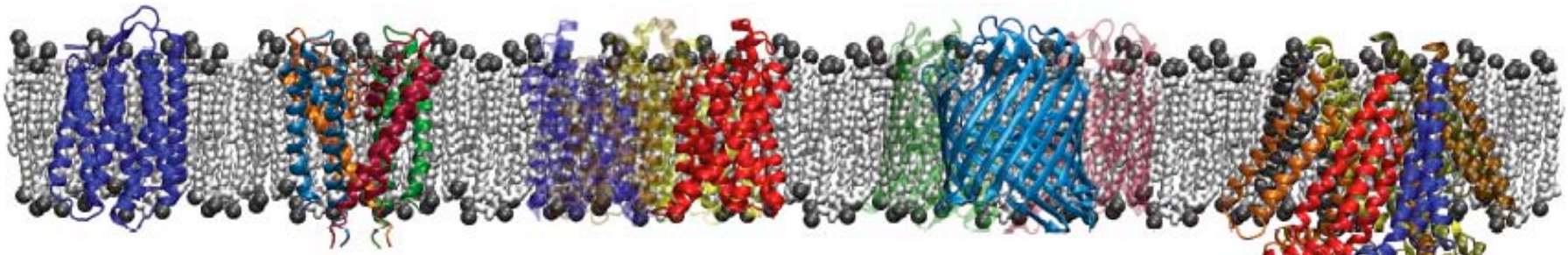
Membrane channels studied by molecular dynamics in our group

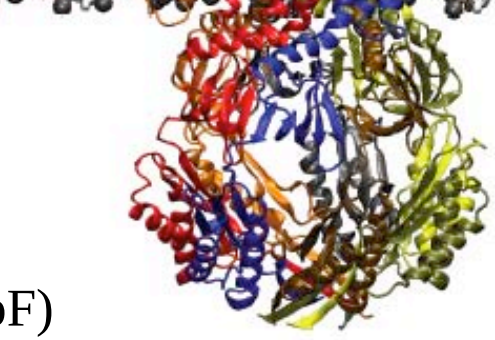


bacteriorhodopsin

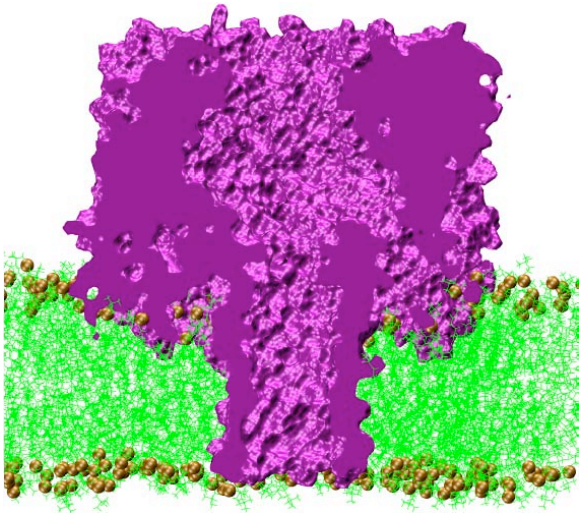
potassium channel KcsA

aquaporin GlpF

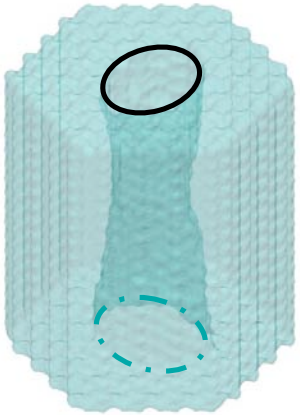
(porin OmpF)



mechanosensitive channel MscS



alpha-hemolysine



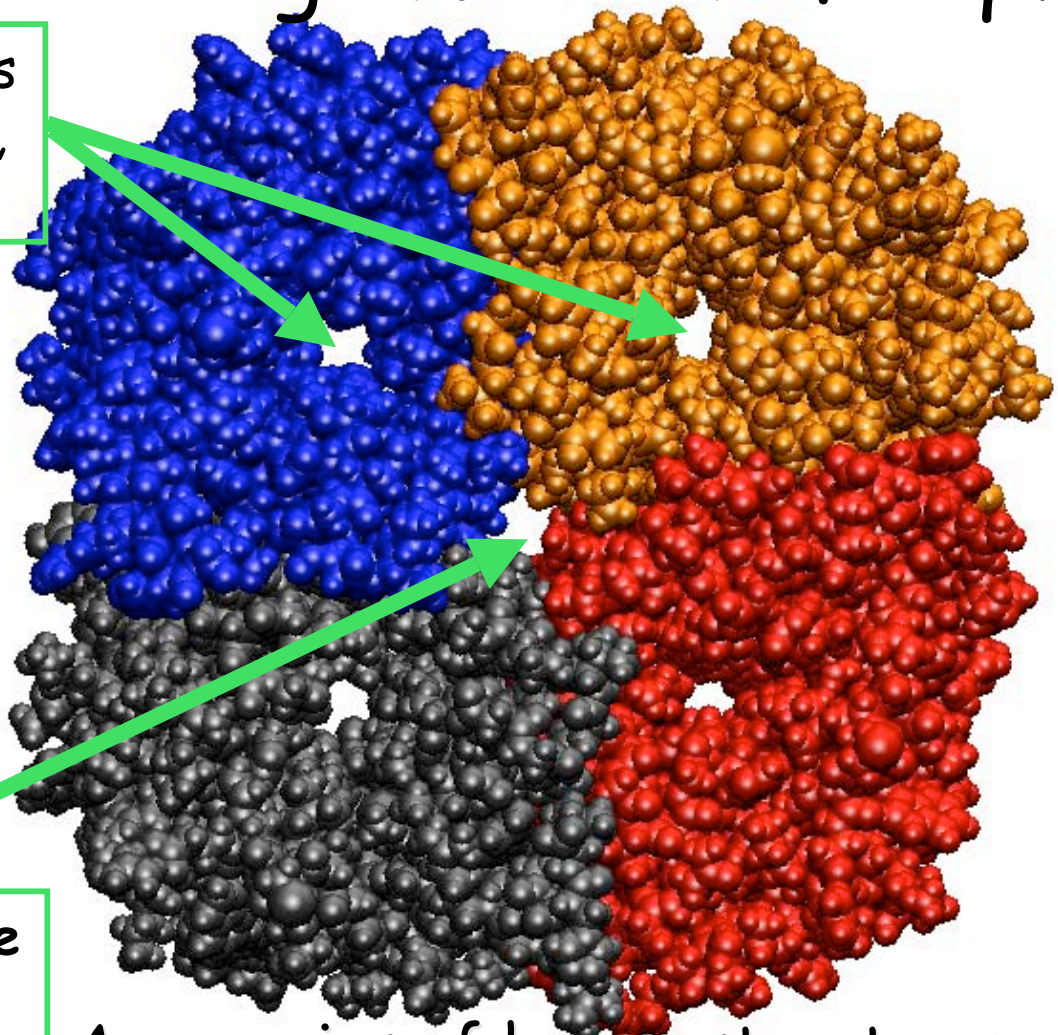
nanopore in Si_3N_4

Other Channels

- SecY
- lactose permease

Structural Organization of Aquaporins

Monomeric pores
Water, glycerol, urea, H_2S, …



Tetrameric pore
????

Aquaporins of known structure:

GlpF – E. coli glycerol channel

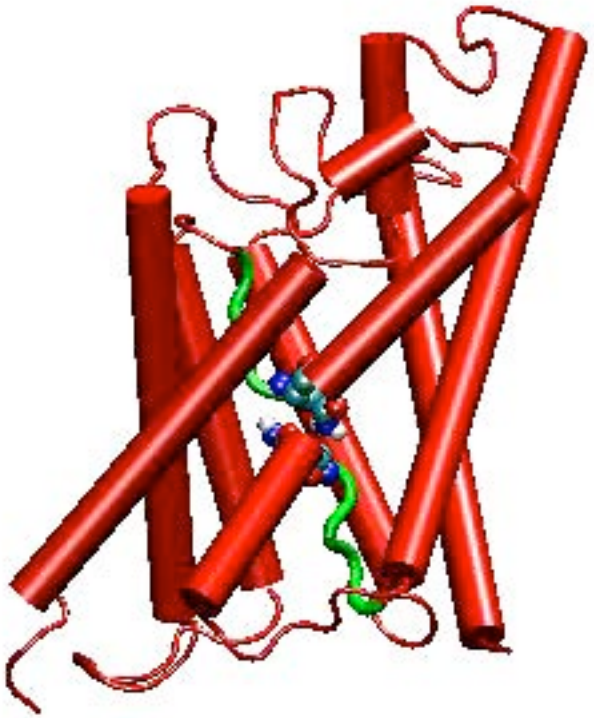
AQP1 – Mammalian aquaporin-1

AqpZ and AQP0 (2004, 2005), AqpM (2005), soPIP2 (2006)

Architecture of Aquaporin Monomer

Issues

- Water and glycerol transport
- Exclusion of ions and protons



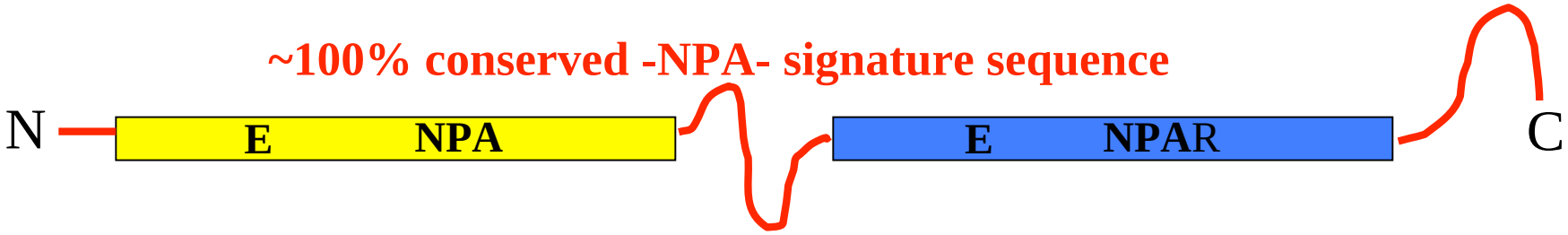
Aquaporins of known structure:

GlpF – E. coli water and glycerol channel

AqpZ - E. coli water channel

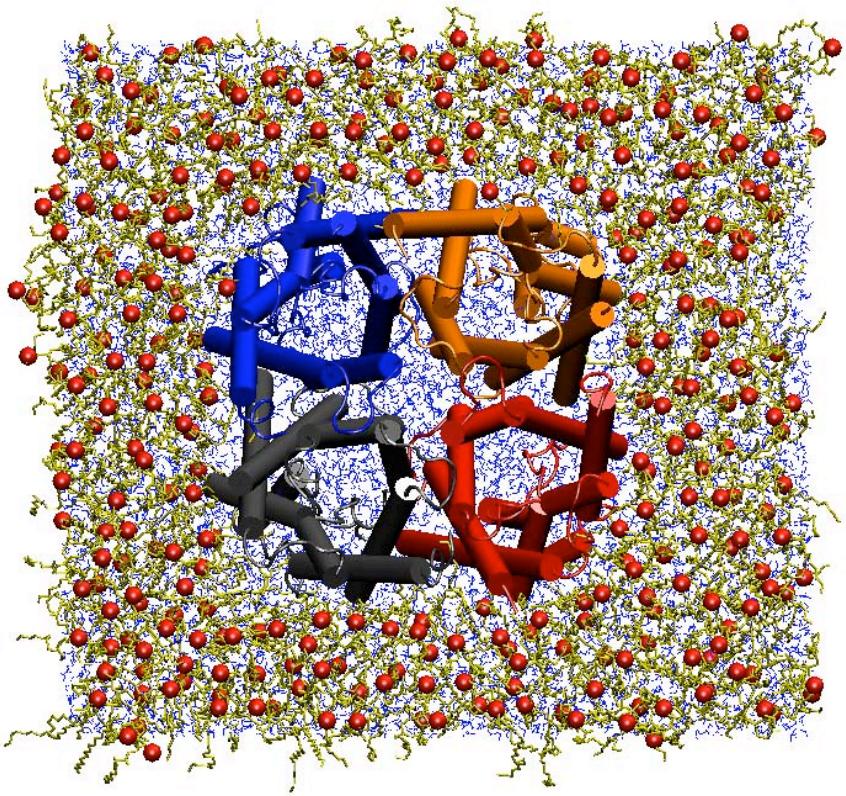
AqP0 - mammalian water and glycerol channel

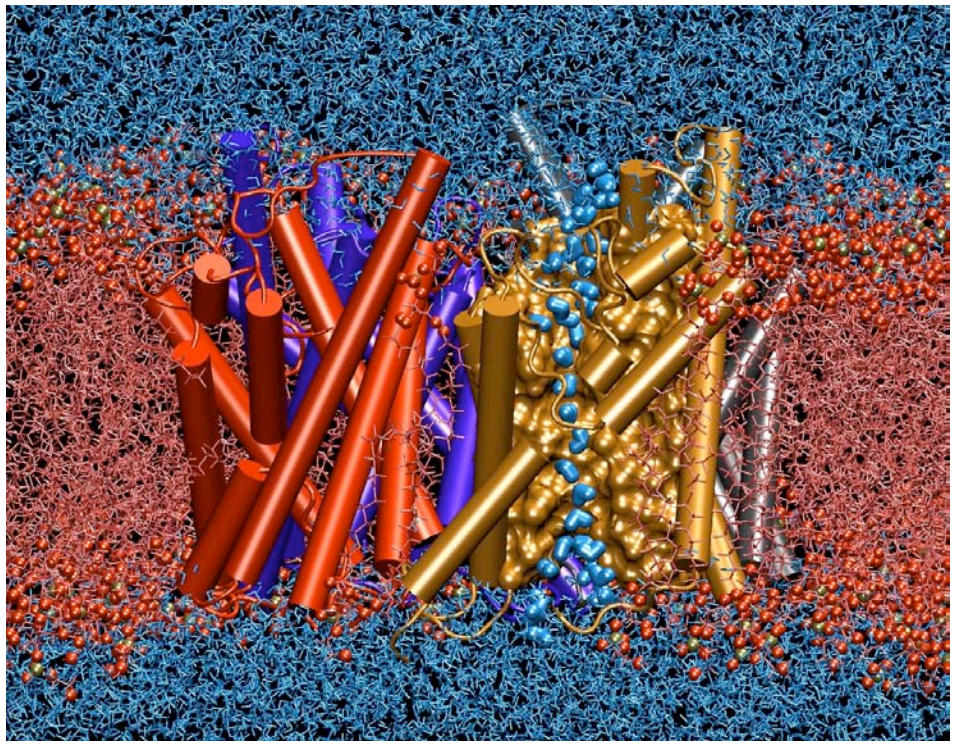
AqP1 – mammalian water channel



Molecular Dynamics Simulations

Protein: ~ 15,000 atoms
Lipids (POPE): ~ 40,000 atoms
Water: ~ 50,000 atoms
Total: ~ 105,000 atoms





