# DNA, RNA, Proteins

Miklós Kellermayer

## 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

## Biological macromolecules: biopolyers

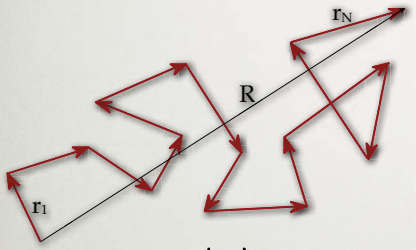
Polymers:
chains built up from monomers

Number of monomers: N>>1;
Typically, N~$10^2$-$10^4$,
but, in DNA, e.g.: N~$10^9$-$10^{10}$

| Biopolymer | Monomer | Bond |
|---|---|---|
| Protein | Amino acid | Covalent (peptide bond) |
| Nucleic acid (RNA, DNA) | Nucleotide (CTUGA) | Covalent (phosphodiester) |
| Polysaccharide (e.g., glycogen) | Sugar (e.g., glucose) | Covalent (e.g., α-glycosidic) |
| Protein polymer (e.g., microtubule) | Protein (e.g., tubulin) | Secondary |

## 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.

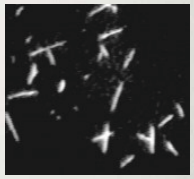
*Entropy: disorder

# Biopolymer elasticity is related to global shape

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

**Rigid chain**
$l >> L$

Microtubule

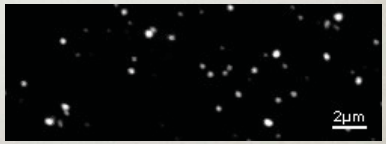


**Semiflexible chain**
$l \sim L$

Actin filament

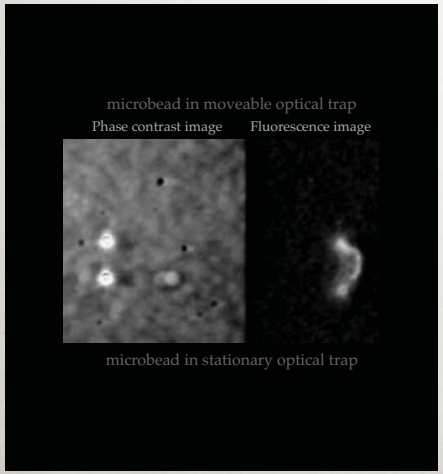
**Flexible chain**
$l << L$

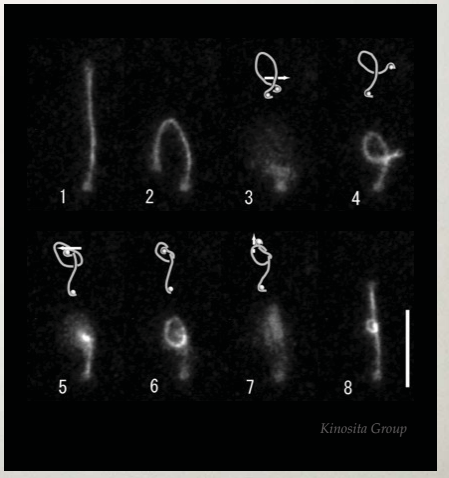
DNA



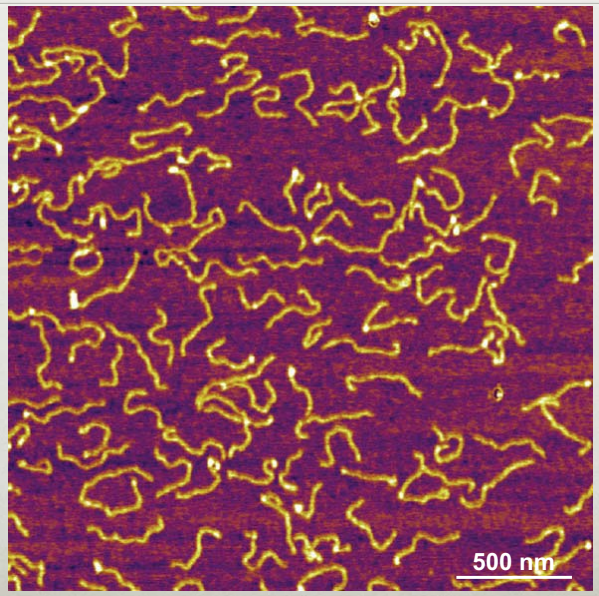

# Visualization of biopolymer elasticity

Tying a knot on a single DNA molecule



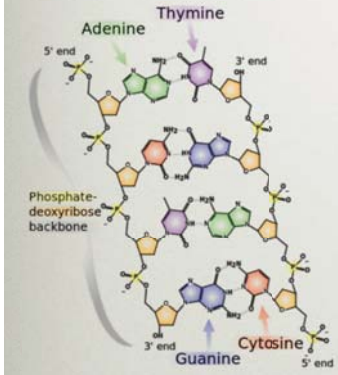

