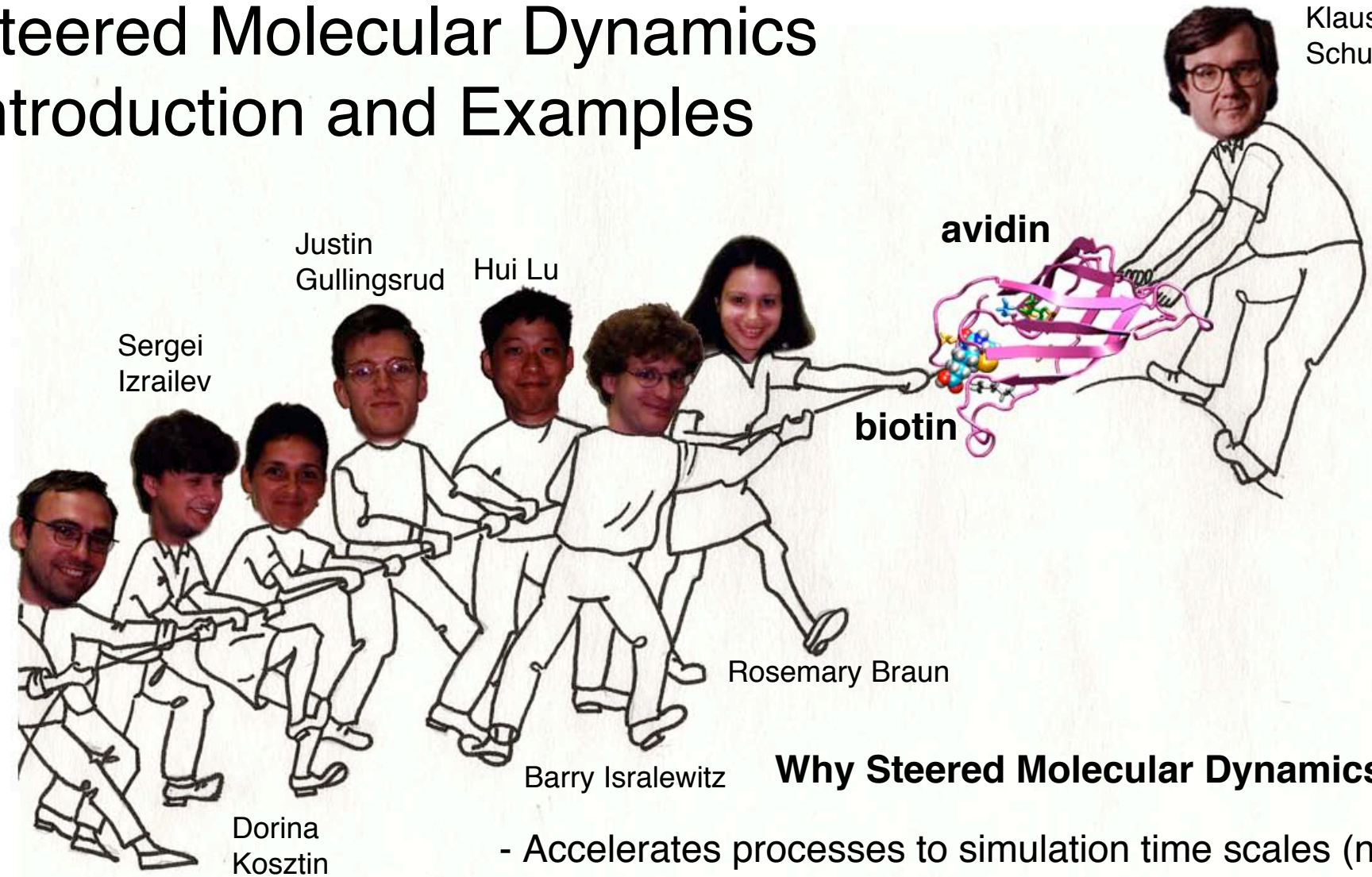
Steered Molecular Dynamics Introduction and Examples



Why Steered Molecular Dynamics?

- Accelerates processes to simulation time scales (ns)
- Yields explanations of biopolymer mechanics
- Complements Atomic Force Microscopy
- Finds underlying unbinding potentials
- Generates and tests Hypotheses

Acknowledgements:
Fernandez group, Mayo C.; Vogel group, U. Washington
NIH, NSF, Carver Trust

Mechanical Functions of Proteins

Forces

naturally arise in cells

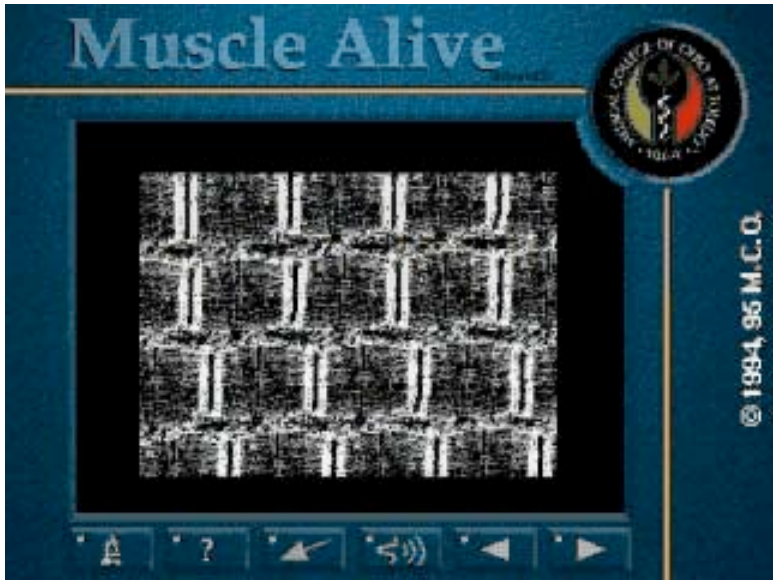
and can also be

substrates (ATPase)

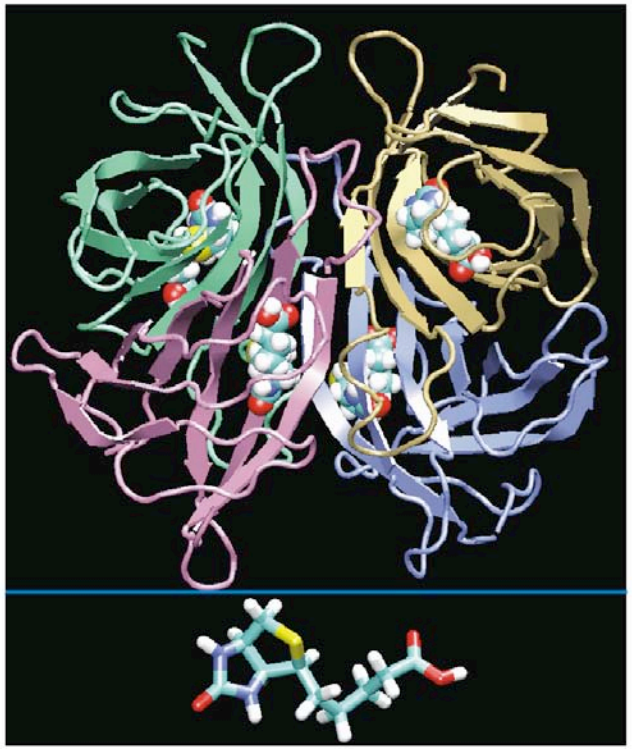
products (myosin)

signals (integrin)

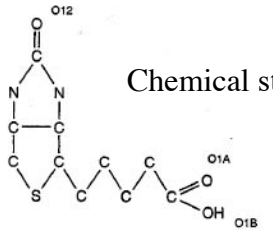
of cellular processes



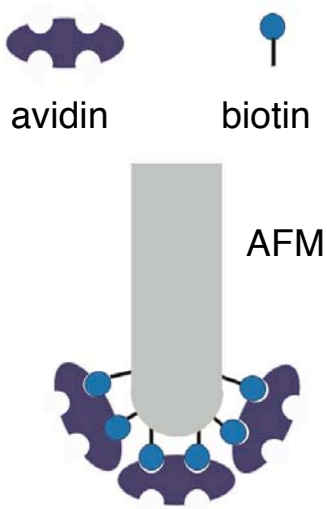
Atomic Force Microscopy Experiments of Ligand Unbinding

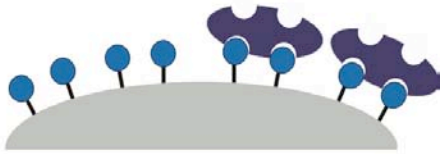


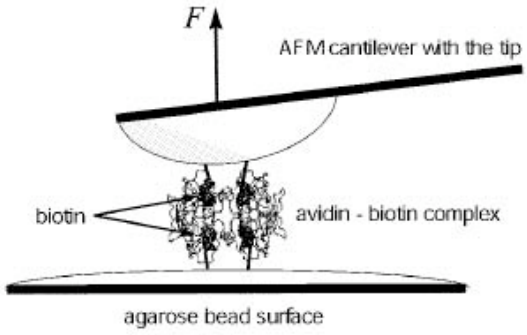
Biotin



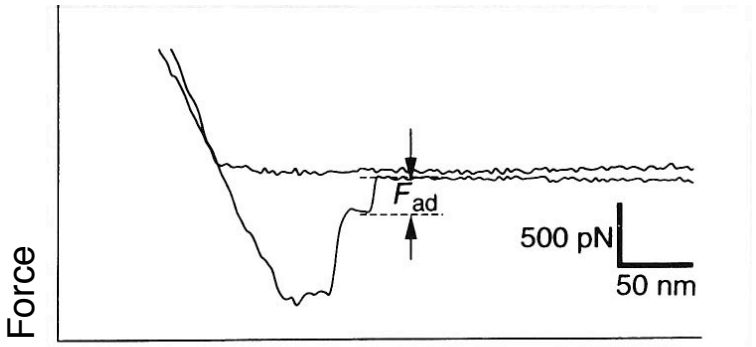
Chemical structure of biotin



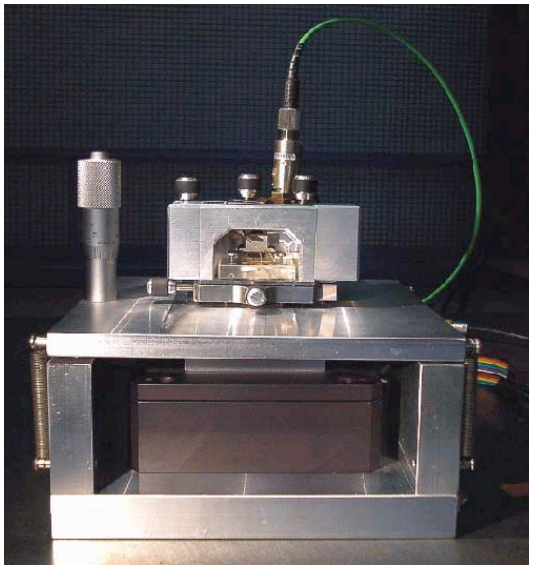




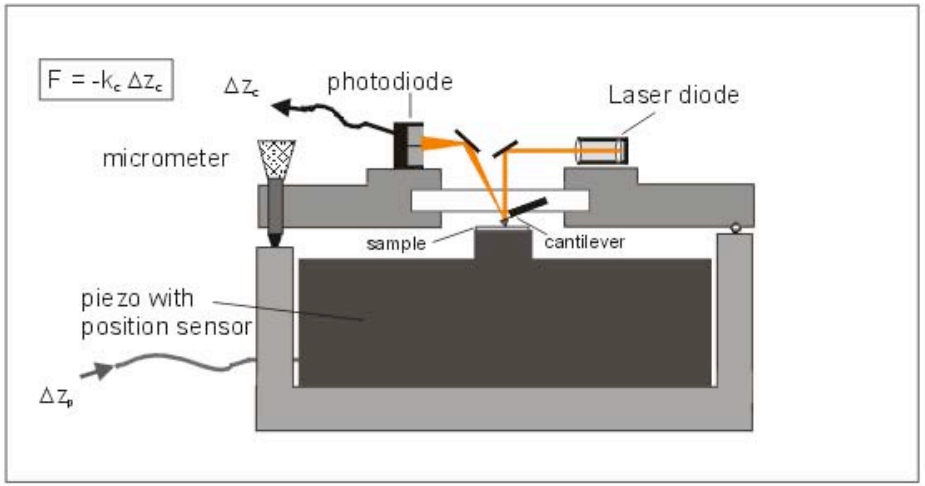
Florin et al., Science 264:415 (1994)

