LECTURE 21: SINGLE CHAIN ELASTICITY OF BIOMACROMOLECULES: THE GIANT PROTEIN TITIN AND DNA

Outline :

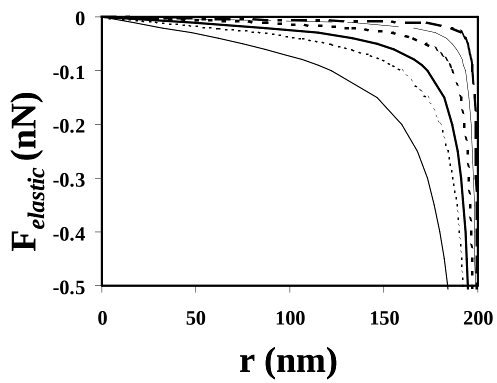
Objectives: To understand the elasticity of biopolymers and they differ from random coil entropic elasticity

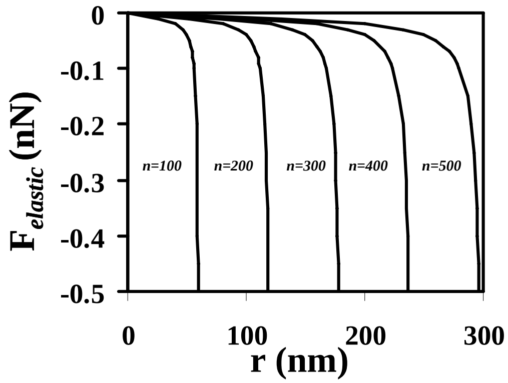
Readings: Course Reader Documents 40-43

Multimedia : Fibronectin and Titin unfolding simulation movies

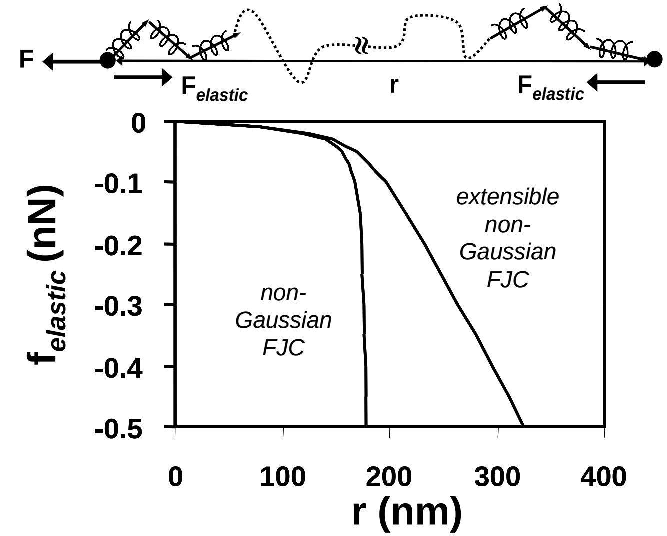
REVIEW LECTURE 20 : EXTENSIBLE FJC AND WLC

-Effect of a and n on the inextensible FJC



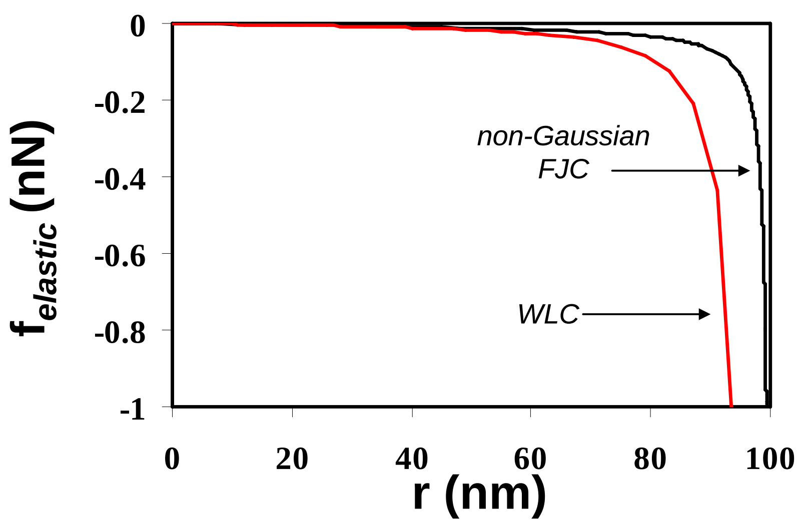


Extensible FJC



$$L_{total} = \underbrace{L_{contour}}_{=na} + \underbrace{n\left(\frac{f}{k_{segment}}\right)}_{\text{extension beyond } L_{contour} \text{ due to enthalpic stretching of chain segments}}$$