# Identical polymer molecules (DNA) captured on a surface




# 1. DNA: deoxyribonucleic acid

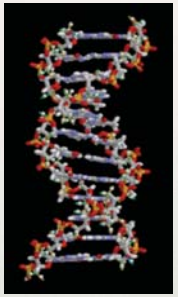
***Function***: molecule of biological information storage

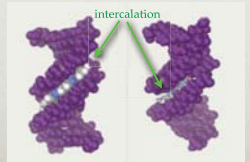
Chemical structure




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

3D structure: double helix



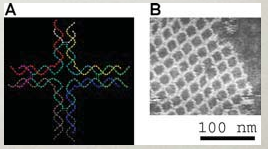



Large groove  Small groove

Various DNA structures

A-DNA  B-DNA  Z-DNA

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

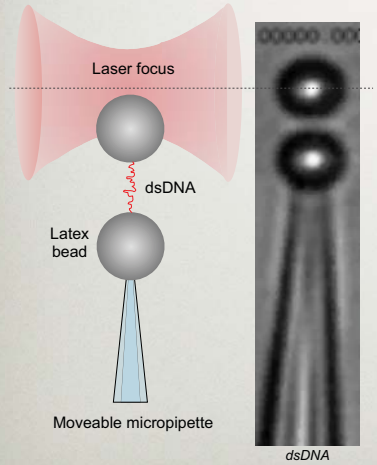



DNA nanostructures

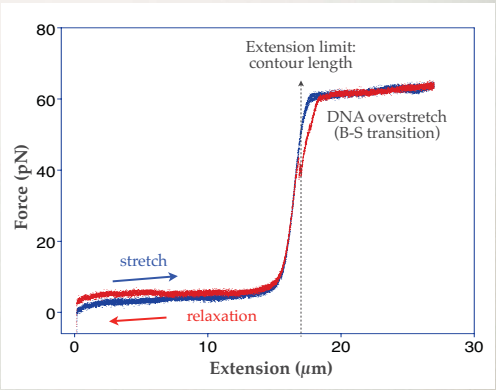
Depends on base-pairing order and hierarchy

# The DNA molecule is elastic!

Force measurement: with optical tweezers

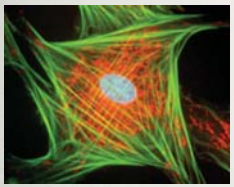



Force versus extension curve of a single dsDNA molecule




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

# How much DNA in a cell?

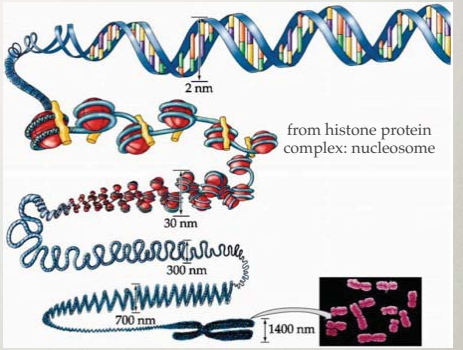


Simplified cell model: cube

| | **Cell:** 20 $\mu$m 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 $\mu$m (!) | ~350 m (!) |
| Volume of fully compacted DNA | ~2 x 2 x 2 $\mu m^3$ | ~2 x 2 x 2 $m^3$ (= 8 $m^3$) |

***Solution***: DNA needs to be packed

Chromosome condensation

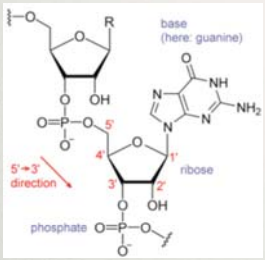



- ***Condensins*** play a role in high-order DNA packaging
- DNA chain: complex linear path with roadblocks!

