20.GEM GEM4 Summer School: Cell and Molecular Biomechanics in Medicine: Cancer
Summer 2007

GEM^4 Summer School on Cell and Molecular Mechanics in Biomedicine

June 25 - July 6 2007

Molecular mechanics

Lecture 1

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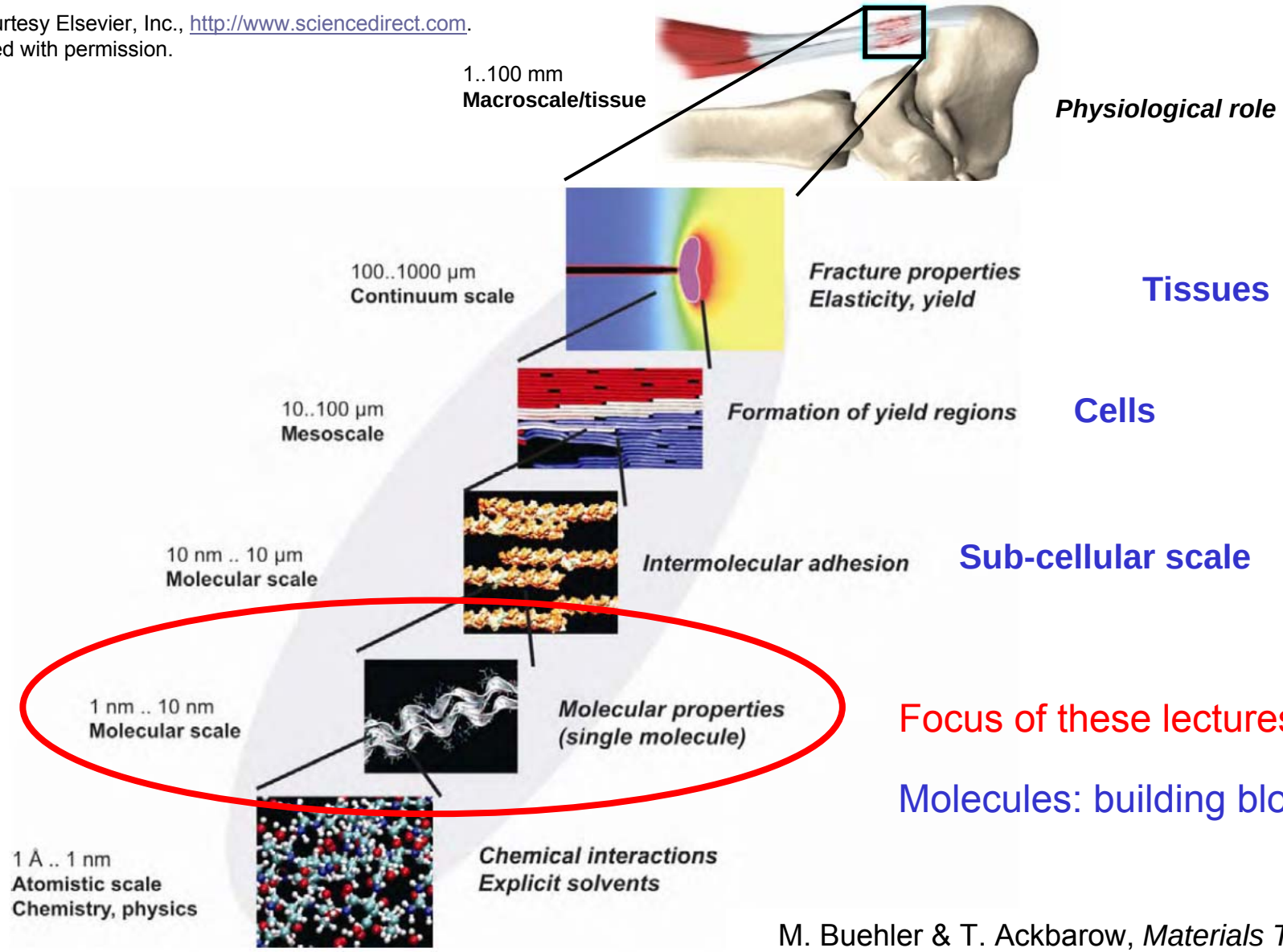
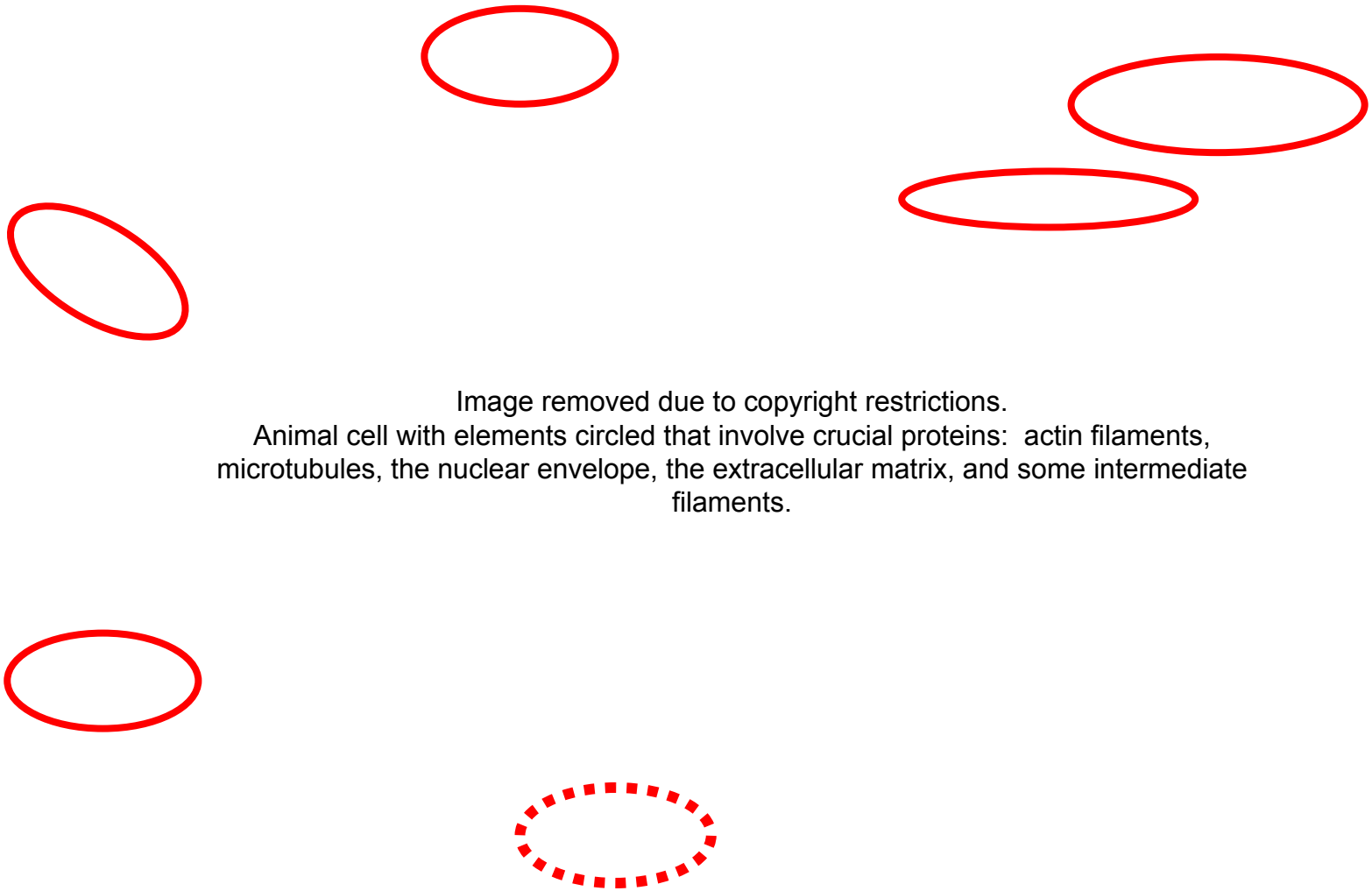
Laboratory for Atomistic and Molecular Mechanics

Molecular mechanics: Definition of scales



M. Buehler & T. Ackbarow, *Materials Today, in press*

Protein (molecules) are crucial for cellular functions



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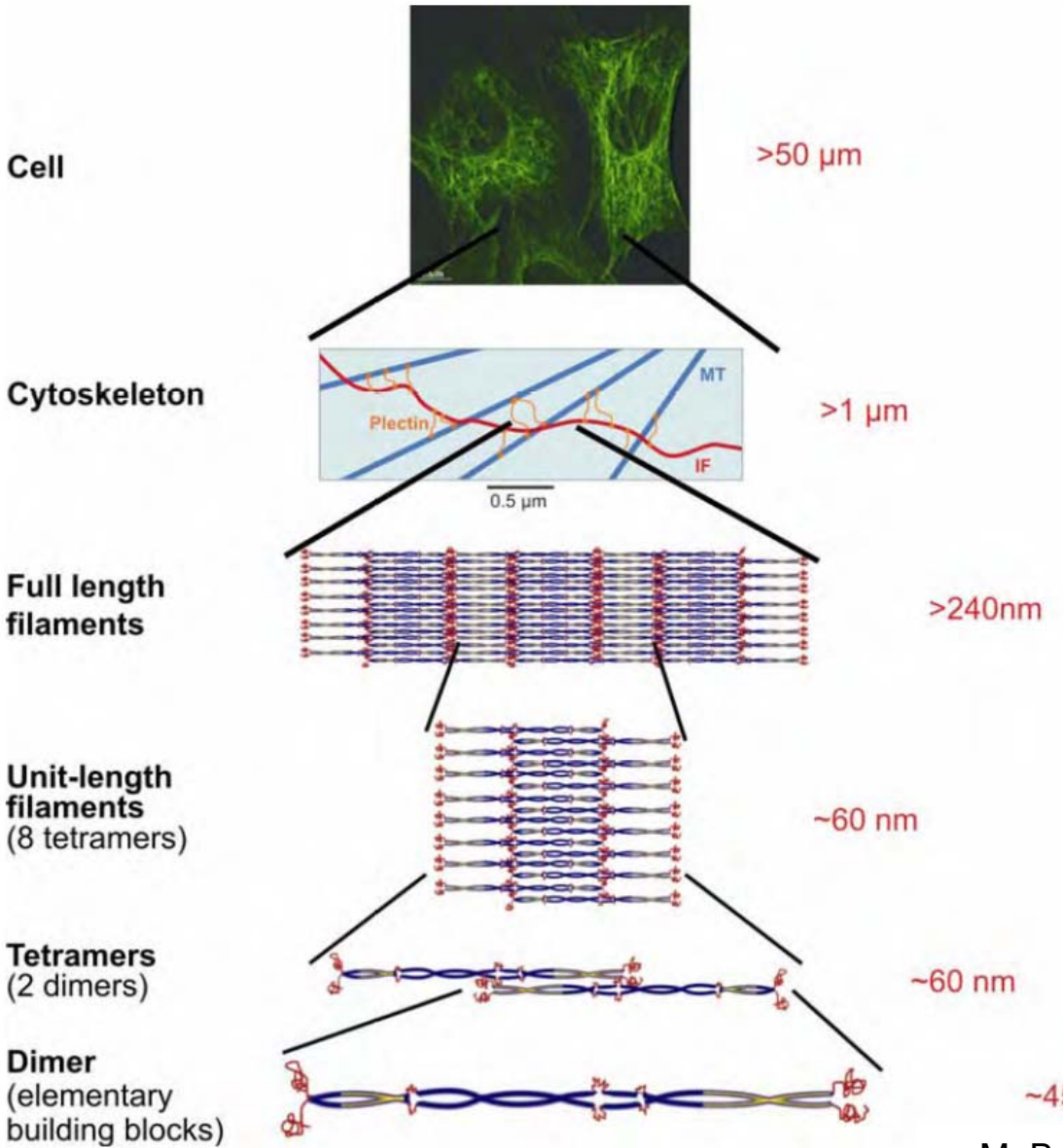
Animal cell with elements circled that involve crucial proteins: actin filaments, microtubules, the nuclear envelope, the extracellular matrix, and some intermediate filaments.

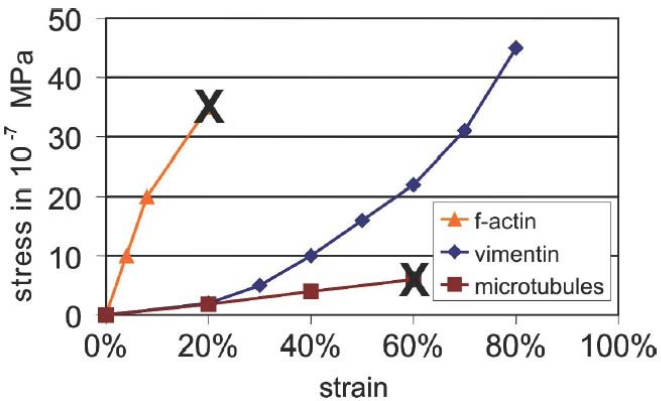
Adapted from Alberts, *The Cell*

Example: Intermediate filament (IF) proteins



Intermediate filaments (IFs)

Structure significant under large deformation (stability, mechanosensation)



Janmey PA, Euteneuer U, Traub P, Schliwa M (1991) J Cell Biol 1134 113:155

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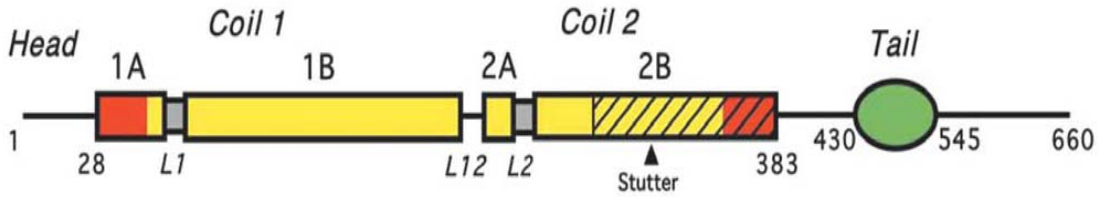
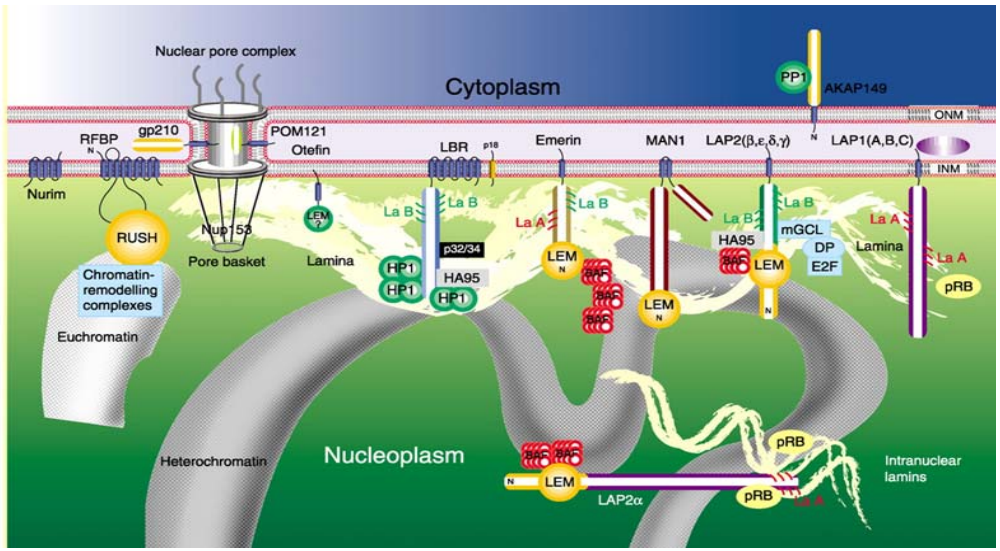
M. Buehler & T. Ackbarow, *Materials Today, in press*

Example: Nuclear envelope



Lamin filament

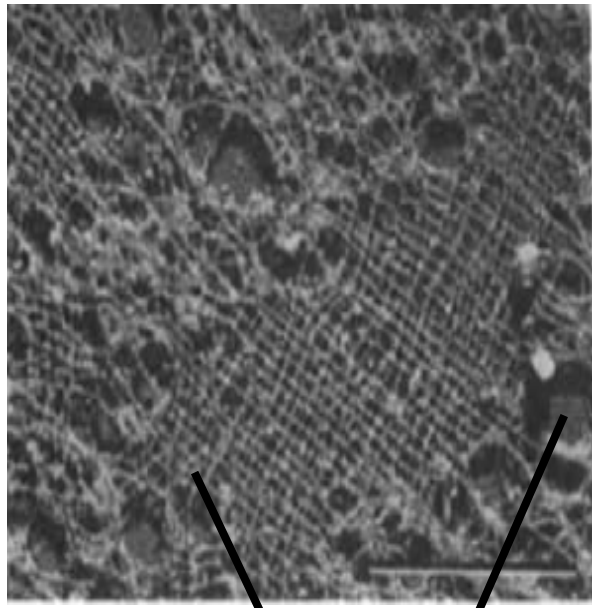


Foisner et al., 2001

Courtesy of the Company of Biologists. Used with permission.

Structural: Maintaining Nuclear Shape, Absorbing Mechanical Shock, Organizing Chromatin, etc
Biological: Regulating Cell Cycle, Controlling DNA replication, Determining Apoptosis, etc

Lamina - a mesh network
U. Aebi *et al.*



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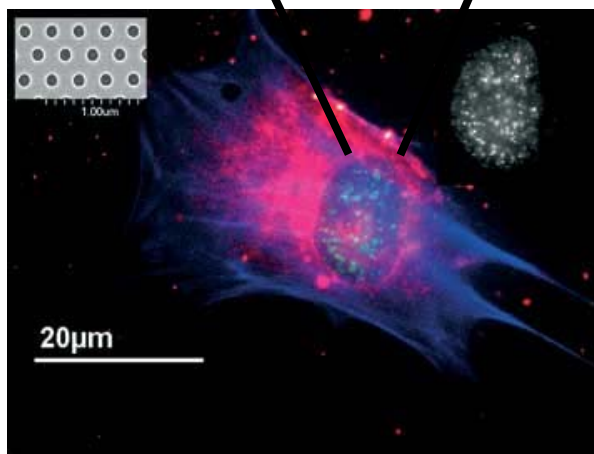
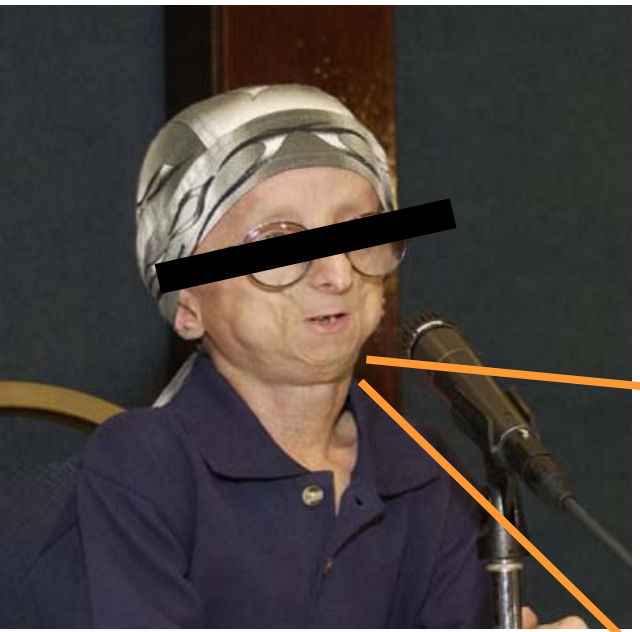


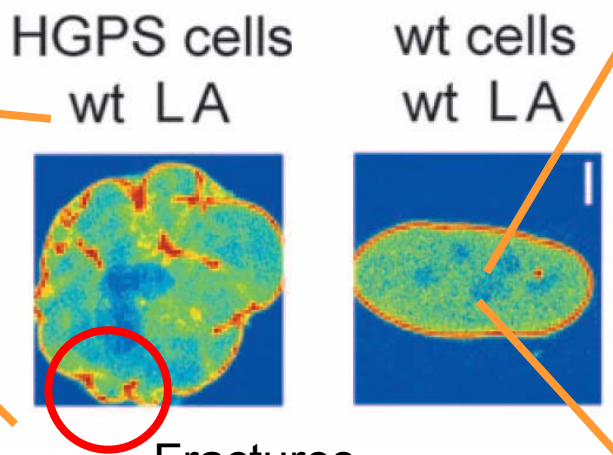
Structural change in protein molecules can lead to fatal diseases

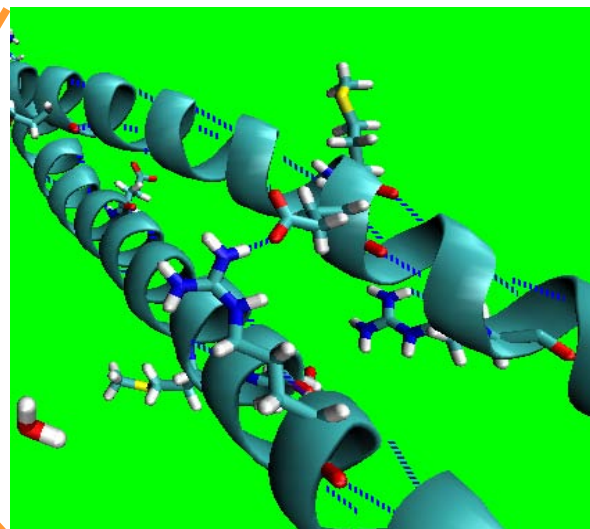
- Single point mutations in IF structure causes severe diseases such as **rapid aging disease progeria – HGPS** (*Nature*, 2003; *Nature*, 2006, *PNAS*, 2006)
- Cell nucleus loses stability under cyclic loading
- Failure occurs at heart (fatigue)



Substitution of a single DNA base: Amino acid guanine is switched to adenine

Experiment suggests that mechanical properties of nucleus change (Dahl *et al*., *PNAS*, 2006)





Role of lamin proteins in cancer

- Many cancer cells have abnormal expression of lamin: For example, HL-60 cells (type of leukemic cells) shows a change expression level of lamin A/C and B and changes in nuclear shape.
- Changes in lamin structure can lead to **abnormal expression of genes** in cancer cells
- A key step of **apoptosis** is the disassembly of lamina and detachment of chromatin: Mutation in Lamin A/C can make the cleavage site of lamina uncleavable, thus hinder apoptosis
- Understanding of molecular mechanics – e.g. assembly, stability, for instance under chemical / enzymatic signals – is vital to understand these processes rigorously

Why is mechanics important in biology?