NAMD, CHARMM27, PME

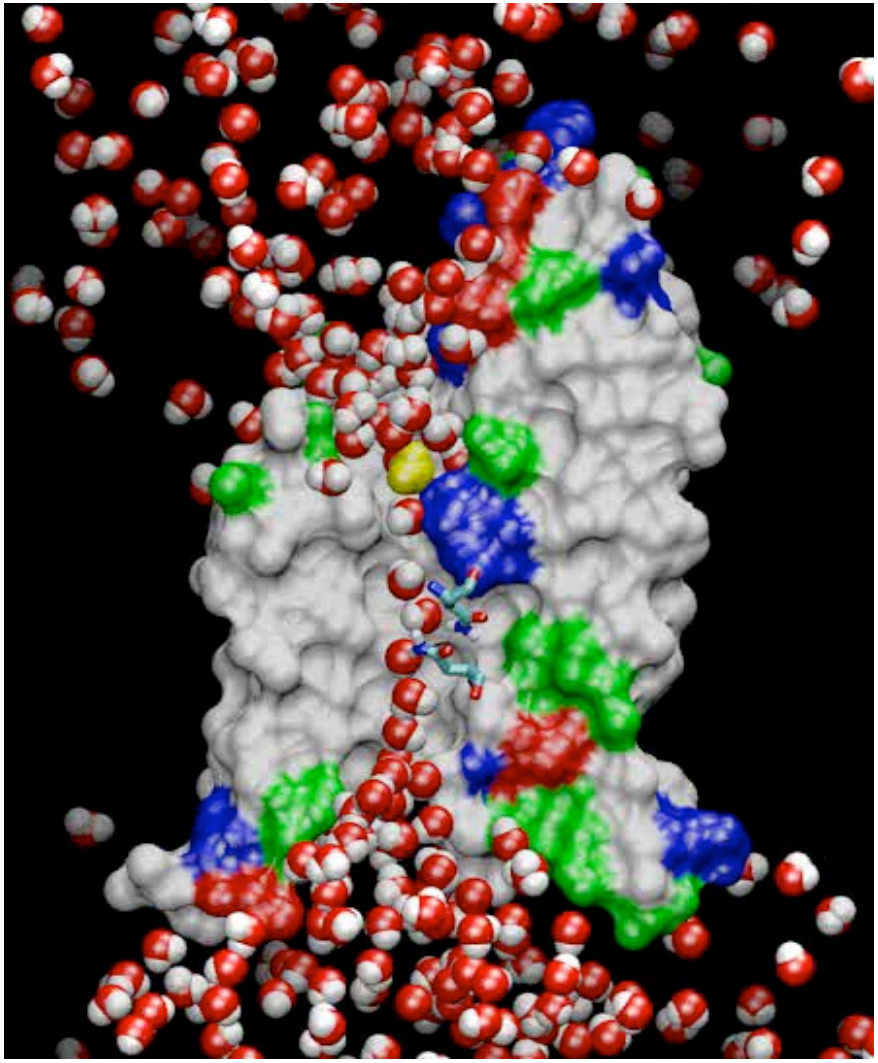
NpT ensemble at 310 K

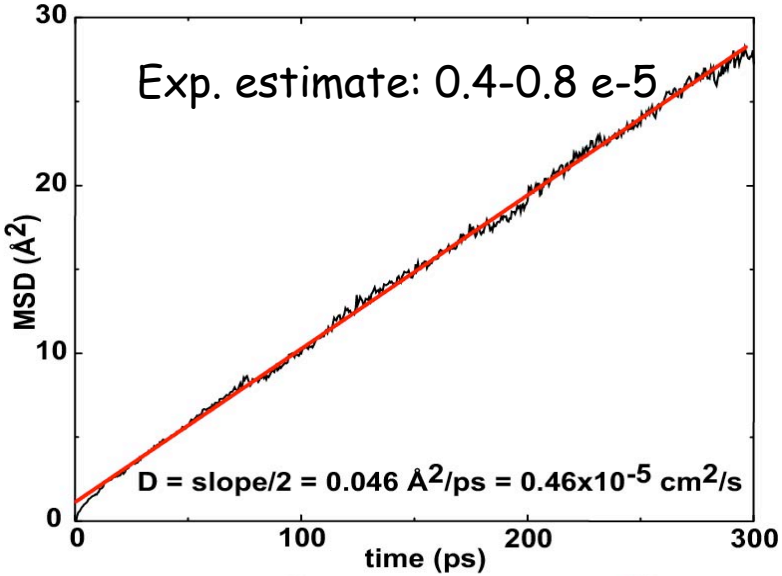
5-35 ns of simulations per system

1.5 ns / day – 48-proc Linux cluster

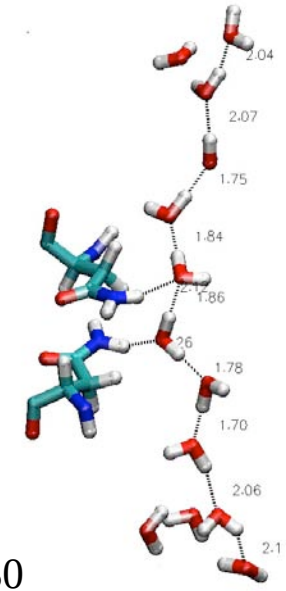
3 ns/day - 128 CPUs on PSC/NCSA

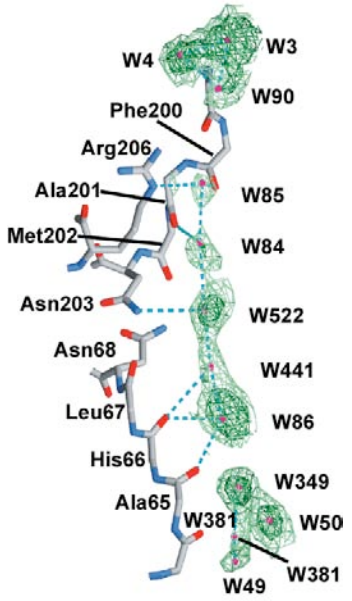
Real Time Simulation of Channel-Mediated Water Permeation





THEORY

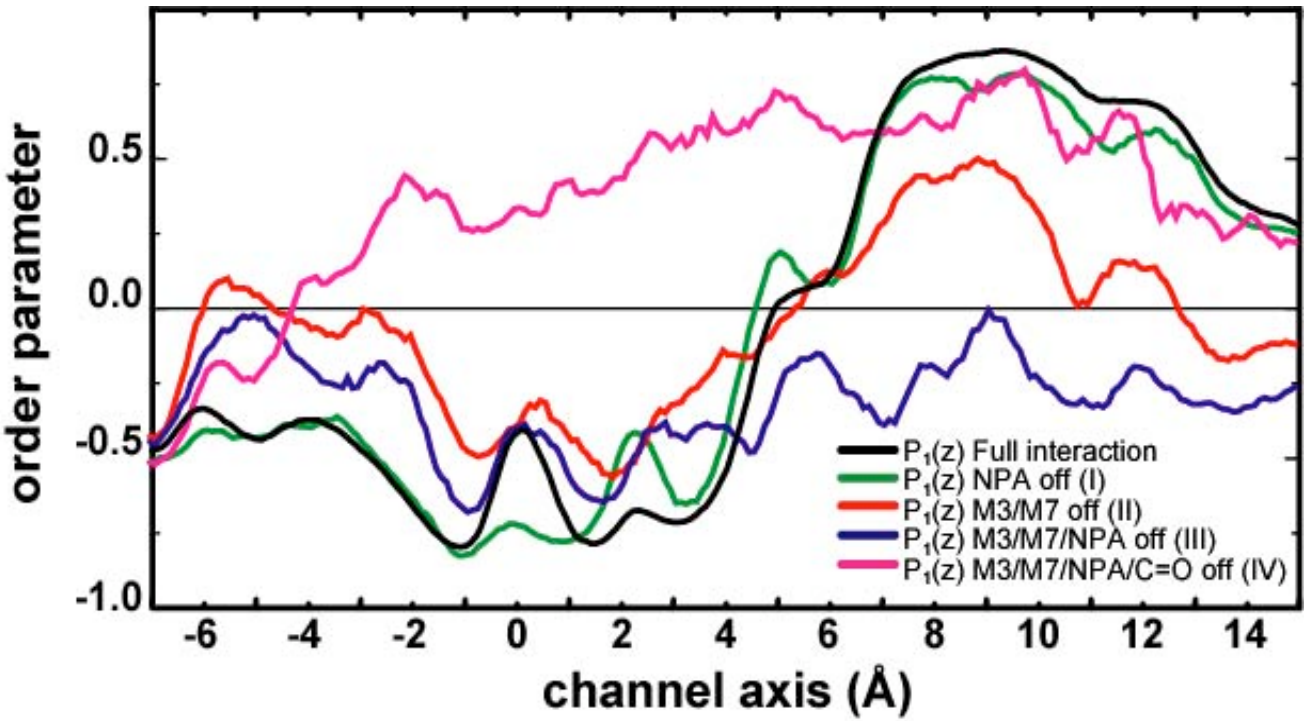


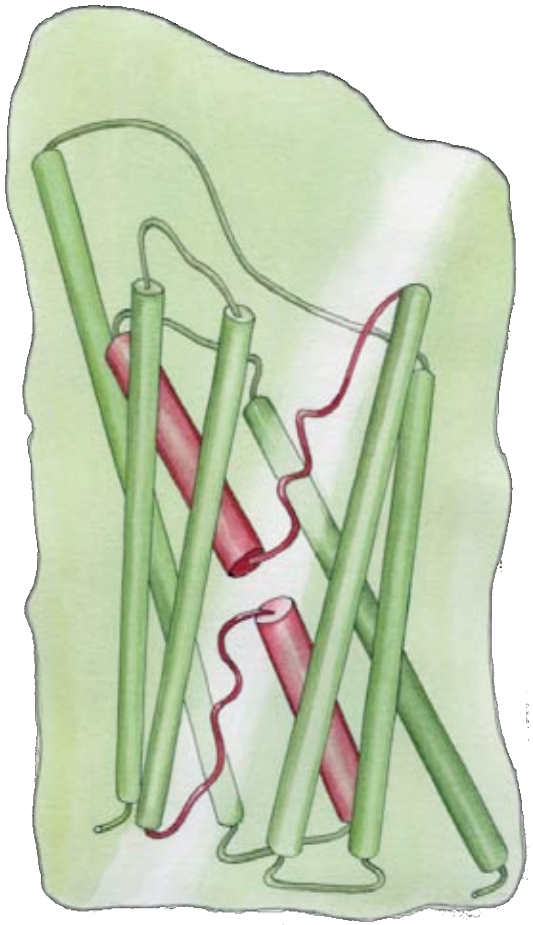


EXPERIMENT

E. Tajkhorshid, P. Nollert, M. Jensen, L. J. W. Miercke, J. O'Connell, R. M. Stroud, and K. Schulten, ***Science*** 296, 525-530

Electrostatic Stabilization of Water Bipolar Arrangement





E. Tajkhorshid, P. Nollert, M. Jensen, L. J. W. Miercke, J. O'Connell, R. M. Stroud, and K. Schulten, ***Science*** 296, 525-530 (2002)

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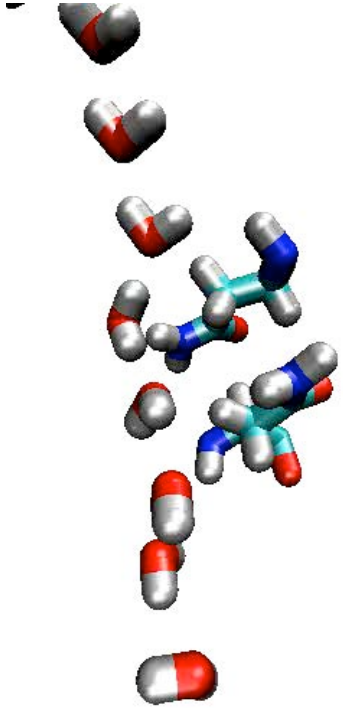
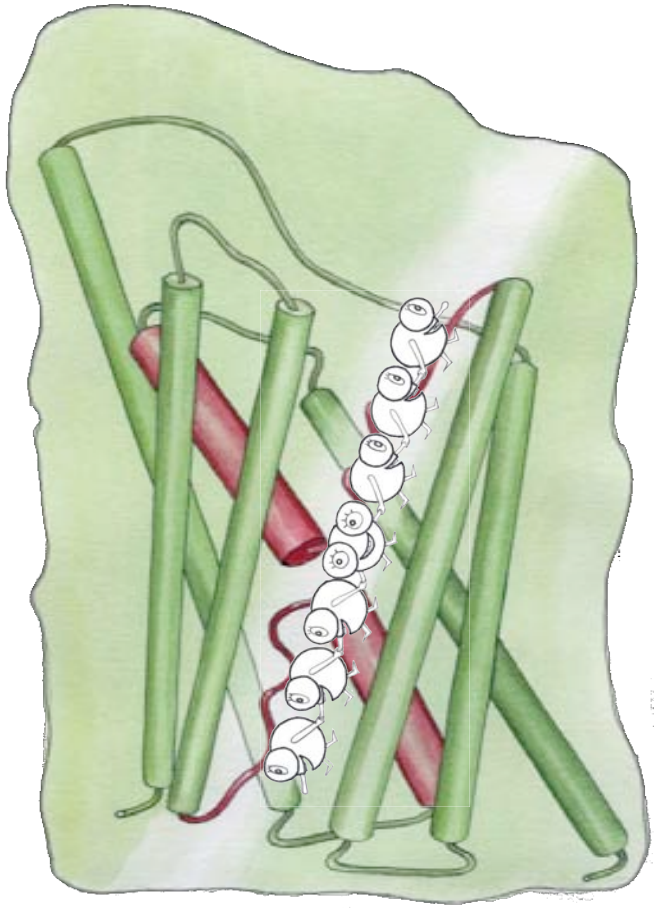
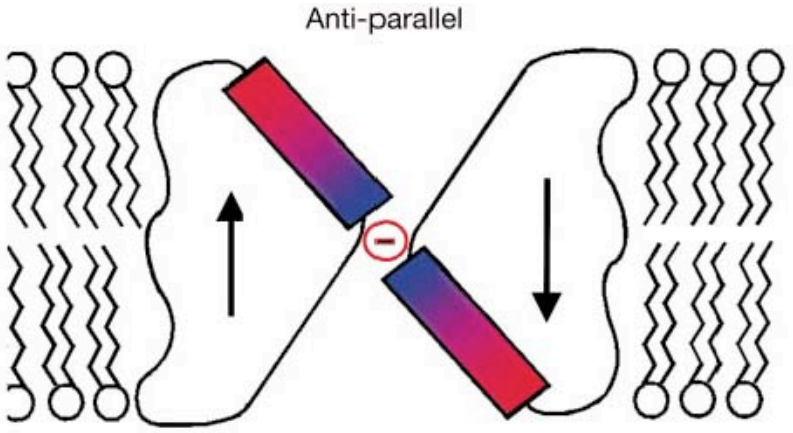


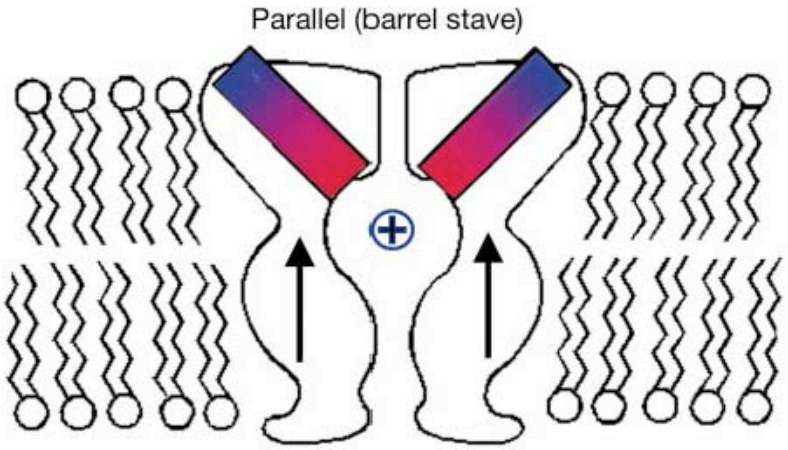


E. Tajkhorshid, P. Nollert, M. Jensen, L. J. W. Miercke, J. O'Connell, R. M. Stroud, and K. Schulten, ***Science*** 296, 525-530 (2002)

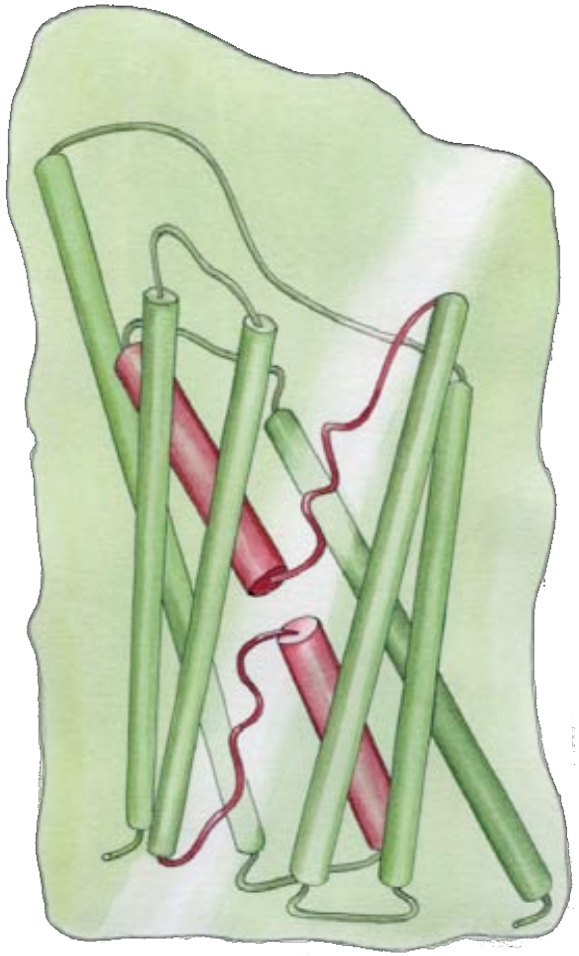
Similarity in Electrostatic Tuning Among Different Membrane Channels

