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ICTP 40th Anniversary

SMR 1564 - 20

SPRING COLLEGE ON SCIENCE AT THE NANOSCALE
(24 May - 11 June 2004)

SINGLE MOLECULE BIOPHYSICS - Part I

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These are preliminary lecture notes, intended only for distribution to participants.

Protein Mechanics

(or, successful nano-mechanics for the past 3.5 billion years)

"Everything in Biology is mechanical"

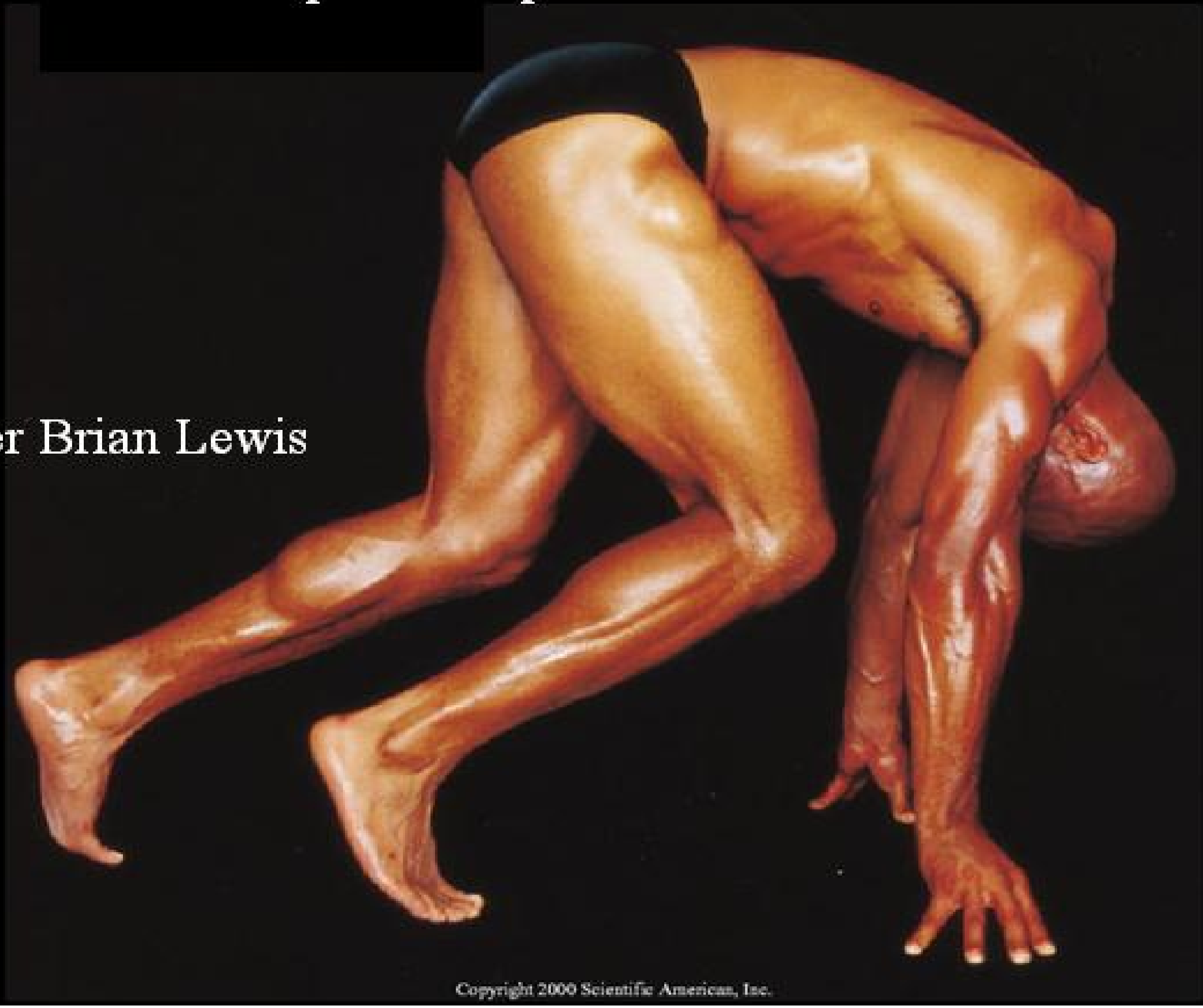
Engineering protein designs using molecular biological tools

Examination of mechanical properties of the resulting molecules using single molecule force probes and electron microscopy

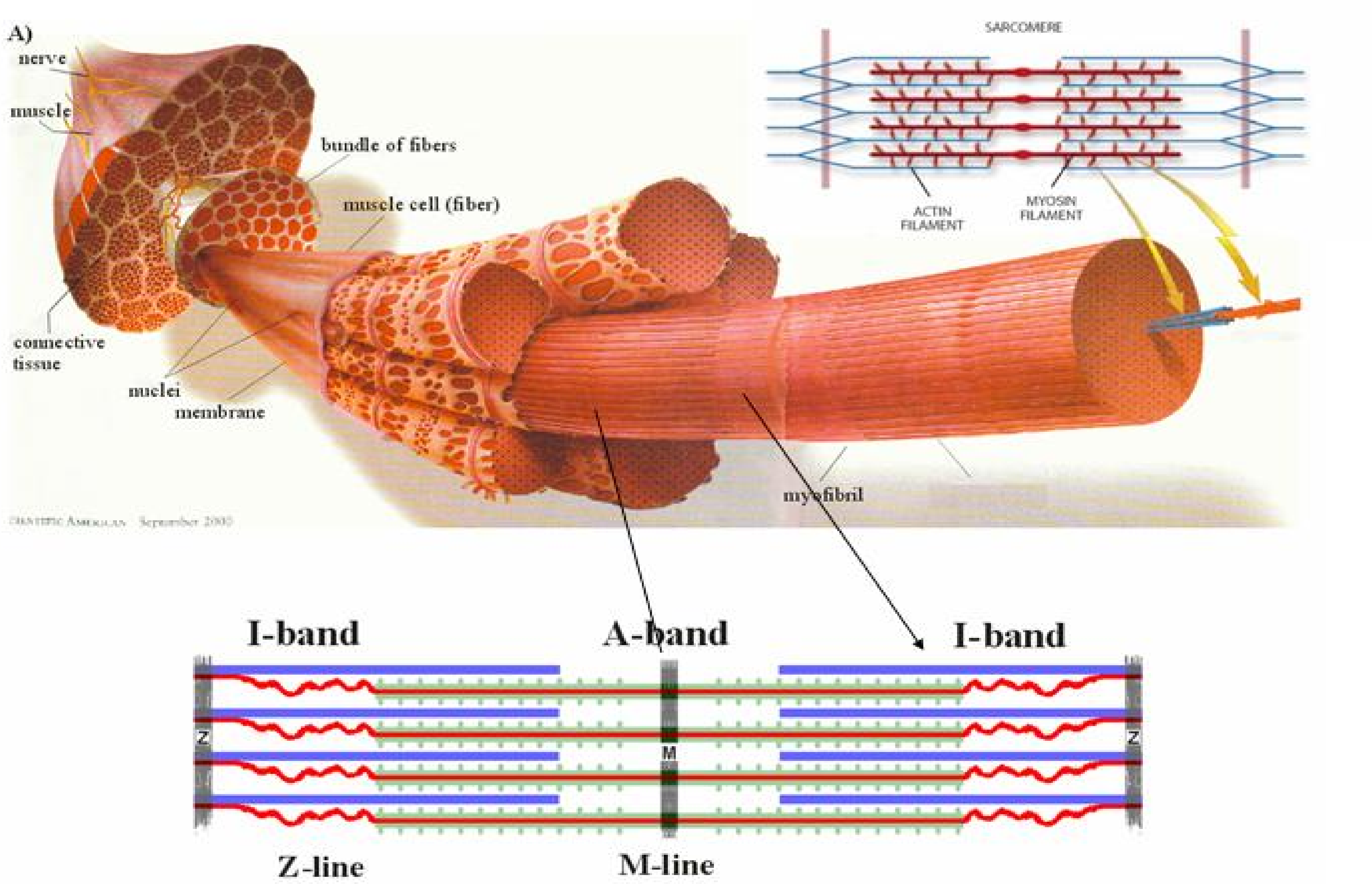
Use this approach to probe the mechanics of biological tissues At the single molecule level.

The elasticity of muscle is due to the Brownian motion driven collapse of a protein named titin

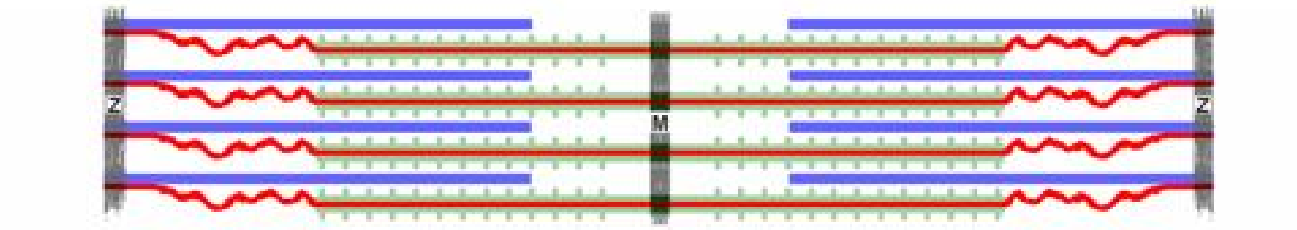
Sprinter Brian Lewis



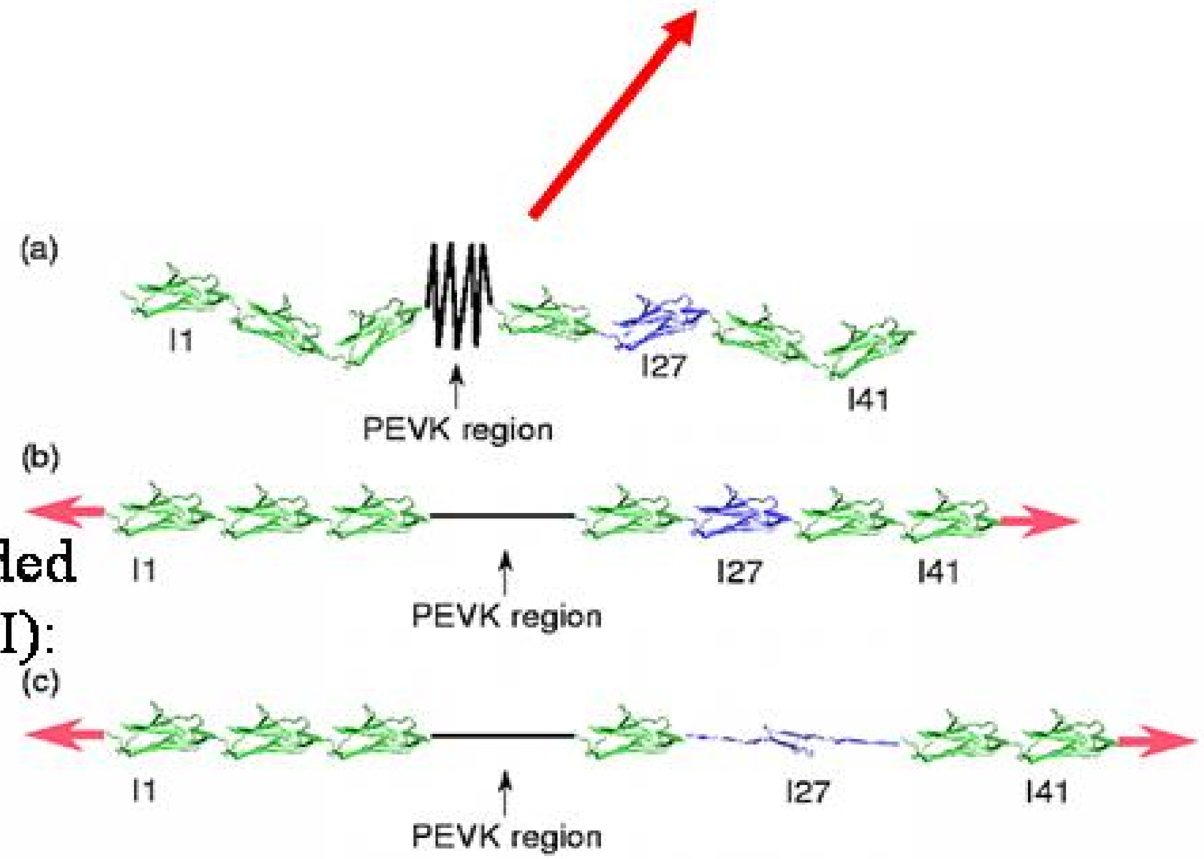
Muscle can contract and also can extend elastically



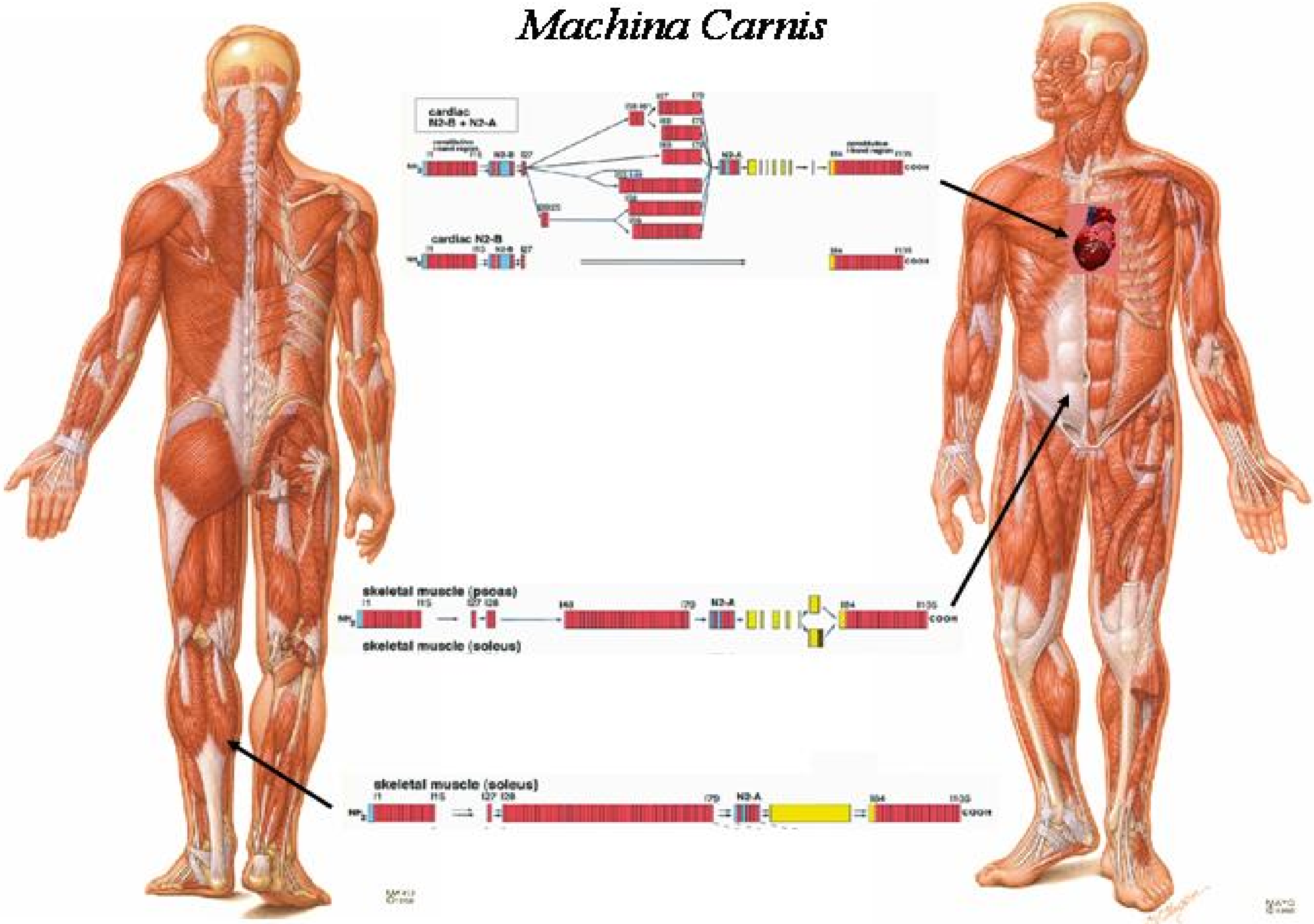
The elasticity of muscle results from the properties of a giant protein named Titin (from Titan!)



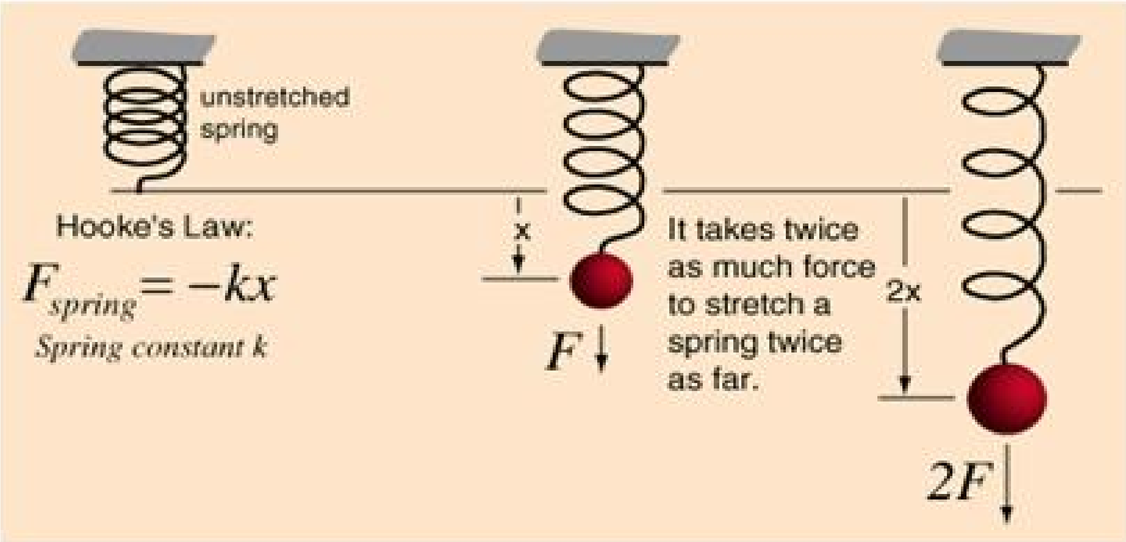
Titin has a coiled region (PEVK) and a region folded into individual modules (I):



Machina Carnis

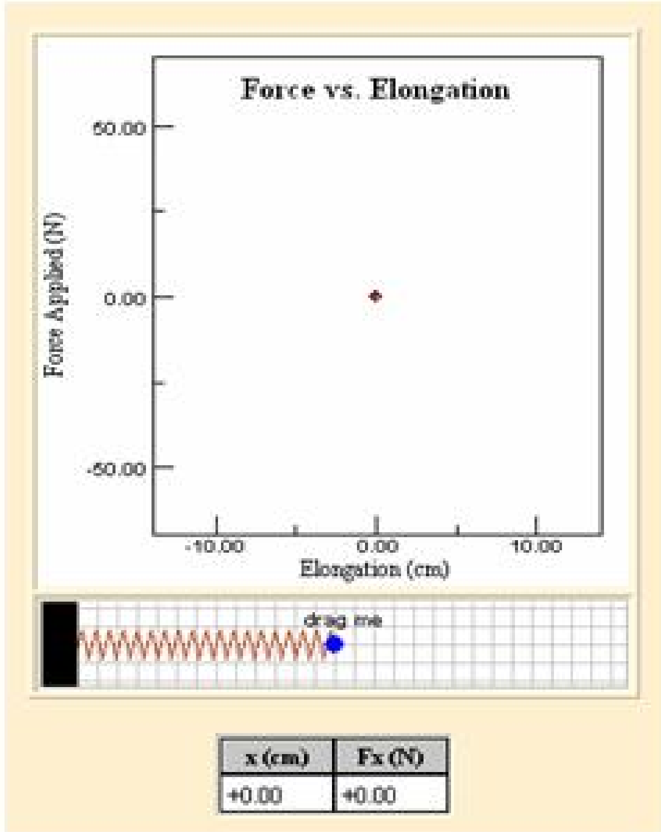


Is the elasticity of titin like that of a spring?