- **Mechanics** describes the relationship between the deformation state of a material or system as a function of the applied load and boundary conditions

- **Mechanical properties are crucial for biological processes**:
  - **Functioning of biological systems** – **passive role** (structural materials such as bone, skin, tendon,…)
  - Mechanical stimulation is utilized to facilitate **signaling in biological processes** (in cells, e.g. via mechanosensation) – **passive role**
  - **Active mechanical stimulation** (protein motors) – **active role**

- **Change in mechanical properties** may interfere with biological processes and **lead to diseases**

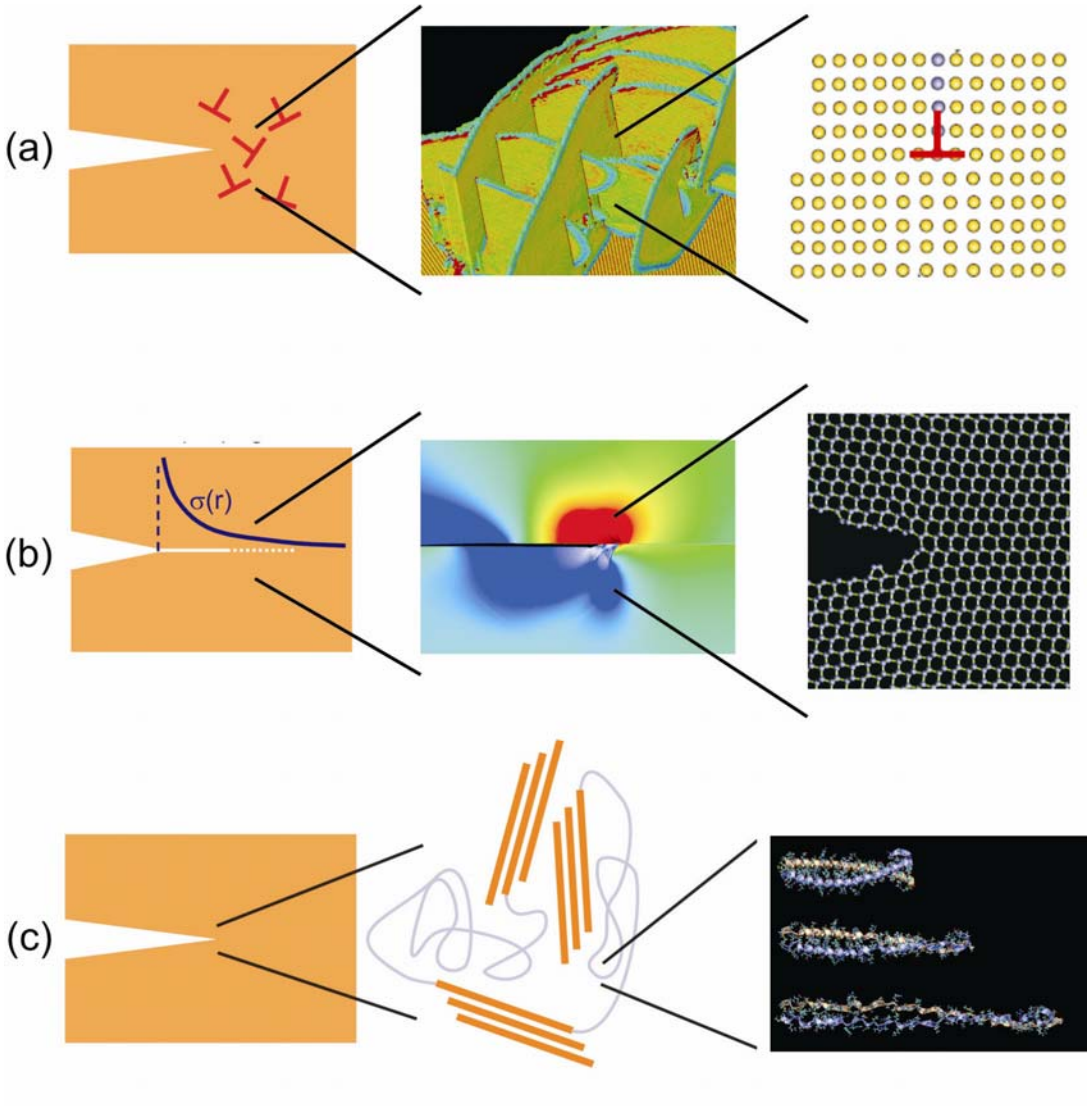
- **Pathological condition may in turn change mechanical behavior**, e.g. cells become stiffer (RBCs in malaria)

Goal of these lectures

- Molecular mechanics with focus on biomolecules (proteins)
- Topics covered:
  - Chemical bonding in molecules & theory
  - Characterization of molecular properties (tensile stiffness, persistence length, adhesion..)
  - Application of continuum mechanical concepts to molecular mechanics
  - Link of molecular properties to tissue properties (collective behavior of many molecules, elasticity, fracture,..)
  - Molecular defects and consequence for diseases: Certain mutation may induce changes in mechanical properties, e.g. molecular defects, mutations; leads to pathological consequence (too soft, too stiff,..)
- Multi-scale modeling
- Emphasis on developing a sensitivity for the significance of molecular mechanics in biology and how atomistic and continuum viewpoints can be coupled

M. Buehler & T. Ackbarow, *Materials Today, in press*

Significance of molecular mechanics from a material scientist's perspective



Metals - dislocation

Brittle materials – crack extension

Protein materials – **unfolding? Sliding?**

M. Buehler & T. Ackbarow, *Materials Today, in press*

From electrons to molecules

A brief review of chemical concepts

Atomic scale

- Atoms are composed of electrons, protons, and neutrons. Electron and protons are negative and positive charges of the same magnitude, 1.6 × 10-19 Coulombs
- Chemical bonds between atoms by interactions of the electrons of different atoms (e.g. sharing of electrons)

"Point" representation

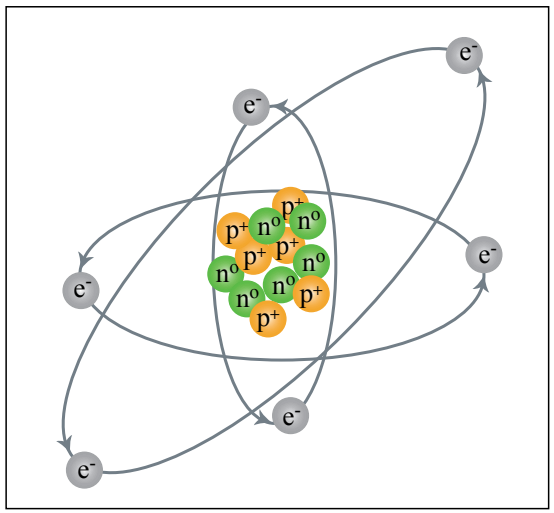
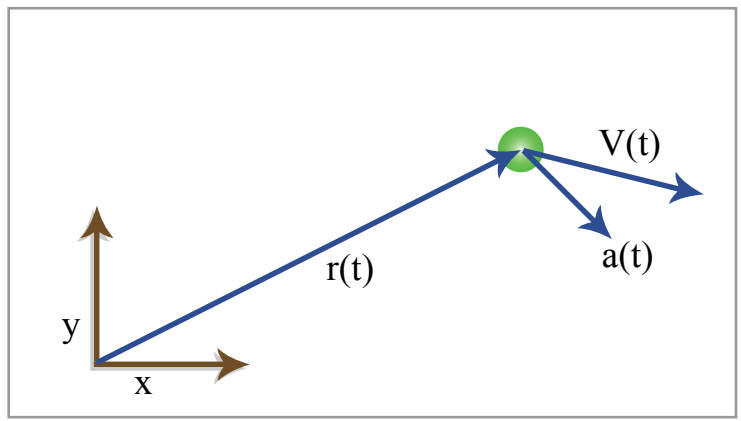


Figure by MIT OpenCourseWare.



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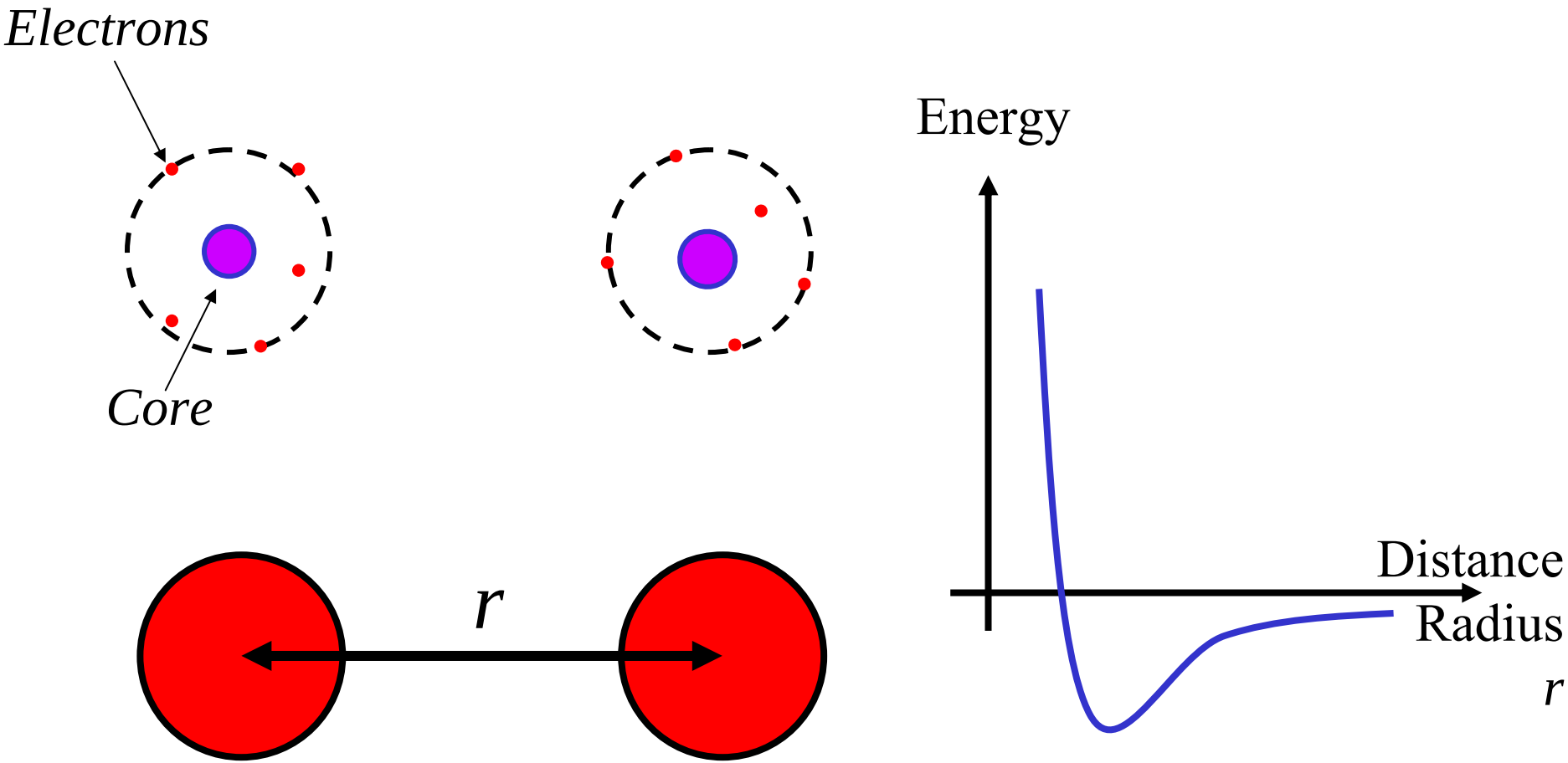
From electrons to atoms



Governed by laws of quantum mechanics: Numerical solution by Density Functional Theory (DFT), for example

Atomic interactions

- **Primary bonds ("strong")**
  - Ionic,
  - Covalent,
  - Metallic (high melting point, 1000-5000K)

Strength: several nN

- **Secondary bonds ("weak")**
  - Van der Waals,
  - Hydrogen bonds (melting point 100-500K)

Strength: 10..100 pN

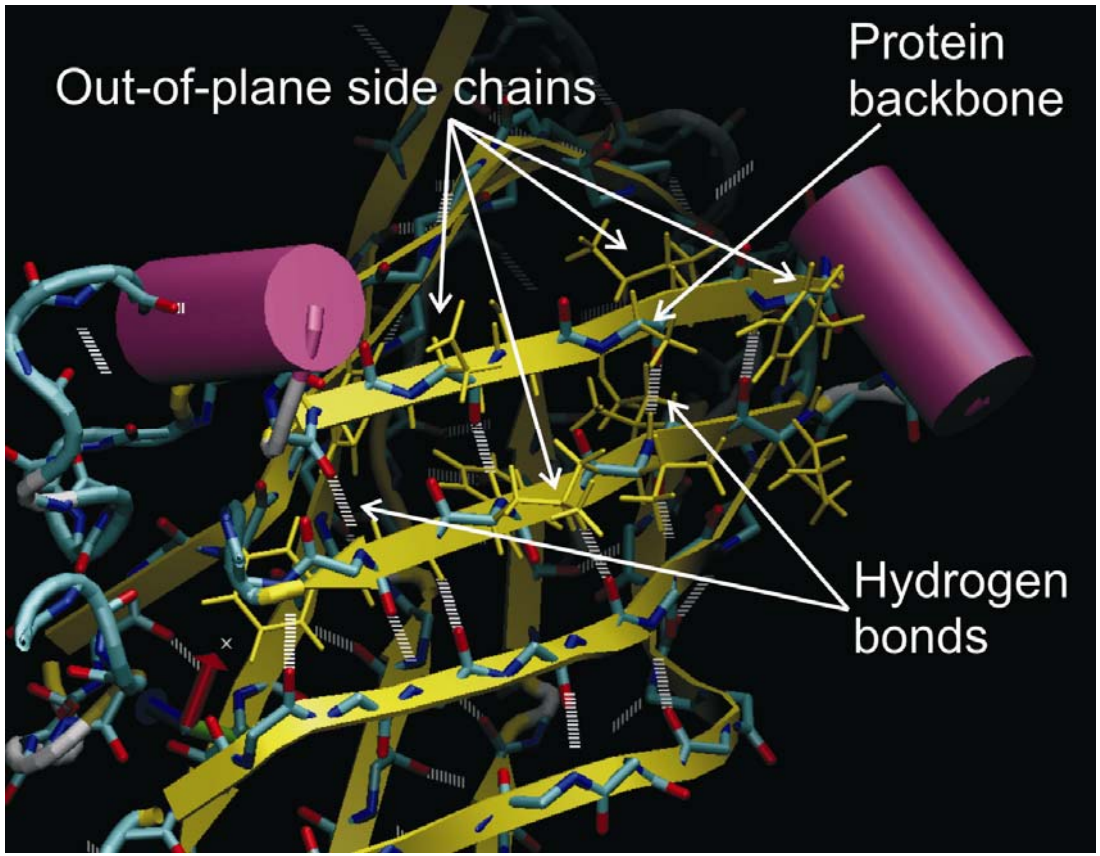
- Ionic: Non-directional
- Covalent: Directional (angles, torsions)
- Metallic: Non-directional

M. Buehler, *J. Comput. Theoretical Nanoscience, 2006*

Chemistry: A fundamental language

M. Buehler *J. Mat. Science, in press*

Proteins often include a variety of atomic interactions



Covalent bonds

Between differently charged species:

Electrostatic interactions

Hydrogen bonds

vdW interactions

Single molecule mechanics

Optical tweezer experiment

Images removed due to copyright restrictions.

Please see Figures 1(f) and 2 in Tskhovrebova, L., J. Trinick, J. A. Sleep and R. M. Simmons. "Elasticity and unfolding of single molecules of the giant muscle protein titin." Nature 387 (1997): 308-312.

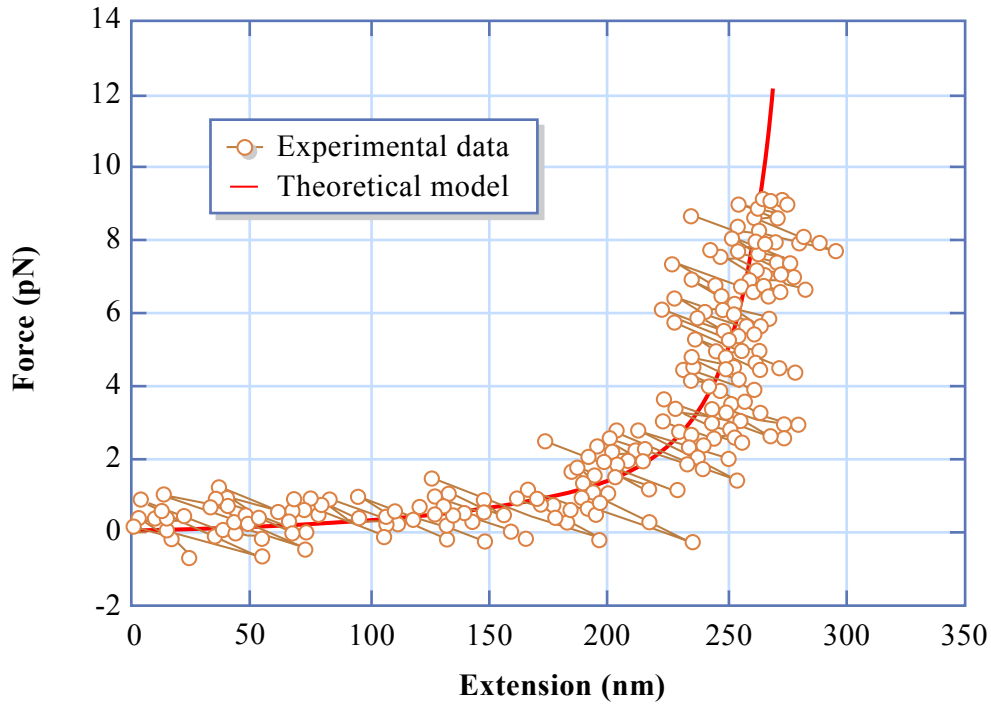
Protein unfolding

Image removed due to copyright restrictions.

Please see Figure 3(a) in Marszalek, Piotr E., et al. "Mechanical unfolding intermediates in titin modules." Nature 402 (1999): 100-103.

http://www.nature.com/nature/journal/v387/n6630/pdf/387308a0.pdf
http://www.nature.com/nature/journal/v402/n6757/pdf/402100a0.pdf

Stretching of tropocollagen molecules



The force-extension curve for stretching a single type II collagen molecule. The data were fitted to Marko-Siggia entropic elasticity model. The molecule length and persistence length of this sample is 300 and 7.6 nm, respectively.

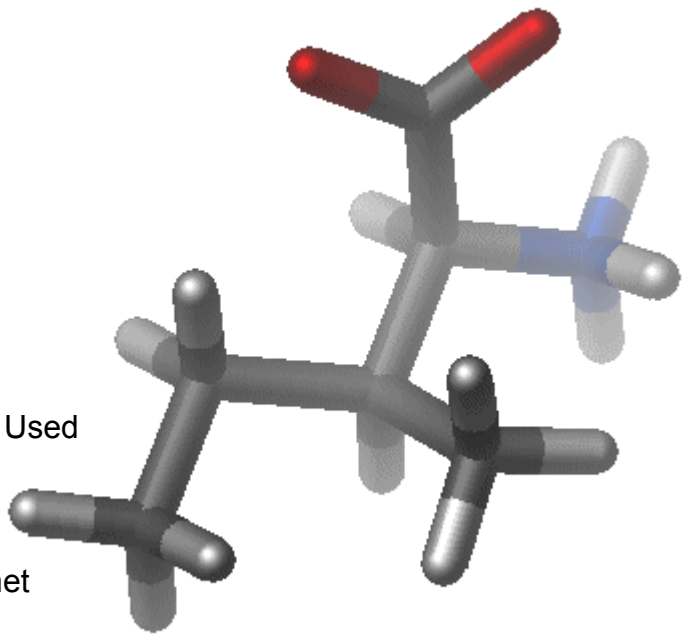
Figure by MIT OpenCourseWare. After Sun, 2004.

Theoretical model for organic chemistry

$$V(R) = E_{bonded} + E_{non-bonded}$$

$$E_{bonded} = E_{bond-stretch} + E_{angle-bend} + E_{rotate-along-bond}$$

Bonding between atoms described as combination of various terms, describing the angular, stretching etc. contributions



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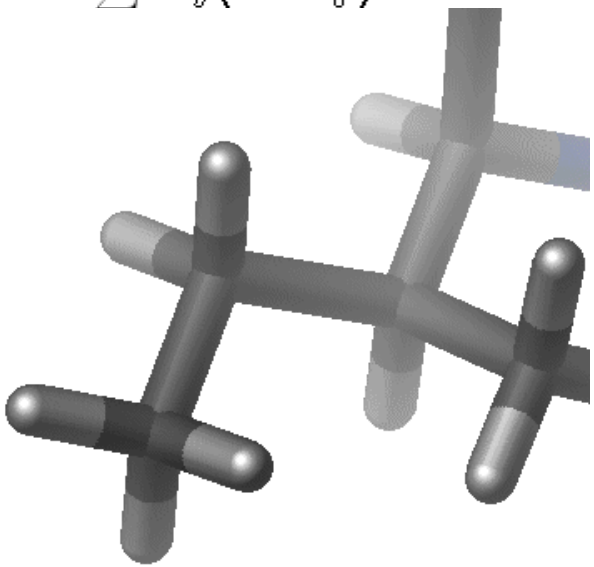
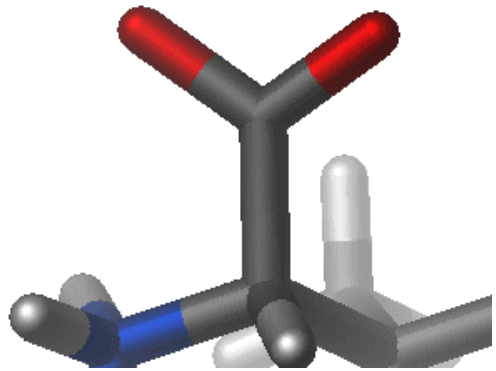
http://www.ch.embnet.org/MD_tutorial/pages/MD.Part2.html
http://www.pharmacy.umaryland.edu/faculty/amackere/force_fields.htm

Model for covalent bonds

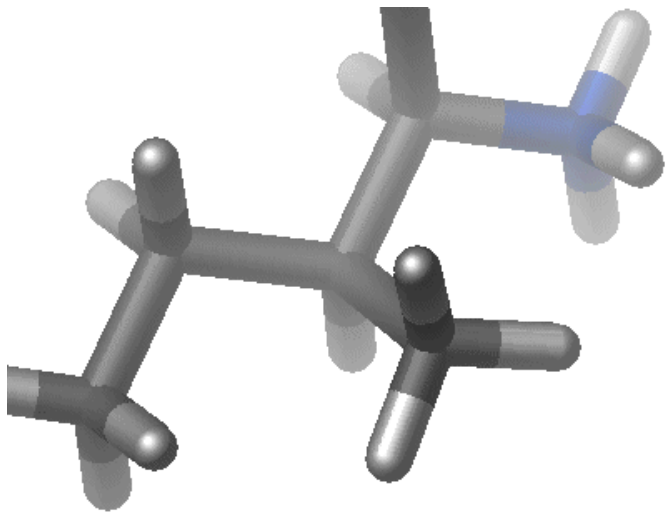
$$E_{bond-stretch} = \sum K_b (b - b_0)^2$$

$$E_{bond-bend} = \sum_{angles} K_\theta (\theta - \theta_0)^2$$





$$E_{rotate-along-bond} = \sum_{1,4\,pairs} K_\phi (1 - \cos(n\phi))$$



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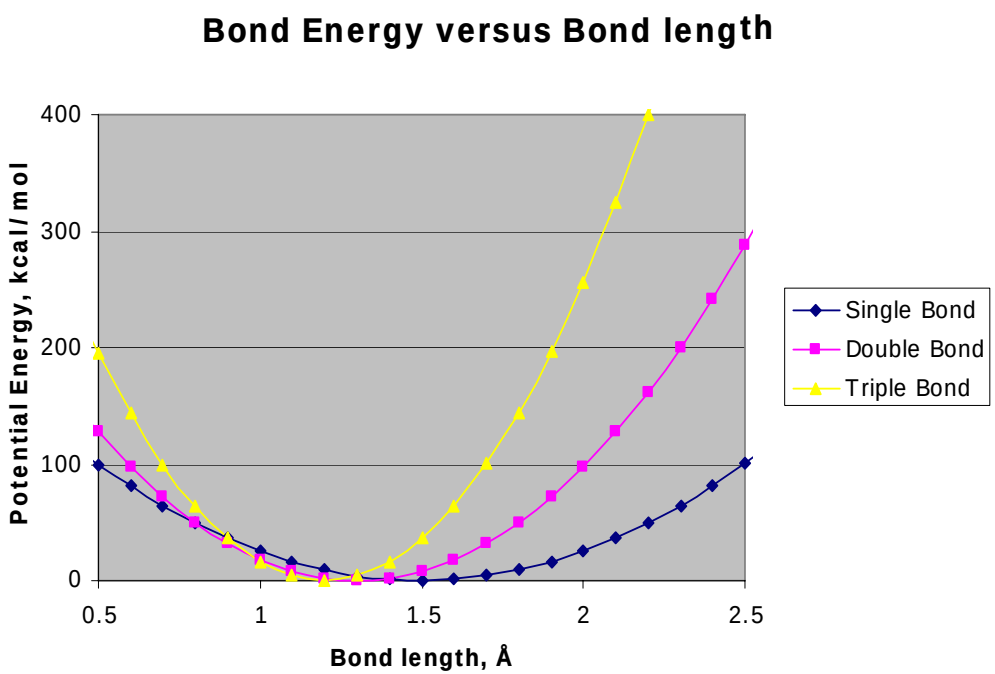
Review: CHARMM potential