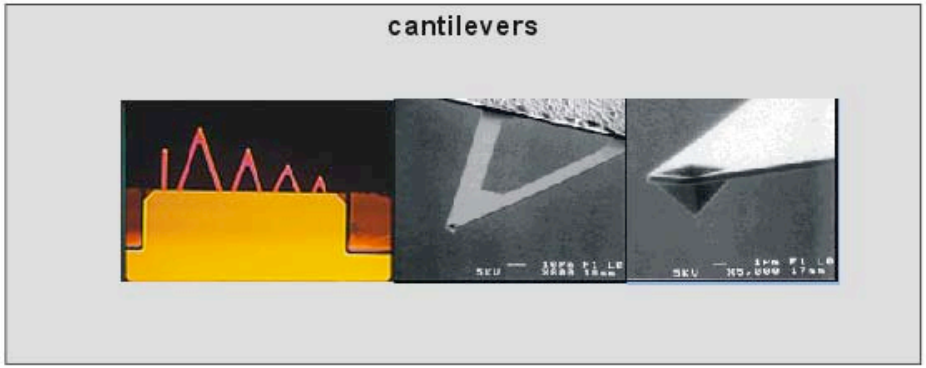


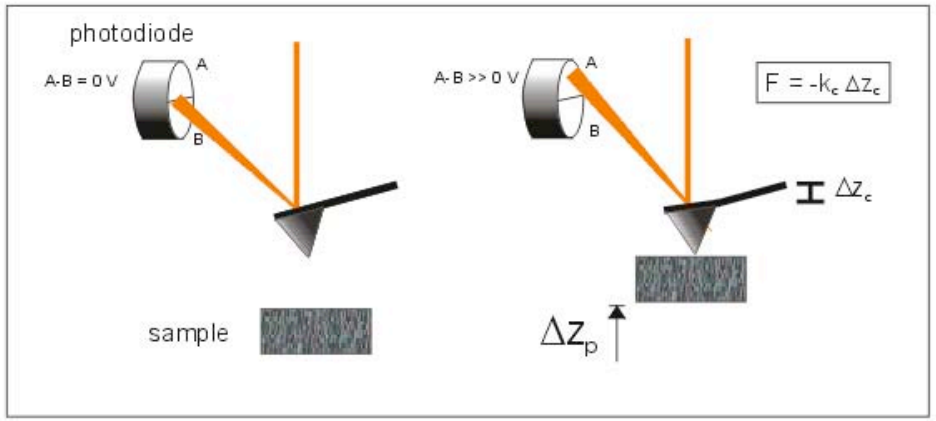
Displacement of AFM tip



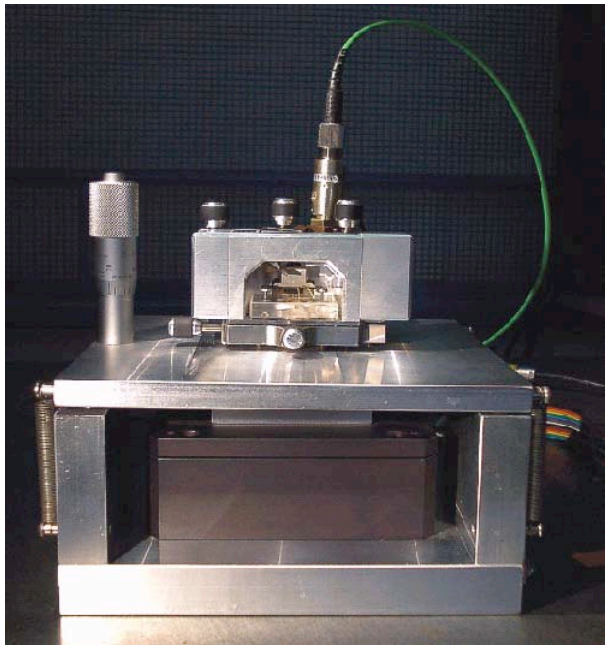
Atomic Force Microscope





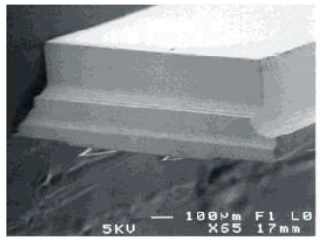


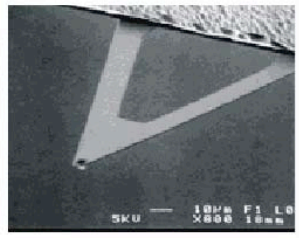
Instrument

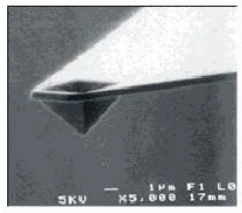


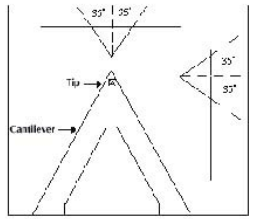
15 cm

AFM cantilevers and tips









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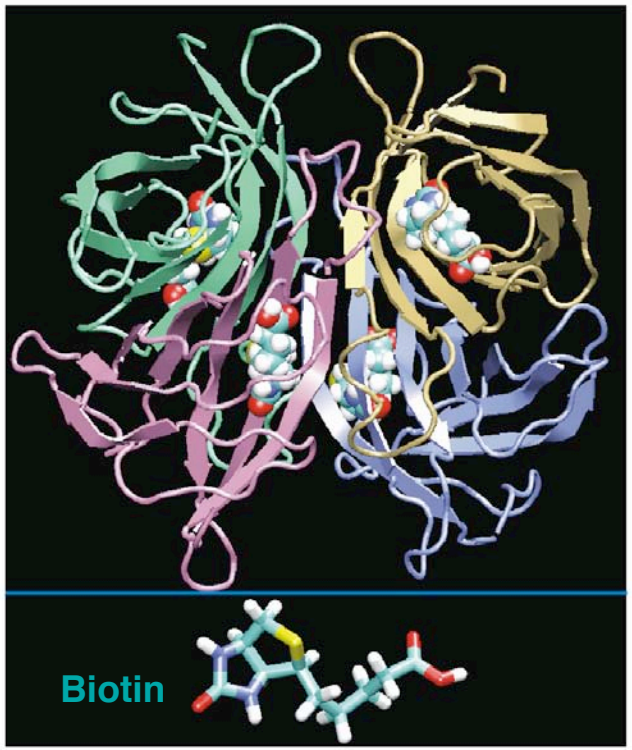
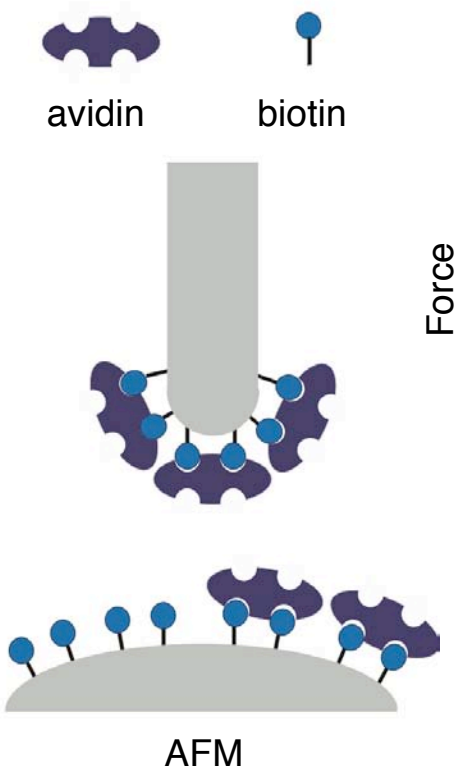
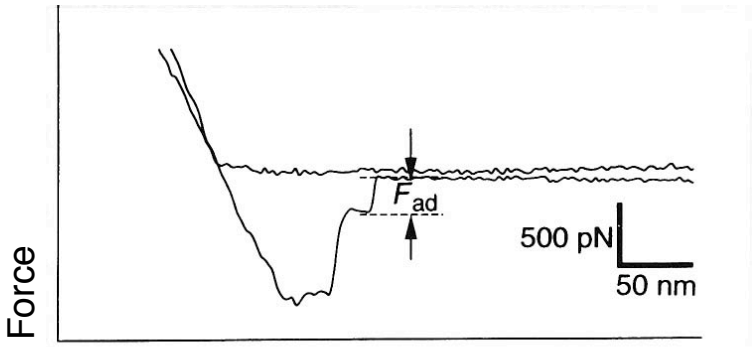
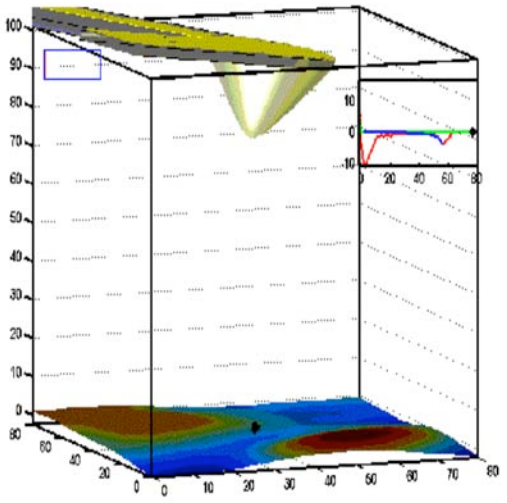
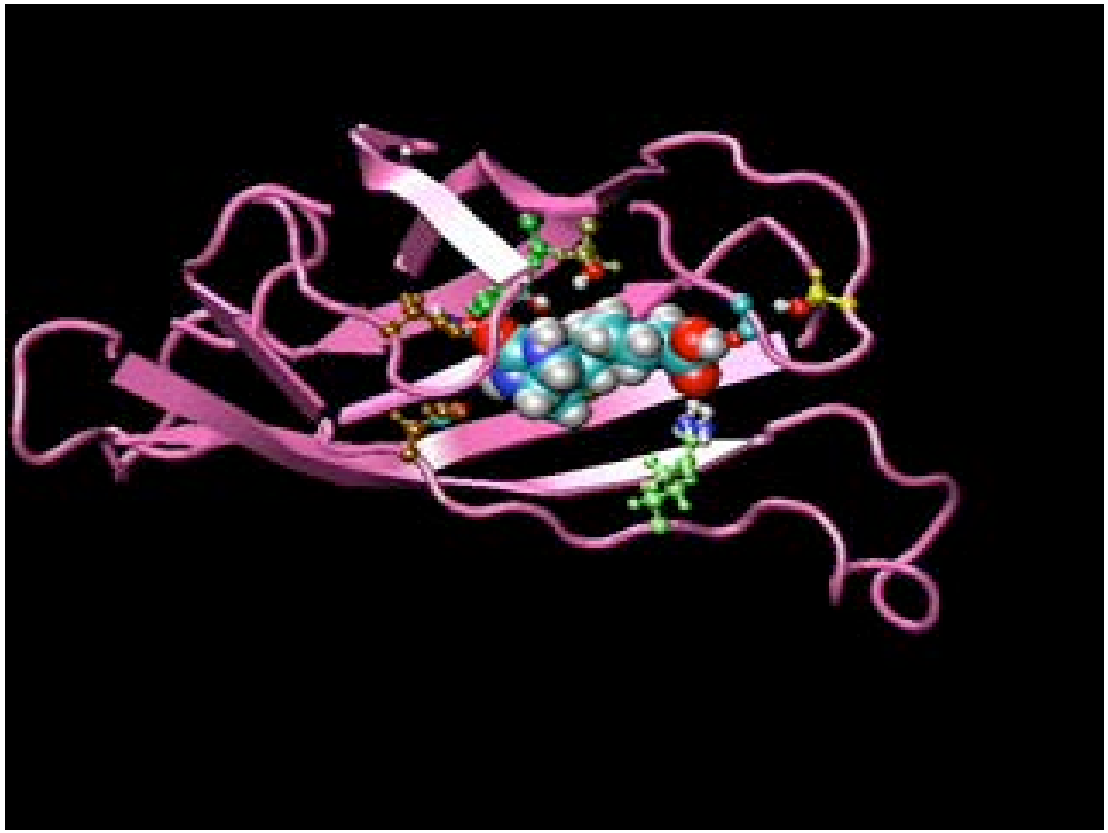








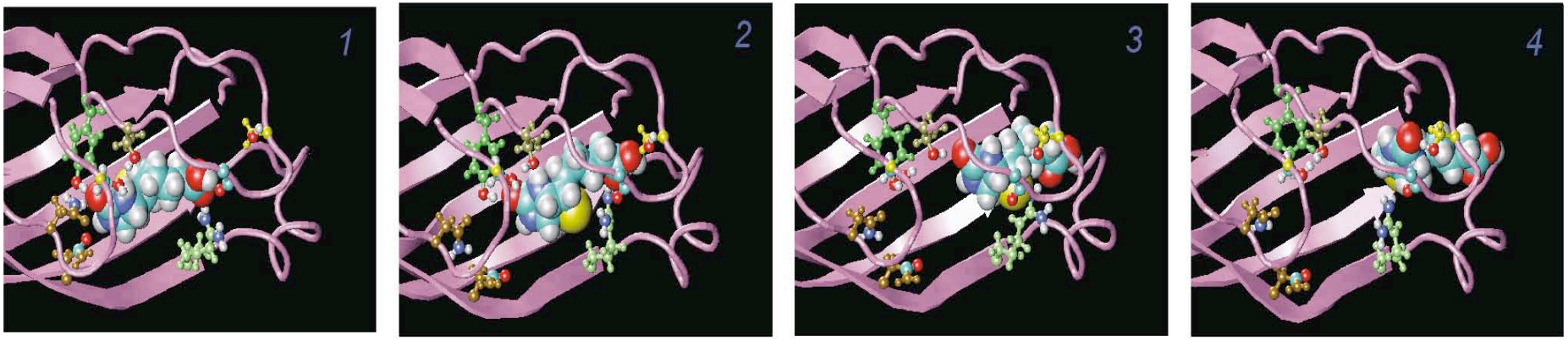
Pulling Biotin out of Avidin

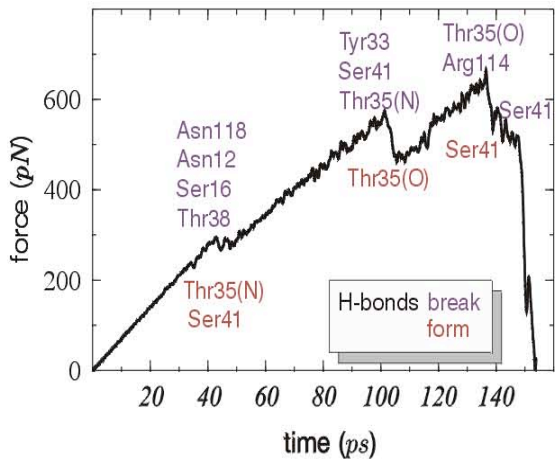


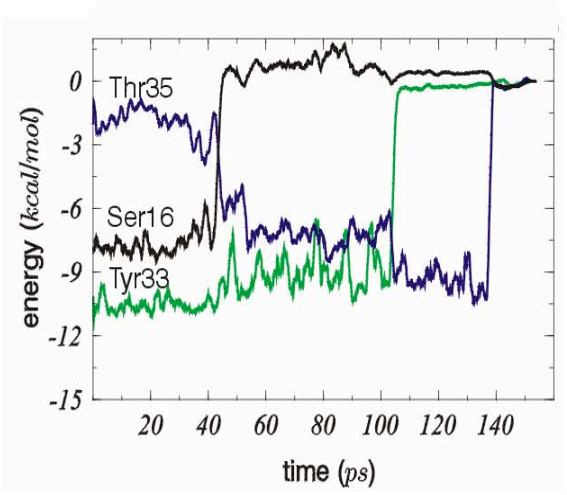
Molecular dynamics study of unbinding of the avidin-biotin complex. Sergei Izrailev, Sergey Stepaniants, Manel Balsera, Yoshi Oono, and Klaus Schulten. *Biophysical Journal*, 72:1568-1581, 1997.

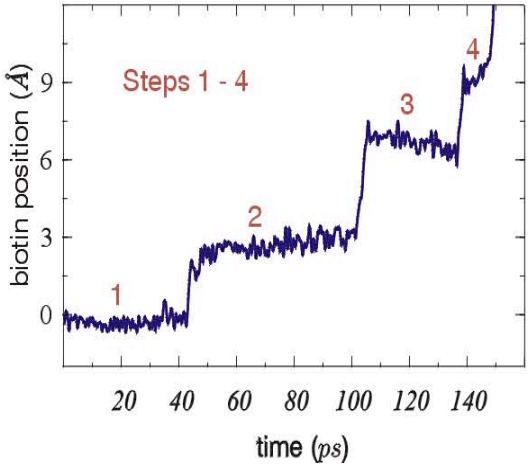
SMD of Biotin Unbinding: What We Learned

biotin slips out in steps, guided by amino acid side groups, water molecules act as lubricant, MD overestimates extrusion force









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Israilev *et al.*, Biophys. J., **72**, 1568-1581 (1997)

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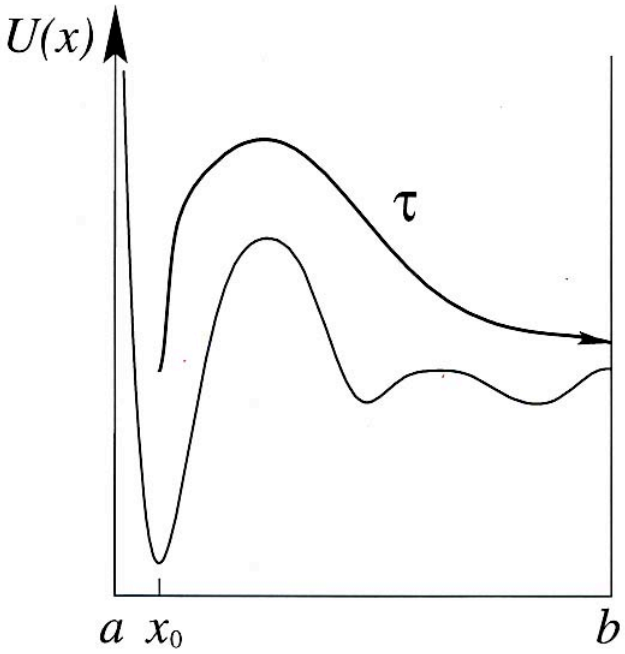
Theory of First Passage Times



- Langevin equation: $\gamma \dot{x} = -\frac{\partial U}{\partial x} + \sigma \xi(t)$
- Fluctuation-dissipation theorem:

$$\sigma^2 = 2k_B T\gamma$$

- Fokker-Plank equation: $(D = \sigma^2/2\gamma^2,\ \beta = 1/k_B T)$

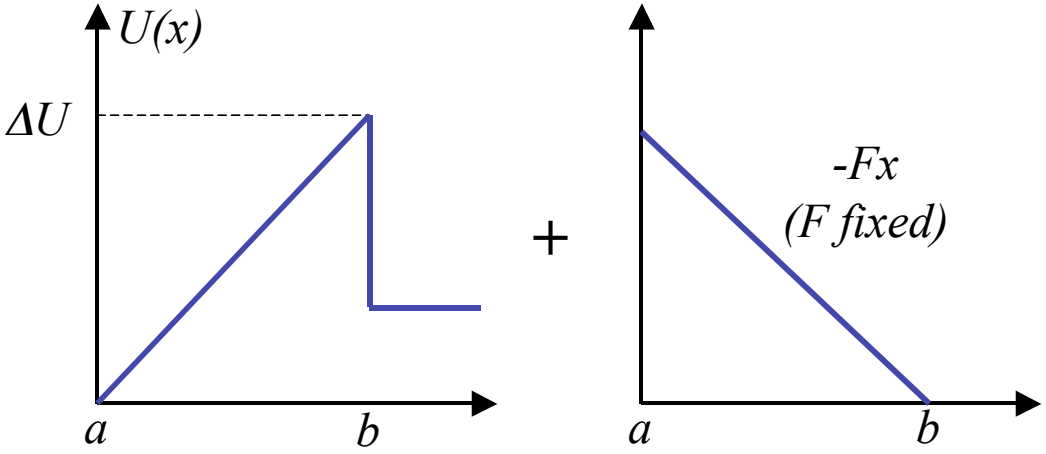
$$\partial_t p(x,t) = \partial_x D\, e^{-\beta U(x)}\, \partial_x e^{\beta U(x)} p(x,t)$$

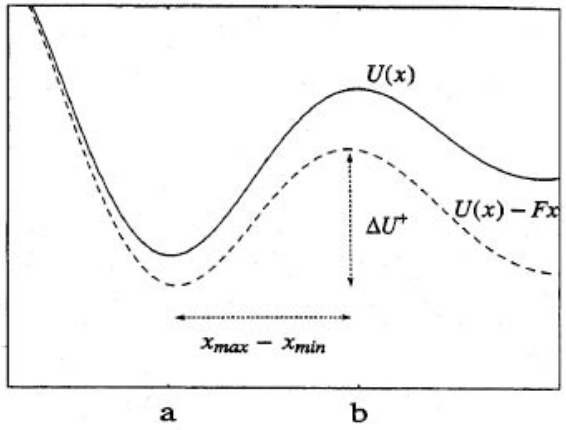
- First passage time:

$$\tau = \int_{x_o}^{b} dx\, e^{\beta U(x)} D^{-1} \int_{a}^{x} dx'\, e^{-\beta U(x')}$$

Schulten *et al*., J. Chem. Phys., **74**, 4426-4432 (1981)
Nadler and Schulten, J. Chem. Phys., **82**, 151-160 (1985)

Linear Binding Potential Model





Exact expression for first passage time

$$\tau(F) = 2\tau_D\delta(F)\,[e^{\delta(F)} - \delta(F) - 1]$$

$$\tau(D) = (b-a)^2/2D \sim 25\ ns$$

(for biotin-avidin)

$$\delta(F) = \beta\,[\Delta U - F(b\text{-}a)]$$

AFM regime

$$e^{\delta(F)} >> 1$$

$$\tau_{AFM} \sim 2\tau_D\delta^{-2}(F)e^{\delta(F)}$$

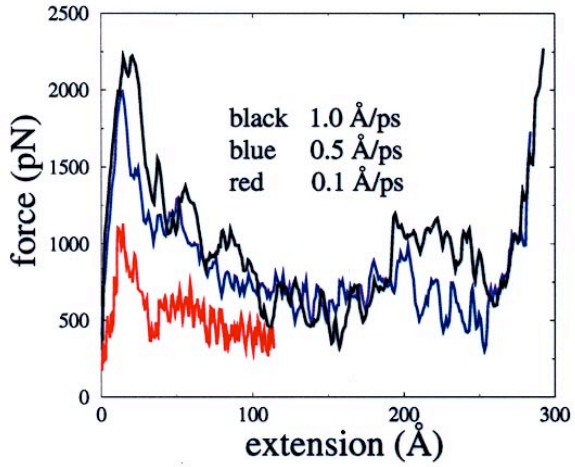
SMD regime

$$e^{\delta(F)} << 1$$

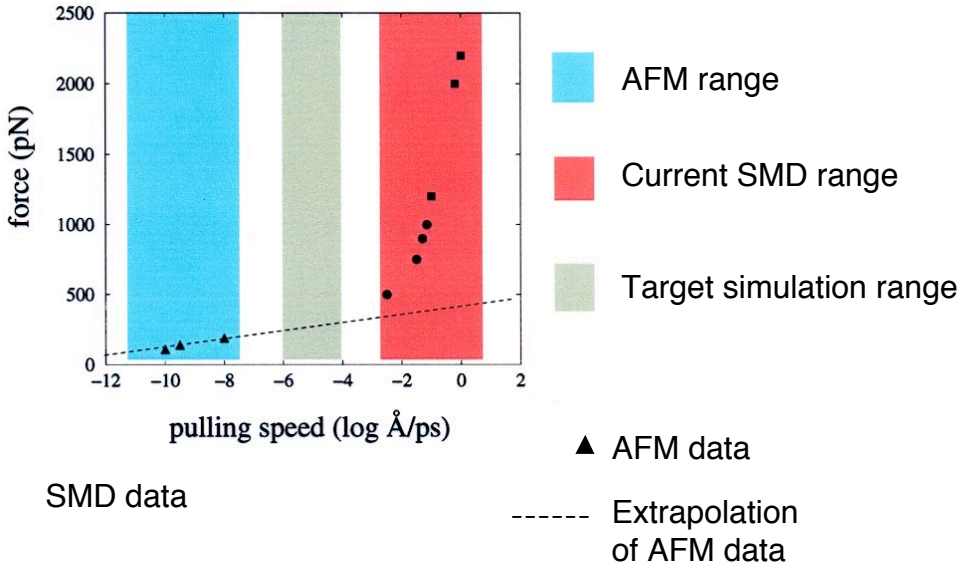
$$\tau_{SMD} \sim 2\tau_D|\delta(F)|^{-1}$$