Cl^- channel





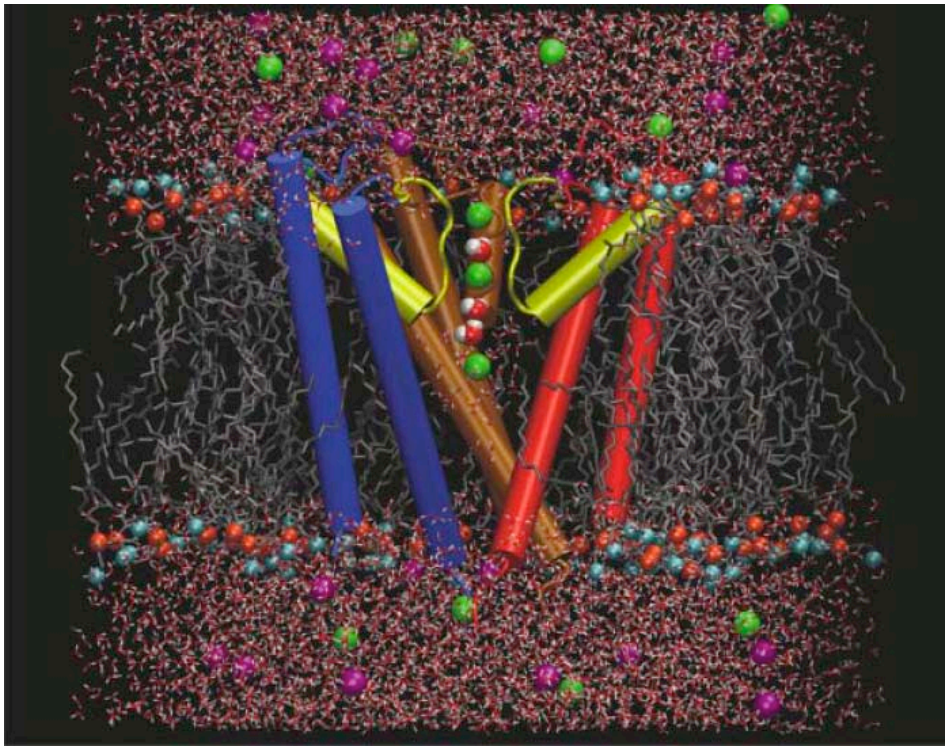
K^+ channel



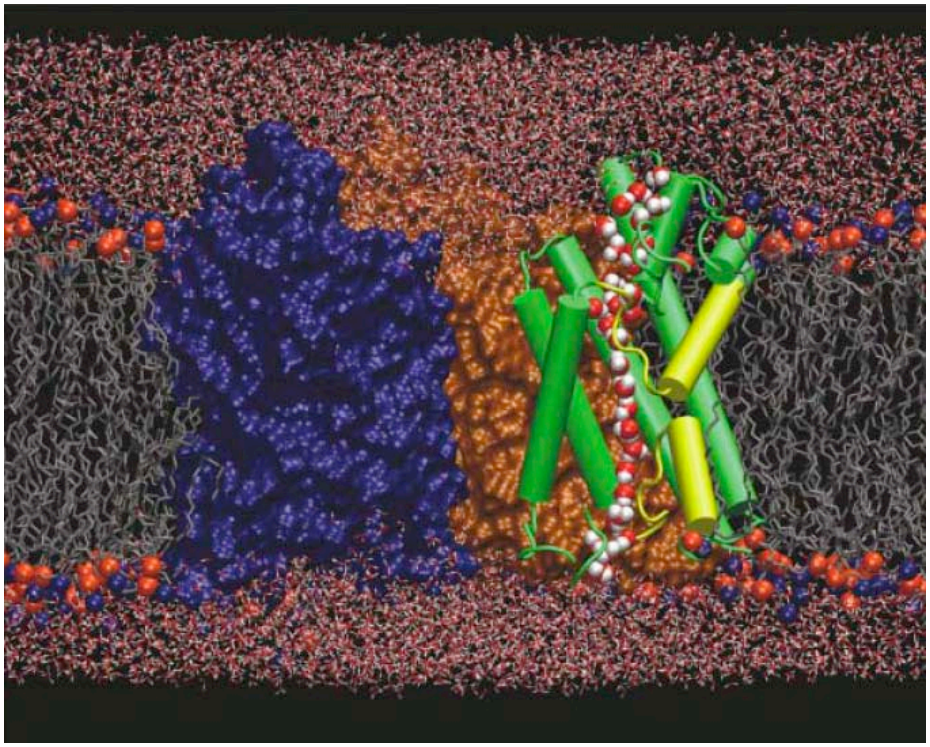
Aquaglyceroporins

Architectural Similarity Among Different Membrane Channels

potassium channel KscA



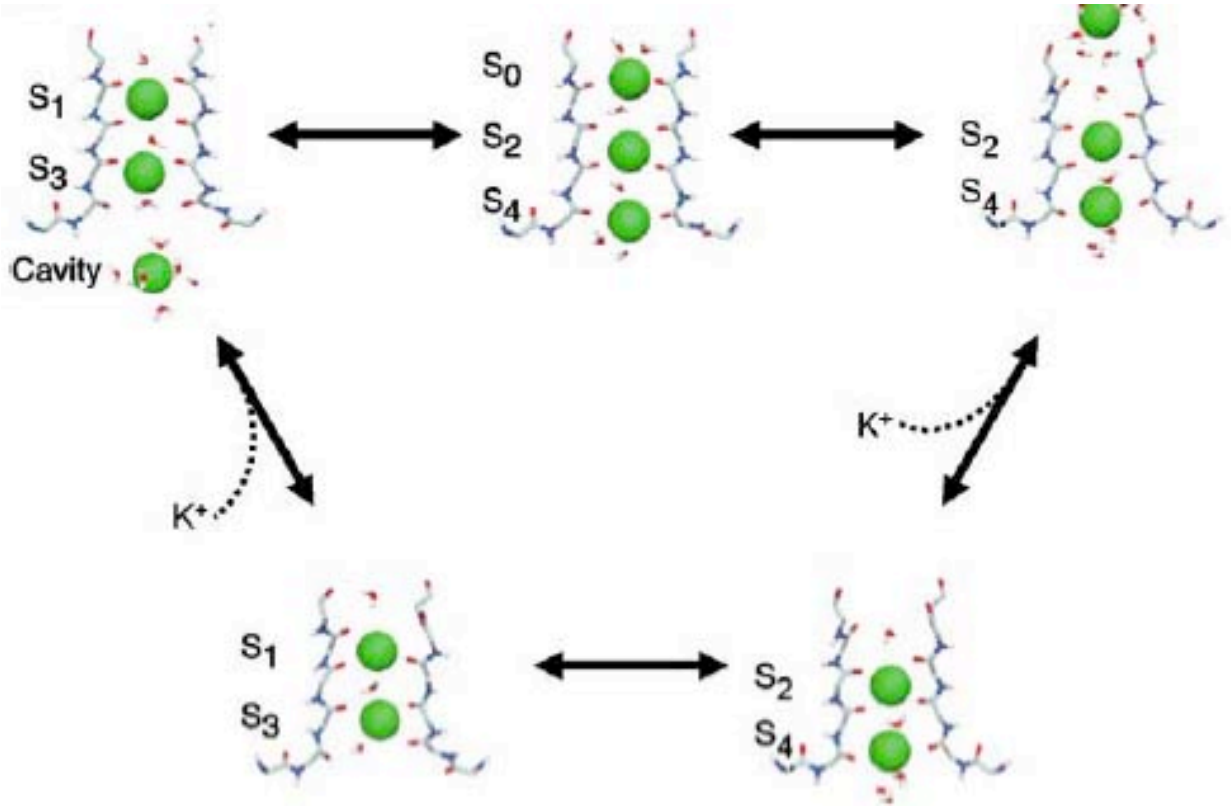
aquaporin GlpF



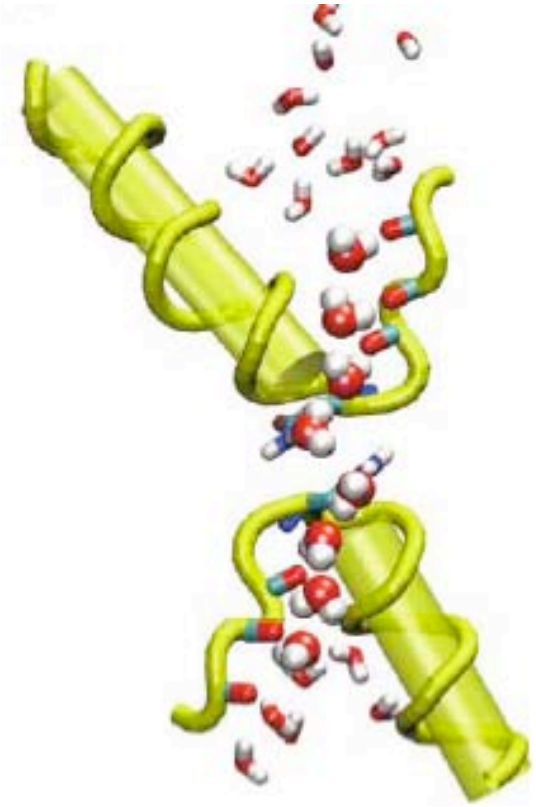
B. Roux and K. Schulten, *Structure* 12: 1343-1351 (2004)

Similarity in Electrostatic Tuning Among Different Membrane Channels

potassium channel KscA

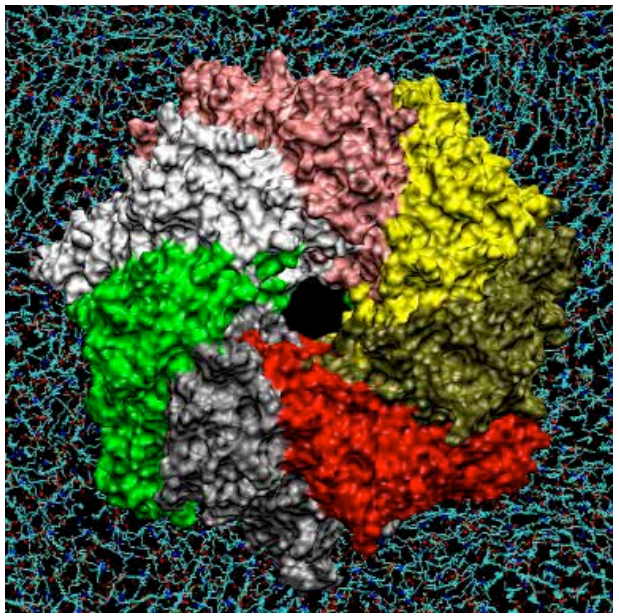


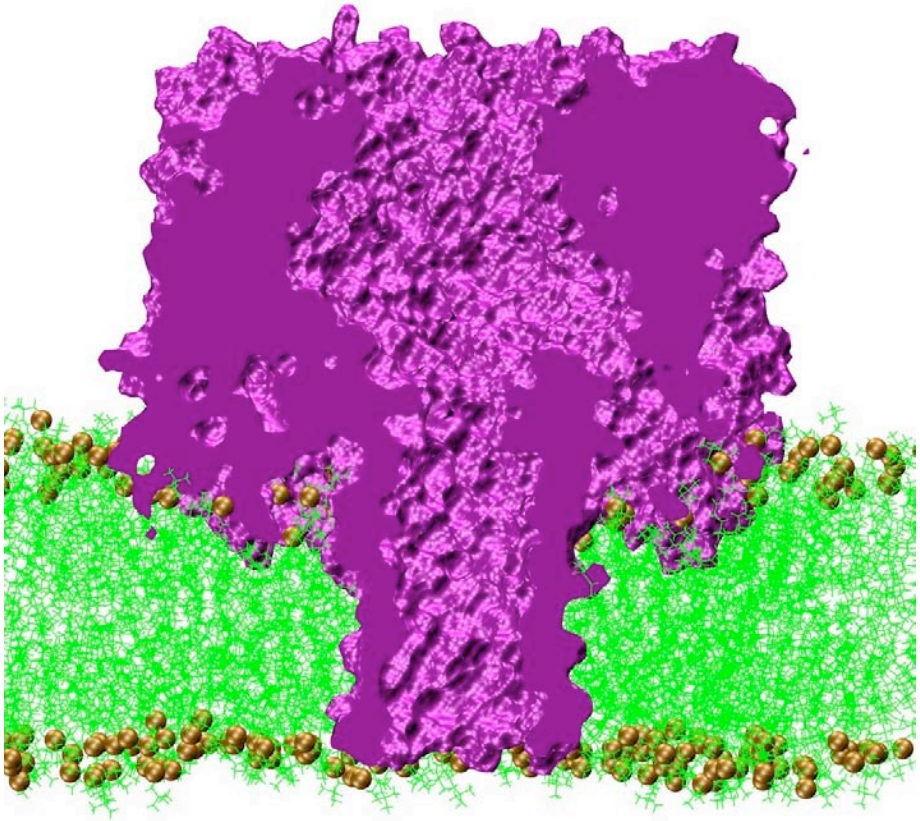
aquaporin GlpF



B. Roux and K. Schulten, *Structure* 12: 1343-1351 (2004)

Imaging α-Hemolysin with Molecular Dynamics



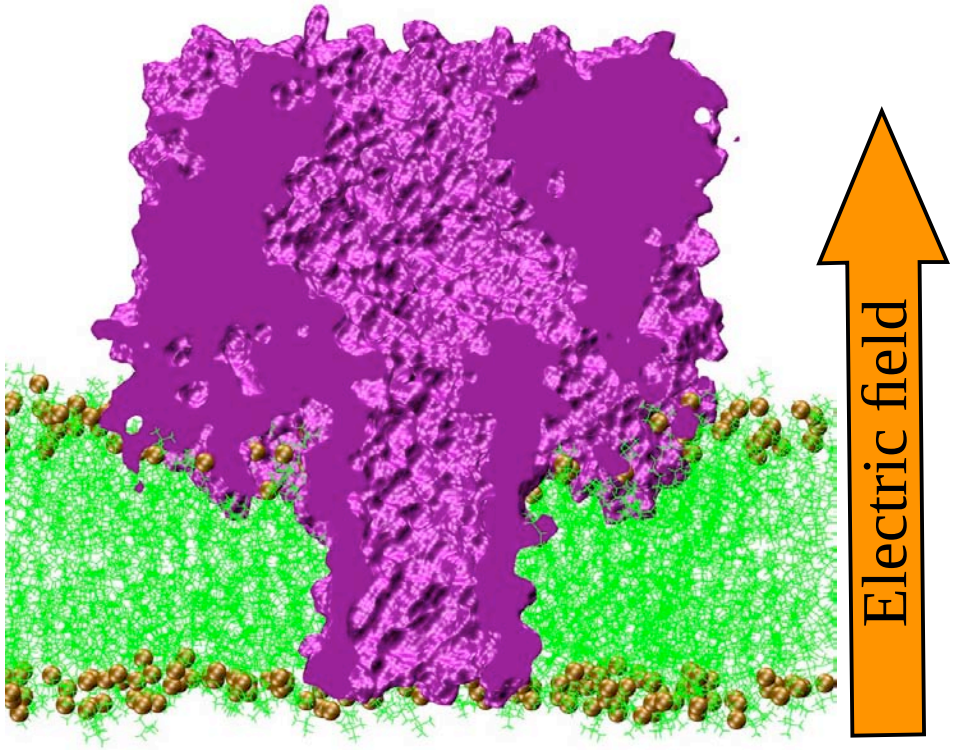


Protein + DPPC lipid membrane + 1M water solution of KCl = 300,000 atoms

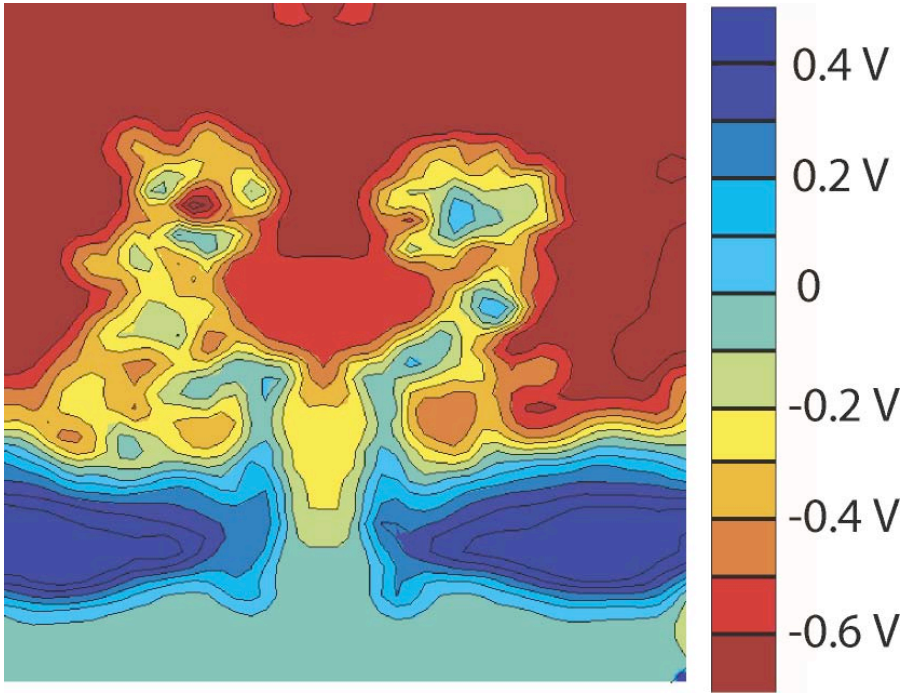
- Structural dynamics
- Ionic currents
- Ionic pathways
- Water conductivity
- Electrostatic potential maps
- pH dependence of ionic conductance

Aleksij Aksimentiev and Klaus Schulten. *Biophys. J.* **88**: 3745-3761 (2005)

Computing conductance of α-hemolysin with molecular dynamics



Electric field



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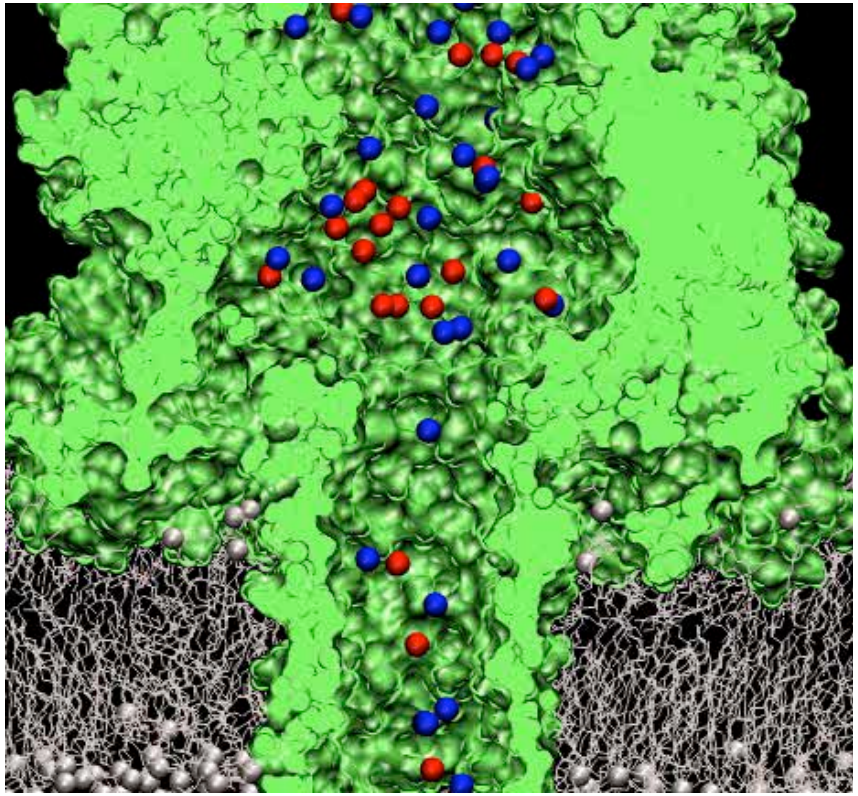
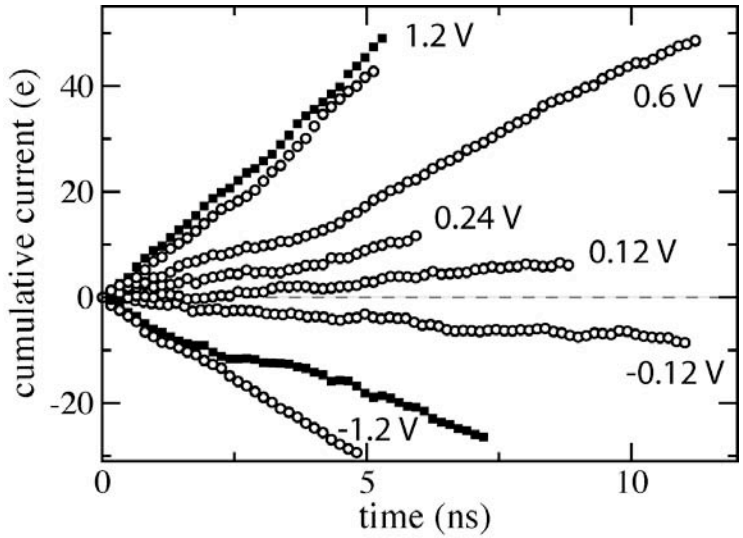
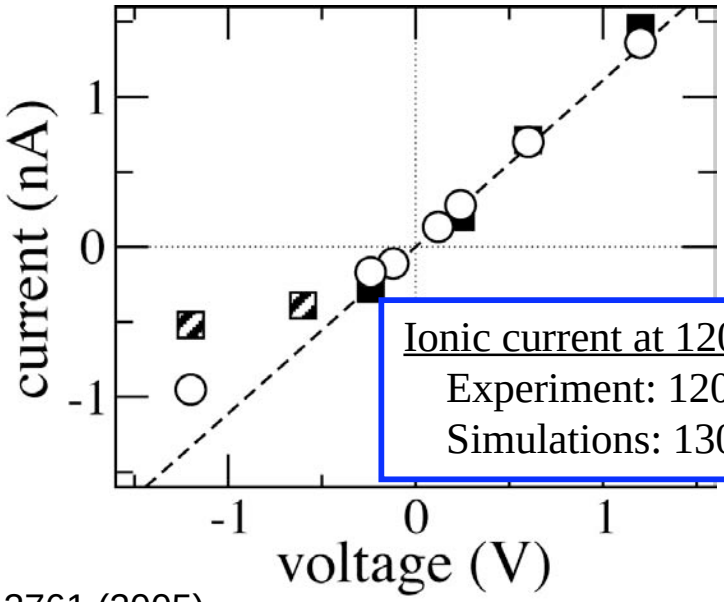
Average electrostatic potential map