Chemical type	K_{bond}	b_o
C−C	100 kcal/mole/Å^2	1.5 Å
C=C	200 kcal/mole/Å^2	1.3 Å
C≡C	400 kcal/mole/Å^2	1.2 Å



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Different types of C-C bonding represented by different choices of b_0 and k_b;

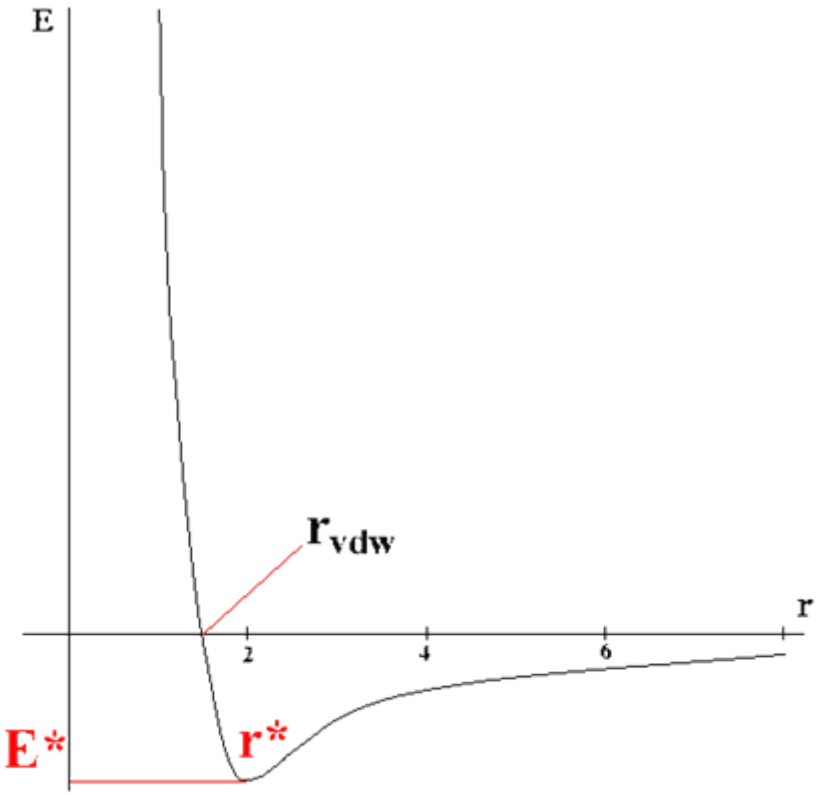
Need to retype when chemical environment changes

$$V_{bond} = K_b\left(b - b_o\right)^2$$

http://www.ch.embnet.org/MD_tutorial/pages/MD.Part2.html
http://www.pharmacy.umaryland.edu/faculty/amackere/force_fields.htm

Review: CHARMM potential

$$E_{non-bonded} = E_{van-der-Waals} + E_{electrostatic}$$



$$E_{van-der-Waals} = \sum_{\substack{nonbonded \\ pairs}} \left(\frac{A_{ik}}{r_{ik}^{12}} - \frac{C_{ik}}{r_{ik}^{6}} \right)$$

$$E_{electrostatic} = \sum_{\substack{nonbonded \\ pairs}} \frac{q_i q_k}{D r_{ik}}$$

Nonbonding interactions

vdW (dispersive)

Coulomb (electrostatic)

H-bonding

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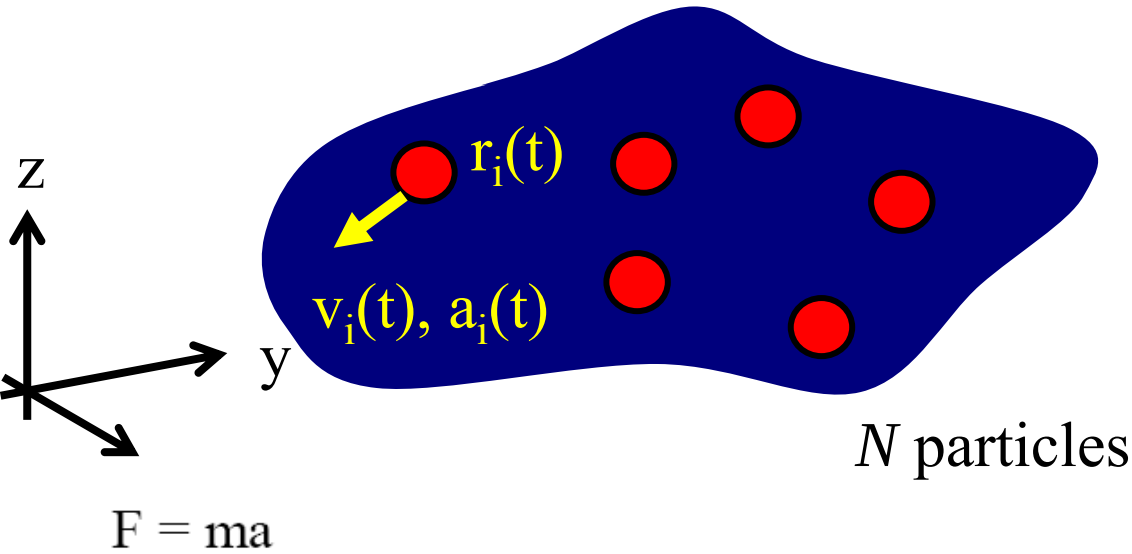
Molecular dynamics

Numerical simulation of stretching experiments

Molecular dynamics



F = ma

Total energy of system

$$E = K + U$$

$$K = \frac{1}{2} m \sum_{j=1}^{N} v_j^2$$

$$U = U(r_j)$$

$$m \frac{d^2 r_j}{dt^2} = -\nabla_{r_j} U(r_j) \qquad j = 1..N$$

Coupled system N-body problem, no exact solution for *N*>2

System of coupled 2nd order nonlinear differential equations

Solve by discretizing in time (spatial discretization given by "atom size")

M. Buehler, *J. Comput. Theoretical Nanoscience, 2006*

Solving the equations

Solve those equations: Discretize in time (*n* steps), Δt time step:

$$r_i(t_0) \rightarrow r_i(t_0+\Delta t) \rightarrow r_i(t_0+2\Delta t) \rightarrow r_i(t_0+3\Delta t) \rightarrow \ldots \rightarrow r_i(t_0+n\Delta t)$$

Taylor series expansion

$$r_i(t_0+\Delta t) = r_i(t_0) + v_i(t_0)\Delta t + \frac{1}{2}a_i(t_0)(\Delta t)^2 + \ldots$$

Adding this expansion together with one for $r_i(t_0-\Delta t)$:

$$r_i(t_0-\Delta t) = r_i(t_0) - v_i(t_0)\Delta t + \frac{1}{2}a_i(t_0)(\Delta t)^2 + \ldots$$

M. Buehler, *J. Comput. Theoretical Nanoscience, 2006*

Steered molecular dynamics

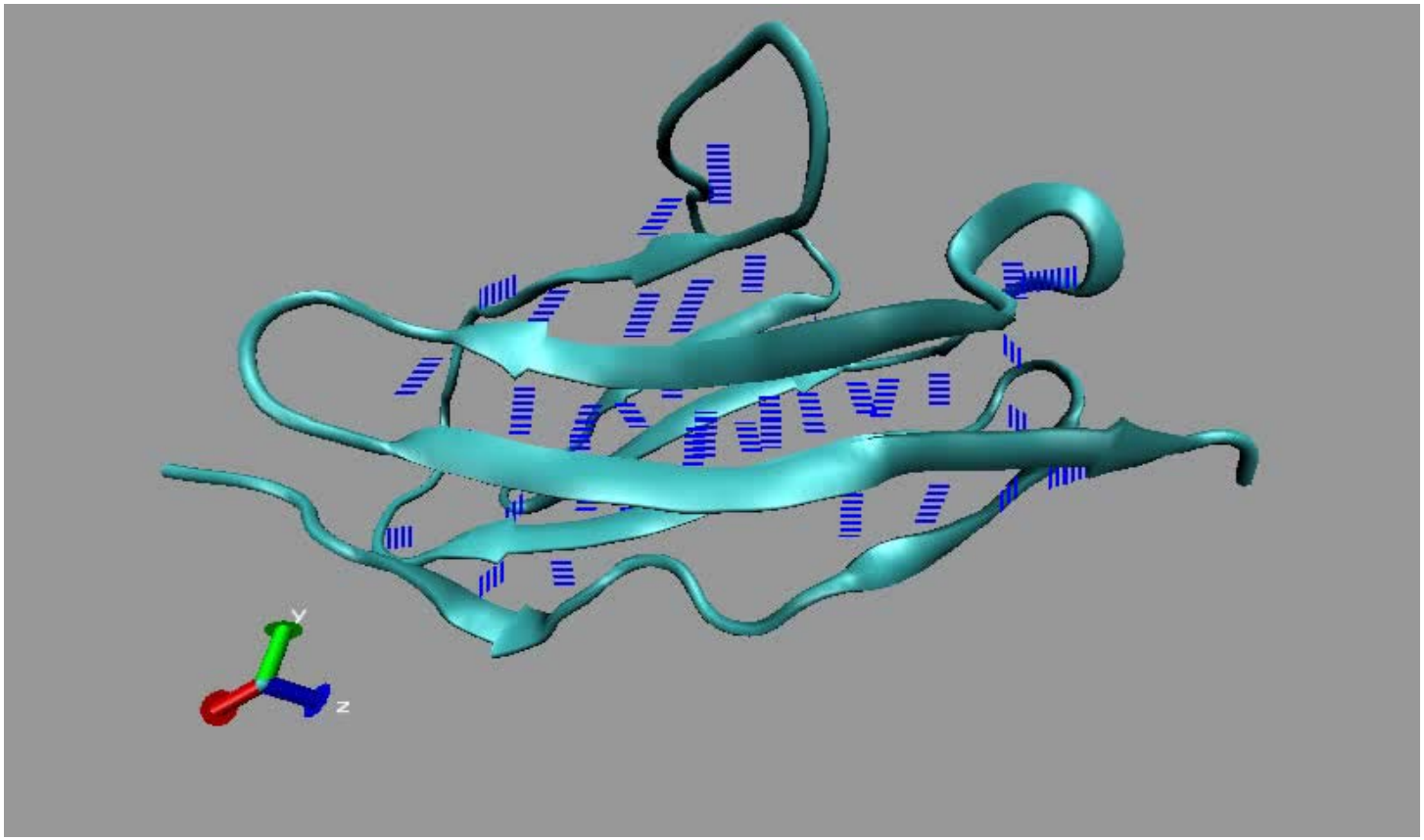
$$F = k(v \cdot t - x)$$

Image removed due to copyright restrictions.

Please see Fig. 6 in Buehler, M., and T. Ackbarow. “Fracture Mechanics of Protein Materials.” M*aterials Today* 10 (2007): 46-58.

M. Buehler & T. Ackbarow, *Materials Today, in press*

Steered molecular dynamics



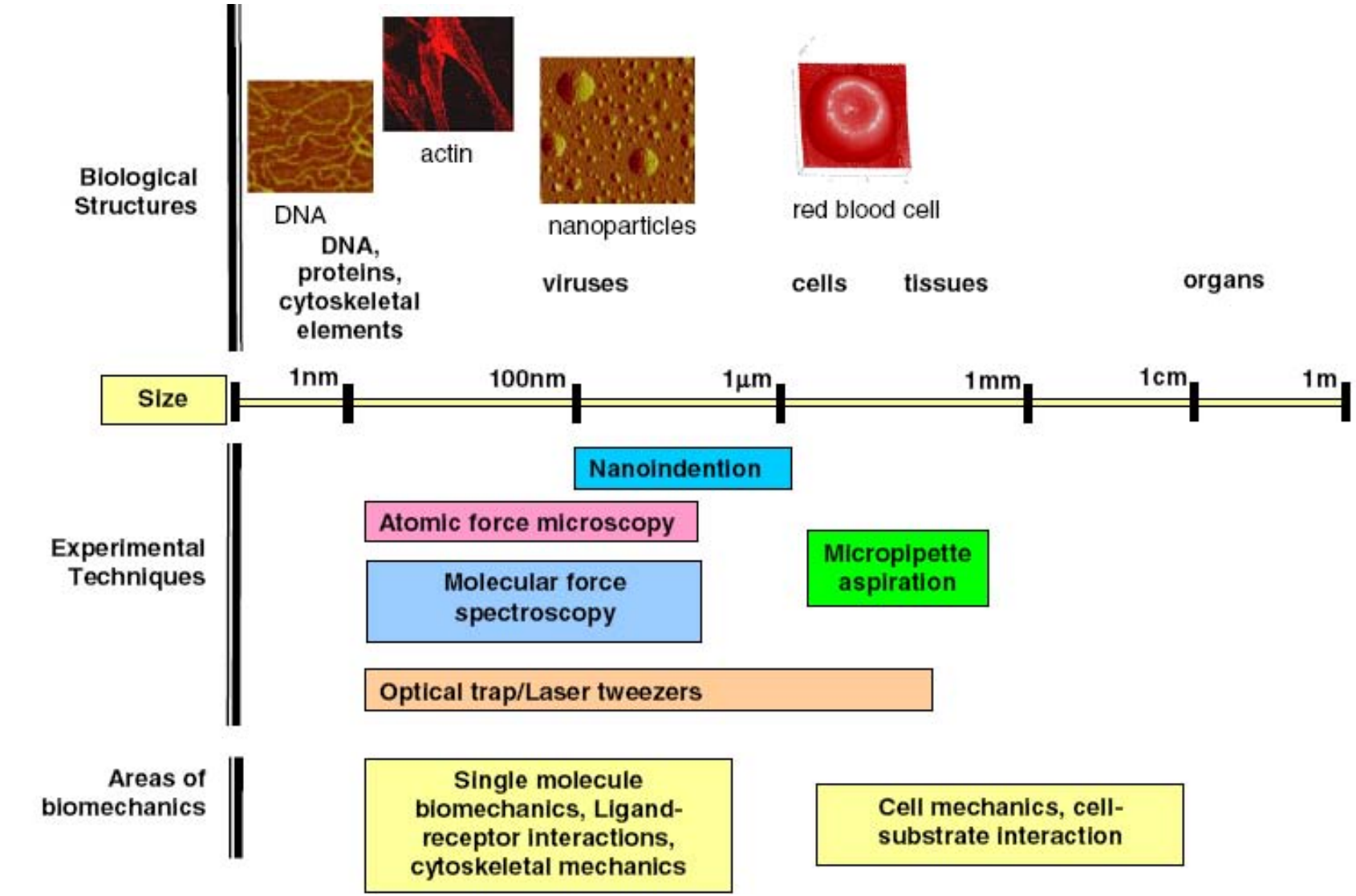
Unfolding of a titin molecule

Multi-scale simulation and experiment

M. Buehler, *J. Mat. Science, in press*

Experimental techniques have progressed very much over the past decade – covering more time- and length-scales …

Experimental techniques

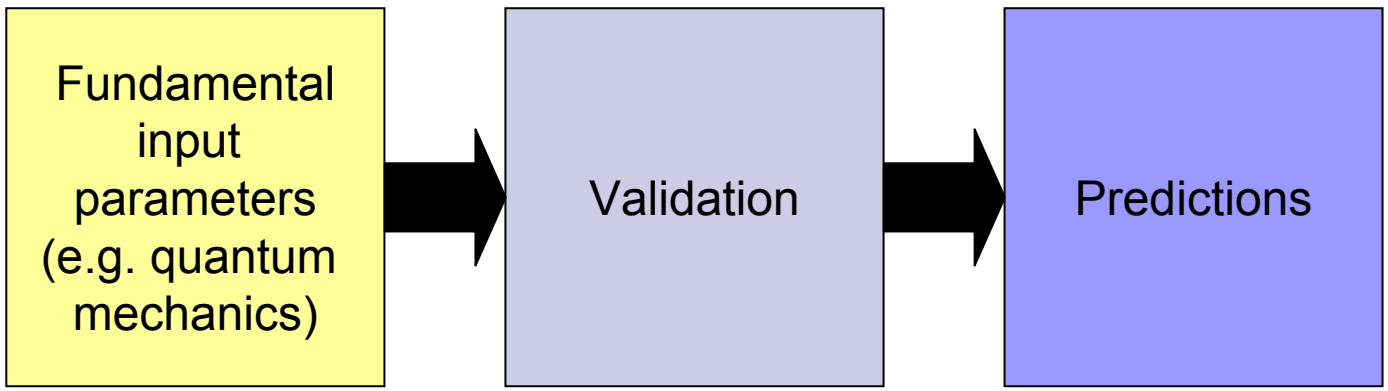


After: C.T. Lim, 2006

New paradigm: Predictive simulation

- Development of quantitative, predictive theoretical models – utilizing large-scale molecular dynamics simulation & multi-scale integration is in reach

- Predict behavior of complex biological materials across many hierarchies and many time- and length scales



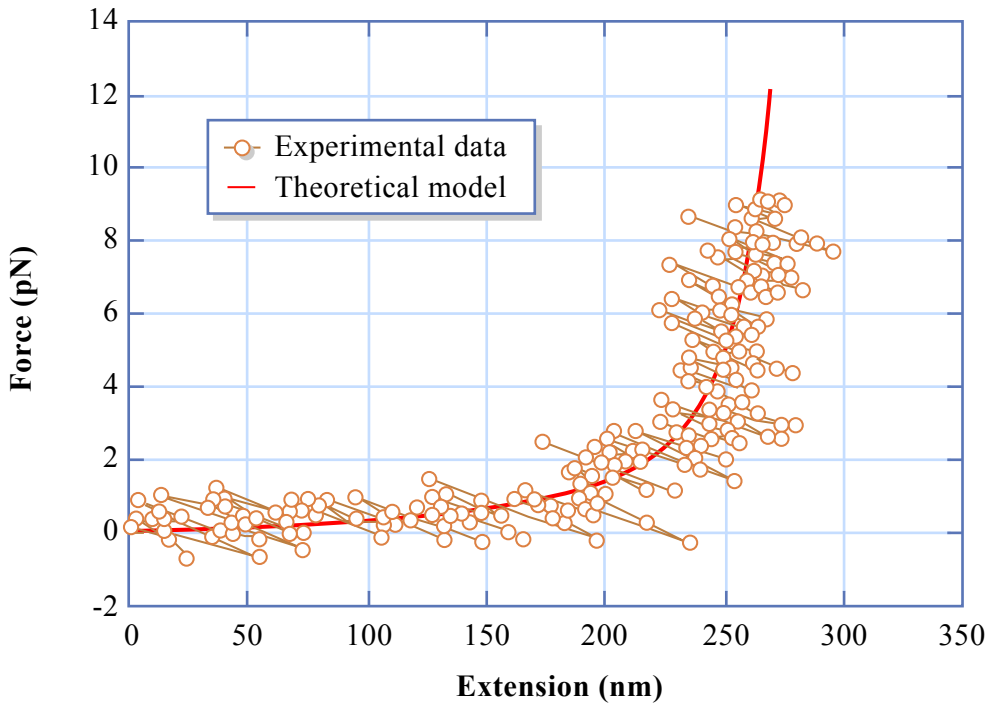
M. Buehler & T. Ackbarow, *Materials Today, in press*

Energy approach to elasticity

Linking atomistic scales to ‘meso’-scale

Elasticity: Reversible deformation

Stretching of tropocollagen molecules

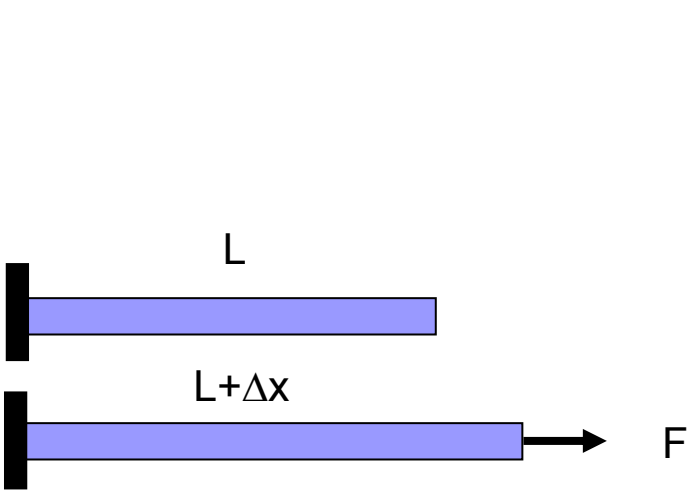


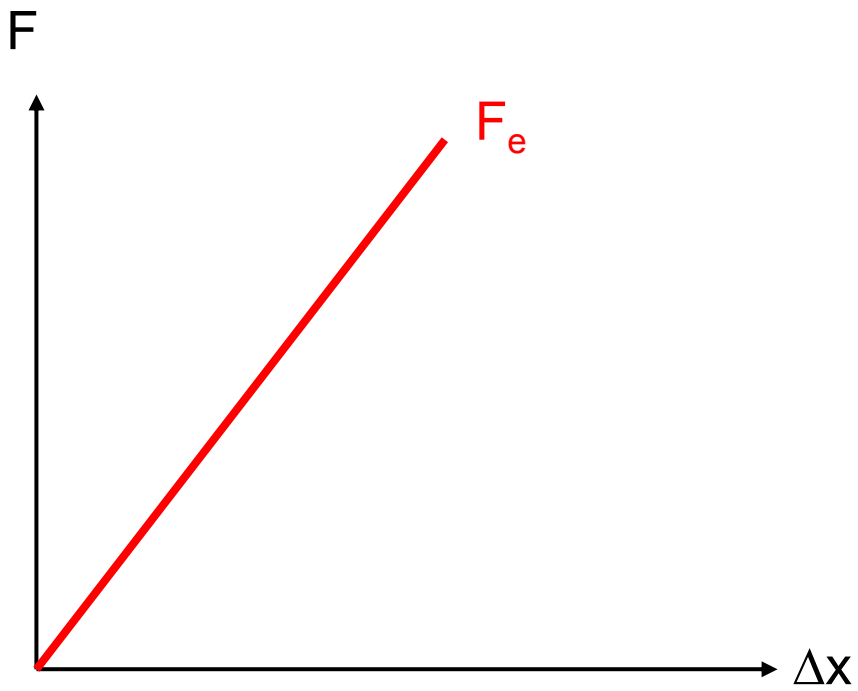
The force-extension curve for stretching a single type II collagen molecule. The data were fitted to Marko-Siggia entropic elasticity model. The molecule length and persistence length of this sample is 300 and 7.6 nm, respectively.