Quantitative Comparison

Force-extension curve



Force-pulling velocity relationship



Bridging the gap between SMD and AFM experiments

AFM regime

$$e^{\delta(F)} >> 1$$

$$\tau_{AFM} \sim 2\tau_D \delta^{-2}(F) e^{\delta(F)}$$

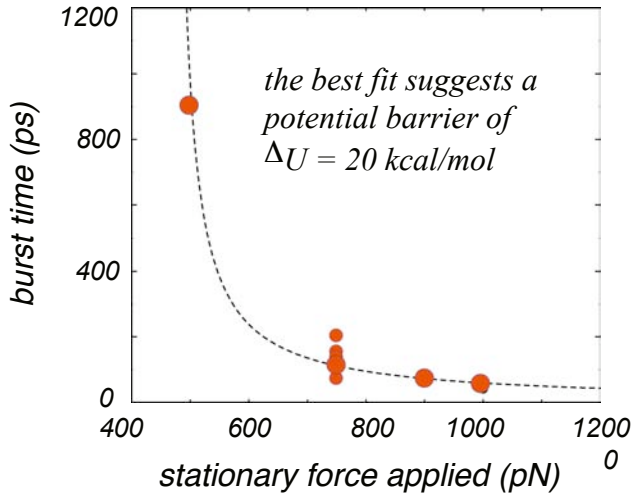
SMD regime

$$e^{\delta(F)} << 1$$

$$\tau_{SMD} \sim 2\tau_D |\delta(F)|^{-1}$$

Rupture/Unfolding Force F_0 and its Distribution

determination of barrier height based on mean first passage time



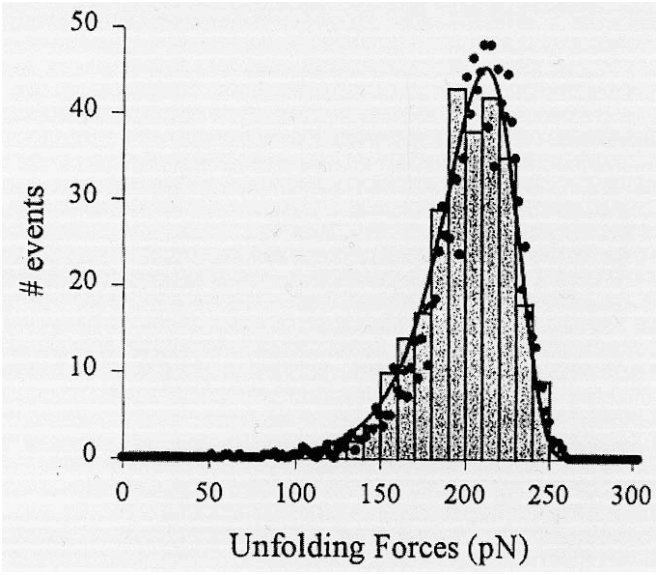
$\tau(F_0)$ = 1 ms time of measurement

=> F_0 rupture/unfolding force

Distribution of rupture/unfolding force

$$p(F_0) = \kappa \exp[\beta F_0(b-a) - \beta\Delta U - \frac{\kappa k_B T}{b-a} e^{-\beta\Delta U}\left(e^{\beta F_0 (b-a)} - 1\right)]$$

$$\kappa = \delta^2(F)/2\tau_D kv$$

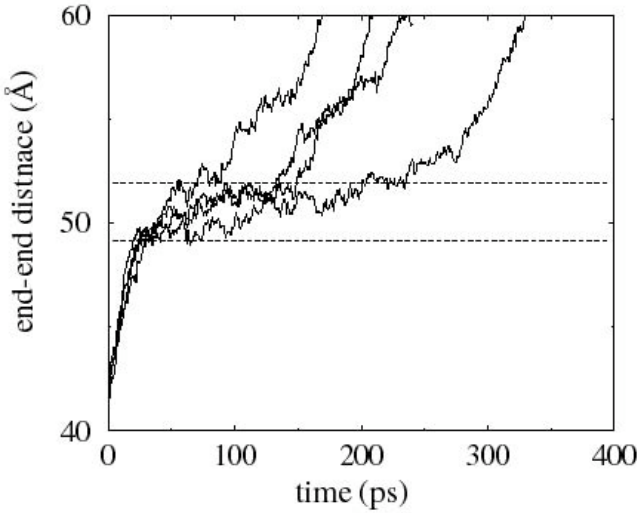


Israilev *et al.,* Biophys. J., **72**, 1568-1581 (1997)

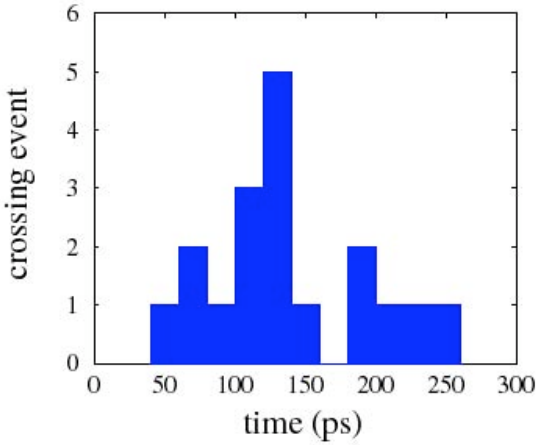
Balsera *et al*., Biophys. J., **73**, 1281-1287 (1997)

Distribution of the Barrier Crossing Time

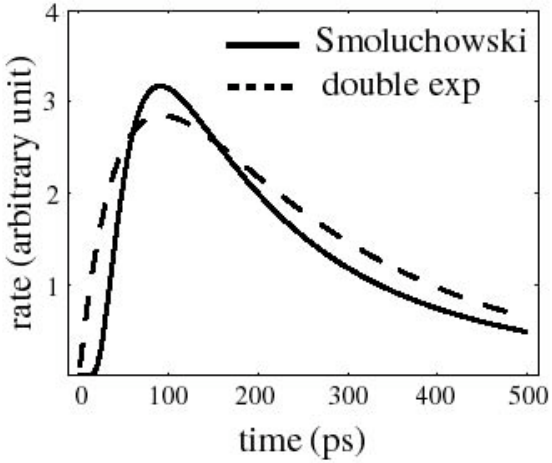
Multiple runs with same force of 750 pN



Barrier crossing times of 18 SMD simulations



Theoretical prediction of the barrier crossing times



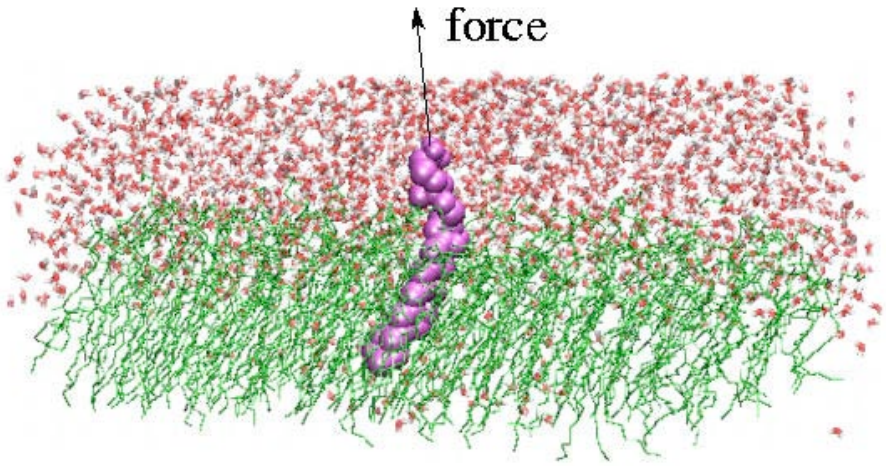
The fraction N(t) that has not crossed the barrier can be expressed through solving the Smoluchowski diffusion equation (linear model potential):

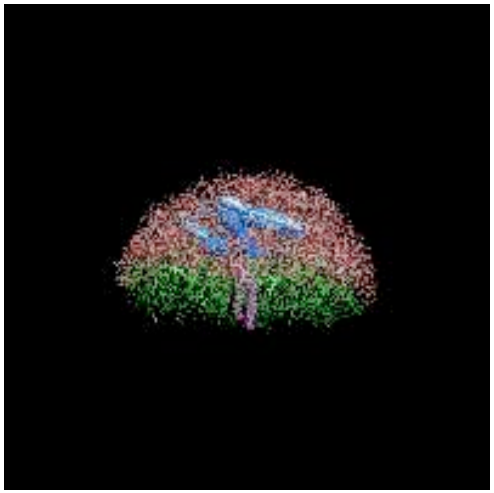
$$N(t) = \frac{1}{2} erfc\left[\frac{-a + \delta(F) D t/(b-a)}{\sqrt{4Dt}}\right] - \frac{1}{2} exp\left[\frac{\delta(F) a}{b-a}\right] erfc\left[\frac{-a + \delta(F) D t/(b-a)}{\sqrt{4Dt}}\right]$$

Or approximated by double exponential (general potential):

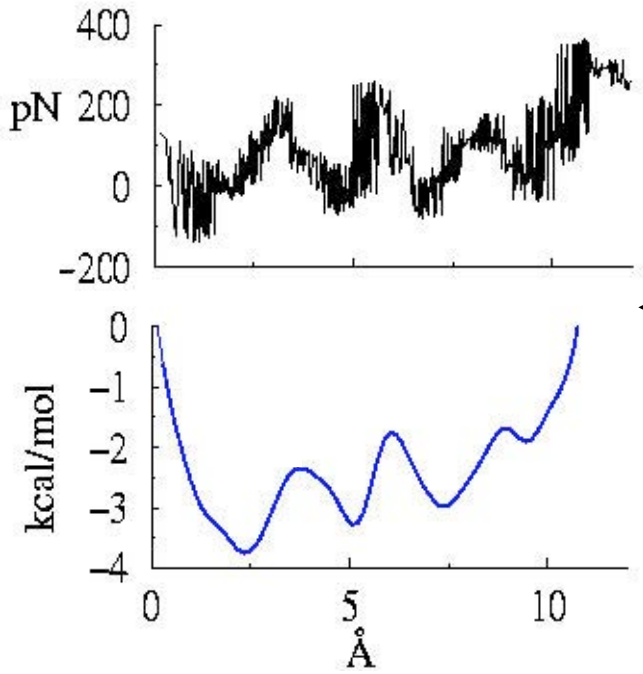
$N(t) = [t_1 \exp(-t/t_1) - t_2 \exp(-t/t_2)]/(t_1-t_2)$, Nadler & Schulten, JCP., **82**, 151-160 (1985)

Quantitative Analysis of SMD





PLA2 pulling a lipid out of membrane



- The potential of mean force (PMF) is reconstructed from time series of applied force and displacement

- Non-equilibrium analysis based on the Langevin equation:

$$\gamma\dot{x} = -dU/dx + k(vt-x) + \sigma\xi(t)$$

- Multiple trajectories can be combined to yield statistically significant results

Stepaniants *et al*., J.Molec. Model., **3**, 473-475 (1997)

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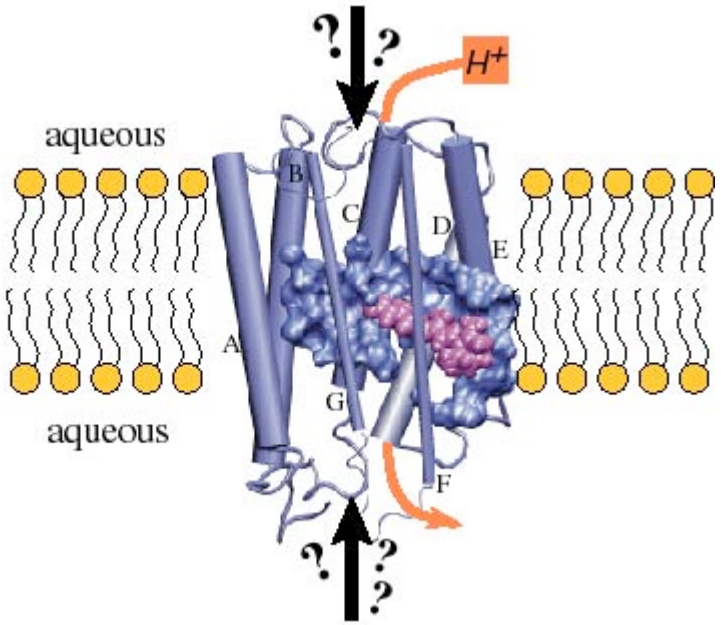
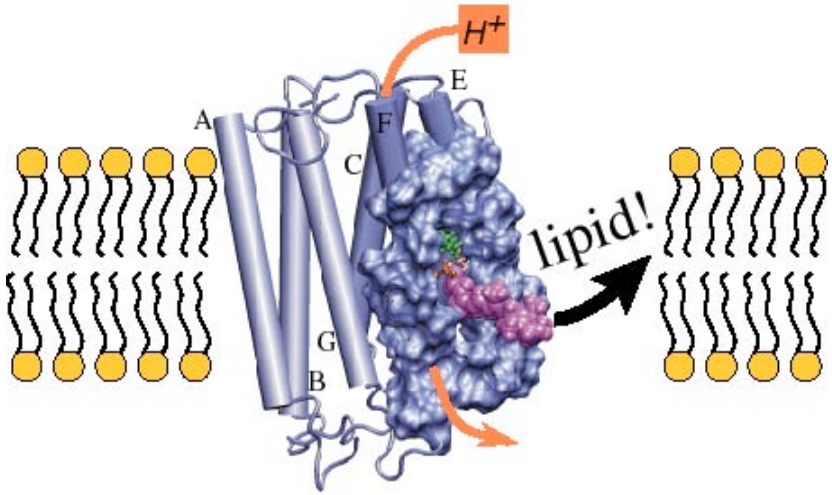
Interactive Modeling

Binding path of retinal to bacterio-opsin (1)



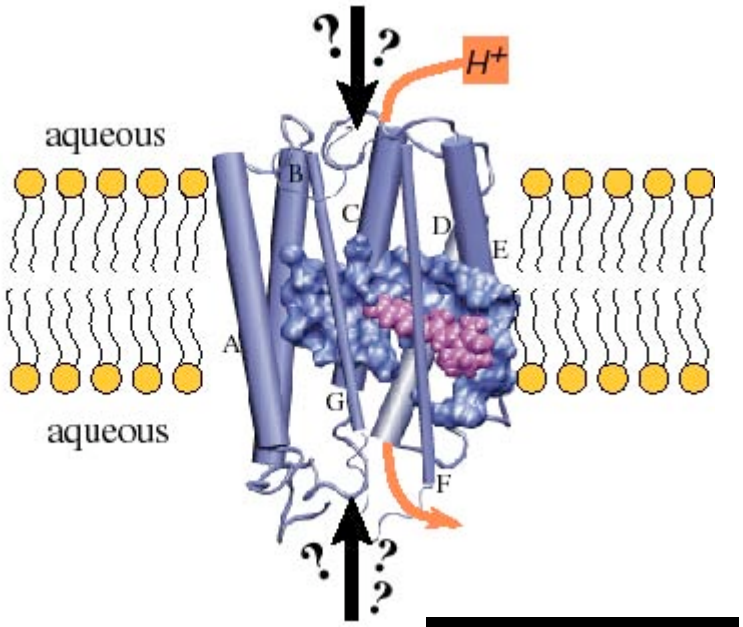
- Retinal deep in bacterio-opsin binding cleft
- How does it get in?
- Use batch mode interactive steered molecular dynamics to pull retinal out of cleft, find possible binding path

- 10 path segments, 3 attempts each
- Choose best attempt at 9 points during pull
- Found path through membrane, and electrostatically attractive entrance window



Interactive Modeling

Binding path of retinal to bacterio-opsin



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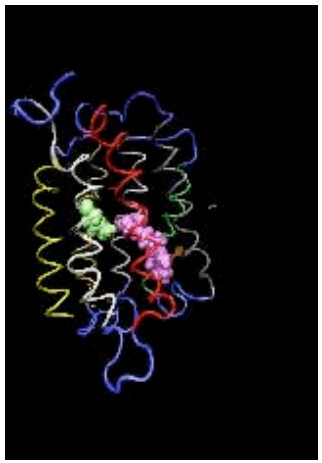
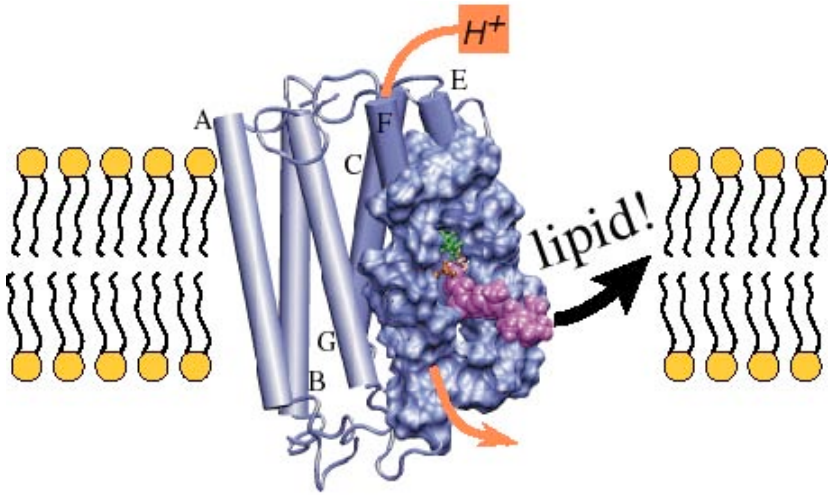
Binding pathway of retinal to bacterio-opsin: A prediction by molecular dynamics simulations. Barry Isralewitz, Sergei Izrailev, and Klaus Schulten. *Biophysical Journal* , 73:2972-2979, 1997.





