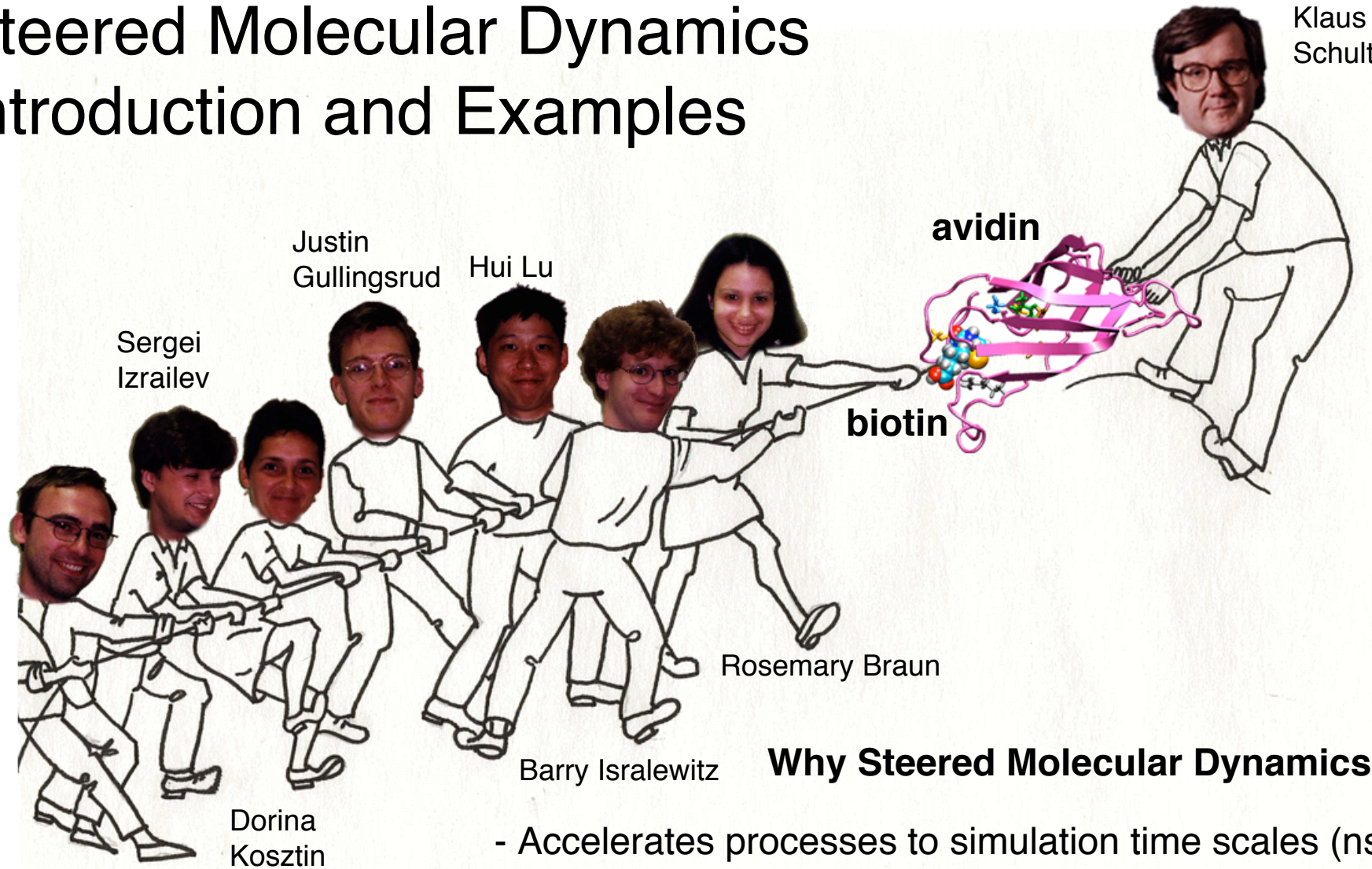
Steered Molecular Dynamics Introduction and Examples



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

Forces

can be

substrates,

products,

signals,

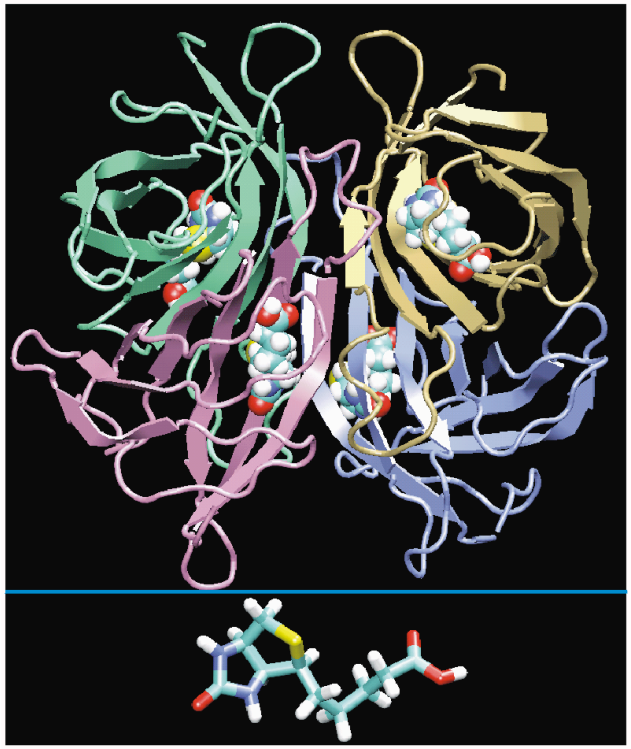
catalysts

of cellular processes

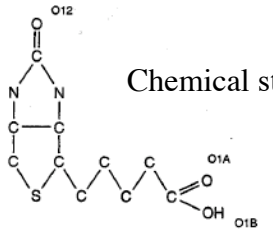
But to what degree can proteins and DNA sustain forces?

How do proteins need to be designed to build machines from them?

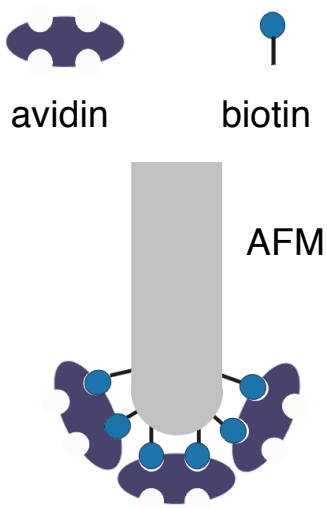
Atomic Force Microscopy Experiments of Ligand Unbinding

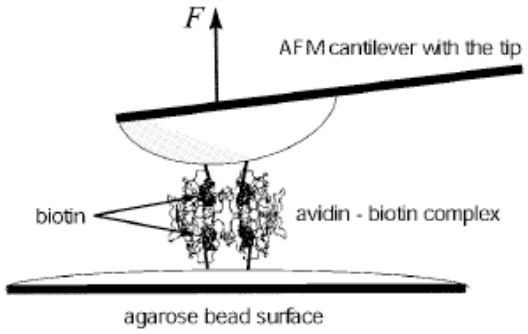


Biotin

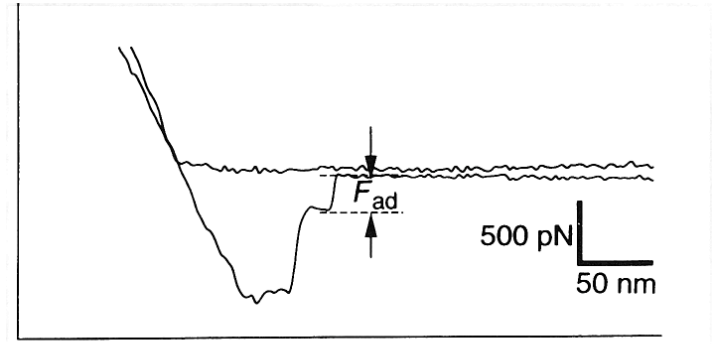


Chemical structure of biotin

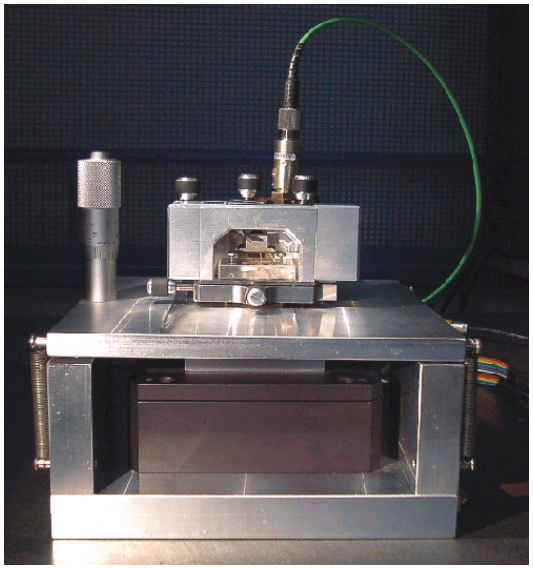




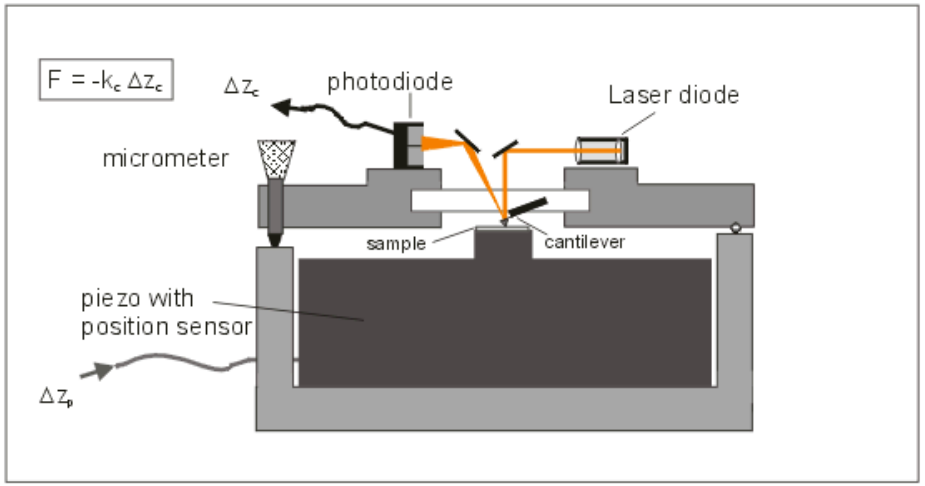
Florin et al., Science 264:415 (1994)