Figure by MIT OpenCourseWare. After Sun, 2004.

Sun *et al.*, 2004

Link between elasticity and energy state





External work rate

$$\delta W = \dot{x} F_e$$

Applied force

Energy approach to elasticity

1st law of TD

$$\frac{\mathrm{d}U}{\mathrm{d}t} = \delta W + \delta Q$$

External work rate

$$\delta W = \dot{x} F_e$$

Applied force

2nd law

$$\frac{\mathrm{d}S}{\mathrm{d}t} \geq \frac{\delta Q}{T}$$

Change in entropy is always greater or equal than the **entropy supplied in form of heat**; difference is due to internal dissipation

$$\frac{\mathrm{d}D}{\mathrm{d}t} = T\frac{\mathrm{d}S}{\mathrm{d}t} - \delta Q \geq 0$$

Dissipation rate

$$\delta Q = \mathrm{d}U/\mathrm{d}t - \delta W$$

Dissipation rate after consider 1st law of TD:

$$\frac{\mathrm{d}D}{\mathrm{d}t} = \delta W - \frac{\mathrm{d}}{\mathrm{d}t}(U - TS)$$

Dissipation rate=External work rate -change in usable energy U-TS

$F = U - TS$ is defined as free energy or Helmholtz energy,

Energy approach to elasticity

Elastic deformation (no dissipation by definition):

$$\frac{\mathrm{d}D}{\mathrm{d}t} = 0 \qquad \delta W - \frac{\mathrm{d}F}{\mathrm{d}t} = 0$$

Assume only internal energy change

$$\delta W = \dot{x} F_e$$

Expand equation
dU/dt = dU/dx dx/dt

$$\dot{x} F_e - \frac{\mathrm{d}F}{\mathrm{d}x}\frac{\mathrm{d}x}{\mathrm{d}t} = 0$$

$$\dot{x}\left(F_e - \frac{\mathrm{d}F}{\mathrm{d}x}\right) = 0.$$

Therefore: If applied force equals change in free energy of the system, have elastic deformation

$$F_e = \frac{\mathrm{d}F}{\mathrm{d}x} \qquad F_e = \frac{\mathrm{d}U}{\mathrm{d}x}$$

With strain energy density: $\Psi = F/V \qquad \Psi = U/V$

$$\sigma_{ij} = \frac{\mathrm{d}\Psi}{\mathrm{d}\varepsilon_{ij}} \qquad c_{ijkl} = \frac{\mathrm{d}^2\Psi}{\mathrm{d}\varepsilon_{ij}\mathrm{d}\varepsilon_{kl}}$$

Energy approach to elasticity

- The equations

$$\sigma_{ij} = \frac{\mathrm{d}\Psi}{\mathrm{d}\varepsilon_{ij}} \qquad c_{ijkl} = \frac{\mathrm{d}^2\Psi}{\mathrm{d}\varepsilon_{ij}\mathrm{d}\varepsilon_{kl}}$$

  are significant since they provide a direct link between energy state and elasticity (stress & moduli)

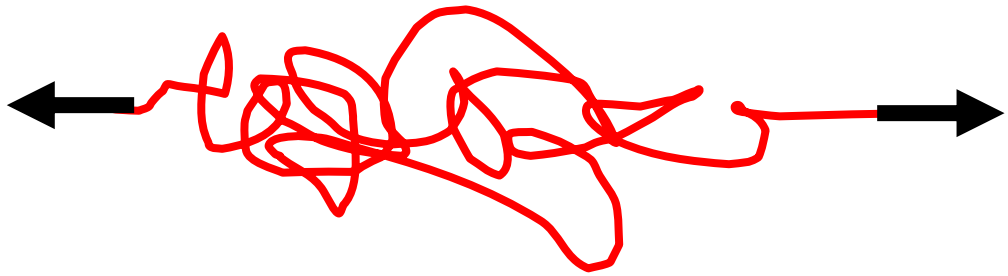
- Physics of deformation:

  How does free energy state change when the deformation changes?

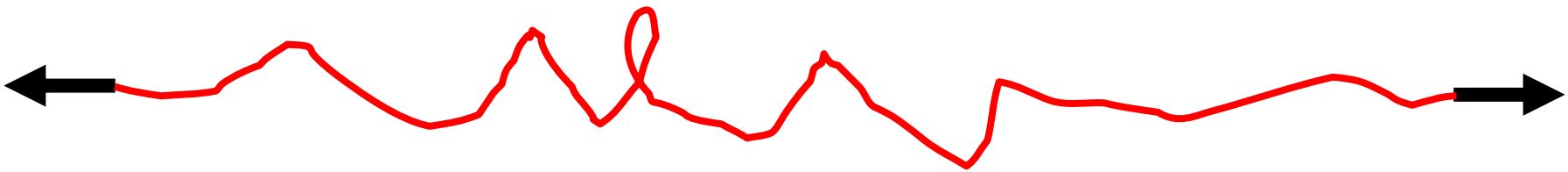
F = U - TS

Change of free energy as a function of deformation provides an intimate link between atomistic/molecular scales and 'continuum' (average)

Entropic change as a function of stretch



High entropy



Low entropy

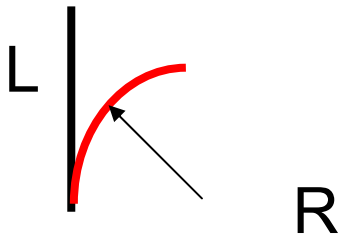
$$F = U - TS$$

M. Buehler, *J. Comput. Theoretical Nanoscience, 2006*

Why are molecules convoluted?

- Bending deformation (R=radius, EI=flexural rigidity of the rod) - energy

$$E_{bend} = EI\frac{L}{2R^2}$$



- Thermal (kinetic) energy per molecule (kinetic theory of gases) - energy

$$E_{kin,mol} = \frac{3}{2}kT$$

- Example: kT~4E-21 J at body temperature

- If *EI* is very small, thermal energy may be enough to bend the molecule

F = U - TS

Persistence length

$$\langle \boldsymbol{t}(s) \cdot \boldsymbol{t}(s') \rangle = e^{-|s-s'|/\ \xi_p}$$

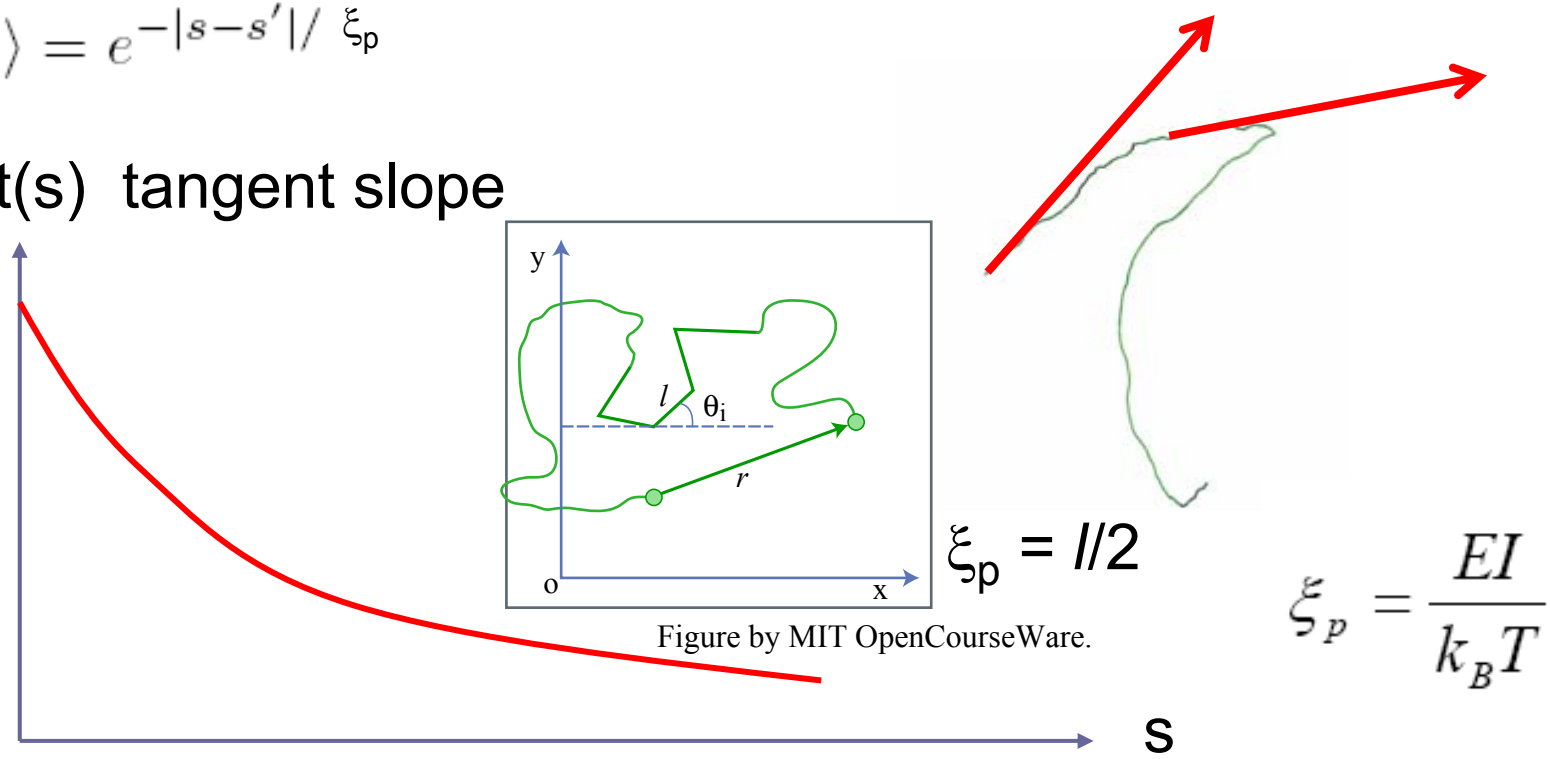


Figure by MIT OpenCourseWare.

$$\xi_p = \frac{EI}{k_B T}$$

The length at which a filament is capable of bending significantly in independent directions, at a given temperature.
This is defined by a autocorrelation function which gives the characteristic distance along the contour over which the tangent vectors **t(s)** become uncorrelated

Bending of tropocollagen molecule

Bending stiffness given by

$$EI = \frac{F_{appl} L^3}{48d}$$

$EI = 9.71 \times 10^{-29}$ Nm²

(5,5) CNT: ~ 1,000 times stiffer

$EI = 6.65 \times 10^{-26}$ Nm²

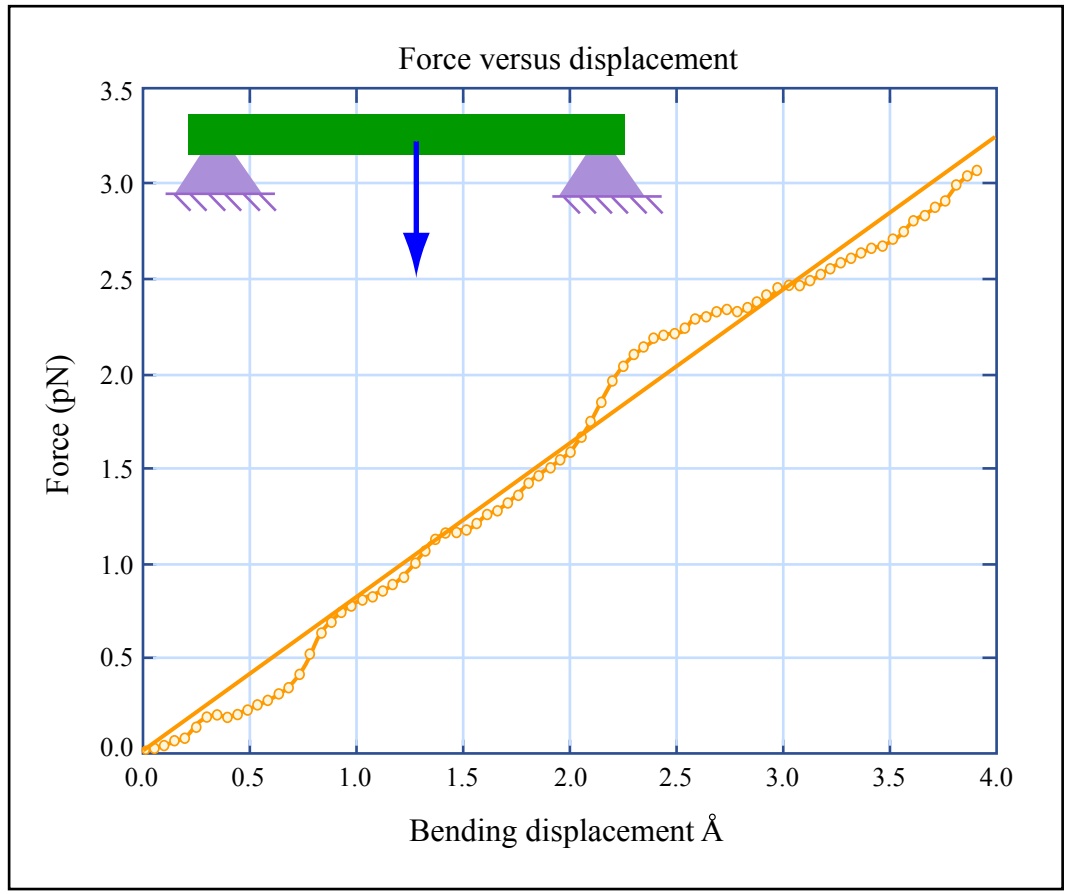


Figure by MIT OpenCourseWare.

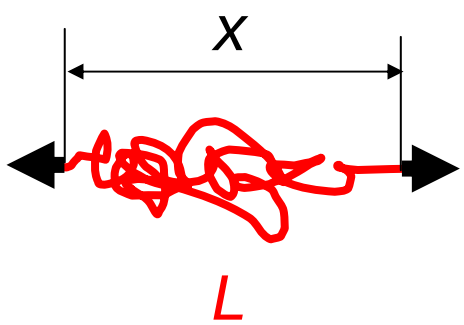
M.J. Buehler, *J. Mater. Res.*, Vol. 21(8), 2006

Contour length of molecules

- The contour length of a molecule is the total length in the stretched configuration, denoted as *L*
- When $L << \xi_p$

  a filament appears relatively straight.
- When $L >> \xi_p$

  a filament adopts more convoluted shapes



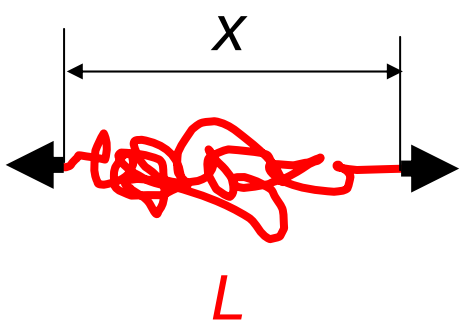
M. Buehler, *J. Comput. Theoretical Nanoscience, 2006*

Entropic spring (single freely jointed chain)

- To pull a highly convoluted molecule apart, a force is necessary; define effective spring constant

No energetic interactions!

$$L >> \xi_p$$



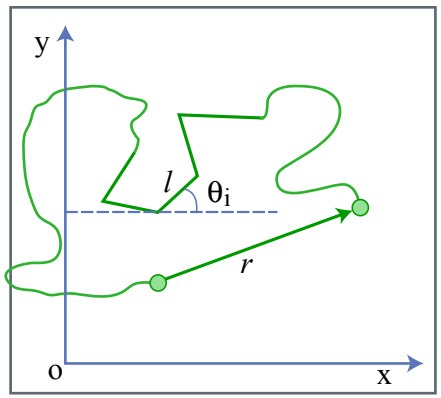


Figure by MIT OpenCourseWare.

$$k_{sp} = \frac{3kT}{2L\xi_p} \qquad F \sim k_{sp} x \qquad x << L$$

M. Buehler, *J. Comput. Theoretical Nanoscience, 2006*

Statistical theory of rubber elasticity

Note: No change in elastic energy of molecules

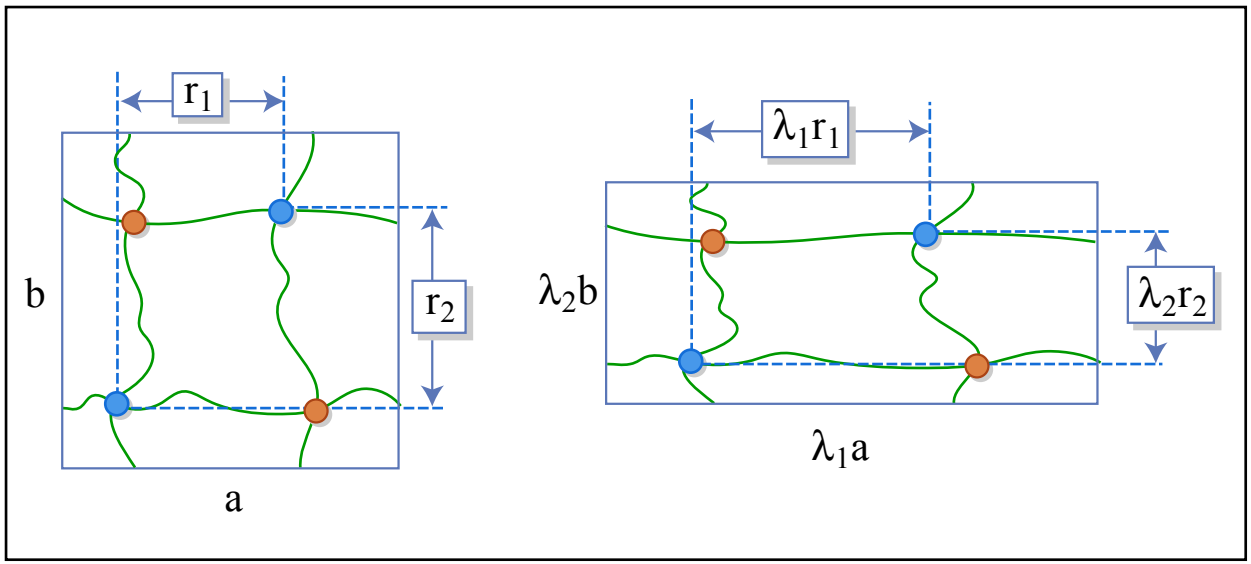


Figure by MIT OpenCourseWare.

Needed to understand elasticity: Expression of free energy as a function of the applied strain!

Entropic elasticity – therefore change in entropy

M. Buehler, *J. Comput. Theoretical Nanoscience, 2006*

Entropic elasticity: Derivation

Freely jointed Gaussian chain with *n* links and length *l* each (same for all chains in rubber)

$$S = c - k_B b^2 r^2 \qquad \text{where} \quad b^2 = \frac{3}{2nl^2}$$

r end-to-end distance of chain

$$\Delta S = -kb^2 \sum_{N_b} \left(\lambda_1^2 - 1\right)x^2 + \left(\lambda_2^2 - 1\right)y^2 + \left(\lambda_3^2 - 1\right)z^2$$

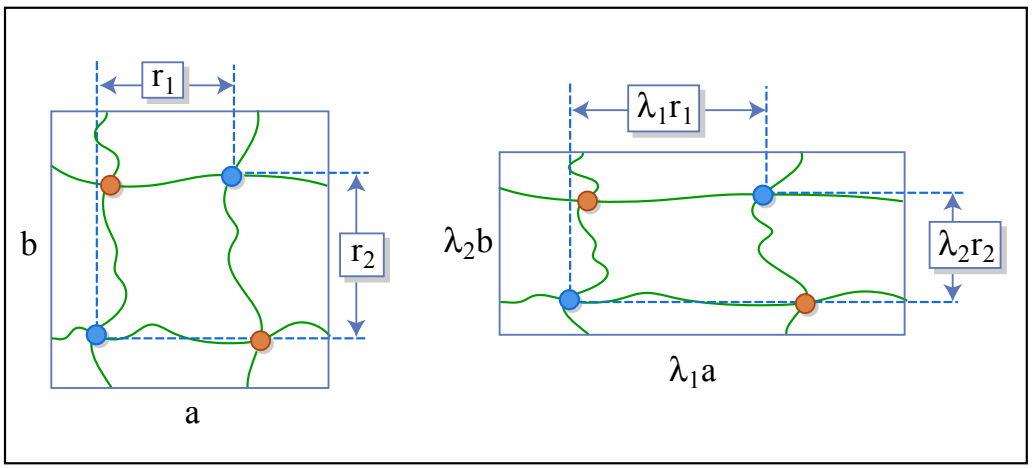


Figure by MIT OpenCourseWare.

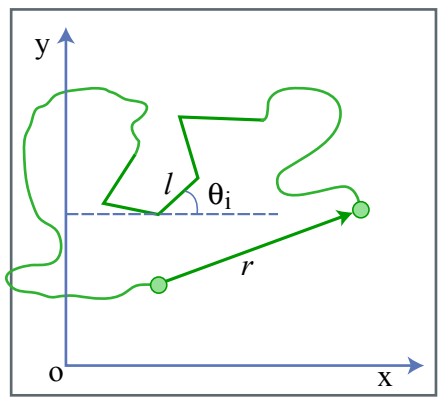


Figure by MIT OpenCourseWare.

