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Mechanisms of Mechanotransduction

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Roadmap

- A brief overview of mechanotransduction
- Some early manifestations -- traveling upstream to find the source
- Current theories -- concept of a mechanical signaling pathway
- State of molecular modeling of MT
- A specific example -- vinculin recruitment to a focal adhesion
- Challenges for future research



Physical factors that elicit a response

- Fluid dynamic shear stress (> 0.5 Pa)
- Cyclic strain of cell substrate ($> 1\%$)
- Osmotic stress
- Compression in a 3D matrix
- Normal stress (> 500 Pa)
- Mechanical perturbations via tethered microbeads (> 1 nN)



Mechanobiology -- some background

Numerous biological processes are associated with mechanical stimulation (Lehoux et al., *J Intern Med*, 2006)

The biochemical signaling pathways that mediate these behaviors have been extensively studied

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Fig. 2 and 3 from Lehoux, S., Y. Castier, and A. Tedgui. "Molecular mechanisms of the vascular responses to haemodynamic forces." *Journal of Internal Medicine* 259 (2006): 381–392



Src activation progresses in a wave from the site of bead forcing

(Wang et al., 2005)

Image removed due to copyright restrictions.

- Response of a membrane-targeted Src reporter.
- Phosphorylation of a domain taken from a c-SRC substrate, P130cas, leads to a conformational change that reduces FRET.
- A wave of activation propagates away from the site of forcing at a speed of ~ 18 nm/s

Neither mechanism -- of force transduction or propagation of activation wave -- are understood



Stretch-activated ion channels constitute one method of mechanotransduction

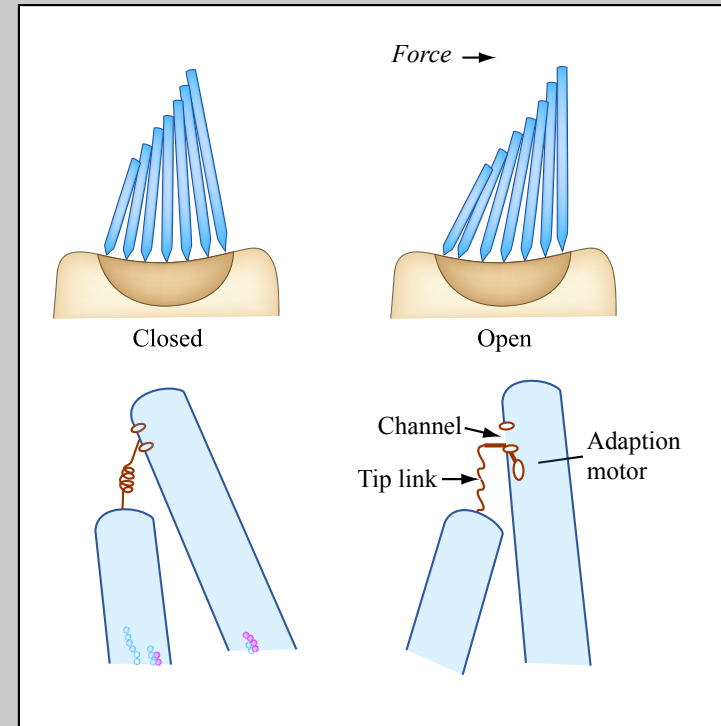


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Please see

http://www.unmc.edu/Physiology/Mann/pix_4b/hair_cell_em.gif

SEM of the stereocilia on
the surface of a single
hair cell (Hudspeth)

Tension in the tip link
activates a stretch-
activated ion channel,
leading to intracellular
calcium ion
fluctuations.

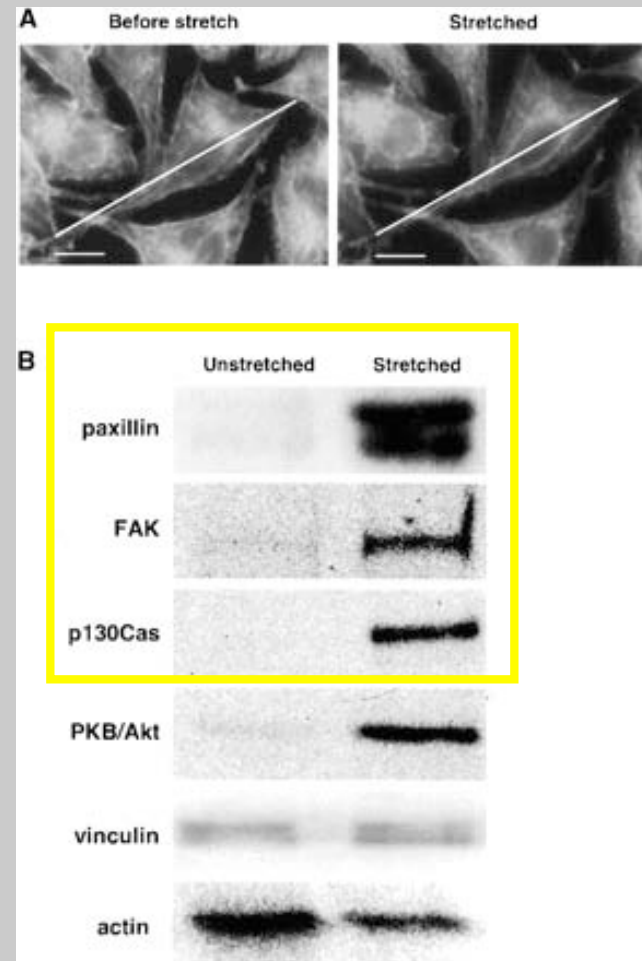


Binding affinity is stretch-dependent; not related to ion channel activity

- Triton X-100 insoluble cytoskeletons
- Incubated with cytoskeletal proteins having a photocleavable biotin tag w/ and w/o 10% stretch
- Focal adhesion kinase, paxillin, p130Cas, PKB/Akt all preferentially bound

Binding of proteins is influenced by stretch of cytoskeleton

Possible role for induced conformational changes?



(Sawada & Sheetz, 2002)

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But we still lack a comprehensive understanding of the links between mechanics and biology/chemistry

- How are forces transmitted at the molecular level?
- How do forces initiate biochemical processes?
- A new approach is needed that recognizes the essential coupling between mechanics and biology



We are just beginning to understand how the proteins are linked, forming pathways for force transmission.

Image removed due to copyright restrictions.

Diagram "Components of Cell-Matrix Adhesions" removed due to copyright restrictions.

Source: Zamir, Eli, and Benjamin Geiger. "Components of Cell-matrix Adhesions." *Journal of Cell Science* 114 (2001): 3577-3579.

We know quite a lot about the *signaling cascade* that follow the initial biochemical event, leading to morphological changes, variations in various biochemical signals, changes in gene expression and protein synthesis.

But we know relatively little about how the initial event is *transduced* from physical force to biochemical reaction.



Intracellular stresses and strains are transmitted through the cell via a complex 3D network of protein filaments.