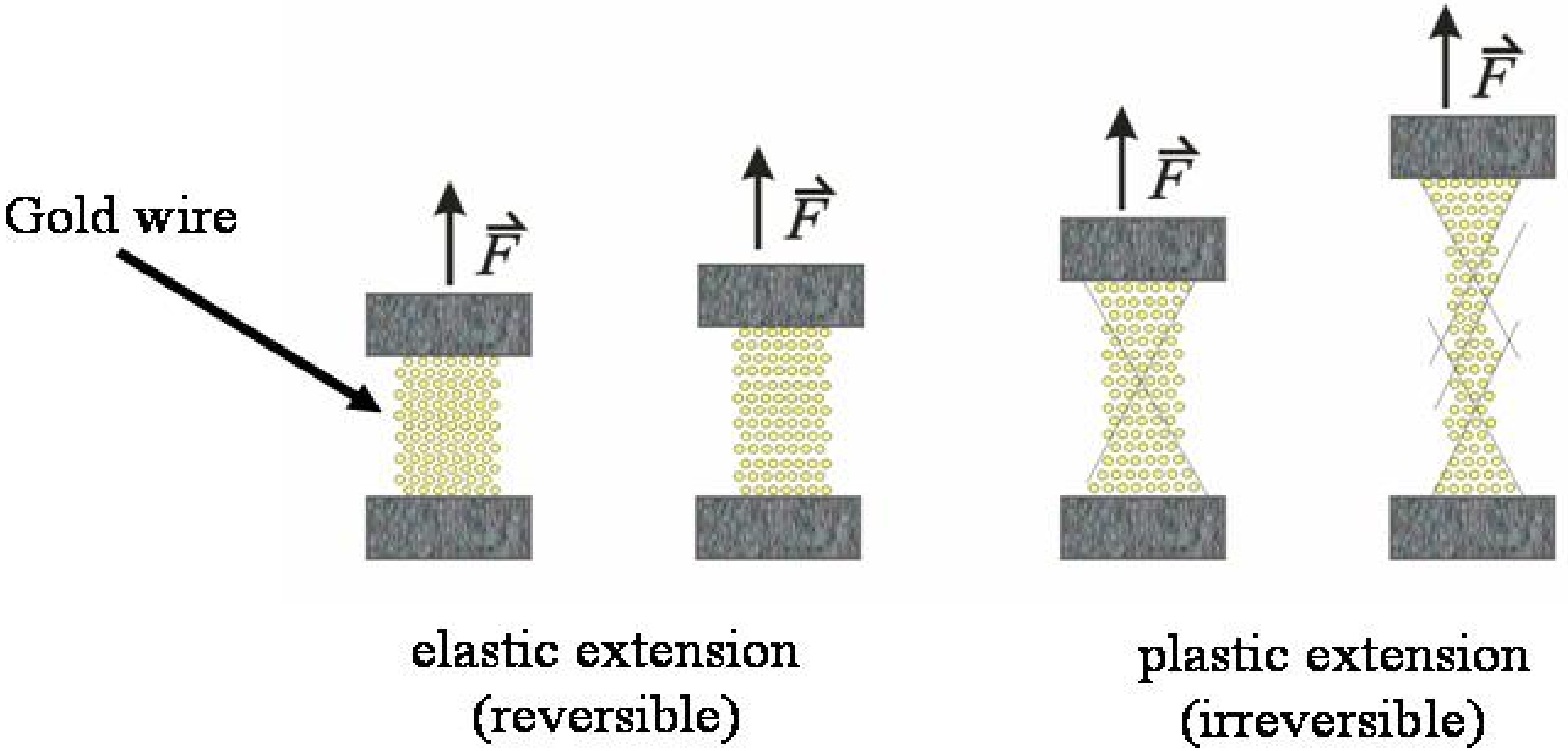
The elasticity of a metal spring results from the stretching of the bonds between the metal atoms.

Is this how titin works?

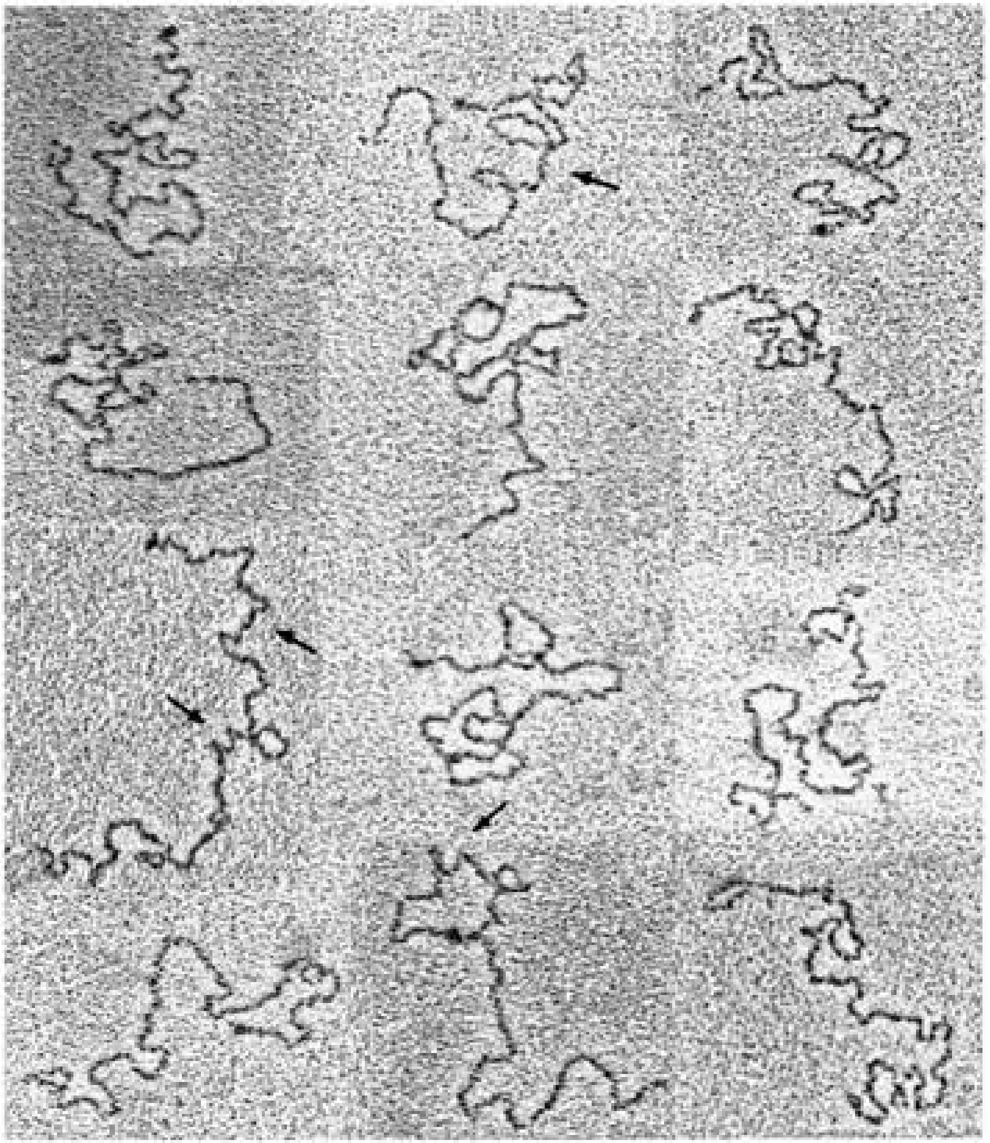


Will titin also break if we pull it too far?

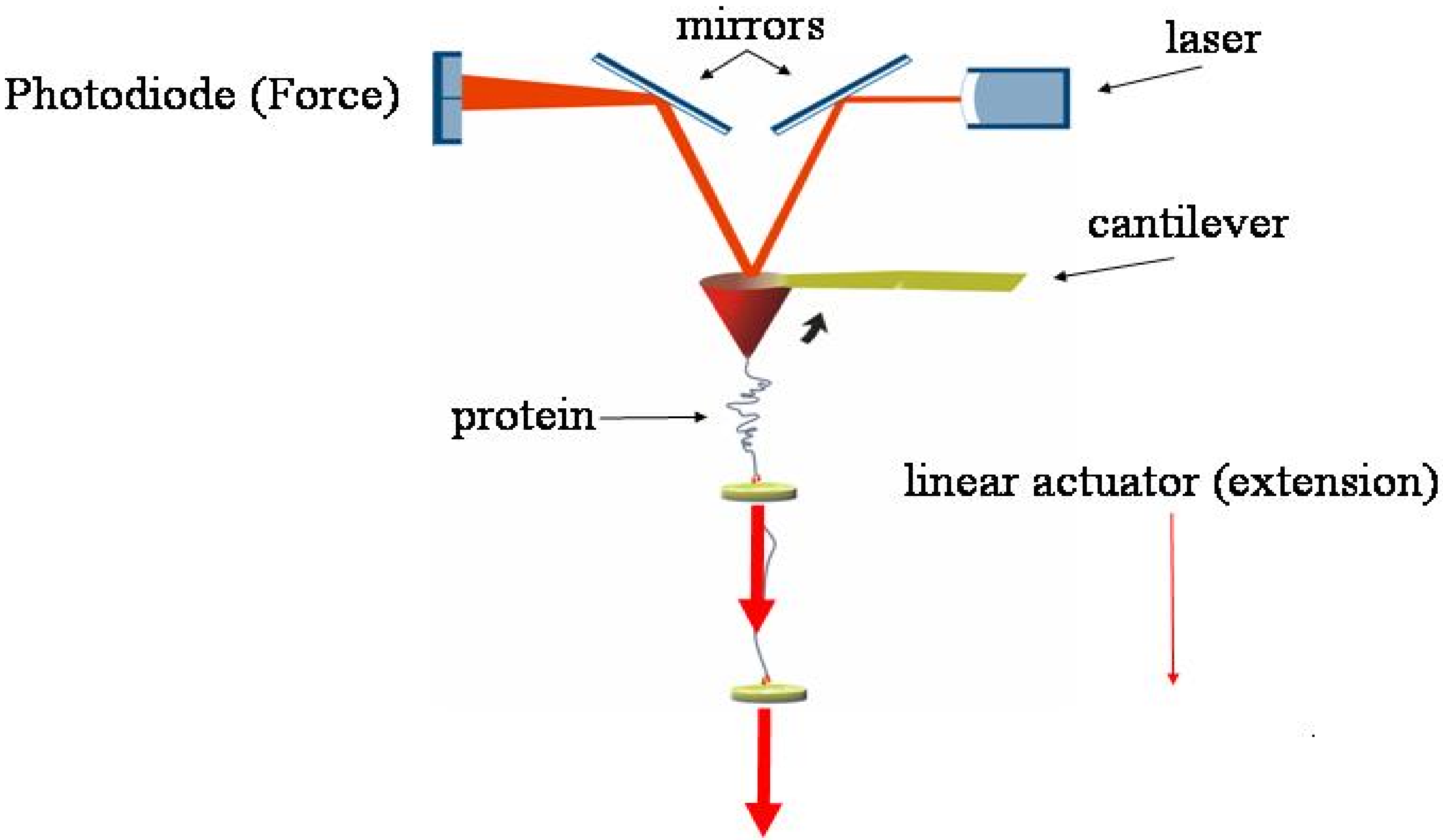
A metal wire in a spring extends by bond stretching and breaks by irreversibly disrupting its atomic arrangements

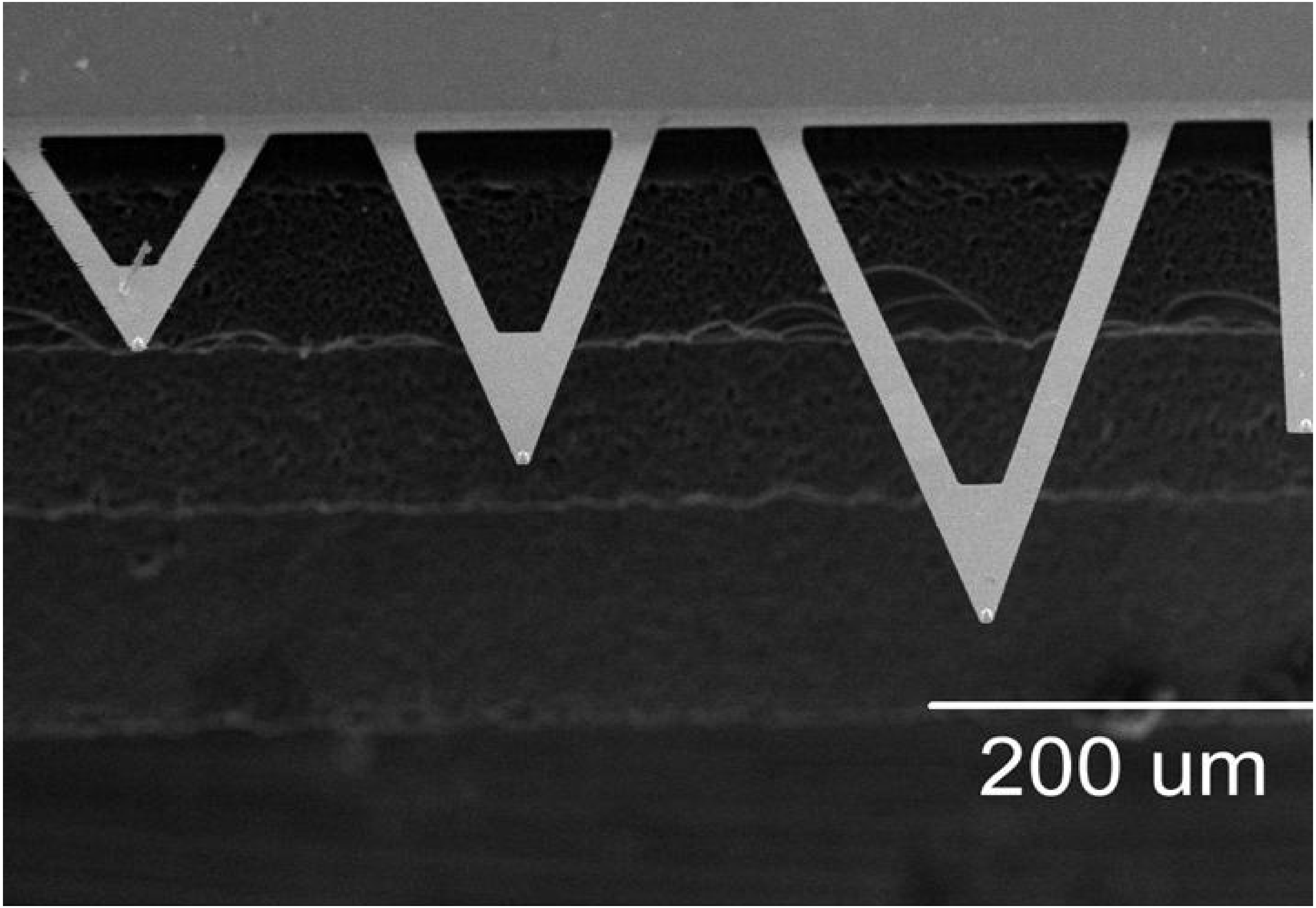


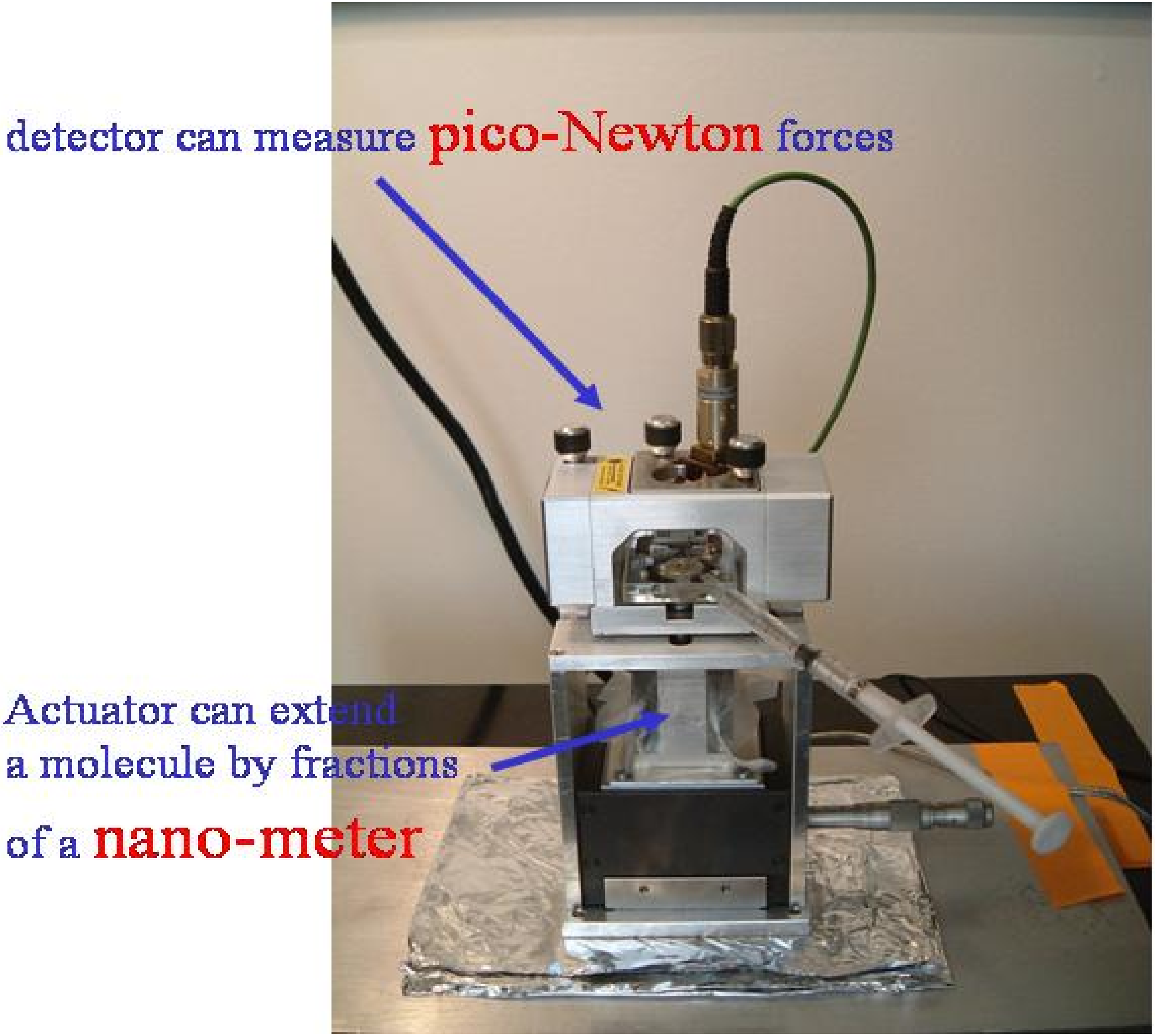
Electron micrographs of isolated titin molecules