F = U - TS

M. Buehler, *J. Comput. Theoretical Nanoscience, 2006*

Entropic elasticity

For SED: *Free energy*

$$F = -T\Delta S = \underbrace{\tfrac{1}{2} N_b kT}_{C = E/6}\left(\lambda_1^2 + \lambda_2^2 + \lambda_3^2 - 3\right)$$

Predictions:

$$E = 3N^* kT \qquad N^* = N_b / V$$

Stiffness is proportional to temperature

$$E \sim T$$

Stiffness is proportional to degree of cross-linking (for ideal network, N^* equals twice the cross-link density)

$$E \sim N^*$$

M. Buehler, *J. Comput. Theoretical Nanoscience, 2006*

Entropic change as a function of stretch

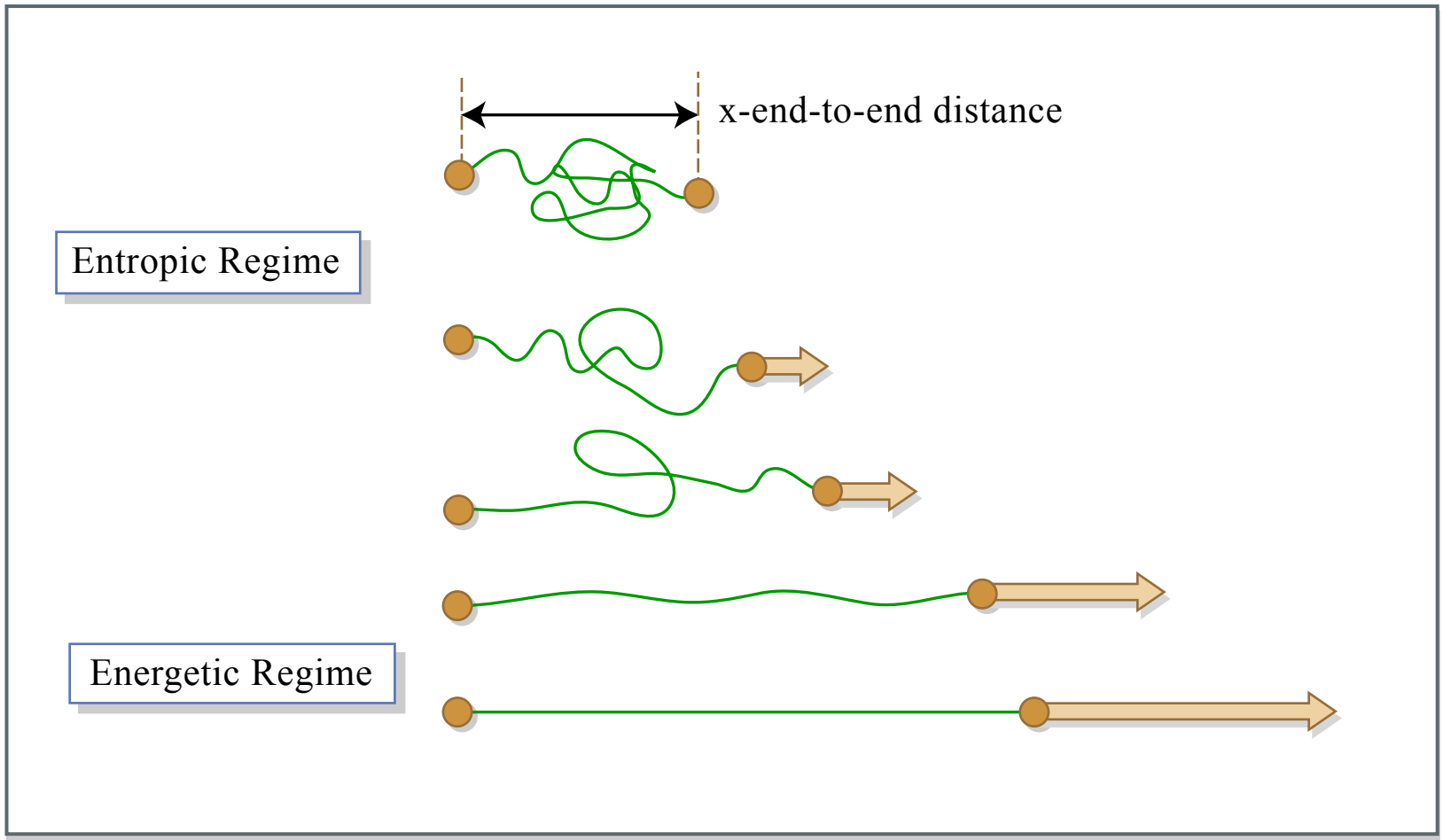


Figure by MIT OpenCourseWare.

M. Buehler, *J. Comput. Theoretical Nanoscience, 2006*

Worm-like chain model

Freely-jointed rigid rods

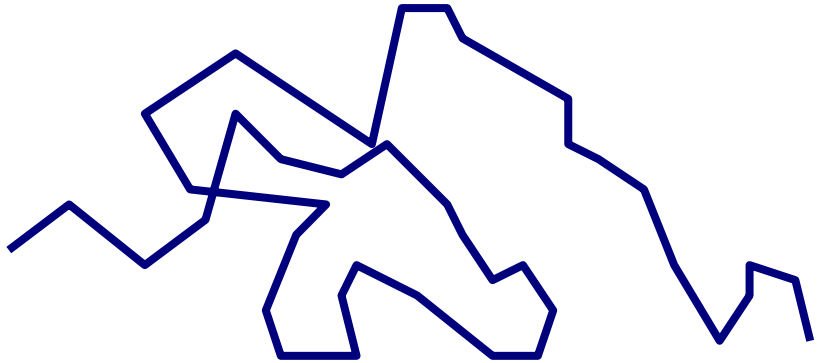
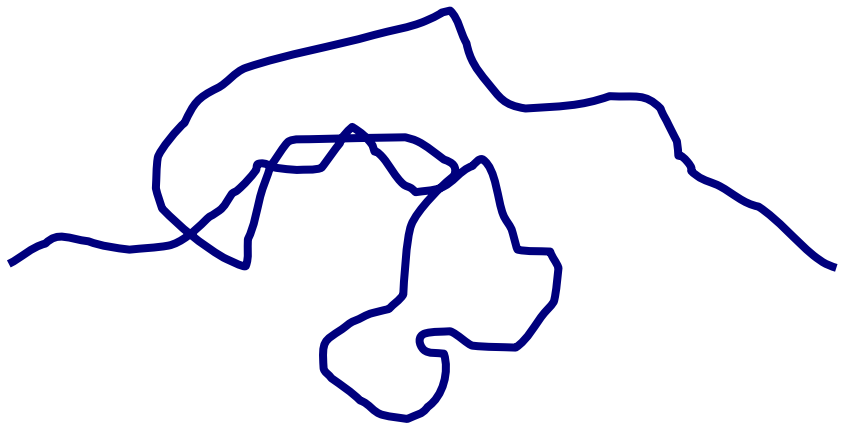


Image removed due to copyright restrictions.

DNA 4-plat electron micrograph (Cozzarelli; Berkeley)

Continuously flexible ropes



Worm like chain model

Worm-like chain model

- This spring constant is only valid for small deformations from a highly convoluted molecule, with length far from its contour length

$$x << L$$

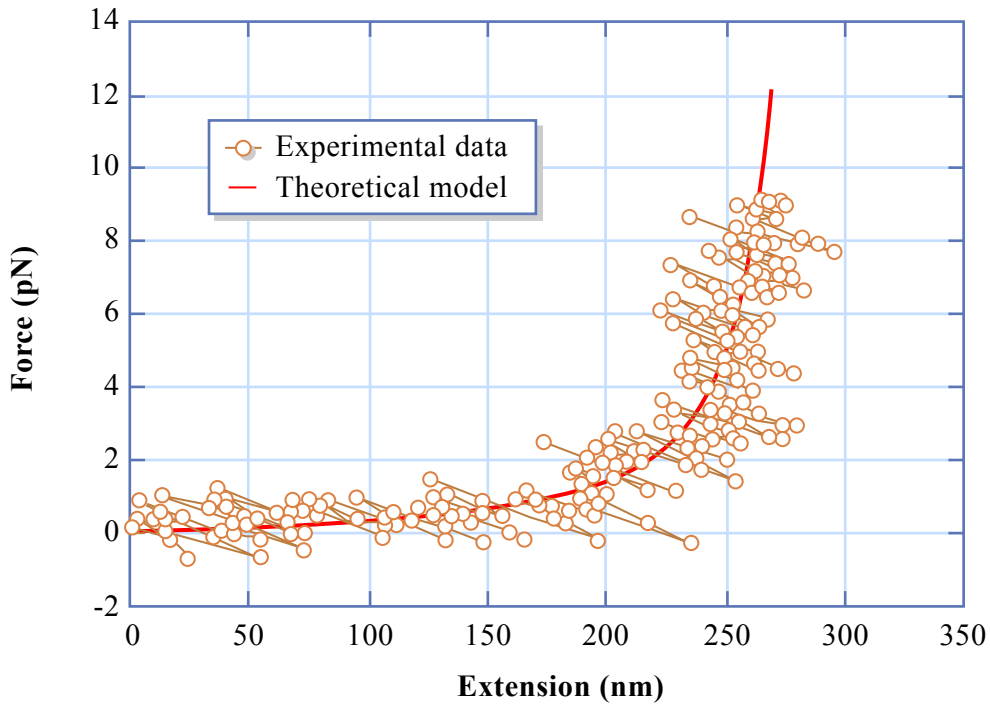
- A more accurate model (without derivation) is the Worm-like chain model (WLC) that can be derived from the Kratky-Porod energy expression

- A numerical, approximate solution of the WLC model:

$$F = \frac{kT}{\xi_p}\left(\frac{1}{4}\frac{1}{\left(1-x/L\right)^2} - \frac{1}{4} + x/L\right)$$

Marko and Siggia, 1995

Applicability of WLC model



The force-extension curve for stretching a single type II collagen molecule. The data were fitted to Marko-Siggia entropic elasticity model. The molecule length and persistence length of this sample is 300 and 7.6 nm, respectively.

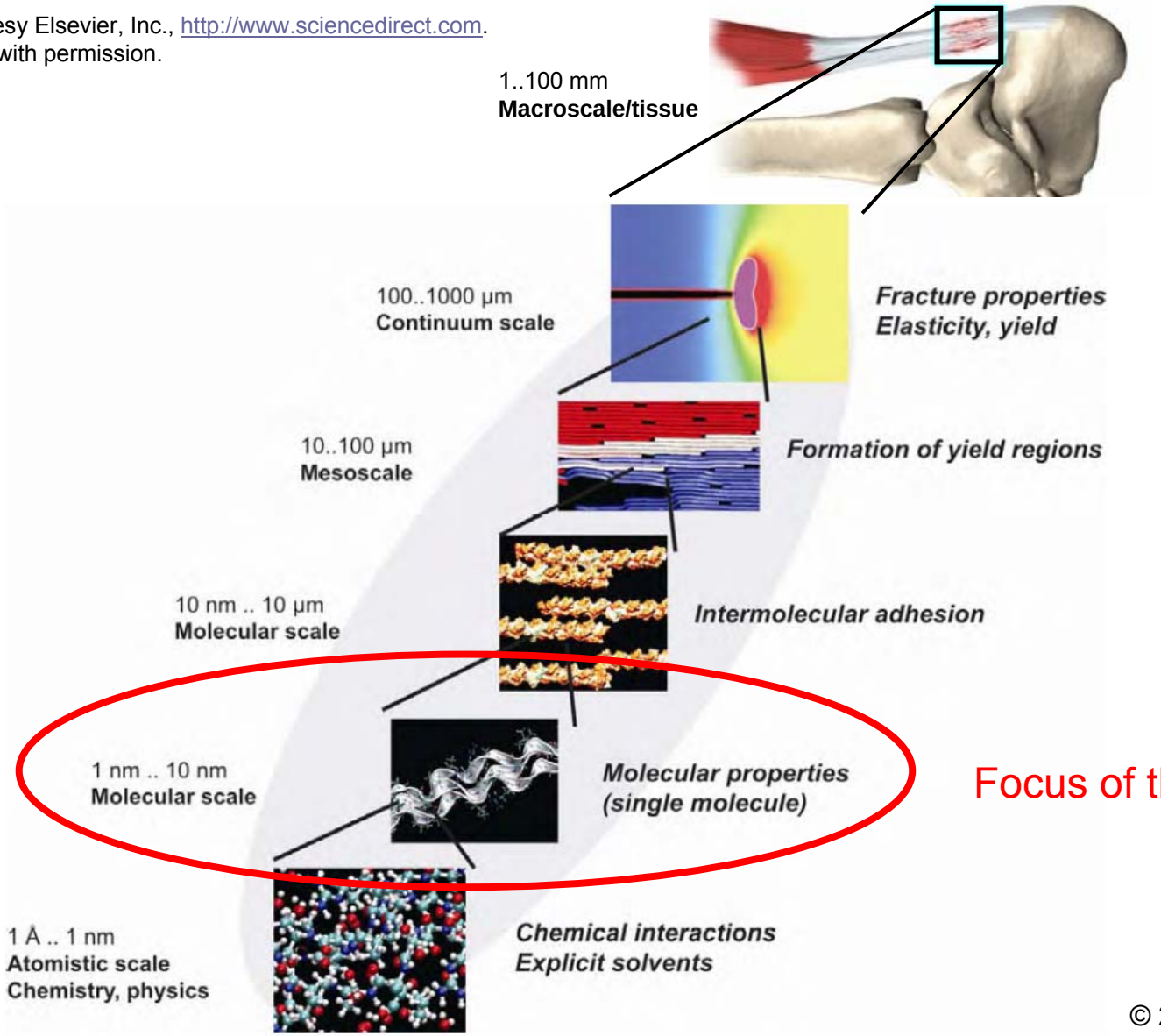
Figure by MIT OpenCourseWare. After Sun, 2004.

Sun *et al.*, *J. Biomechanics,* 2004

Summary and review

Lecture 1

Molecular mechanics: Definition of scales



Focus of these lectures

Goal of these lectures

- Molecular mechanics with focus on biomolecules (proteins)
- Topics covered:
  - Chemical bonding in molecules & theory
  - Characterization of molecular properties (tensile stiffness, persistence length, adhesion..)
  - Application of continuum mechanical concepts to molecular mechanics
  - Link of molecular properties to tissue properties (collective behavior of many molecules, elasticity, fracture,..)
  - Molecular defects and consequence for diseases: Certain mutation may induce changes in mechanical properties, e.g. molecular defects, mutations; leads to pathological consequence (too soft, too stiff,..)
- Multi-scale modeling
- Emphasis on developing a sensitivity for the significance of molecular mechanics in biology and how atomistic and continuum viewpoints can be coupled

M. Buehler & T. Ackbarow, *Materials Today, in press*

GEM^4 Summer School on Cell and Molecular Mechanics in Biomedicine

June 25 - July 6 2007

Molecular mechanics

Lecture 2

Markus J. Buehler

Massachusetts Institute of Technology

Laboratory for Atomistic and Molecular Mechanics

Proteins and protein structures

- Proteins are made up of amino acids
- 20 amino acids carrying different side groups (R)
- Amino acids linked by the amide bond via condensation; formation of proteins controlled by genes
- Proteins have four levels of structural organization: primary, secondary, tertiary and quaternary

Fascinating for a material scientist:

Understand structure-function relationship for protein materials
20 building blocks; machinery of material synthesis from DNA
molecular scale control of structure, multi-functionality, …

20 natural amino acids

Images removed due to copyright restrictions.

Table of amino acid chemical structures.
See similar image:
http://web.mit.edu/esgbio/www/lm/proteins/aa/aminoacids.gif

Protein structure

- **Primary structure:** Sequence of amino acids
- **Secondary structure:** Protein secondary structure refers to certain common repeating structures found in proteins. There are two types of secondary structures: alpha-helix and beta-pleated sheet.
- **Tertiary structure:** Tertiary structure is the full 3-dimensional folded structure of the polypeptide chain.
- **Quartenary Structure:** Quartenary structure is only present if there is more than one polypeptide chain. With multiple polypeptide chains, quartenary structure is their interconnections and organization.

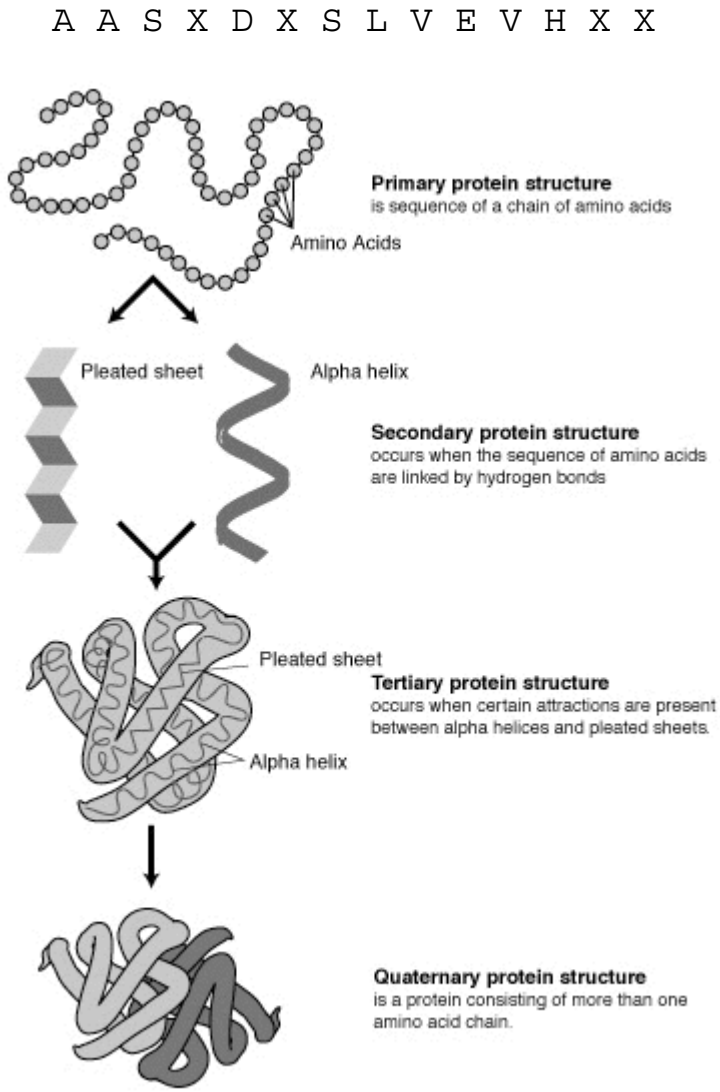
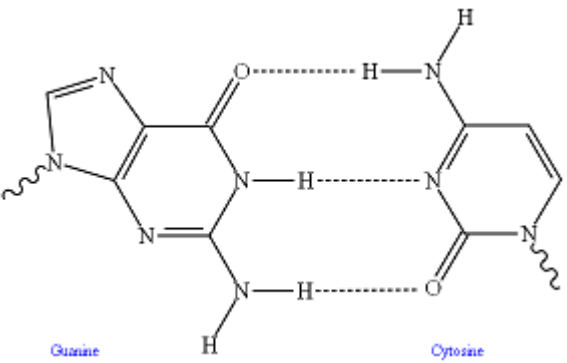


Image courtesy of NIH.

Despite the existence of a GREAT variety of protein materials, observe dominance of 'universal building blocks'

Alpha-helix (AH)



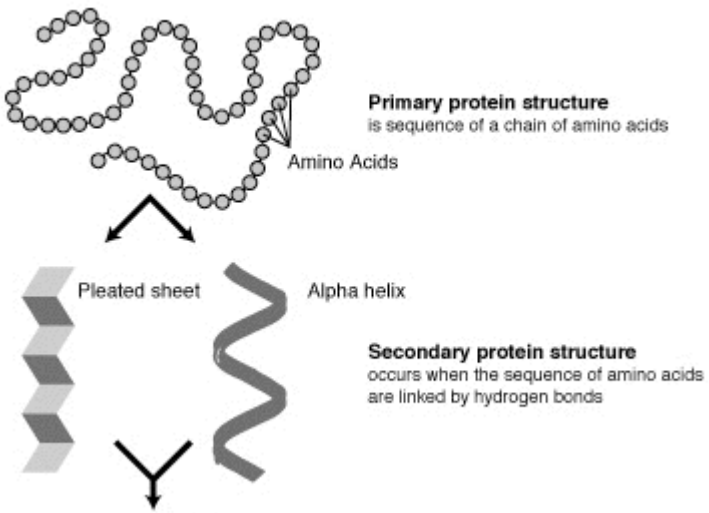
Images courtesy of Wikimedia Commons and NIH.

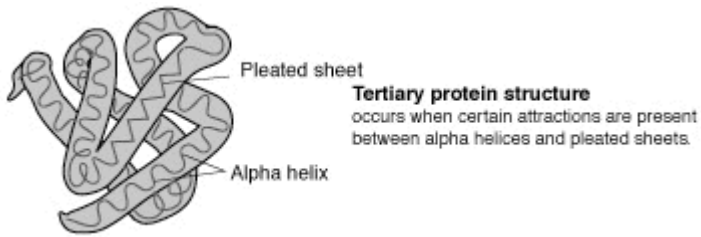
Hydrogen bonding
e.g. between O and H in H_2O
Between N and O in proteins…

Formation of AH induced due to hydrophobic character of side chains

-

Image removed due to copyright restrictions.
For an image of the classic hydrogen bond example, hydrogen bonds in water, please see http://upload.wikimedia.org/wikipedia/commons/f/f9/3D_model_hydrogen_bonds_in_water.jpg.





Beta-sheets (BS)

Beta sheet

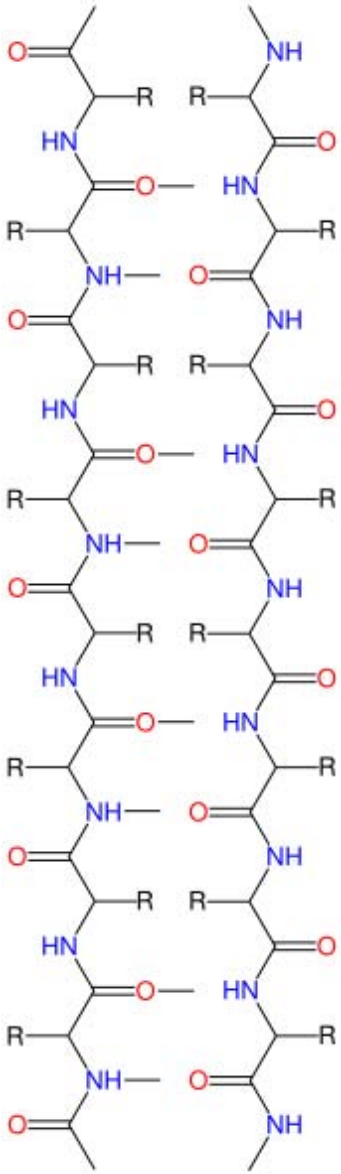


Image removed due to copyright restrictions.

For an image of the classic hydrogen bond example, hydrogen bonds in water, please see

http://upload.wikimedia.org/wikipedia/commons/f/f9/3D_model_hydrogen_bonds_in_water.jpg.