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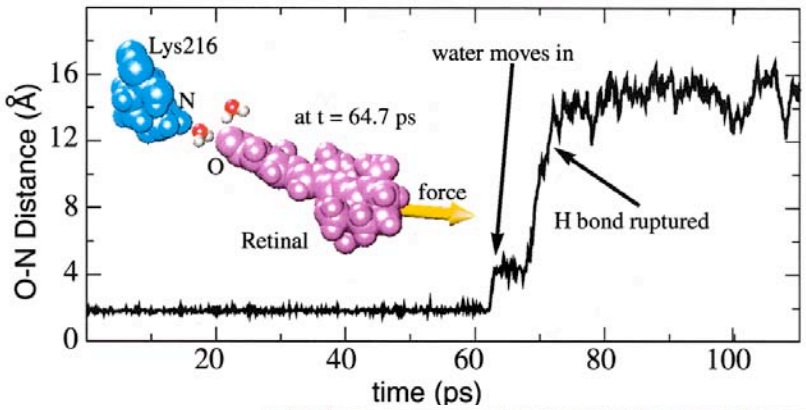
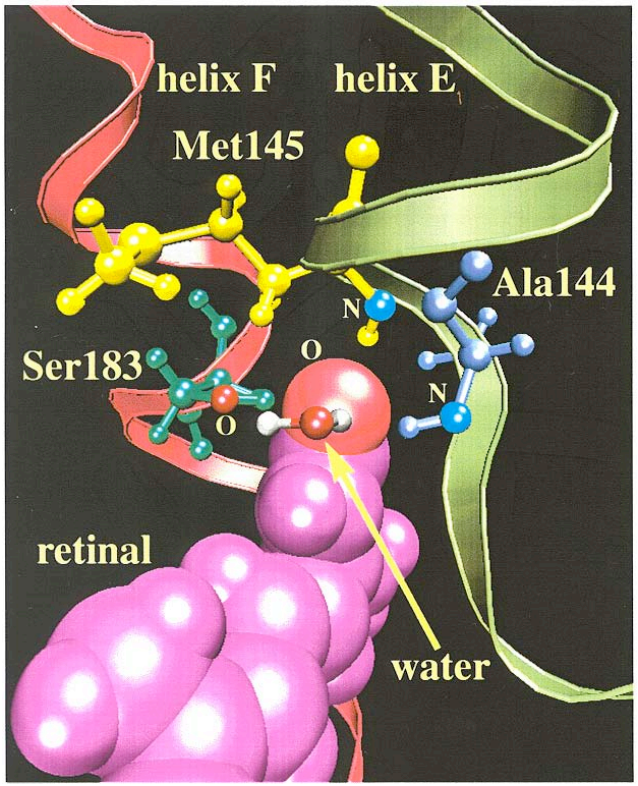
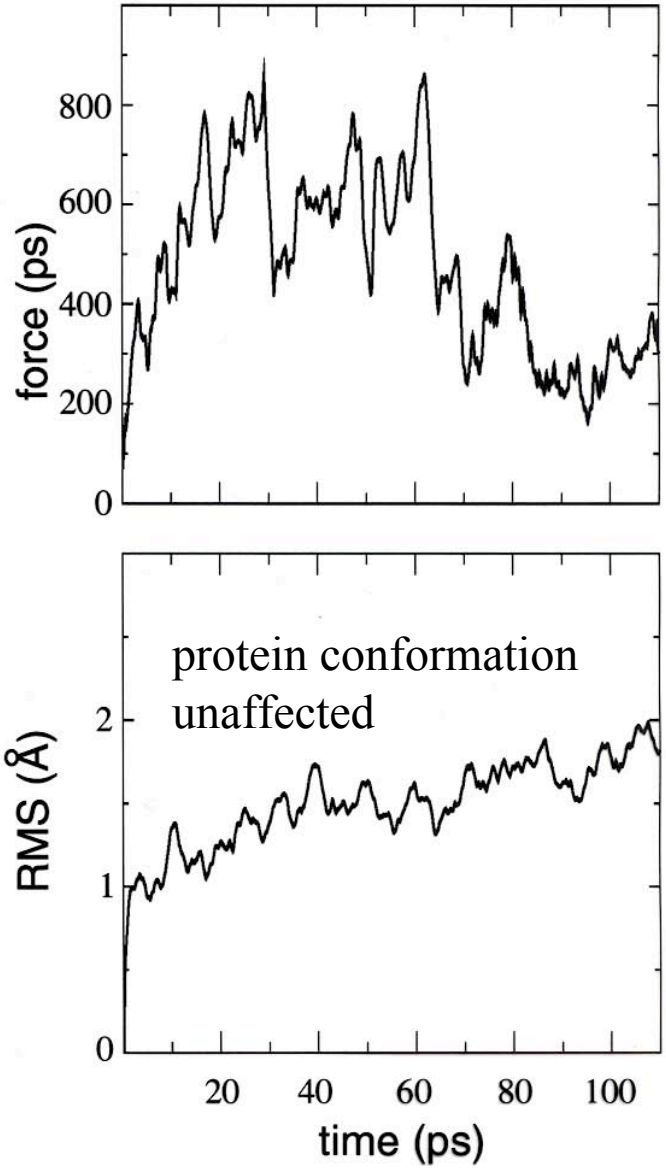
Stepwise Unbinding of Retinal from bR



water needed to shield lys – retinal interact.

Retinal's exit and entrance "door" attracts its aldehyde group





Israelewitz *et al.*, Biophys. J., **73**, 2972-2979 (1997)

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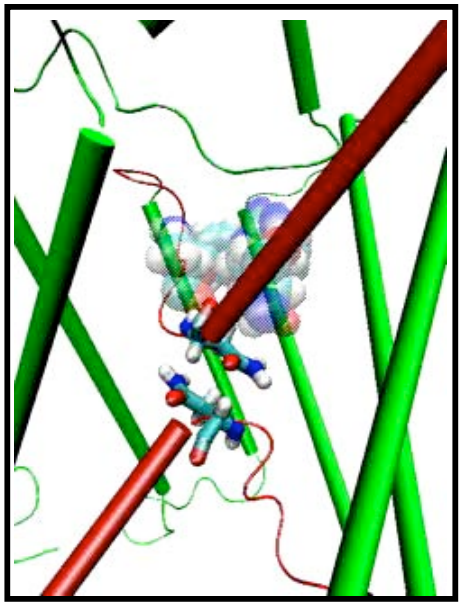
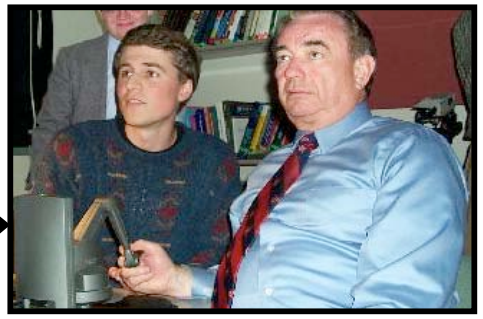
Interactive Molecular Dynamics

NAMD

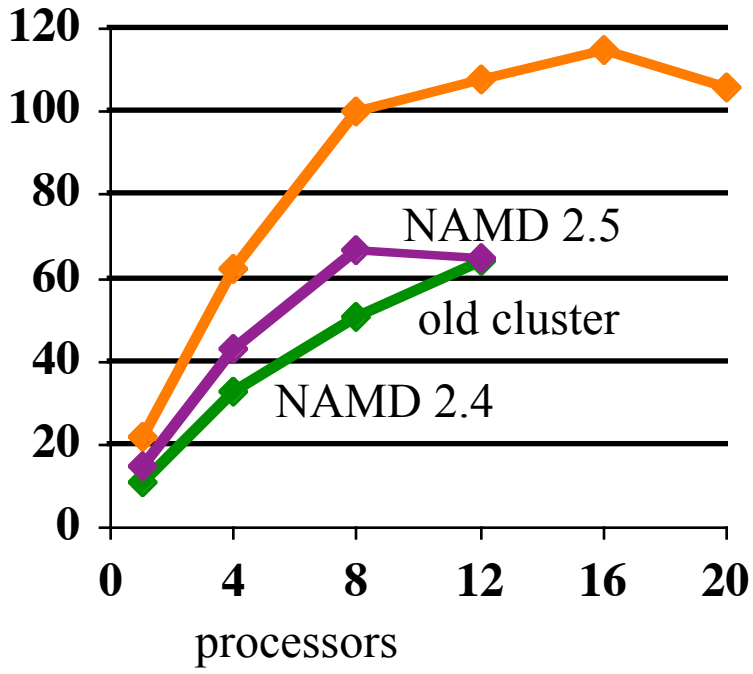


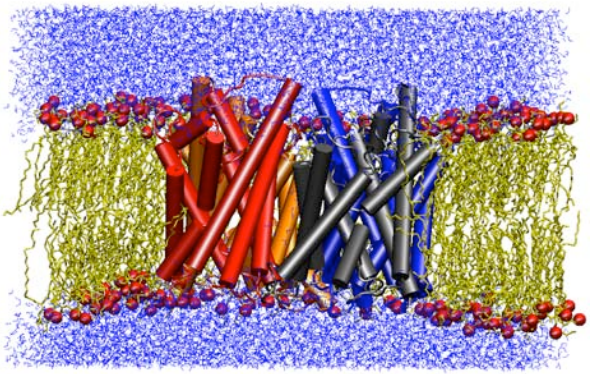
User



(HHS Secretary Thompson)

steps per second (more is better)





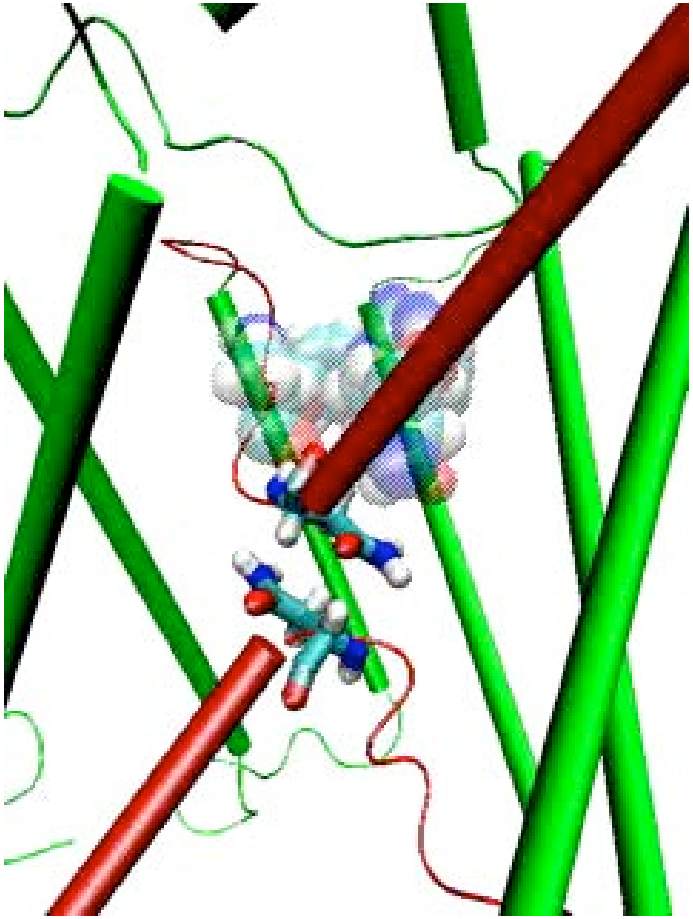
GlpF IMD Benchmark:

- 4210 atoms
- 3295 fixed atoms
- 10A cutoff, no PME
- Limited by network latency

Interactive Molecular Dynamics

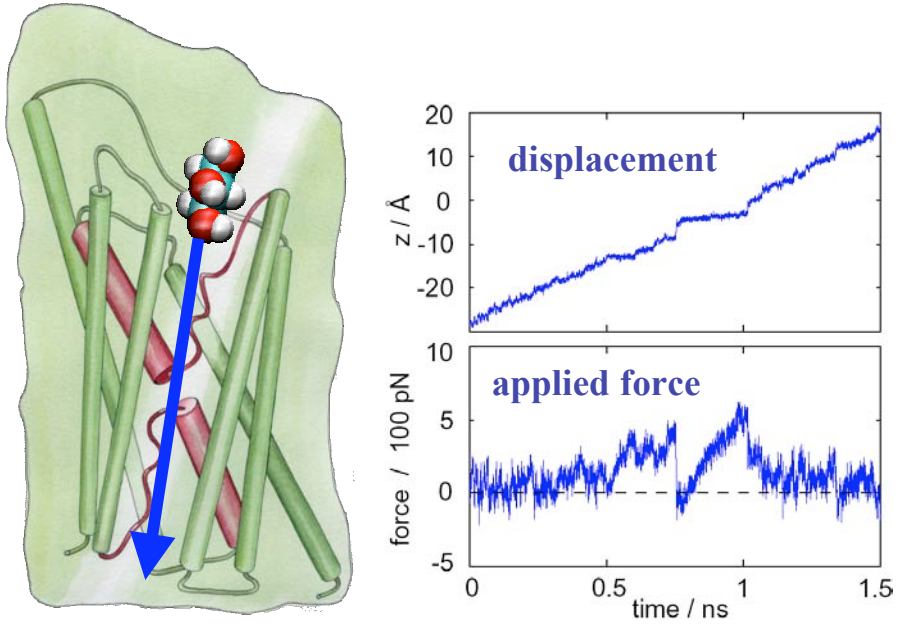
VMD ◄·····► **NAMD**

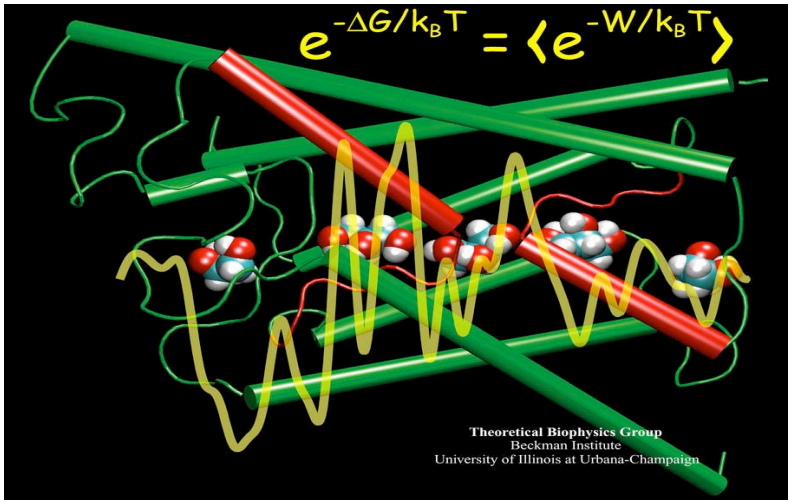
- Any PC/Workstation
- Supports 3D force-feedback devices for interaction



J. Stone, J. Gullingsrud, K. Schulten, and P. Grayson.
A System for Interactive Molecular Dynamics Simulation.
2001 ACM Symposium on Interactive 3D Graphics, pp.191-194, ACM SIGGRAPH
P. Grayson, E. Tajkhorshid, and K. Schulten.
Biophysical J, **83**: 36 (2003)

Quantitative Analysis of Substrate Permeation





Jensen et al, *PNAS* **99:** 6731-6736 (2002)

Calculation of the free energy profile of sugar transport from SMD simulations by Jarzynski's identity

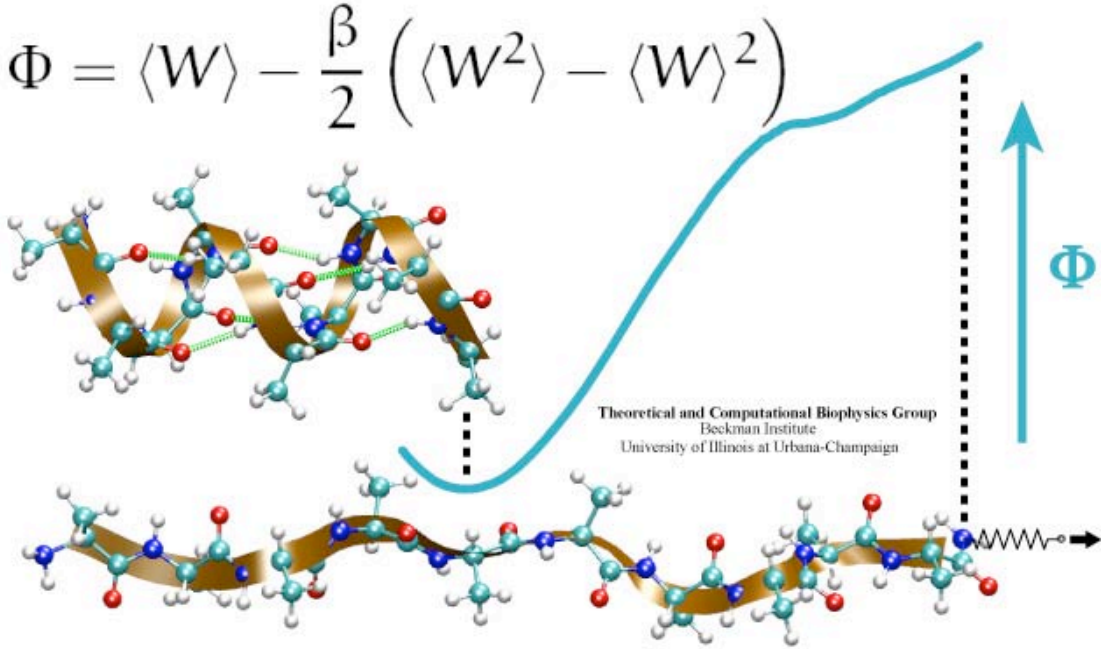
Thermodynamics: $\Delta G \leq \langle W \rangle$

Is there any chance to discount the irreversible work? Yes!

