DNA, RNA, Proteins

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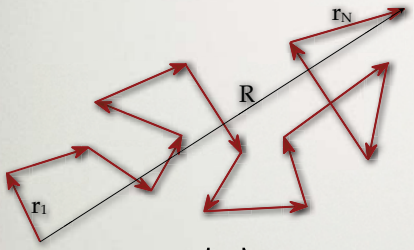
Biophysics of macromolecules

- Space
  Size, shape, local and global structure
- Time
  Fluctuations, structural change, folding
- Interactions
  Internal and external interactions, bonds, bond energies
  Mechanics, elasticity

Shape of the polymer chain resembles random walk

Brown movement: random walk



"Square-root law": $\langle R^2 \rangle = Nl^2 = Ll$

R = end-to-end distance
r_i = elementary vector
N = Number of elementary vectors
$l = |\vec{r}_i| =$ correlation length ("persistence length", describes bendingn rigidity)
$Nl = L$ = contour length

In case of Brown-movement R=displacement, N=number of elementary steps, L=total path length, és l=mean free path length.

Tendency for entropy maximization results in chain *elasticity*

Entropic* elasticity:
Thermal fluctuations of the polymer chain

Configurational entropy (orientational disorder of elementary vectors) increases.

The chain shortens.

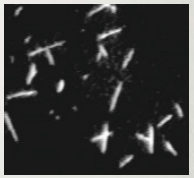
*Entropy: disorder

Biopolymer elasticity is related to global shape

l = persistence length: measure of bending rigidity
L = contour length

Rigid chain
$l >> L$

Microtubule



Semiflexible chain
$l \sim L$

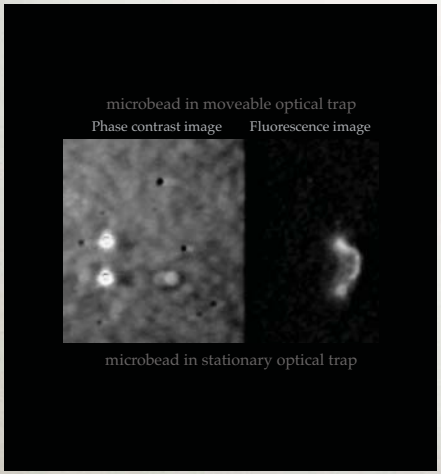
Actin filament

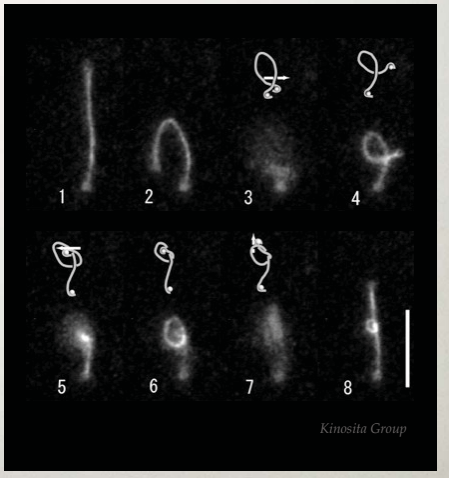
Flexible chain
$l << L$

DNA

Visualization of biopolymer elasticity

Tying a knot on a single DNA molecule

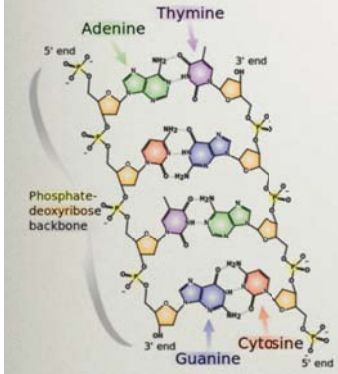




1. DNA: deoxyribonucleic acid

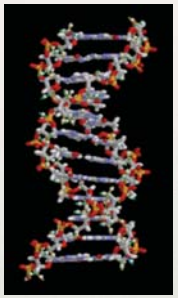
Function: molecule of biological information storage

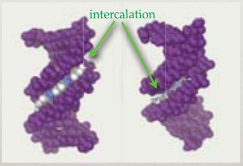
Chemical structure



"Watson-Crick" base pairing: via H-bonds
Gene sequence is of central significance in molecular genetics

