Steered Molecular Dynamics Studies of Titin Domains

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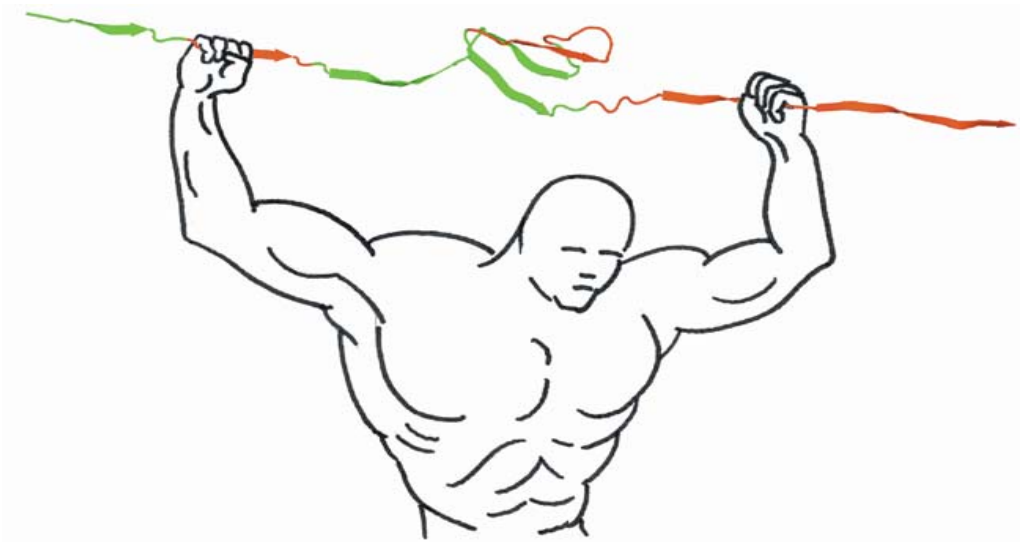
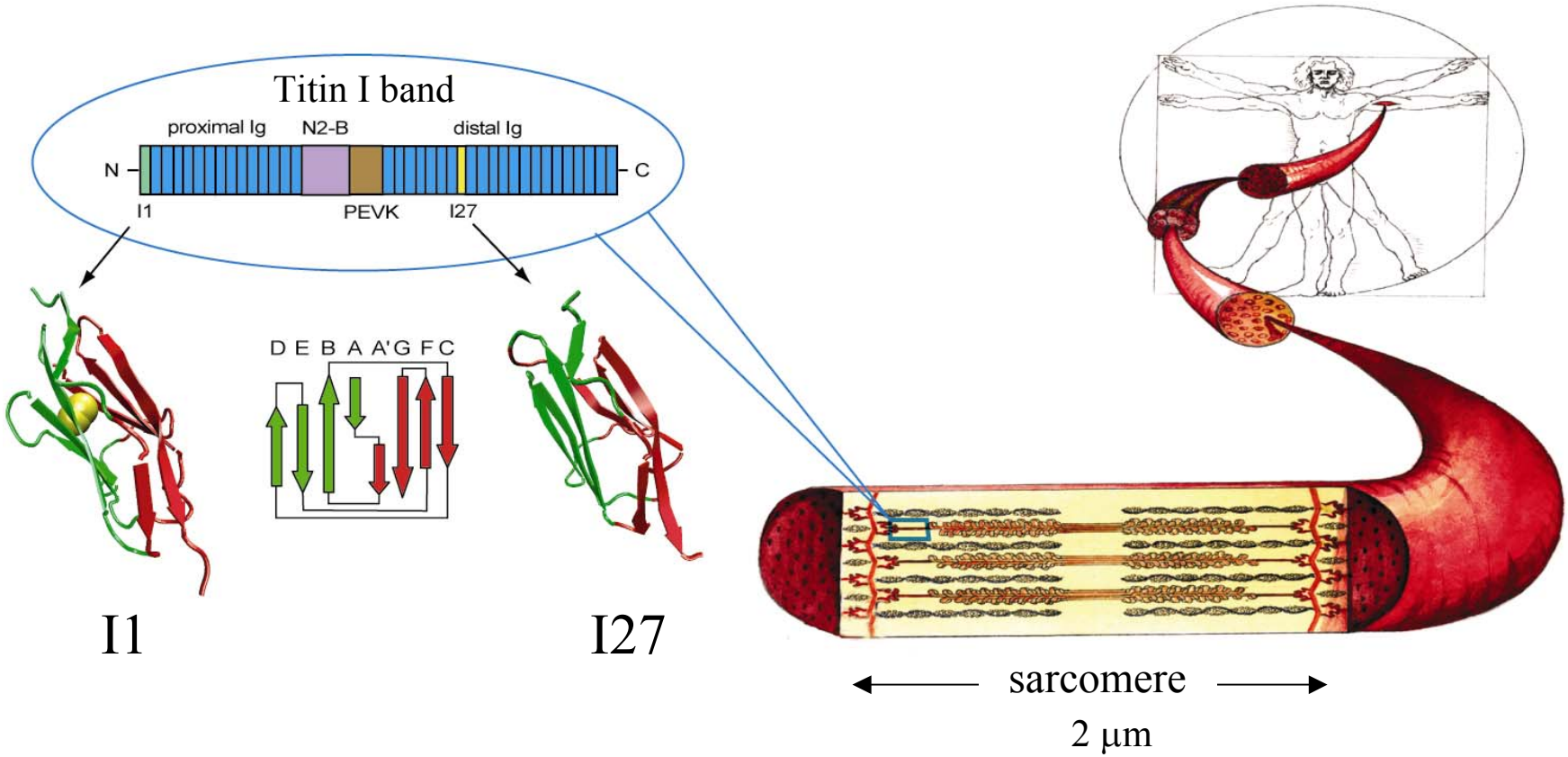
Overview

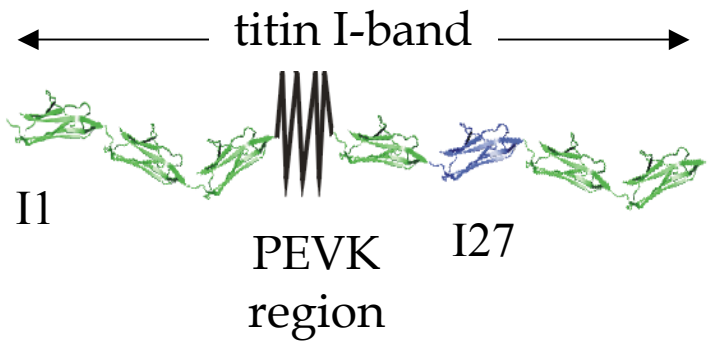
- Biology Background
- AFM experiments
- Modeling and simulation protocols
- SMD simulations of titin domain I27
- SMD simulations of the oxidized and reduced I1 domains