Free Energy of Stretched Alpha-Helix

Thermodynamics: $\Delta G \leq \langle W \rangle$

Jarzynski (1997): $e^{-\Delta G / k_B T} = \left\langle e^{-W / k_B T} \right\rangle$

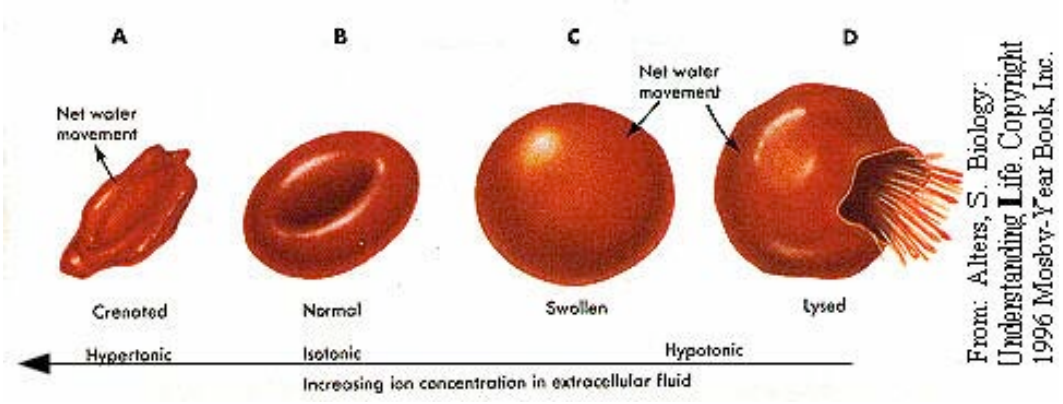


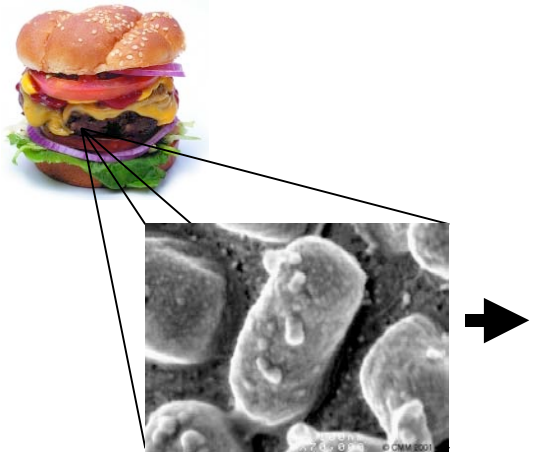
Free energy calculation from steered molecular dynamics simulations using Jarzynski's equality. S. Park, F. Khalili-Araghi, E. Tajkhorshid, and K. Schulten. *Journal of Chemical Physics* , 119:3559-3566, 2003

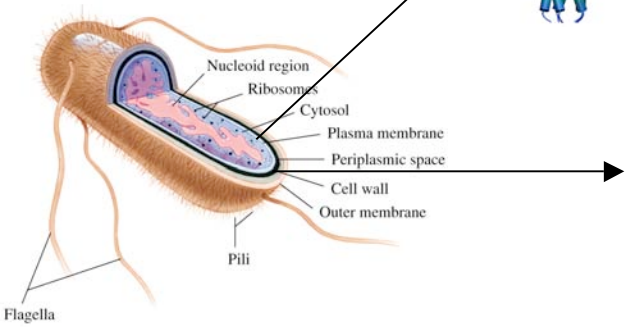
Calculating potentials of mean force from steered molcular dynamics simulations. S. Park and K. Schulten. *Journal of Chemical Physics* , 120: 5946-5961, 2004

Mechanosensitive Channels of Large and Small Conductance

- Mechanosensitive channels: one of the major mechanisms by which mechanical forces are sensed.
- MscL and MscS: Specific MSCs in bacteria. **Safety valves** located in their inner membrane (prevention of cell lysis)

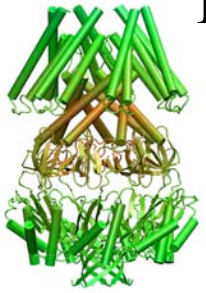






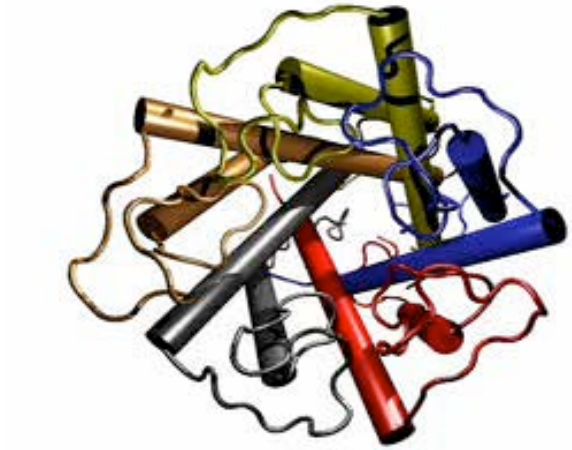
MscL

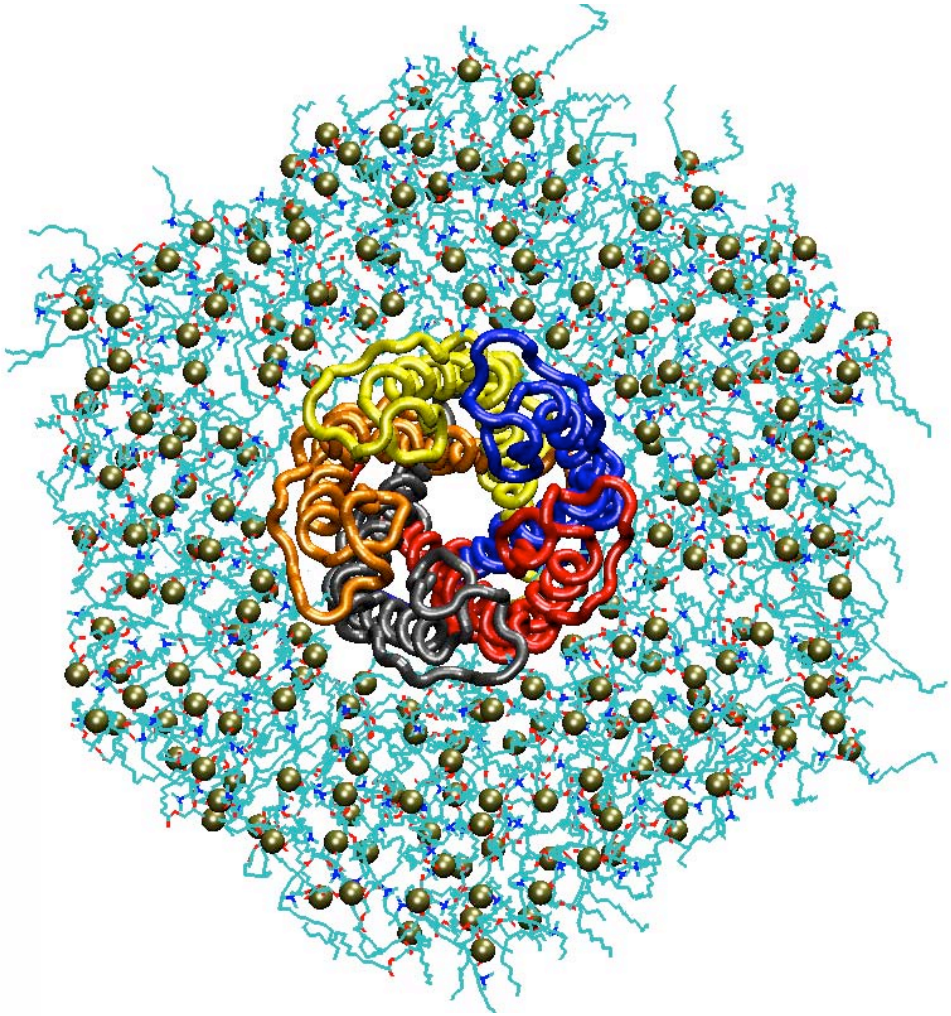
MscS



Gating Mechanism of a Mechanosensitive Channel

- Inserted MscL protein from crystal structure into equilibrated POPC membrane – 242 lipids, 16,148 water molecules, **88,097 atoms**
- Program NAMD, periodic boundary conditions, full electrostatics (PME), NpT ensemble, anisotropic pressure to describe surface tension, **2.4 days on 128 T3E CPUs**



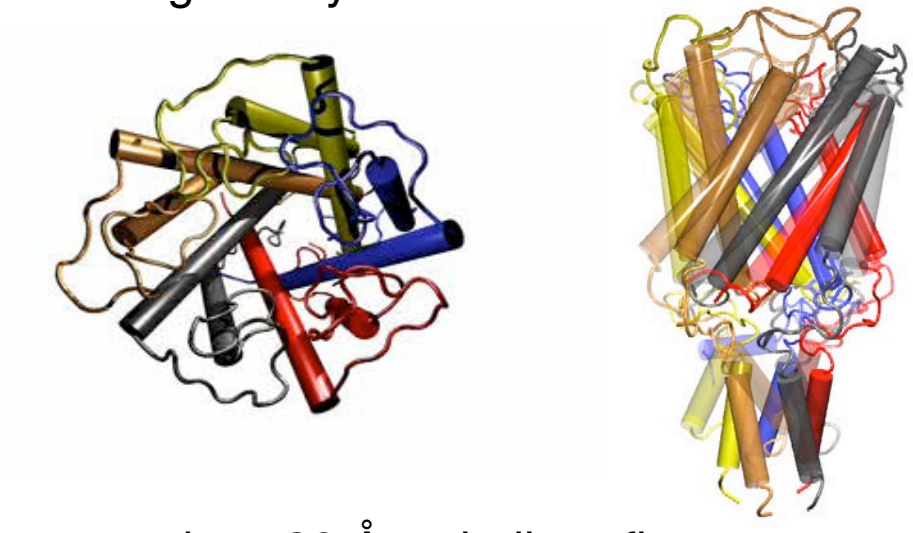


Biophys. J. 80:2074-2081, 2001.

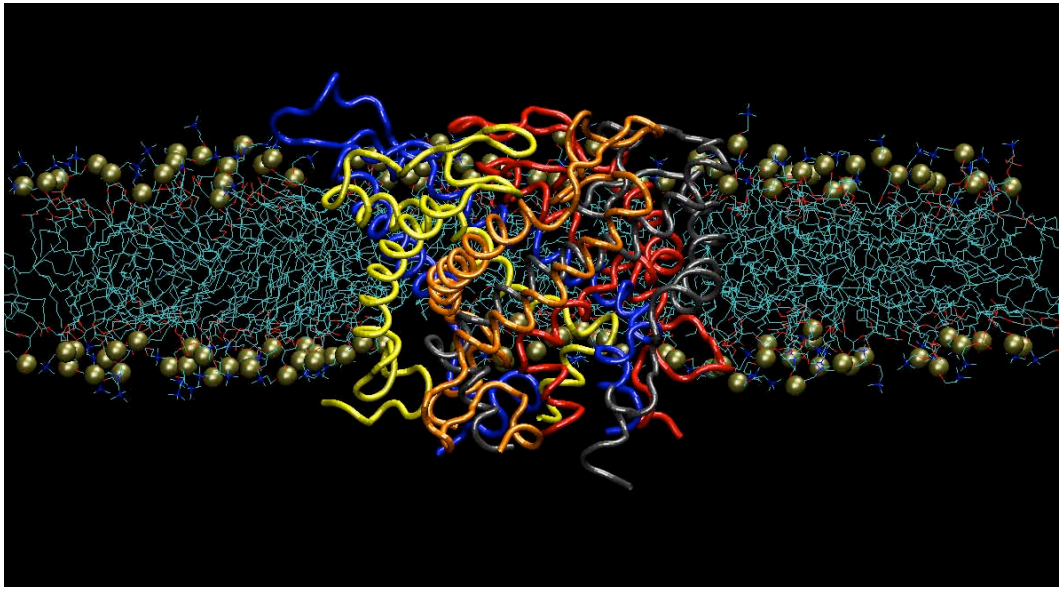
Justin Gullingsrud

Mechanosensitive Ion Channel

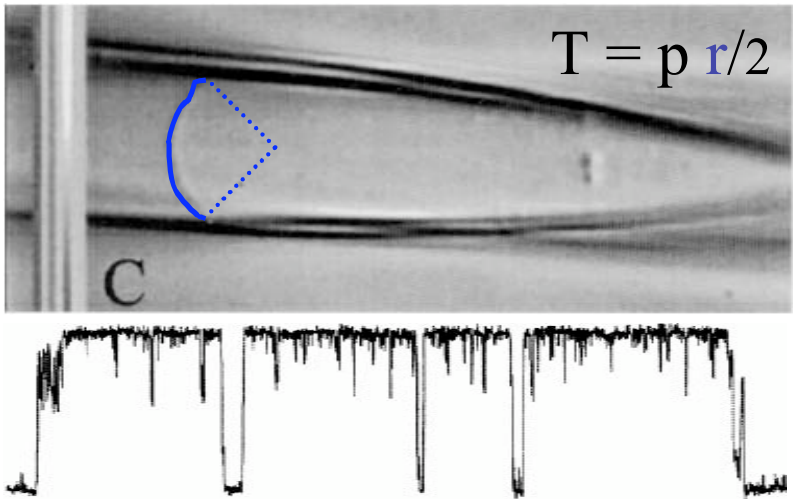
MscL gates by membrane tension



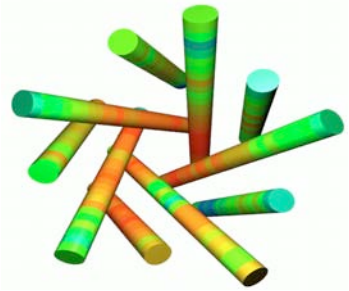
Pore expands to 30 Å as helices flatten out



Justin Gullingsrud



Patch-clamp measurements relate membrane tension to channel gating

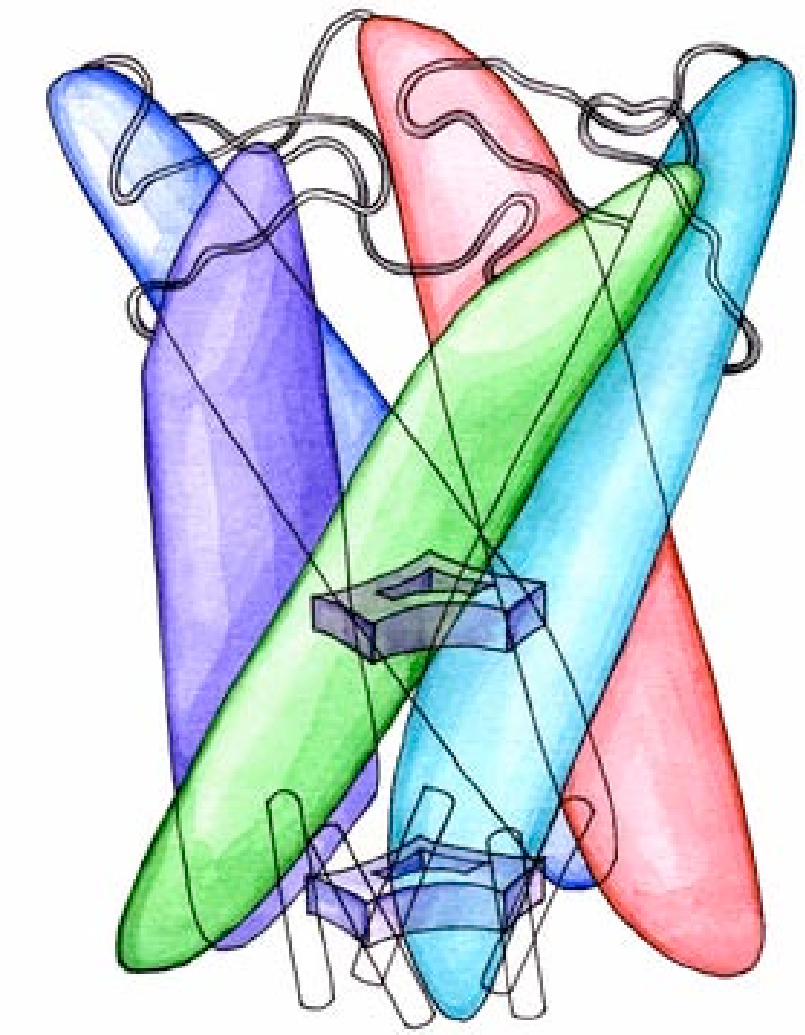


The protein is stiffest in the pinched gating region, in agreement with EPR measurements (Martinac et al, unpublished results)

Biophys. J. 80:2074-2081, 2001.