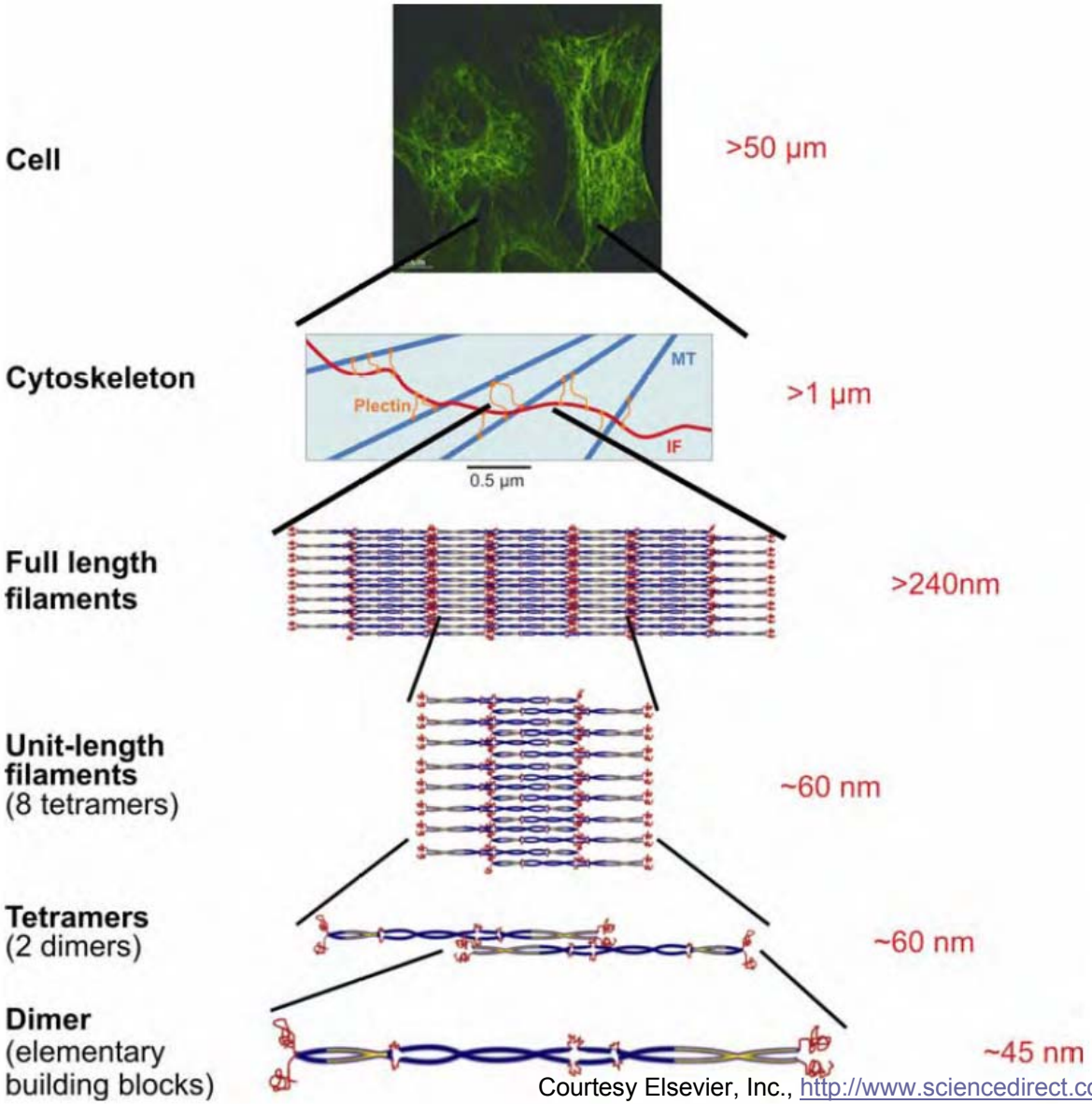
Present in mechanically relevant proteins

Spider silk

Fibronectin

Titin (muscle tissue)

Case study: Intermediate filament proteins



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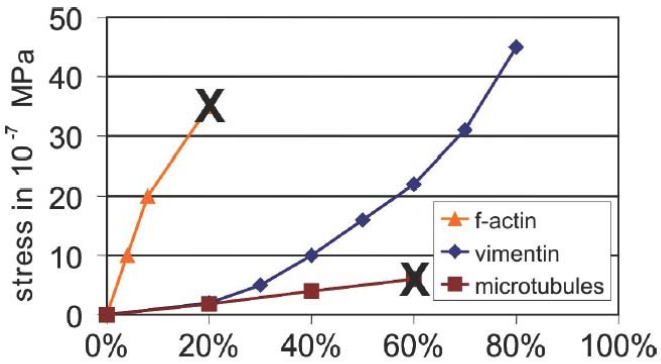
Intermediate filaments (IFs)

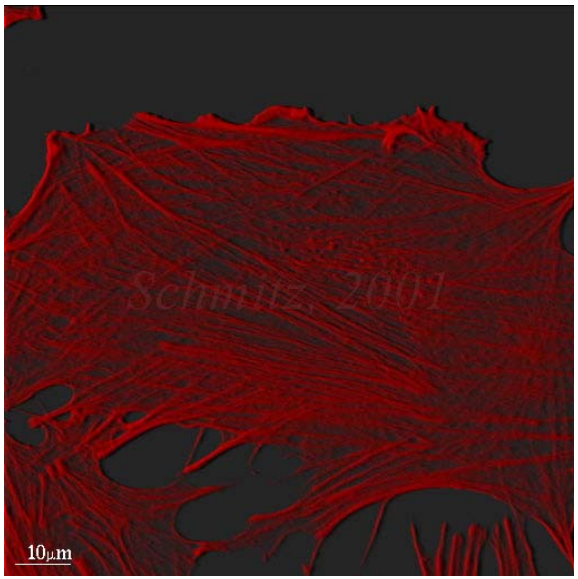
Structure significant under large deformation
Forms nuclear envelope (lamin)



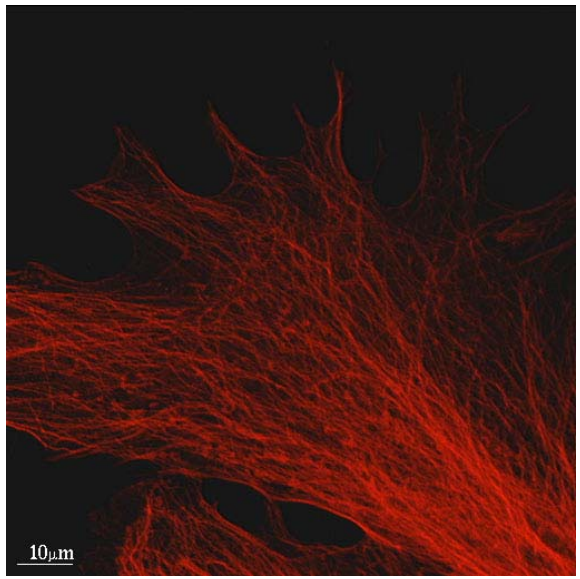
Janmey PA, Euteneuer U, Traub P, Schliwa M (1991) J Cell Biol 1134 113:155

Courtesy of Rockefeller University Press via CC BY-NC-SA license.

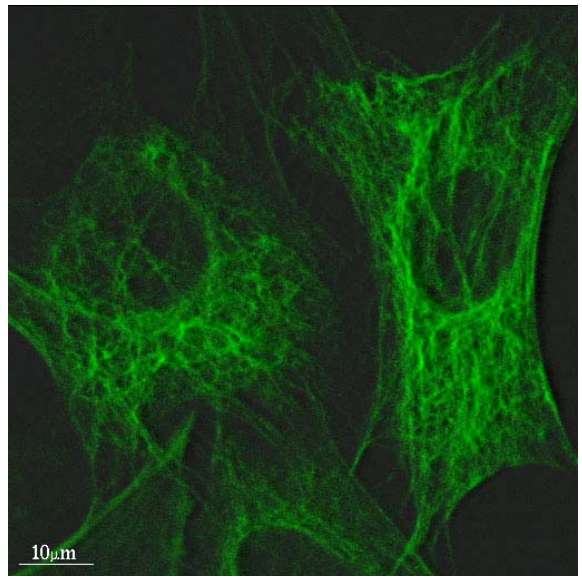
The cell's cytoskeleton



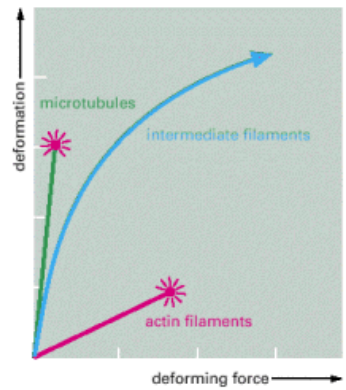
Actin



Tubulin

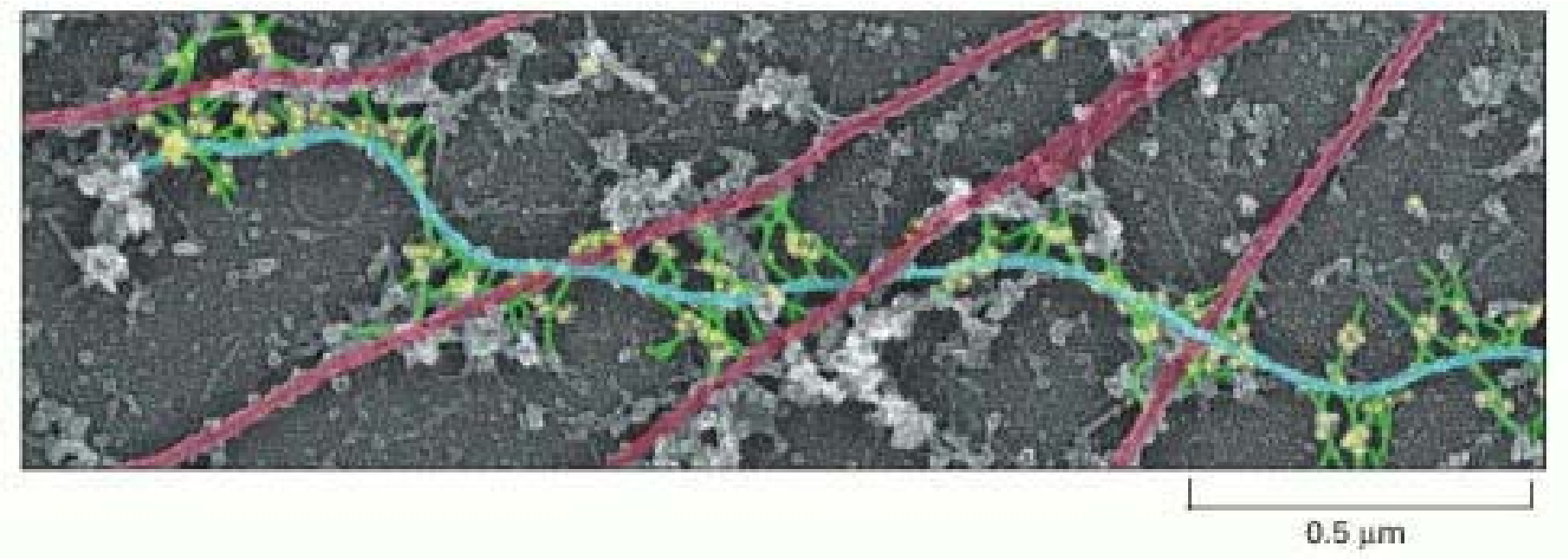


Vimentin



Fluorescent staining for different protein networks

Cross-linking of cytoskeletal elements

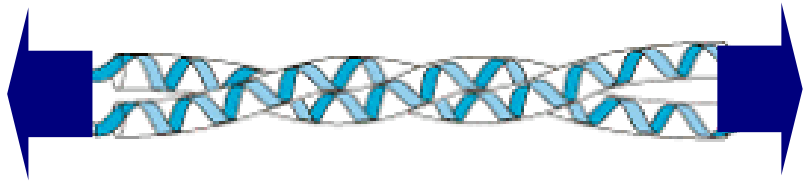


Plectin cross-linking of diverse cytoskeletal elements. Plectin *(green)* makes cross-links from **intermediate filaments *(blue)*** to other intermediate filaments, to **microtubules *(red),*** and to **myosin thick filaments**. In this electron micrograph, the dots *(yellow)* are gold particles linked to anti-plectin antibodies. The entire actin filament network was removed to reveal these proteins.

(From T.M. Svitkina and G.G.Borisy, *J. Cell Biol.* 135:991–1007, 1996)

Mechanics of vimentin dimers

Stage I: Uncoiling of each alpha helix
Stage II: Uncoiling of coiled-coil
Stage III: Stretching of protein backbone



F, v

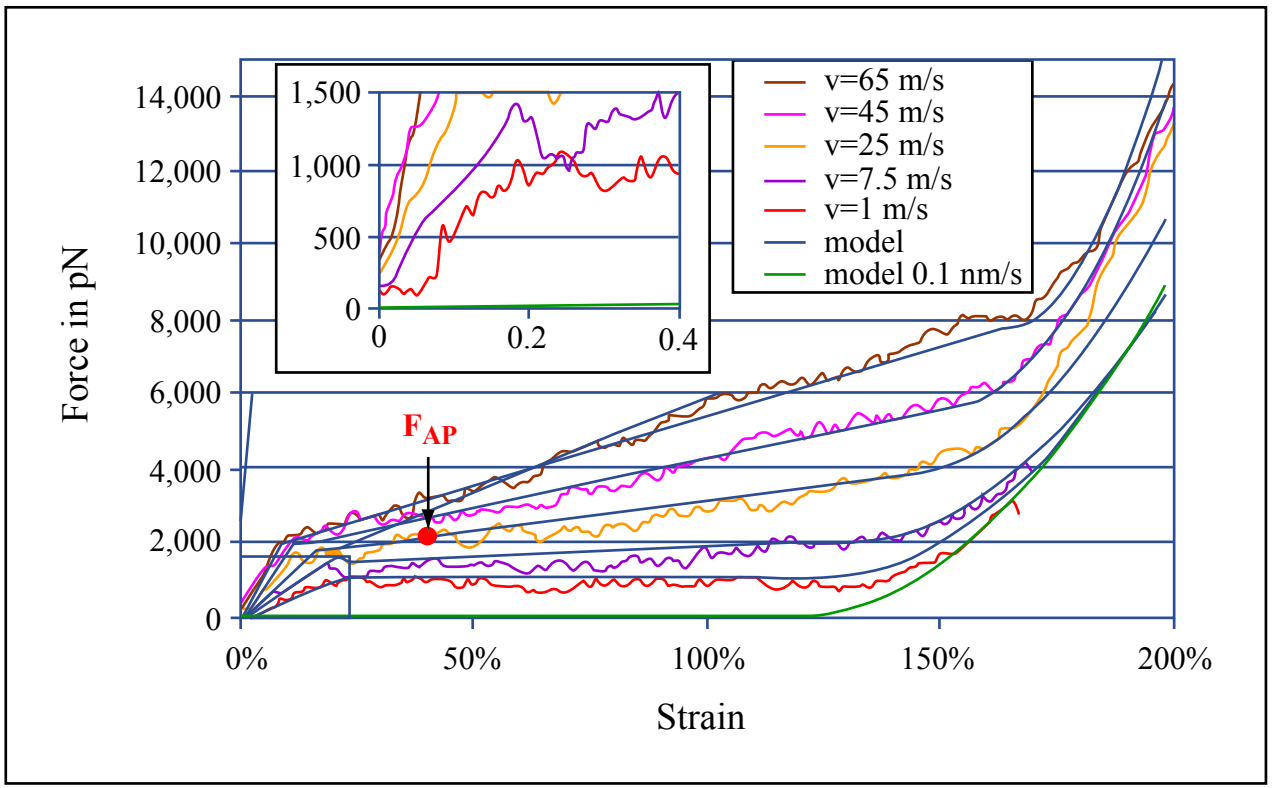


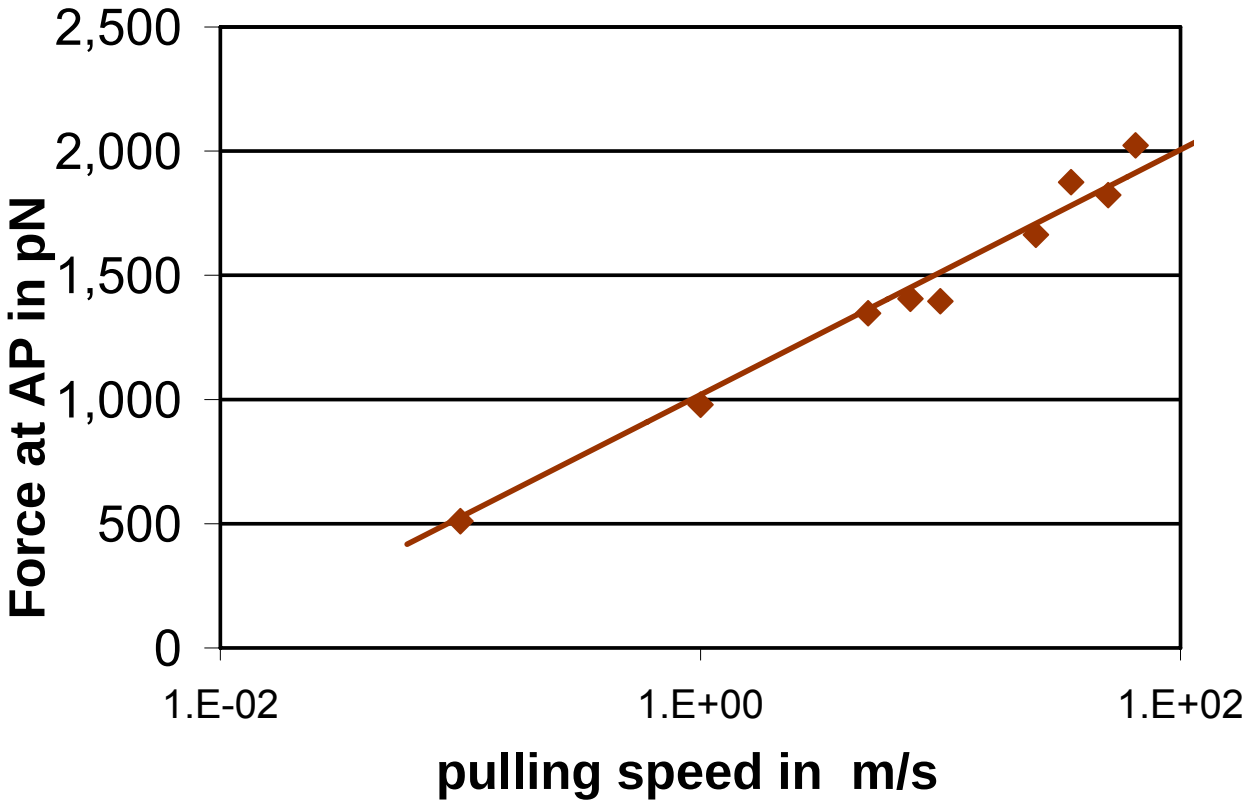
Figure by MIT OpenCourseWare. After Ackbarow and Buehler, 2006.

$$\sigma = F/A$$

A: Cross-sectional area of molecule

Ackbarow and Buehler, MRS Proc. 2006; JMS, in press (to appear 2007)

Unfolding force depends logarithmically on pulling speed



Larger pulling speed leads to larger resistance against unfolding

M. Buehler & T. Ackbarow, *Materials Today, 2007*

Studies on other protein structures

MD simulation

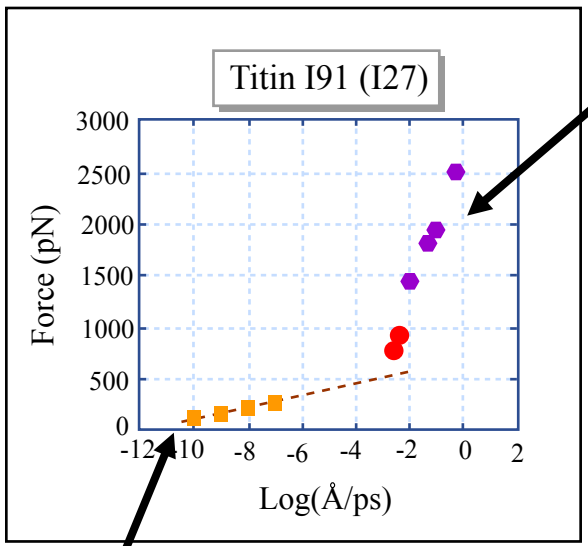


Figure by MIT OpenCourseWare. After Schulten, 2007.

Experiment

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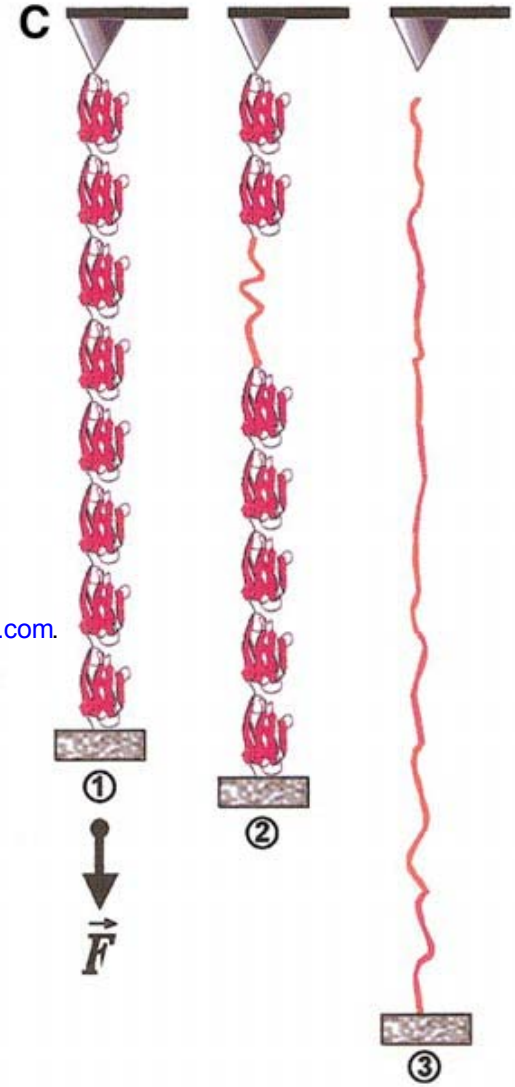


Molecular defects in vimentin

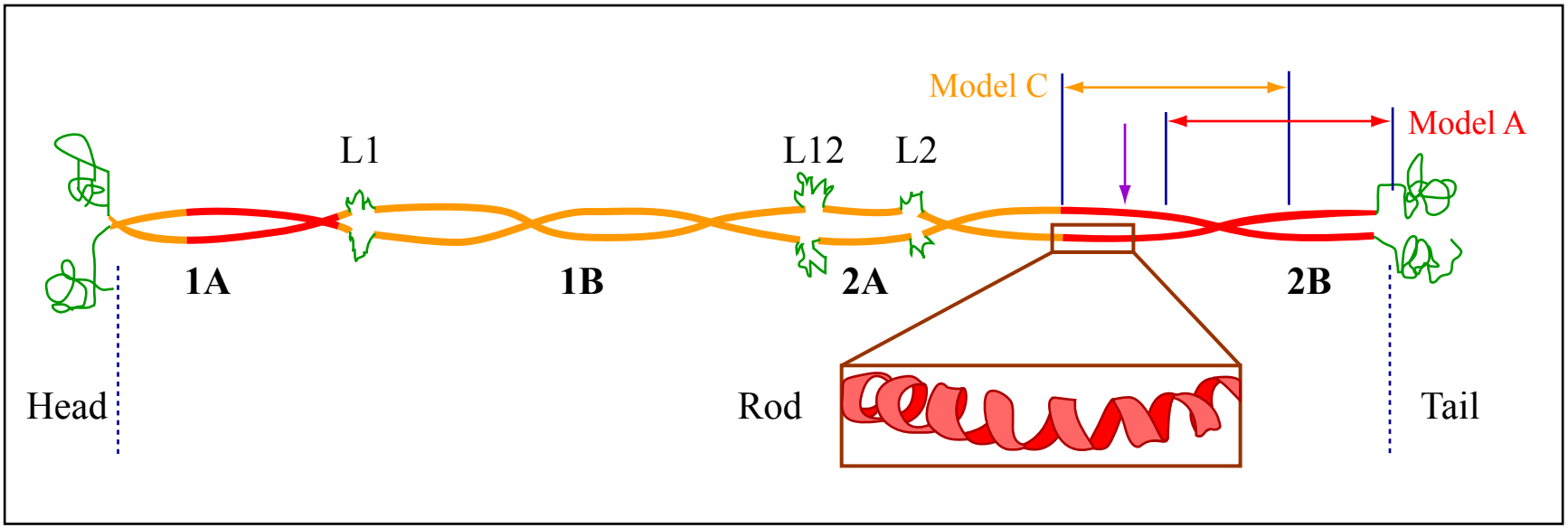


Figure by MIT OpenCourseWare. After Ackbarow and Buehler, 2008.

'**Skips**' are insertions of one residue into the heptad pattern

'**Stammers**' result through an insertion of three additional residues

'**Stutters**' appear if four additional residues interrupt the heptad sequence: Presence of a stutter results in an almost parallel run of both AHs without interrupting the CC geometry.