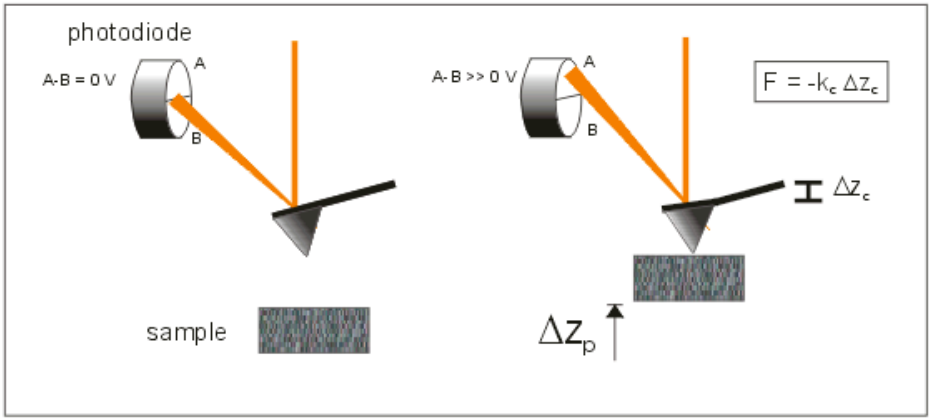


Displacement of AFM tip

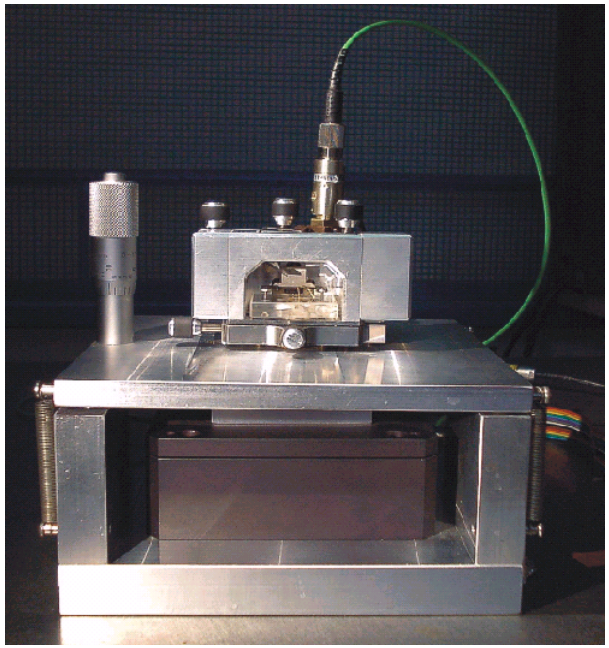


Atomic Force Microscope



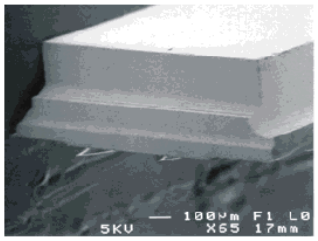


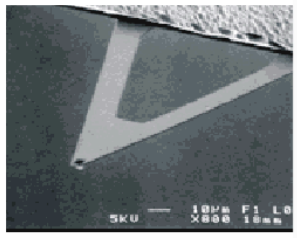
Instrument

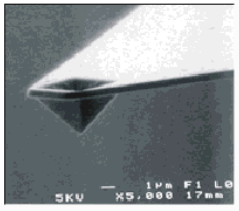


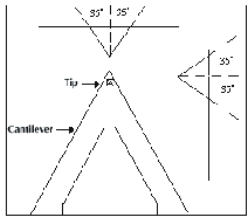
15 cm

AFM cantilevers and tips









Atomic Force Microscopy Experiments of Ligand Unbinding

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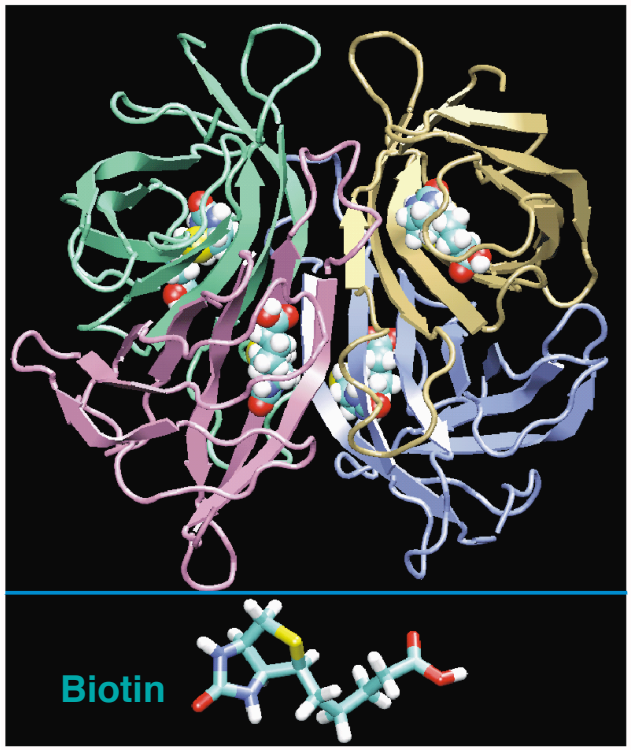
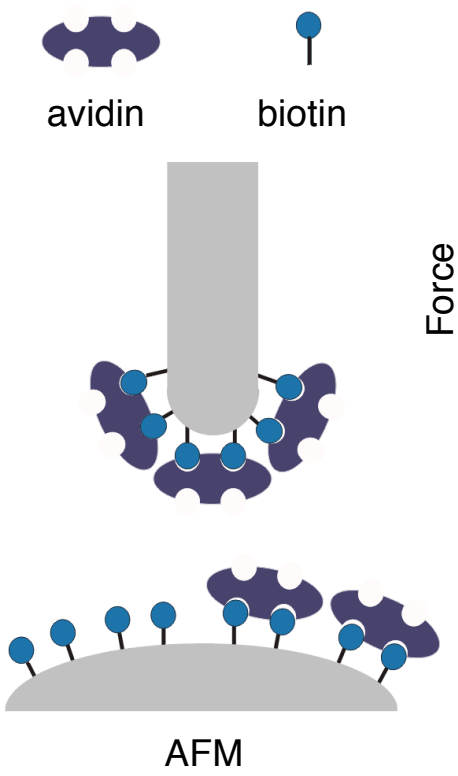
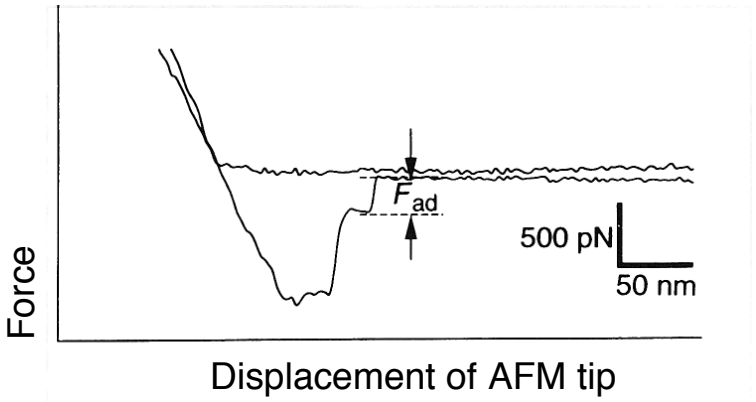
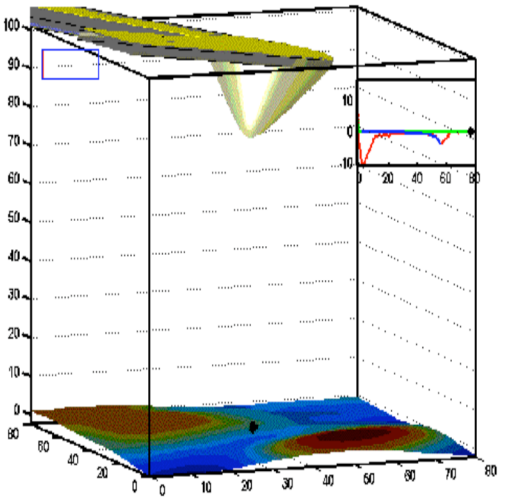
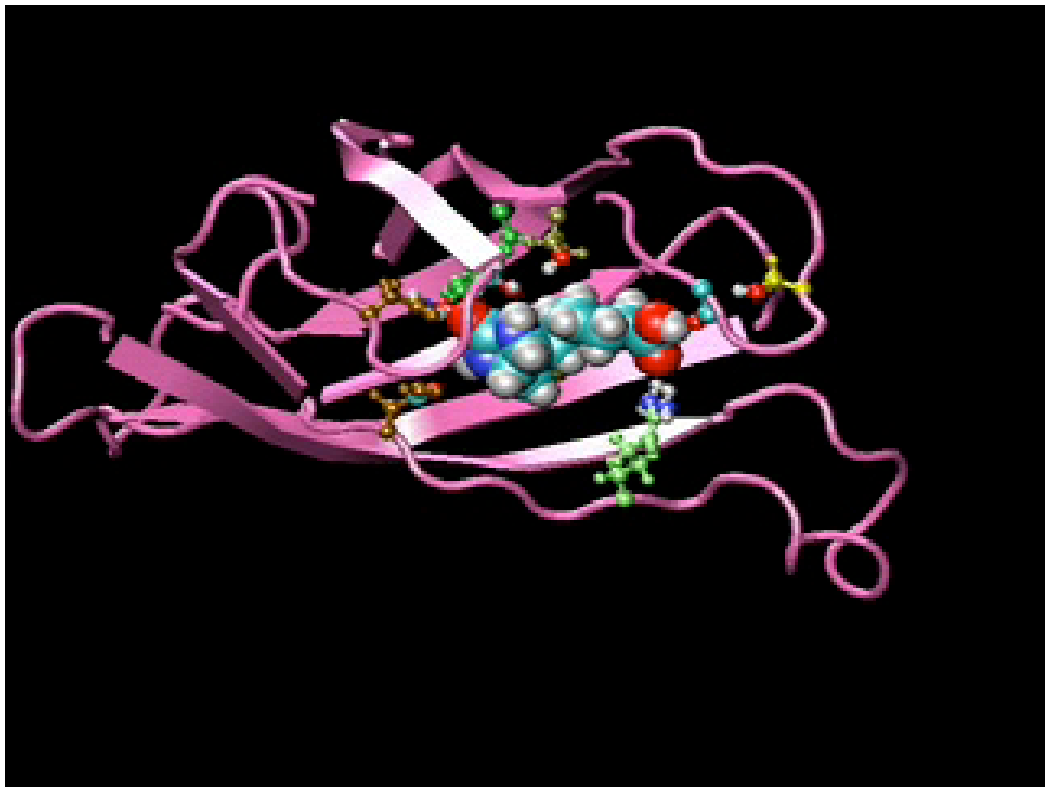