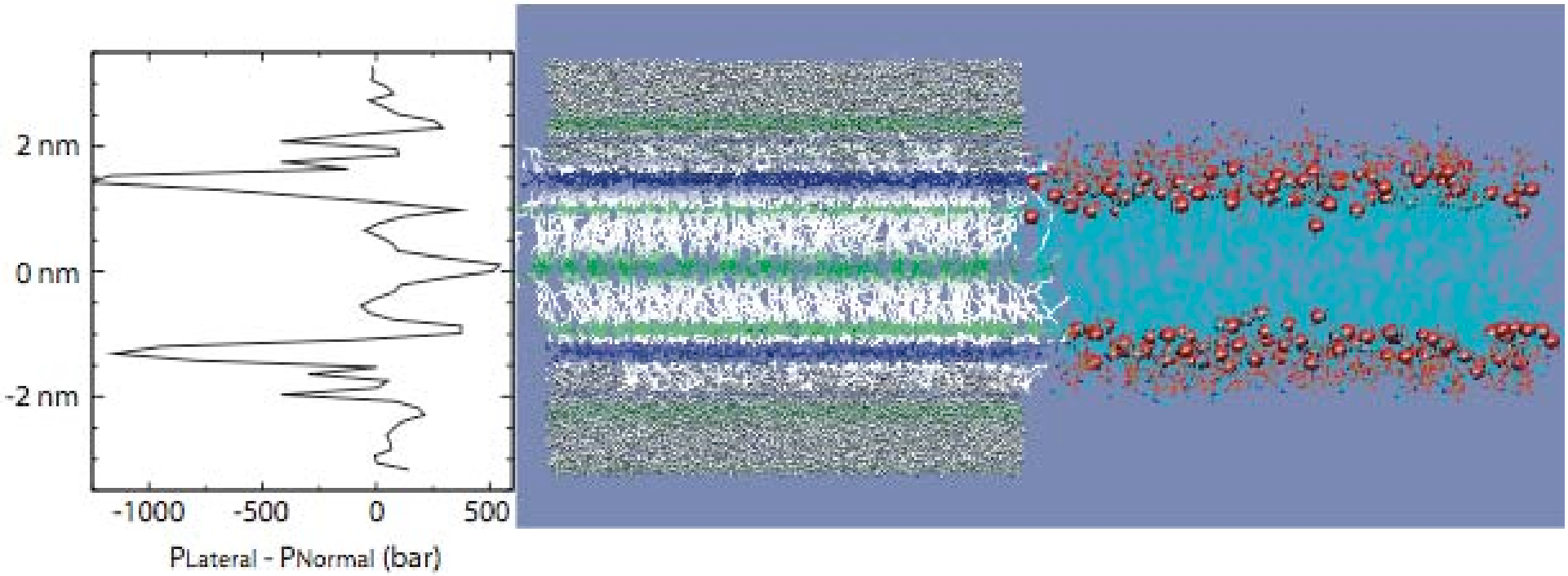
SMD Simulations of MscL

- How can we understand the interaction between the MscL and the surrounding bilayer? How can bilayer-derived forces open the channel?
- What does the open state of the channel look like? What is the opening pathway?
- Since there is no “signature sequence” for MS channels, what controls the gating sensitivity?



J. Gullingsrud

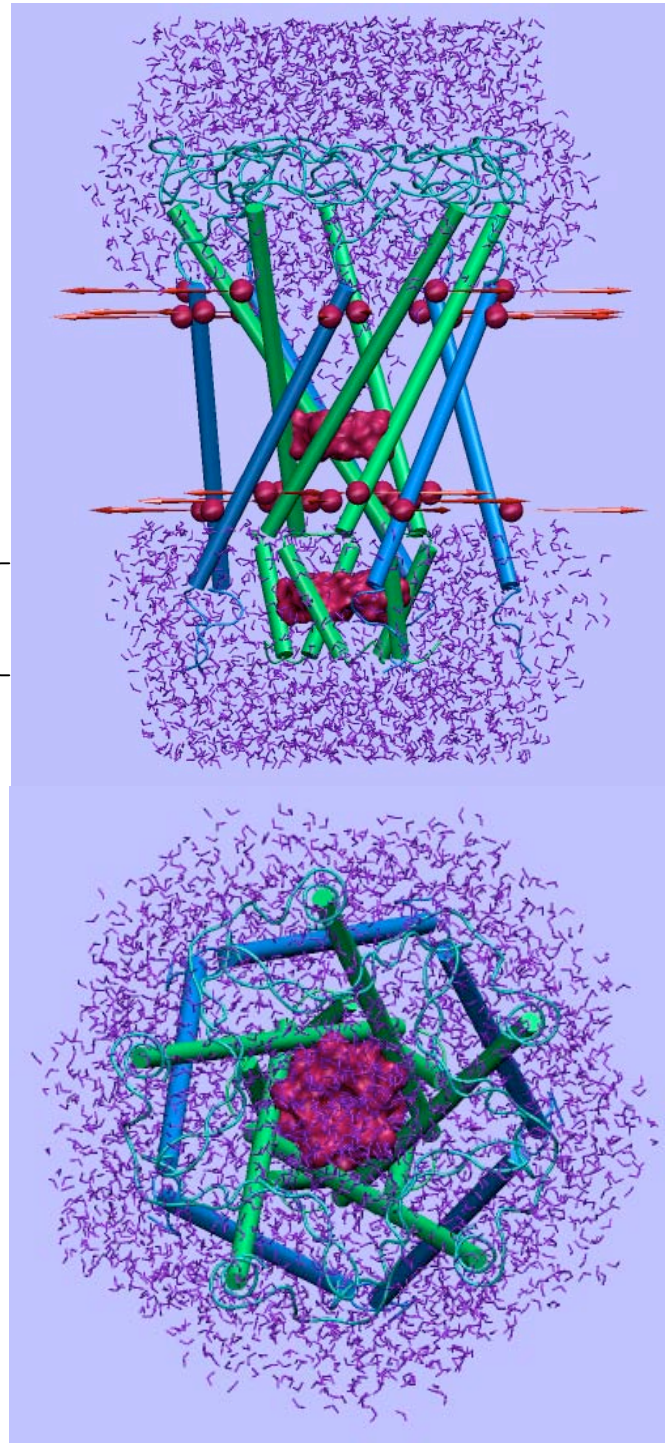
Gating Forces Derived from Bilayer Pressure Profile



Pressure profile calculations similar to those of Lindahl & Edholm showed that the interfacial tension of the membrane may be simulated by applying external forces of about 40 pN to the protein.

Simulation Setup

- MscL from *E. coli* based on homology model.
- Eliminated C-terminal helices; these are nonessential for gating.
- Sufficient water for full hydration of loops and N-terminal helix bundle.
- Constant radial force applied to residues at the ends of M1 and M2 (16, 17, 40, 78, 79, 98).
- 10 ns simulation time.



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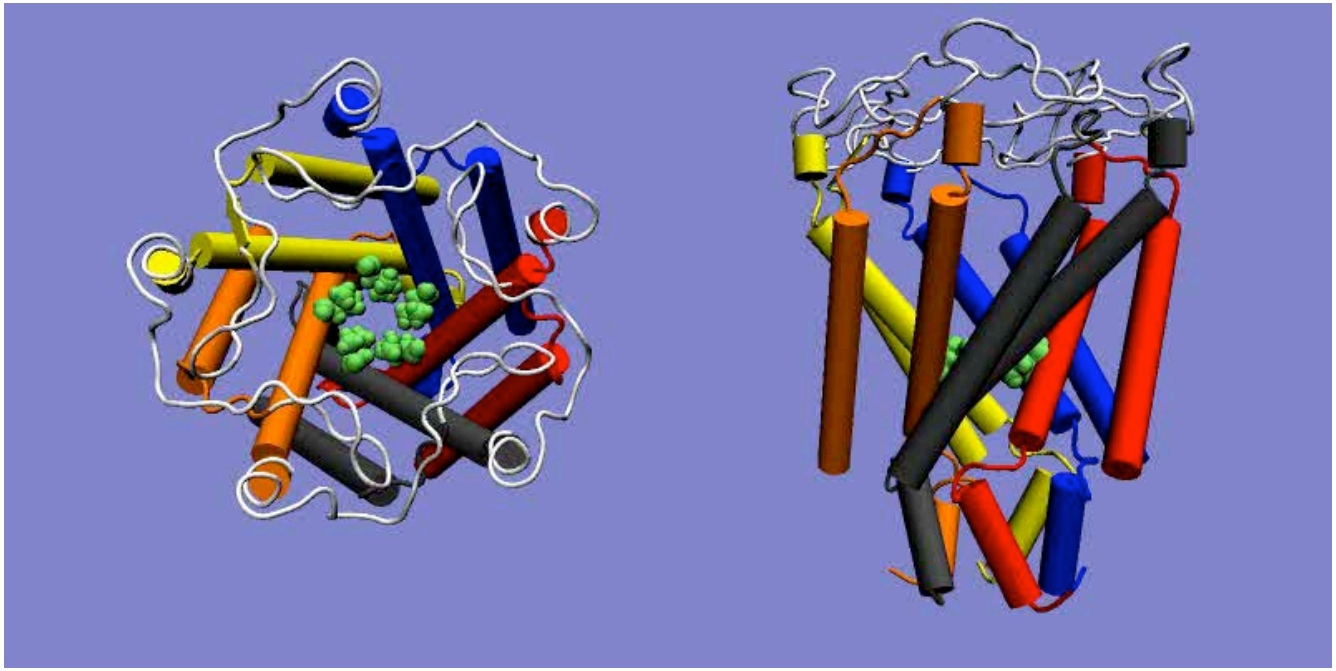
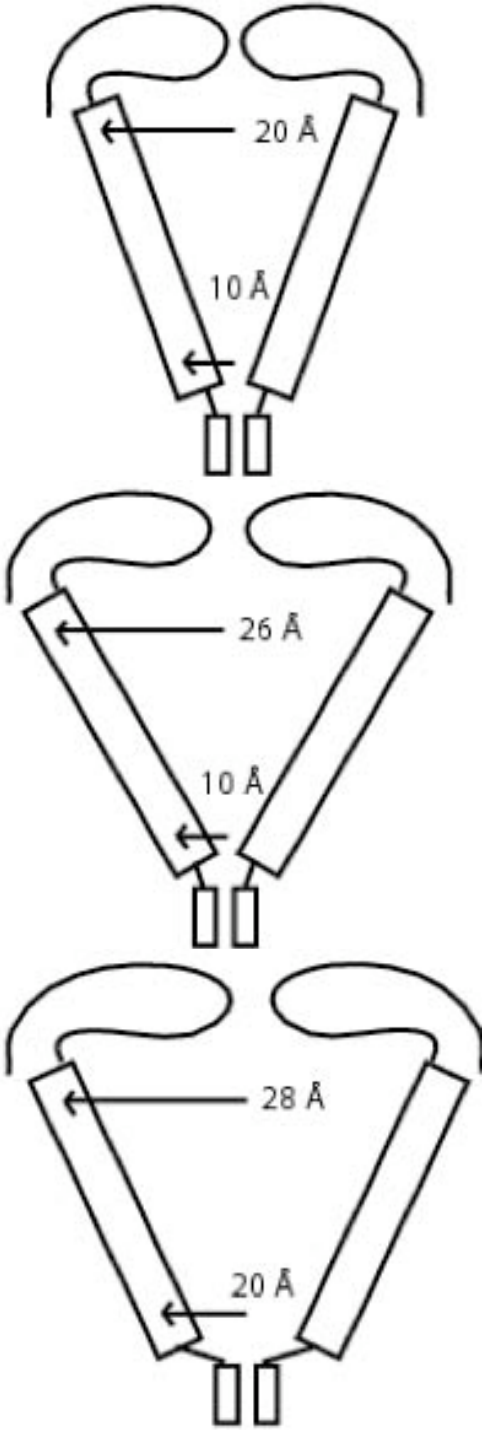
MscL Expanded State





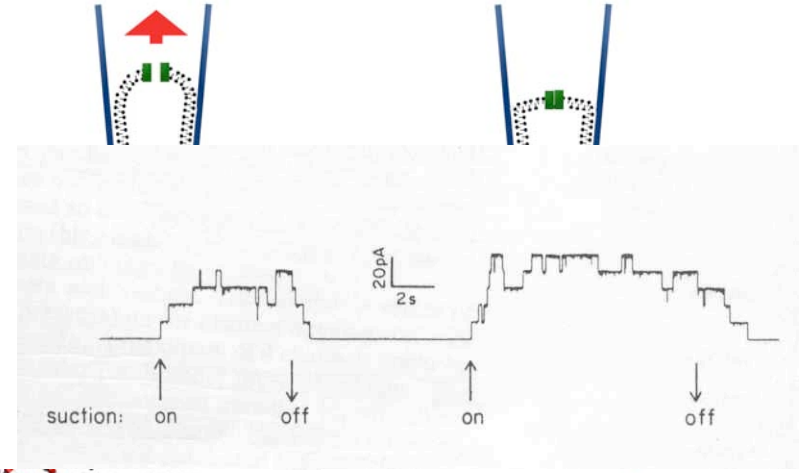
- 0-2 ns: expansion of the periplasmic ends of M1 and M2.
- 2-6 ns: slippage of conserved Ala20 past Ile25 and Phe29.
- 6-10 ns: continued expansion; stretching of linker residues.

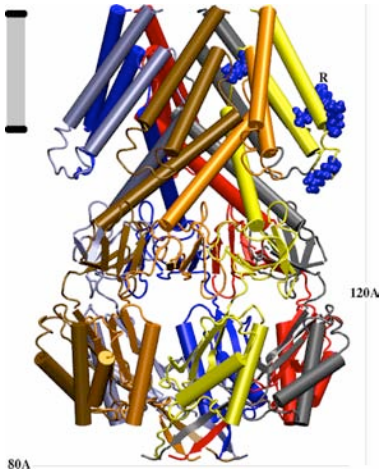
J. Gullingsrud

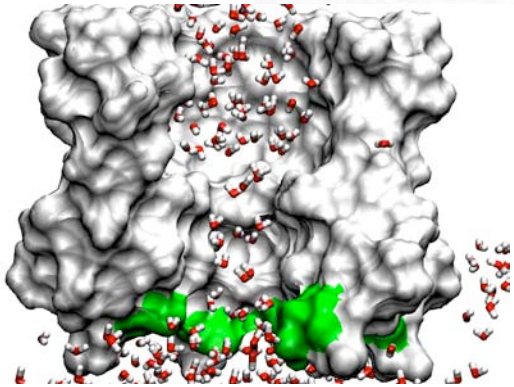
Mechanisensitive Channel of Small Conductance MscS

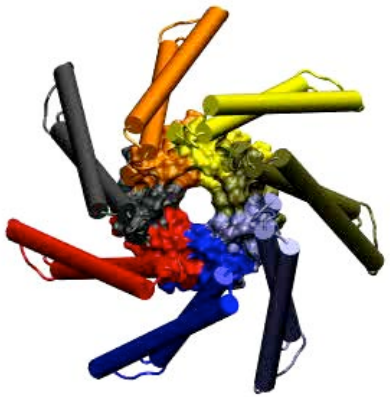
Marcos Sotomayor

- Patch Clamp Experiments (B. Martinac et al 1986)
- Crystal Structure in a putative open state (D.C. Rees et al 2002)
- Simulations: closed state

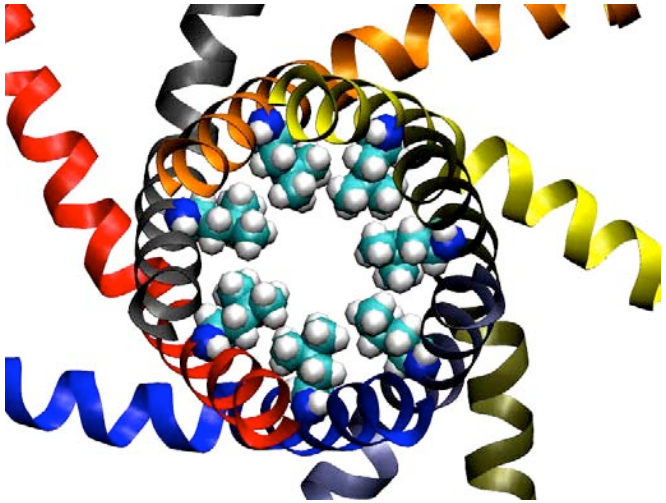


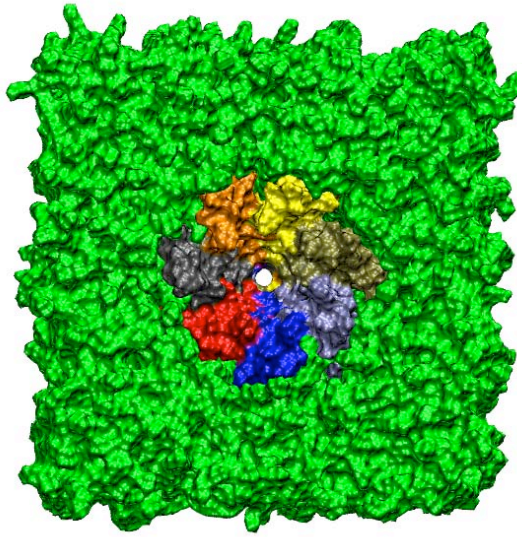


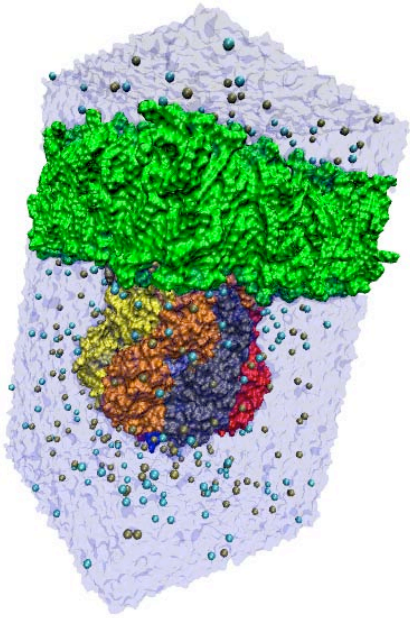




Mechanosensitive Channel of Small Conductance



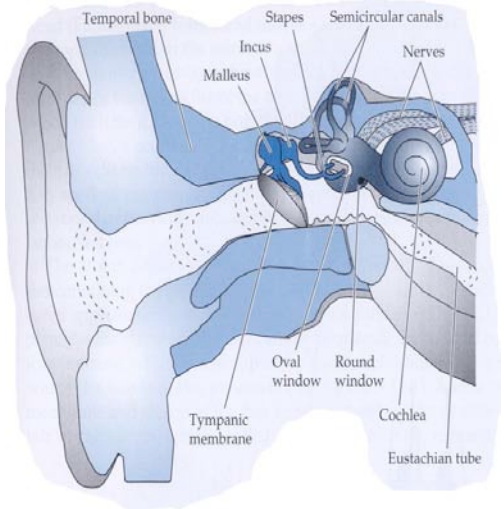




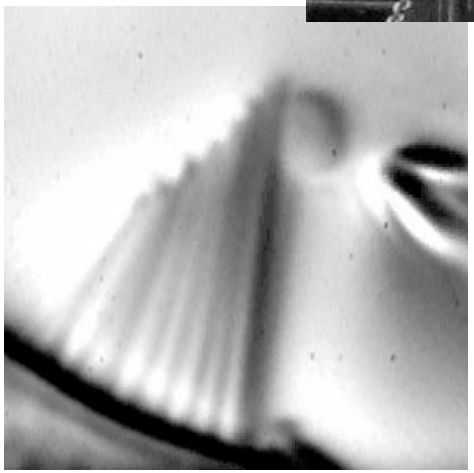
- 225, 000 atoms including explicit water molecules
- CHARMM27 force-fields
- Periodic boundary conditions
- Constant pressure and temperature
- PME for full electrostatic calculation
- Teragrid benchmark: 0.5 day/ns on 128 Itanium 1.5GHz processors