WLC



"Directed random walk"- segments are correlated, polymer chains intermediate between a rigid rod and a flexible coil (e.g. DNA)

- takes into account both local stiffness and long range flexibility

-chain is an isotropic, homogeneous elastic rod whose trajectory varies continuously and smoothly through space as opposed to the jagged contours of FJC

p= persistence length, length over which statistical segments remain directionally correlated in space

STRUCTURE OF MUSCLE AND TITIN

(*MARSZALEK, et. al *Nature* 402, 100 - 103 (**1999**))

Image removed due to copyright restrictions.

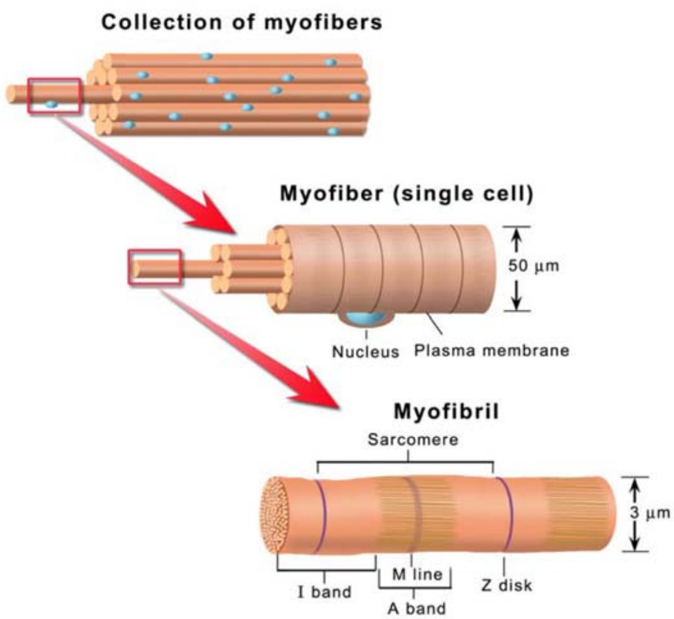


Figure by MIT OCW.

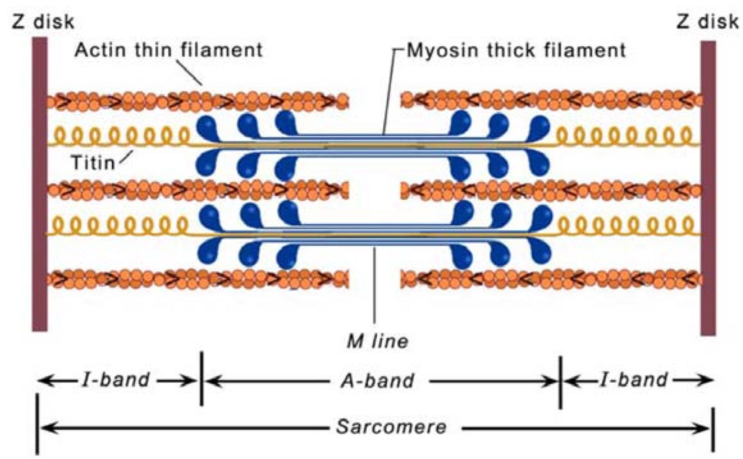
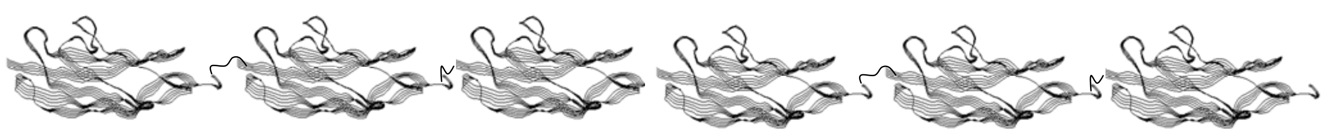


Figure by MIT OCW.



Sarcomere- fundamental contractile unit of muscle

-many proteins exhibit a modular motif (spectrin, fibronectic, seashell nacre, bone)

Titin:

- largest known protein (1-3 mm in length)≈ 25,000 amino acids (a.a.)