Ackbarow and Buehler, *Experimental Mechanics*, 2008

Pulling rate dependence: Bell model

Probability for bond rupture

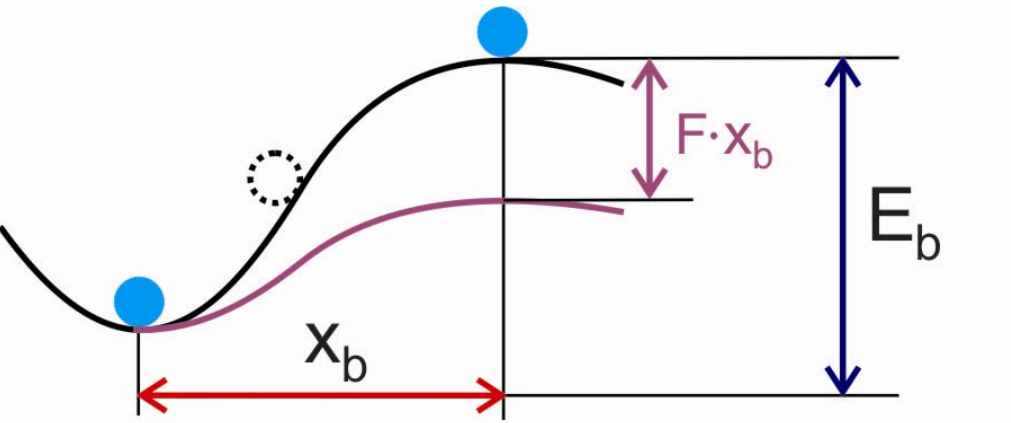
$$p = \exp\left[-\frac{E_b - Fx_B}{k_B T}\right]$$

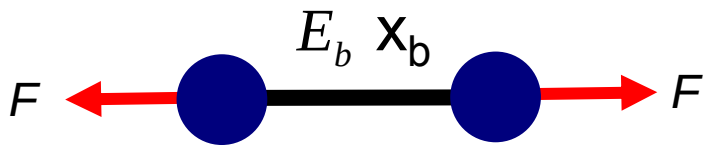
"off rate"

$$\chi = \omega_0 \cdot \exp\left(-\frac{(E_b - F \cdot x_b)}{k_b \cdot T}\right)$$

$$p = \tau^{-1}\omega^{-1}$$

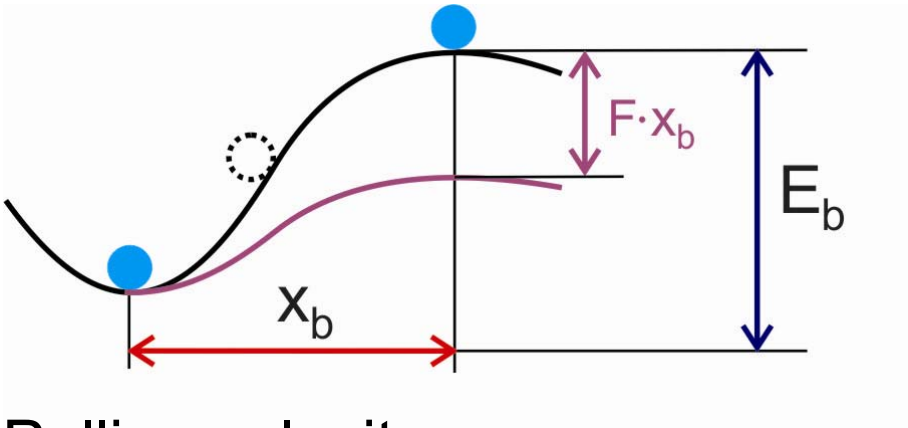
$$\omega = 1 \times 10^{13} / \sec$$

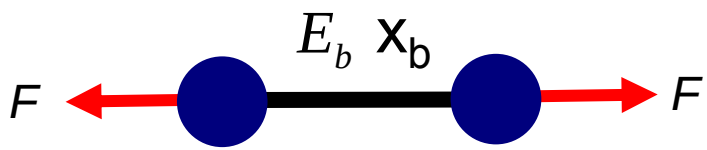




Bell, 1978; M. Buehler & T. Ackbarow, *Materials Today, 2007*

Pulling rate dependence: Bell model



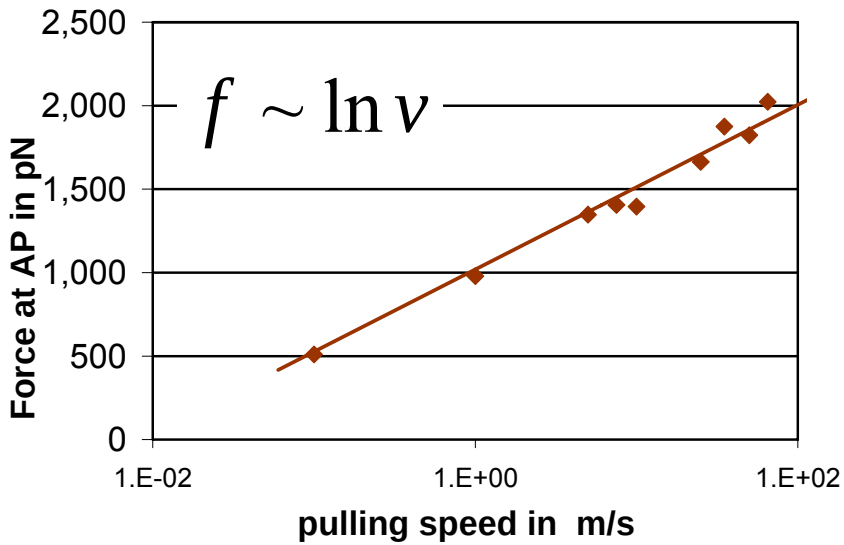


Pulling velocity

$$v = \omega_0 \cdot x_b \cdot \exp\left(-\frac{(E_b - F \cdot x_b)}{k_b \cdot T}\right)$$

Rupture force

$$F(v) = \frac{k_b \cdot T}{x_b} \cdot \ln v - \frac{k_b \cdot T}{x_b} \cdot \ln v_0 = a \cdot \ln v + b$$



Energy barrier: $E_b \approx 5.6$ kcal/mol

$x_b \approx 0.17$ Å: Suggests rupture of single H-bond

Ackbarow and Buehler, JMS, 2007)

Change in deformation mechanism

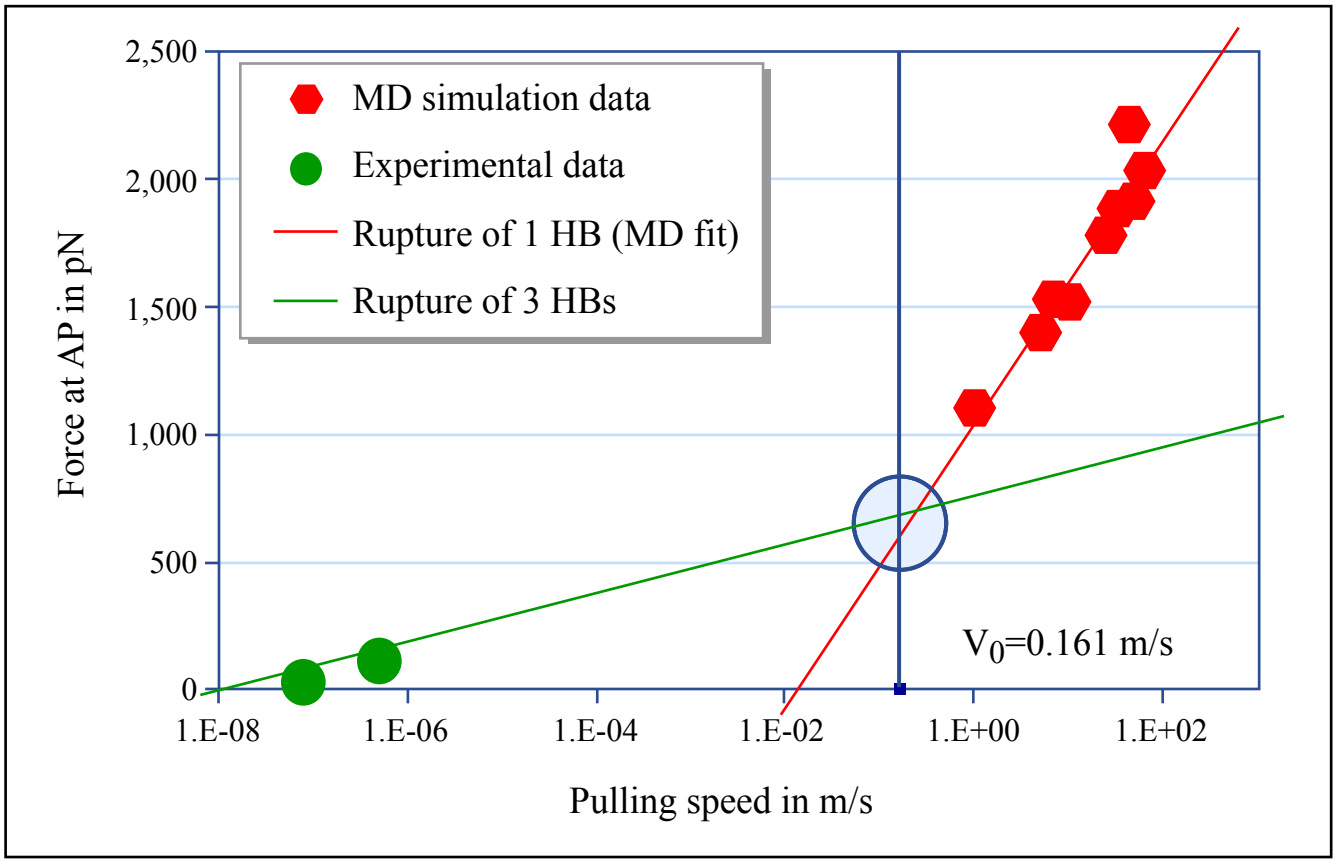


Figure by MIT OpenCourseWare. After Ackbarow and Buehler, 2006.

Ackbarow and Buehler, *MRS Proc.* 2006; *JMS*, 2007

Studies on other protein structures

MD simulation

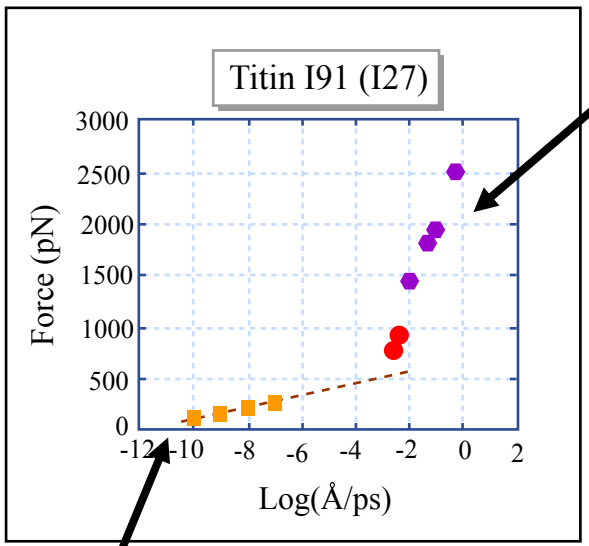
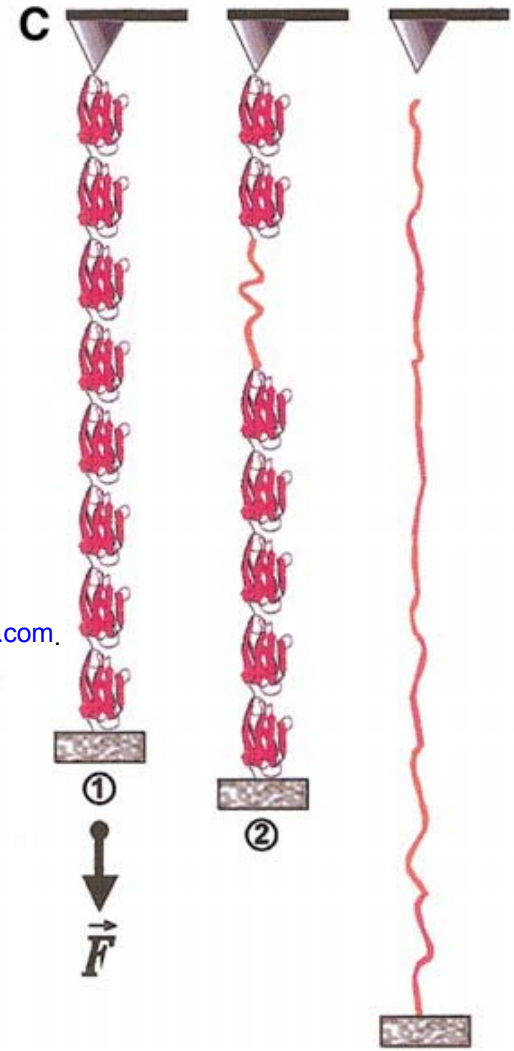


Figure by MIT OpenCourseWare. After Schulten, 2007.

Experiment



Linke *et al.*, *Journal of Structural Biology*, 2002; Schulten, *Science,* 2007

Change in deformation mechanism

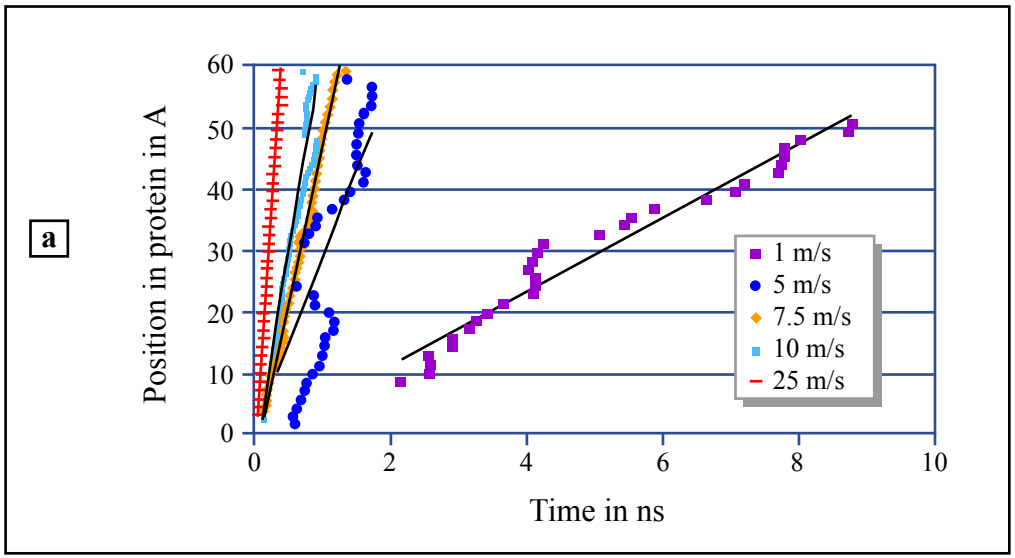


Figure by MIT OpenCourseWare. After Ackbarow and Buehler, 2006.

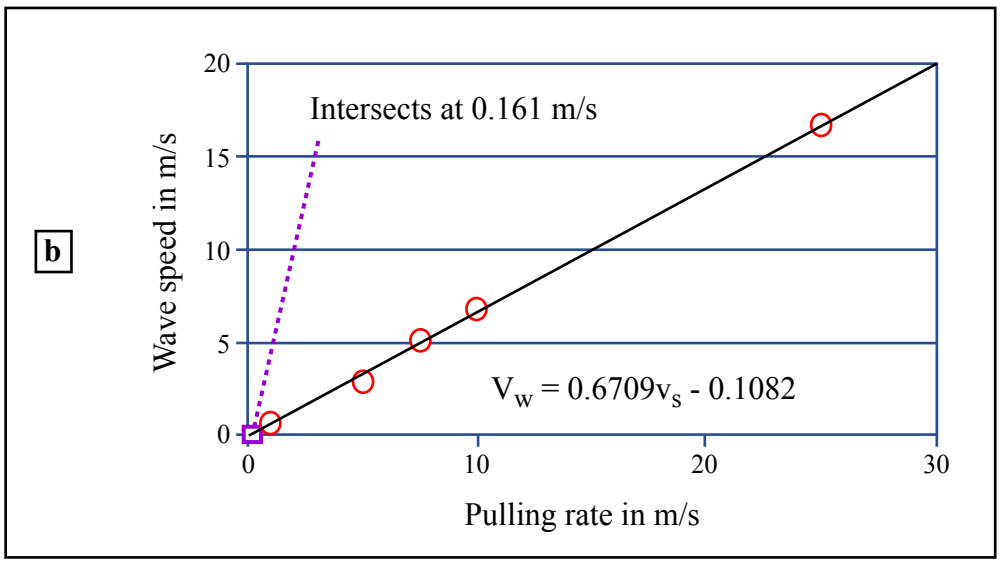
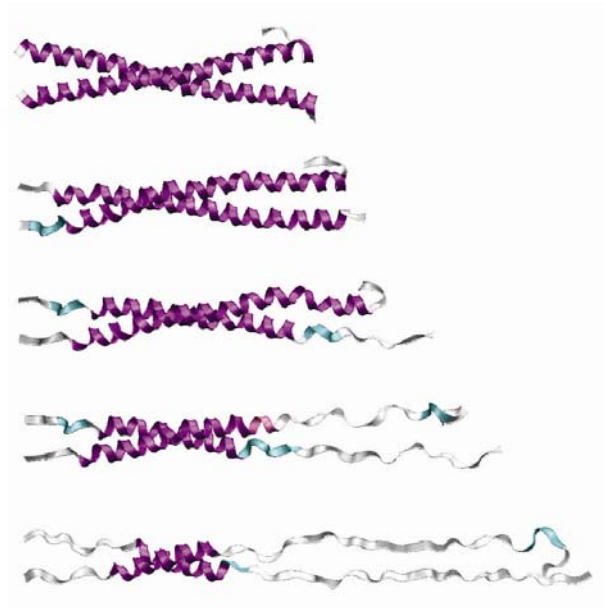


Figure by MIT OpenCourseWare.



Slower rates – deformation 'wave' vanishes

Change in deformation mechanism

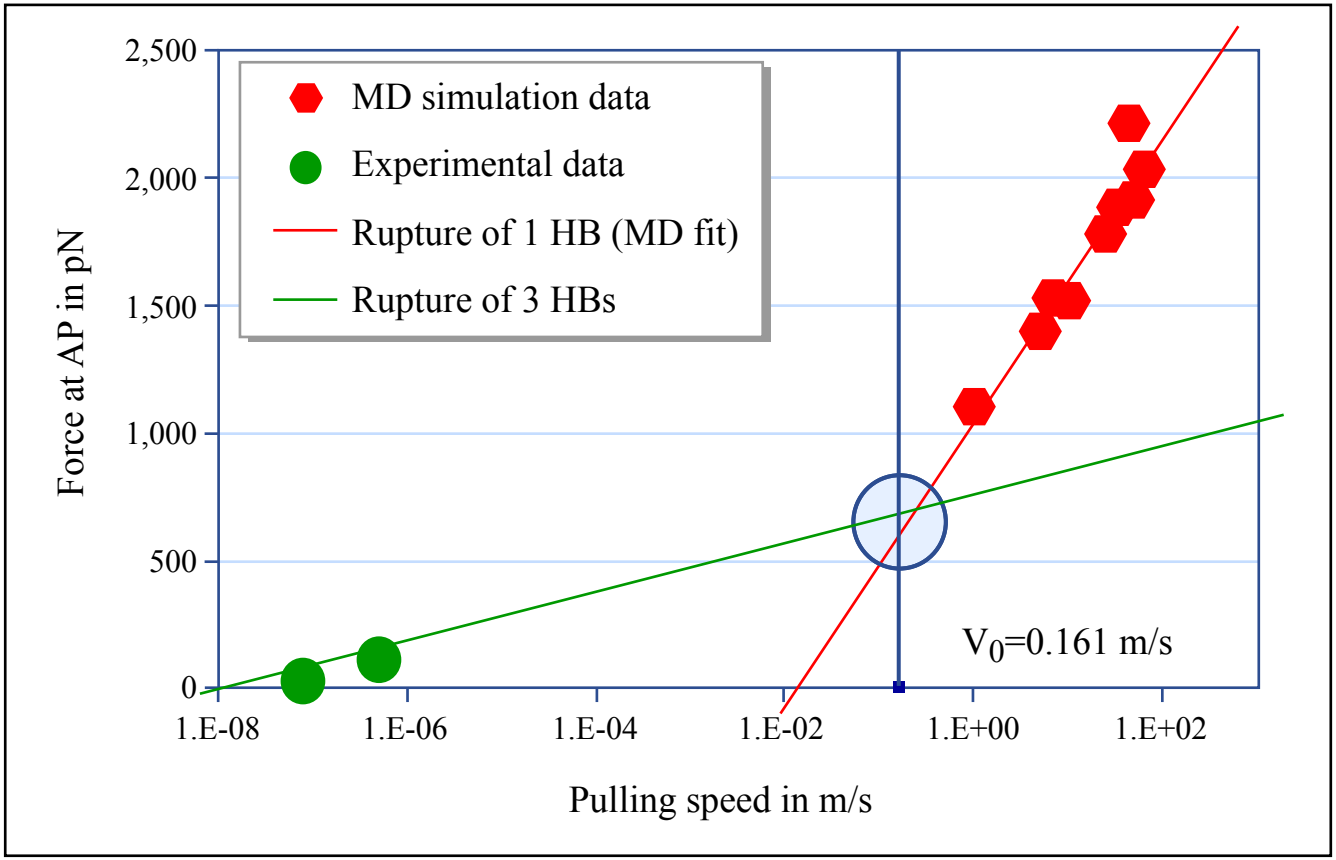


Figure by MIT OpenCourseWare. After Ackbarow and Buehler, 2006.