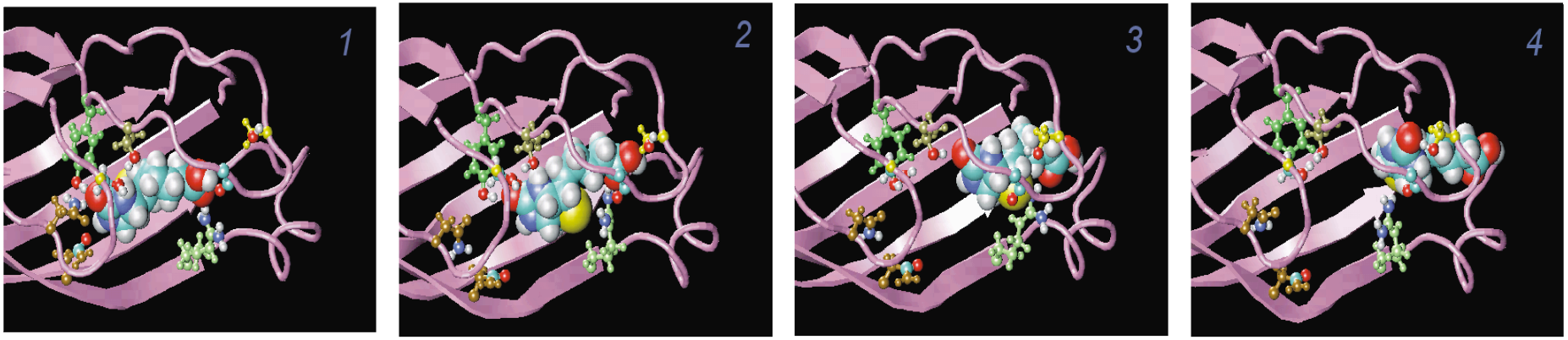
Pulling Biotin out of Avidin

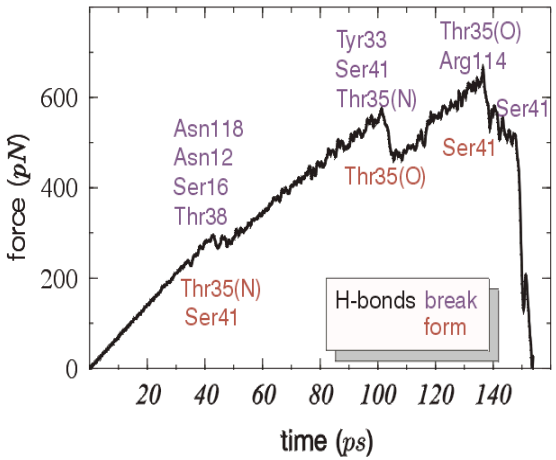


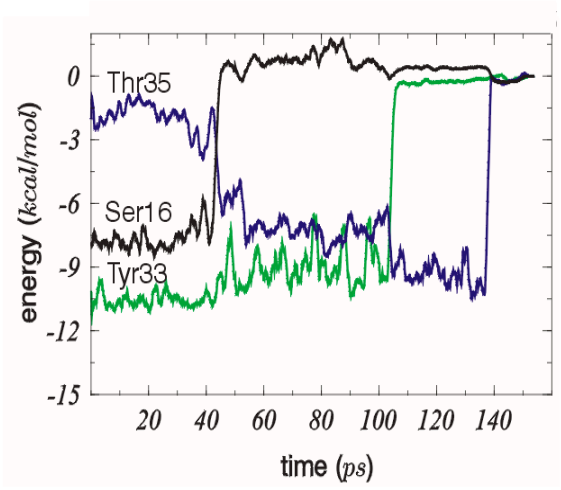
Molecular dynamics study of unbinding of the avidin-biotin complex. Sergei Izrailev, Sergey Stepaniants, Manel Balsera, Yoshi Oono, and Klaus Schulten. *Biophysical Journal*, 72:1568-1581, 1997.

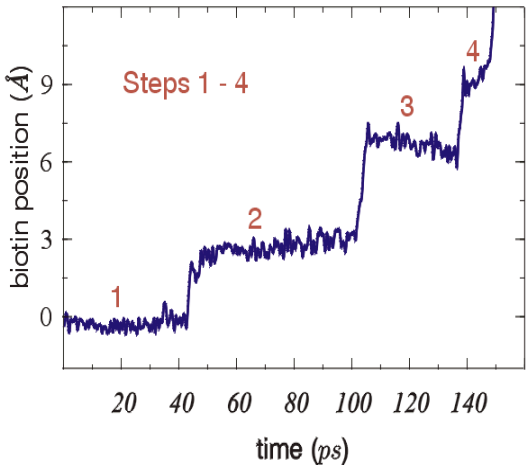
SMD of Biotin Unbinding: What We Learned

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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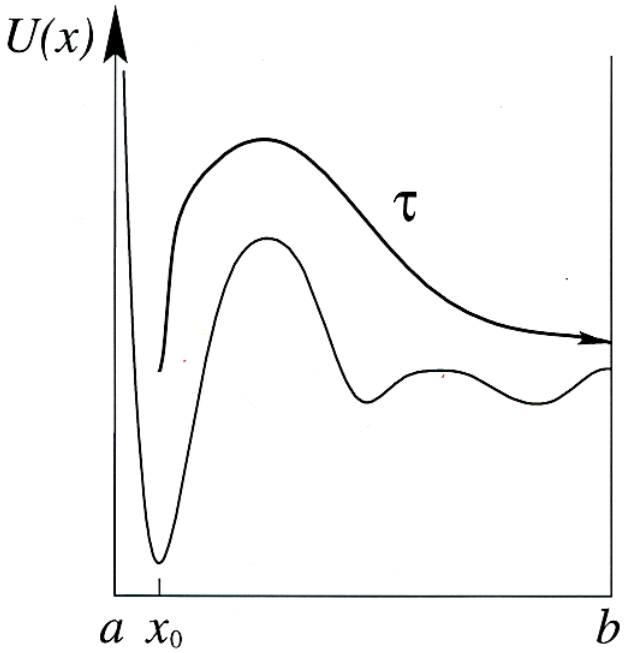
Theory of First Passage Times



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

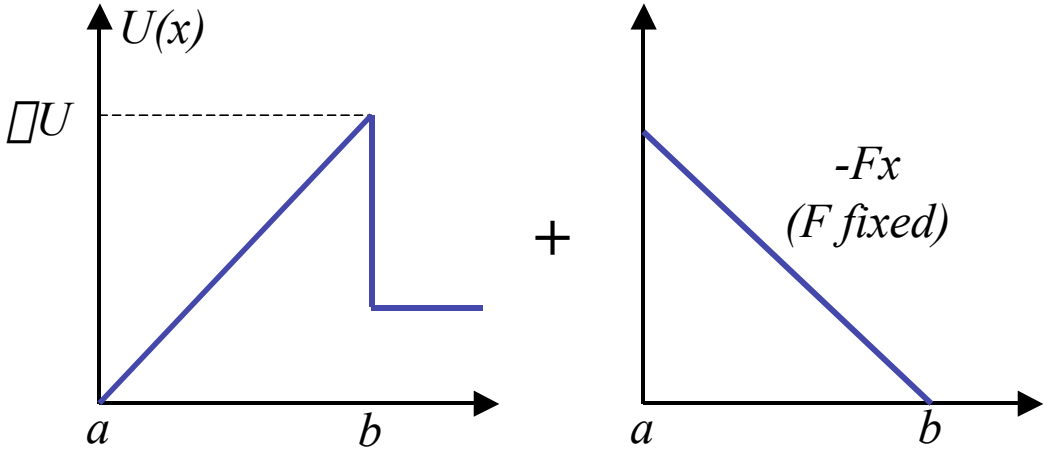
$$\partial_t p(x,t) = \partial_x D\, e^{-\beta U(x)}\, \partial_x e^{\beta U(x)} p(x,t)$$

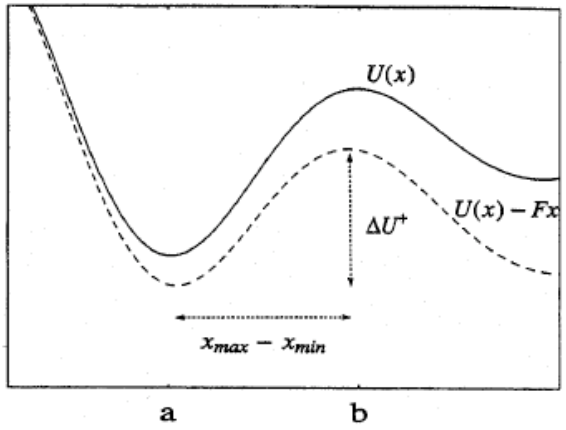
- First passage time:

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

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

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

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

(for biotin-avidin)