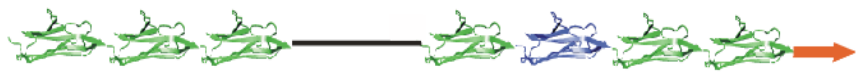
Architecture of Titin Molecules



Titin Under Tension

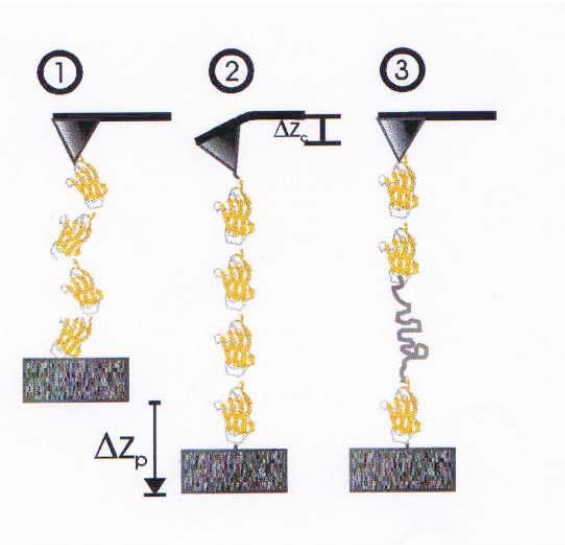


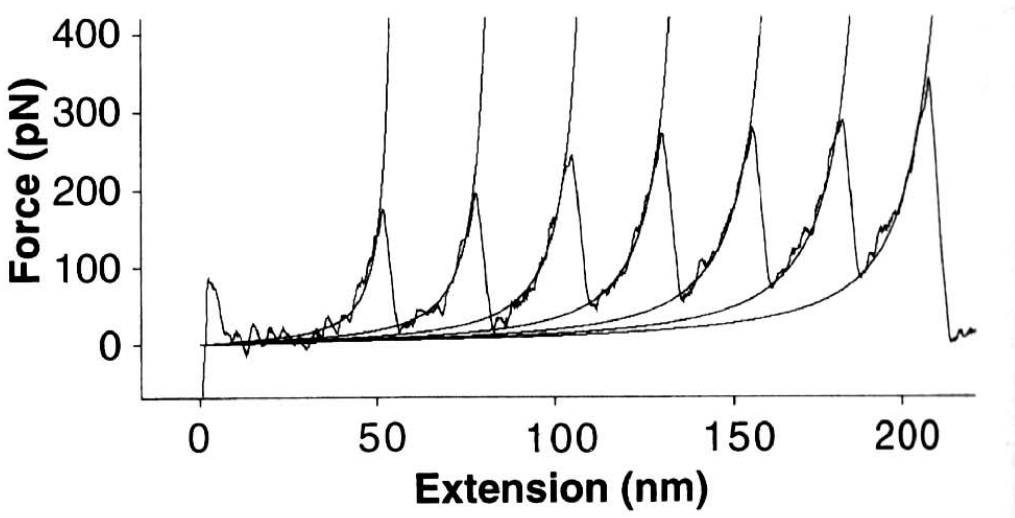
Under external forces up to 20 pN, the PEVK region extends



Under stronger external forces, Ig domains unfold one by one

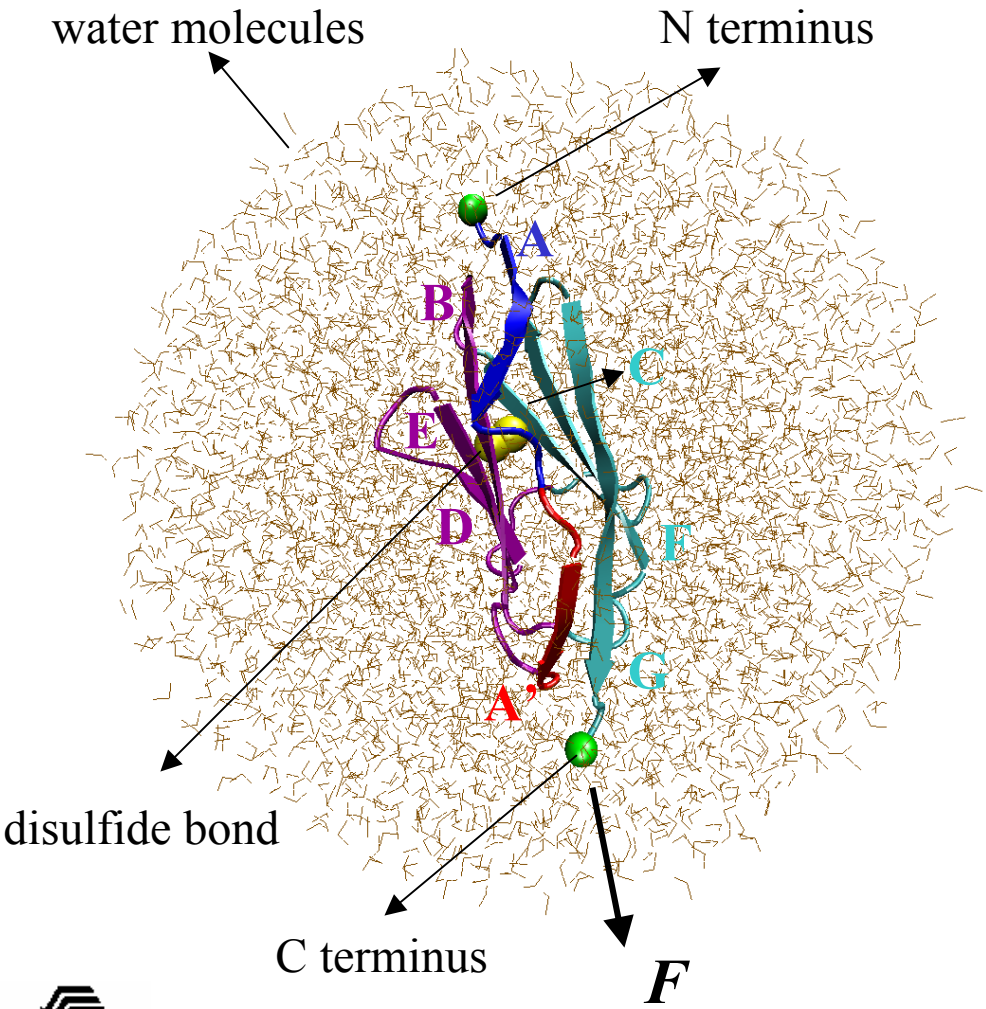
Unfolding Titin Domains With Atomic Force Microscopy





AFM extension of titin Ig multimers, Reif *et al*., Science **276**, 1109 (1997)

Models and Simulation Procedures



- A titin module is solvated in a water sphere of 60 - 70 Å in diameter
- The whole system has 12,000 - 18,000 atoms
- After 1 ns equilibration, Steered Molecular Dynamics is employed to stretch the domains, using constant velocity and constant force protocols
- NAMD benchmark on local cluster of 32 1.1 GHz Athlon processors: 24 -29 hours/ns

SMD Simulation Protocols

(i) Constant velocity Stretching

$$F = -k(x - vt)$$

*Sample **NAMD** script*

```
fixedAtoms          on
fixedAtomsFile      I27.fix

SMD                 on
SMDFile             I27.smd
SMDk                7
SMDVel              0.0001
SMDDir              -0.0997 0.9195 -0.3825
SMDOutputFreq       10
```