Aleksij Aksimentiev and Klaus Schulten. *Biophys. J.* **88**: 3745-3761 (2005)

Current-Voltage curve:
excellent agreement with experiment







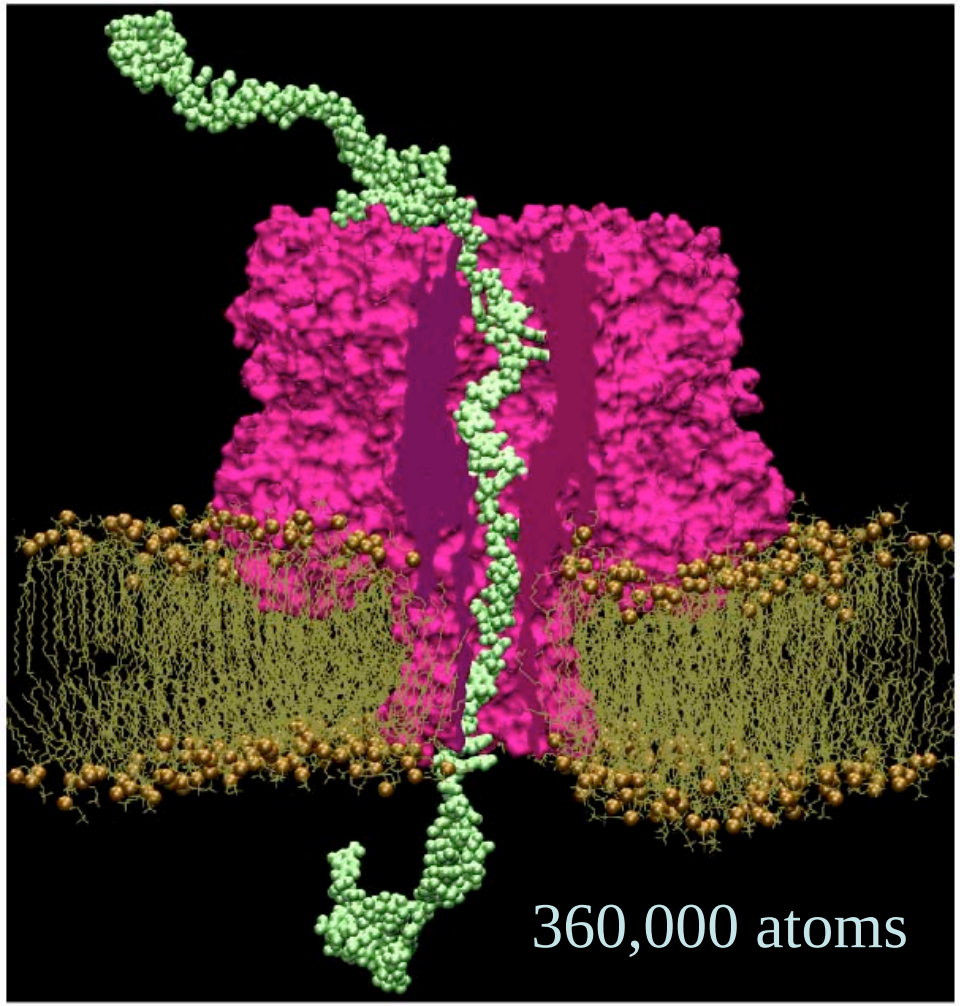
Ionic current at 120mV:
Experiment: 120 pA
Simulations: 130 pA

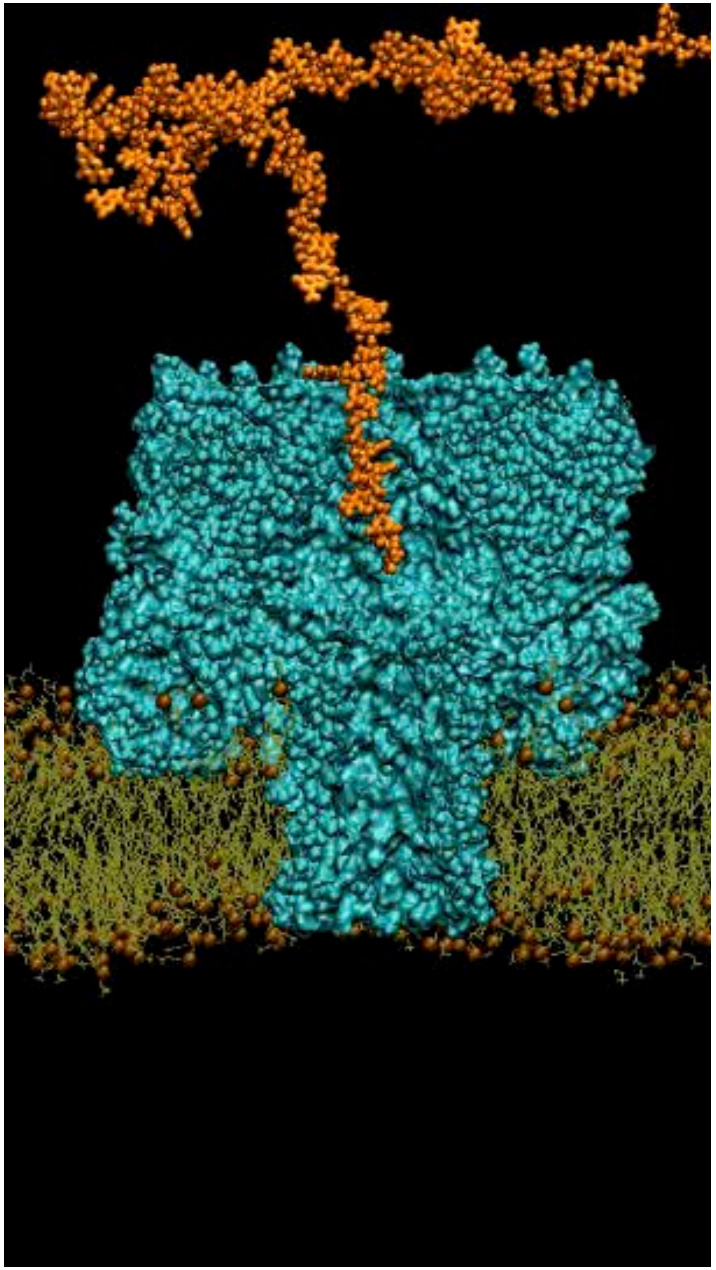
$$I(t) = \frac{1}{\Delta t L_z} \sum_{i=1}^{N} q_i (z_i(t + \Delta t) - z_i(t))$$

Instantaneous current

Aleksij Aksimentiev and Klaus Schulten. *Biophys. J.* **88**: 3745-3761 (2005)

In confined geometry bases align toward the 5’ end





Jerome Mathé, Aleksei Aksimentiev, David R. Nelson, Klaus Schulten, and Amit Meller. Orientation discrimination of single stranded DNA inside the α-hemolysin membrane channel. Proceedings of the National Academy of Sciences, USA, 102:12377-12382, 2005

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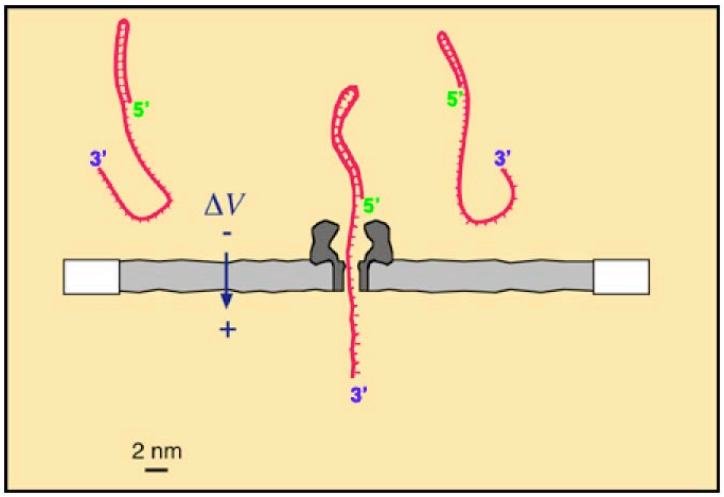

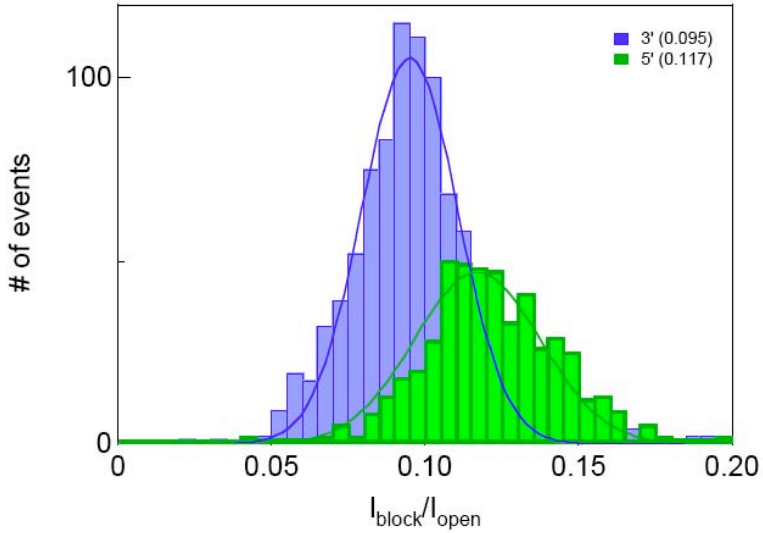

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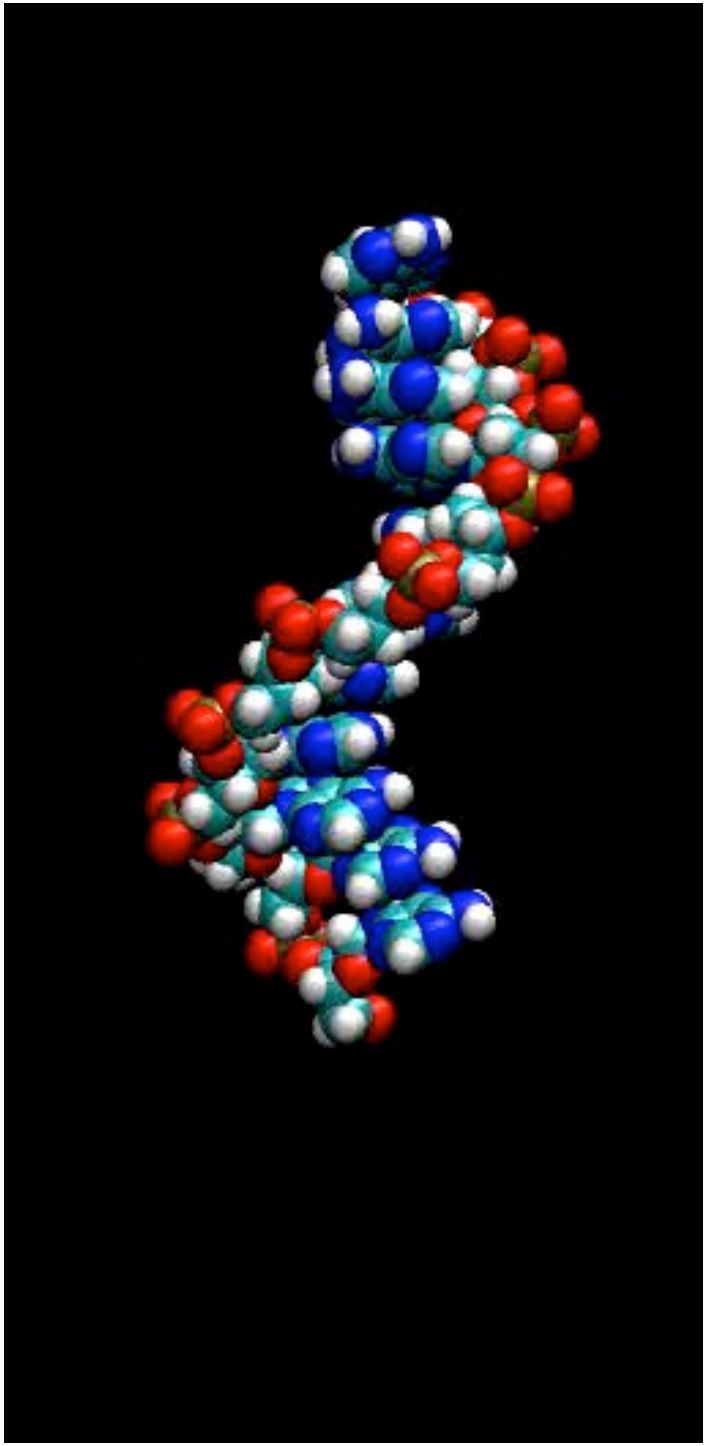
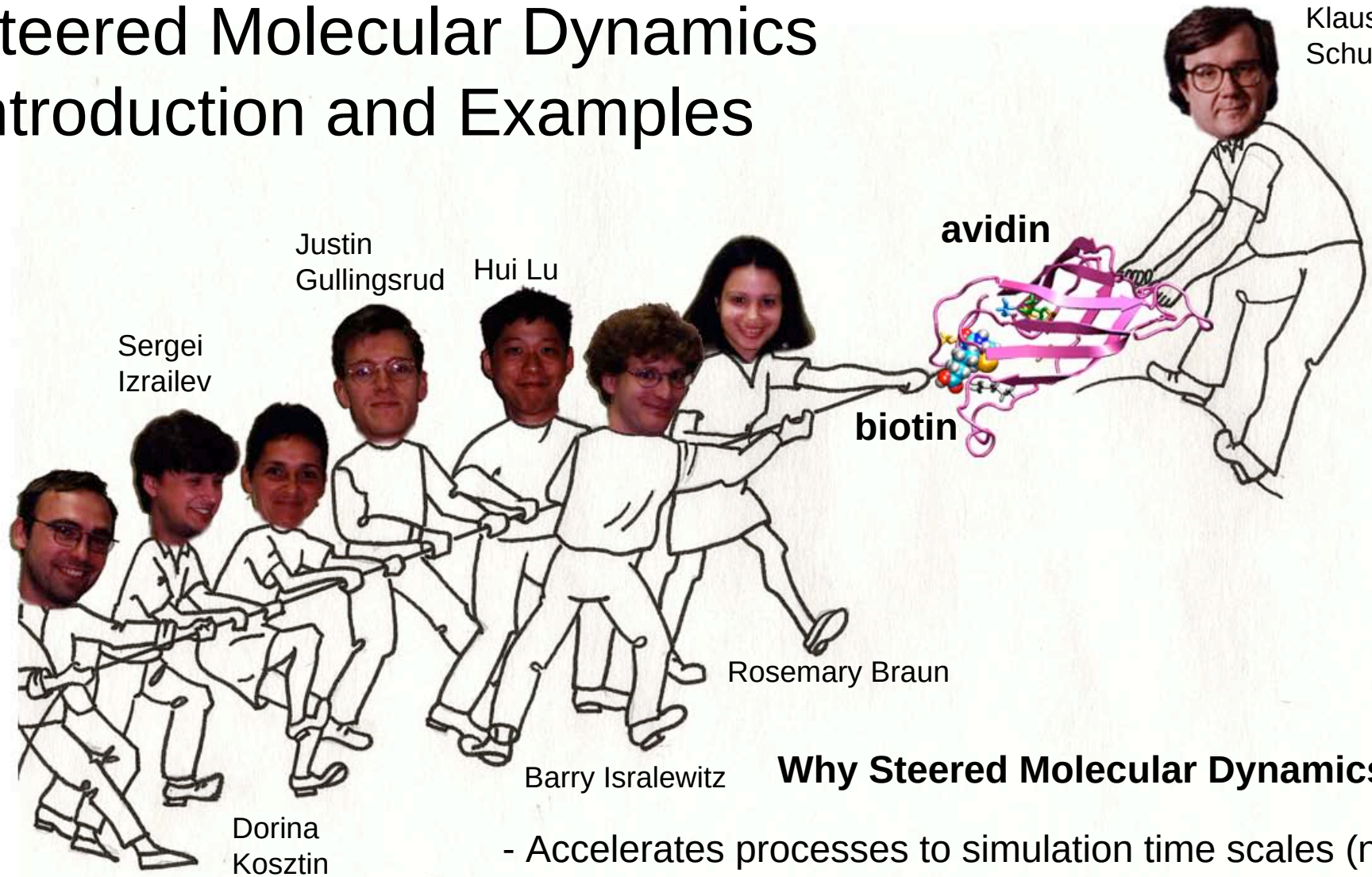