$$\tau(F) = \tau\,[\Delta U - F(b-a)]$$

AFM regime

$$e^{\Delta U(F)} >> 1$$

$$\tau_{AFM} \sim 2\tau_D\tau^2(F)e^{\Delta U(F)}$$

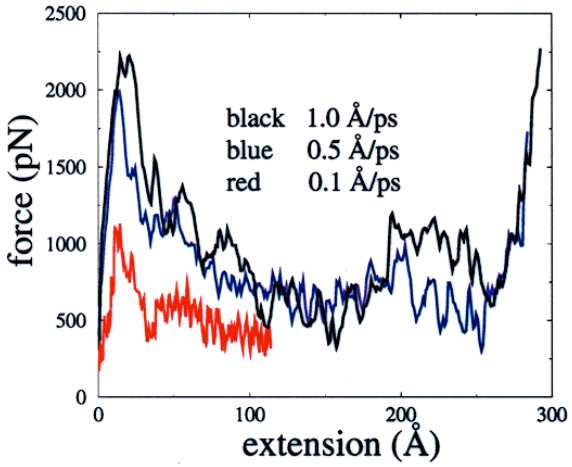
SMD regime

$$e^{\Delta U(F)} << 1$$

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

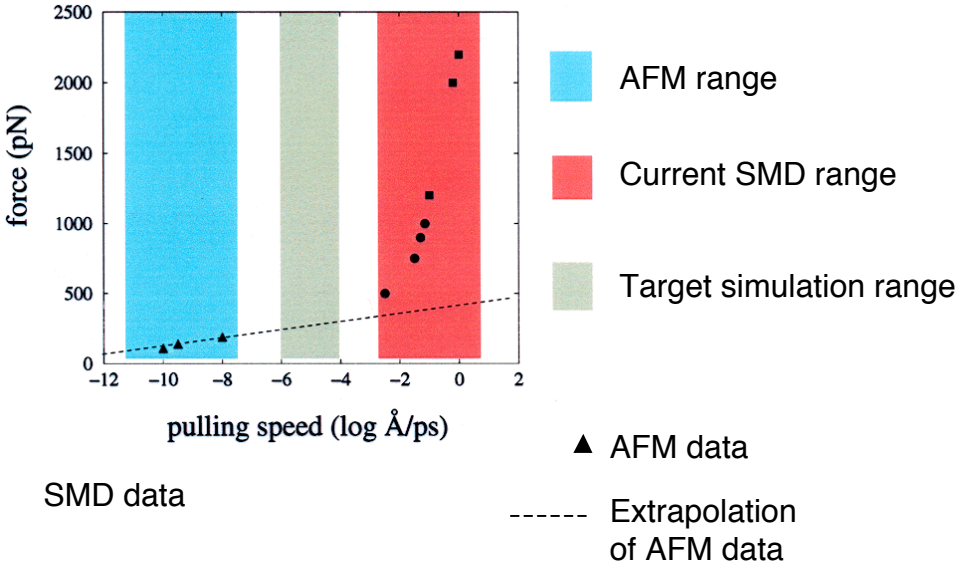
Quantitative Comparison

Force-extension curve



Bridging the gap between SMD and AFM experiments

Force-pulling velocity relationship



AFM regime

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

$$\Delta_{AFM} \sim 2\Delta_D\Gamma^2(F)e^{\Delta(F)}$$

SMD regime

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

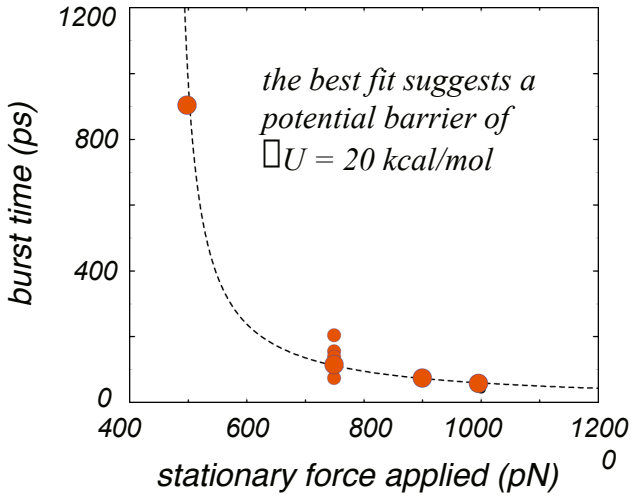
$$\Delta_{SMD} \sim 2\Delta_D|\Delta(F)|^{-1}$$

Rupture/Unfolding Force F_0 and its Distribution

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

=> F_0 rupture/unfolding force

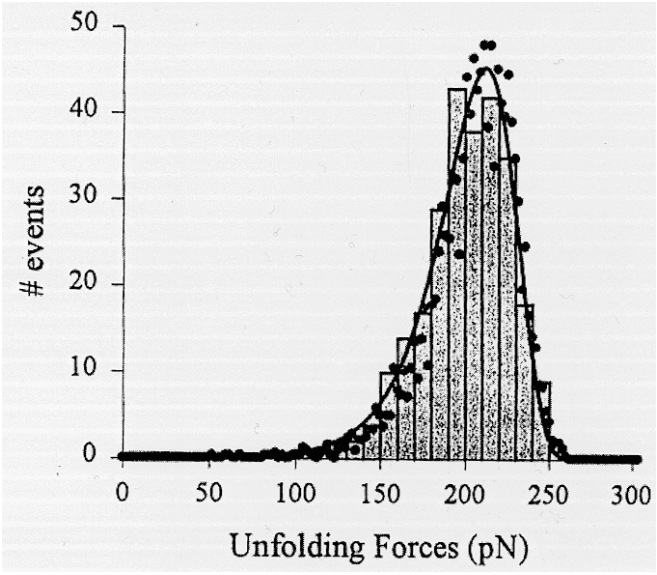
determination of barrier height based on mean first passage time



Distribution of rupture/unfolding force

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

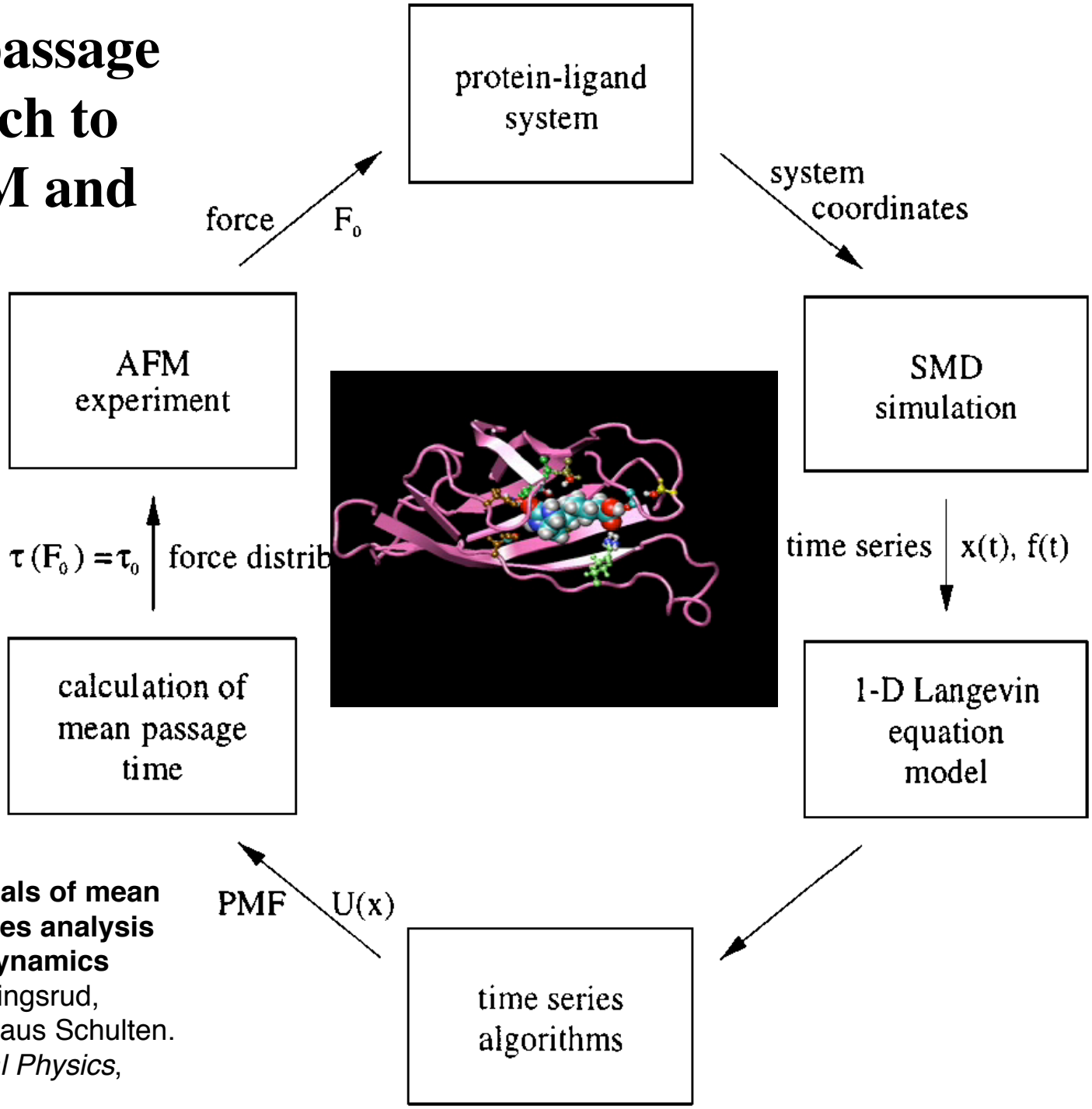
$$\tau = \tau^2(F)/2\tau_b kv$$



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

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

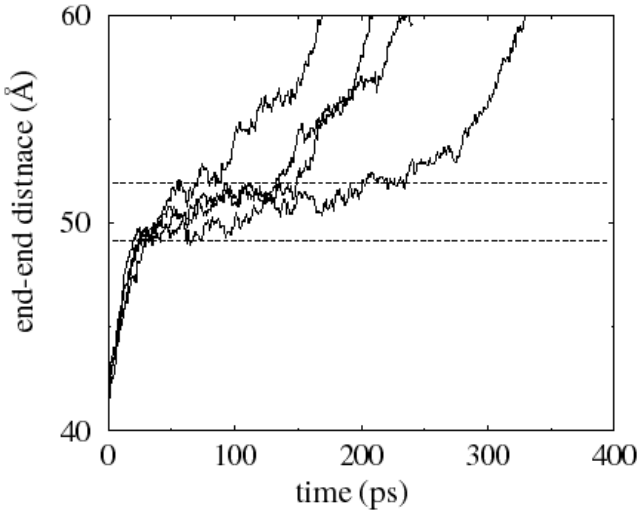
Mean first passage time approach to analyze AFM and SMD data



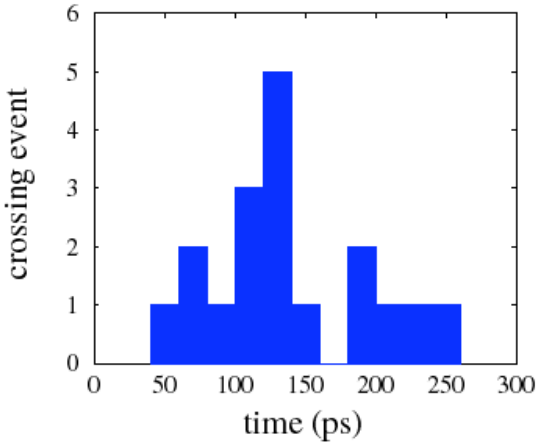
Reconstructing potentials of mean force through time series analysis of steered molecular dynamics simulations. Justin Gullingsrud, Rosemary Braun, and Klaus Schulten. *Journal of Computational Physics*, 151:190-211, 1999.