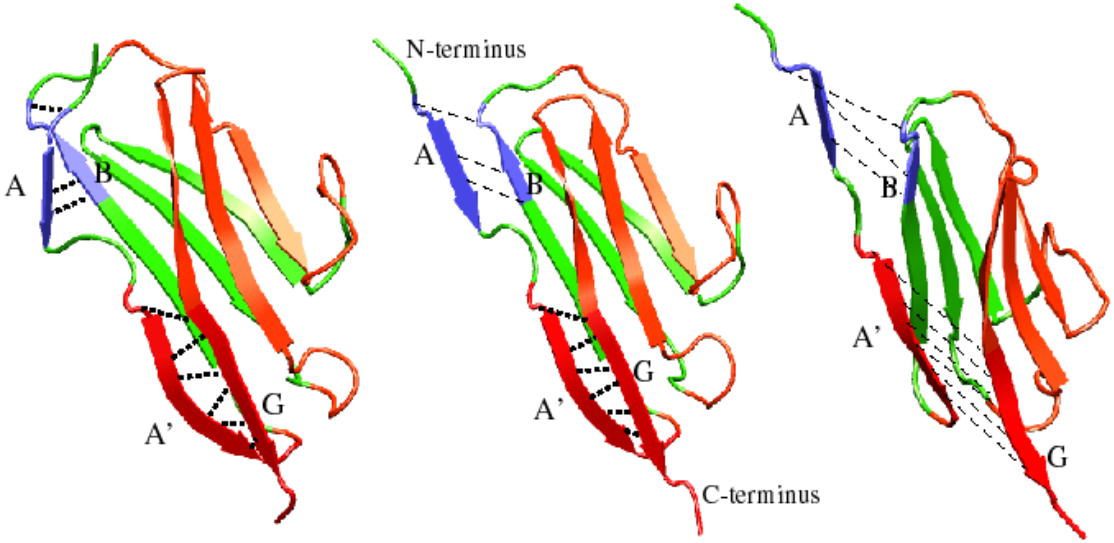
(ii) Constant force Stretching

*Sample **tcl** script*

```
# select the carbon atom that we apply the force to
set carbon [atomid I27 89 CA]
addatom $carbon
# set force vector
set magnitude 2.8786
set force_x [expr -0.0997 * $magnitude ]
set force_y [expr 0.9185 * $magnitude ]
set force_z [expr -0.3825 * $magnitude ]
lappend force_vec $force_x $force_y $force_z

# SMD part
proc calcforces {} {
  global carbon force_vec
  addforce $carbon $force_vec
return}
```

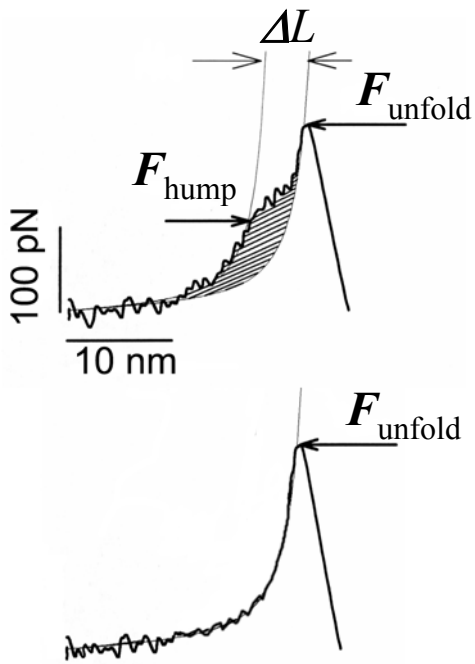
Two-step Unfolding of Titin I27

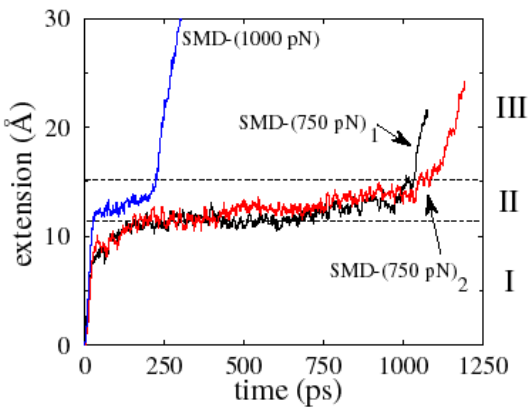


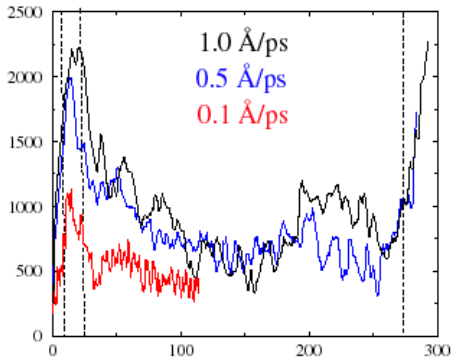
I. Native structure
(0 Å of extension)

II. Mechanical unfolding intermediate
(10 Å of extension)

III. Unfolded state
(25 Å of extension)

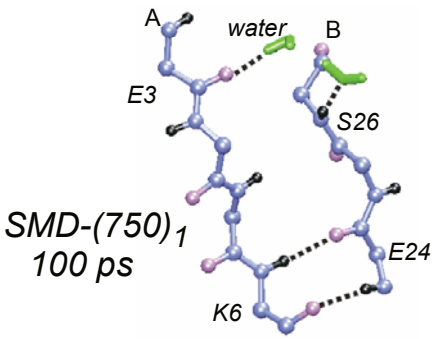




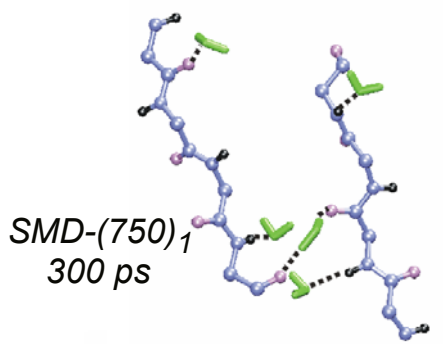


- Step I: disruption of two hydrogen bonds between β-strands A and B
- Step II: rupture of a cluster H-bonds between A' and G

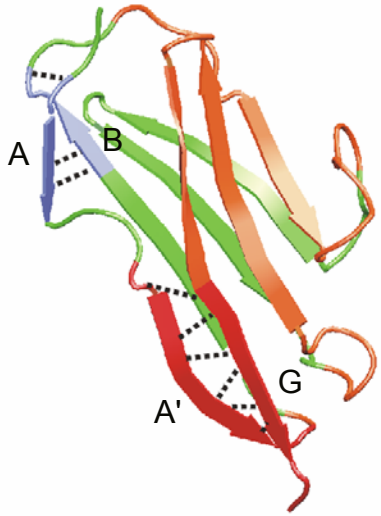
Water Backbone Interactions in Titin Ig Domain Unfolding