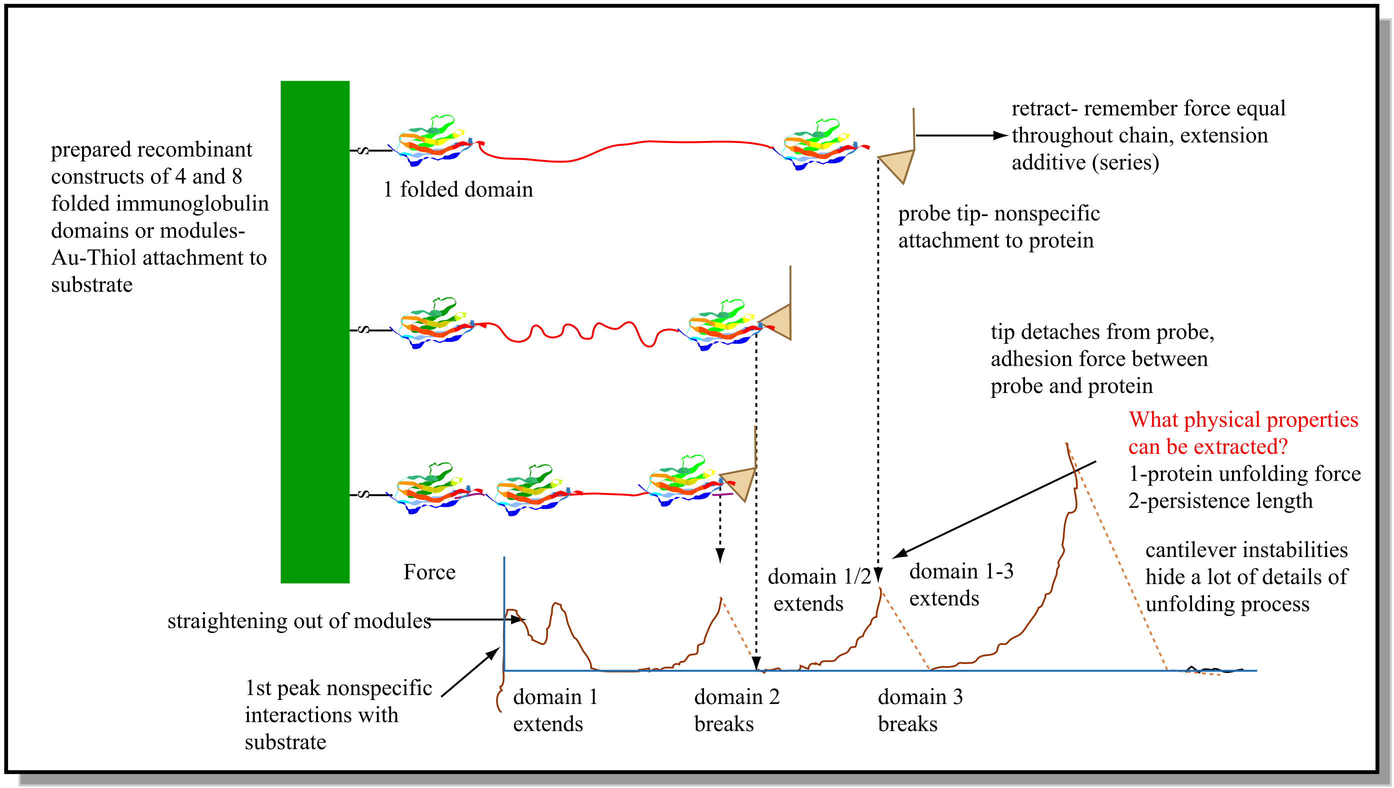
-modular structure, linear array of folded immunoglobulin domains covalently attached in series ("beads on a string")

-subunit 7-stranded β-barrel

-highly extensible, "giant rubberband" (L_{folded} domain = 3 nm (~90 a.a.), $L_{unfolded}$ domain = 30 nm)

-plays a major role in the passive elasticity of muscle; serves as an anchoring spring to keep myosin aligned on actin tracks, resist large sarcomere lengths, allows for overstretching of muscles without permanent damage to the sarcomere

SINGLE MOLECULE ELASTICITY OF TITIN-AFM *(Rief, et al. CHEMPHYSCHEM 2002, 3, 255-261)*



Distance = r chain end to end distance

Figure by MIT OCW.