Distribution of the Barrier Crossing Time

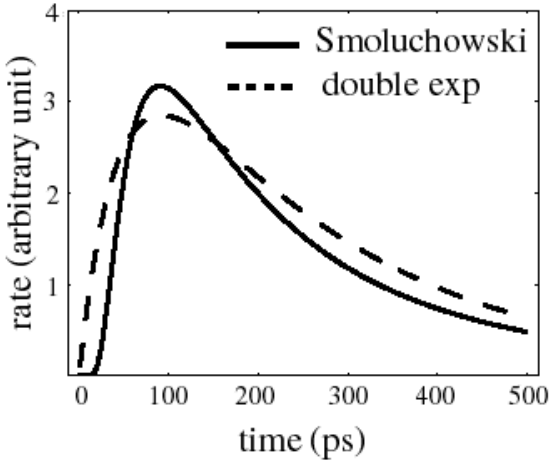
Multiple runs with same force of 750 pN



Barrier crossing times of 18 SMD simulations



Theoretical prediction of the barrier crossing times



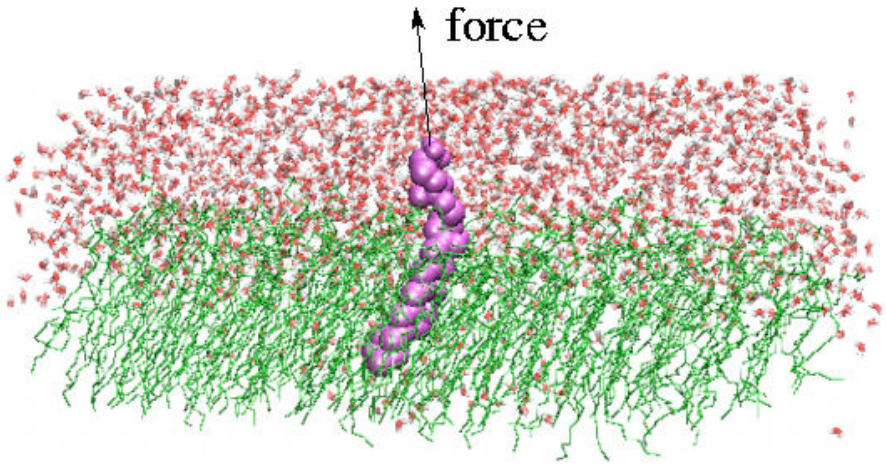
The fraction N(t) that has not crossed the barrier can be expressed through solving the Smoluchowski diffusion equation (linear model potential):

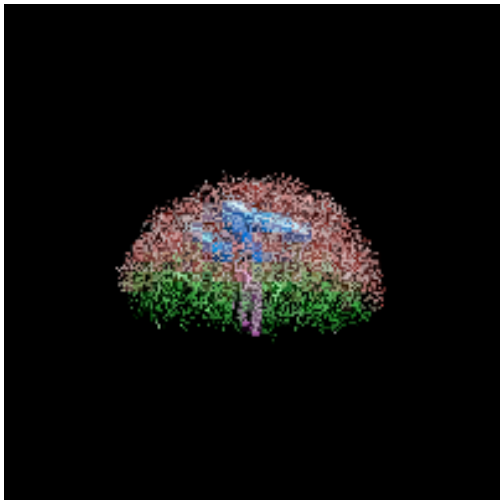
$$N(t) = \frac{1}{2} erfc\left[\frac{-a + \delta(F) D t/(b-a)}{\sqrt{4Dt}}\right] - \frac{1}{2} exp\left[\frac{\delta(F) a}{b-a}\right] erfc\left[\frac{-a + \delta(F) D t/(b-a)}{\sqrt{4Dt}}\right]$$

Or approximated by double exponential (general potential):

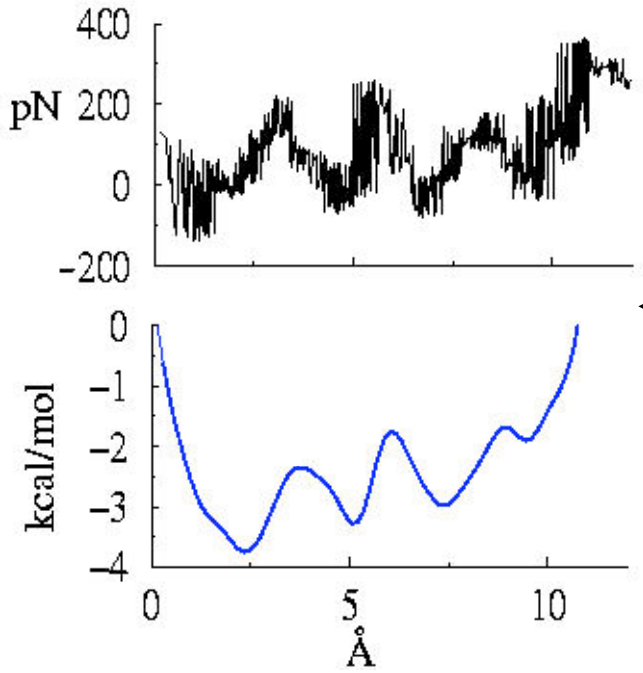
$N(t) = [t_1 \exp(-t/t_1) - t_2 \exp(-t/t_2)]/(t_1-t_2)$, Nadler & Schulten, JCP., **82**, 151-160 (1985)

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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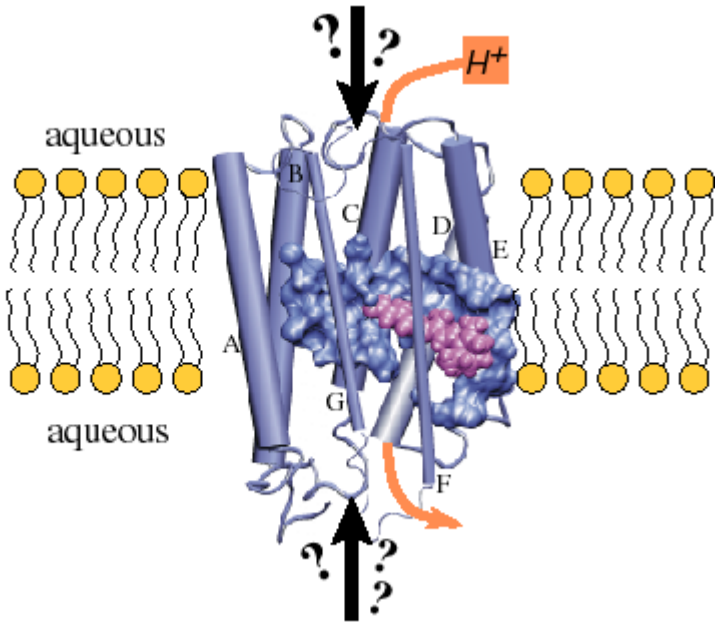
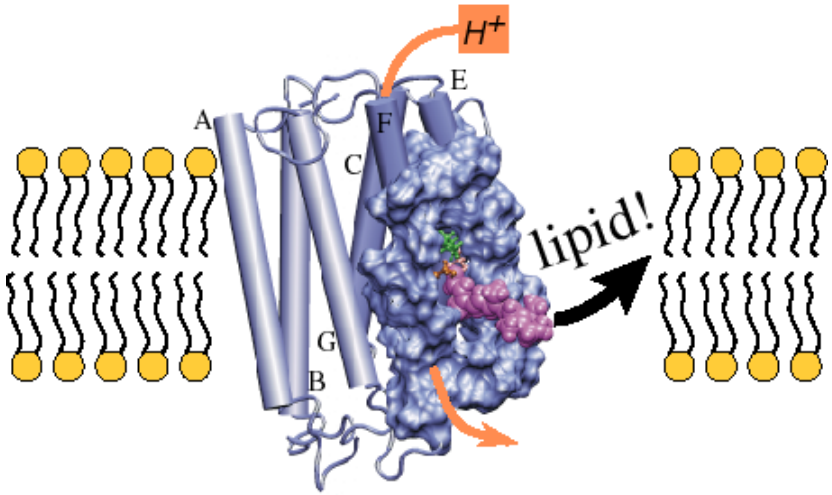
Interactive Modeling

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



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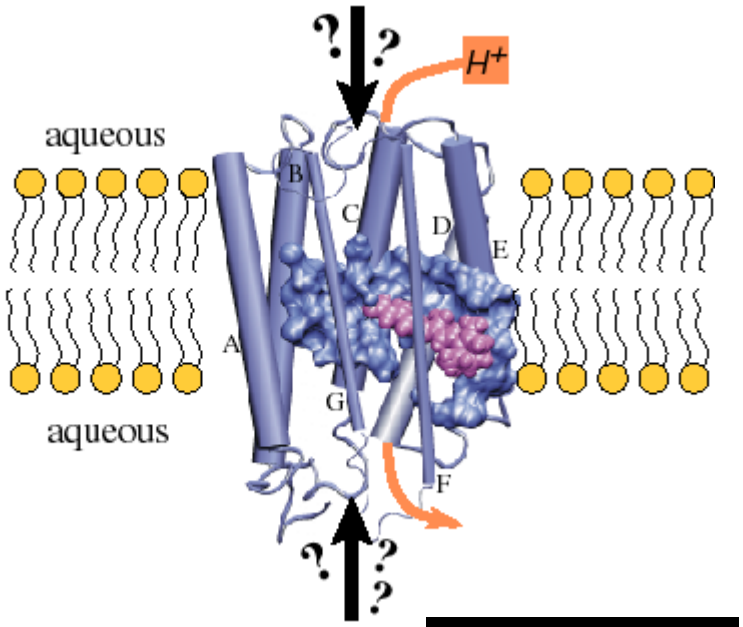
Interactive Modeling

Binding path of retinal to bacterio-opsin



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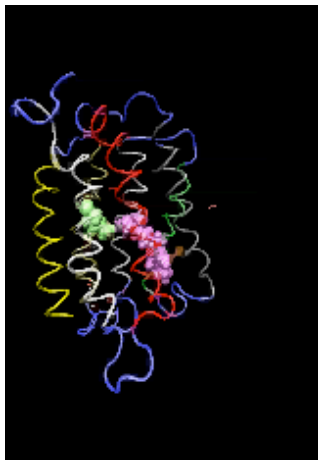
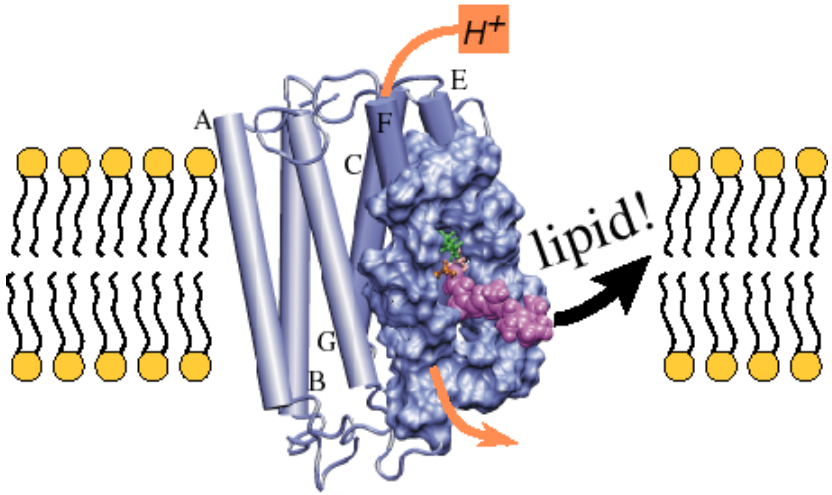
Binding pathway of retinal to bacterio-opsin: A prediction by molecular dynamics simulations. Barry Isralewitz, Sergei Izrailev, and Klaus Schulten. *Biophysical Journal* , 73:2972-2979, 1997.