Ankyrin Repeats: Springs in the Inner Ear

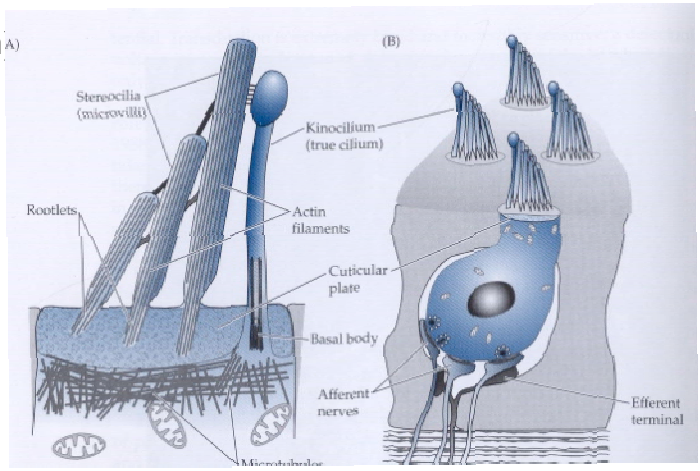
Marcos Sotomayor



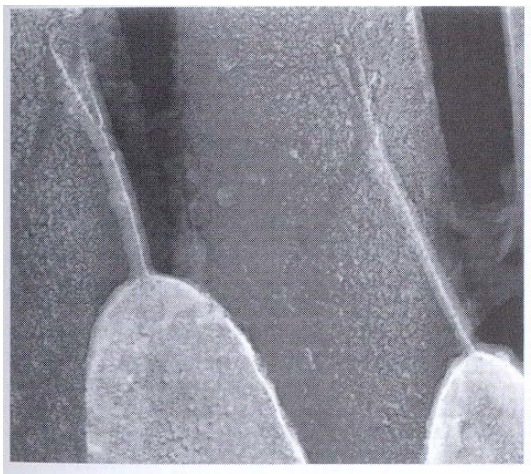
Mammalian Inner Ear (from Sensory Transduction, G. L. Fain)



Hair bundle (D.P. Corey)

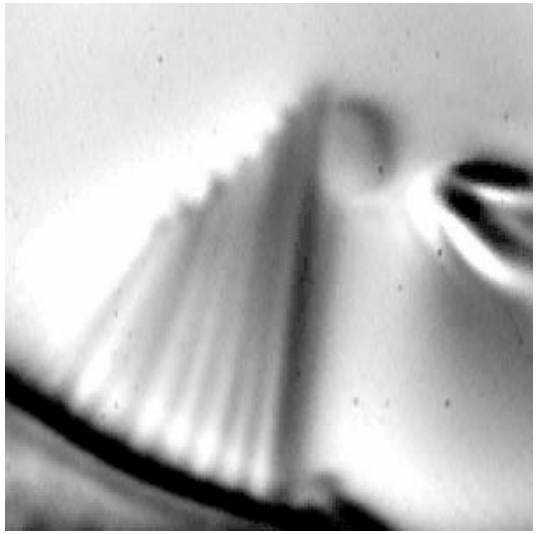


Cuticular plate, stereocilia and kinocilium in hair cells (from Sensory Transduction, G. L. Fain)



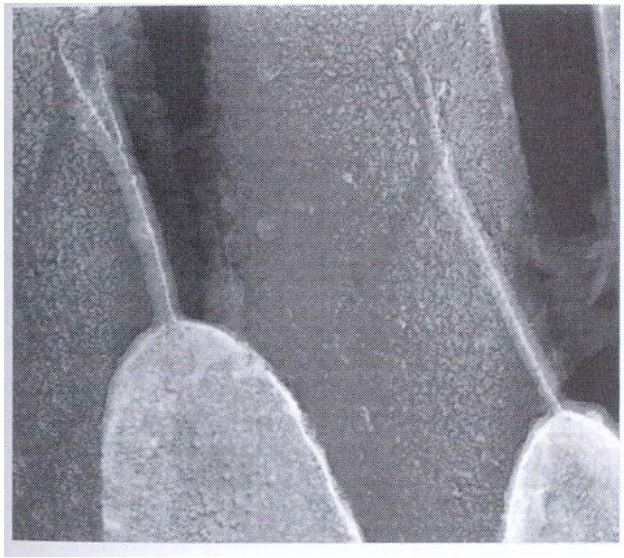
Tip Links (Kachar et al., 2000)

340,000 atom simulation of 24 repeat ankyrin



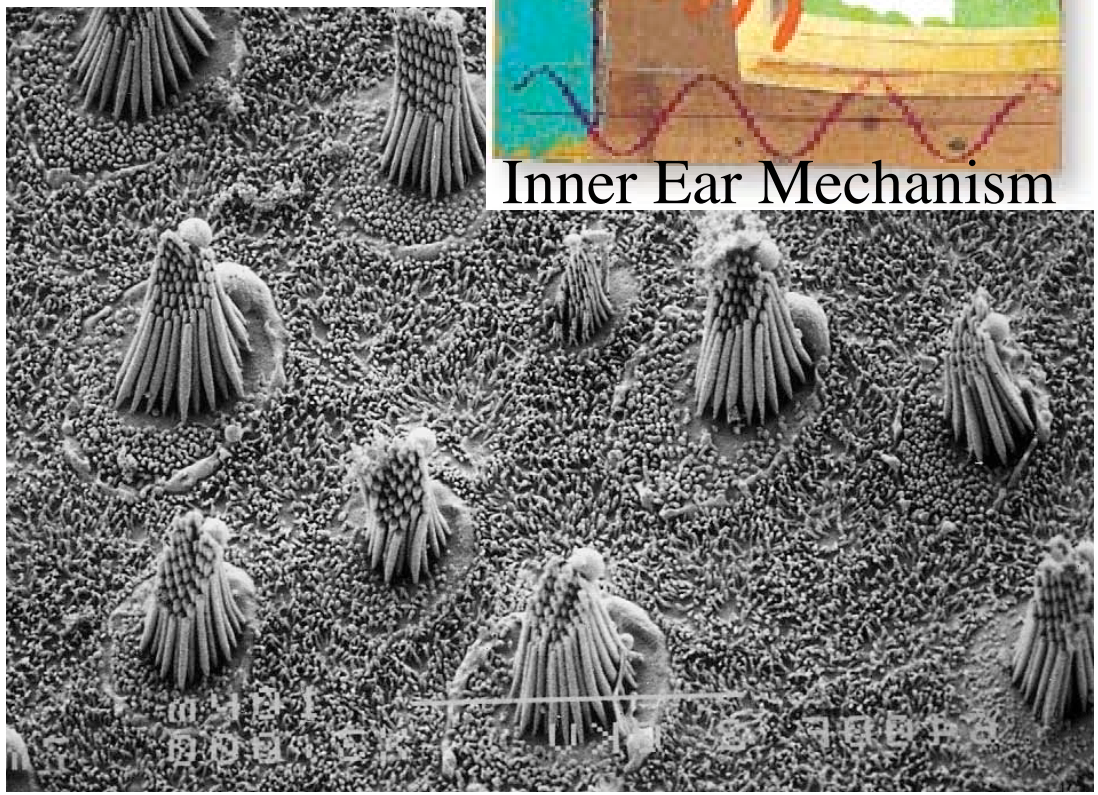
- 340,000 atoms including explicit water molecules
- CHARMM27 force-field
- Periodic boundary conditions
- Steered MD (25-75 pN)
- PME for full electrostatic calculation
- Teragrid benchmark: 0.7 day/ns on 128 Itanium 1.5GHz processors.

NAMD: 128 processors NCSA teragrid



Tip Links (Kachar et al., 2000; Corey Lab)

Hair bundle (Assad and Corey, from Sensory Transduction, G. L. Fain).



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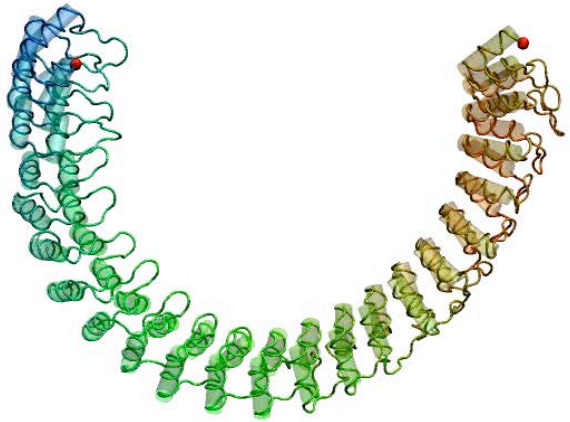
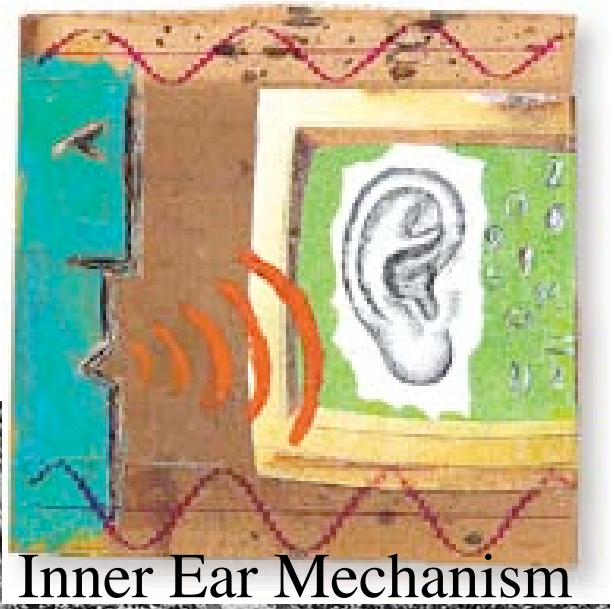
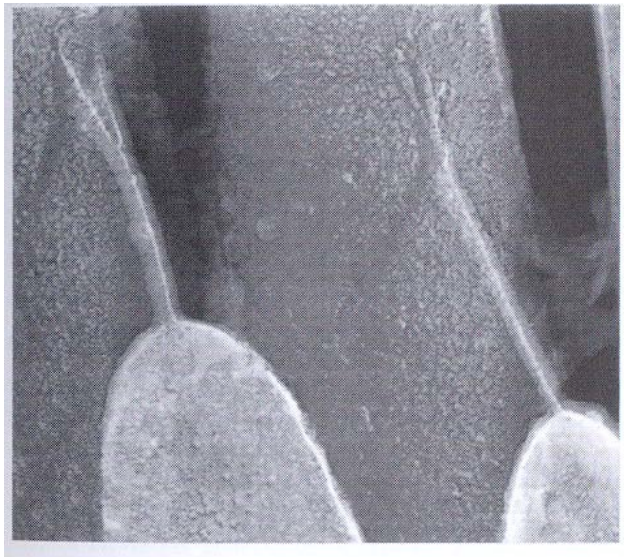


NAMD: 128 processors NCSA teragrid





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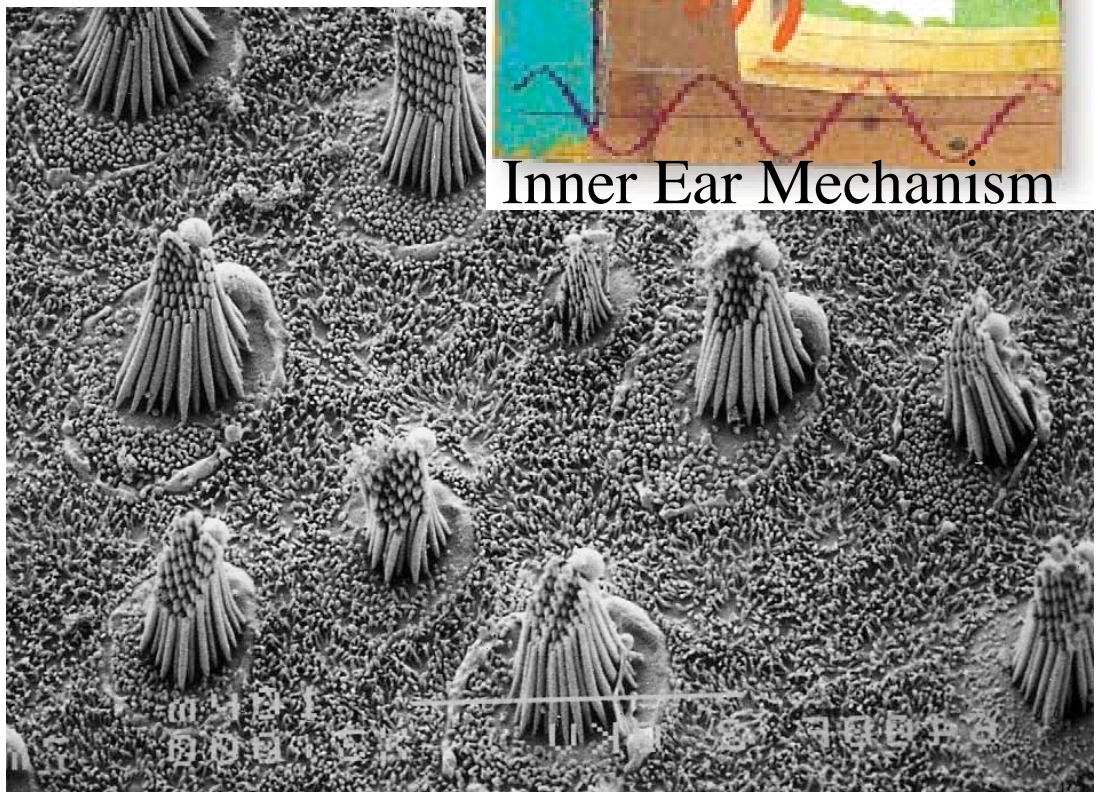
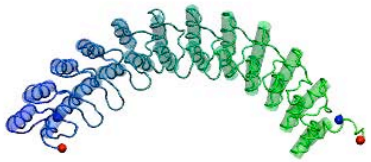
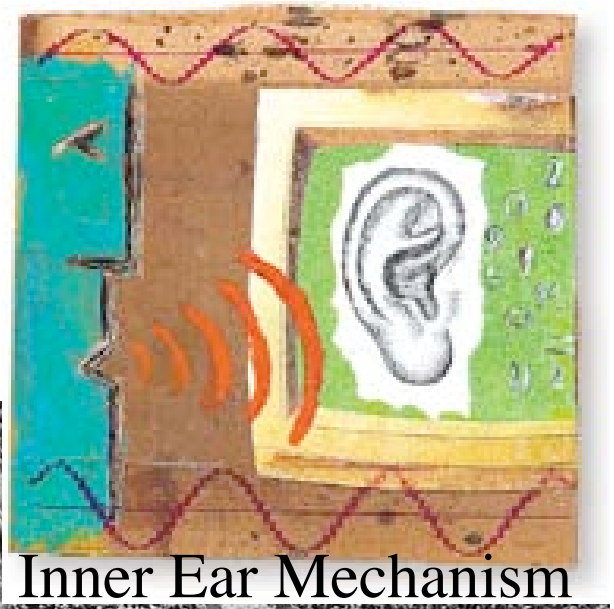


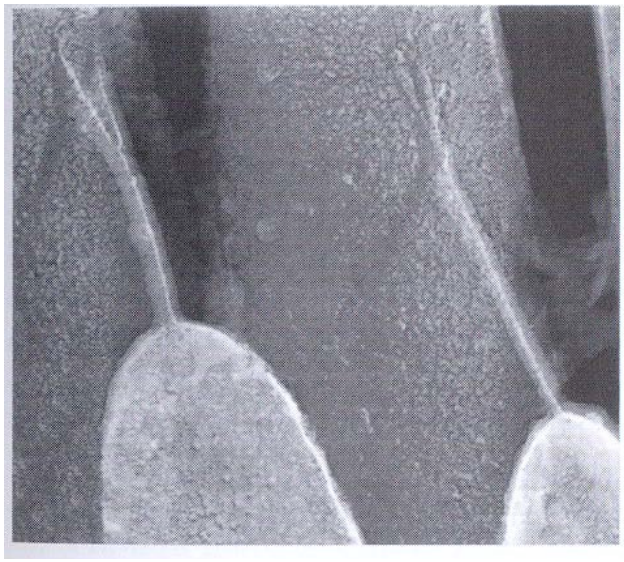
340,000 atom simulation of 24 repeat ankyrin