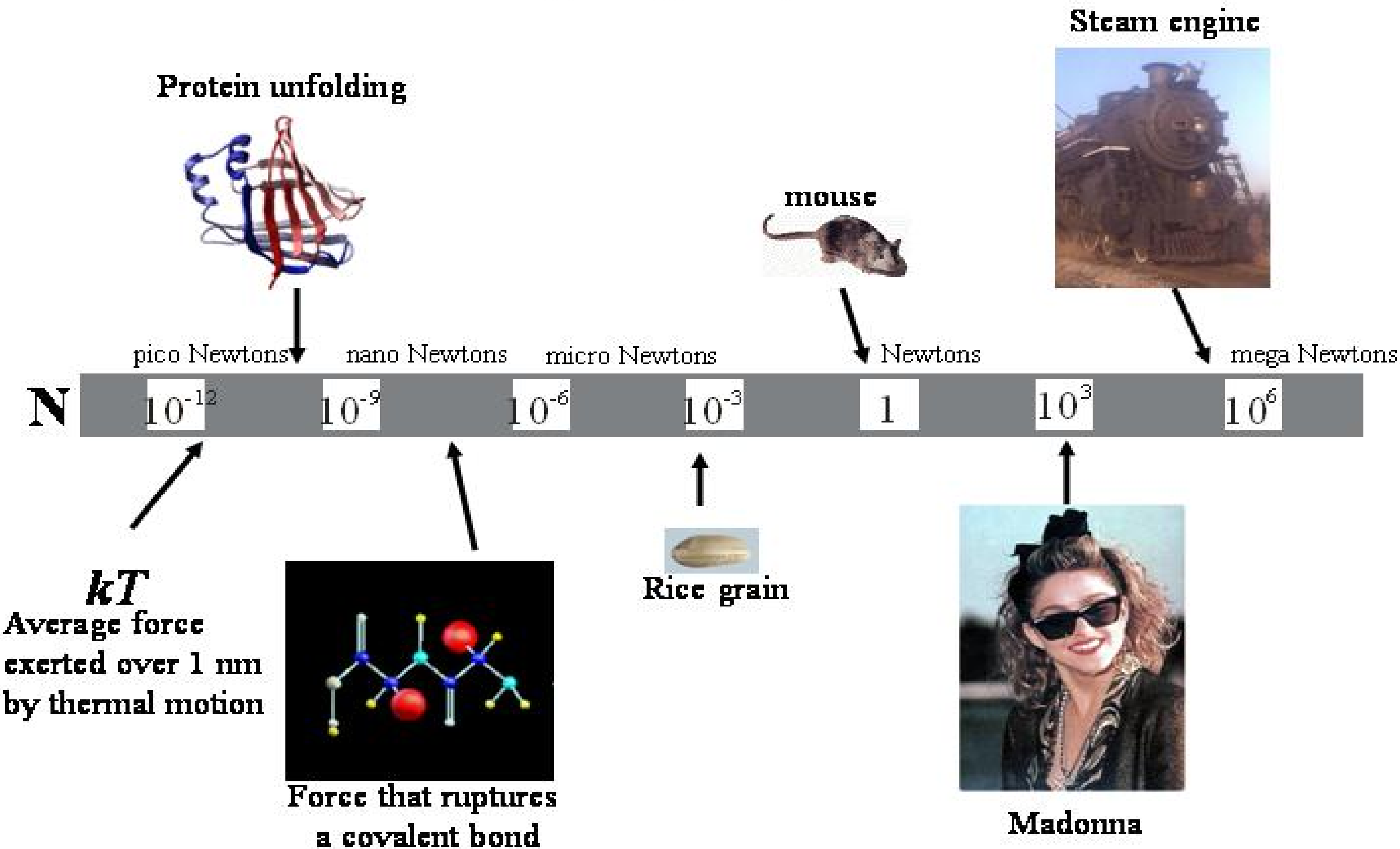
We can stretch a single protein and measure how does the restoring force changes with the extension.



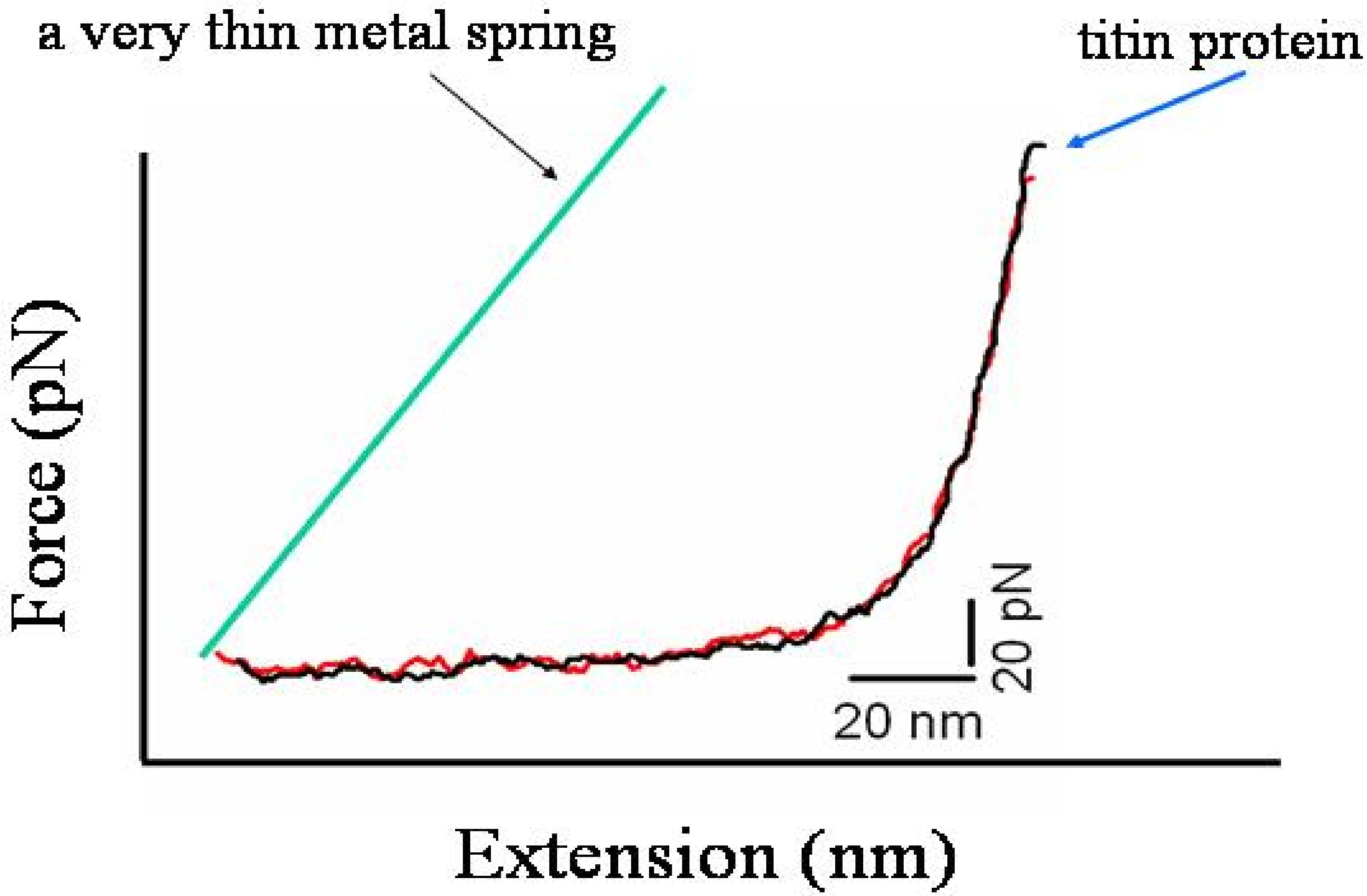

200 um


detector can measure pico-Newton forces
Actuator can extend
a molecule by fractions
of a nano-meter

How much is a pico (10^{-12}) Newton of Force?

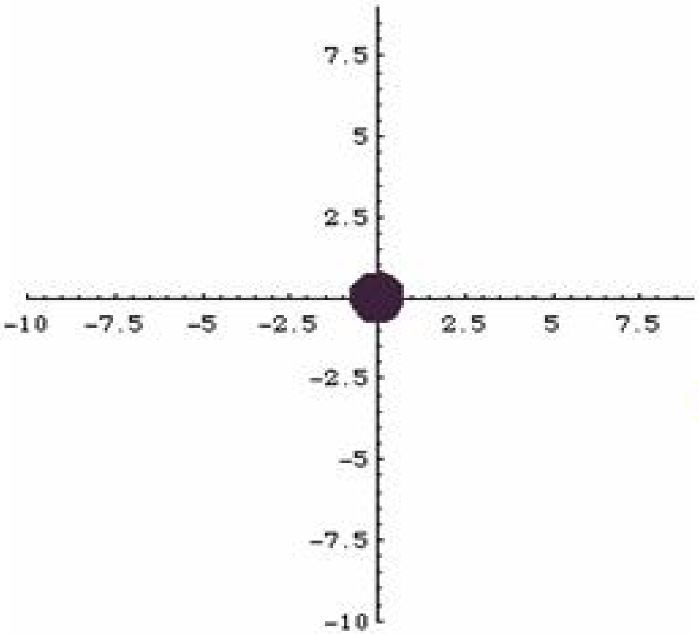


If we stretch a single titin protein we obtain force-extension curves that is very different from Hooke's law.



To understand how titin works,
we must first understand Brownian motion.

Robert Brown (1773-1858), a botanist, reported in 1828 his observation that pollen grains in water underwent incessant motion.



A single pollen grain observed with a microscope is being moved about by mysterious forces.

Albert Einstein, *Annalen der Physik (Paris)*, 17:549, 1905

“In this paper it will be shown that, according to the molecular kinetic theory of heat, bodies of a microscopically visible size suspended in liquids must, as a result of thermal molecular motions, perform motions of such magnitudes that they can be easily observed with a microscope. “

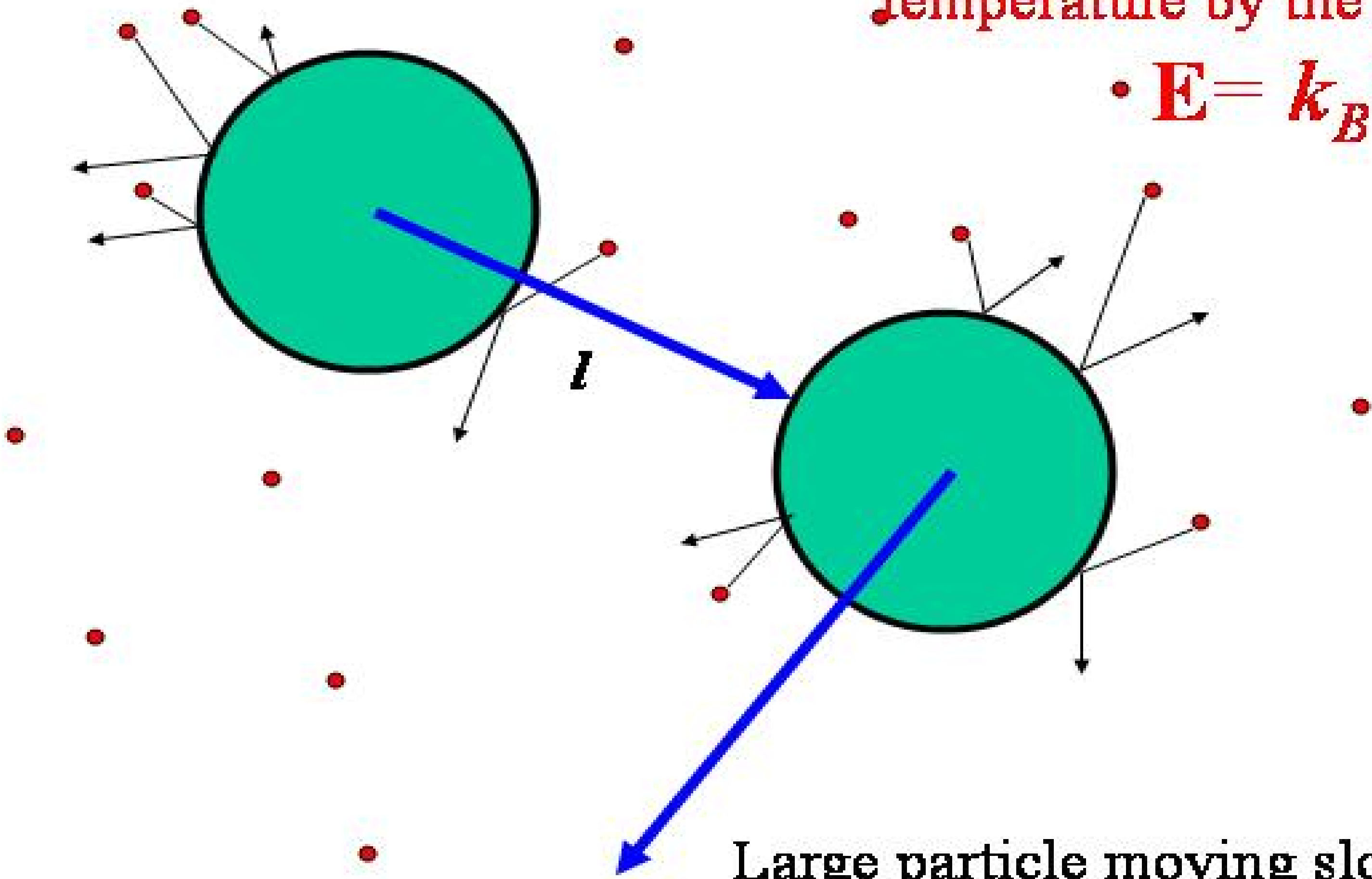
Smoluchowski was a Polish physicist that published a paper in 1906 where he also arrived at a very similar conclusion.

Marian Smoluchowski , *Annalen der Physik (Paris)*, 21:772, 1906

Small particles moving fast (red water molecules)

The average kinetic energy of each molecules is related to temperature by the relationship:

- $\mathbf{E} = k_B T$



Large particle moving slowly at an average velocity ***v***, on a random path of steps of length ***l*** (green pollen grain)