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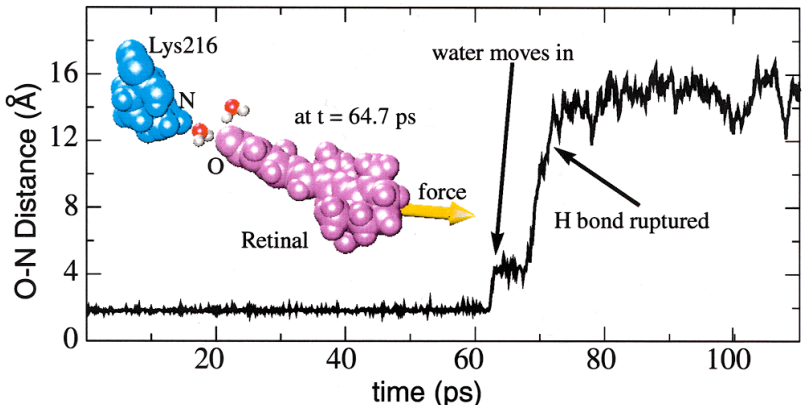
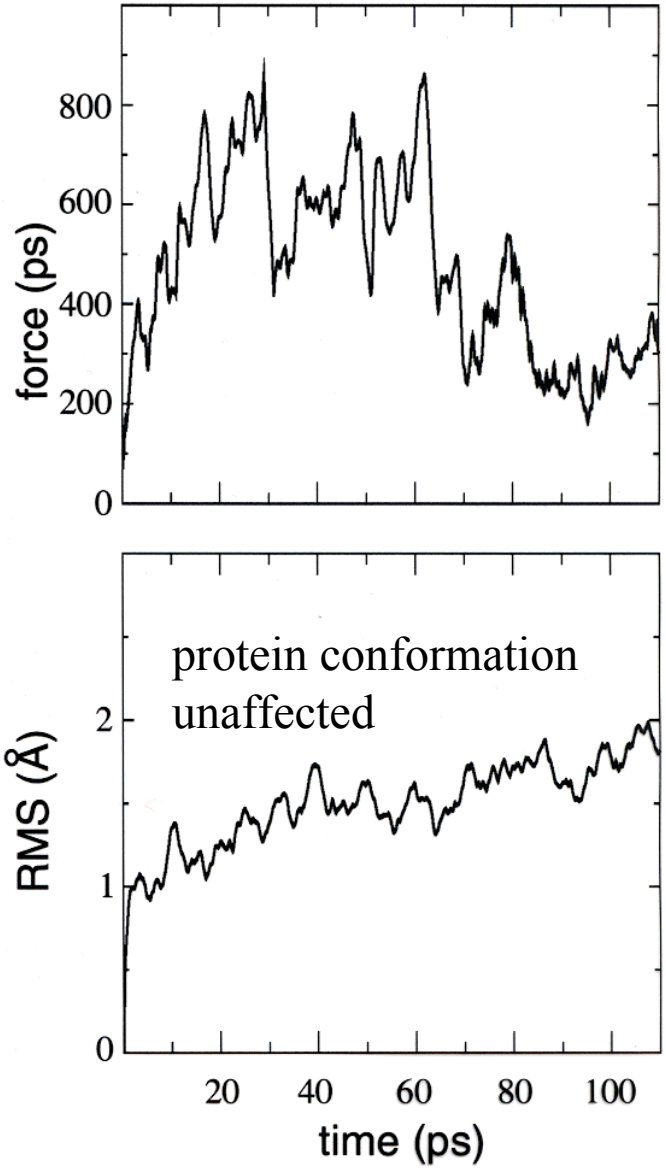
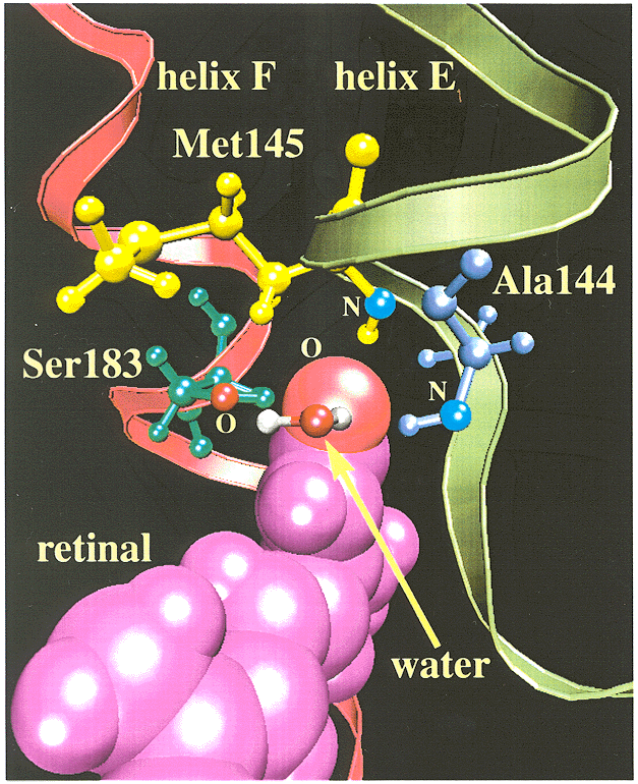
Stepwise Unbinding of Retinal from bR



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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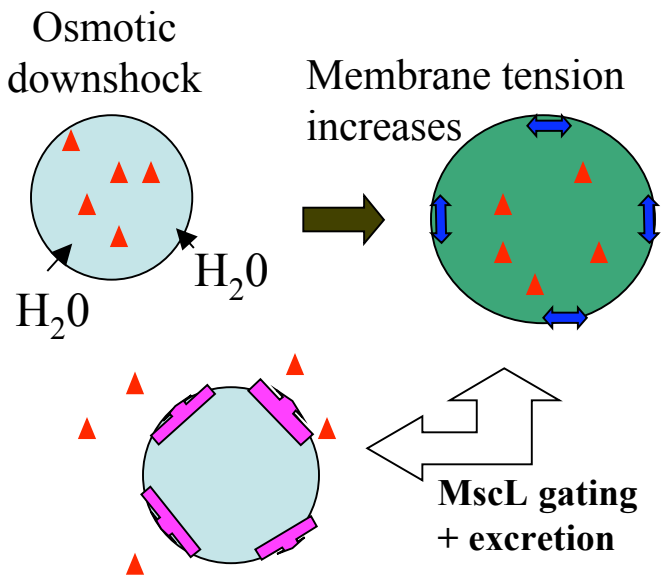
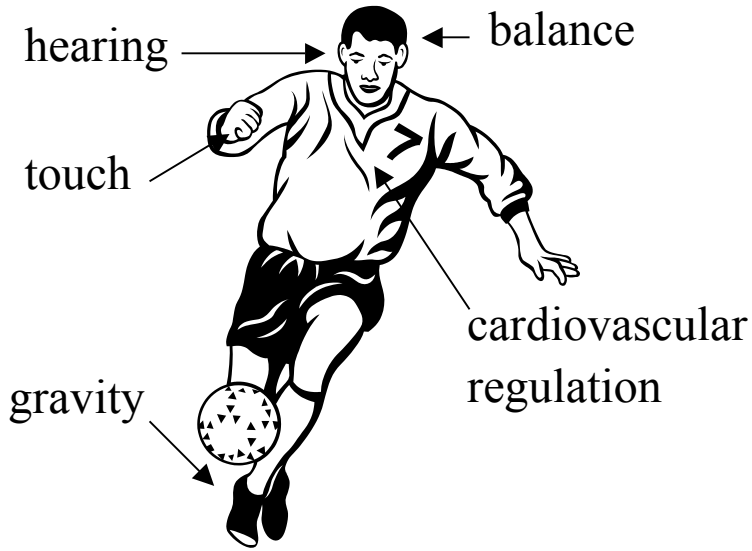
Ubiquitous Mechanosensitive Channels

MscL is a bacterial safety valve



Bacterial MscL is functional in reconstituted lipid bilayers (Sukharev et al., 1994).

Roles in Higher Organisms

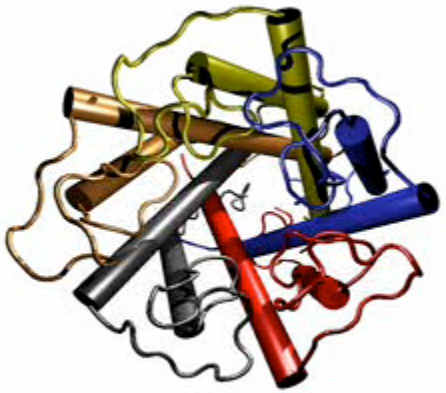


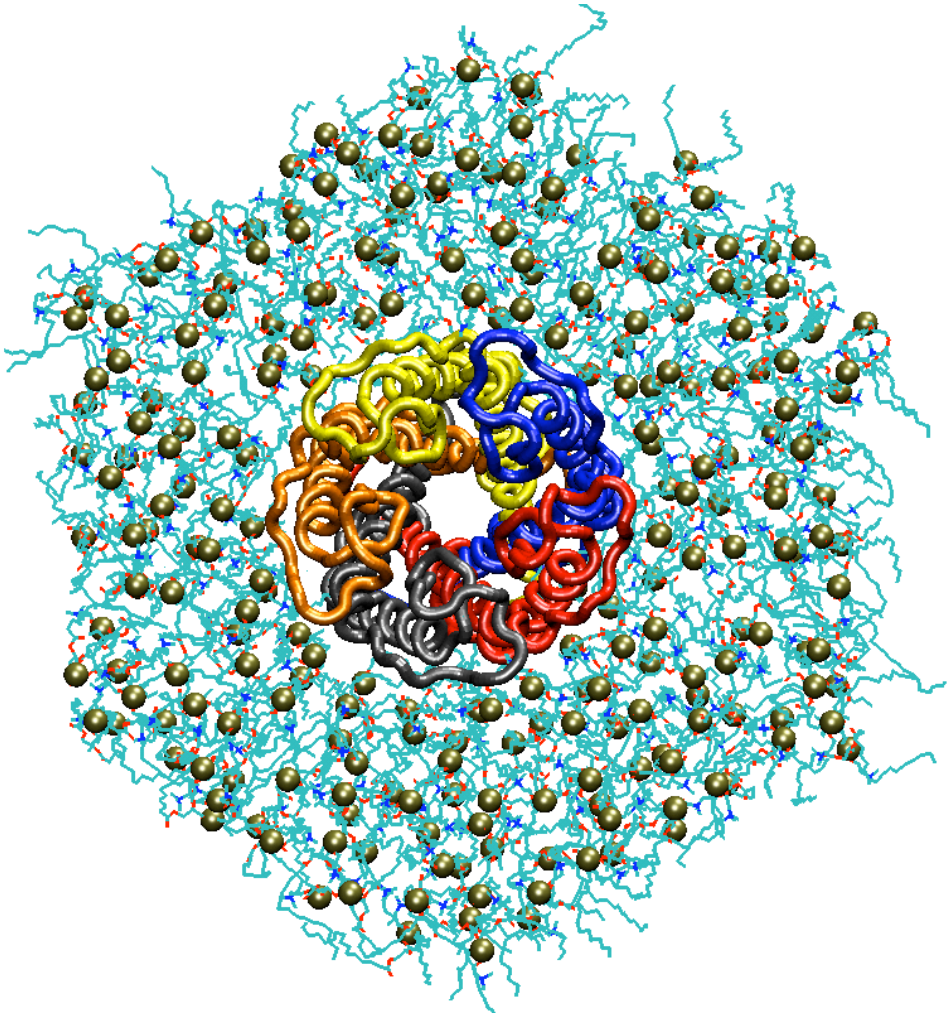
Most eukaryotic MS channels require coupling to the cytoskeleton and/or the extracellular matrix (Sachs and Morris, 1998).