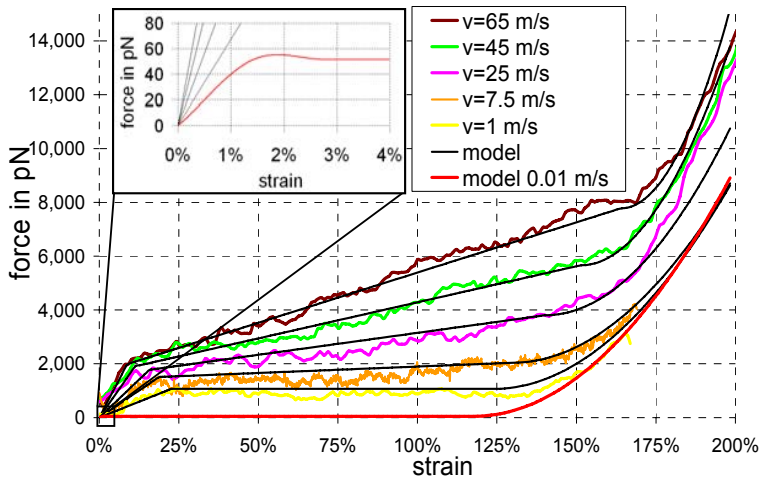
Predict: Strain distribution homogeneous below v_0, no localization

Ackbarow and Buehler, *MRS Proc.* 2006; *JMS*, 2007

Atomistically informed continuum theory

Three regimes

$$F(x,v)=\begin{cases} F_1=(F_a\cdot x)/x_a & \text{if } x<x_a \\ F_2=s_2\cdot(x-x_a)+F_a & \text{if } x_a\le x<x_s \\ F_3=s_3\cdot(x-x_s)^2+F_s & \text{if } x\ge x_s \end{cases}$$



$$s_1=c_5\cdot v+c_6,\quad s_2=\max(c_7\cdot v+c_8;0),\quad F_a=c_1\cdot\ln(v)+c_2,\ \text{and}\ \ x_a=F_a/s_1$$

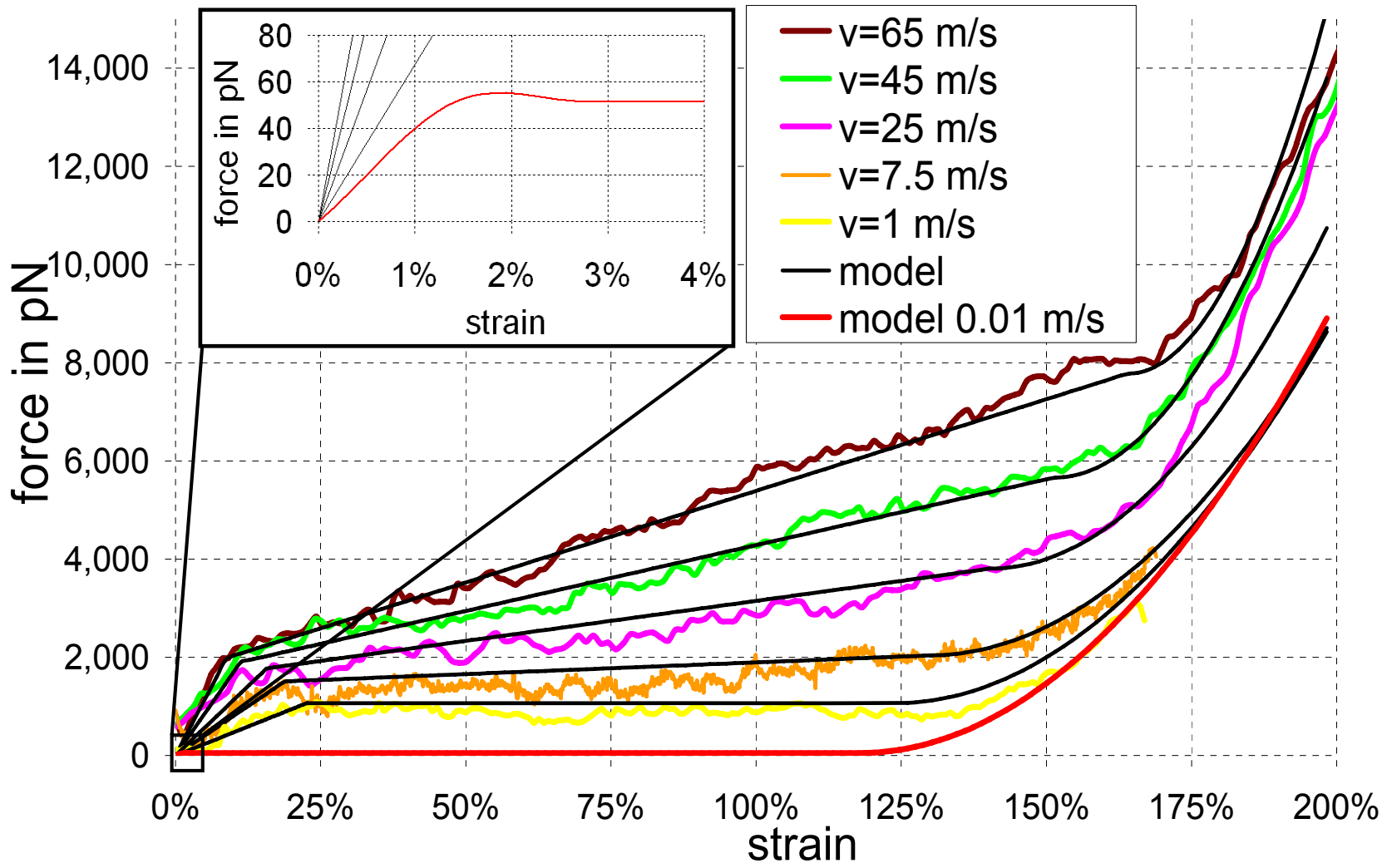
$$F_s=c_3\cdot x_s+c_4,$$

$$s_3=c_9\cdot v^2+c_{10}\cdot v+c_{11}\ \text{and}\ \ x_s=\left(F_a\cdot\left(1-\frac{s_2}{s_1}\right)-c_4\right)/(c_3-s_2)$$

Numerical parameters and its units										
c_1	c_2	c_3	c_4	c_5	c_6	c_7	c_8	c_9	c_{10}	c_{11}
220	3,604	237	-19,65	723,894	59.6	173,519	-6.3	2.4E7	-4.55	2.7
pN	pN	pN/Å	pN	pN·fs/Å^2	pN/Å	pN·fs/Å^2	pN/A	pN·fs^2/Å^3	pN·fs/Å^2	pN/Å

Ackbarow and Buehler, MRS Proc. 2006; JMS, 2007

Atomistically informed continuum theory



Ackbarow and Buehler, MRS Proc. 2006; JMS, 2007

‘Impact shielding’ superelastic material

“cell’s safety belt”

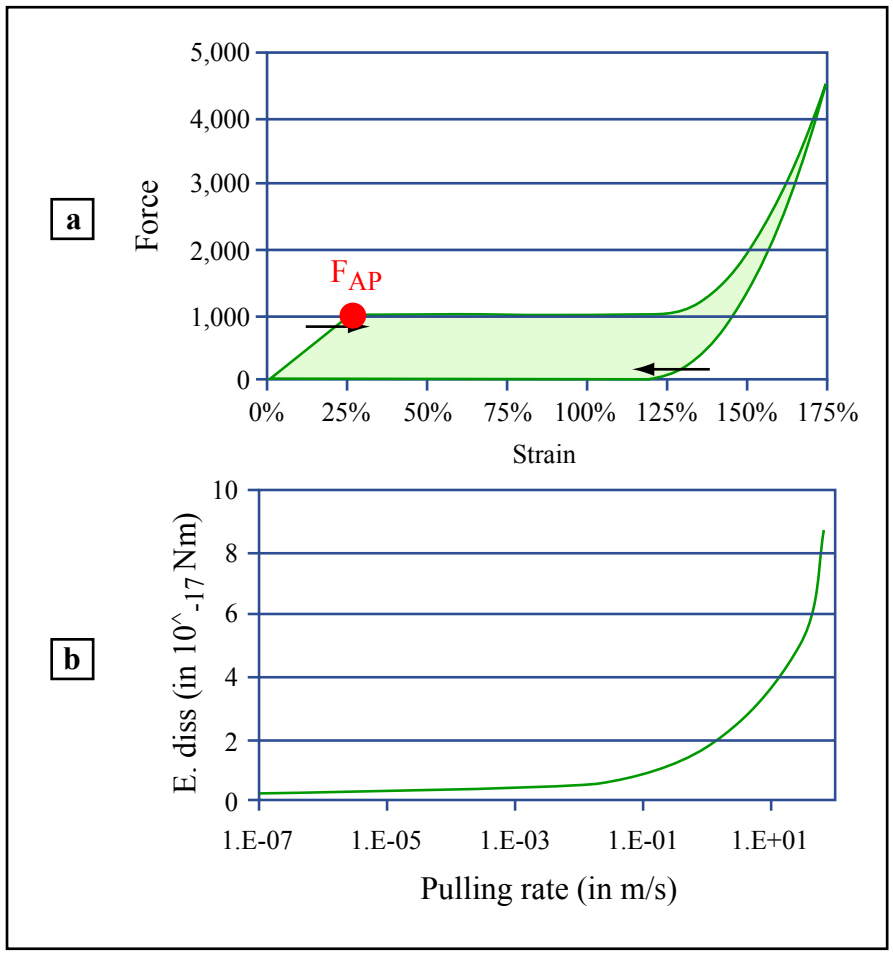


Figure by MIT OpenCourseWare. After Buehler and Ackbarow.

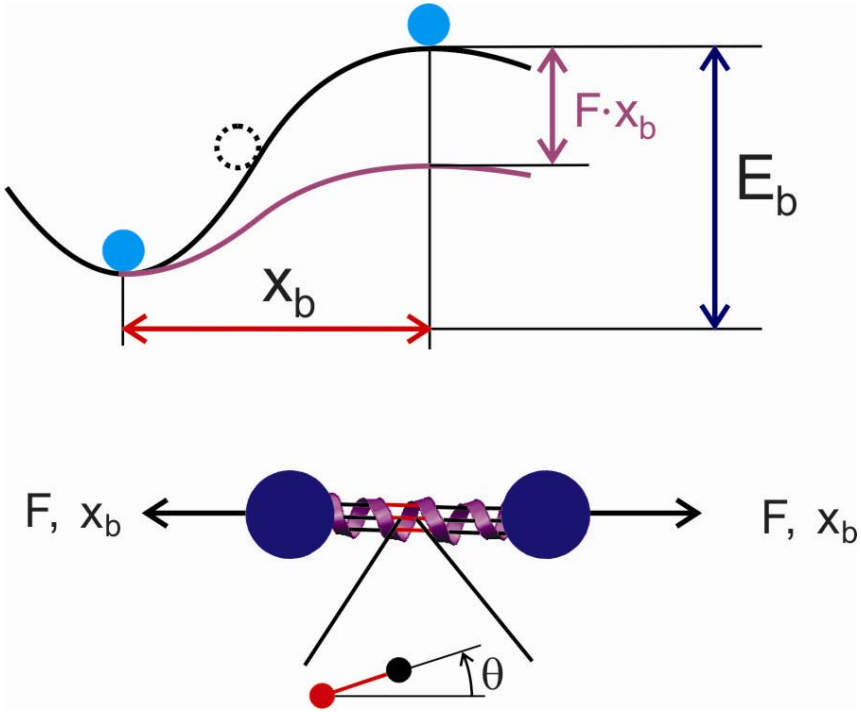
Superelastic material

High impact resistance

- **Coiled-coil:** Enables large superelastic strains (low forces, since H-bonds break easily) – scale ~ 10..100 nm
- **Different mechanisms**: Breaking of single H-bond leads to large slope of AP w.r.t. strain rate (significant strengthening at large deformation rates)
- **At small deformation rates:** strengthening not as strong, as three H-bonds break simultaneously
- **Refolding possible**, since sequence has not been destroyed (if forces below rupture of covalent bonds)

Ackbarow and Buehler, *JMS, in press*

Predictive Bell Model



"off rate"

$$\chi = \omega_0 \cdot \exp\left(-\frac{(E_b - F \cdot x_b \cdot \cos(\theta))}{k_b \cdot T}\right)$$

Pulling velocity

$$v = \chi \cdot x_b$$

$$v = \omega_0 \cdot x_b \cdot \exp\left(-\frac{(E_b - F \cdot x_b \cdot \cos(\theta))}{k_b \cdot T}\right)$$

Rupture force

$$F(v) = \frac{k_b \cdot T}{x_b \cdot \cos(\theta)} \cdot \ln v - \frac{k_b \cdot T}{x_b \cdot \cos(\theta)} \cdot \ln v_0 = a \cdot \ln v + b$$

M. Buehler & T. Ackbarow, *Materials Today, in press*

Molecular defects in vimentin

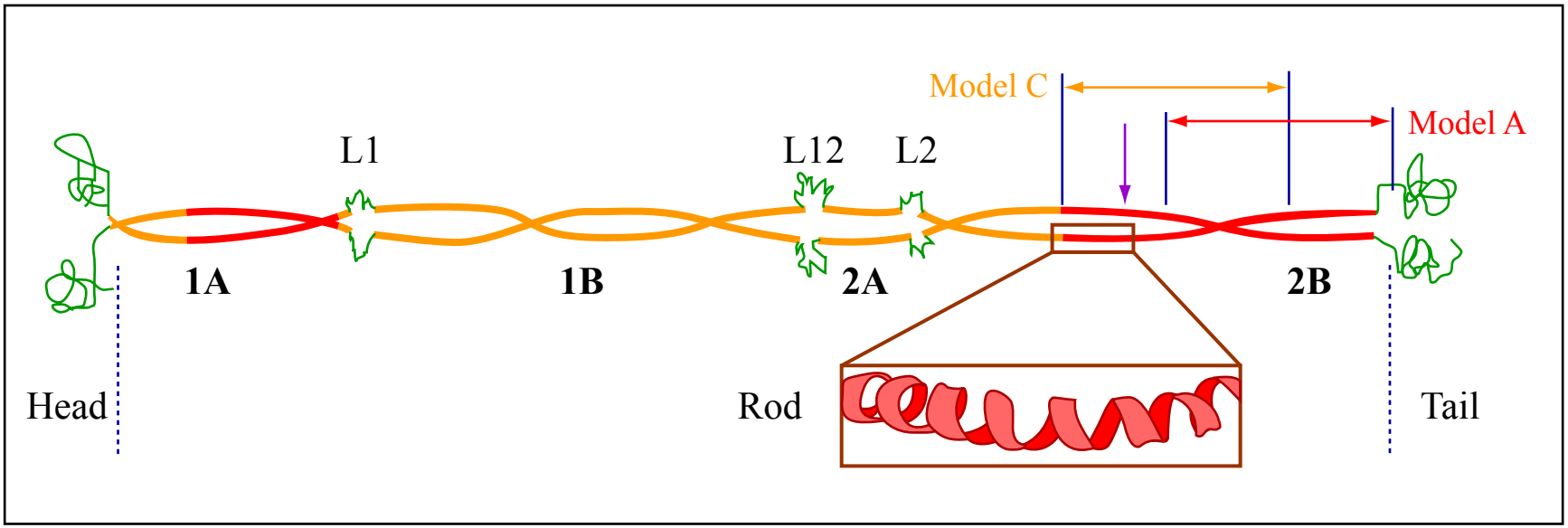


Figure by MIT OpenCourseWare. After Ackbarow and Buehler, 2008.

'**Skips**' are insertions of one residue into the heptad pattern

'**Stammers**' result through an insertion of three additional residues

'**Stutters**' appear if four additional residues interrupt the heptad sequence: Presence of a stutter results in an almost parallel run of both AHs without interrupting the CC geometry.

Governing irregularities and defects in proteins with HPT

Stutter: insert of residues into the heptad repeat periodicity

→local uncoiling of the coiled-coil

→different angles within one structure

alpha helix and stutter: θ_1=16°

coiled-coil : θ_2=23°

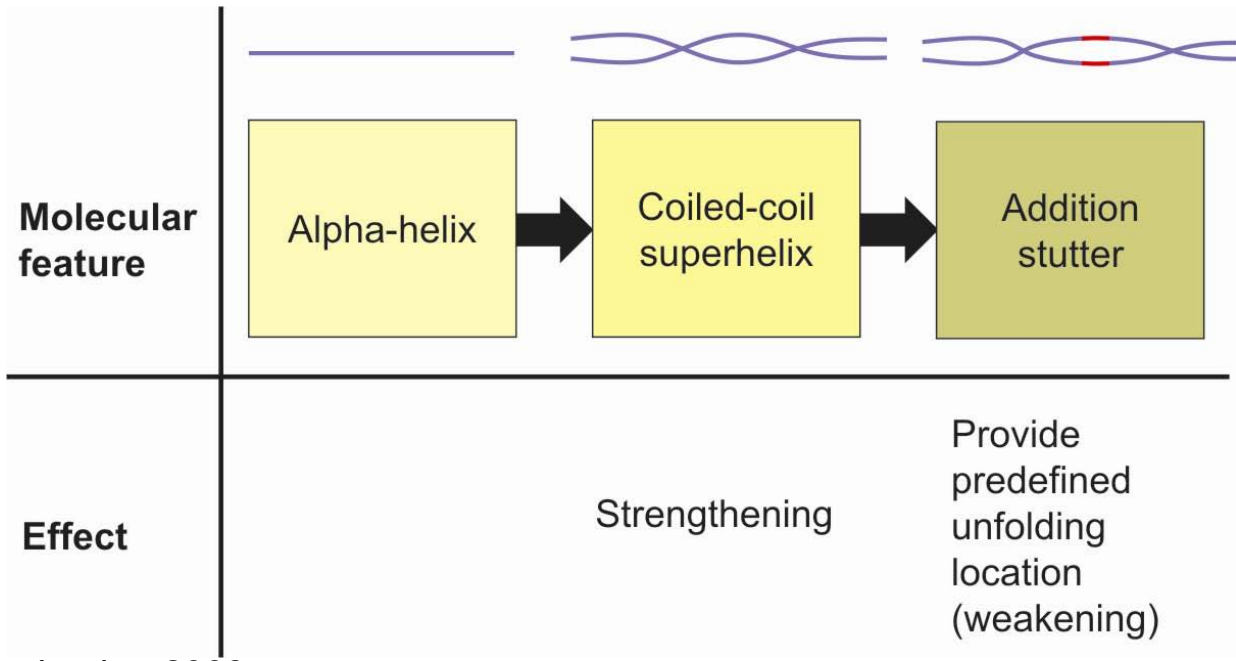
$$f(v) = \frac{k_b \cdot T}{x_b \cdot \cos(\theta)} \cdot \ln v - \frac{k_b \cdot T}{x_b \cdot \cos(\theta)} \cdot \ln v_0$$

Difference in unfolding force
5% weaker at stutter

$$\theta_2 > \theta_1$$

$$\cos\theta_2 < \cos\theta_1$$

$$f_2(v) = \frac{\cos\theta_1}{\cos\theta_2} \cdot f_1(v)$$

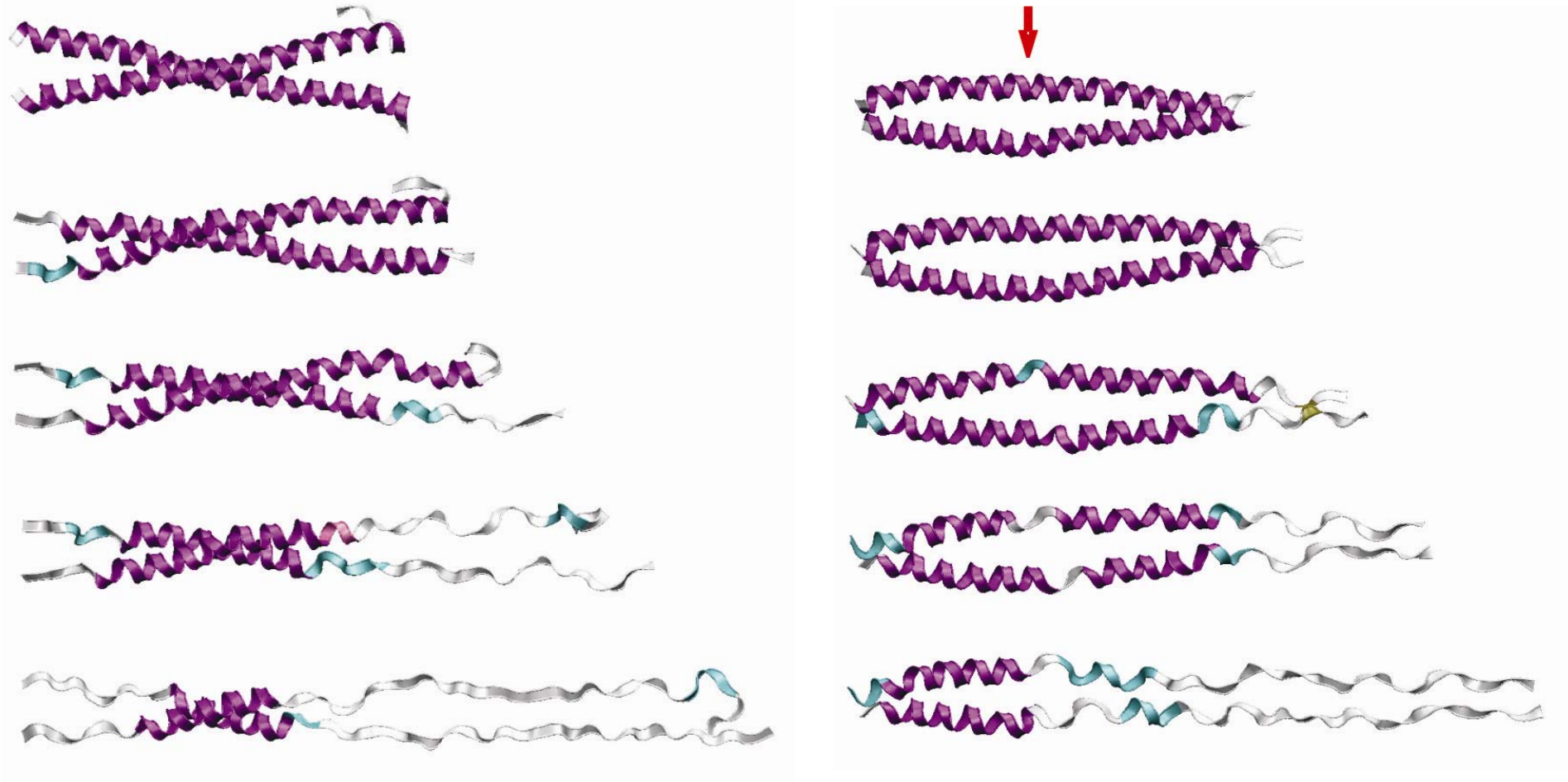


Ackbarow and Buehler, *Experimental Mechanics*, 2008

Stutter: location of predetermined unfolding

Without stutter

With stutter



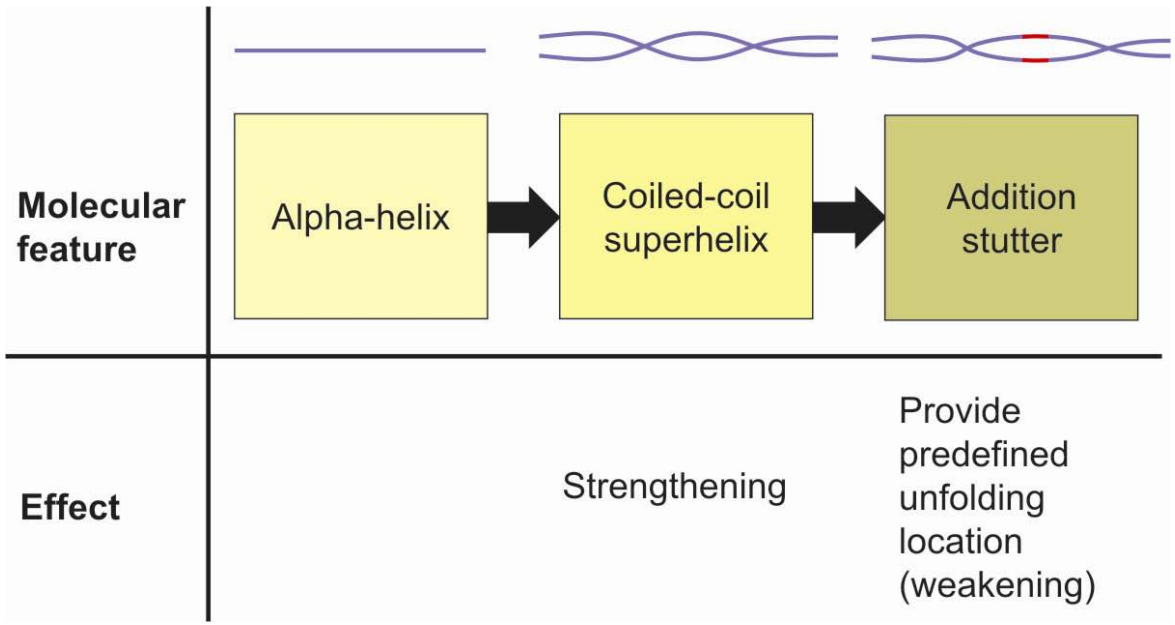
Ackbarow and Buehler, Experimental Mechanics, 2008

Summary: Effects of the stutter

• Renders the molecular structures softer, that is, unfolding occurs at lower tensile forces,

• Introduces predefined locations of unfolding, and

• Thus leads to a more homogeneous distribution of plastic strains throughout the molecular geometry.



Ackbarow and Buehler, *Experimental Mechanics*, 2008

Summary: 'Take home message'

- Significance of protein mechanics for biological processes (signaling, cancer, mechanical integrity..)
- Chemical bonds within protein & theoretical description (force field)
- Free energy source of elasticity: Link atomistic/molecular concepts with continuum theory (rubber elasticity, WLC model)
- Case study: Vimentin dimer – response to mechanical cue (stretching, unfolding, rate dependence)

  Bell model – statistical concept to predict unfolding force 'onset of plasticity' – permanent deformation

  Structure-function relationship to describe role of molecular defects in protein mechanics (example: stutter defect)