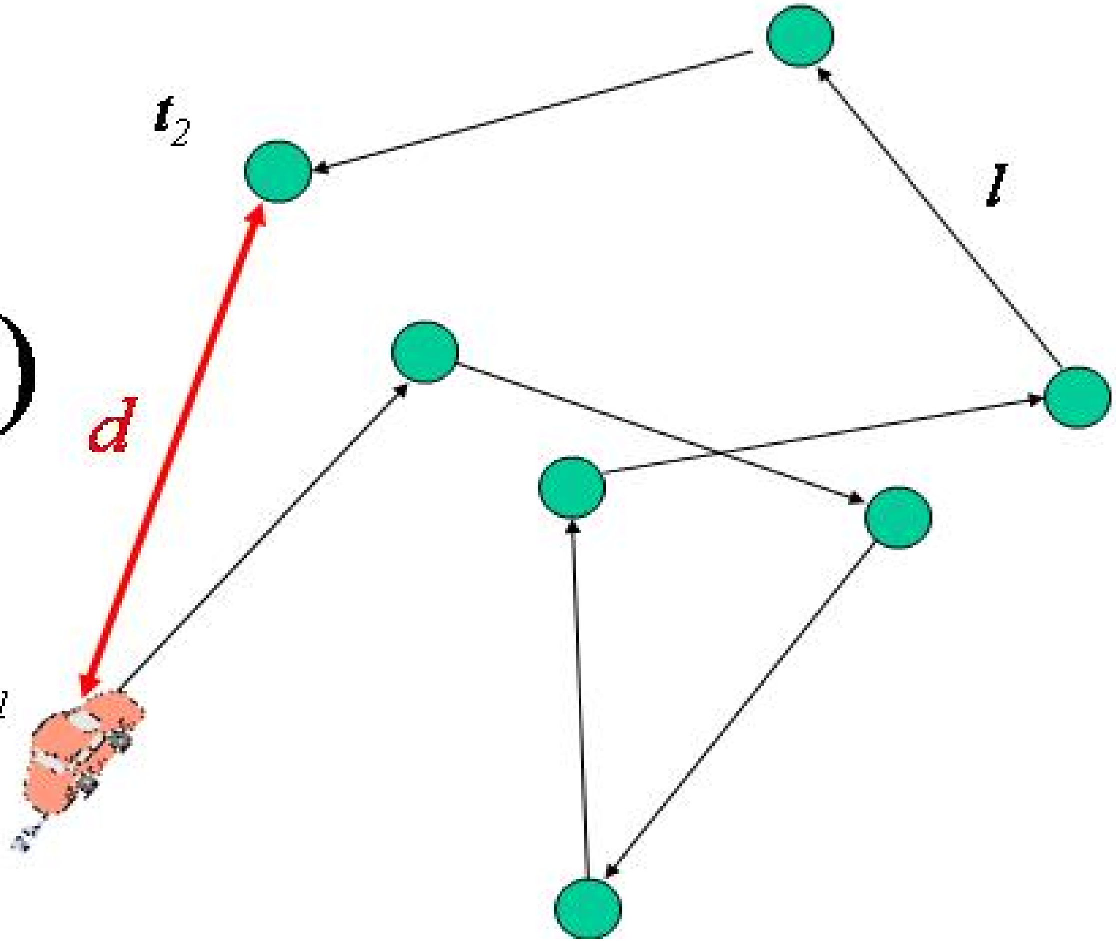
Motion in a straight line

$$d = v(t_2 - t_1)$$

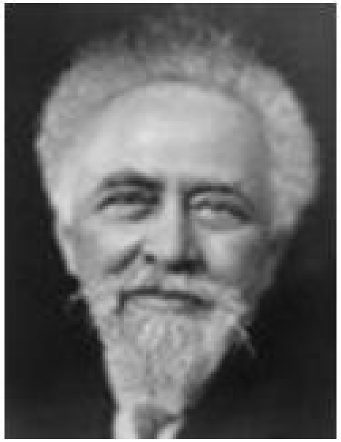
Brownian motion

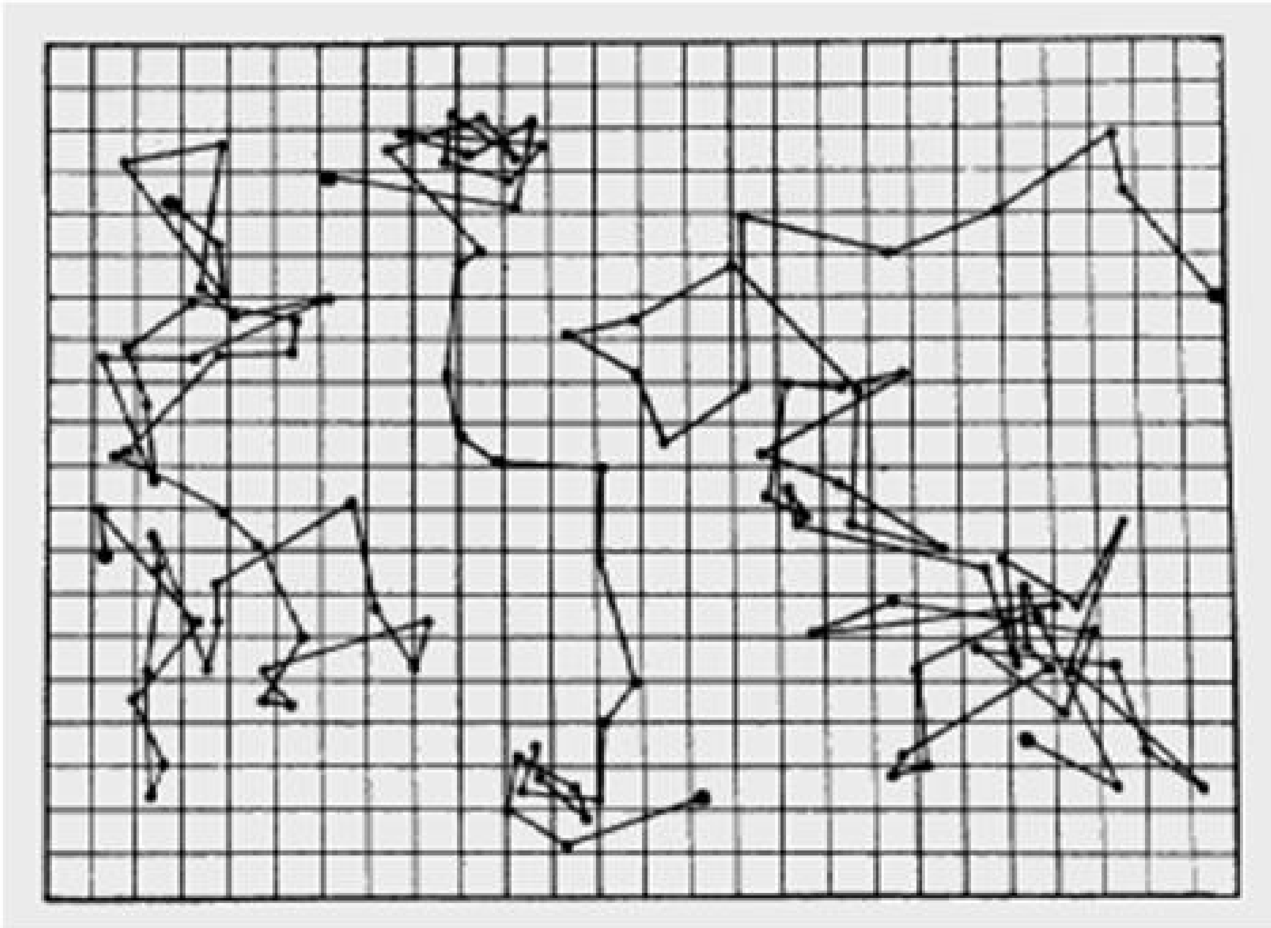
$$d \cong \sqrt{lv(t_2 - t_1)}$$

A. Einstein, 1906



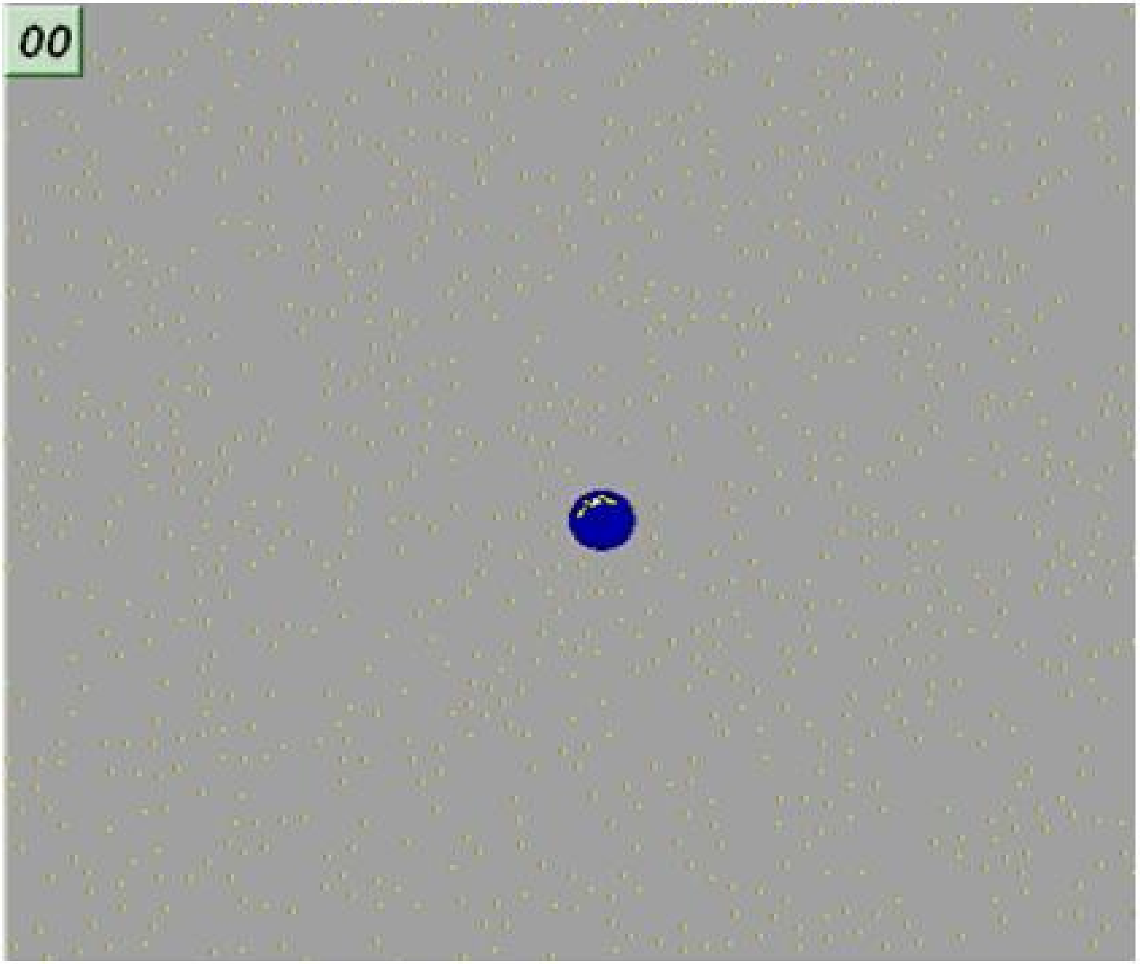
In 1908, the French scientist Jean Babtiste Perrin (who was awarded the Nobel prize in 1926), experimentally confirmed the predictions made by Einstein and Smoluchowski.





Three tracings of the motion of particles of radius 0.53μm, as seen under the microscope by Perrin. Positions every 30 seconds are joined by lines (the mesh size is 3.2μm).

A computer simulation of the Einstein-Smoluchowski view of Brownian motion

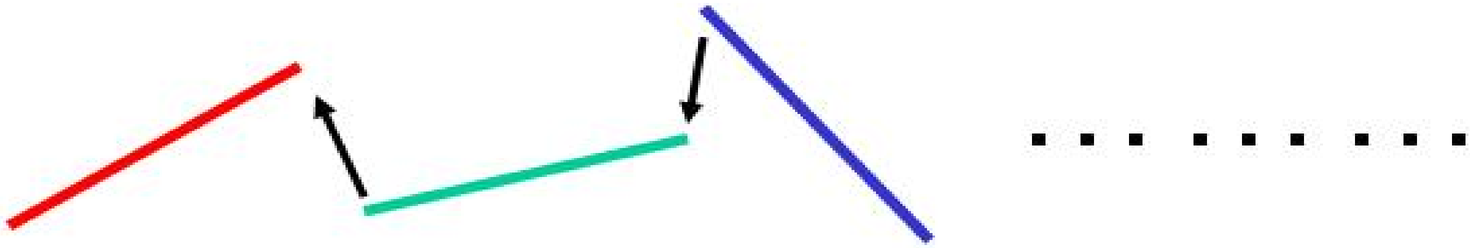


$$d \sim \sqrt{\Delta t}$$

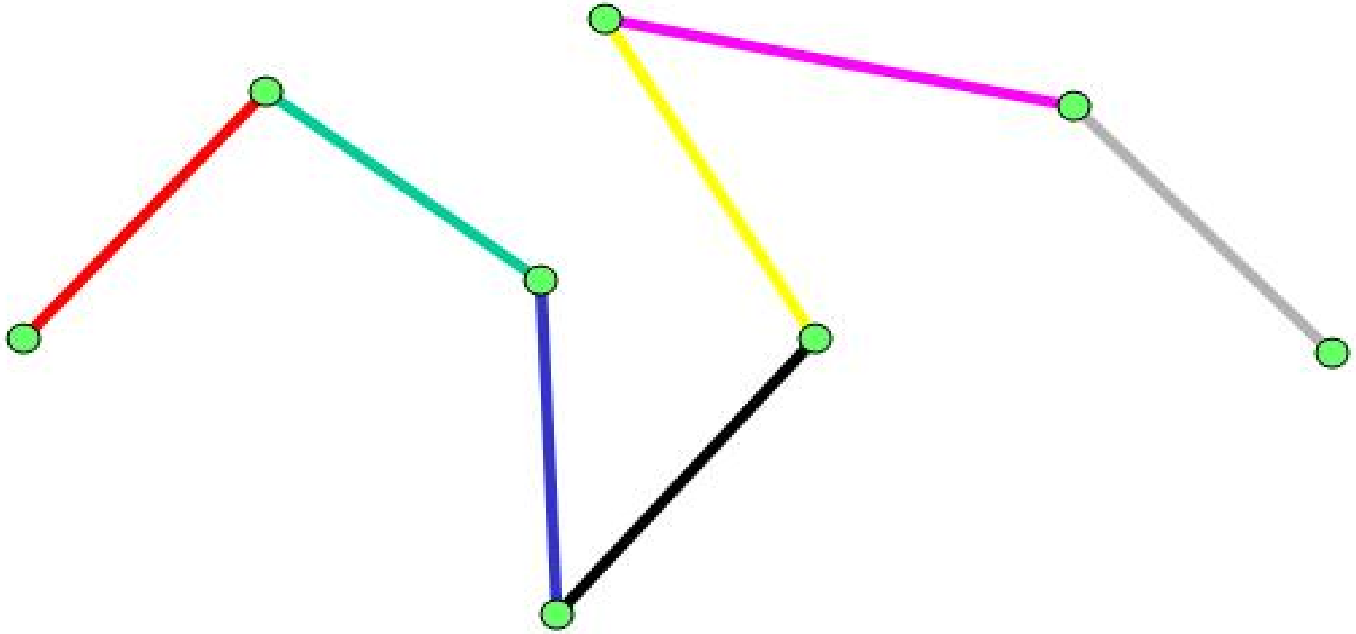
Titin is a polymer.

What does Brownian motion do to a polymer?

The linking of many individual molecules called monomers



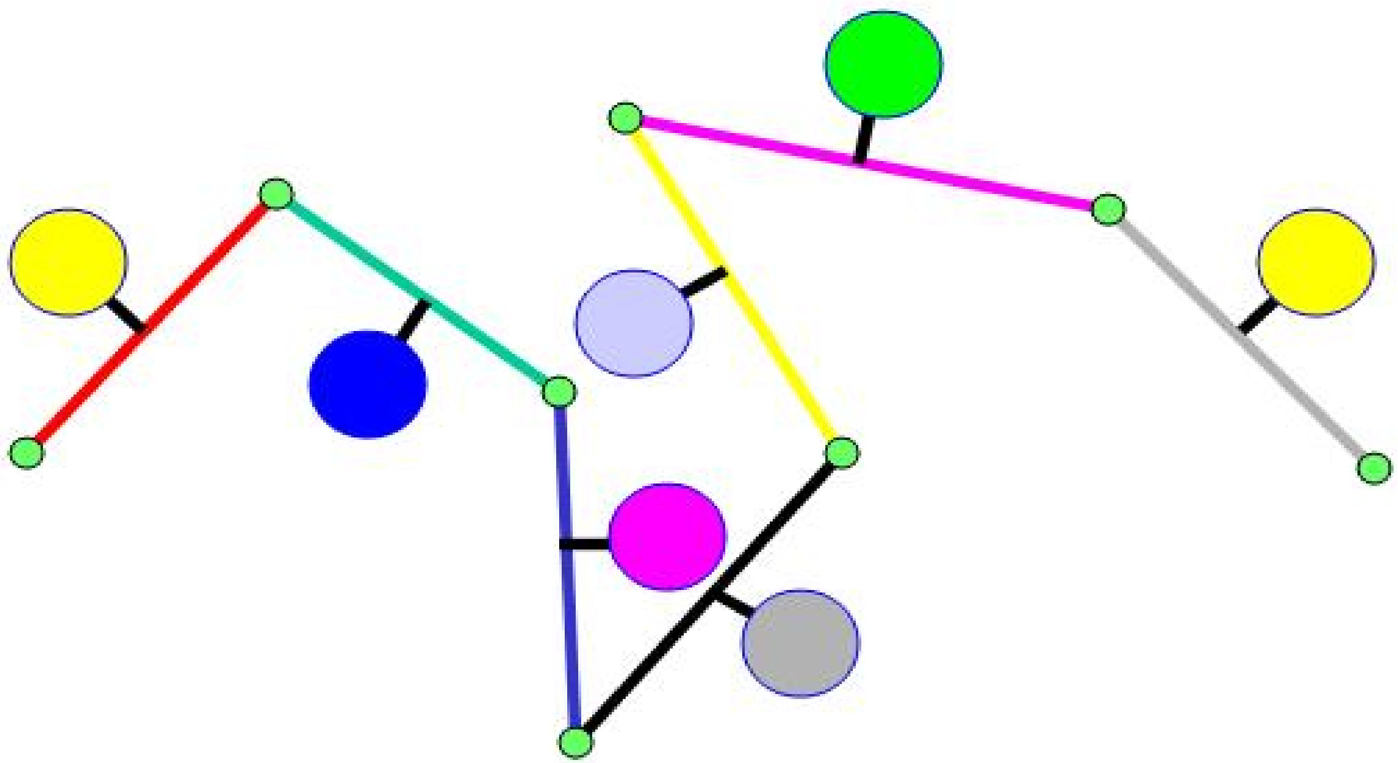
Results in the formation of a **POLYMER**



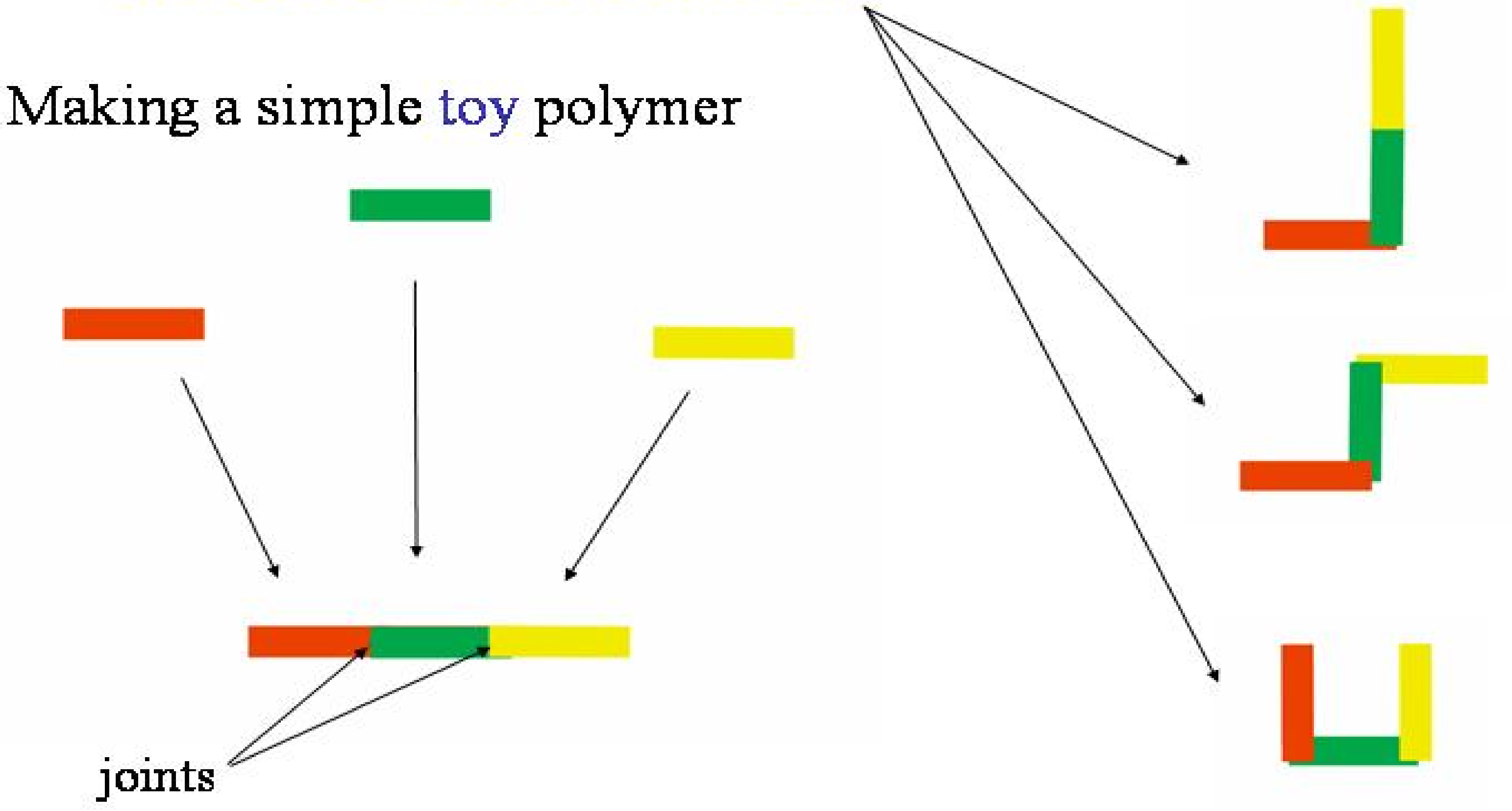
Building a very simple polymer: polyethylene

Proteins are polymers of individual amino-acids. There are 20 different amino-acids. Combinations of these 20 amino acids can give rise to extremely complex polymers called polypeptides.