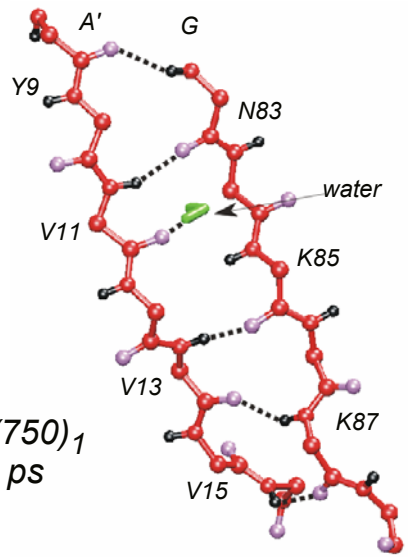


SMD-(750)$_1$ 100 ps

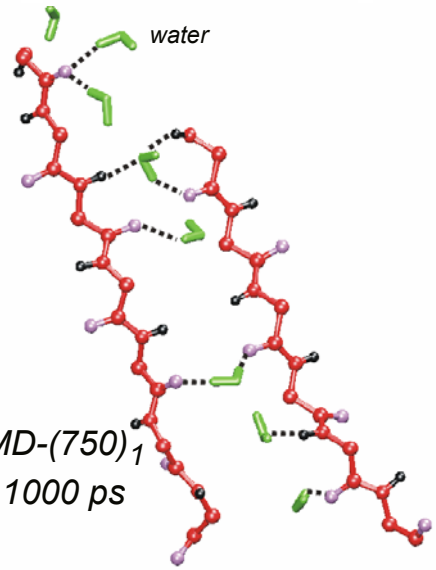


SMD-(750)$_1$ 300 ps

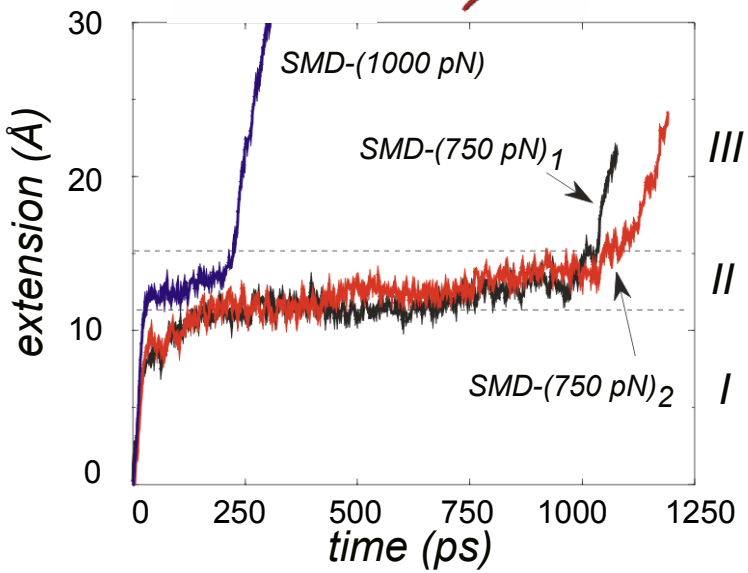




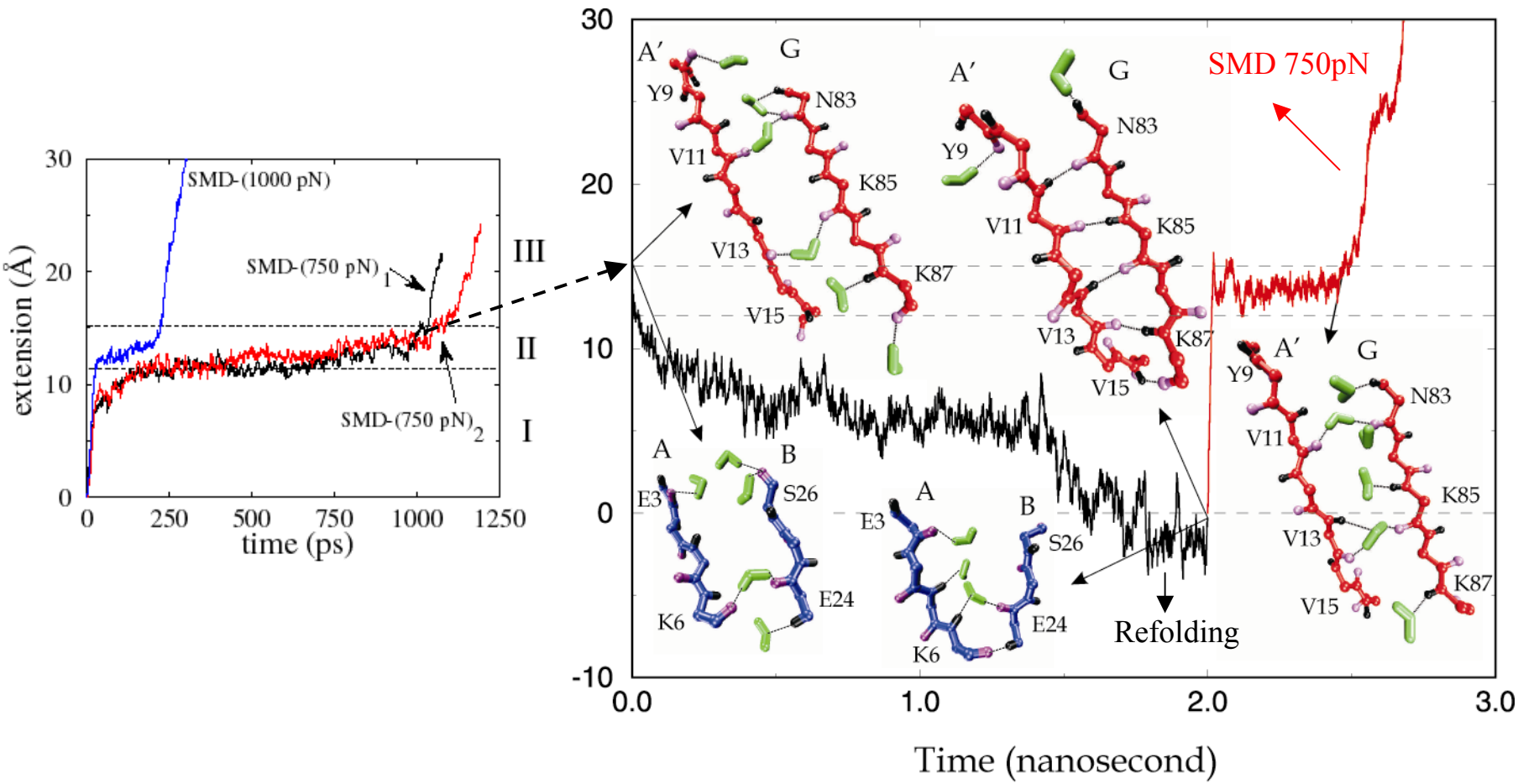
SMD-(750)$_1$ 230 ps