Non-entropic, nearly indistructable molecular spring

NAMD: 128 processors NCSA teragrid



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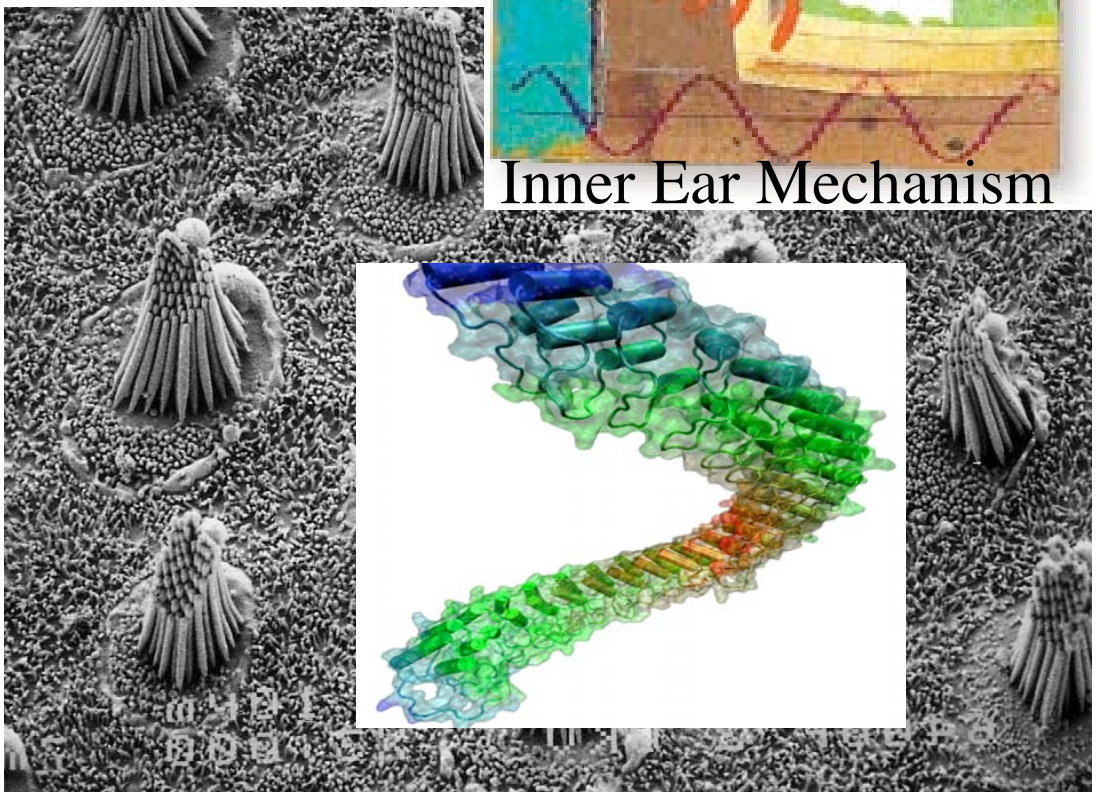
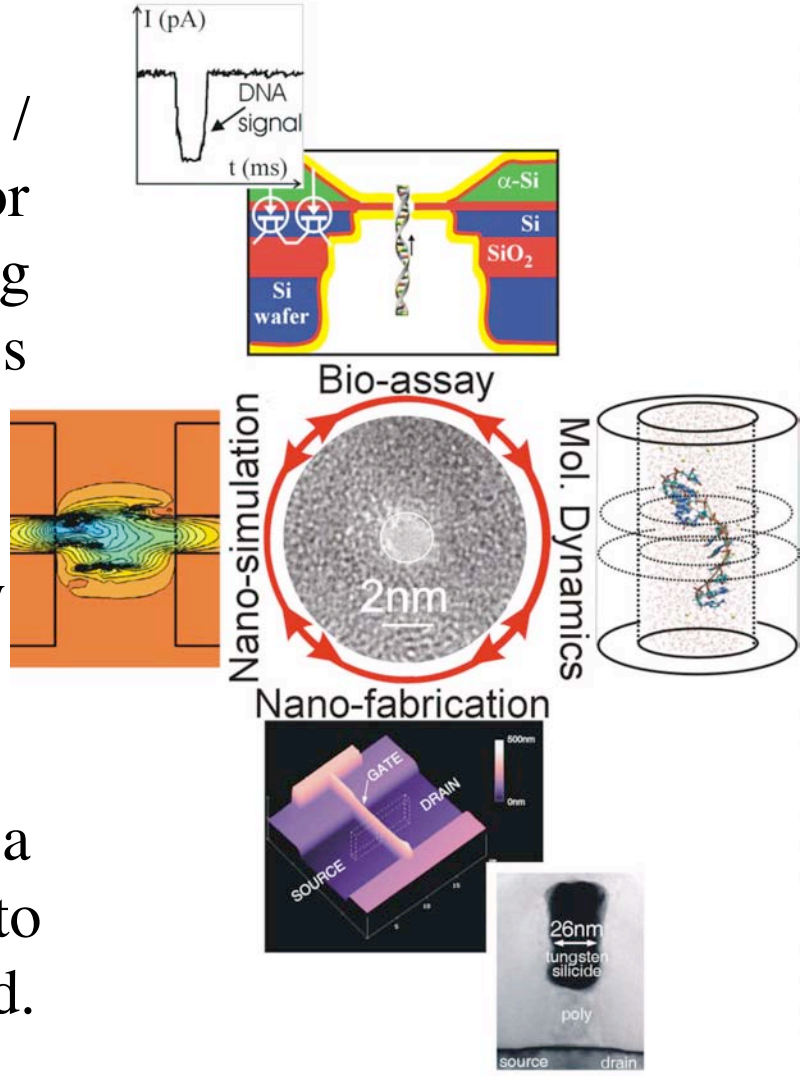
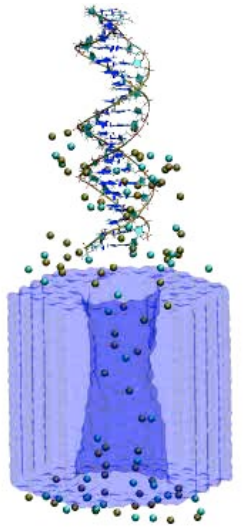
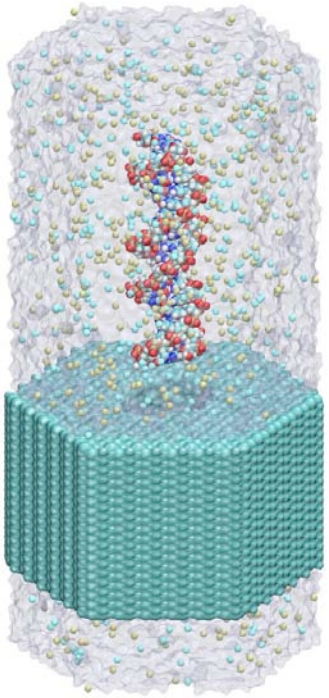


Nano-pore Detection of DNA Sequence

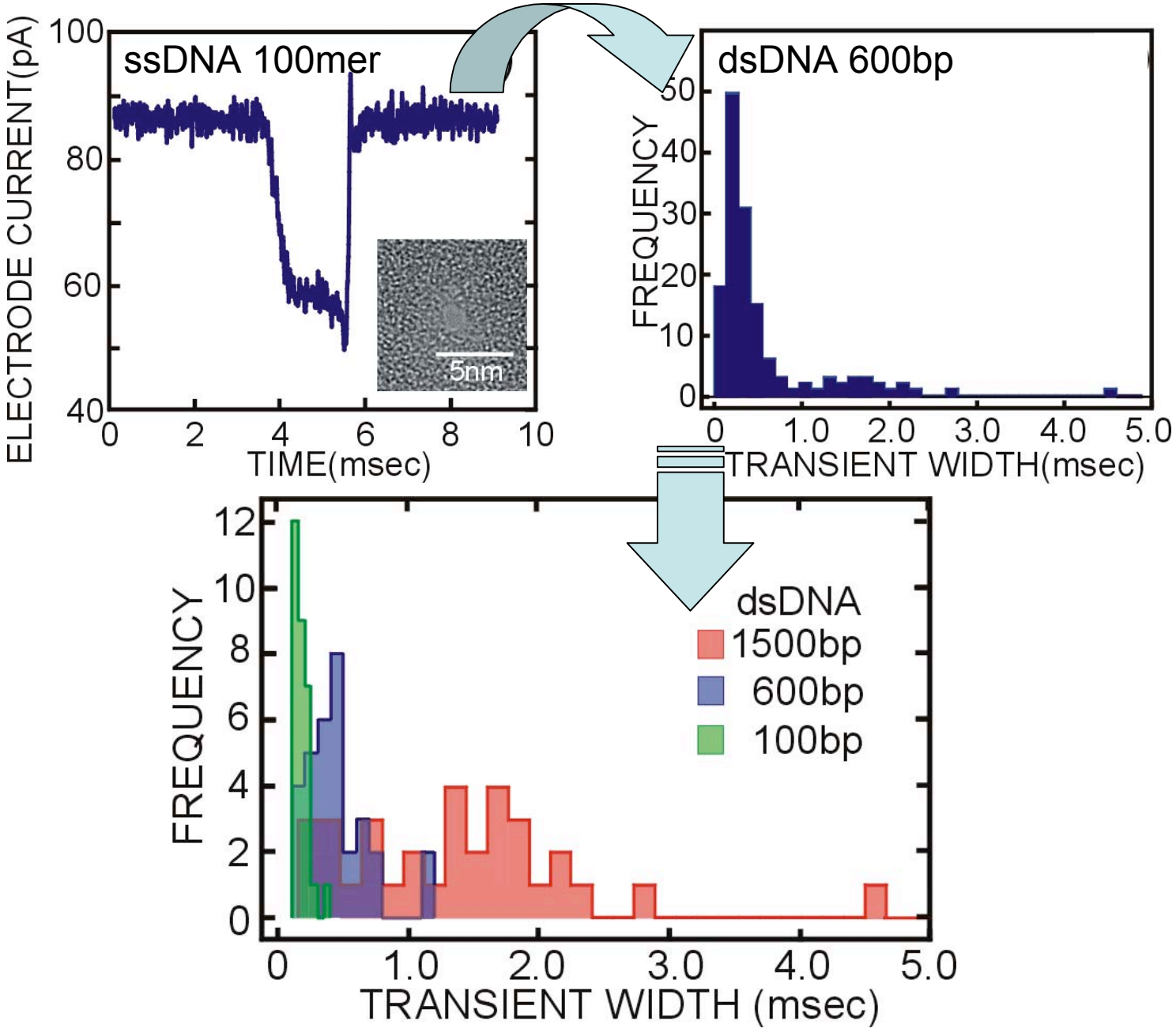
- An integrated nano-pore / nano-transistor device for DNA sequencing is being built in our collaborator's lab (G. Timp, UIUC).
- Our group investigates translocation of DNA by MD simulations.
- The goal is to identify a signature response from a specific DNA sequence to an applied electrical field.

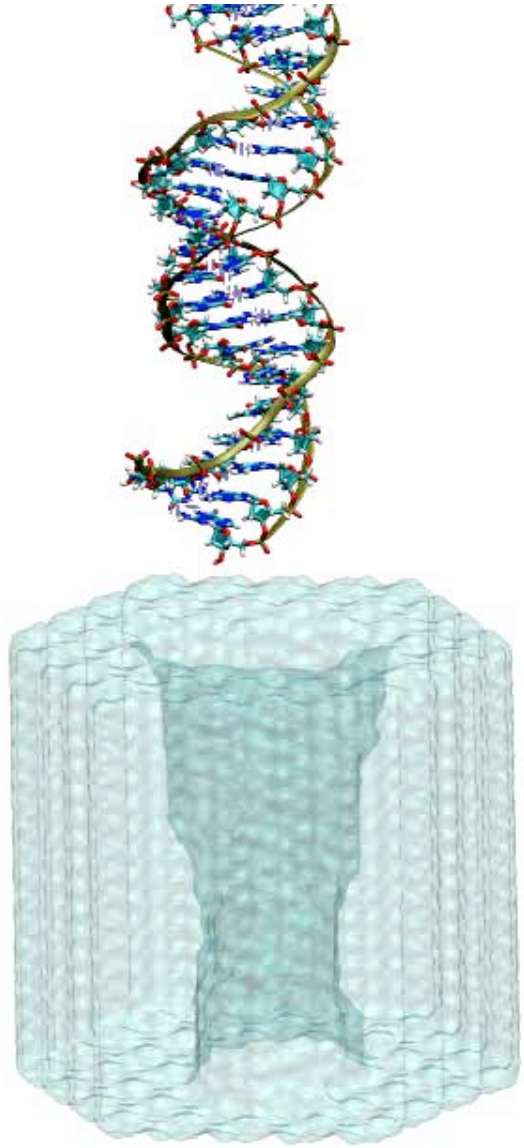






Sizing DNA using a Silicon Nanopore



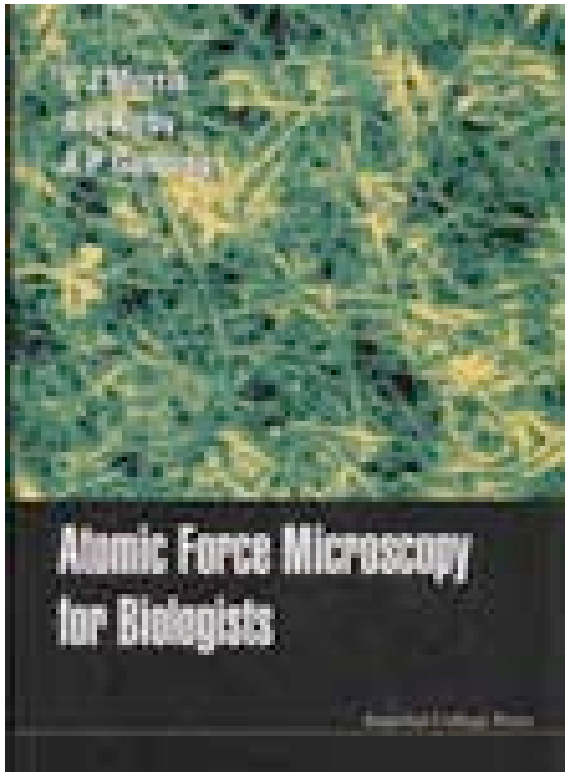


Capturing DNA by strong electrical field

Applied field

Electrical field = 2.8×10^9 V/m (equivalent to by 14 eV over 5nm in vacuum).

Simulation time 4.5 ns



ATOMIC FORCE MICROSCOPY FOR BIOLOGISTS

by V J Morris, A R Kirby & A P Gunning

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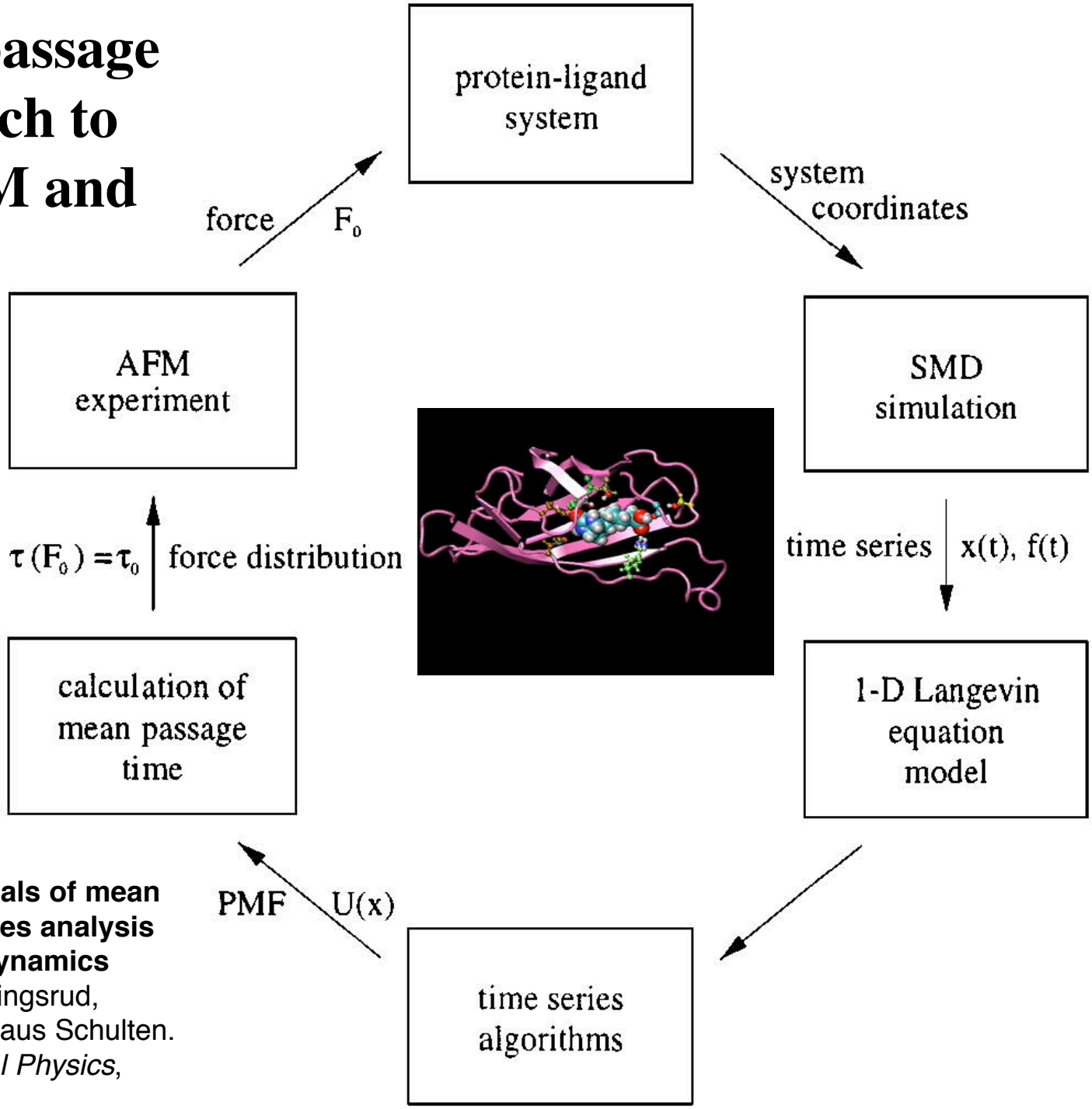
ISBN 1-86094-199-0 US$51 / £32

Contents:

Atomic force microscopy (AFM) is part of a range of emerging microscopic methods for biologists which offer the magnification range of both the light and electron microscope, but allow imaging under the 'natural' conditions usually associated with the light microscope. To biologists AFM offers the prospect of high resolution images of biological material, images of molecules and their interactions even under physiological conditions, and the study of molecular processes in living systems. This book provides a realistic appreciation of the advantages and limitations of the technique and the present and future potential for improving the understanding of biological systems.

Mean first passage time approach to analyze AFM and SMD data



Reconstructing potentials of mean force through time series analysis of steered molecular dynamics simulations. Justin Gullingsrud, Rosemary Braun, and Klaus Schulten. *Journal of Computational Physics*, 151:190-211, 1999.