-domain "breaks" - rupture of some critical noncovalent interactions needed to keep stability of folded structure
-domain "extends" - even though there is some noncovalent rupture, entropic elasticity dominates
# of unfolded domains = # of peaks - 1 (last peak) - any short range nonspecific substrate peak
$L_{contour}$ (entire folded protein) = D at first unfolding peak
$L_{contour}$ (unfolded module) = D at 2nd unfolding peak -3 (folded domain lengths) - distance between peaks
- **Sawtooth force profile** : sequential unfolding (weakest to strongest) of domains where each peak corresponds to the unfolding (mechanical denaturation) nanomechanical properties of an individual module or domain (many domains in series lead to huge extensibility)

SINGLE MOLECULE ELASTICITY OF DNA - MOTIVATION

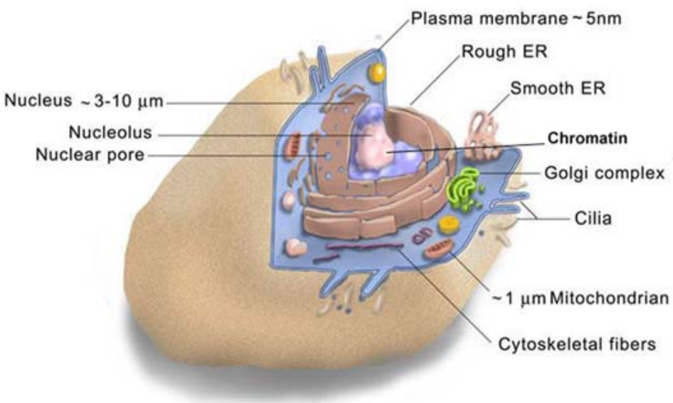


Figure by MIT OCW.

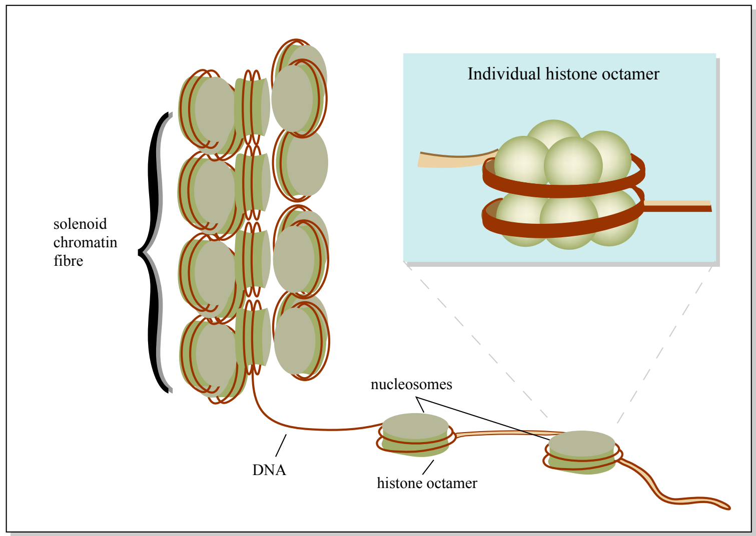
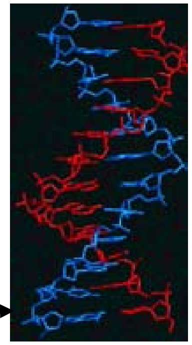