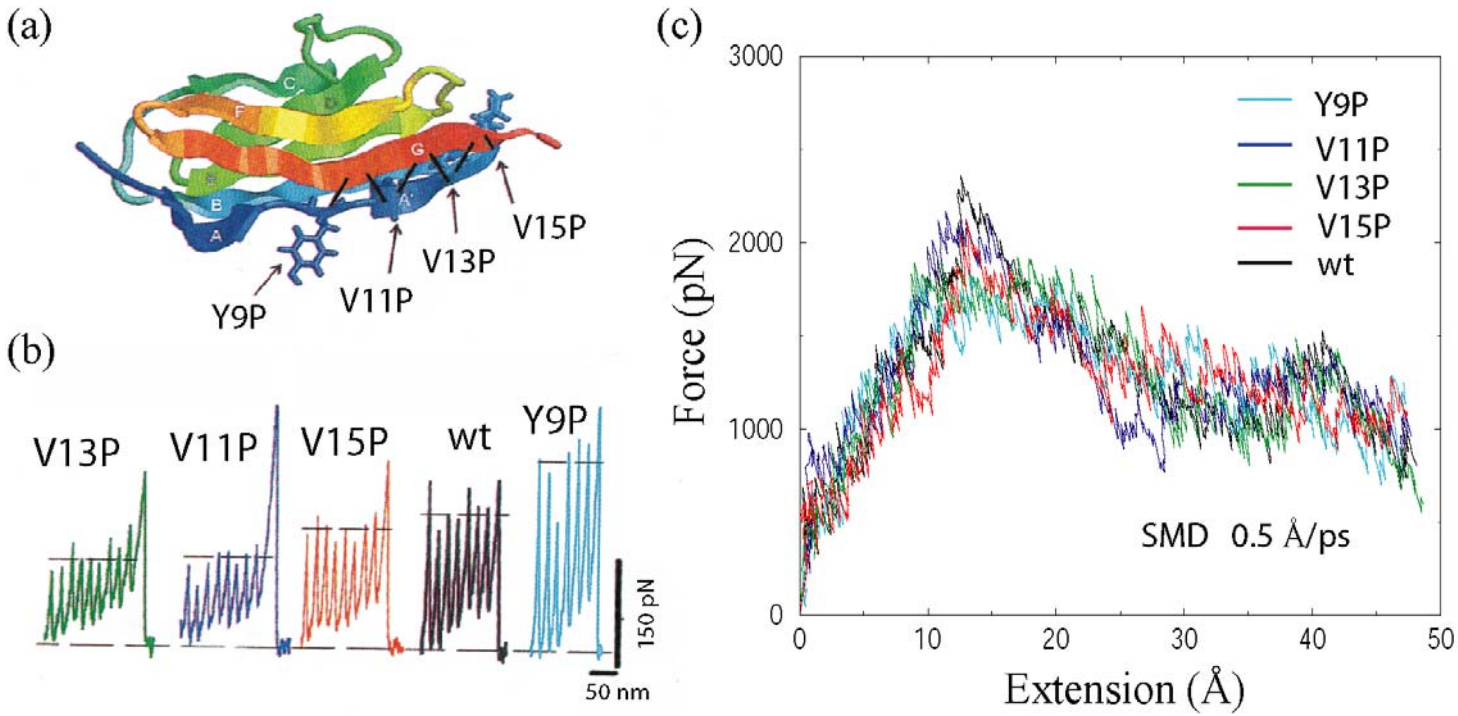
SMD-(750)$_1$ 1000 ps



Refolding of I27 Intermediates

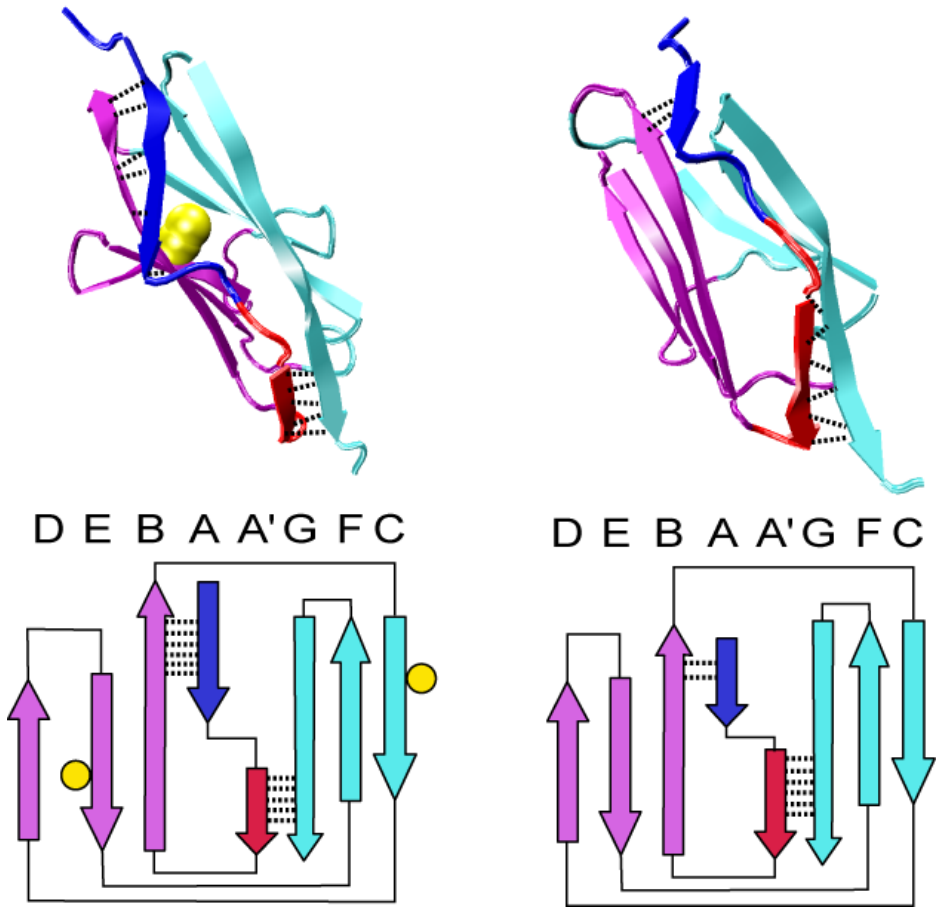


Mechanical Stability of I27 Mutants



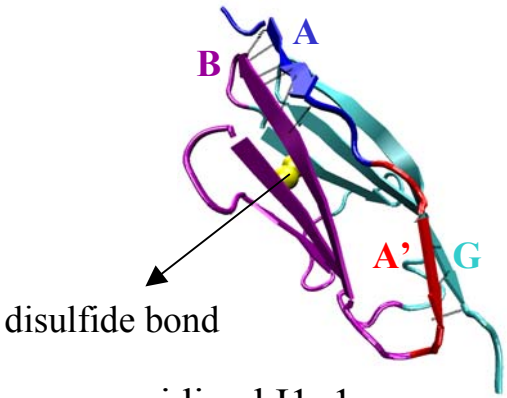
Li *et al*. *Nature Struct. Biol.* **7**:1117-1120 (2001)