3D structure: double helix



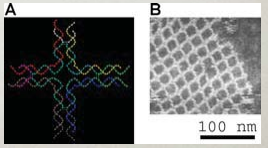


Large groove Small groove

Various DNA structures

A-DNA B-DNA Z-DNA

Depends on hydration, ionic environment, chemical modification (e.g., methylation), direction of superhelix

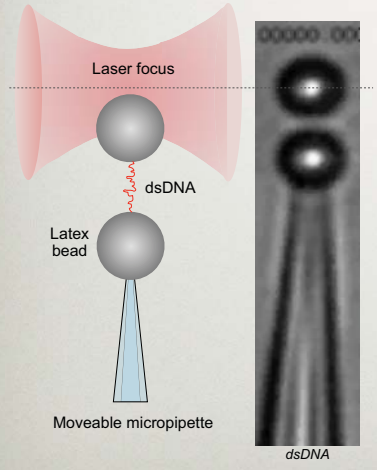


DNA nanostructures

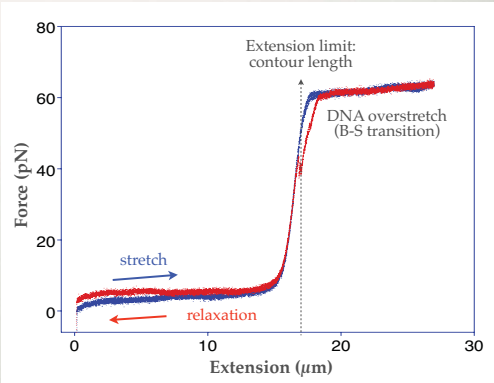
Depends on base-pairing order and hierarchy

The DNA molecule is elastic!

Force measurement: with optical tweezers

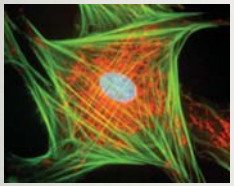


Force versus extension curve of a single dsDNA molecule



Peristence length of dsDNA: ~50 nm
Overstretch transition at ~65 pN

How much DNA in a cell?

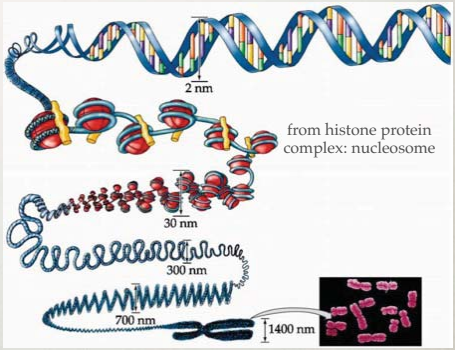


Simplified cell model: cube

	Cell: 20 μm edge cube	Analog - **Lecture hall:** 20 m edge cube
DNA thickness	2 nm	2 mm
Full length of human DNA	~2 m	~2000 km (!!!)
Persistence length of dsDNA	~50 nm	~50 cm
End-to-end distance (R)	~350 μm (!)	~350 m (!)
Volume of fully compacted DNA	~2 x 2 x 2 μm^3	~2 x 2 x 2 m^3 (= 8 m^3)

Solution: DNA needs to be packed

Chromosome condensation

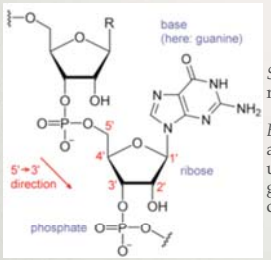


- ***Condensins*** play a role in high-order DNA packaging
- DNA chain: complex linear path with roadblocks!

2. RNA:
Ribonucleic acid

Function: information transfer (transcription), structural element (e.g., ribosome), regulation (turning gene expression on and off)

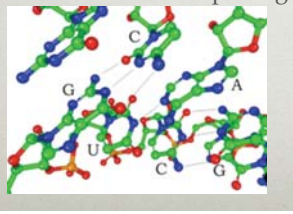
Chemical structure



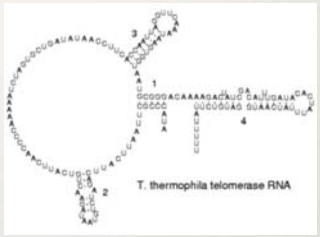
Sugar: ribose

Bases: adenine uracyl guanine cytosine

"Watson-Crick" base pairing

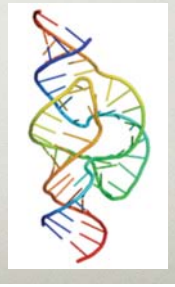


The RNA moleucule is not paired!