Steered Molecular Dynamics Introduction and Examples



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

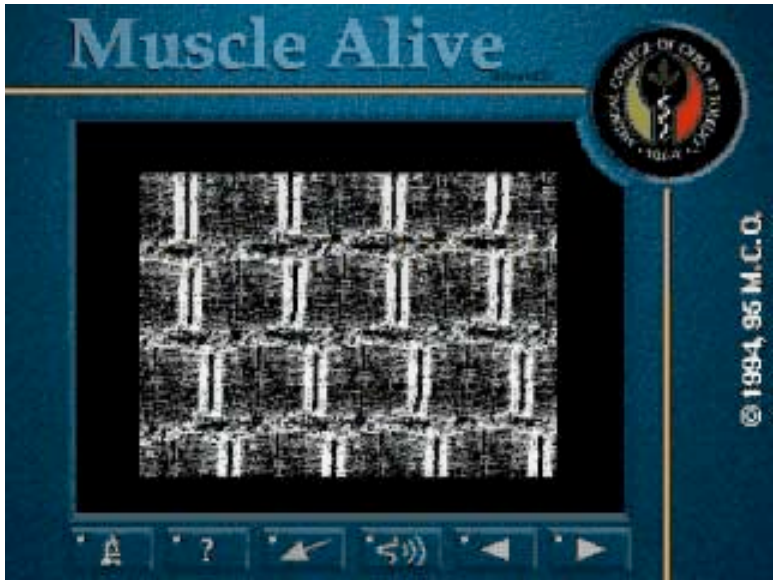
and can also be

substrates (ATPase)

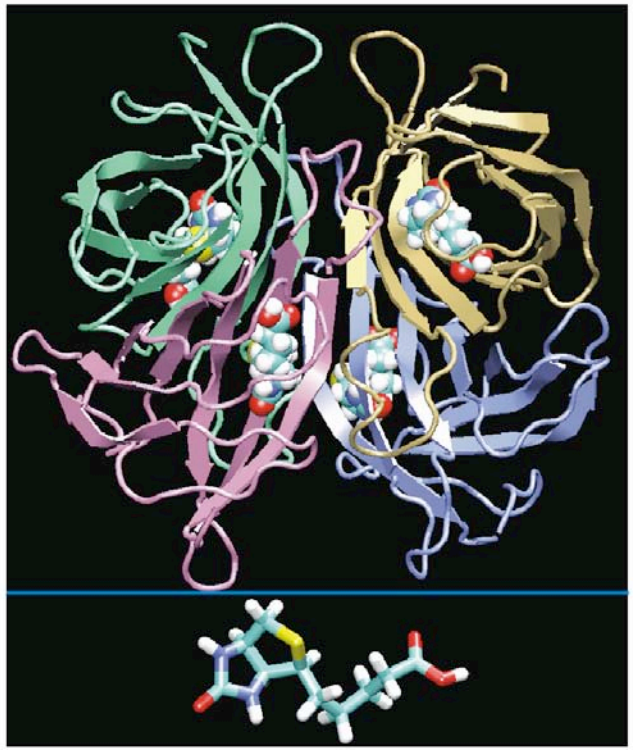
products (myosin)

signals (integrin)

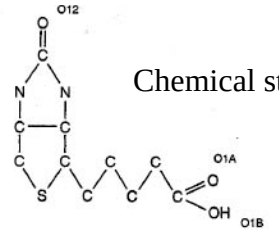
of cellular processes



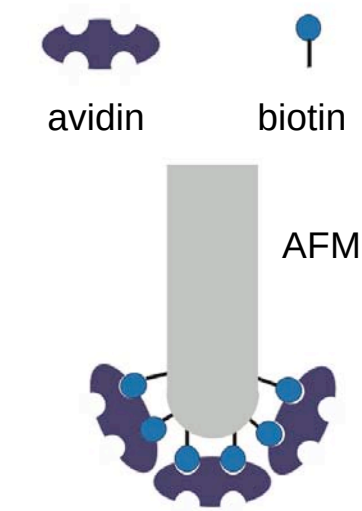
Atomic Force Microscopy Experiments of Ligand Unbinding

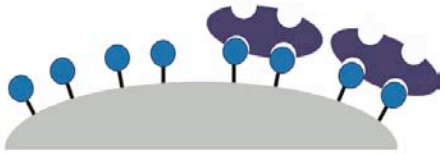


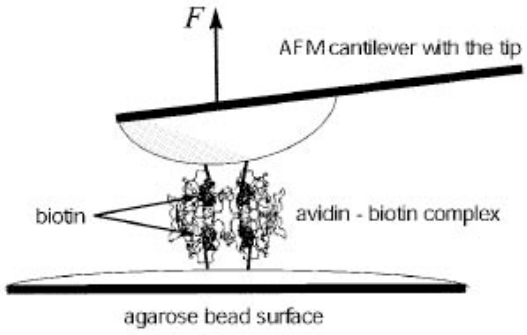
Biotin



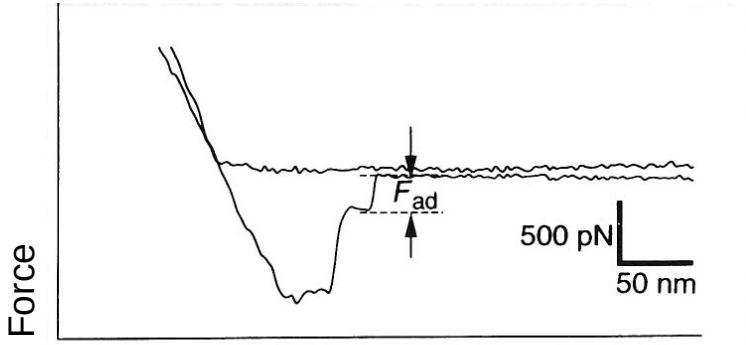
Chemical structure of biotin



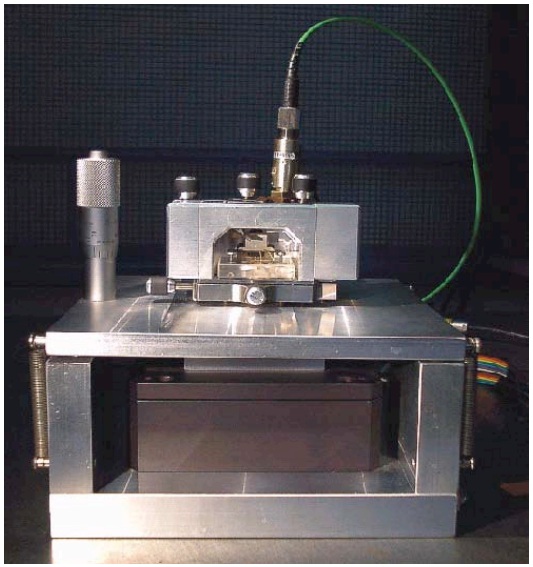




Florin et al., Science 264:415 (1994)



Displacement of AFM tip



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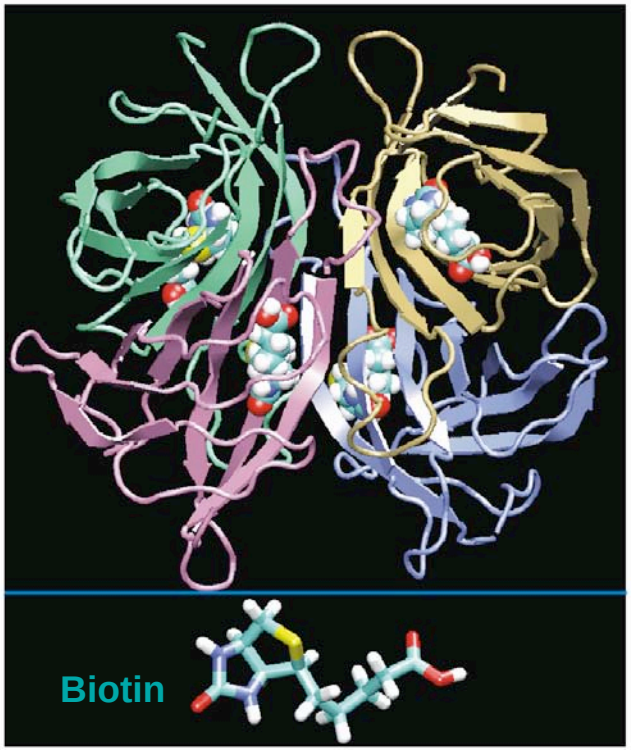

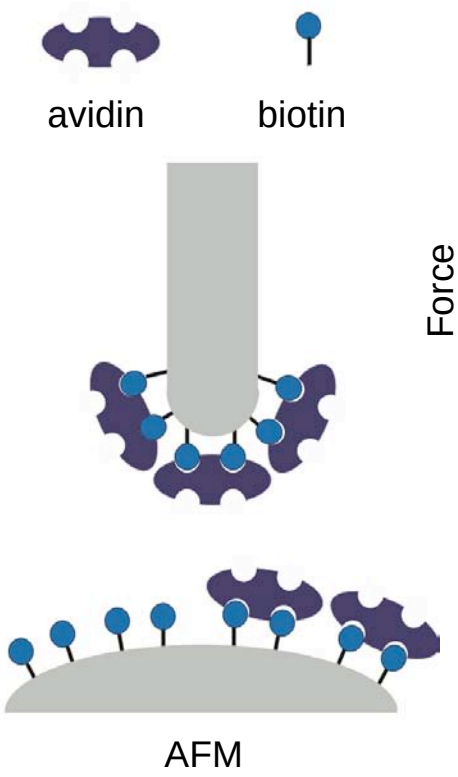

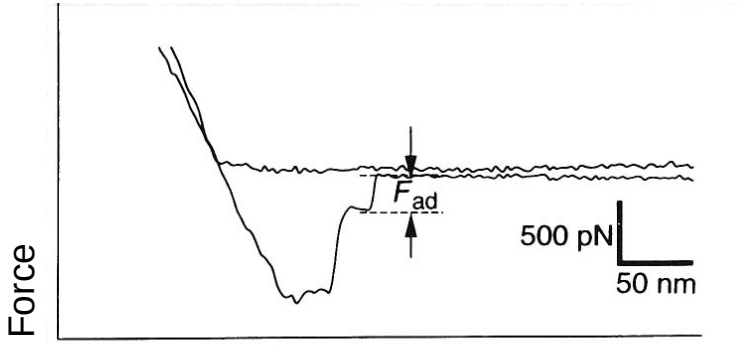

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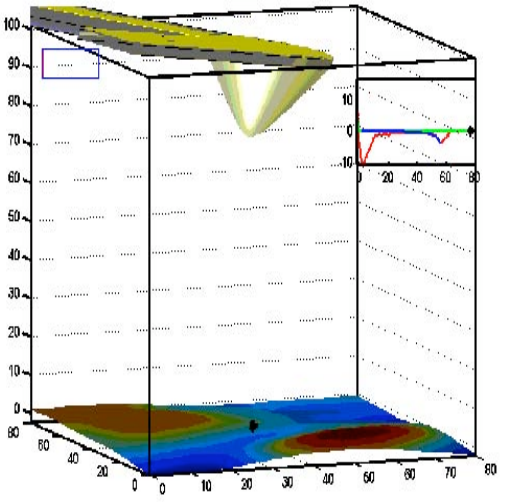
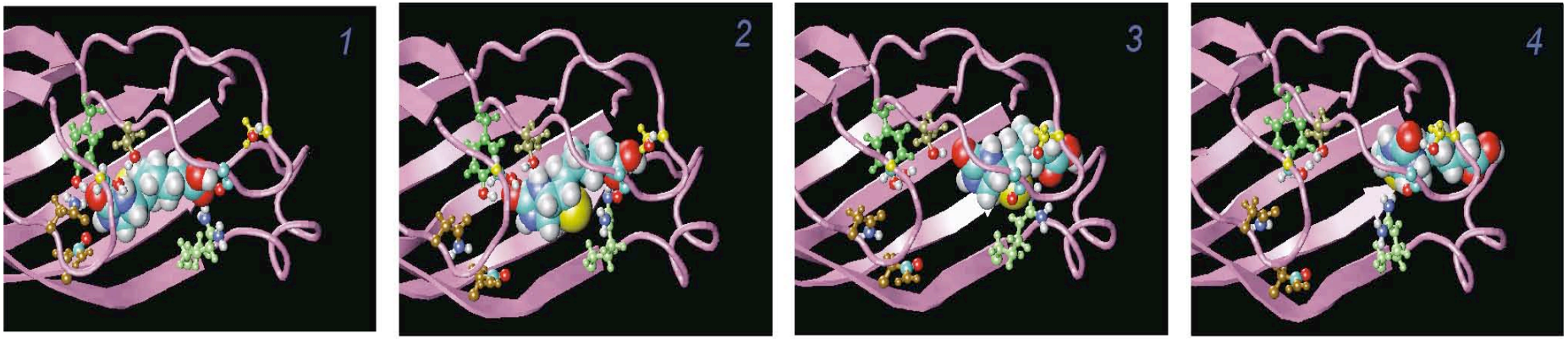


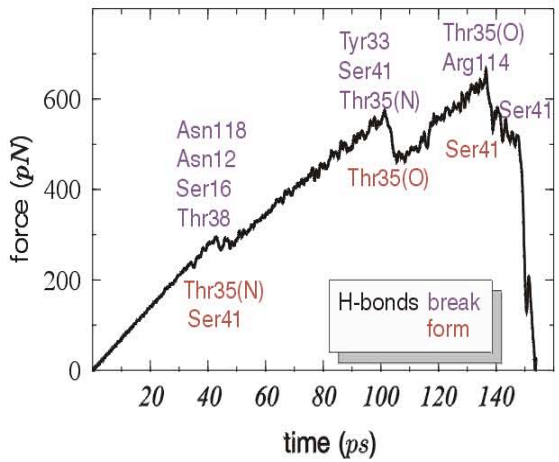
Pulling Biotin out of Avidin

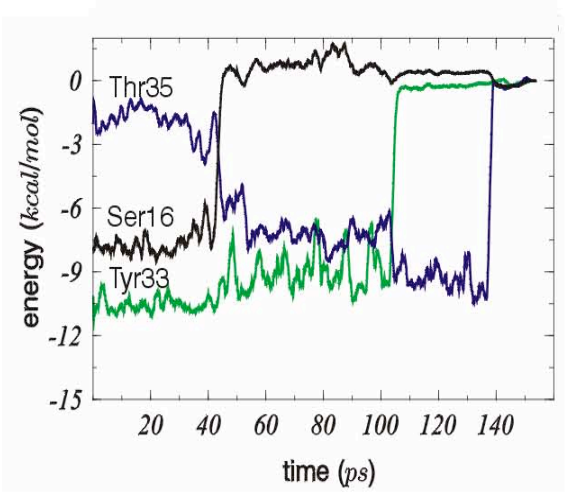
Molecular dynamics study of unbinding of the avidin-biotin complex. Sergei Izrailev, Sergey Stepaniants, Manel Balsera, Yoshi Oono, and Klaus Schulten. *Biophysical Journal*, 72:1568-1581, 1997.

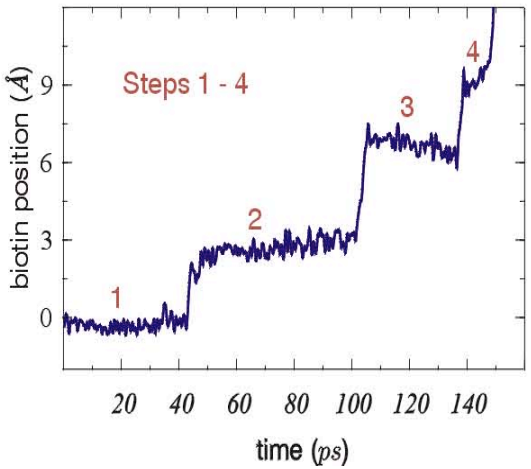
SMD of Biotin Unbinding: What We Learned

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









http://www.ks.uiuc.edu

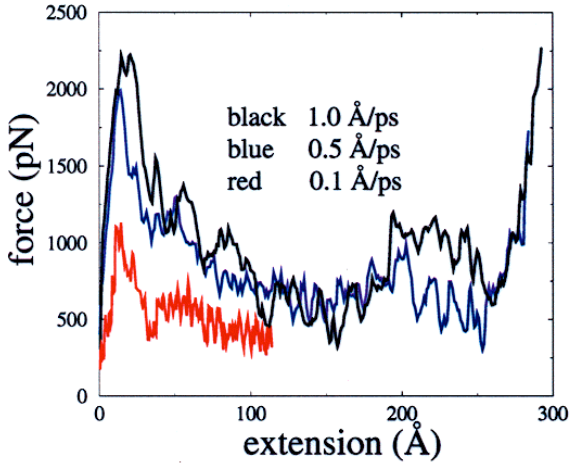
Israilev *et al.*, Biophys. J., **72**, 1568-1581 (1997)

NIH Resource for Macromolecular Modeling and Bioinformatics
Theoretical Biophysics Group, Beckman Institute, UIUC

Quantitative Comparison

Bridging the gap between SMD and AFM experiments

Force-extension curve



Schematic potentials

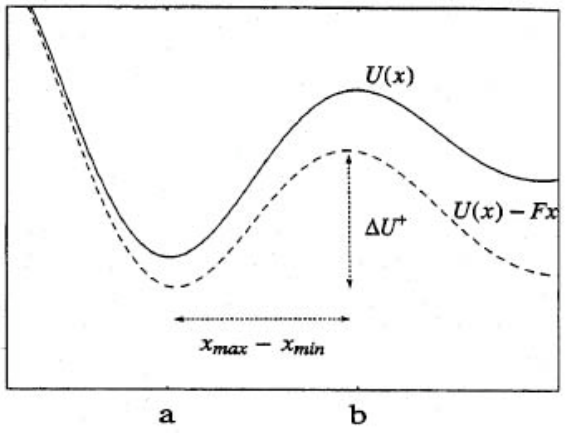
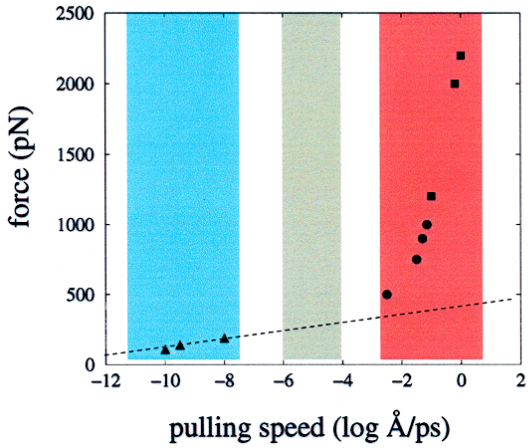


FIGURE 9 Schematic potentials $U(x)$, and $U(x) - Fx$.