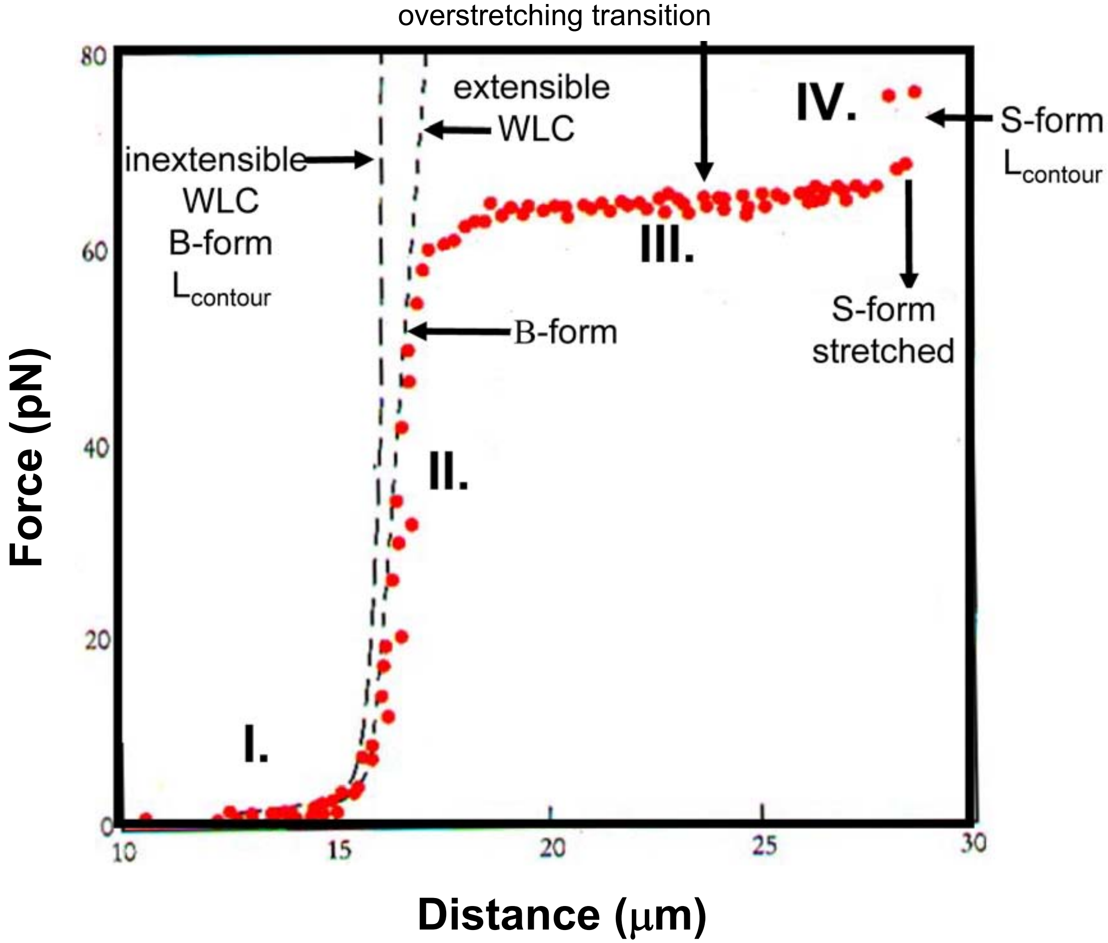
Figure by MIT OCW.



In an average human cell **2 meters** of DNA (hydrated!) has to fit into a 10 μm diameter while still maintaining accessibility to proteins and enzymes.
- The compaction of DNA is achieved by winding it around small proteins called **histones**
- Histones are composed of many positively charged amino acids that form ionic bonds to the negatively charged groups on DNA (polyelectrolyte).
-Elasticity of DNA is critically important to this process-has to be just the right stiffness (too stiff- will be too hard to bend around histones).

SINGLE MOLECULE ELASTICITY OF DNA - OPTCAL TWEEZERS

(Bustamante, et al. Science 1999, 271, 795)



I. low stretched behaves like WLC (p ≈ 50 nm under physiological conditions, much larger than most polymers ~ 1nm, hence much smaller forces, need optical tweezers)

II. intermediate stretches -some extensibility as apparent by finite slope beyond $L_{contour}$ (B-form)

III. At 65 pN ~ 0.06 nN, reversible strain-induced conformational transition; chain "yields" and stretches out almost 2× its native B-form contour length at relatively constant force (plateau in force region)
-All of hydrogen bonding and binding between 2 strands is still in tact, tilting of base pairs, tightened helix, reduction in diameter
"overstretching transition"

IV. entropic elasticity of S-form

V. can't see here - if you go to high enough stretches, separation between strains (mechanical "melting")

AFM SINGLE MOLECULE FORCE SPECTROSCOPYOF DNA

(Rief, et al. Nature Structural Biology 6, 346, 1999)

Biological Relevance of Overstretching Transition? Ability to switch between different structures is critical to the processes of transcription, replication, condensaton, e.g. the base pairs are much more exposed in S-DNA than normal DNA, the transition may be biologically significant for accessing information contained in the DNA code

V. At 150 pN another transition is found- force induced melting in which the double strands are split apart into single strands, which in many cases is reversible