Structure Comparison of I1 and I27

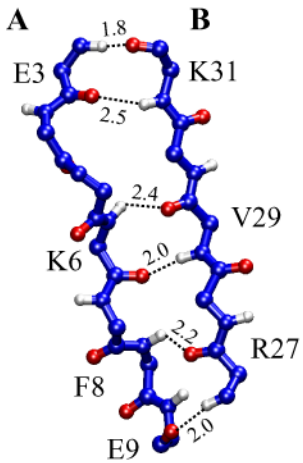


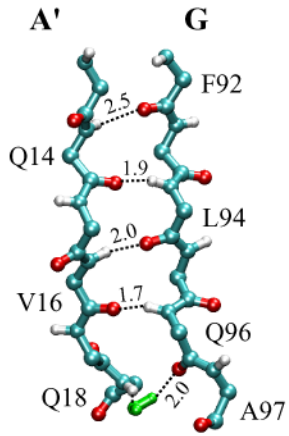
I1 I27

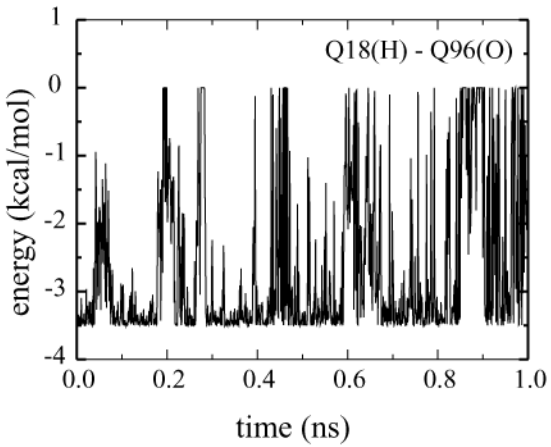
Equilibration of I1 Domains

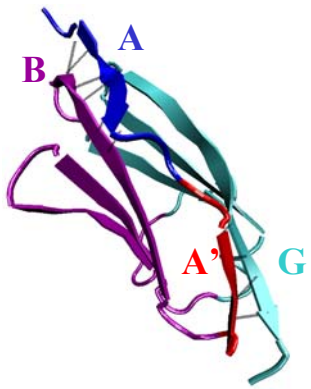


oxidized I1, 1 ns eq.

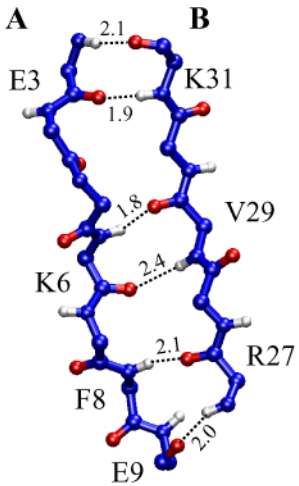


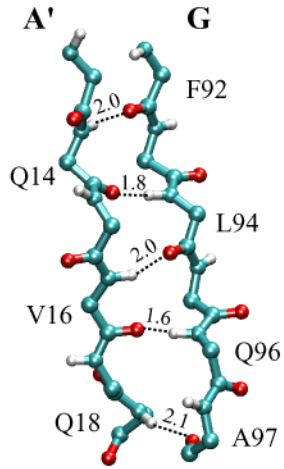


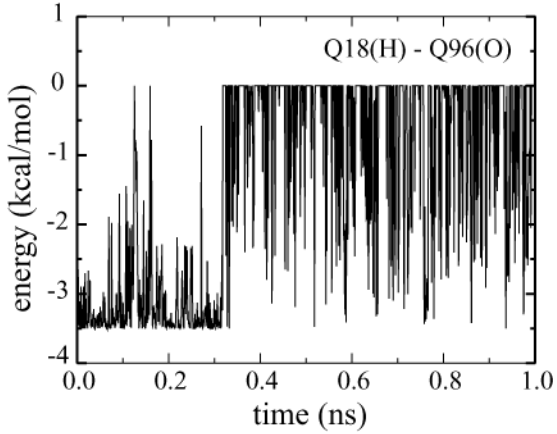




reduced I1, 1 ns eq.

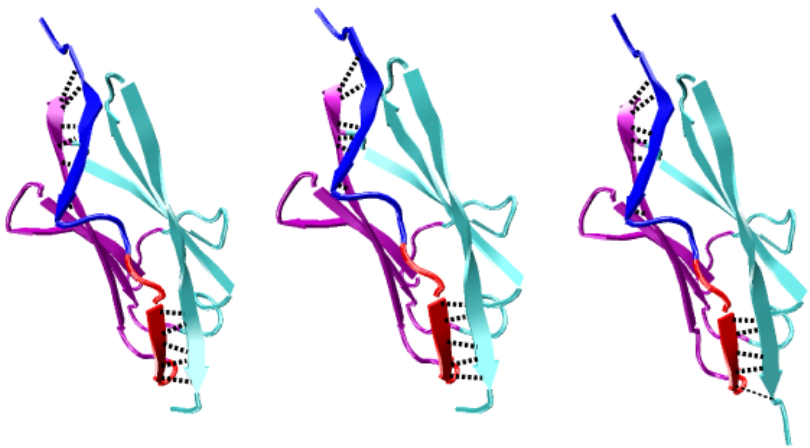




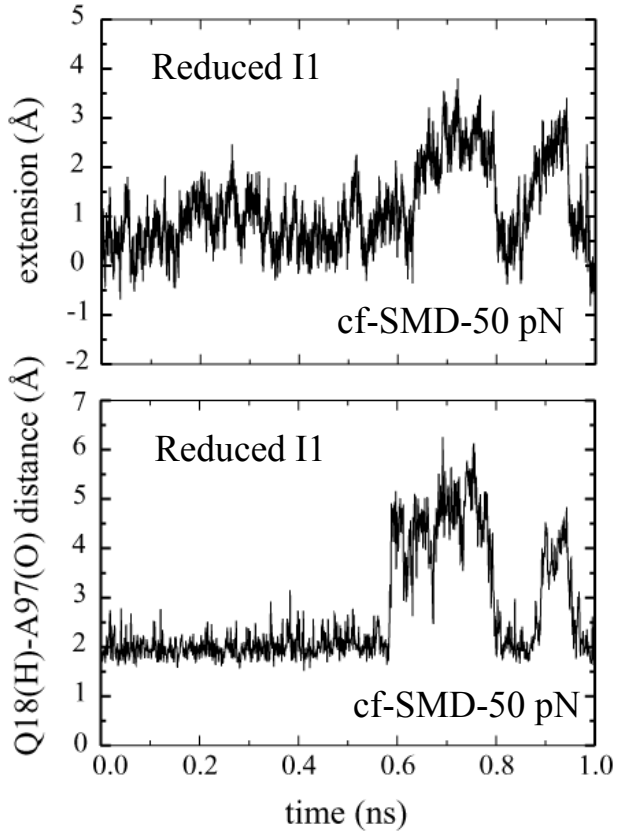


Stretching I1 Domains with Small Constant Forces

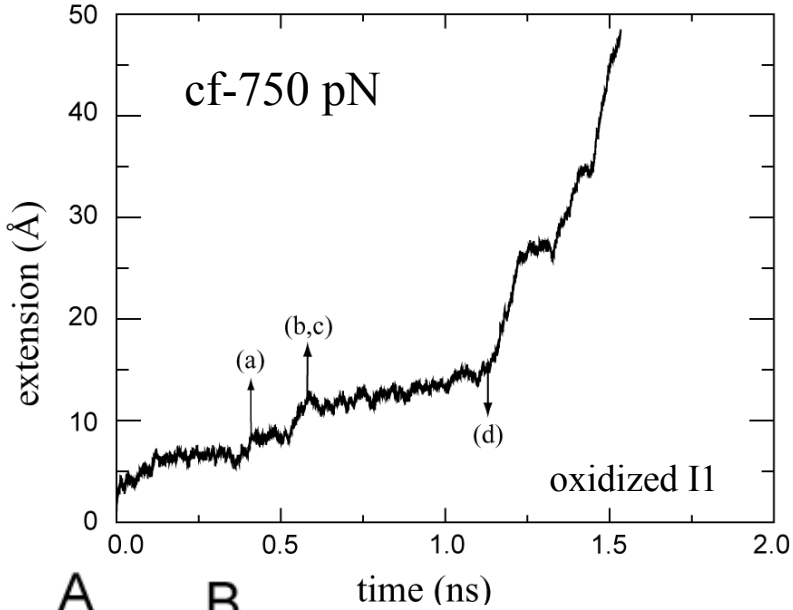
Titin Domains	extension (Å) at small forces		
	50 pN	100 pN	200 pN
Oxidized I1	0.5	1.4	1.6
Reduced I1	1.3	3.1	3.4
I27	2.2	6.1	7.8