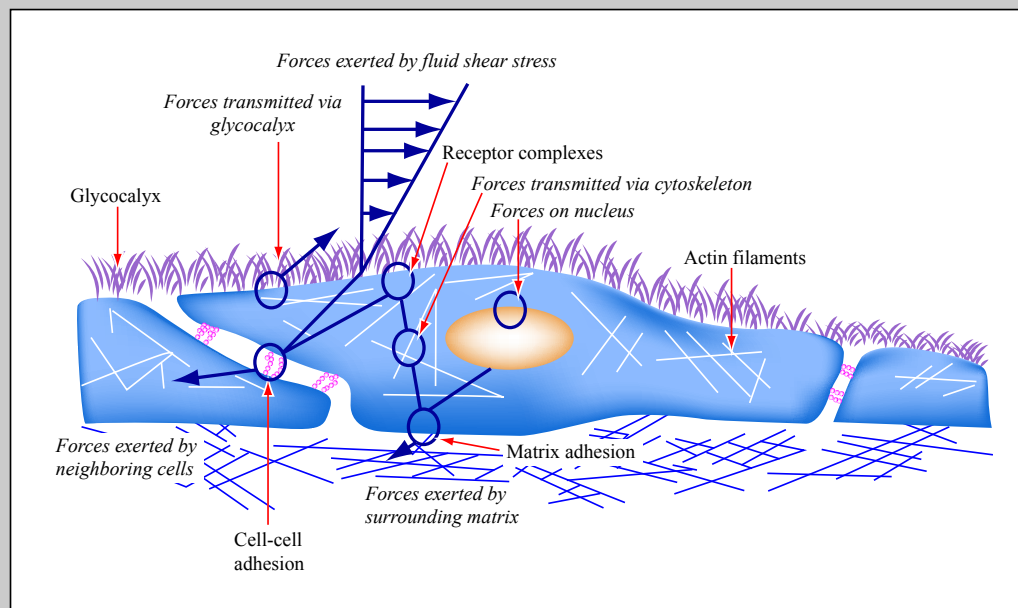


Figure by MIT OpenCourseWare.

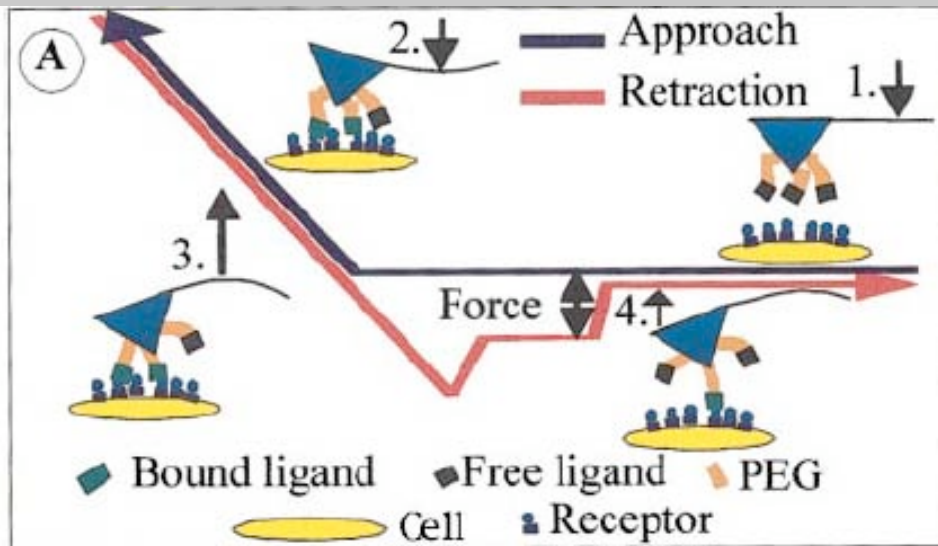
Forces can then be transduced into a biochemical signal leading to changes in cell morphology, gene expression and protein synthesis.

Mechanotransduction: Current theories

- Changes in membrane fluidity and the diffusivity of transmembrane receptors --> receptor clustering (Butler, 2002, Wang, 2004)
- Direct mechanical effects on the nuclear membrane, DNA, and gene expression (Ingber)
- Stretch-activated ion channels (Gullinsgrud, 2003, 2004)
- Glycocalyx deformation coupling to the cortical cytoskeleton (Weinbaum, 2003)
- Force-induced changes in the conformation of load-bearing proteins (Schwartz, 2001, Jiang, 2003, Bao, 2002)
- Constrained autocrine signaling (Tschumperlin, et al., 2004)



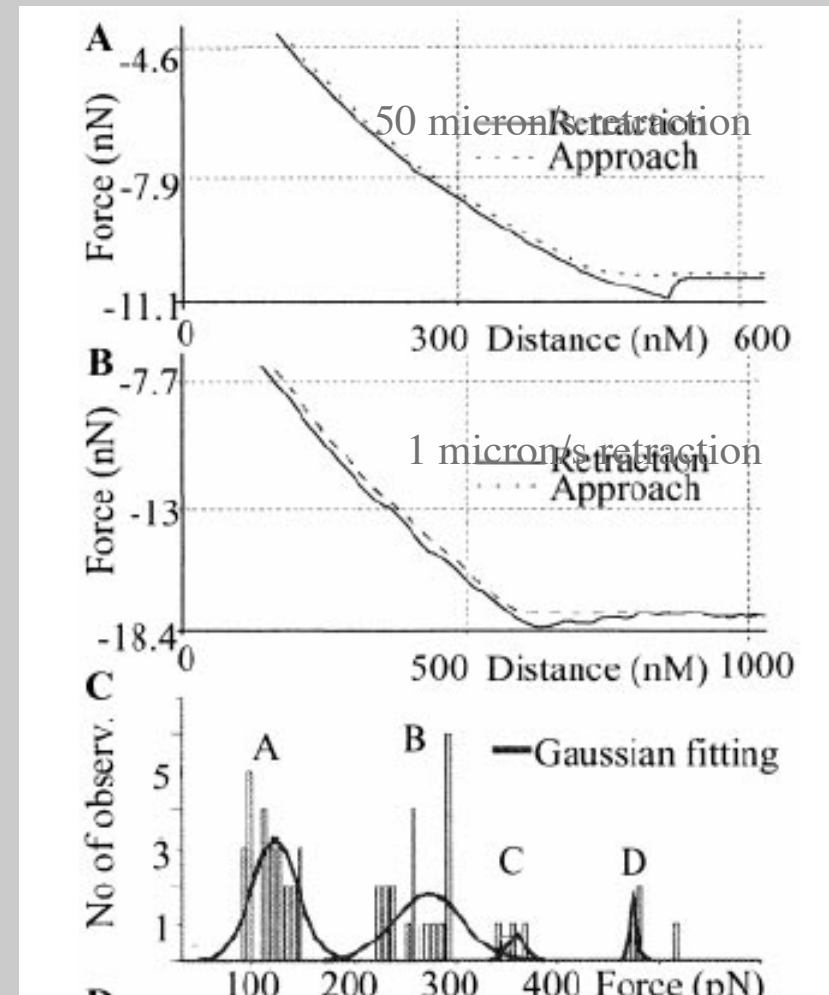
Bond rupture forces: Strength of integrin bonds to ECM ligands



AFM used to measure the strength of integrin bonds to various RGD ligands.