# 2. RNA: Ribonucleic acid

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

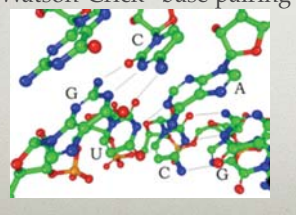
Chemical structure



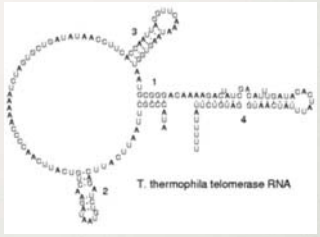

*Sugar*: ribose

*Bases*: adenine uracyl guanine cytosine

"Watson-Crick" base pairing

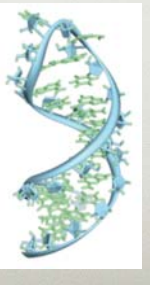


The RNA moleucule is not paired!




Secondary and tertiary structural elements

RNA hairpin

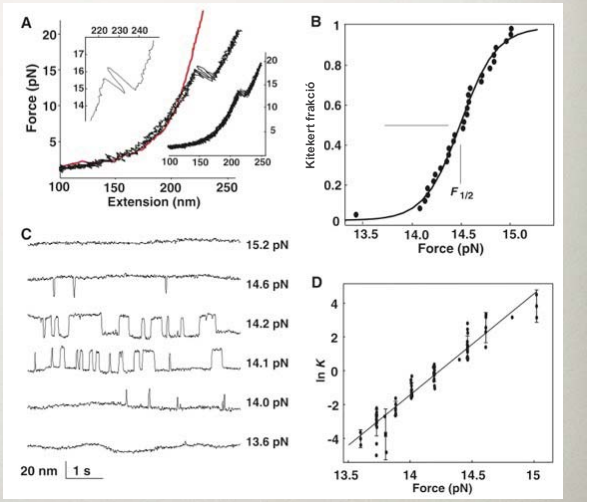


Complex structure (ribozyme)

# RNA structure can be perturbed with mechanical force

Stretching with optical tweezers

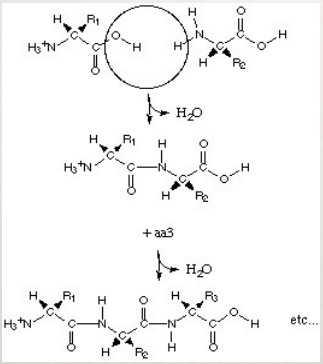

Unfolding of RNA hairpin: near reversible process - the RNA hairpin refolds rapidly

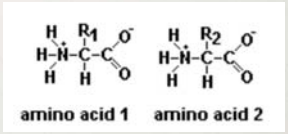


# 3. Proteins: Biopolymers interconnected with peptide bonds

*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

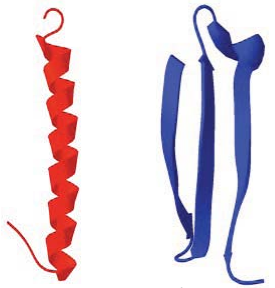
**Primary**

**Amino acid sequence**

Determines spatial structure as well.