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

Force-pulling velocity relationship



AFM range

Current SMD range

Target simulation range

■ ● SMD data

▲ AFM data

------ Extrapolation of AFM data

AFM regime

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

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

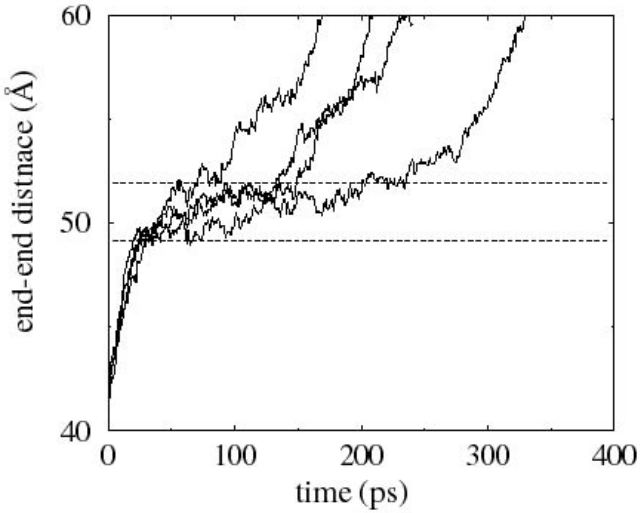
SMD regime

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

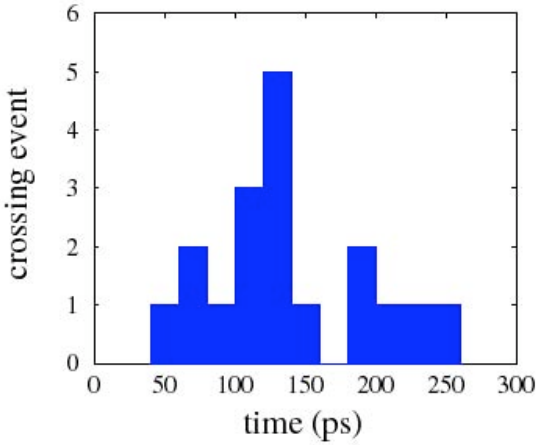
$$\tau_{SMD} \sim 2\tau_D |\delta(F)|^{-1}$$

Distribution of the Barrier Crossing Time

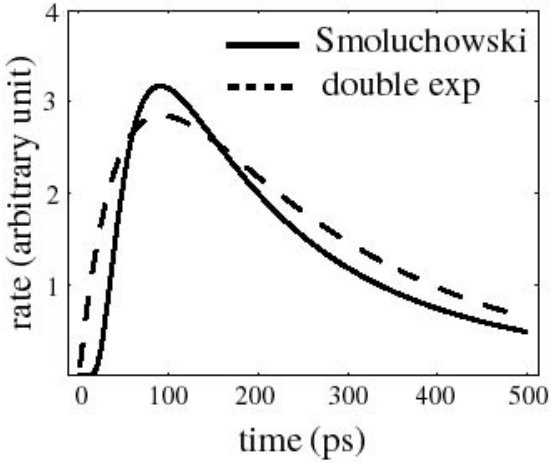
Multiple runs with same force of 750 pN



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):

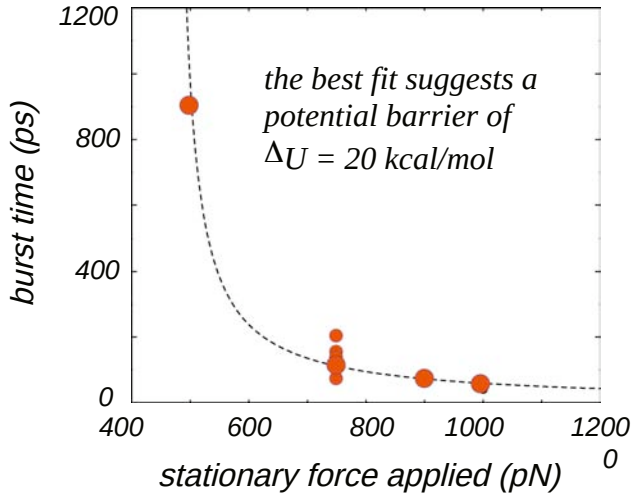
$$N(t) = \frac{1}{2} erfc\left[\frac{-a + \delta(F) D t/(b-a)}{\sqrt{4Dt}}\right] - \frac{1}{2} exp\left[\frac{\delta(F) a}{b-a}\right] erfc\left[\frac{-a + \delta(F) D t/(b-a)}{\sqrt{4Dt}}\right]$$

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)

Rupture/Unfolding Force F_0 and its Distribution

determination of barrier height based on mean first passage time



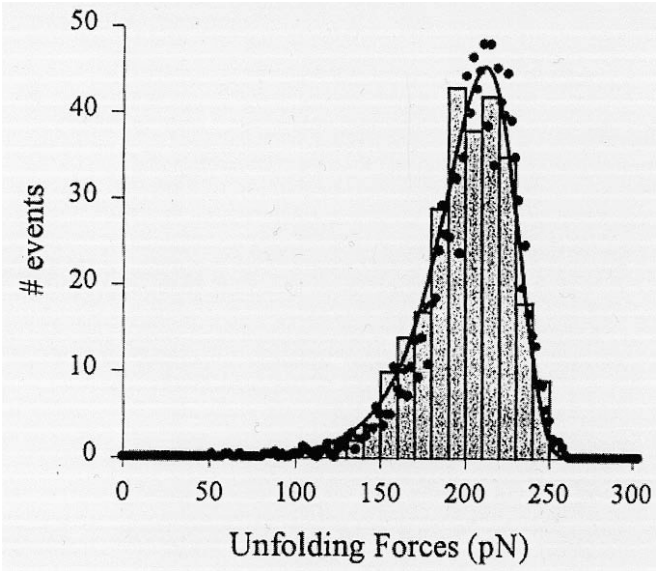
$\tau(F_0)$ = 1 ms time of measurement

=> F_0 rupture/unfolding force

Distribution of rupture/unfolding force

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

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



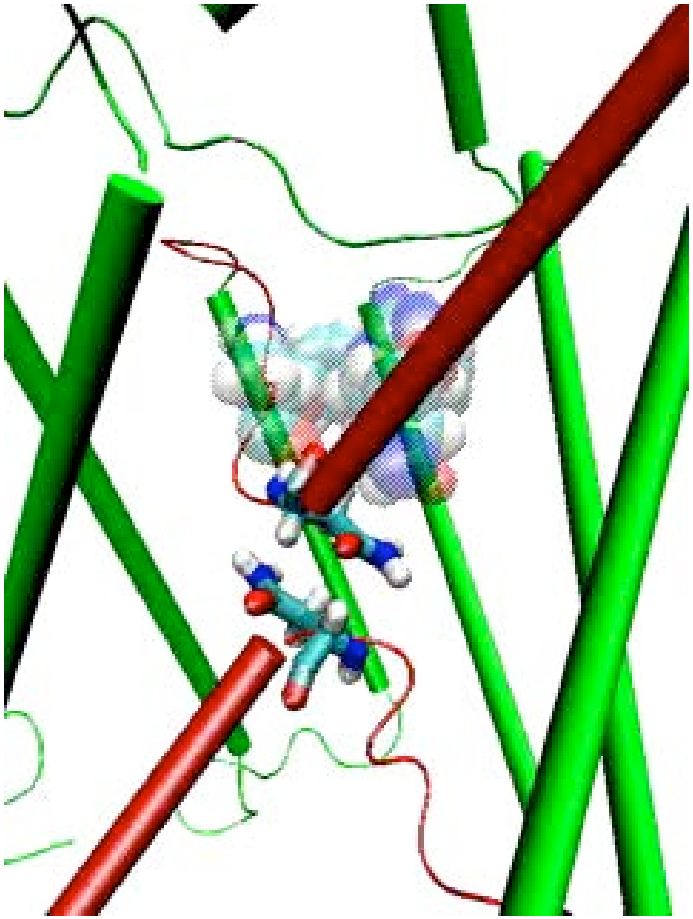
Israilev *et al.,* Biophys. J., **72**, 1568-1581 (1997)

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

Interactive Molecular Dynamics

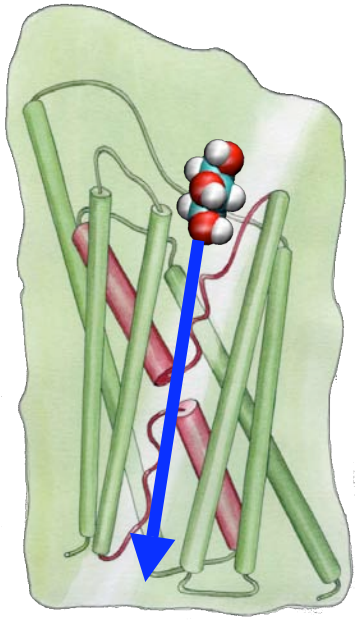
VMD ◄·····► **NAMD**

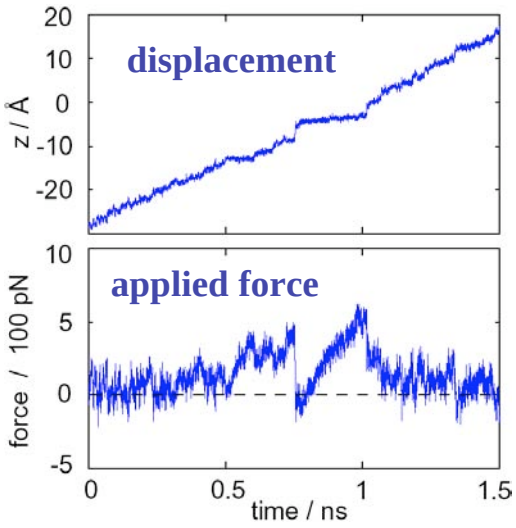
- Any PC/Workstation
- Supports 3D force-feedback devices for interaction

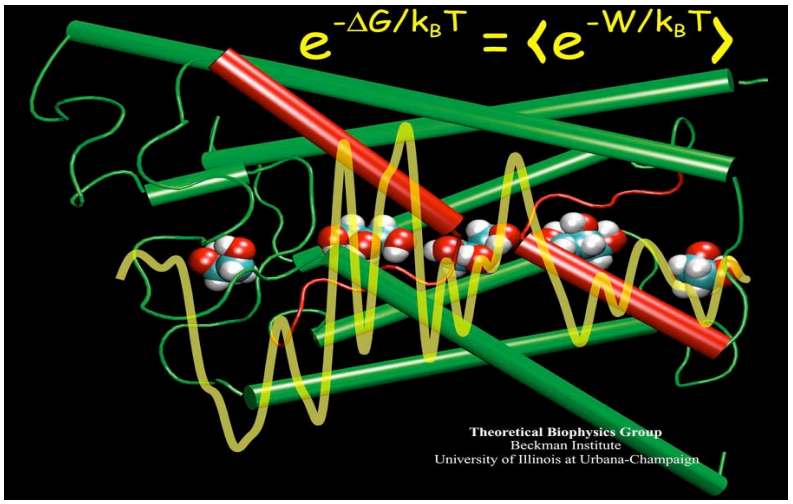


J. Stone, J. Gullingsrud, K. Schulten, and P. Grayson.
A System for Interactive Molecular Dynamics Simulation.
2001 ACM Symposium on Interactive 3D Graphics, pp.191-194, ACM SIGGRAPH
P. Grayson, E. Tajkhorshid, and K. Schulten.
Biophysical J, **83**: 36 (2003)

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

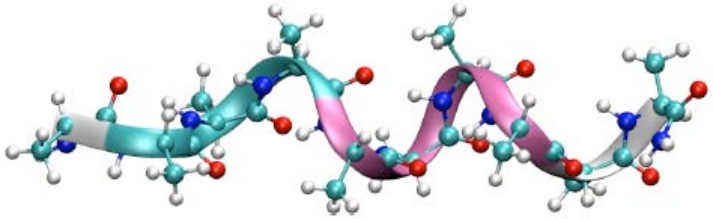
Thermodynamics: $\Delta G \leq \langle W \rangle$

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

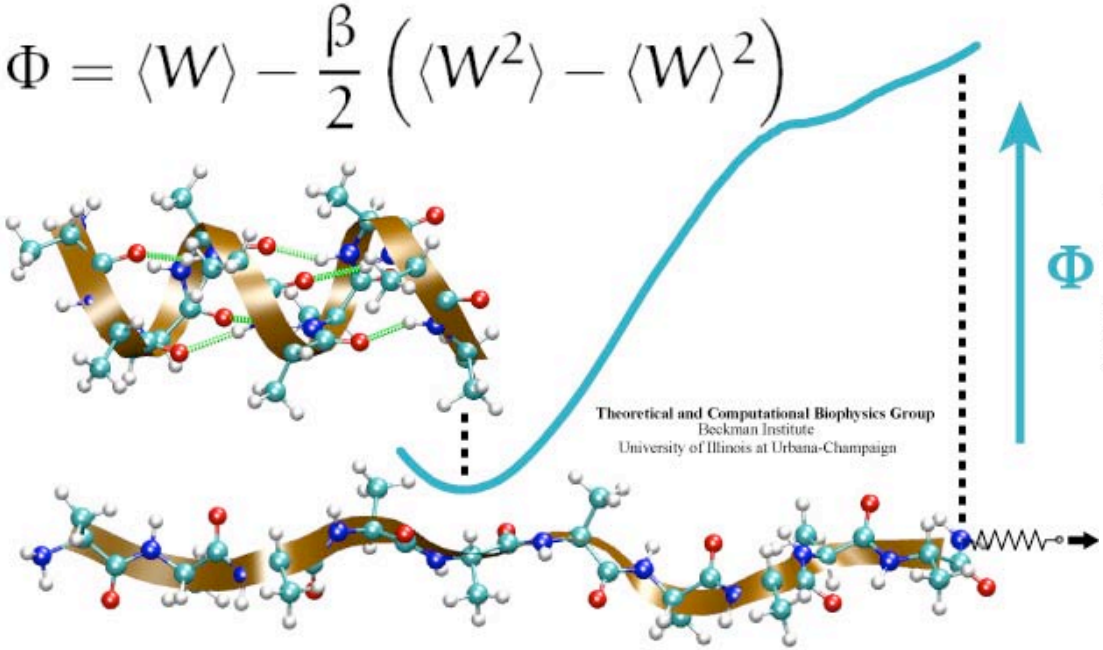
Free Energy of Stretched Alpha-Helix (Deca-alanin)

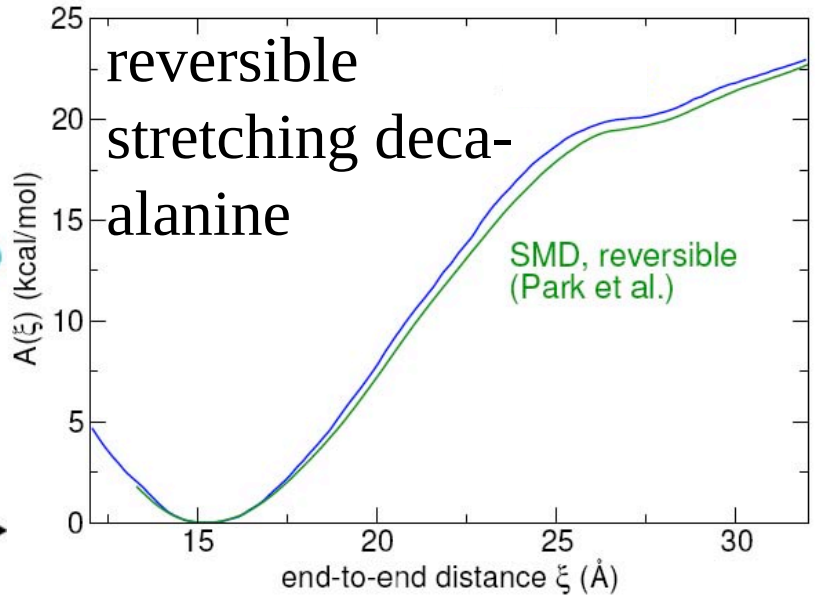
Thermodynamics: $\Delta G \le \langle W \rangle$

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



$$\Phi = \langle W \rangle - \frac{\beta}{2}\left(\langle W^2 \rangle - \langle W \rangle^2\right)$$



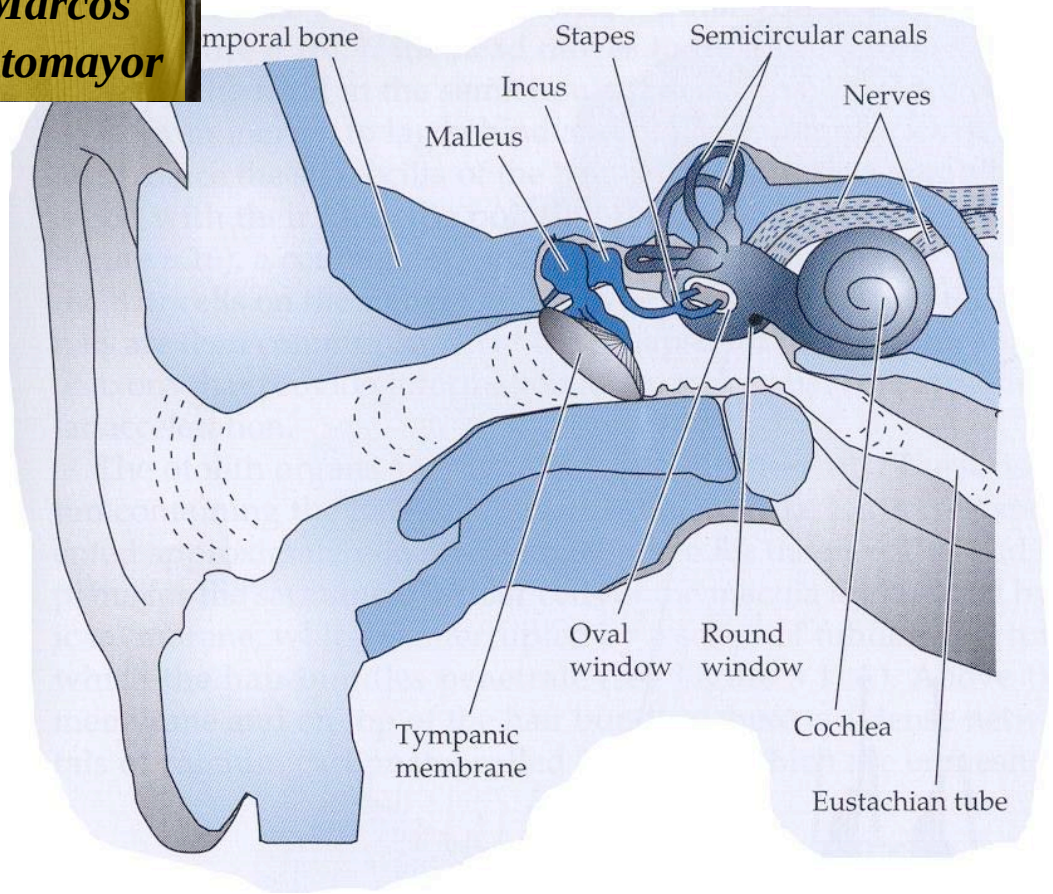


Free energy calculation from steered molecular dynamics simulations using Jarzynski's equality. S. Park, F. Khalili-Araghi, E. Tajkhorshid, and K. Schulten. *Journal of Chemical Physics* , 119:3559-3566, 2003