Secondary and tertiary structural elements

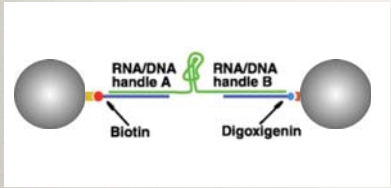
RNA hairpin



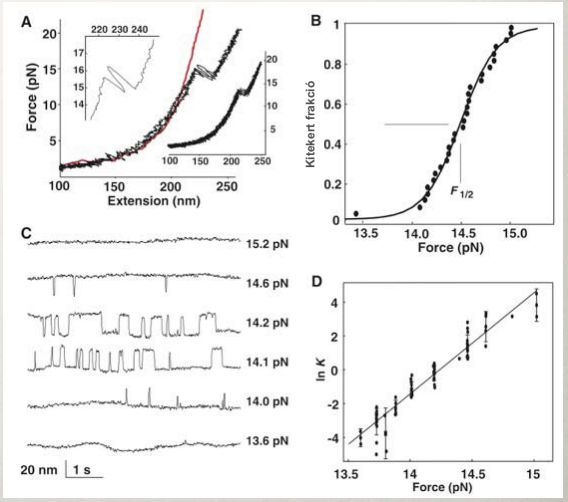
Complex structure (ribozyme)

RNA structure can be perturbed with mechanical force

Stretching with optical tweezers



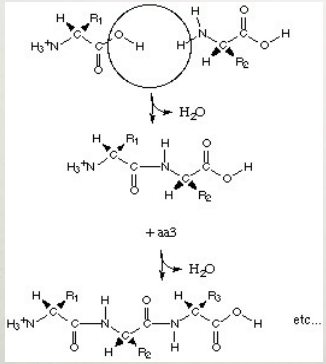
Unfolding of RNA hairpin: near reversible process - the RNA hairpin refolds rapidly

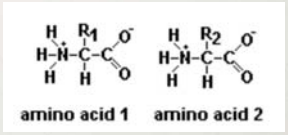


3. Proteins: Biopolymers interconnected with peptide bonds

Function: most important molecules of the cell.
Highly diverse functions - structure, chemical catalysis energy transduction, motoric functions, etc.

Formation of the peptide bond





Condensation reaction followed by the relase of water

Protein structure

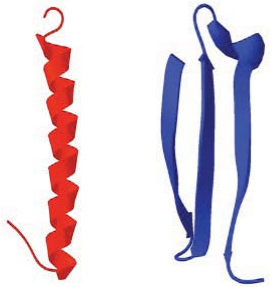
Primary

Amino acid sequence

Determines spatial structure as well.

Secondary

α-helix
β-sheet
β-turn (β-hairpin)