Single bond forces were 32-97 pN.

Lower forces (of order 10 pN) are likely adequate to produce conformational changes.



Lehenkari & Horton, BBRC, 1999



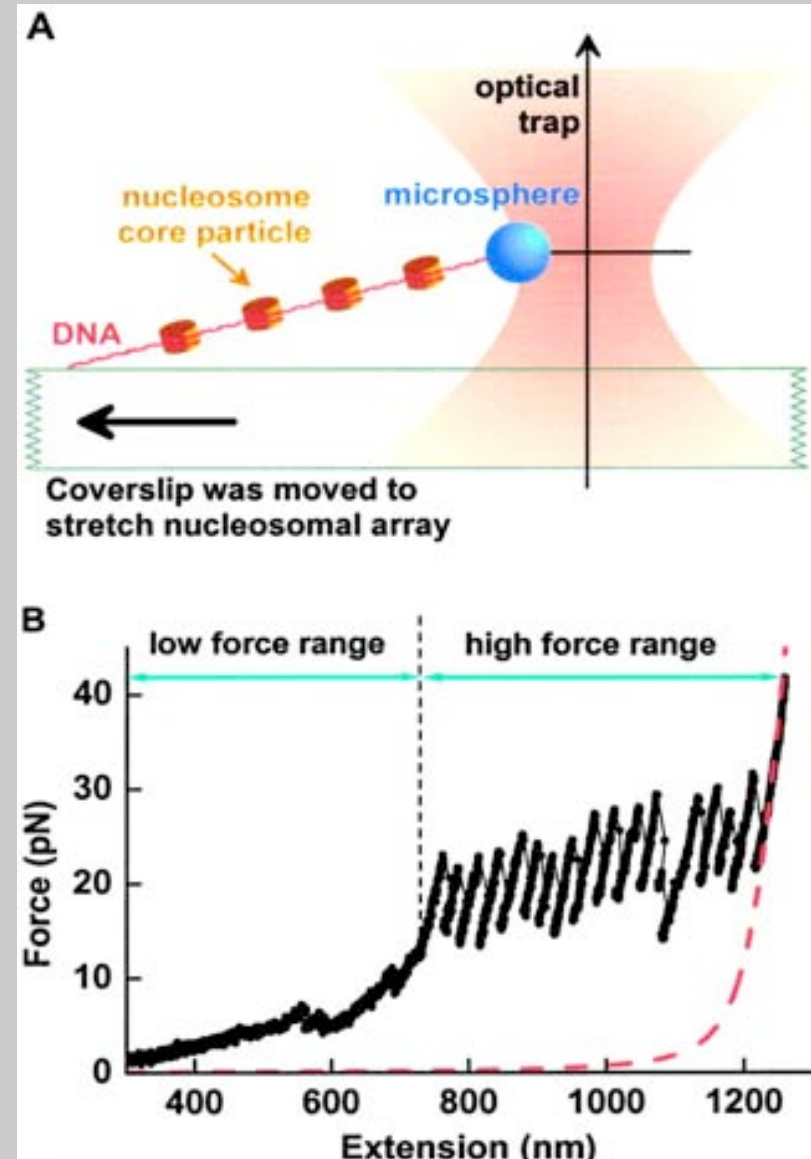
Unbinding forces: force can also be induced even when forces do not act across the binding site

- Histones can be shed from DNA by force
- Forces on the order of 15-30 pN cause unbinding

Courtesy of National Academy of Sciences, U. S. A.
Used with permission.

Brower-Toland, Brent, et al. "Mechanical Disruption of Individual Nucleosomes Reveals a Reversible Multistage Release of DNA." *Proc Natl Sci Acad* 99 (2002): 1960-1965.

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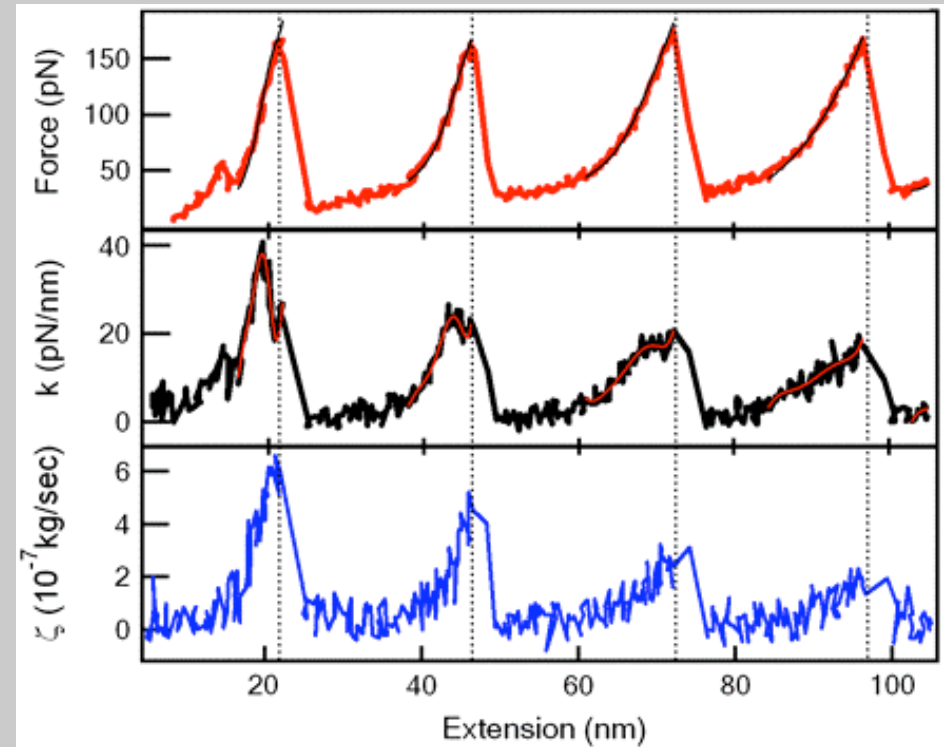


Brower-Toland, PNAS, 2002



Single molecule viscoelasticity

- AFM can be used to measure both the elastic (k) and viscous (ζ) properties of a single molecule as a function of extension
- Sequential unfolding of the immunoglobulin (Ig) domains of titin during oscillations to measure viscoelasticity
- Single molecule elastic and viscous properties appear to scale with each other



Kawakami et al., BJ, 2006

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Typical dimensions and time-scales for conformational changes in globular proteins

- Local Motions (0.01 to 5 Å, 10^{-15} - 10^{-1} s, ~ 1 pN)
(Atomic fluctuations, sidechain motions, loop motions)
- Rigid-Body Motions (1-10 Å, 10^{-9} -1s, ~ 10 pN)
(Helix motions, Domains (Hinge-bending) motions, Subunit motions)
- Larger-Scale Motions (>5 Å, 10^{-7} to 10^4 s, ~ 100 pN)
(Helix-coil transitions, unfolding, dissociations, associations)