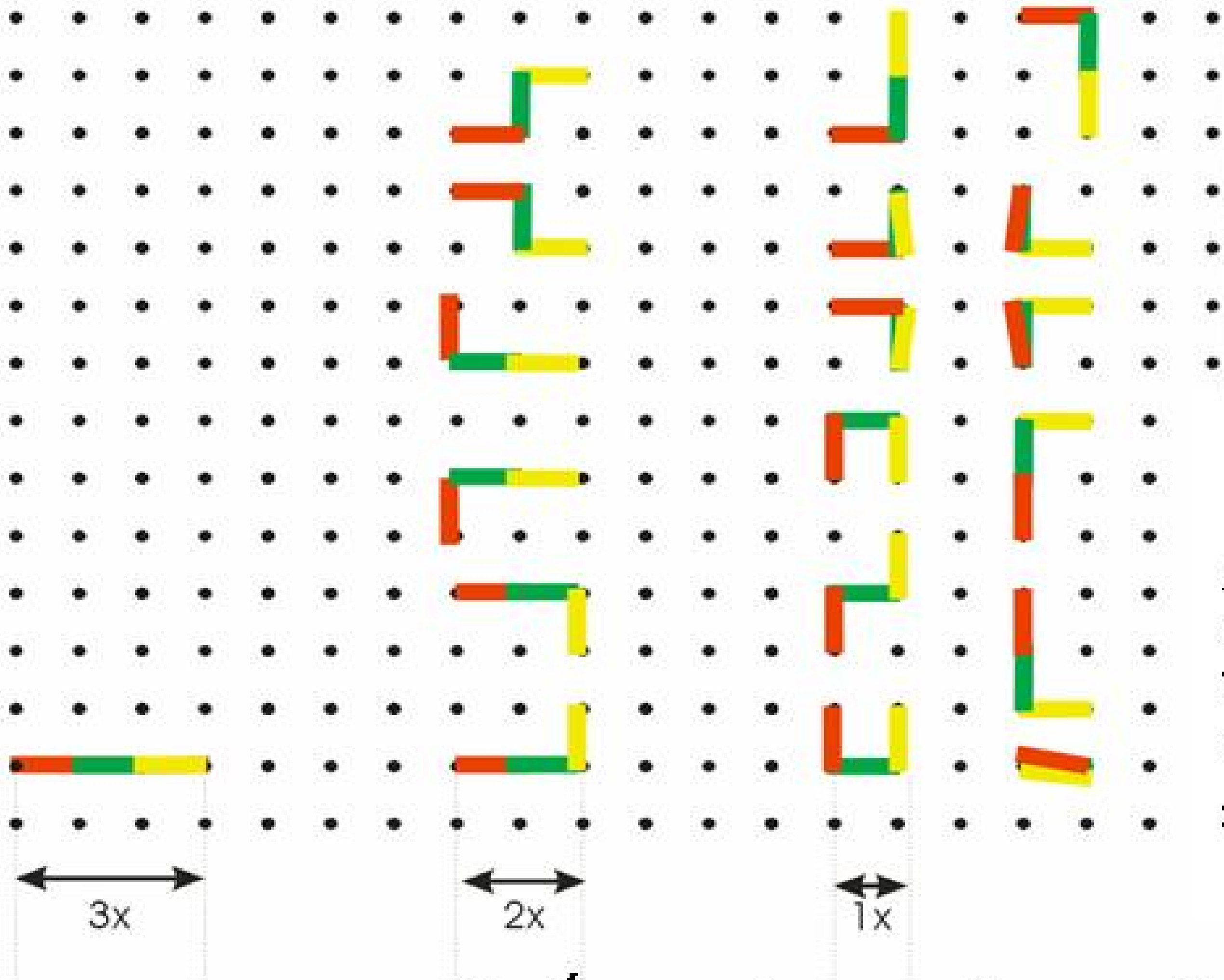
Polymers then, are freely jointed segments with complex chemical groups branching out on the sides.



Polymers can have many different conformations



Polymers move towards situations that permit the largest number of conformations

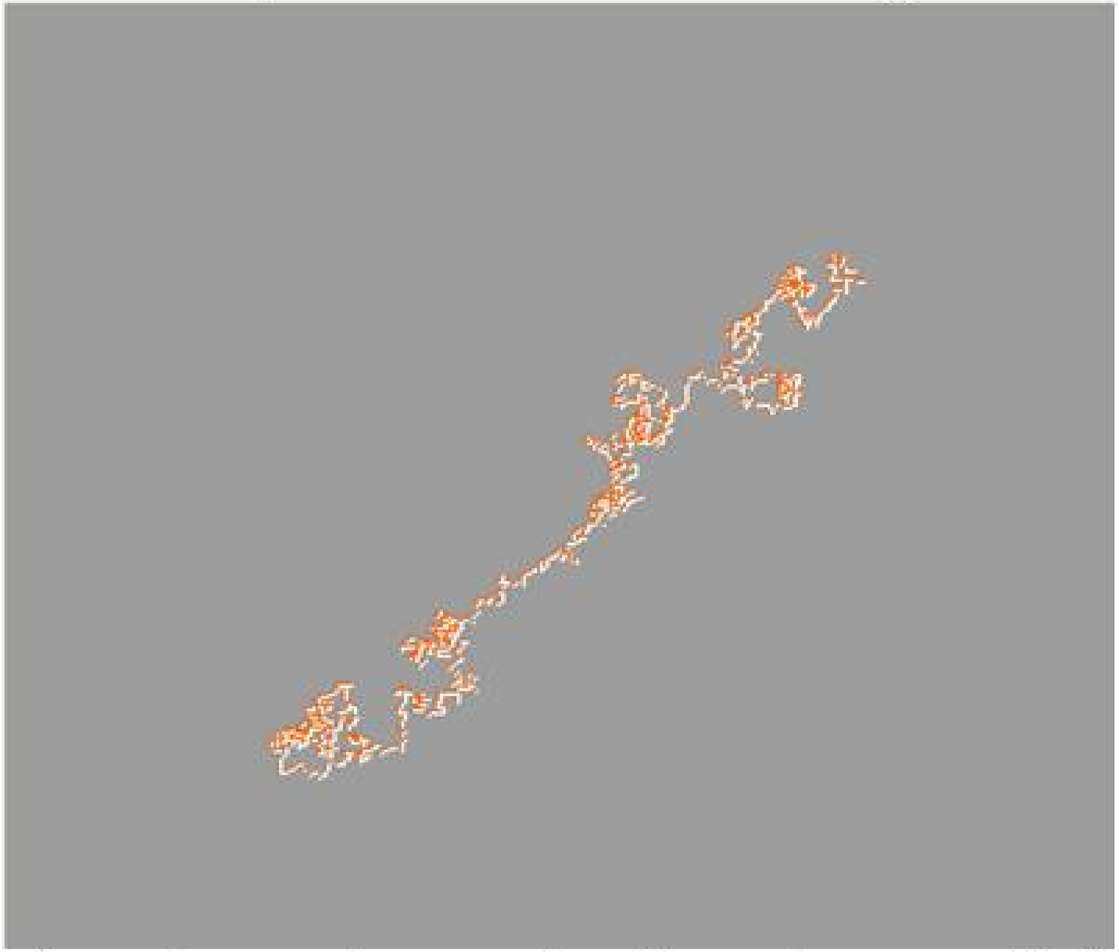


Ludwig Boltzman
(1844-1906)

Austrian physicist who established the relationship between entropy and the statistical analysis of molecular motions

$$Entropy \cong \ln(number\ \ of\ \ conformations)$$

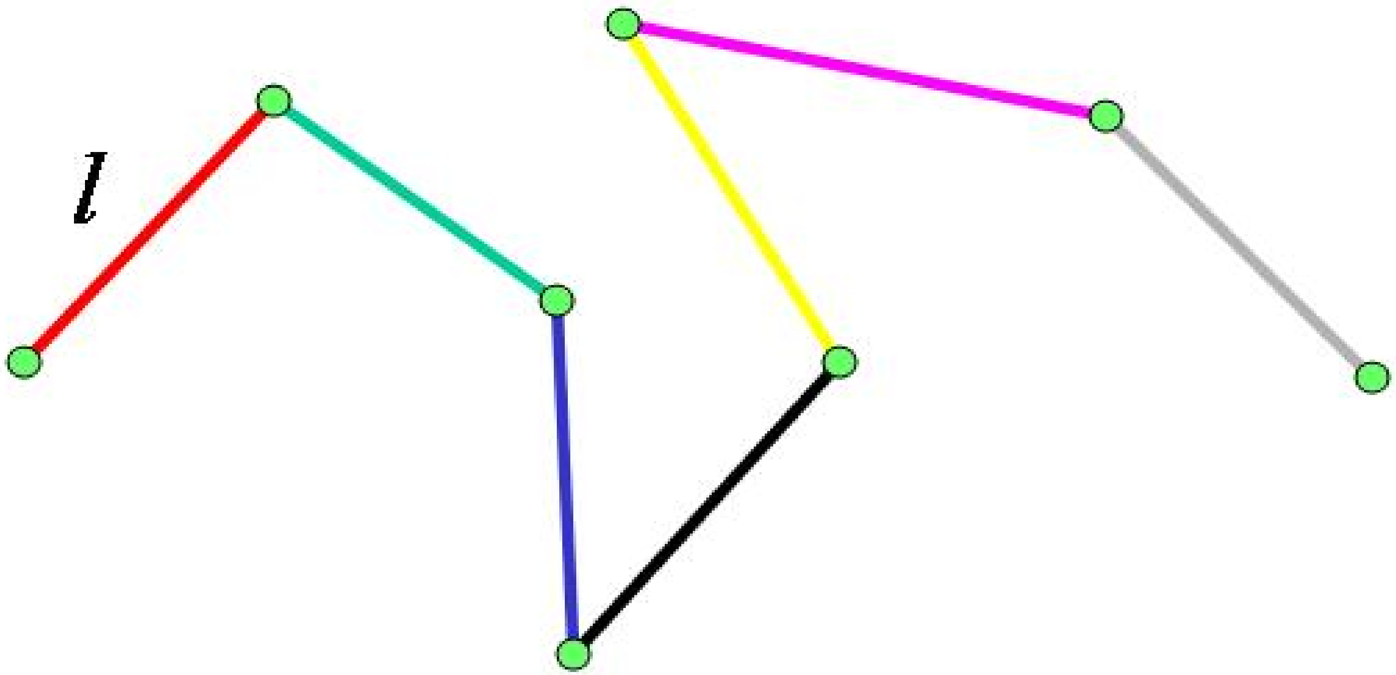
Increased entropy makes polymers look crooked and pushes them to collapse



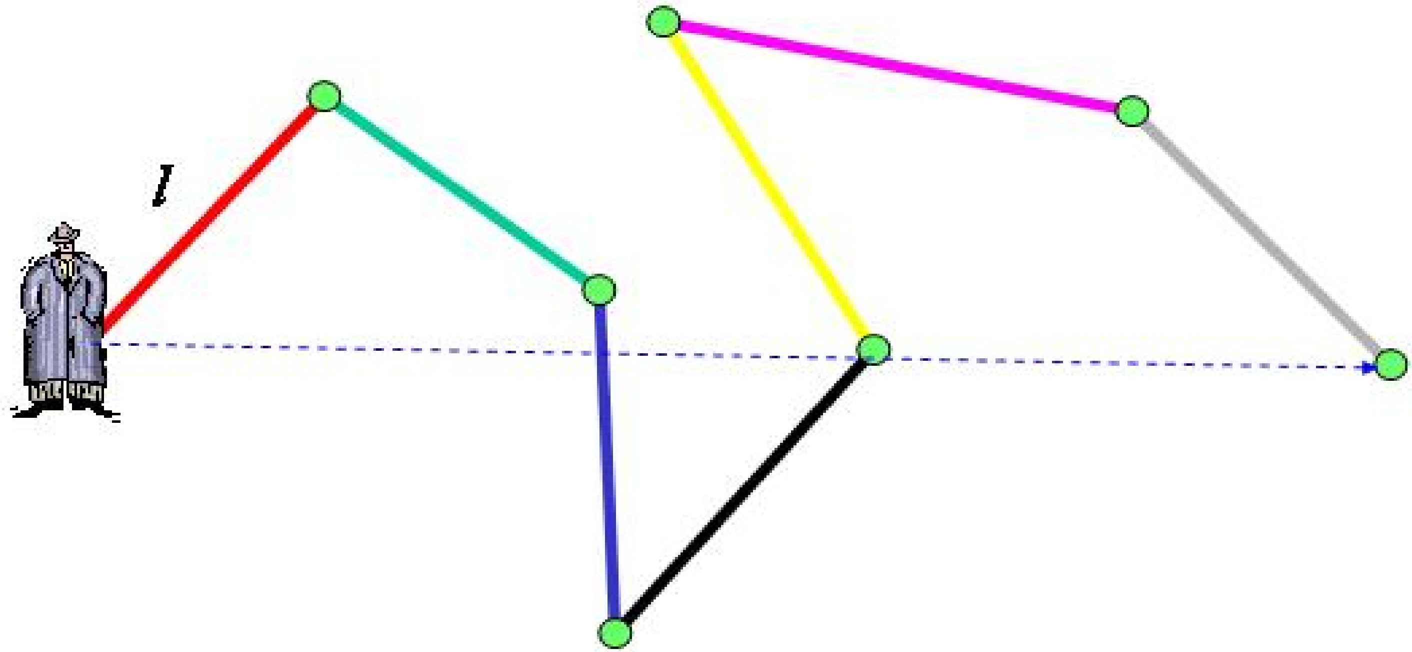
Simulation of a polymer increasing its entropy as it shrinks from a more extended conformation

For a polymer made of N segments of equal length l, the contour length is defined as:

$$L_c = N \cdot l$$

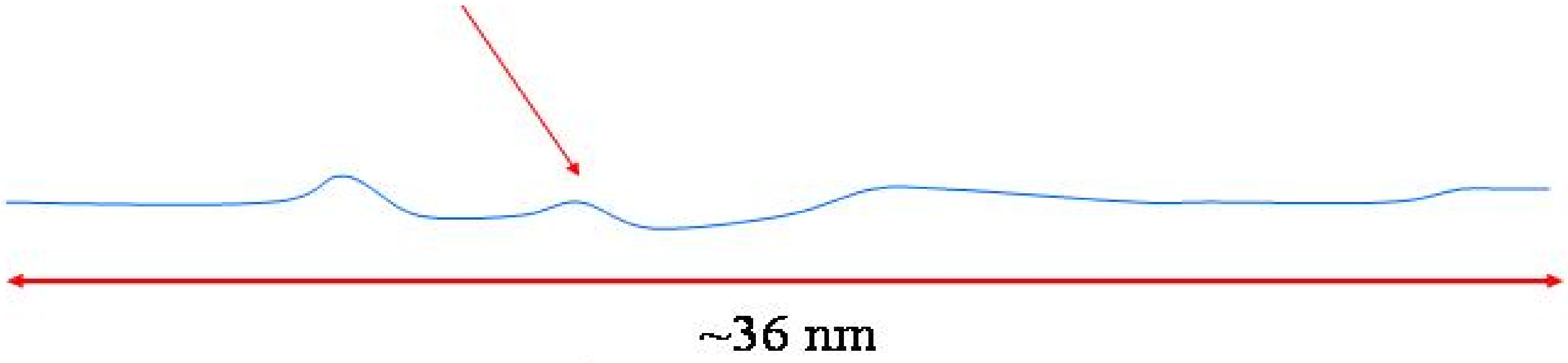


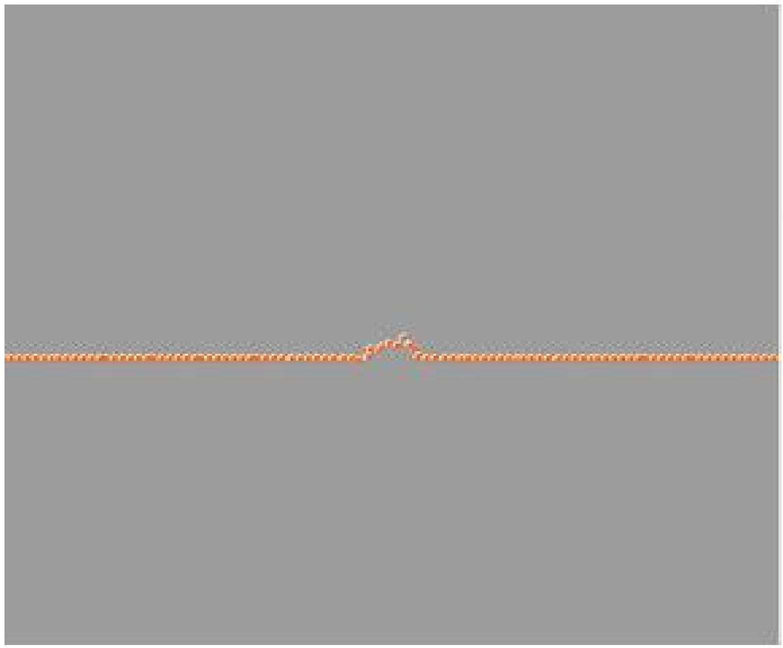
A polymer looks like the path of a particle in Brownian motion



$$d = \sqrt{lv(t_2 - t_1)} = \sqrt{lL_c}$$

The contour length, L_c, of a single protein molecule composed of 100 amino acids is 36 nm





Answer:

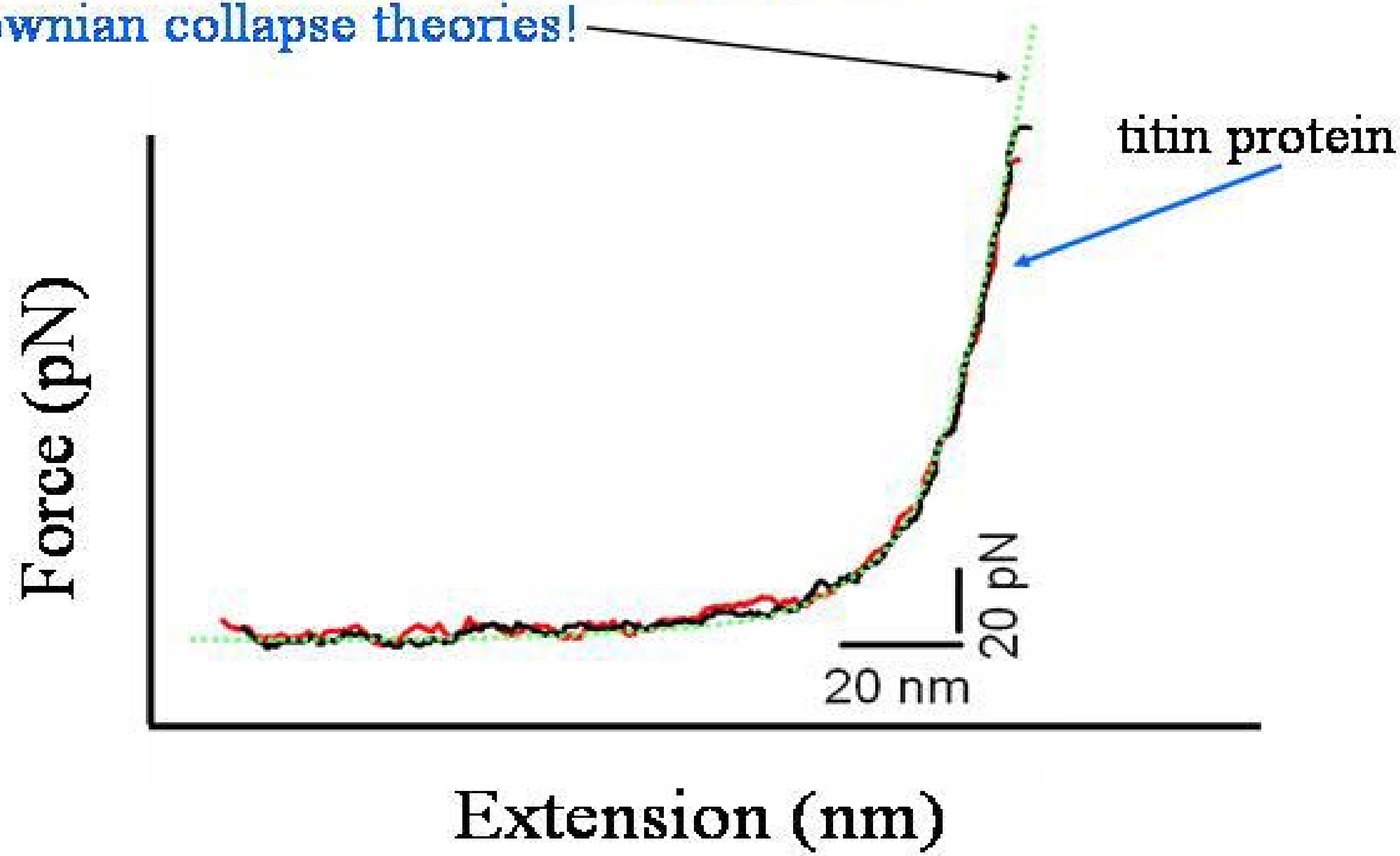
$$d = \sqrt{L_c l} \cong 3.6nm$$

Derivation of the elasticity of a freely jointed polymer chain

We calculate how much a polymer extends at a given pulling force.