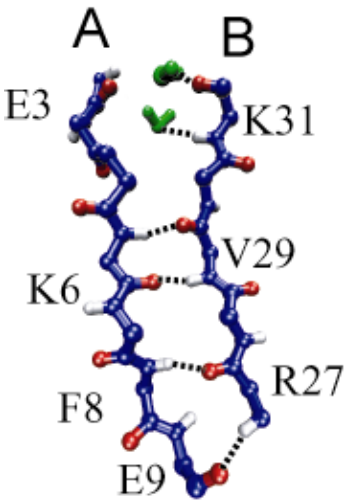


reduced I1, 1ns eq. 50 pN 200 pN

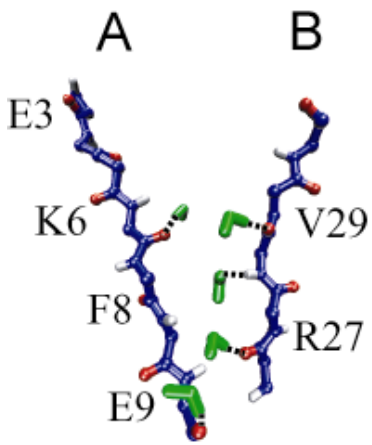


Backbone Hydrogen Bonds and Key Events In Unfolding I1

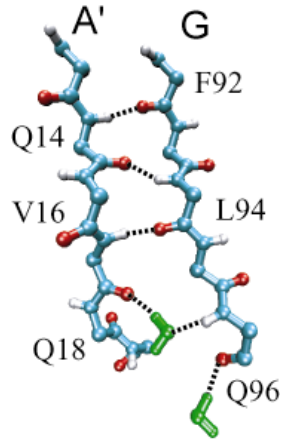




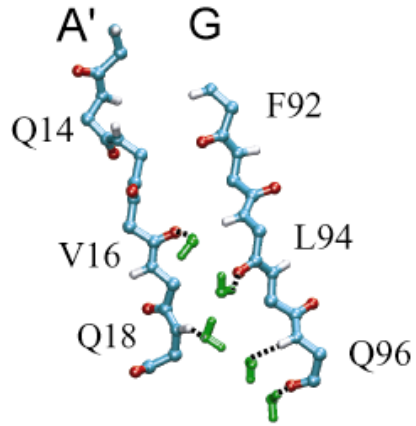
(a) 410 ps, 8 Å



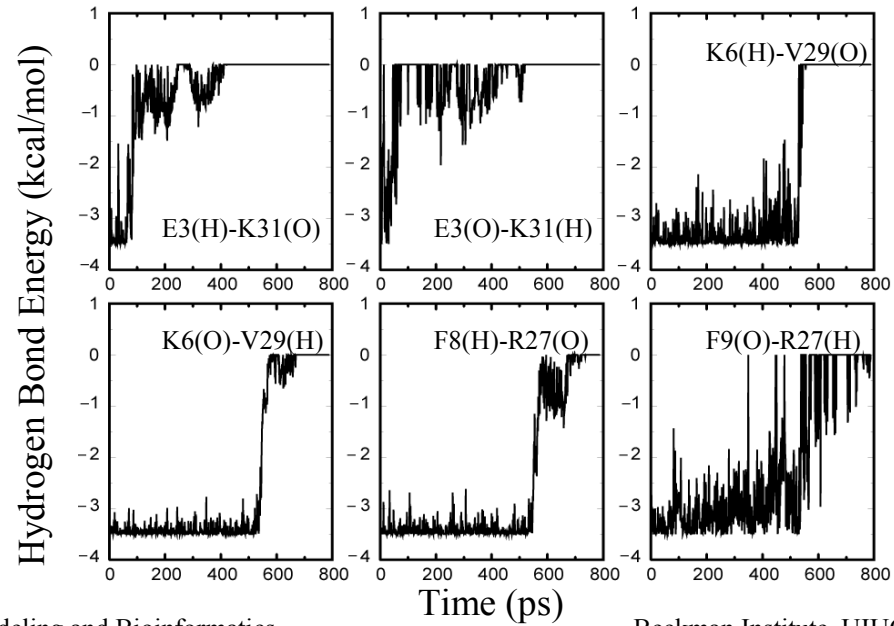
(b) 585 ps, 12 Å



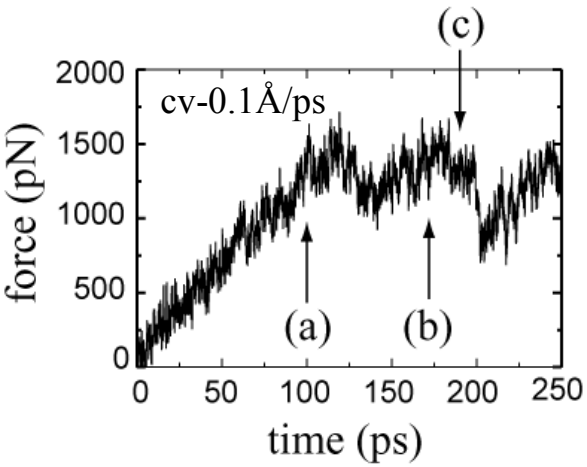
(c) 585 ps, 12 Å

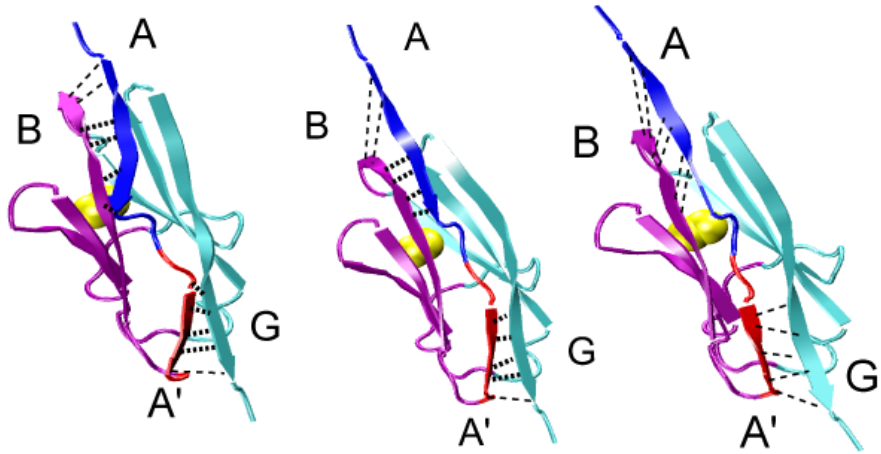


(d) 920 ps, 13 Å

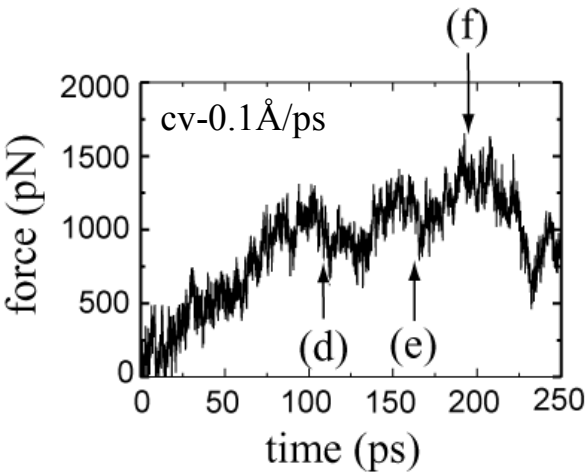


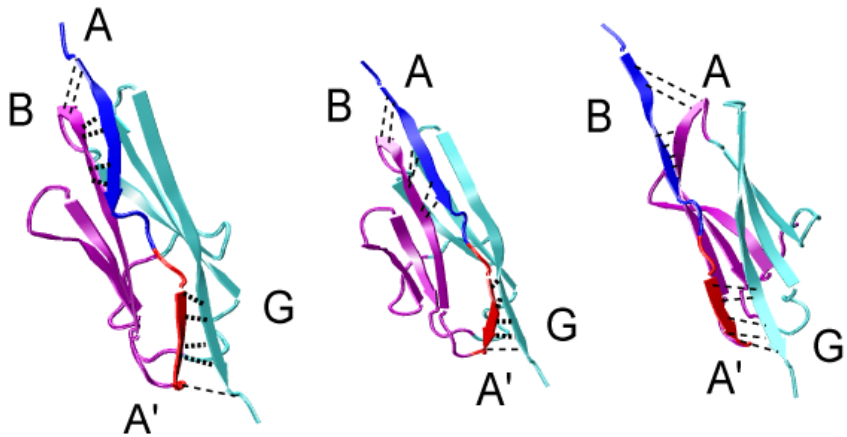
Three step unfolding of I1





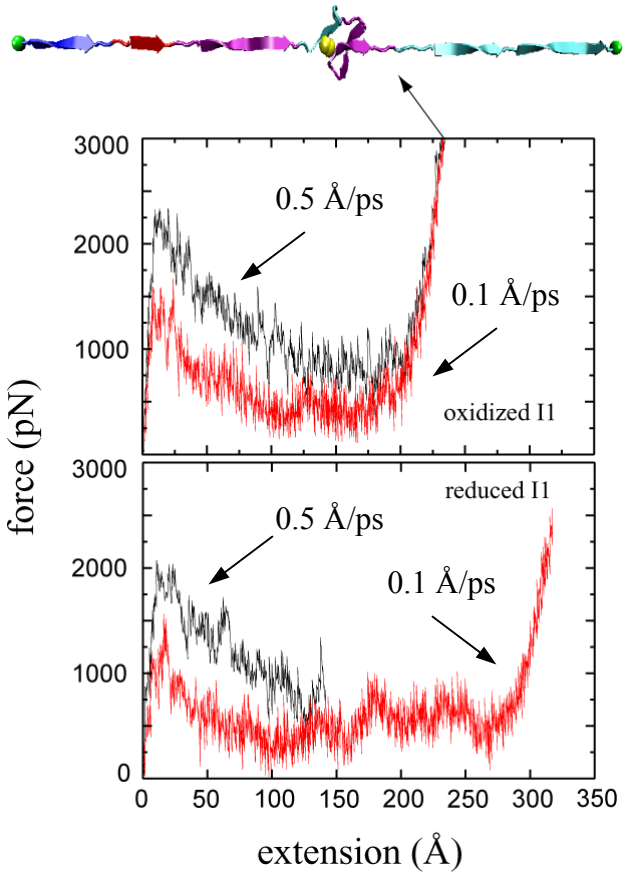