In: Proteins, A theoretical perspective of dynamics, structure and thermodynamics
Brooks, Karplus, Pettitt, John Wiley & Sons (1988)



Energetics of mechanotransduction

Sensitivity -- activation must occur at energy levels greater than, but comparable to, thermal energy ($\sim kT$)

Level of force required will depend on scale of conformational change

E.g., if $\Delta E_{\text{conf}} \sim 10kT$:

$F = 100 \text{ pN}$ for $\Delta x = 0.1 \text{ nm}$

$F = 10 \text{ pN}$ for $\Delta x = 1 \text{ nm}$

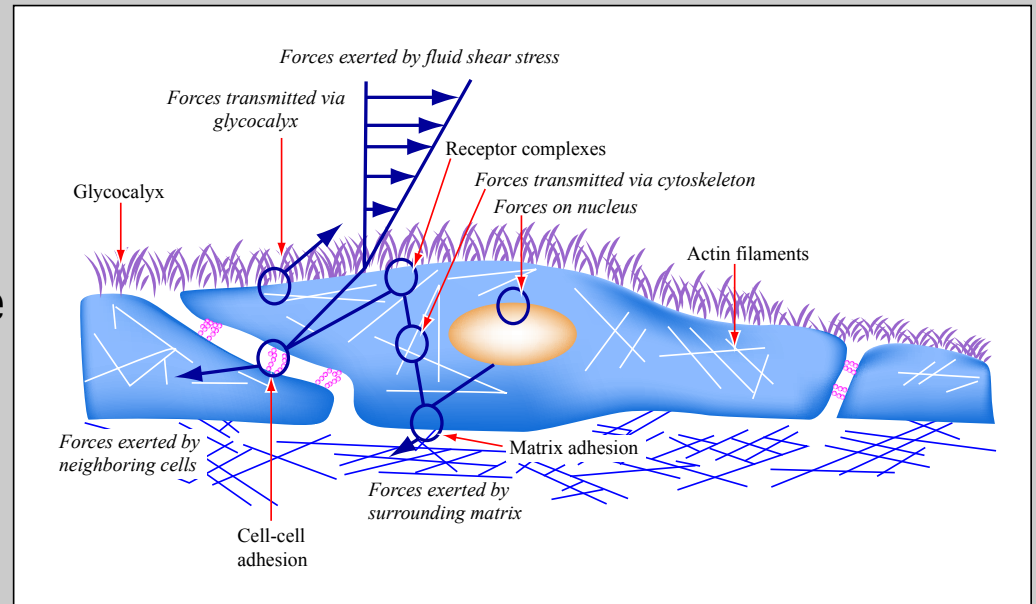


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- Forces for bond rupture ~ 10 - 200 pN (but also depend on pulling rate)
- Proteins change conformation under forces $< 100 \text{ pN}$
- Proteins “live” on the edge of mechanical activation

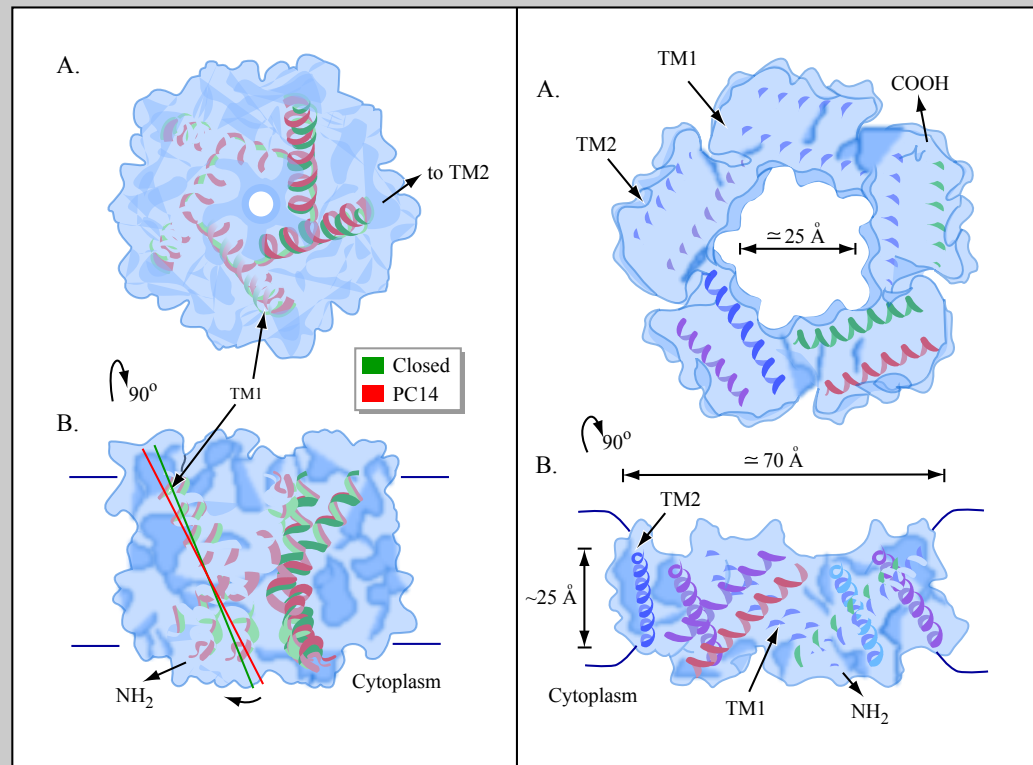


Structure of a Mechano-Sensitive Ion Channel (MscL, large conductance)