- Mammals: TRAAK (Maingret, JBC 274, 1999.
- Haloferax volcanii, a halophilic archaeon.
- Prokaryotes: MscL in E. coli, Mycobacterium tuberculosis, many others.
- Eukaryotes: Mid1 gene in yeast (Kanzaki et al, Science (1999), **285**, 882-886.

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.

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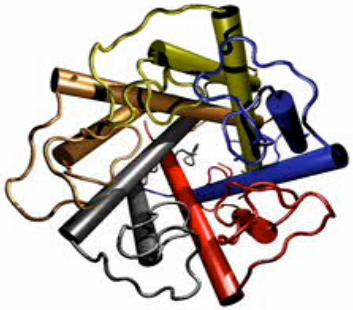
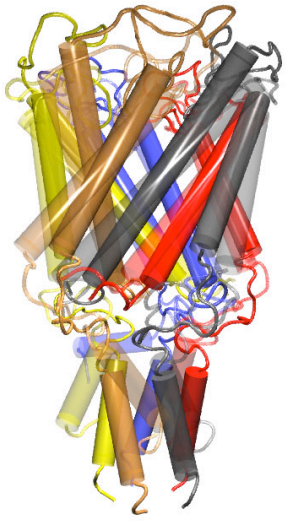
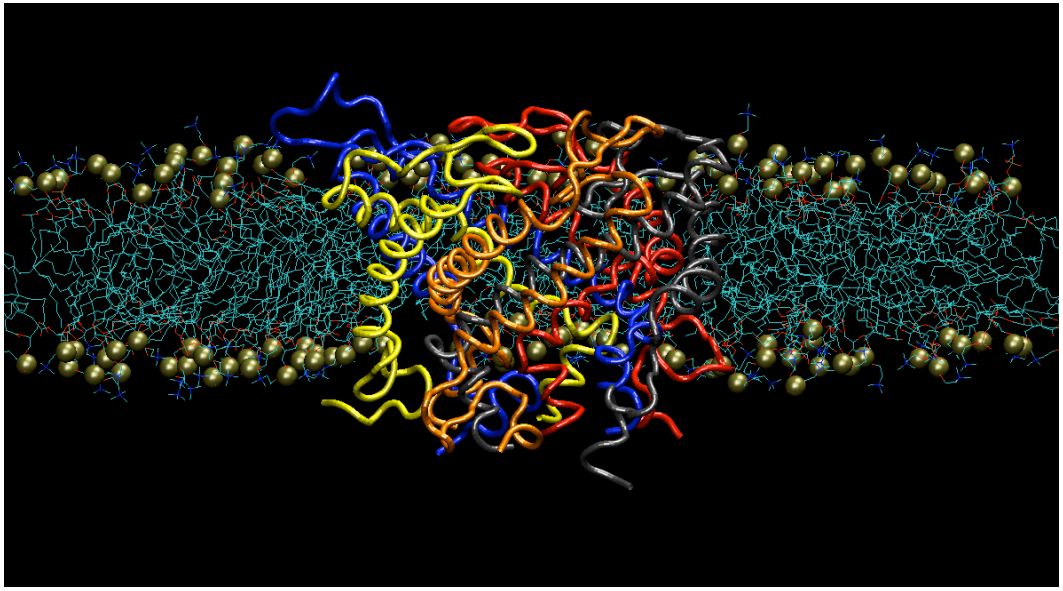
Mechanosensitive Ion Channel

MscL gates by membrane tension





Pore expands to 30 Å as helices flatten out



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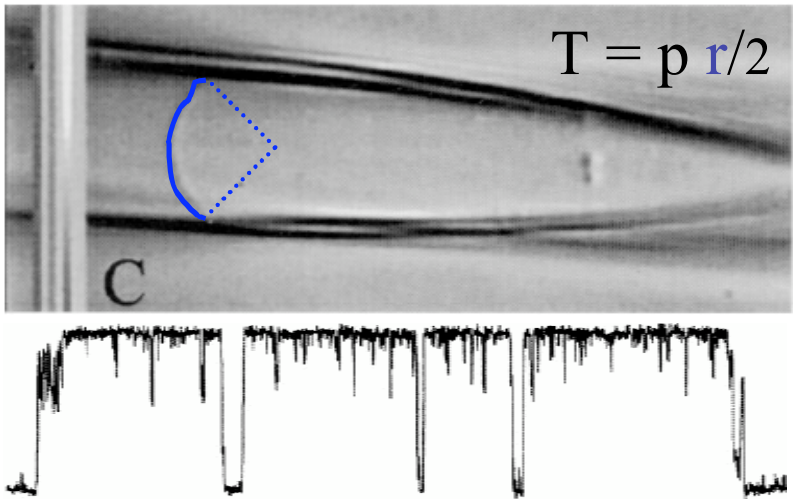
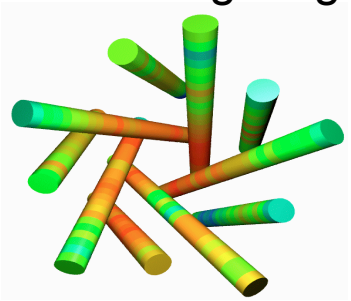


Patch-clamp measurements relate membrane tension to channel gating

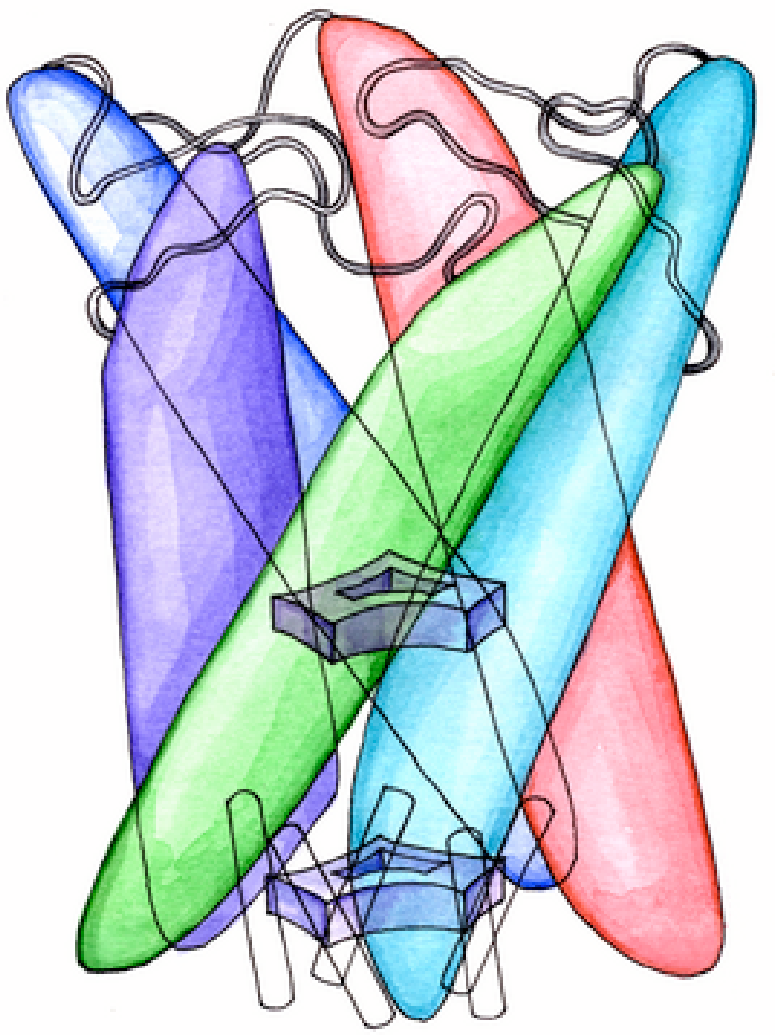


The protein is stiffest in the pinched gating region, in agreement with EPR measurements (Martinac et al, unpublished results)

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?