We assume that the polymer chain is in equilibrium, with randomly oriented segments.

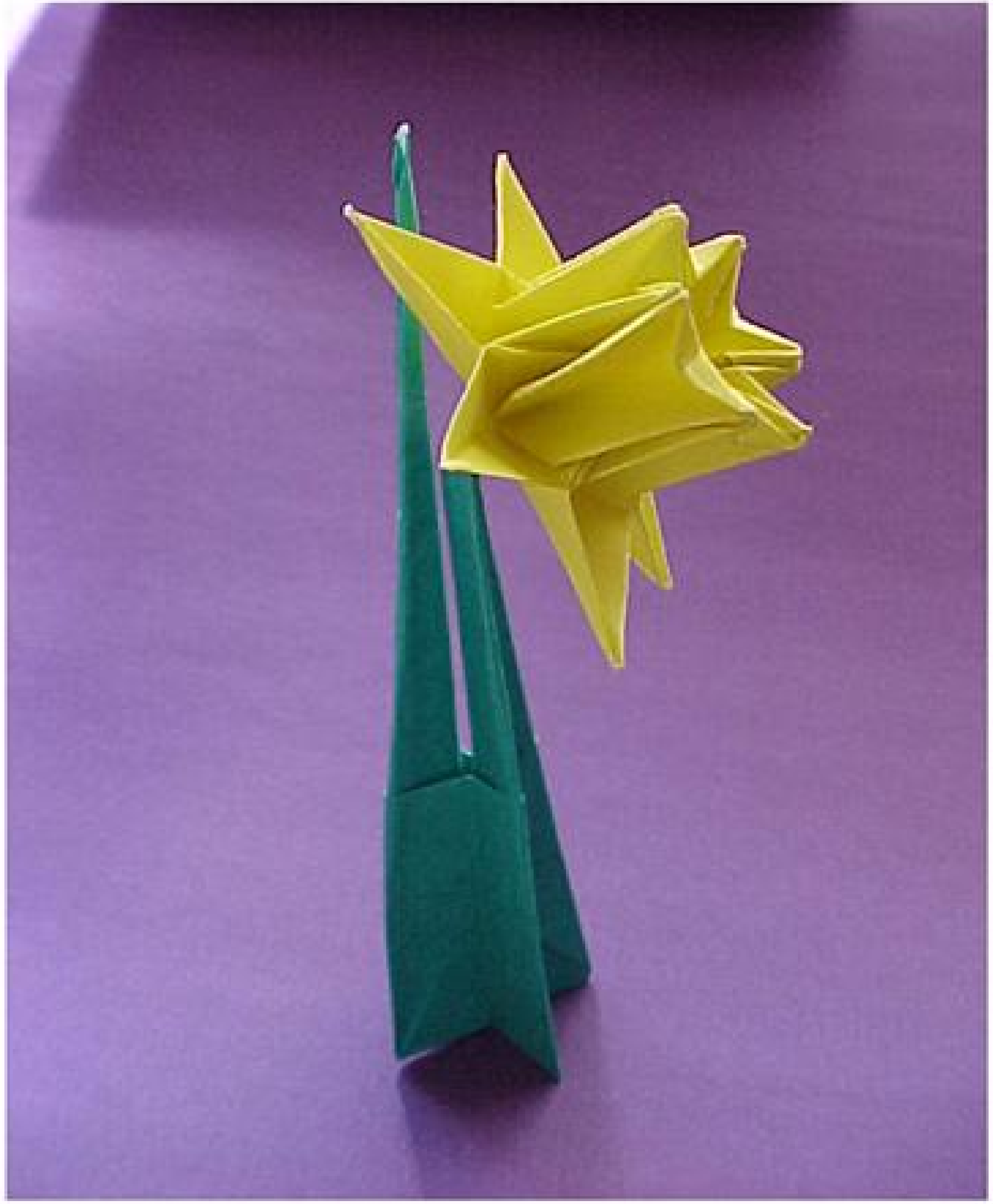
If we stretch a single titin protein we obtain force-extension curves that are perfectly explained by the Brownian collapse theories!



Ok, so we have discovered that muscle elasticity results from the Brownian motion of titin.

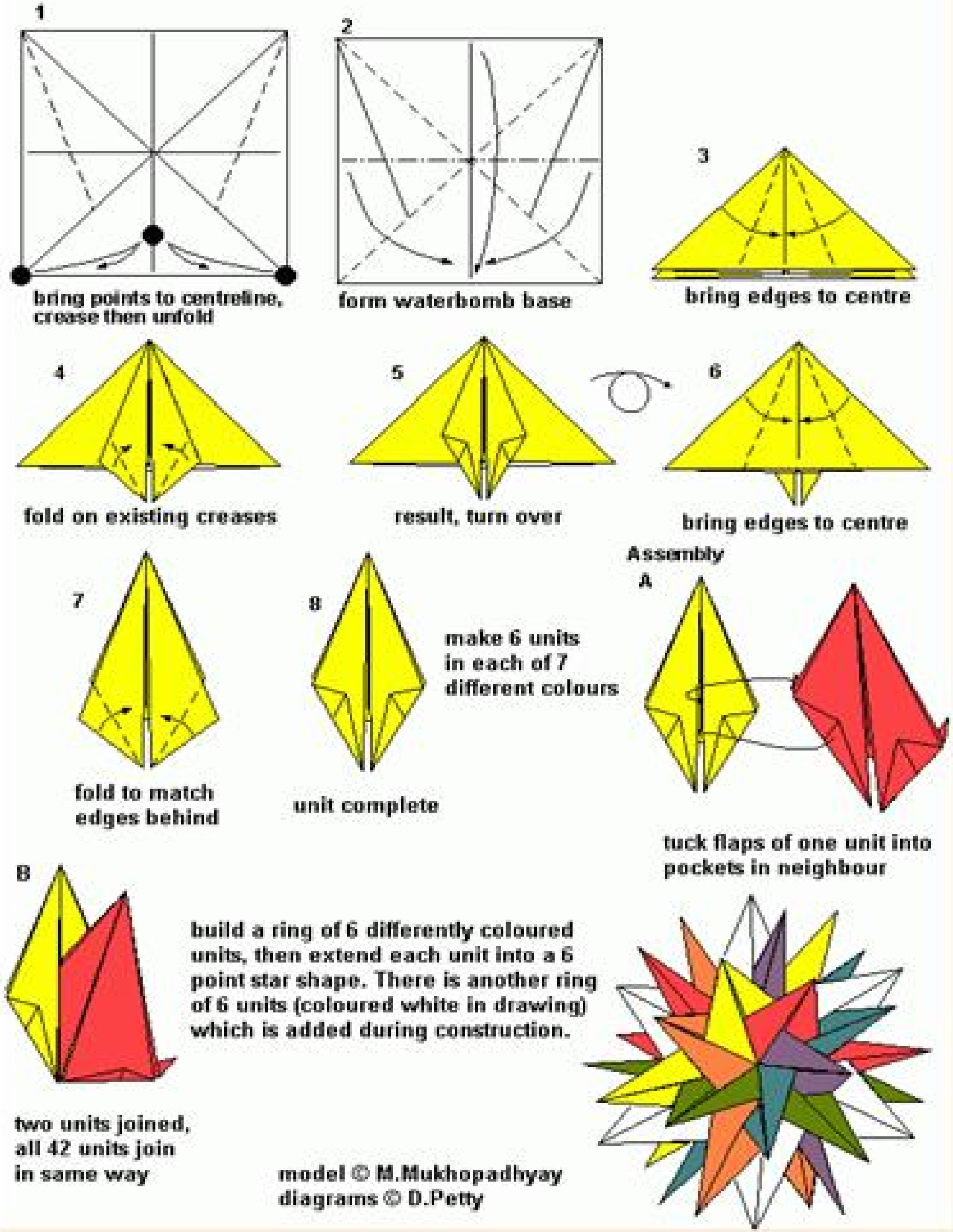
What happens to titin if we stretch it too much? Will it brake like the metal spring?

No! Certain parts of titin will just reversibly unfold.

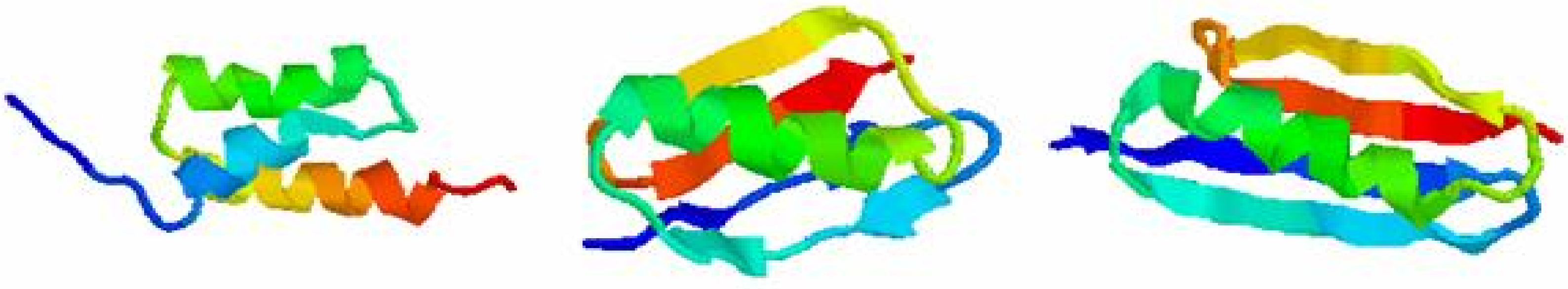


Origami: The art of paper folding

TUVWXYZ

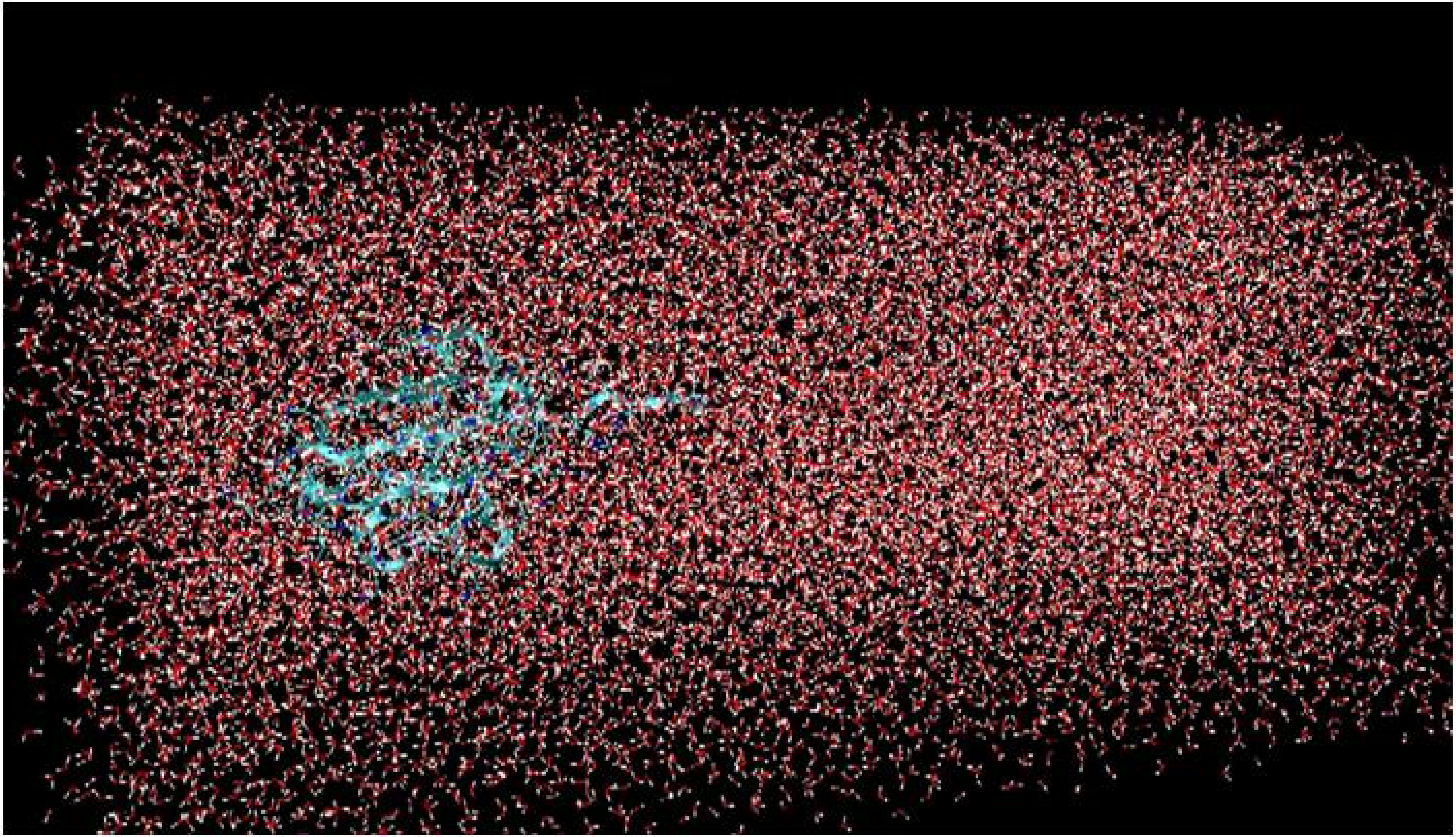

1
bring points to centreline, crease then unfold
2
form waterbomb base
3
bring edges to centre
4
fold on existing creases
5
result, turn over
6
bring edges to centre
7
fold to match edges behind
8
unit complete
make 6 units in each of 7 different colours
Assembly
A
tuck flaps of one unit into pockets in neighbour
B
two units joined, all 42 units join in same way
build a ring of 6 differently coloured units, then extend each unit into a 6 point star shape. There is another ring of 6 units (coloured white in drawing) which is added during construction.
model © M.Mukhopadhyay
diagrams © D.Petty

Likewise there are folding instructions for proteins

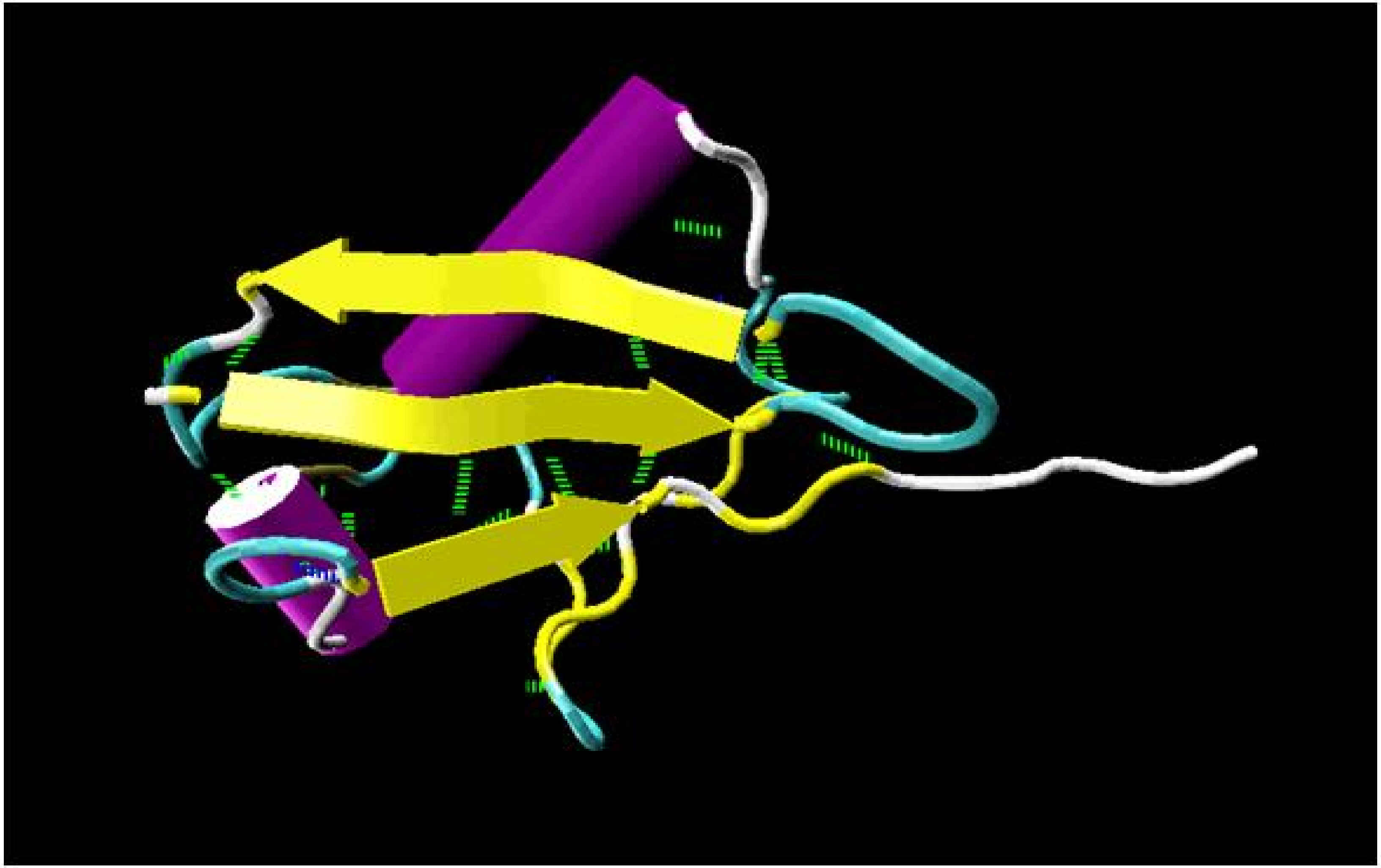


We know they exist, but we do not know what they are.

