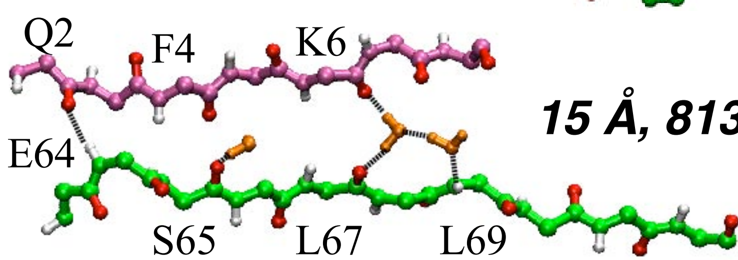




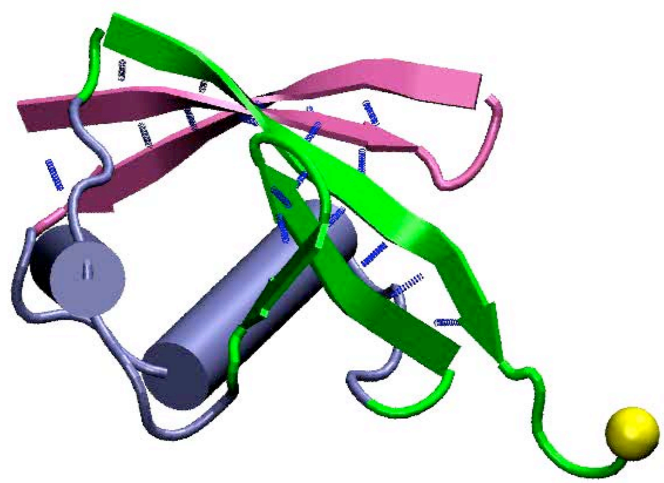
0 Å, 1 ps



14 Å, 700ps

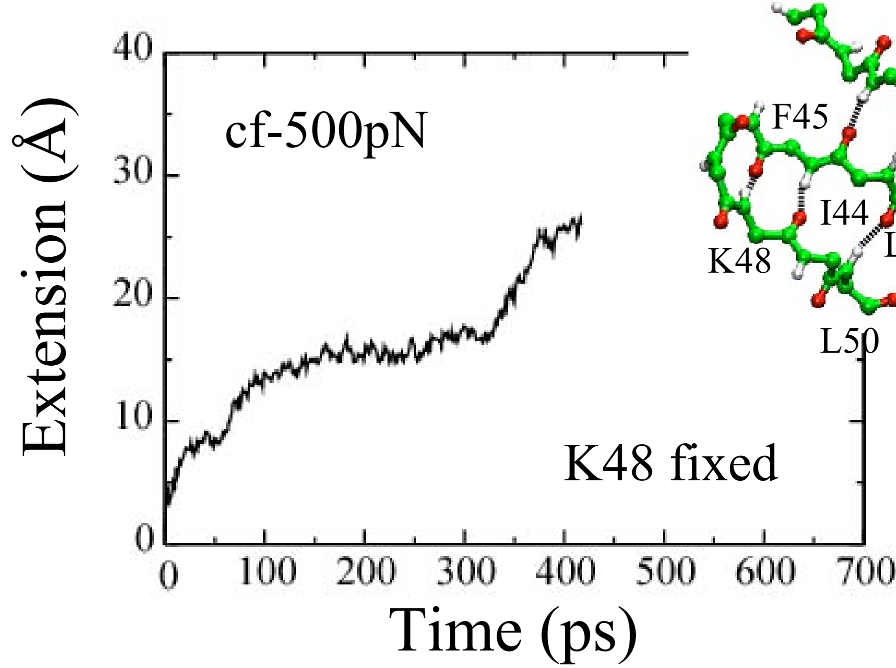


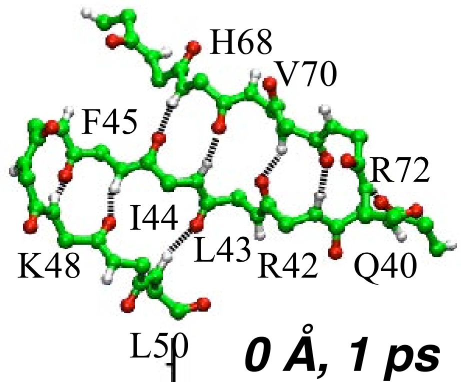
15 Å, 813ps

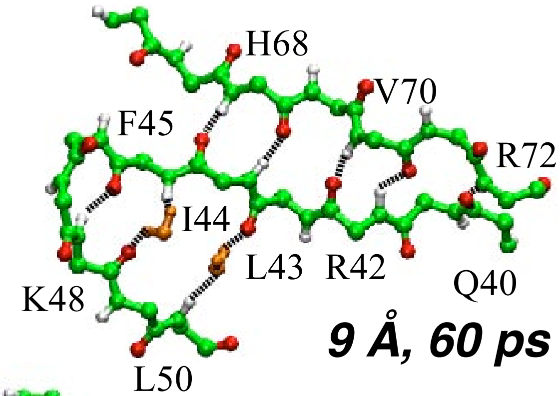