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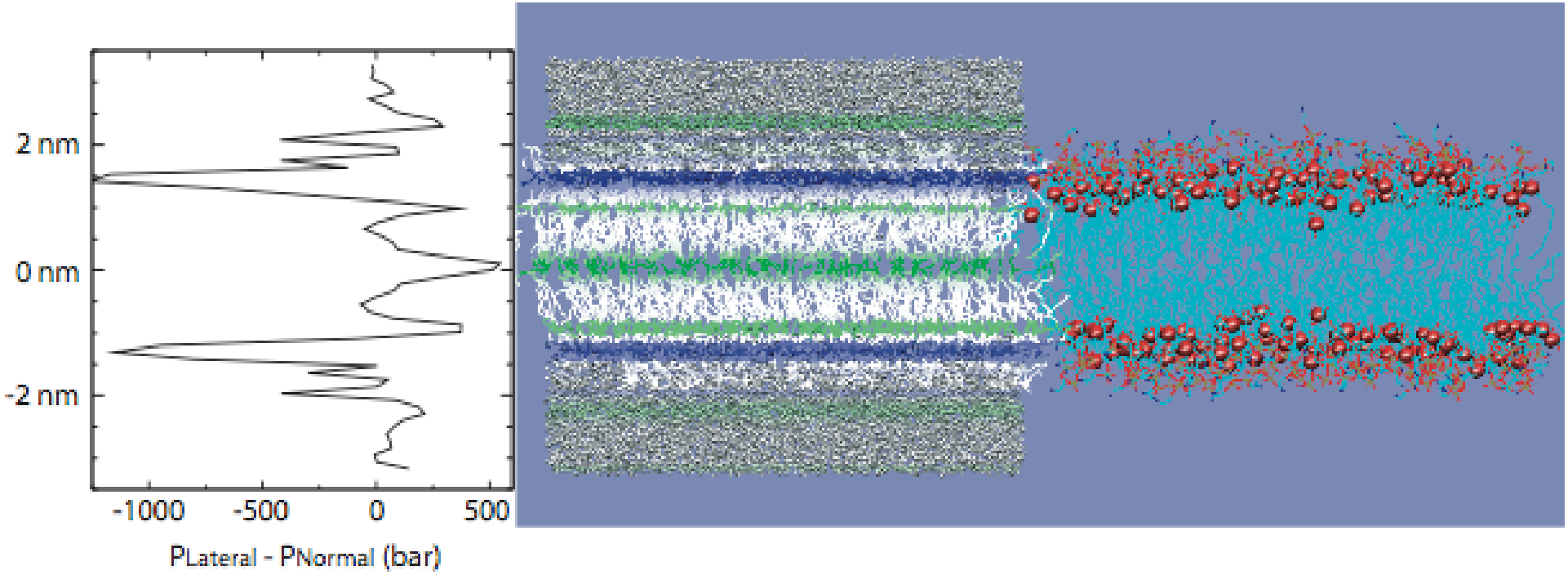
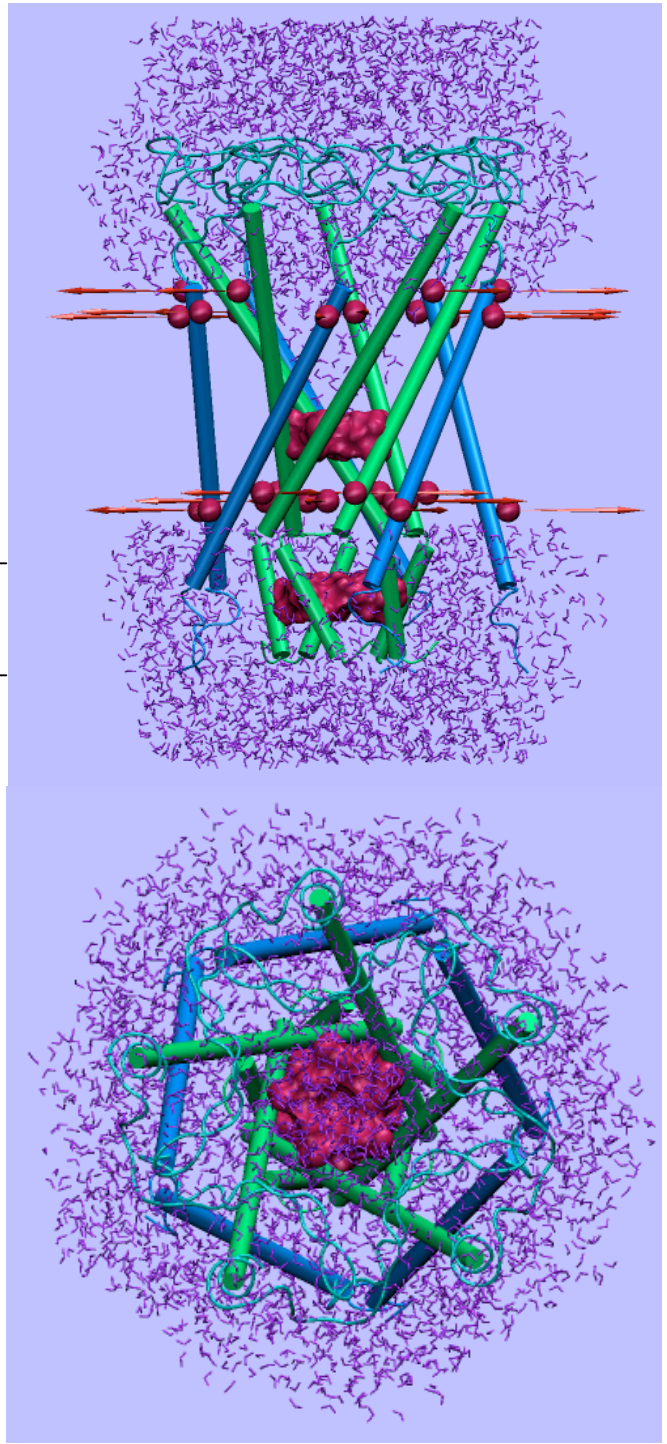
Gating Forces Derived from Bilayer Pressure Profile



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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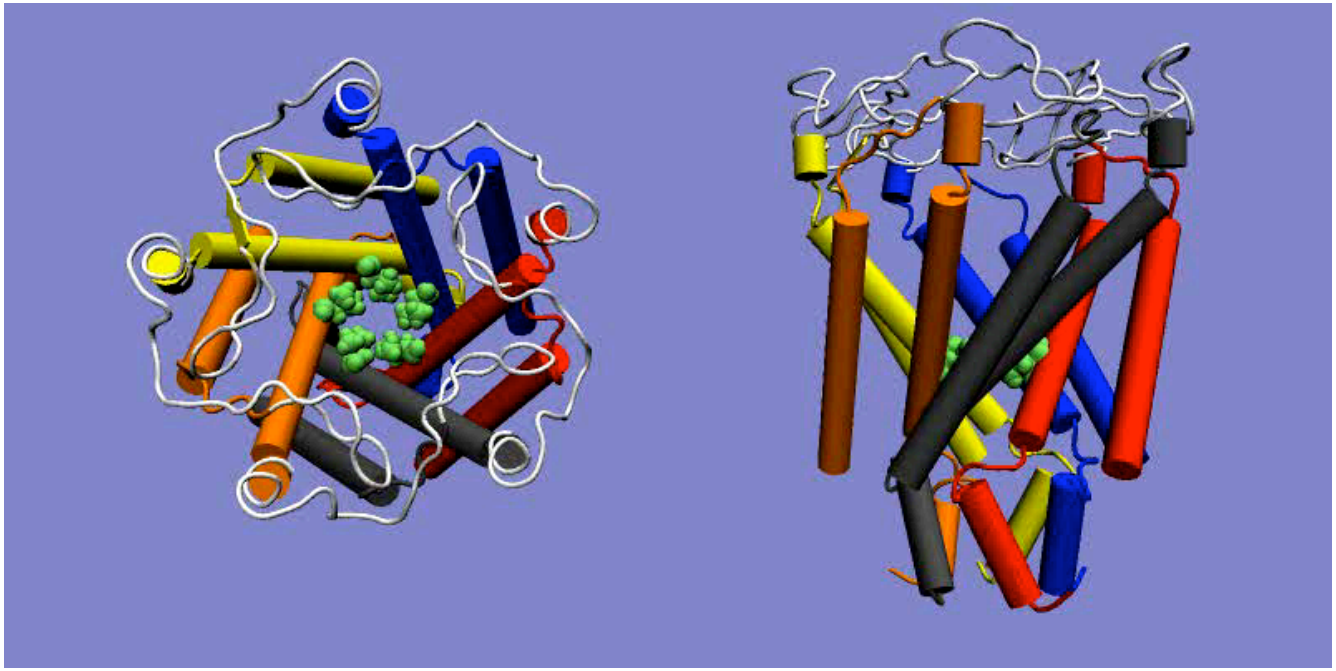
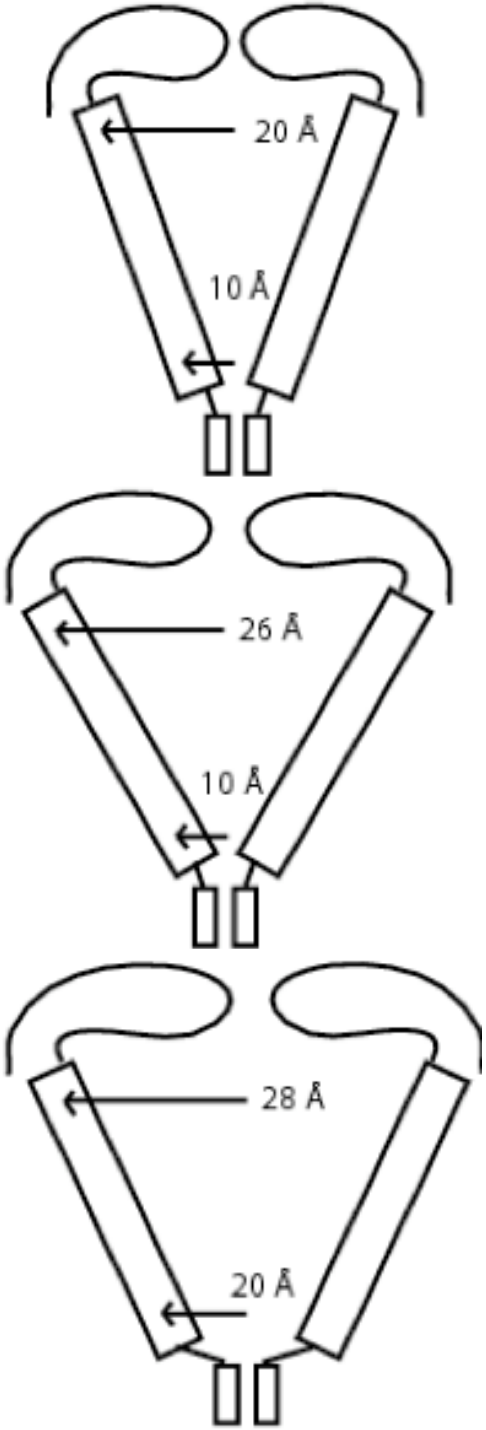
MscL Expanded State





- 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.

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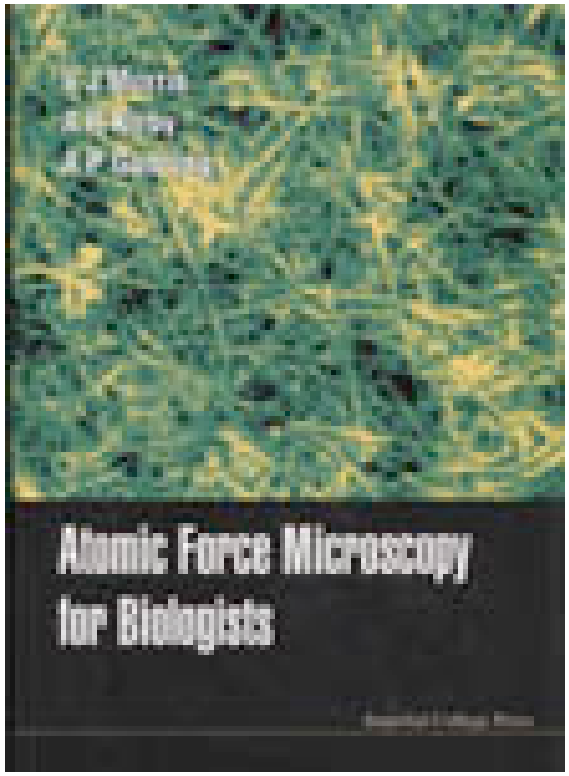

ATOMIC FORCE MICROSCOPY FOR BIOLOGISTS

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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.