Structural studies suggest a diameter of $\sim 2.5\text{nm}$

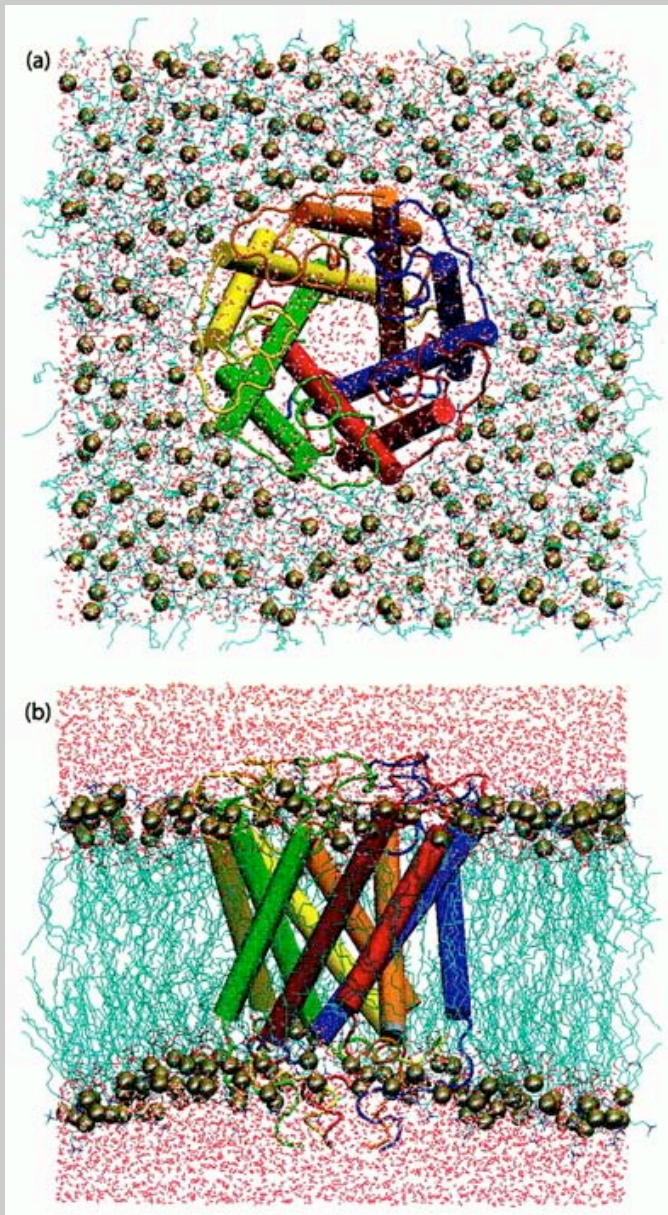
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Please see Fig. 5 in Chang, Geoffrey, Robert H. Spencer, Allen T. Lee, Margaret T. Barclay, and Douglas C. Rees. "Structure of the MscL Homolog from *Mycobacterium tuberculosis*: A Gated Mechanosensitive Ion Channel." *Science* 282 (1998): 2220-2226.



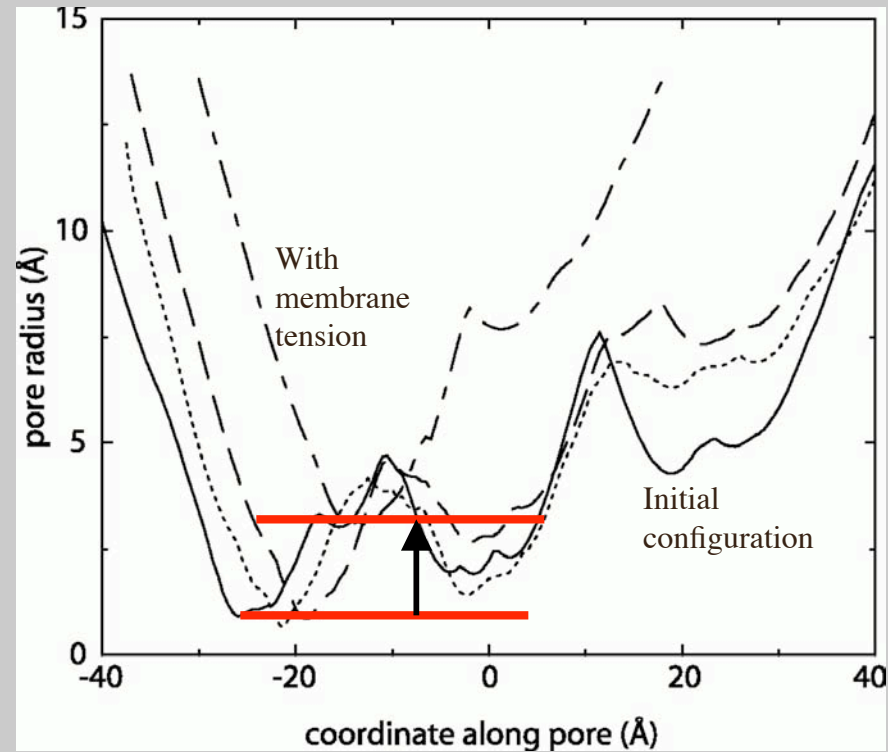
Pore diameter increases to $\sim 2.5\text{ nm}$





Molecular dynamics simulation: MscL channel regulation by membrane tension

(Gullingsrud, et al., Biophys J, 2001)



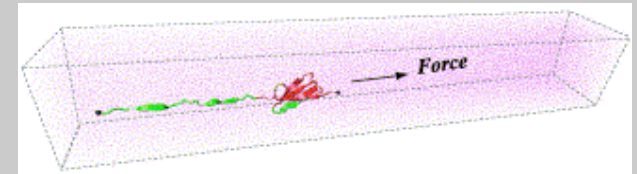
Simulation shows a maximum pore diameter of ~0.6nm



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Simulations of fibronectin unfolding under force



For each atom:

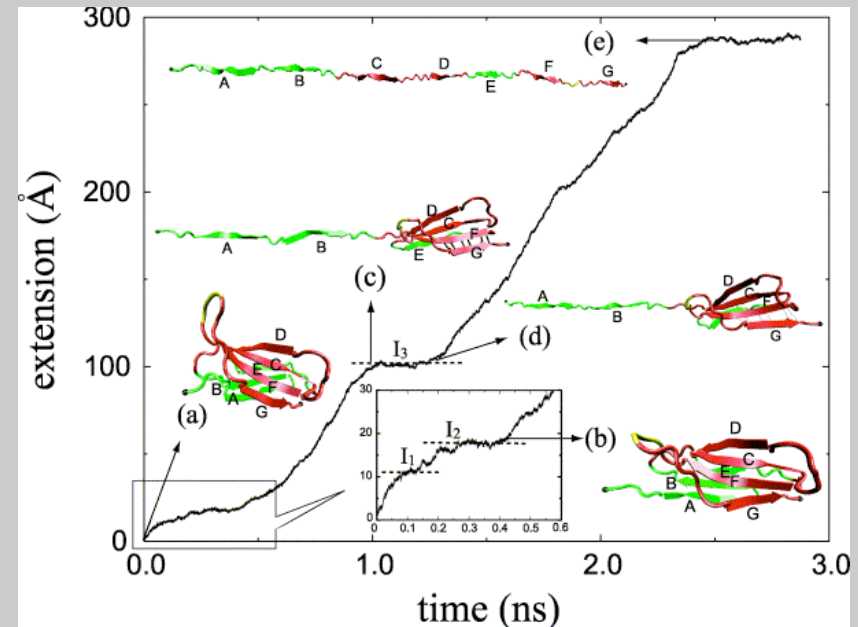
$$m_i \frac{d^2 x_i}{dt^2} = \sum_{j=1}^N F_{ij}$$

Interaction forces F_{ij} are determined as the gradient of the potential energy.

$$E(\bar{r}) = \frac{1}{2} \sum_{\text{bonds}} K_b (b - b_0)^2 + \frac{1}{2} \sum_{\text{bond angles}} K_\theta (\theta - \theta_0)^2$$

$$+ \frac{1}{2} \sum_{\text{bond rotation}} K_\phi [1 + \cos(n\phi - \delta)] + \sum_{\text{non-bond pairs}} \left(\frac{A}{r^{12}} - \frac{B}{r^6} + \frac{q_1 q_2}{Dr} \right)$$

Steered molecular dynamics (SMD) is the forced unfolding of a protein to reveal new conformational states.