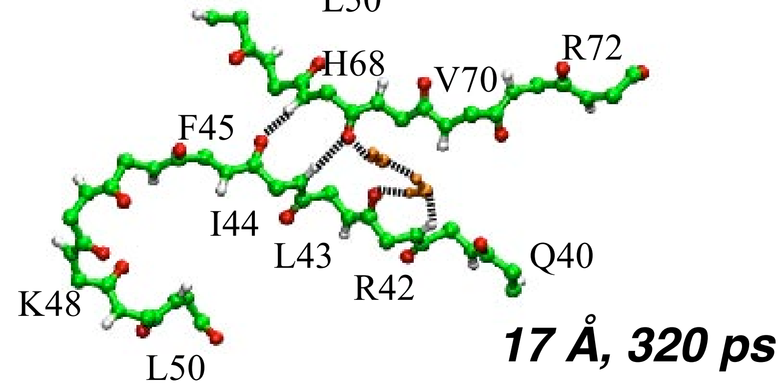
Mu Gao

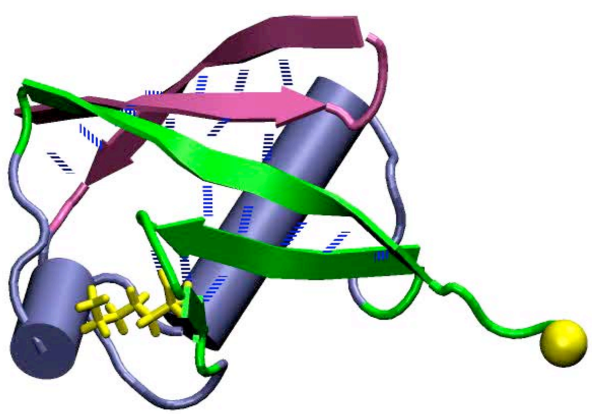
Ubiquitin Unfolding II

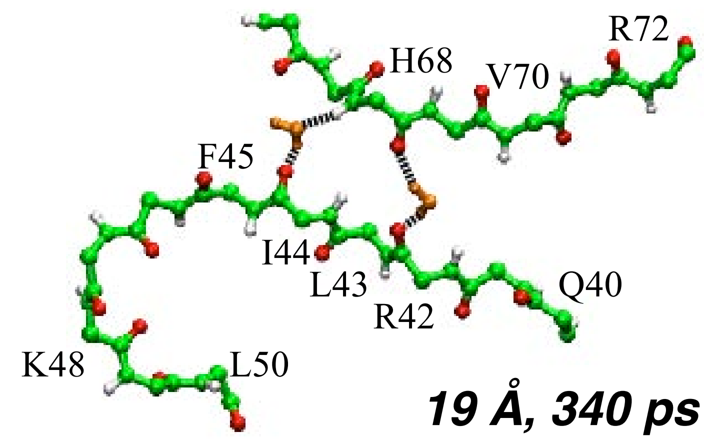












Mu Gao

Pulling Dimer

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