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

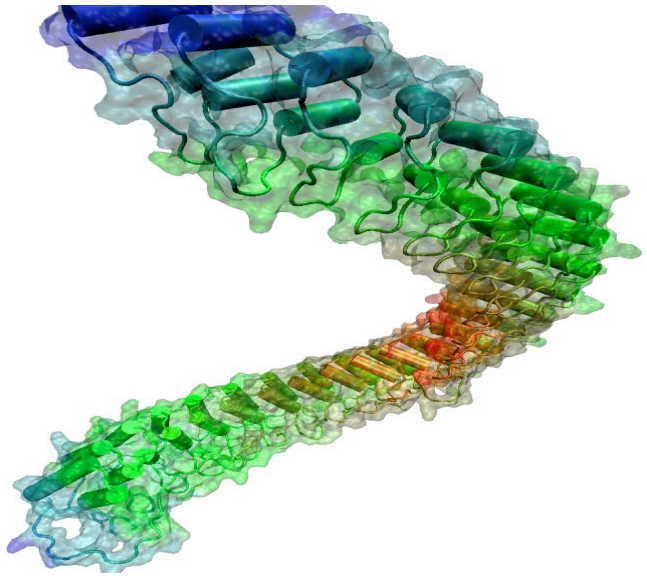
Molecular Basis of Hearing

Molecular Modeling Ahead of Observation



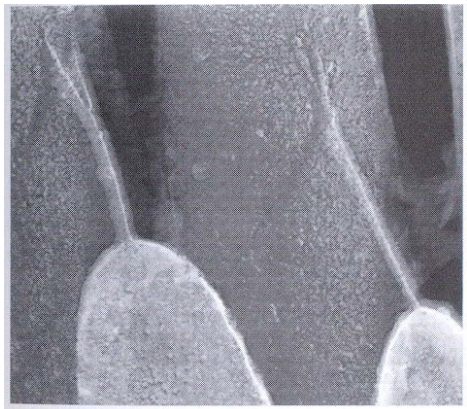
Mammalian Inner Ear (from Sensory Transduction, G. L. Fain).

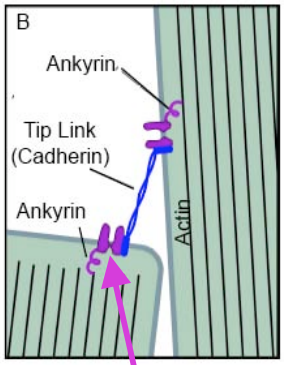
340,000 atoms

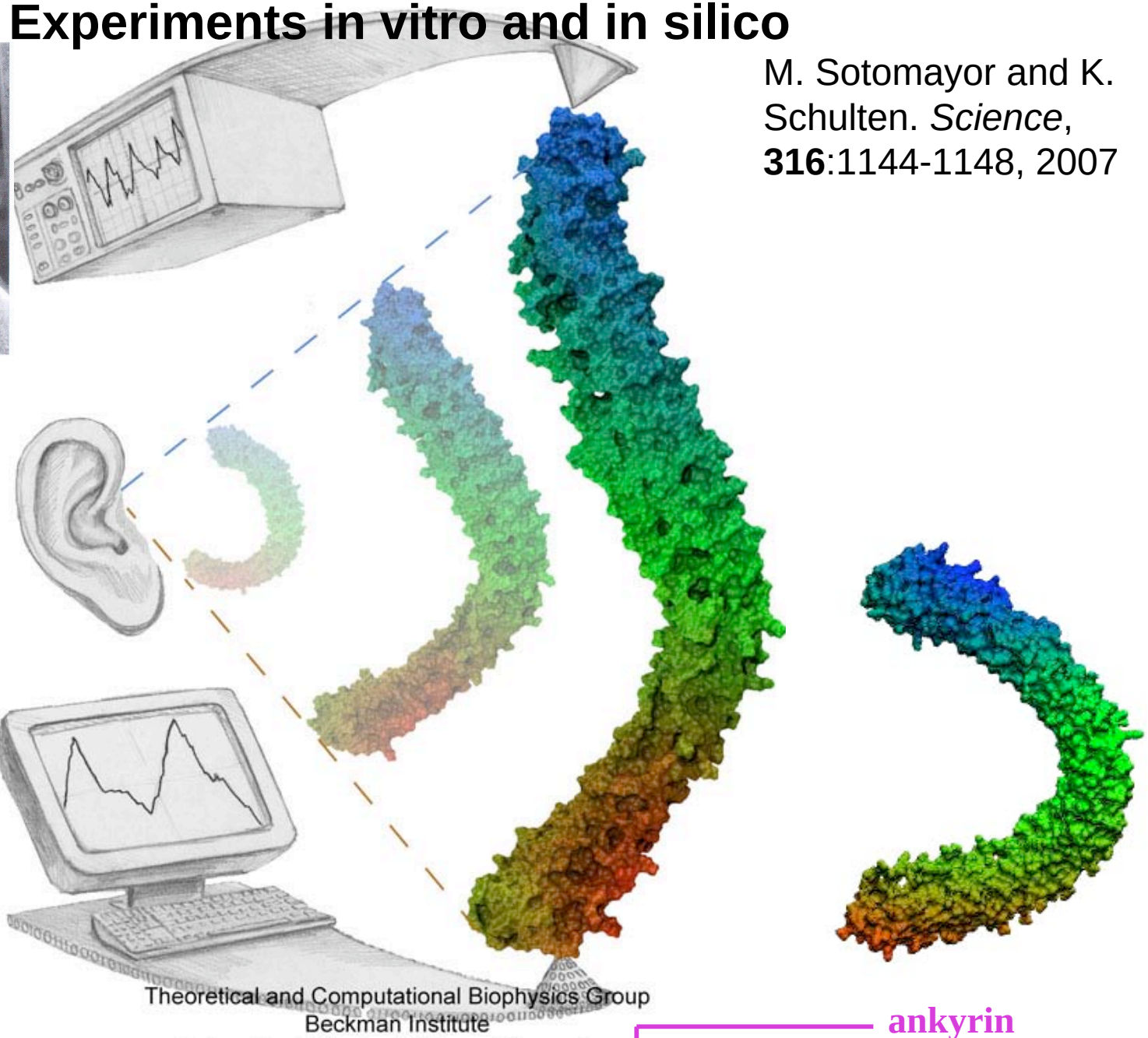


Ankyrin -
gating spring in the inner ear hair cells

Protein Elasticity and Unfolding by Single Molecule Experiments in vitro and in silico







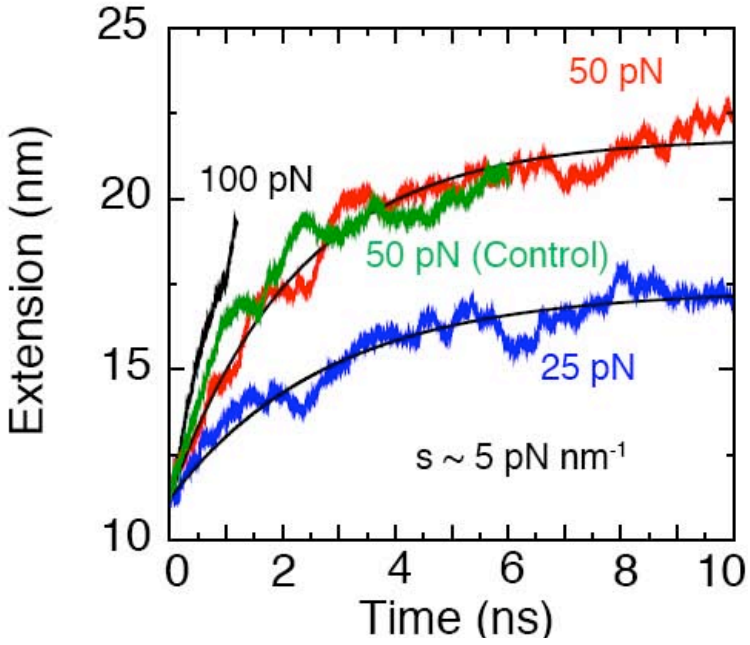
M. Sotomayor and K. Schulten. *Science*, **316**:1144-1148, 2007

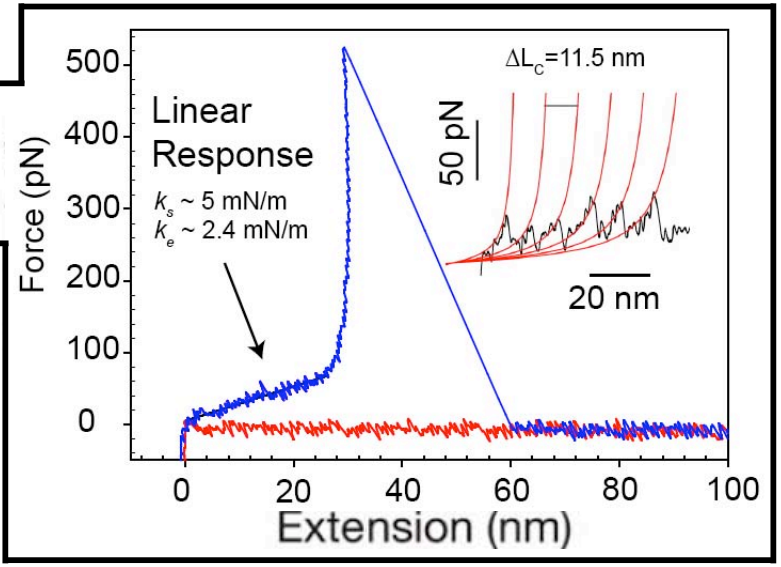
ankyrin

Hookean Elasticity of 24 repeats of Ankyrin measured by Single Molecule Force Spectroscopy by AFM

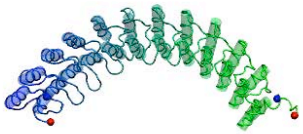
Simulation: M. Sotomayor, D. P. Corey, and K.Schulten. In search of the hair-cell gating spring: Elastic properties of ankyrin and cadherin repeats. *Structure*, 13:669-682, **2005.**

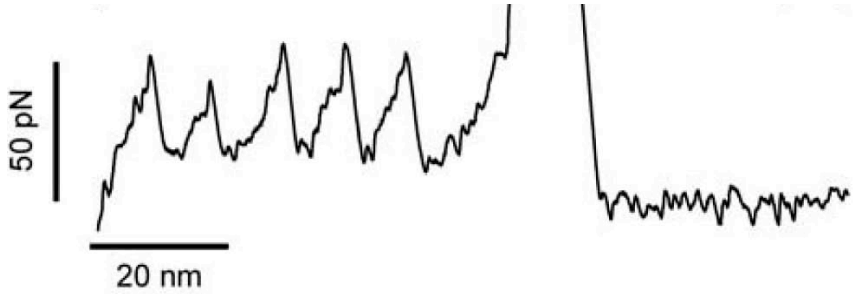
Experiment: G. Lee *et al.*, *Nature* **440**, 246-249, **2006**; L. Li, S. Wetzel, A. Pluckthun, and J. M. Fernandez, Biophys. J. 90, L30–L32, **2006**.



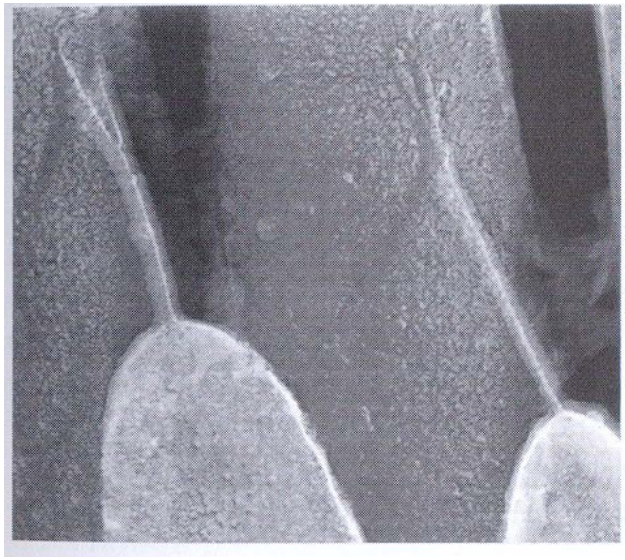


Ankyrin - Secondary Structure Spring at Large Force



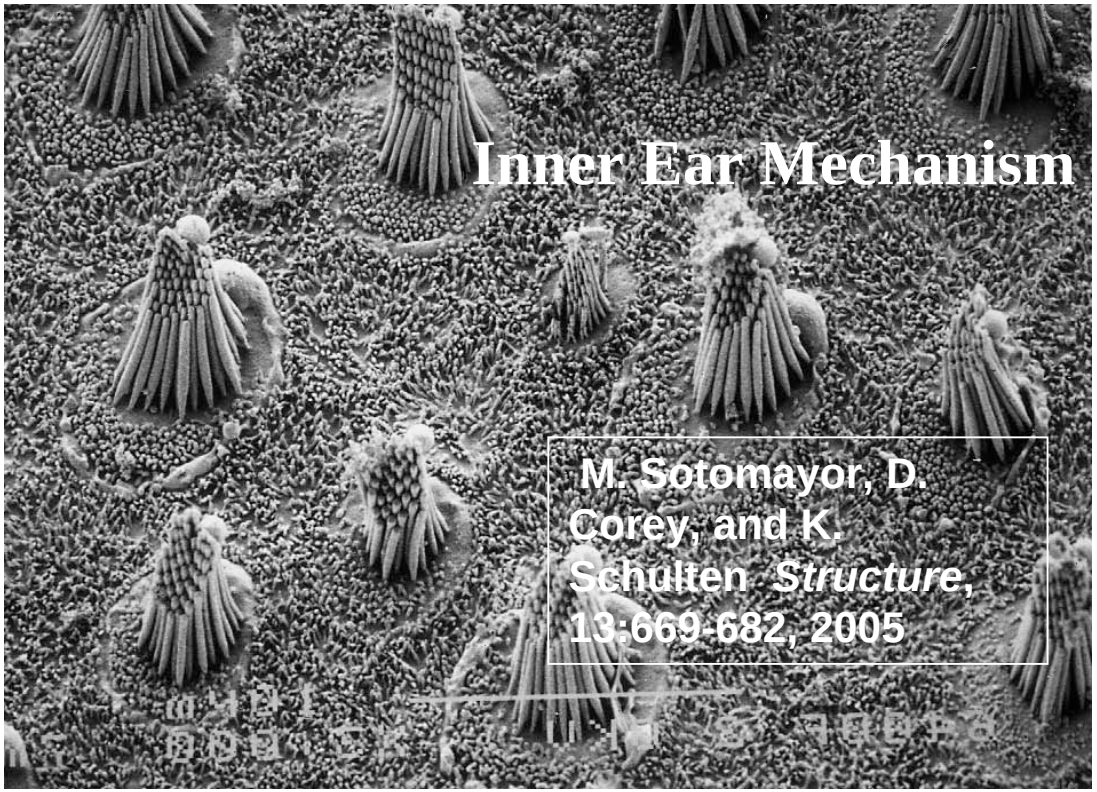


Experiment:
L. Li, S. Wetzel, A. Pluckthun, and J. M. Fernandez, Biophys. J. 90, L30–L32, 2006.