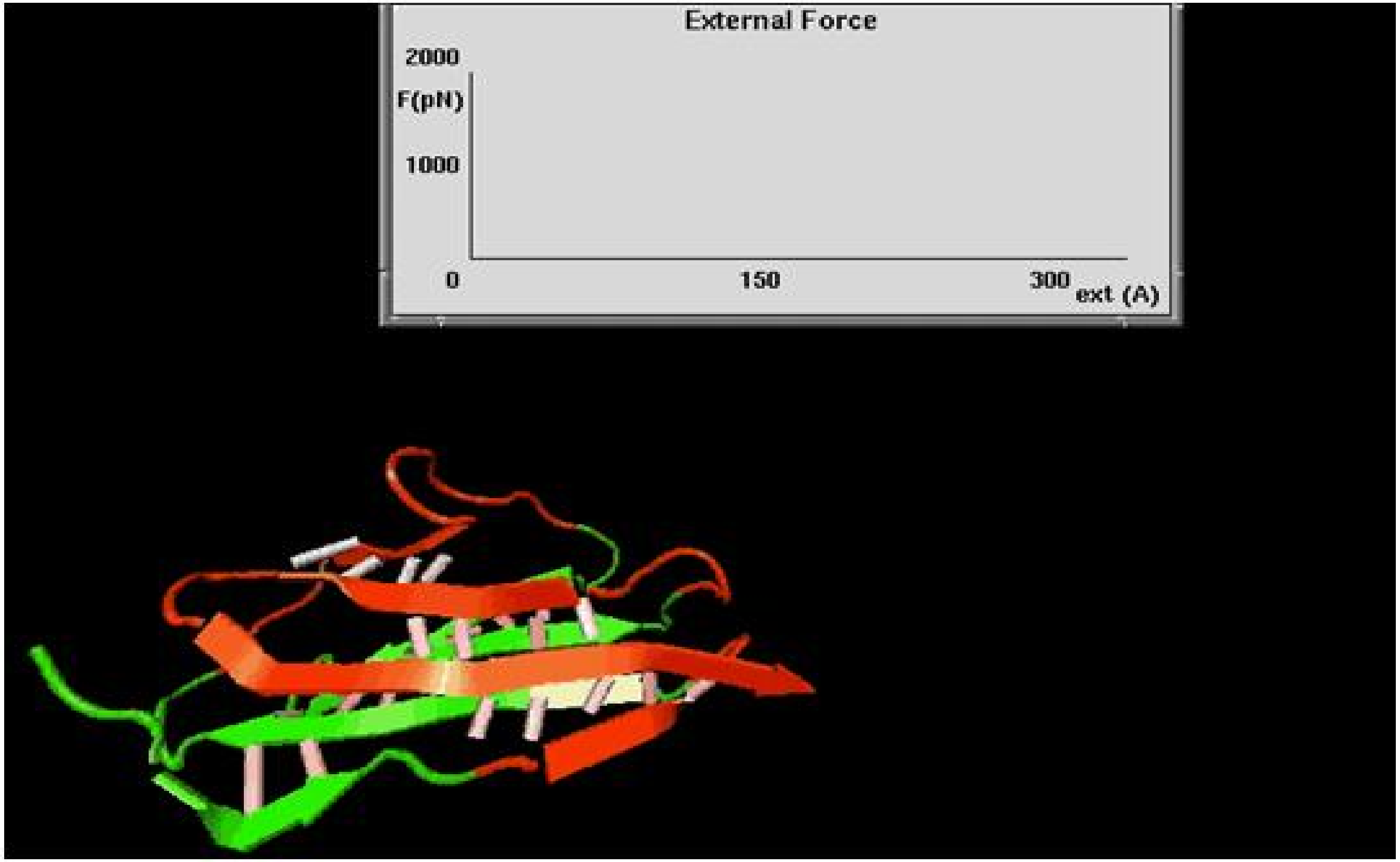
Folded proteins have a **bonded structure** that helps them resist the constant bombardment caused by Brownian motion.



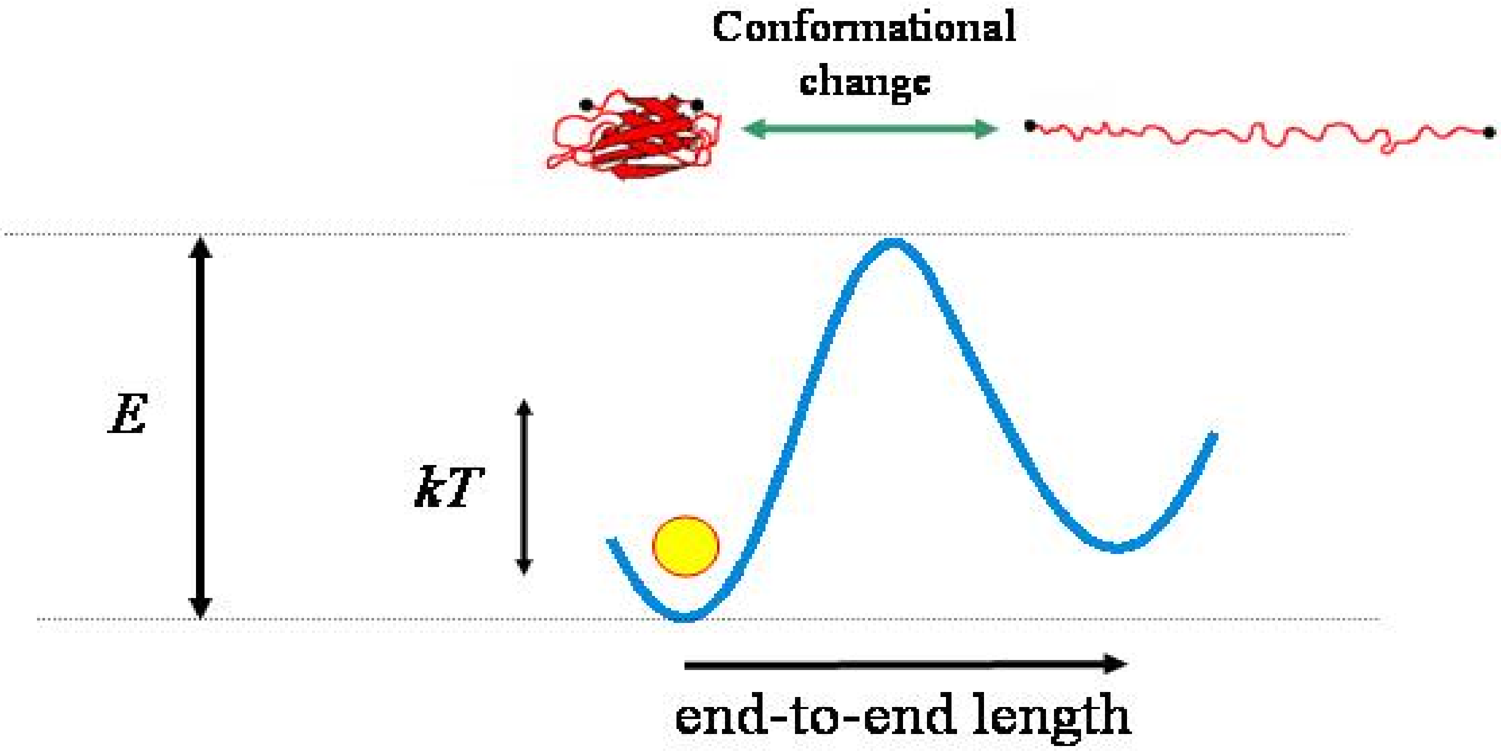
The Bonded structure is dynamic, as it gets bombarded, the structure bends and bonds break and reform



The bonds create an energy barrier



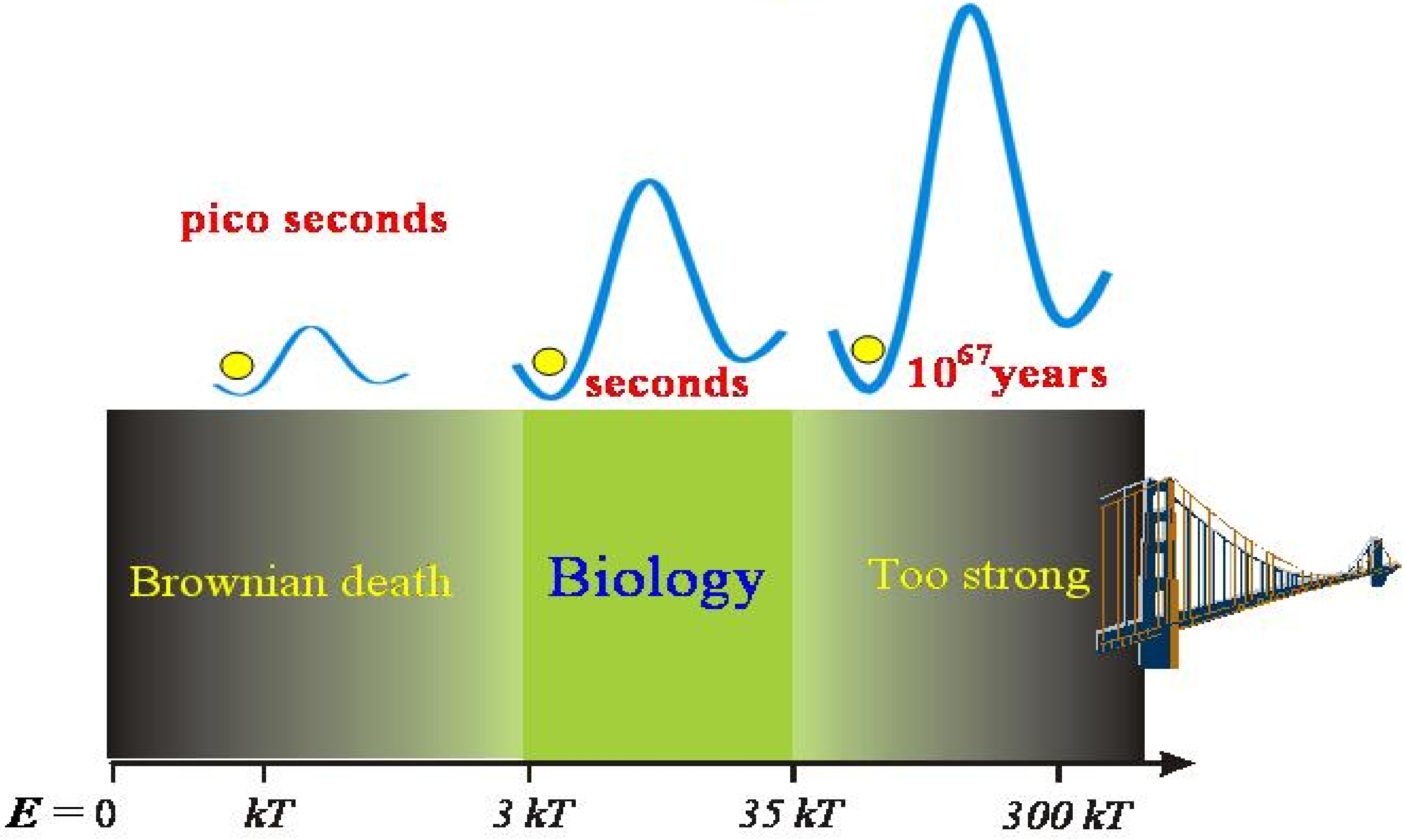
How often does Brownian motion overcomes the energy barrier and causes the protein to unfold spontaneously ?



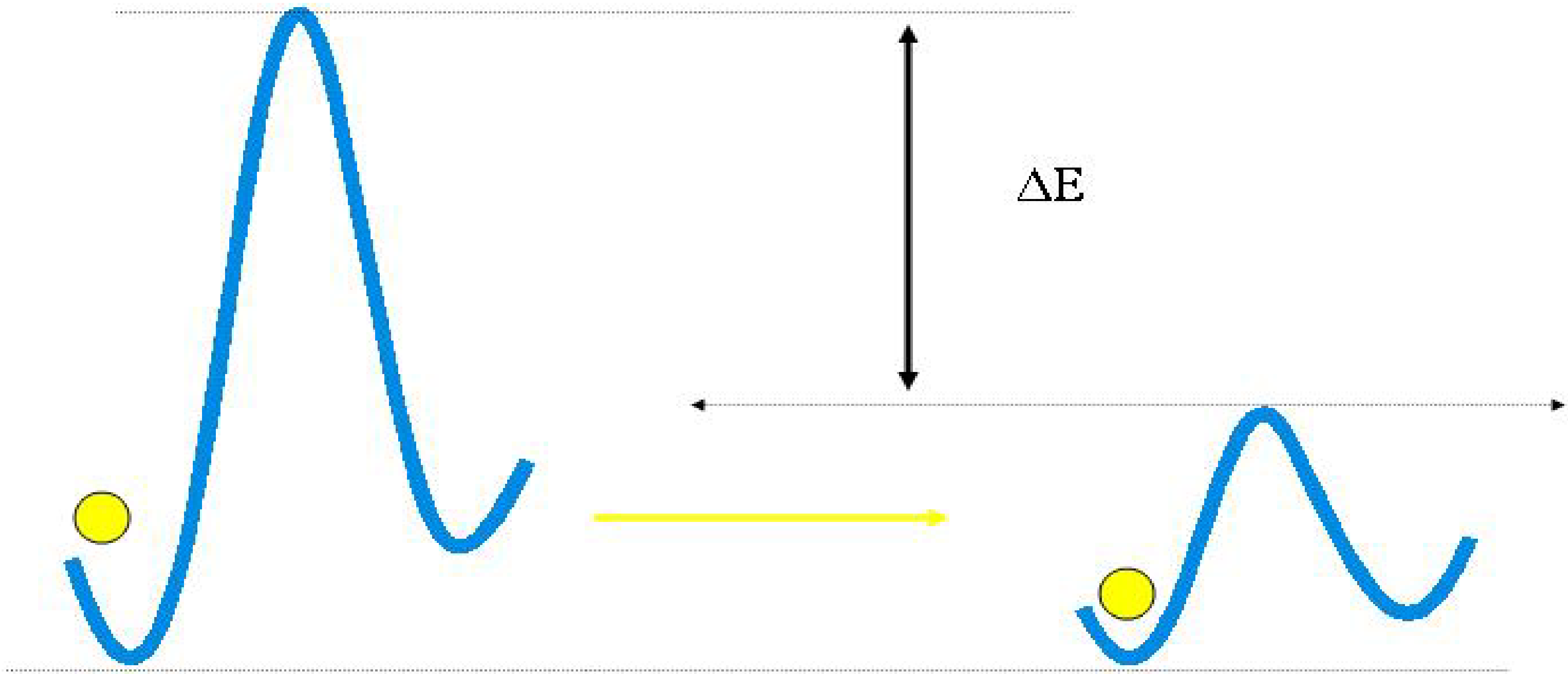
$$t \cong t_0 \cdot e^{\frac{E}{kT}}$$

$t_0 \sim 10^{-12}$ seconds

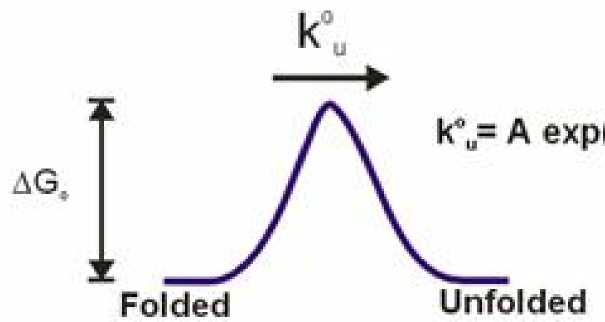
How long do we have to wait for Brownian motion to overcome an energy barrier ?