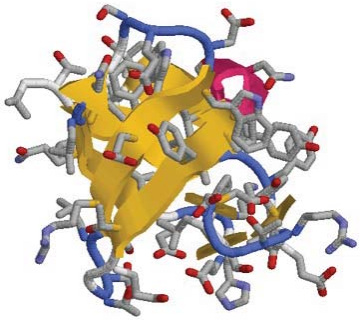


α-helix:
•right handed
•3.4 residue/turn
•H-bridges

β-sheet:
•parallel or
•antiparallel
•H-bridges between distant residues

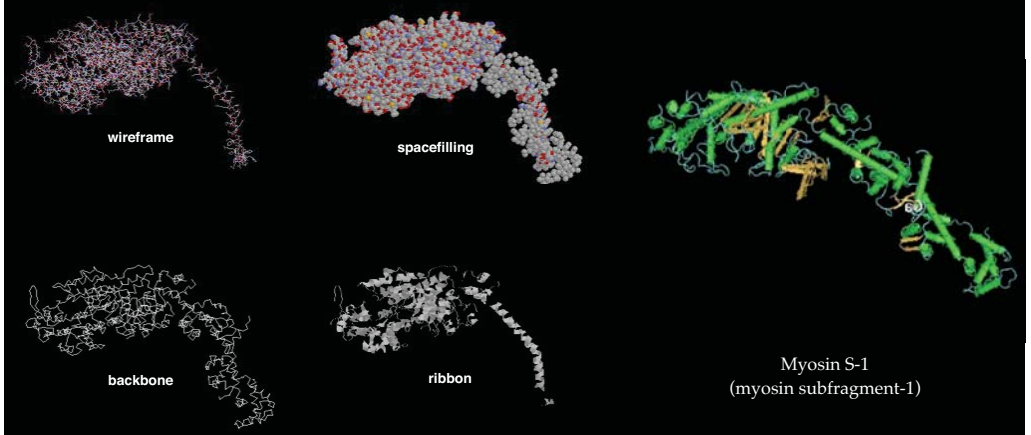
Tertiary

3D structure of single-chain protein



*Quaternary structure: binding of independent subunits into a complex

Display of protein structure



Myosin S-1
(myosin subfragment-1)

Bonds holding protein structure together

Weak (secondary) bonds

1. ***Hydrogen bond***: proton sharing between proton-donor side chains.

2. ***Electrostatic interaction*** (salt bridge): between oppositely charged residues.

3. ***van der Waals bond***: weak interaction between atoms (molecules) with closed electron shells.

4. ***Hydrophobe-hydrophobe interaction***: between hydrophobic residues (in the interior of the molecule).

Covalent bond

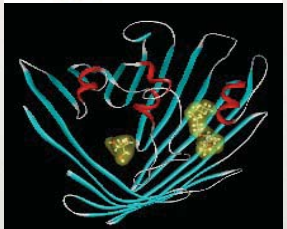
5. ***Disulfide bridge***: between cysteine side chains; connects distant parts of the protein chain.

Protein structure classes

1. All alpha

calmodulin

2. All beta



porin

(3. Alpha-beta)

4. Multidomain

Domain:
folding subunit

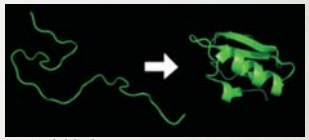
myosin

Although there are as many sequences as proteins, the spatial structures are classified into a surprisingly small number of classes!

How is the three-dimensional structure acquired?

Christian Anfinsen
(1916-1995)

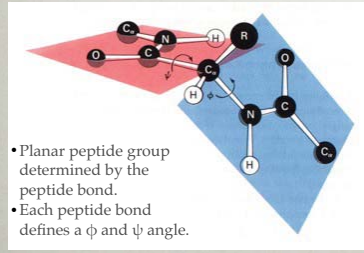
Anfinsen: proteins fold spontaneously (sequence determines structure)



Unfolded state

Native state (N)
Lowest energy

Levinthal's paradox (Cyrus Levinthal, 1969)**:**
Are all available conformations explored?

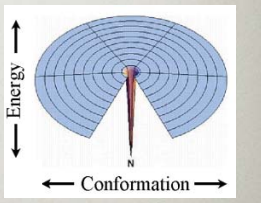


- Planar peptide group determined by the peptide bond.
- Each peptide bond defines a ϕ and ψ angle.

Number of possible conformations (degrees of freedom): i^n

i = number of possible angular positions of a given ϕ or ψ angle
n = total number of ϕ and ψ angles

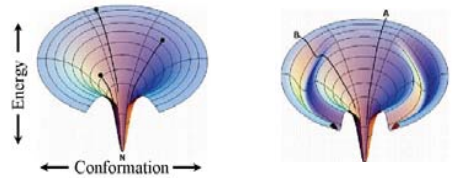
Example: in a peptide composed of 100 residues the number of possible ϕ or ψ angles is 2.
n=198m.
Number of possible conformations: 2^{198}(!!!)

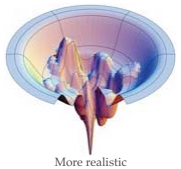


What is the probability that a billiards ball will find the hole merely via random motion?

Protein folding is guided by the shape of its conformational space

Shape of conformational space:
"Folding funnel"



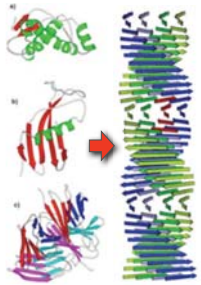


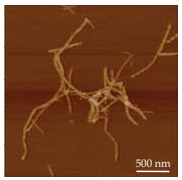
More realistic funnel shape

- Proteins "slide down" the wall of the funnel.
- Folding funnel shape can be complex (determination of the shape is usually very difficult).
- A protein may get stuck at intermediate states (pathology).
- In the living cell chaperones assist folding.