(a) 7 Å (b) 14 Å (c) 16 Å



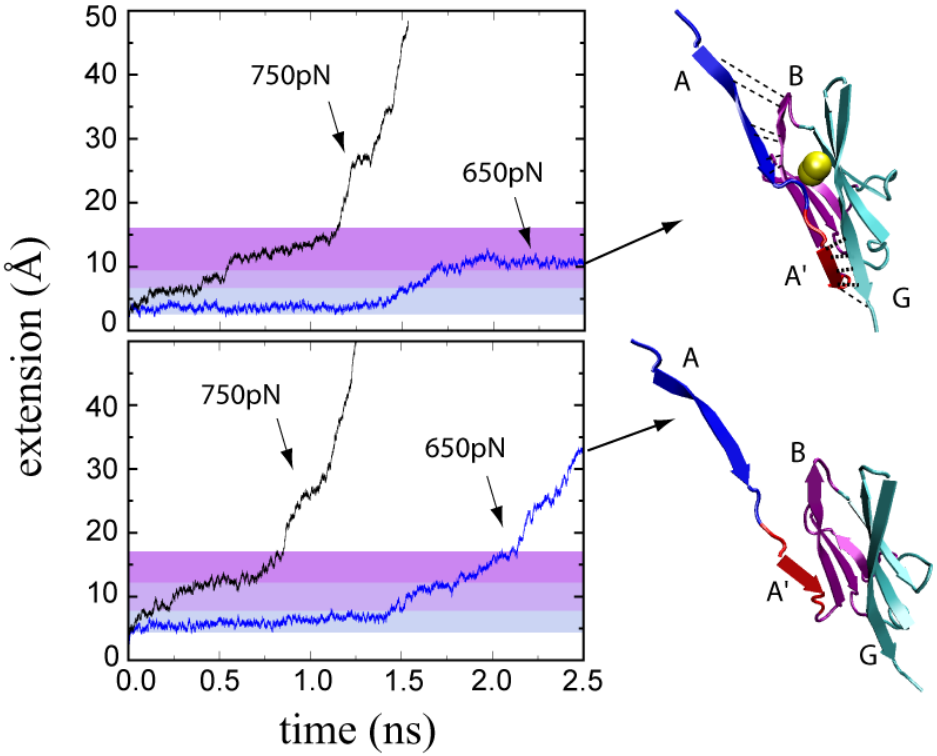


(d) 9 Å (e) 14 Å (f) 17 Å

Roles of the Disulfide Bond



(i) limit the extension of oxidized I1 within 220 Å



(ii) stabilize backbone hydrogen bonds between A'- and G-strands

Conclusion

- Backbone hydrogen bonds between A- and B-strands and between A'- and G-strands are the major force bearing elements of titin domains
- I27 exhibits a mechanical intermediate but I1 does not
- Water molecules are involved in the unfolding processes
- The disulfide bond can limit the extension of I1 and increases its mechanical stability