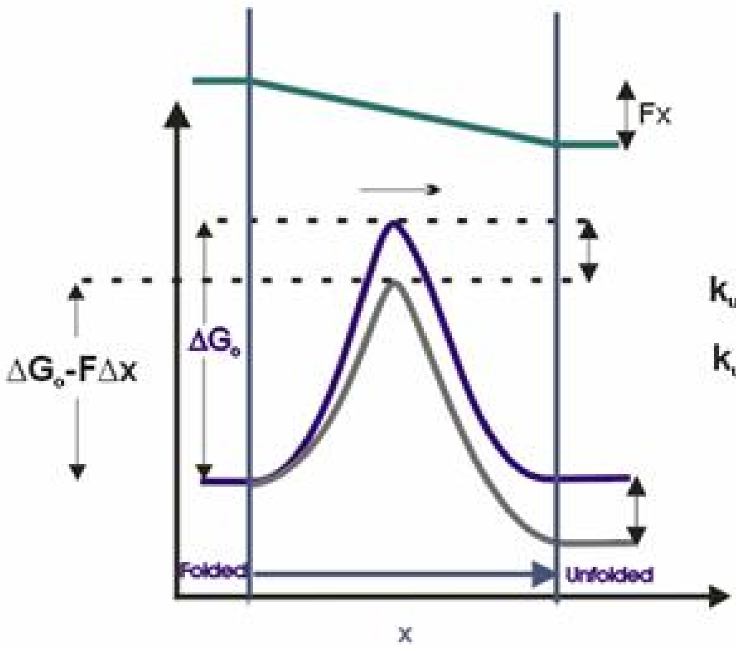
Control in Biology is accomplished by reducing energy barriers. Then, unlikely events will occur over short time periods



Effect of an external force on a rate constant



$$k^{o}_{u} = A\ \exp(-\Delta G_{o}/kT)$$



$$k_{u} = A\ \exp\text{-}(\Delta G_{o} - F\Delta x)/kT)$$

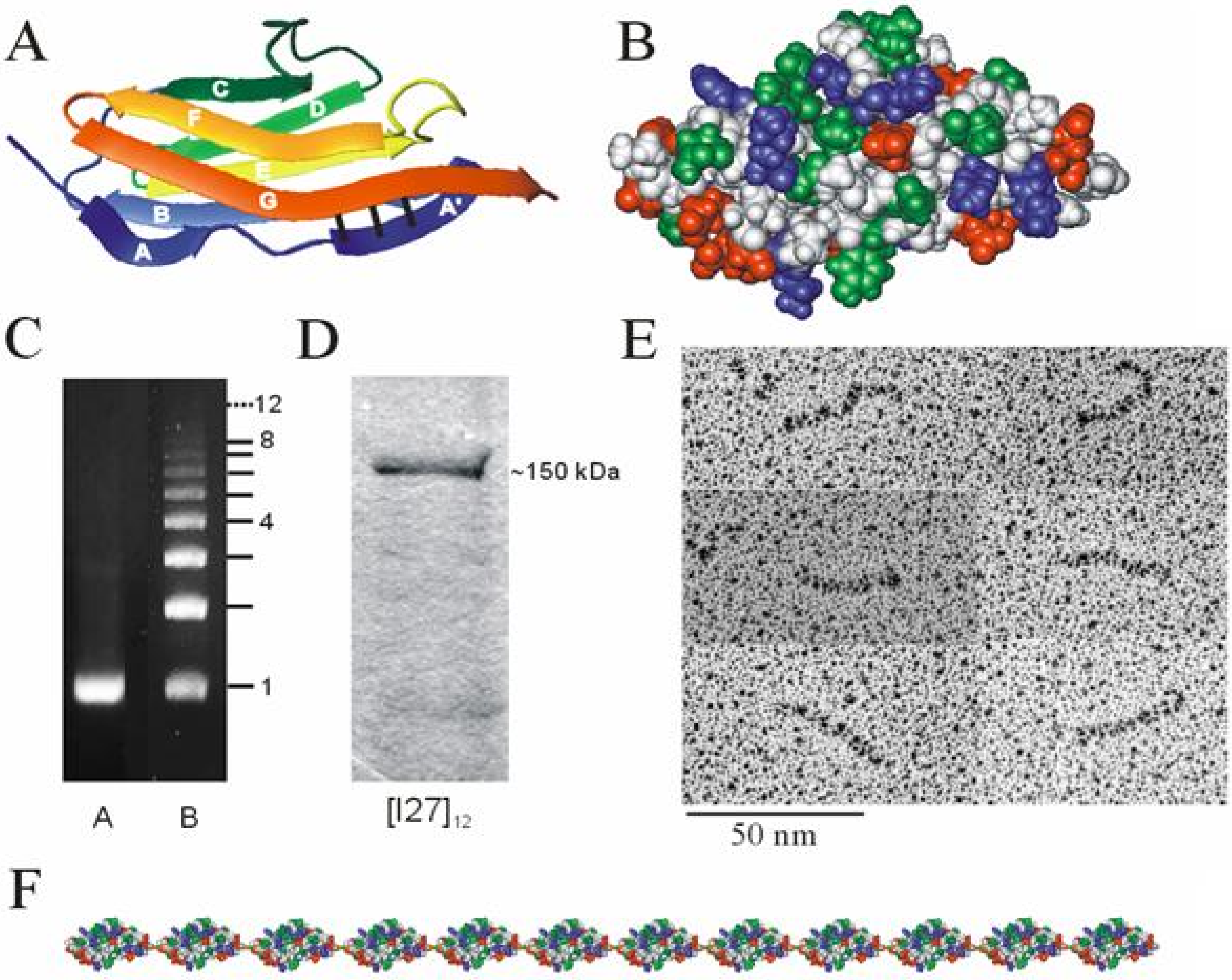
$$k_{u} = k^{o}_{u}\ \exp(F\Delta x)/kT)$$

If we apply a mechanical force to the protein, we can accelerate unfolding by reducing the energy barrier.

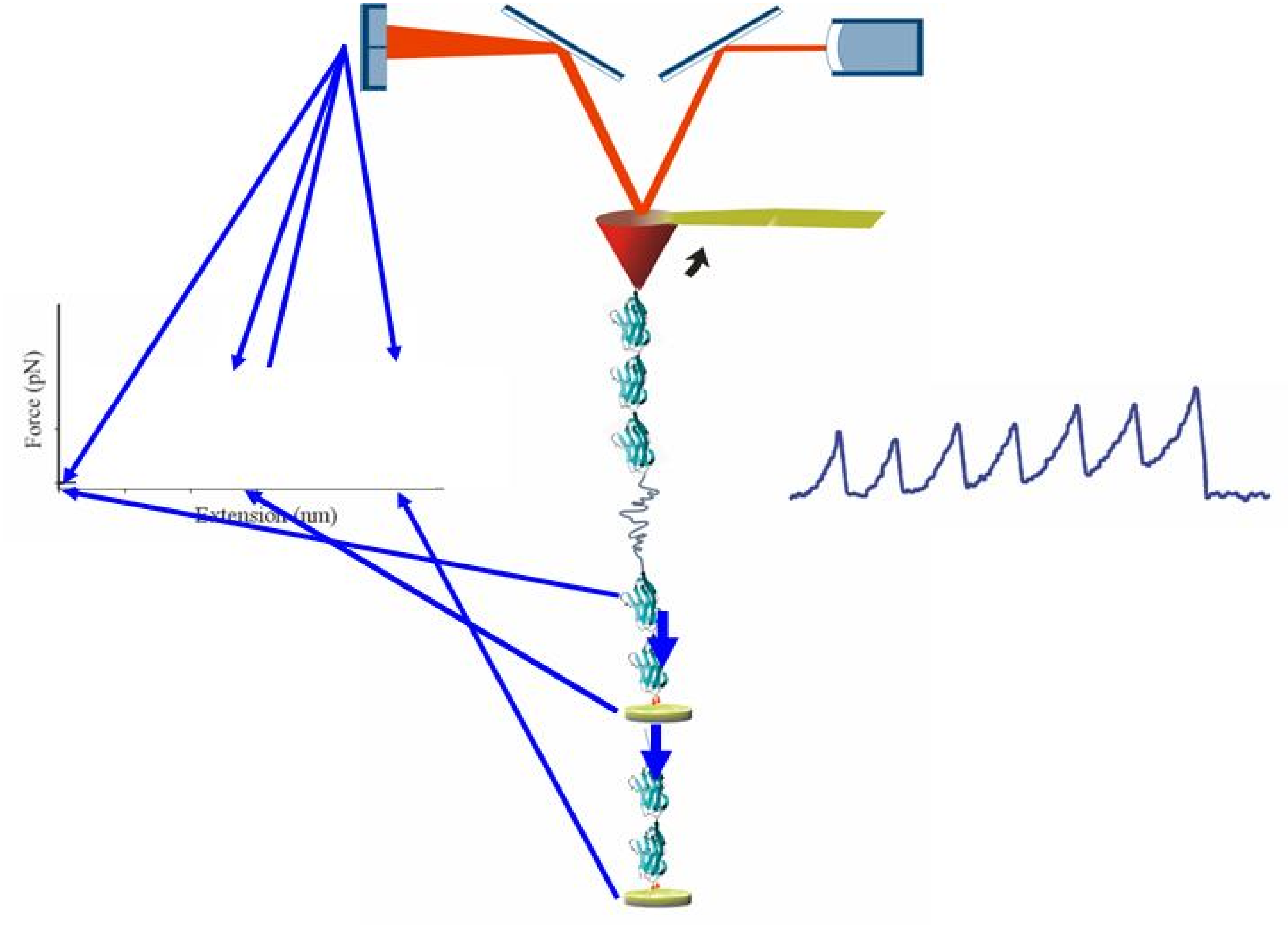
If $F\Delta x = 0$ then t=40 minutes!

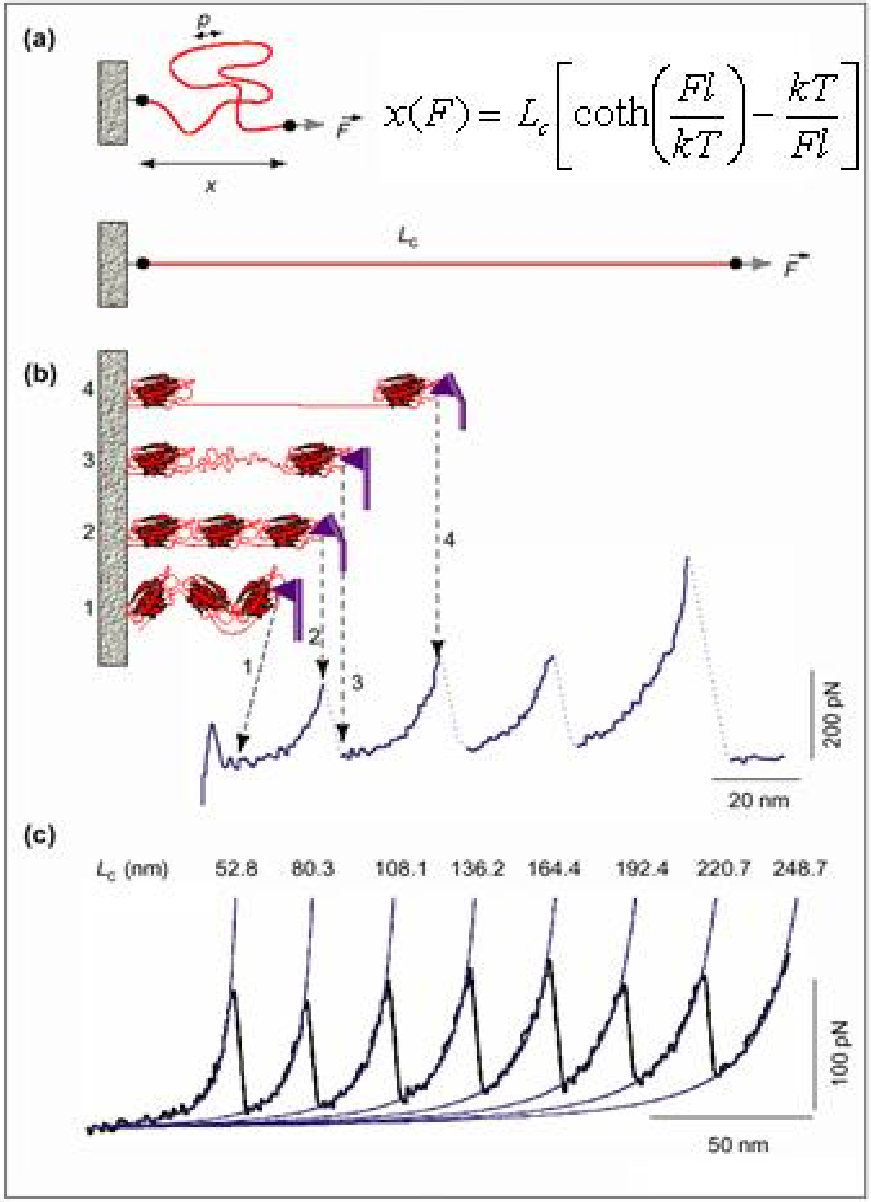
If $F\Delta x = 3kT$ then t=2 minutes!

If $F\Delta x = 35kT$ then t < picosecond ~0



A
C
F
D
E
B
G
A'
A
B
C
D
E
12
8
4
1
A
B
~150 kDa
[I27]12
50 nm
F

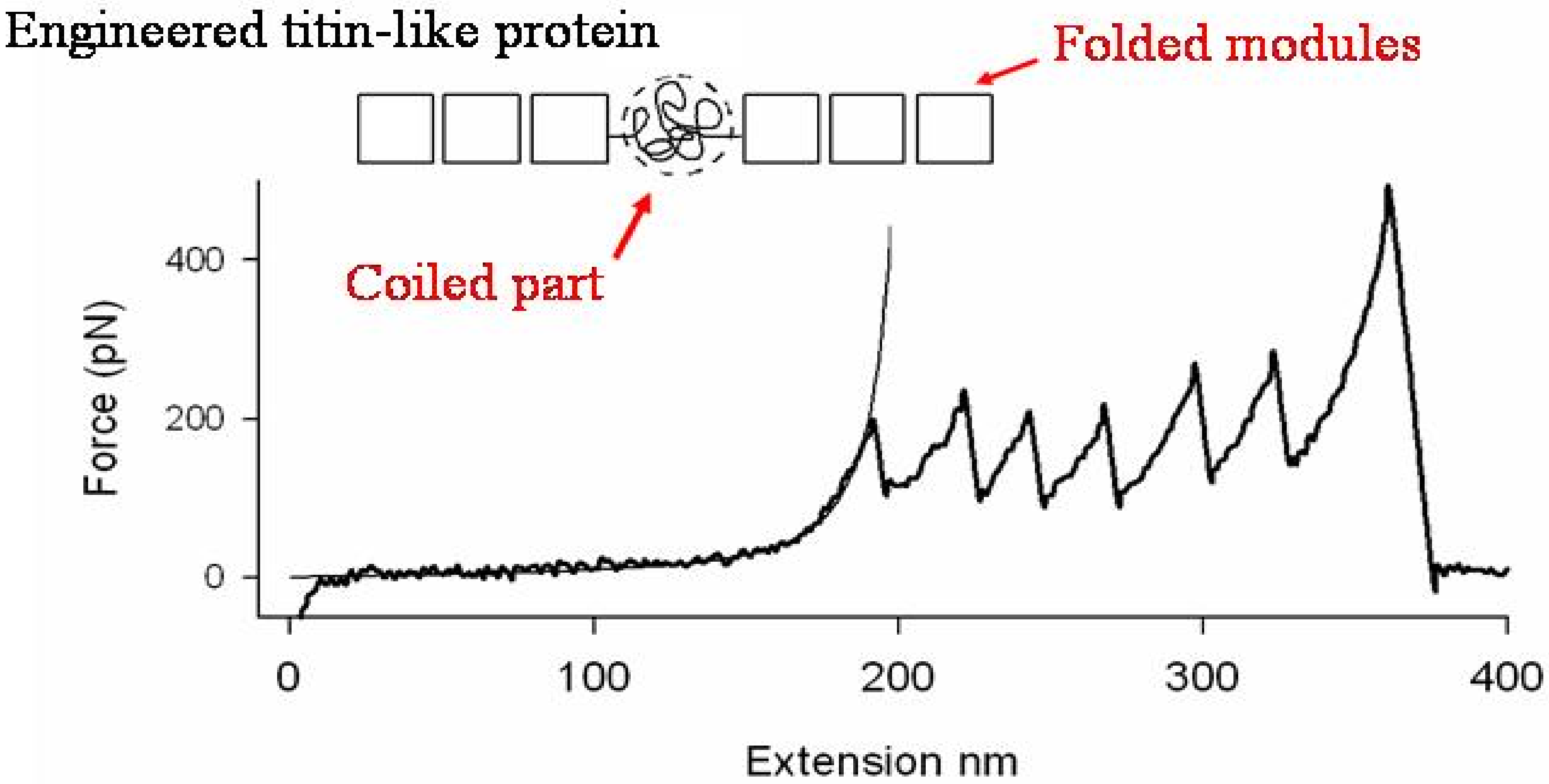

Force (pN)
Extension (nm)



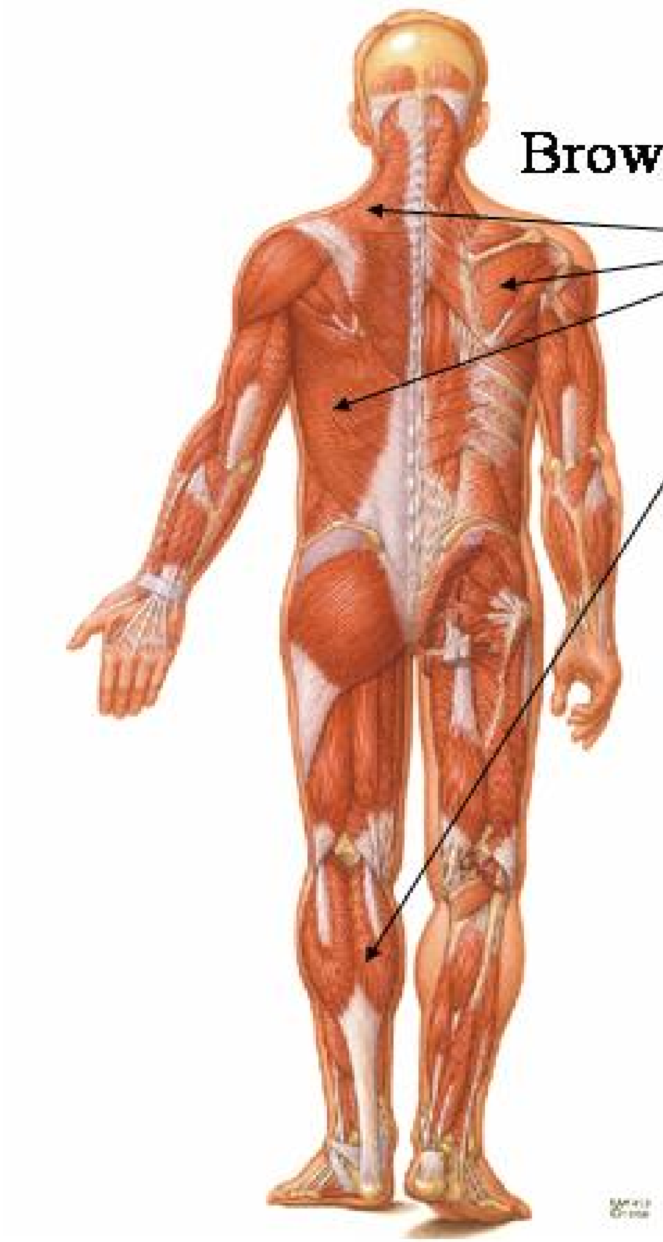
(a)
p
x
$x(F) = L_c\left[\coth\left(\frac{Fl}{kT}\right) - \frac{kT}{Fl}\right]$
F
L_c
F
(b)
4
3
2
1
4
2
3
1
200 pN
20 nm
(c)
L_c (nm) 52.8 80.3 108.1 136.2 164.4 192.4 220.7 248.7
100 pN
50 nm

Igor Demonstration of actual data and its analysis with models of polymer elasticity

We can understand how titin works by engineering a protein that imitates its properties.

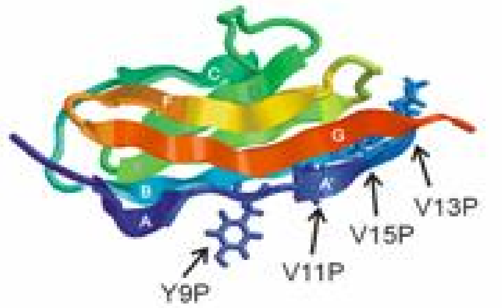


Conclusions



Brownian motion explains muscle elasticity in humans

Helps understand the effect of mutations on the elasticity



We may be able to test and design better titin molecules