(Gao, JMB, 2002)

Fibronectin links the ECM to the cell via integrin receptors

Applied force = **500 pN**

Unfolding is important in the exposure of buried cryptic binding sites.



Vinculin recruitment to an initial contact

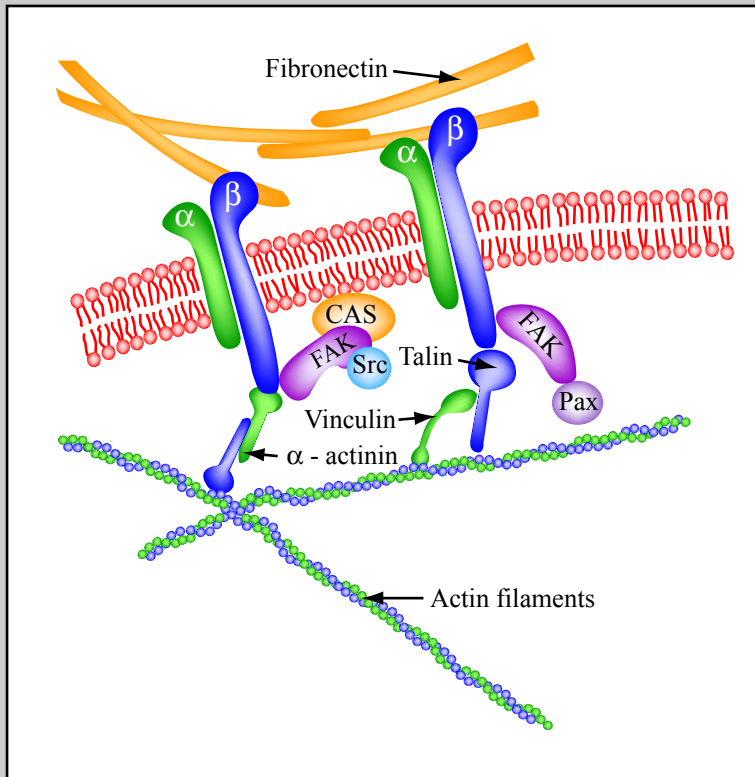
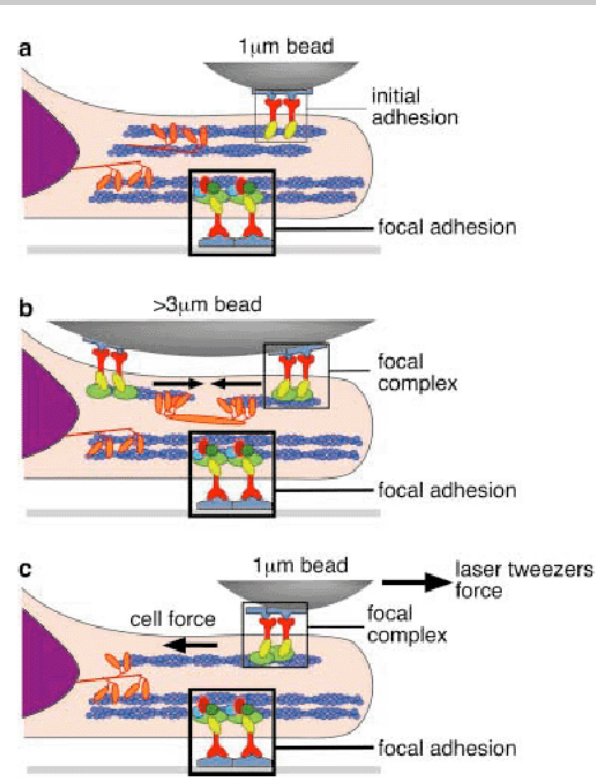


Figure by MIT OpenCourseWare.



Large FN-coated beads induce vinculin recruitment through binding to talin and internally-generated forces

Small (< 1 mm dia) beads only recruit vinculin with externally-applied force

(Galbraith, et al. *JCB*, 2002)

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- Talin bridges between β -integrin and actin
- Low levels of force applied to initial contacts recruit vinculin



Can forces acting through focal adhesion proteins lead to activation and vinculin recruitment?

- Forces are transmitted via fibronectin, integrin, talin, actin connections
- Talin undergoes a conformational change in response to transmitted stresses
- Conformational change enhances the binding affinity of vinculin to talin VBS1 recruiting vinculin and reinforcing the initial contact