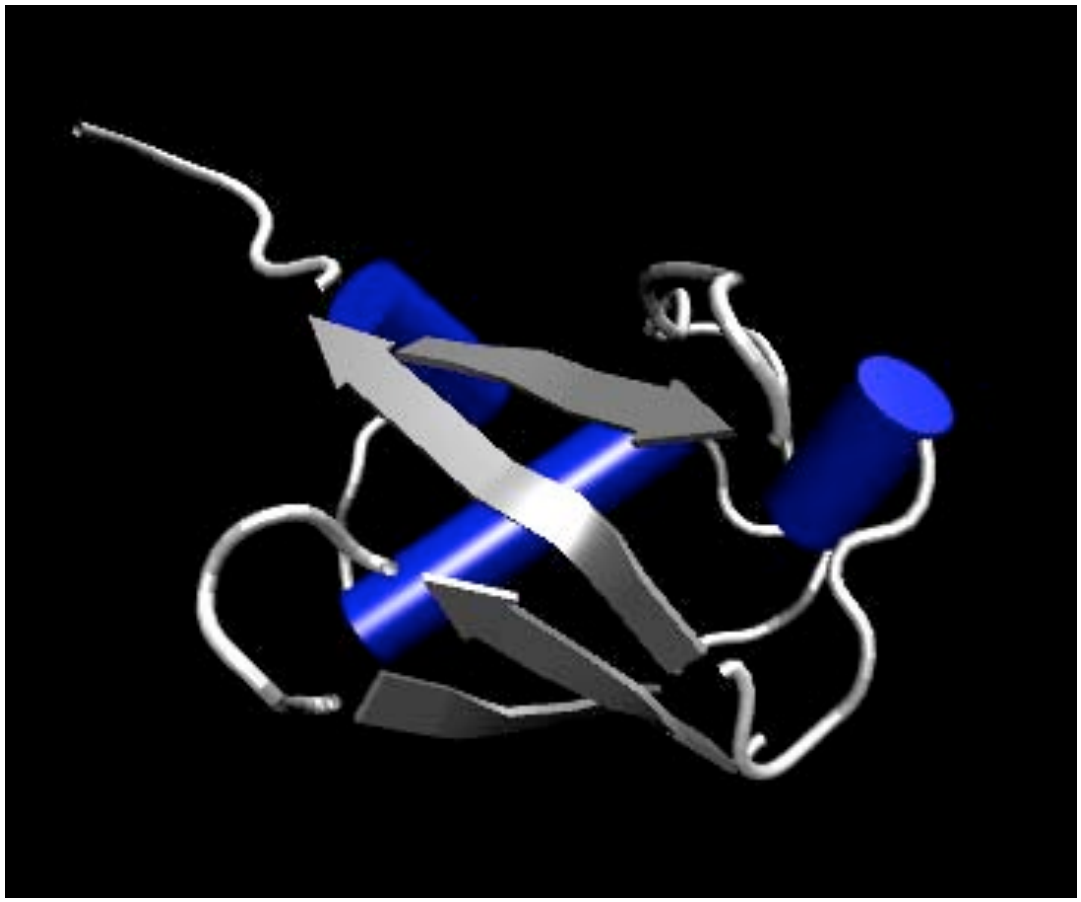


Tip Links (Kachar et al., 2000; Corey Lab)

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

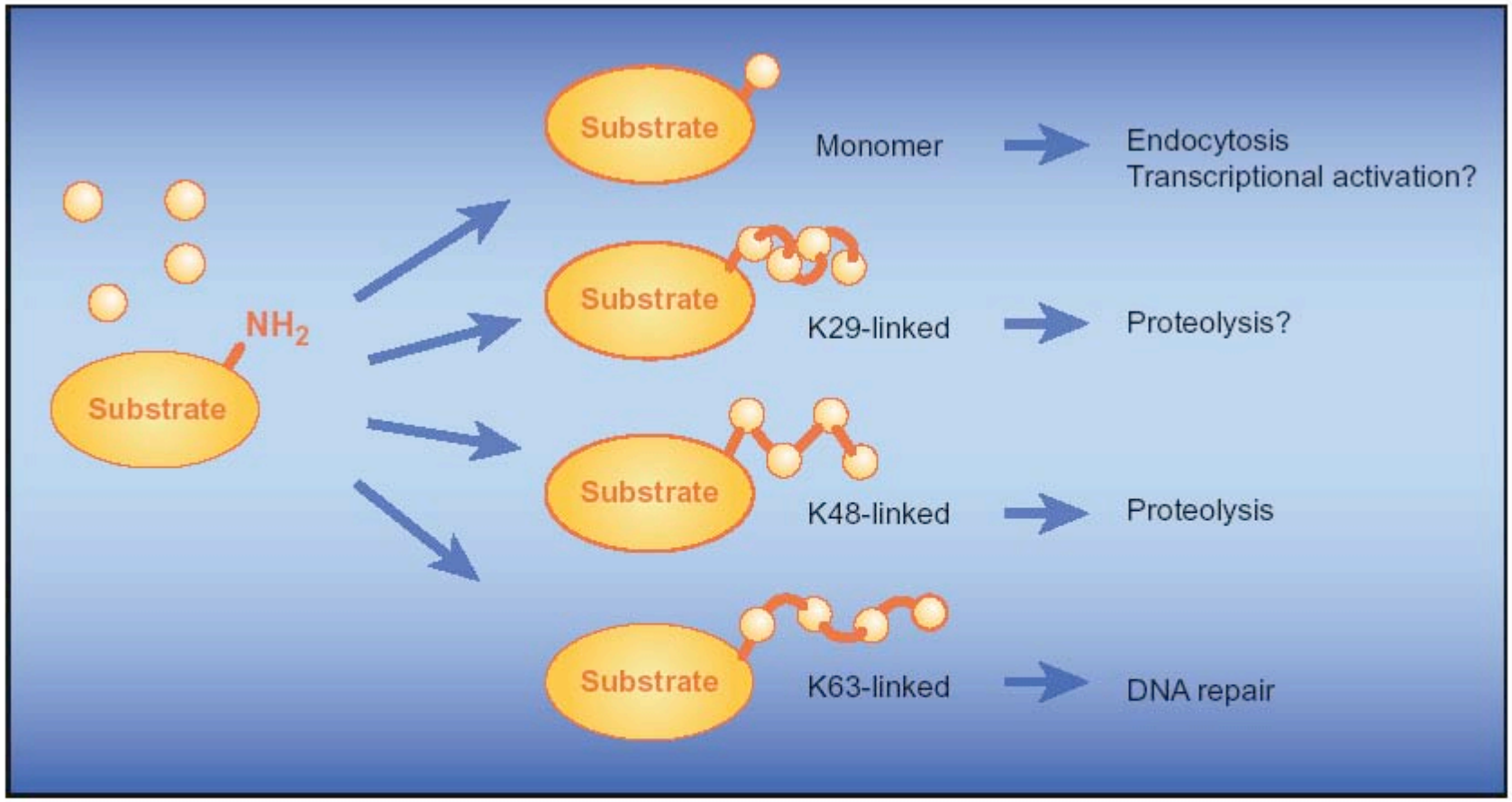


Ubiquitin



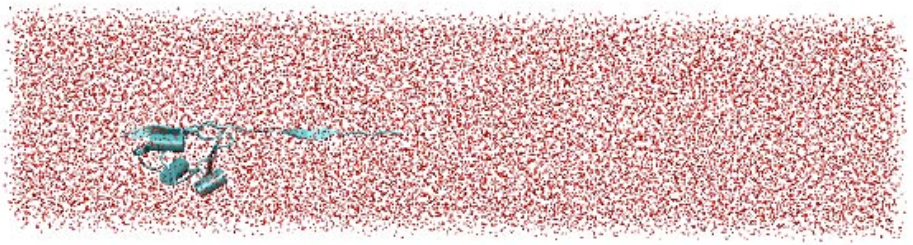
Fatemeh Araghi, Timothy Isgro, Marcos Sotomayor

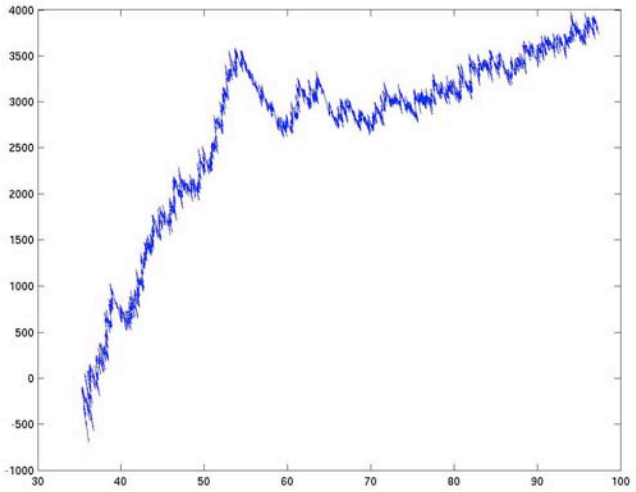
Monoubiquitylation versus multi-ubiquitylation



Multifaceted. Ubiquitin can attach to its various substrate proteins, either singly or in chains, and that in turn might determine what effect the ubiquitination has. (K29, K48, and K63 refer to the particular lysine amino acid used to link the ubiquitins to each other.)

First SMD Simulation

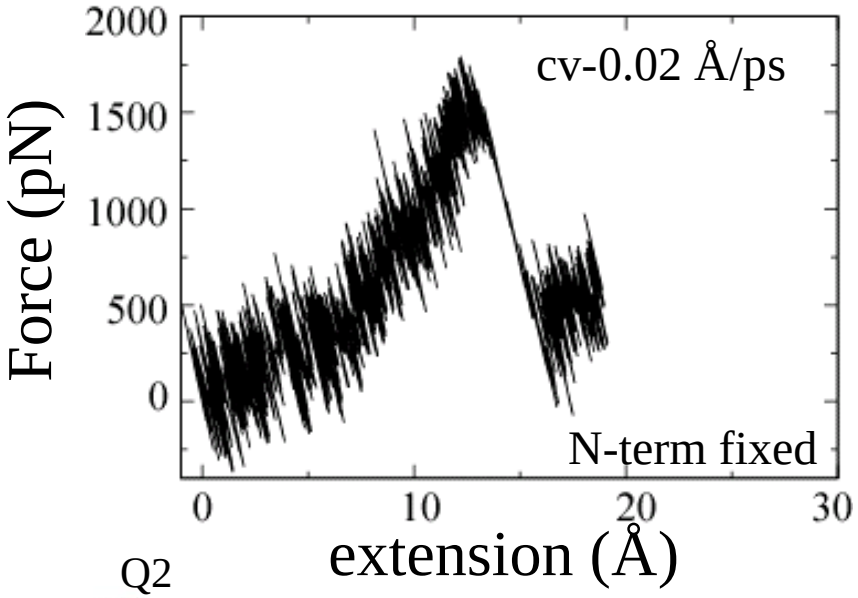


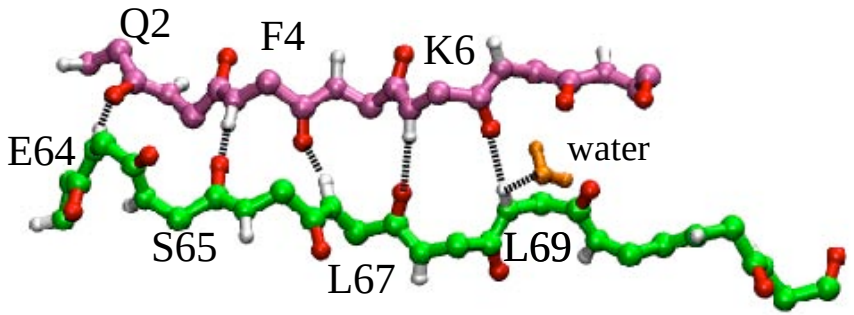


First peak when the first beta strand is stretched out

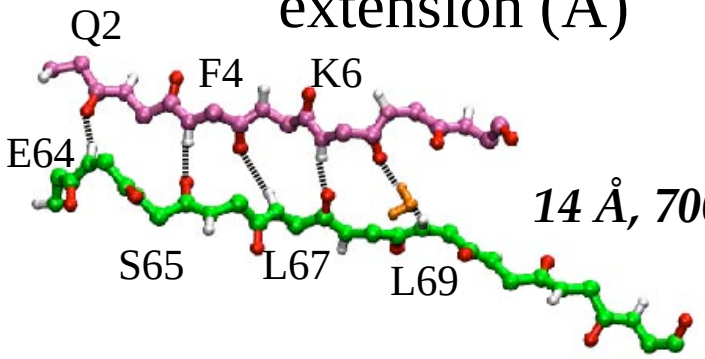
- SMD simulation, with constant velocity
- Box of water 70x240x70 A ~81K atoms
- smd velocity 0.4 A/ps
- smd spring constant 7 kcal/mol A^2

Ubiquitin Unfolding I

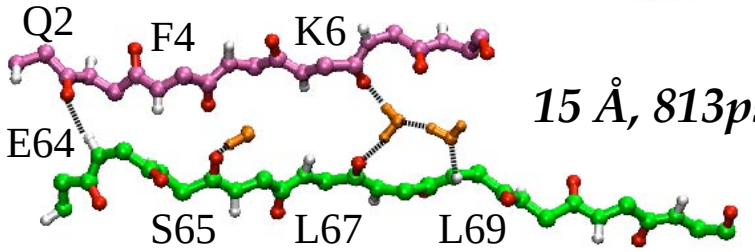




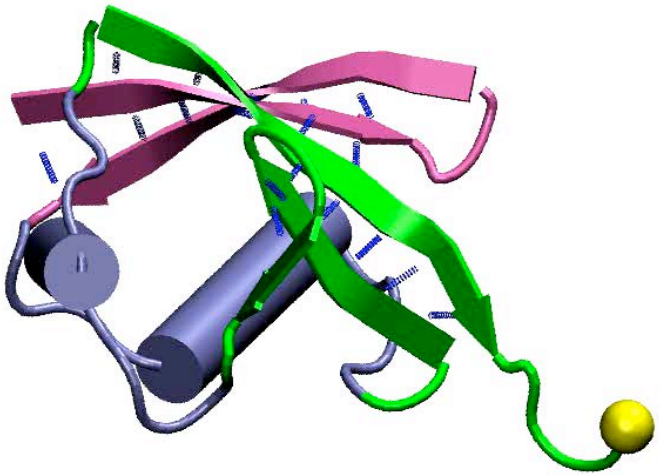
0 Å, 1 ps



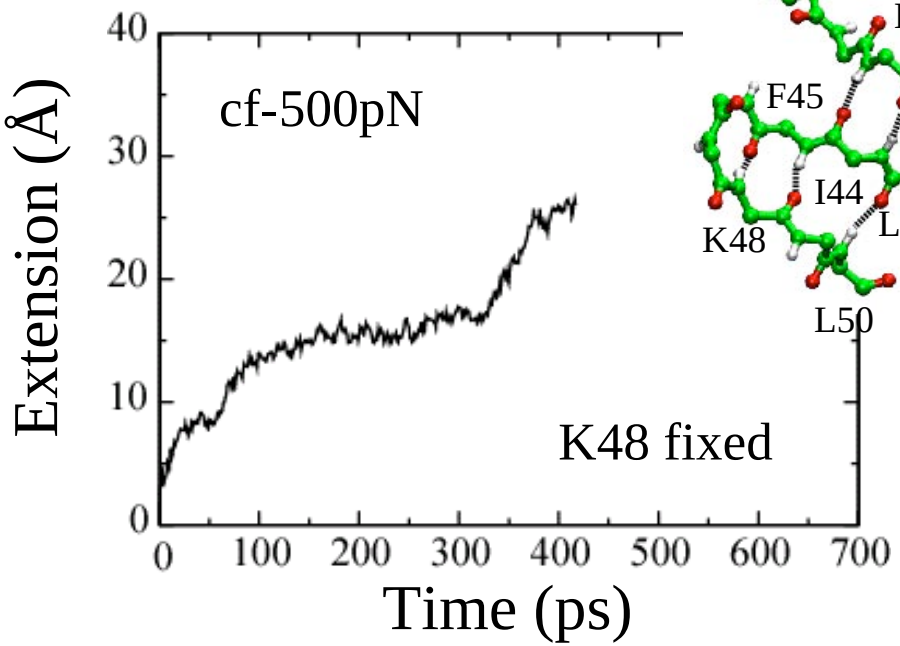
14 Å, 700ps

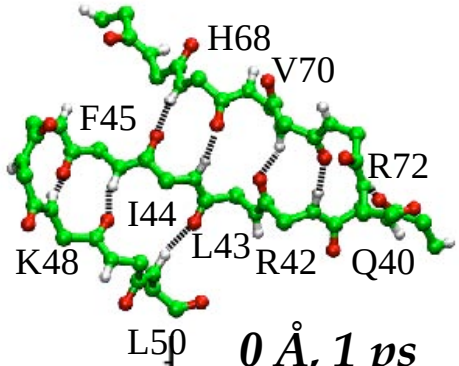


15 Å, 813ps

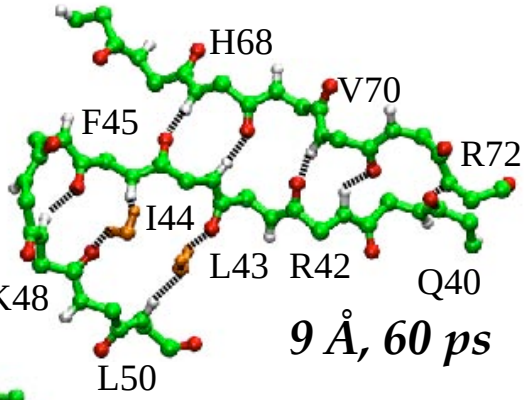


Ubiquitin Unfolding II

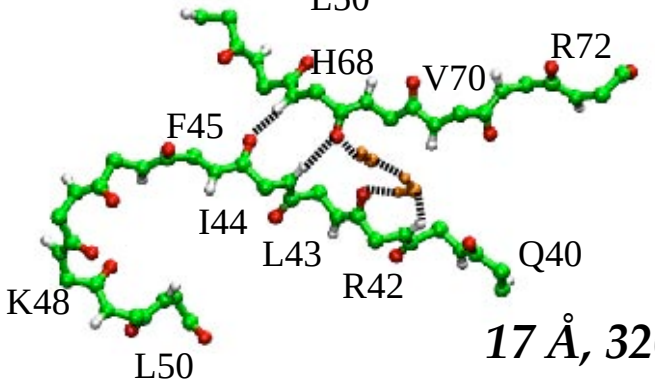




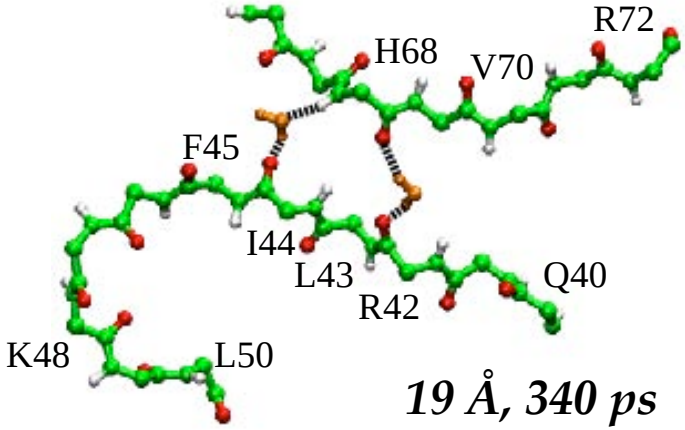
0 Å, 1 ps



9 Å, 60 ps



17 Å, 320 ps



19 Å, 340 ps