Pathology

- Protein "folding diseases"
- Alzheimer's disease
- Parkinson's disease
- II-type diabetes
- Familial amyloidotic neuropathy





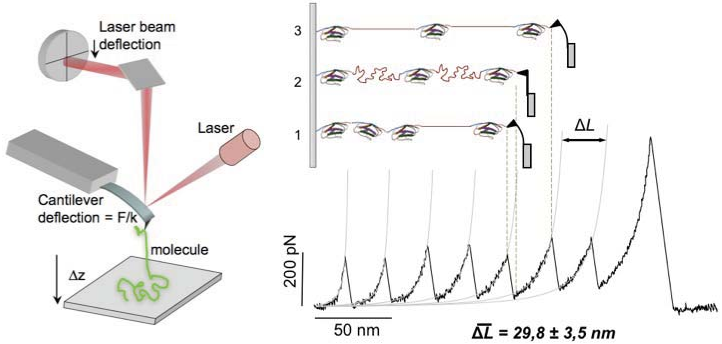
β-fibrils:
undissolved precipitate
cross-β structure

Methods of protein unfolding (denaturation)

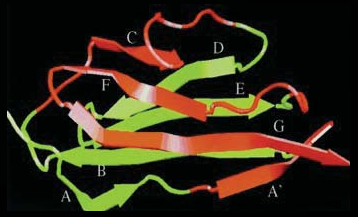
- Heat
- Chemical agent
- Mechanical force

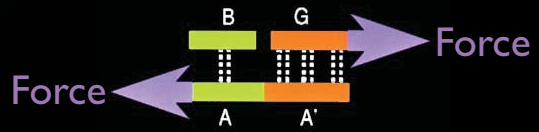
Break secondary chemical bonds
Disrupt secondary and tertiary structure

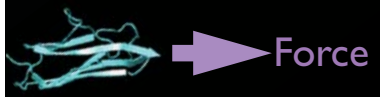
Mechanical unfolding of a single protein with atomic force microscope



Titin's Ig domains are mechanically stable



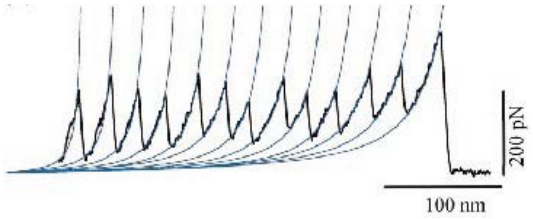




Basis of mechanical stability: parallel coupling of H-bonds

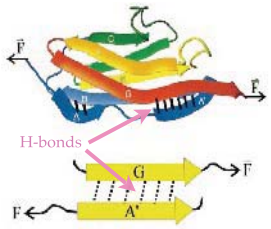
Mechanical stability provided by shear pattern of H-bond patch

Force spectrum



Domain unfolding force > 200 pN

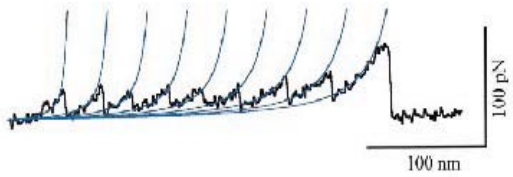
Structure-stabilizing H-bonds:



Low mechanical stability: H-bonds are coupled in series

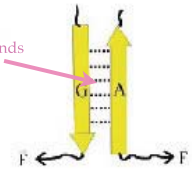
Low mechanical stability due to zipper pattern of H-bond patch

Force spectrum of $C2A_9$



Domain unfolding forces ~ 20 pN

Structure-stabilizing H-bonds:

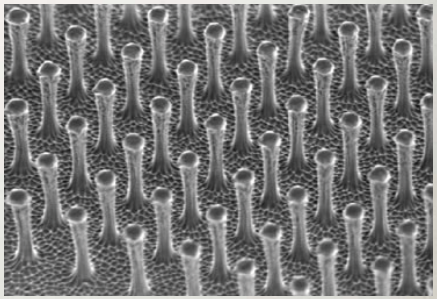


Logic of mechanical stabilization in macrosopic systems

Principle of parallel mechanical coupling

Gecko foot stickiness: Bristles (setae) coupled in parallel

Application:



Artificial gecko foot