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 I band

proximal Ig  N2-B  distal Ig

I1  PEVK  I27

I1  I27

sarcomere

2 μm
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)
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

\[ F = -k(x - vt) \]

(ii) Constant force Stretching

Sample Tcl script

```tcl
# 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}
```

Sample NAMD script

```nasm
fixedAtoms on
fixedAtomsFile I27.fix

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

SMD-(750)\(_2\) 1000 ps

SMD-(1000 pN)

SMD-(750 pN)\(_1\)

SMD-(750 pN)\(_2\)

Y9 V11 K85

V13

K87

extension (Å)

time (ps)

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http://www.ks.uiuc.edu/ Beckman Institute, UIUC
Refolding of I27 Intermediates
Mechanical Stability of I27 Mutants

Structure Comparison of I1 and I27
Equilibration of I1 Domains

oxidized I1, 1 ns eq.

reduced I1, 1 ns eq.

disulfide bond
## Stretching I1 Domains with Small Constant Forces

<table>
<thead>
<tr>
<th>Titin Domains</th>
<th>extension (Å) at small forces</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 pN</td>
</tr>
<tr>
<td>Oxidized I1</td>
<td>0.5</td>
</tr>
<tr>
<td>Reduced I1</td>
<td>1.3</td>
</tr>
<tr>
<td>I27</td>
<td>2.2</td>
</tr>
</tbody>
</table>

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**Graphs:**
- **Reduced I1**
  - cf-SMD-50 pN

**Images:**
- **Reduced I1, 1ns eq.**
  - 50 pN
  - 200 pN

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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

Hydrogen Bond Energy (kcal/mol)

Time (ps)

extension (Å)

0.0 0.5 1.0 1.5 2.0

0 10 20 30 40 50

cf-750 pN

oxidized I1

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Three step unfolding of I1

(a) 7 Å  
(b) 14 Å  
(c) 16 Å  
(d) 9 Å  
(e) 14 Å  
(f) 17 Å

force (pN)

0  50  100  150  200  250

time (ps)

cv-0.1Å/ps

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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.