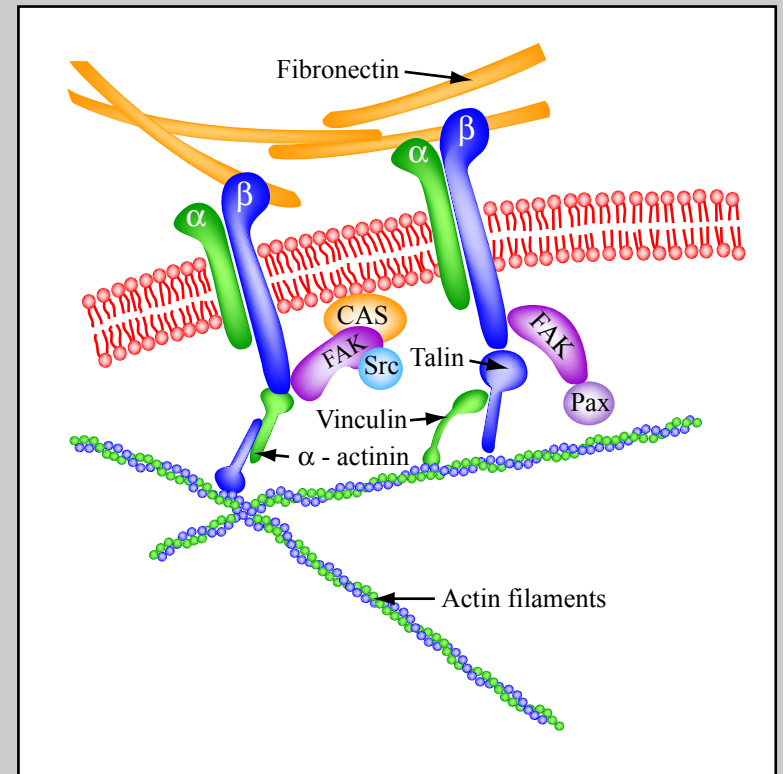
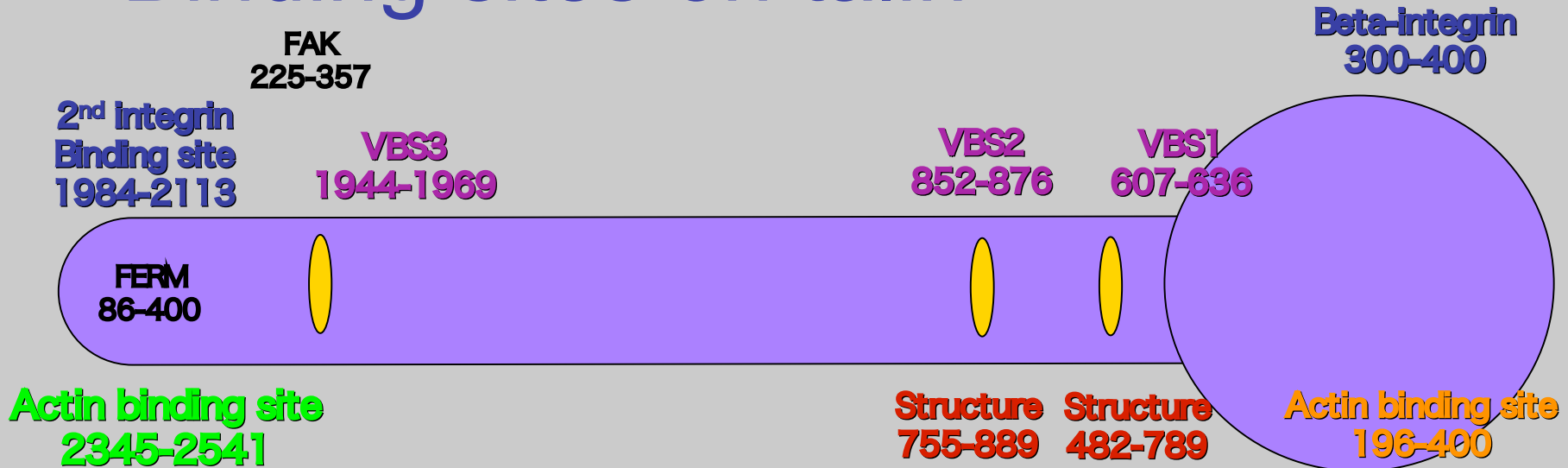
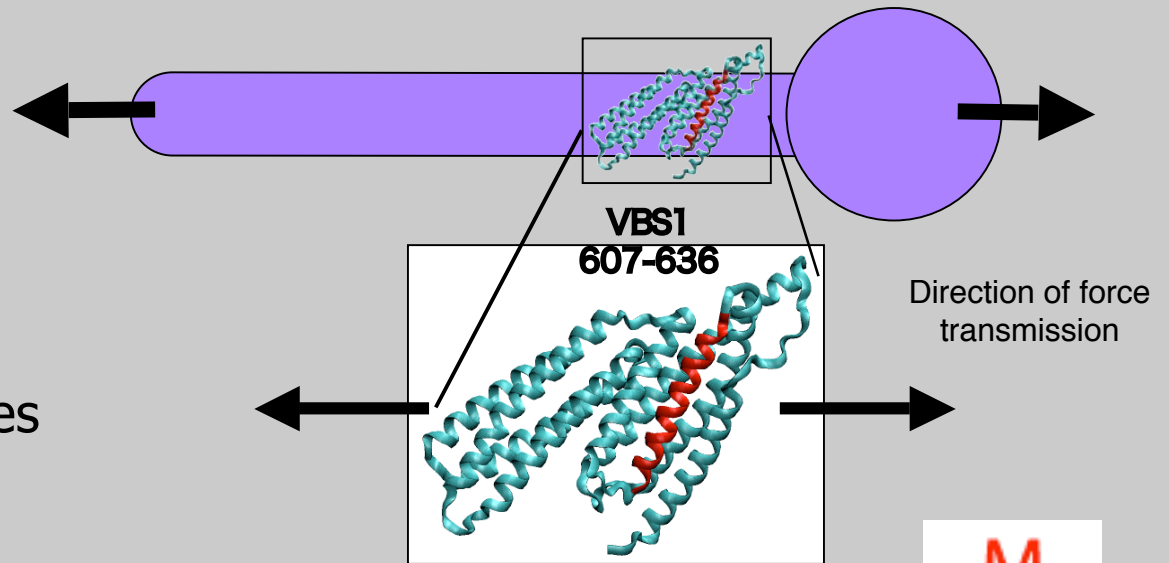


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Binding sites on talin



- 11 vinculin binding sites (VBS1-11)
- Two actin binding sites
- Two integrin binding sites



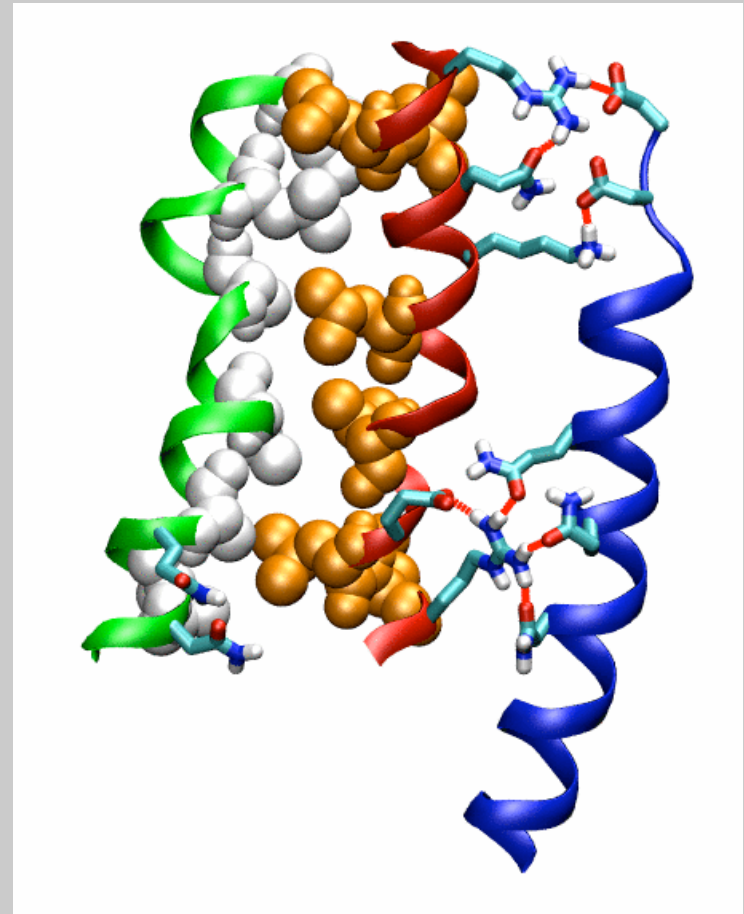
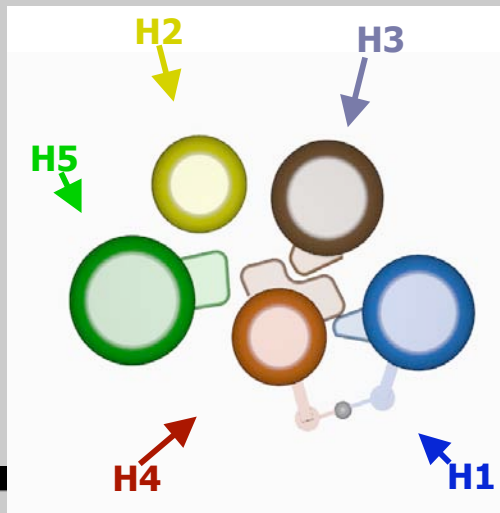
Simulations of VBS1 activation by force

- Apply forces in a distributed manner to carbon atoms at the two ends of the 9- or 5-helix bundle (*not* at N- and C-termini)
- Use MD (CHARMM) with one of several implicit (EEF1, GBSW) models and selected explicit simulations
- Pull either at constant rate or constant force
- Map energy landscape
- Probe internal conformational changes that lead to activation of VBS1



Focusing on the 5-helix bundle that contains VBS1, the critical residues can be identified

- Torques applied to helix-4 (H4) via H-bonds with helix-1 cause H-4 to rotate and expose VBS1
- Hydrophobic interactions along VBS1 with H-5 and the H-bonds and salt bridges with H-1 are critical in force transmission

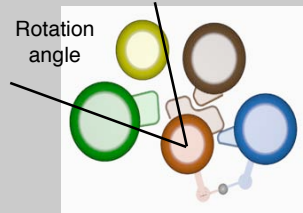


Simulations focusing on the 5-helix bundle reveals a more consistent pattern

Activation appears to be a two-step process

Applied forces < 80 pN for a pulling rate of 0.125 \AA/ns

Force peaks coincide with transition states



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Activation energy ~ 0.7 kcal/mo, $\Delta x \sim 0.2$ nm,
force ~ 25 pN. ($kT \sim 4$ pN.nm)



MD simulations identify a potential activation mechanism

(S. Lee, J Biomech, 2007)

- Torques applied to helix-4 (H4) via H-bonds with helix-1 cause H-4 to rotate and expose VBS1
- Hydrophobic interactions along VBS1 with H-5 and the H-bonds and salt bridges with H-1 are critical in force transmission

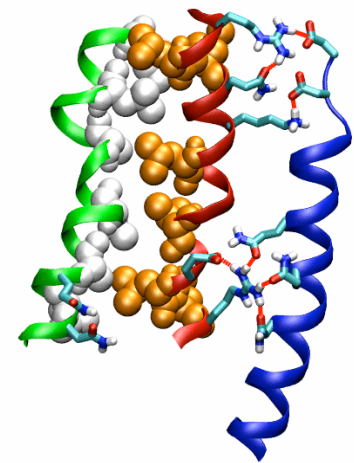
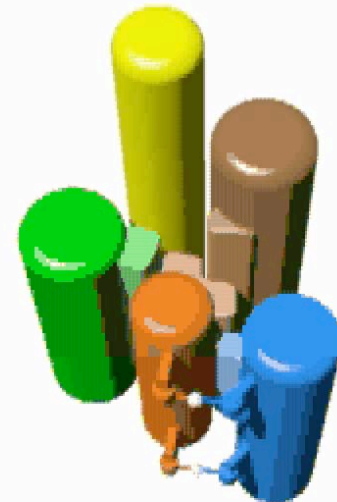


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V V

Is this a generic mechanism for protein activation?

- Sequence homology with other VBSs in talin
- 4- and 5-helix bundles common in many proteins thought to be involved in mechanotransduction
- Complete denaturing is likely not necessary for activation in most proteins due to the need for refolding



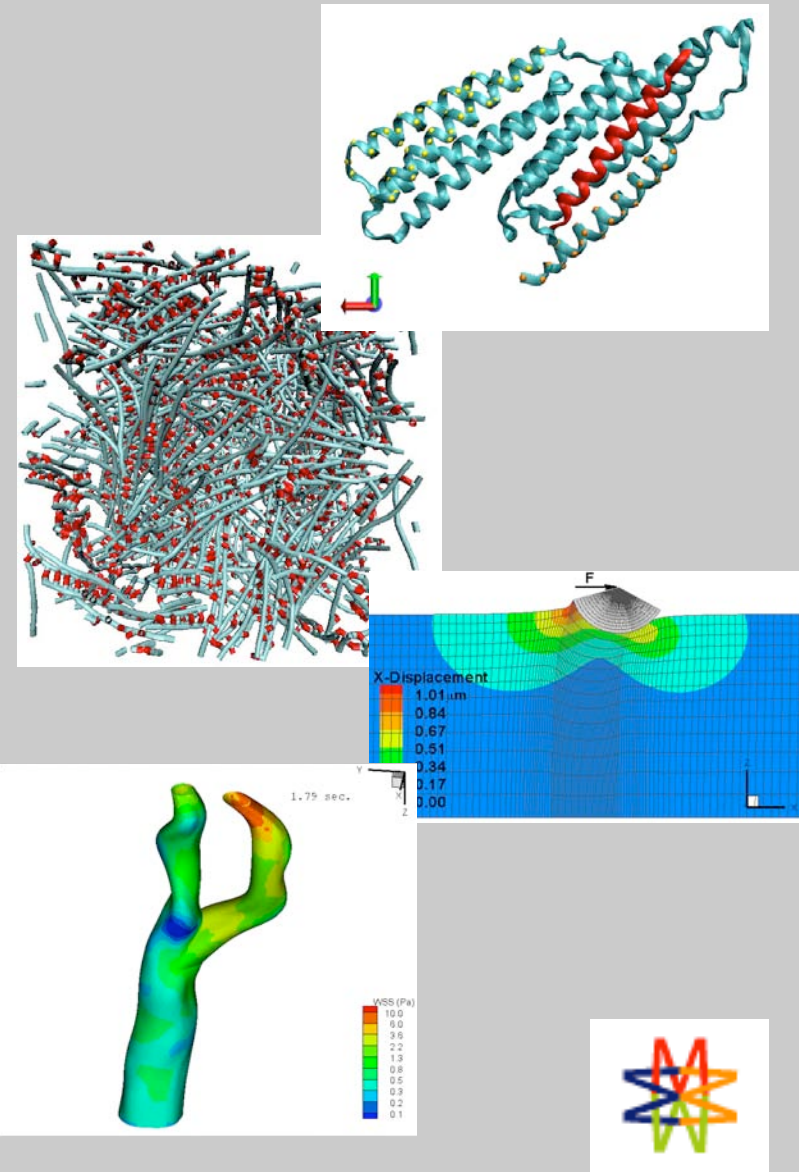
In this age of “-omics” we need to add one more

- The “*Mechanome*” (M. Lang, MIT):
 - The complete state of stress existing from tissues to cells to molecules
 - The biological state that results from the distribution of forces
 - Knowledge of the mechanome requires:
 - the distribution of force throughout the cell/organ/body
 - the functional interactions between these stresses and the fundamental biological processes
 - “Mechanomics” is then the study of how forces are transmitted and the influence they have on biological function



Connecting back to the macro-level

- Conformational changes in single proteins can be computed (e.g., MD, ns time-scales)
- Forces transmitted by single proteins need to be determined from network-level models (e.g., Brownian dynamics, μ s times)
- Intra- and extra-cellular stress distributions can be determined from continuum models that employ appropriate constitutive models (FEM, seconds to minutes)
- Vessel-level or organ level behavior (hours to years)



Where to from here? What is needed to make progress?

- Atomic structures of signaling proteins
- Force mappings down to the level of single molecules
- Methods to interface mechanical and biochemical signaling pathways
- Multi-scale models to link organ-level behavior with molecular phenomena



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