**Secondary**

**α-helix**
**β-sheet**
**β-turn (β-hairpin)**

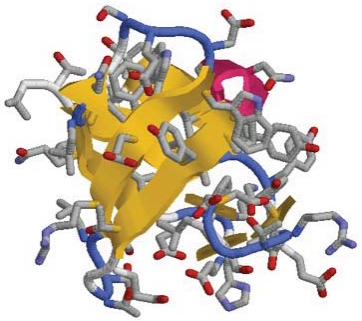


α-helix:
•right handed
•3.4 residue/turn
•H-bridges

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

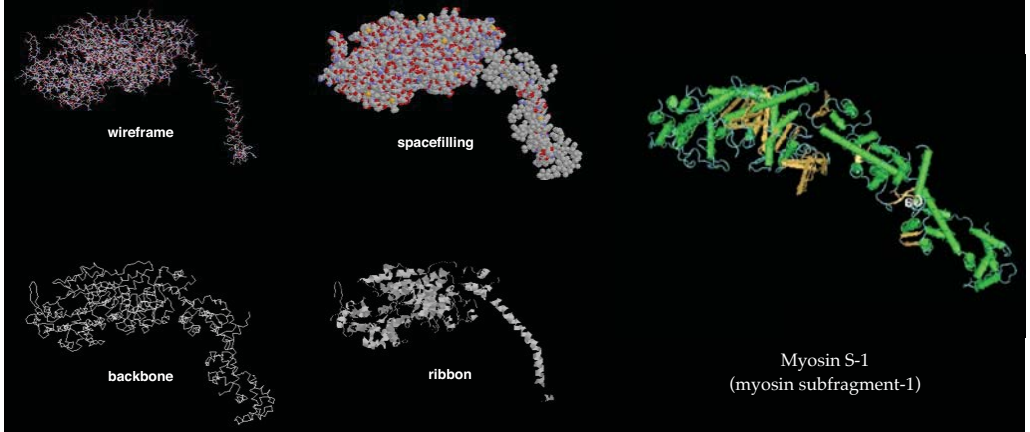
**Tertiary**

**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

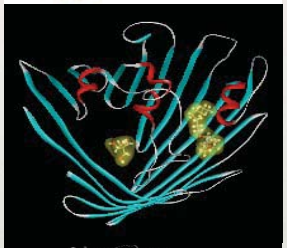
5. ***Disulfide bridge***: between cysteine side chains; connects distant parts of the protein chain.

# Protein structure classes

1. All alpha

calmodulin

2. All beta

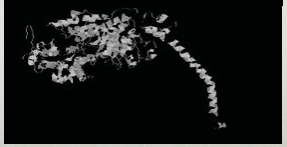


porin

(3. Alpha-beta)

4. Multidomain

*Domain*:
folding subunit



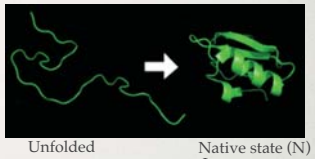
myosin

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)

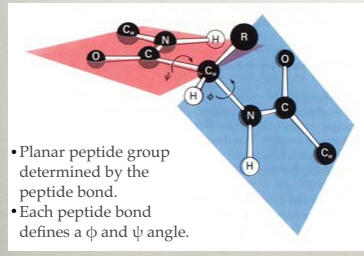
**Anfinsen:** proteins fold spontaneously (sequence determines structure)



Unfolded state

Native state (N)
Lowest energy

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

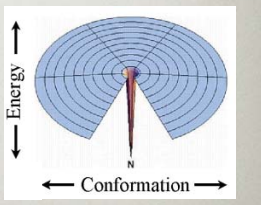


- Planar peptide group determined by the peptide bond.
- Each peptide bond defines a ϕ and ψ angle.

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

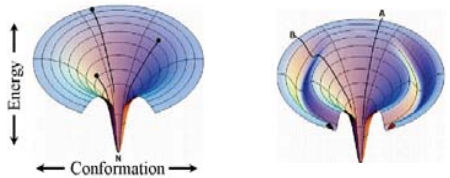
Example: in a peptide composed of 100 residues the number of possible ϕ or ψ angles is 2.
n=198m.
Number of possible conformations: $2^{198}$(!!!)



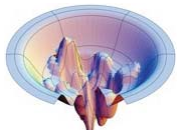

What is the probability that a billiards ball will find the hole merely via random motion?

# Protein folding is guided by the shape of its conformational space

Shape of conformational space:
"Folding funnel"





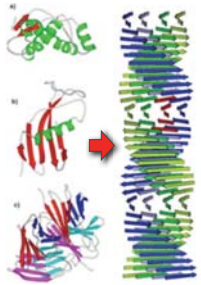



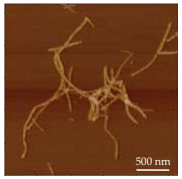
More realistic funnel shape

- 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





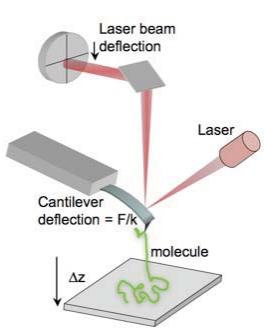

β-fibrils:
undissolved precipitate
cross-β structure

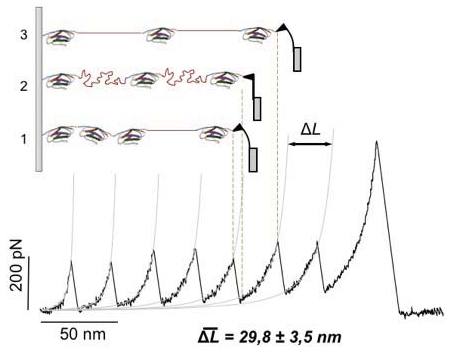
# Methods of protein unfolding (denaturation)

- Heat
- Chemical agent
- Mechanical force

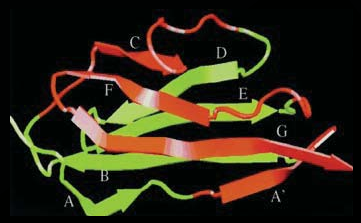
Break secondary chemical bonds
Disrupt secondary and tertiary structure

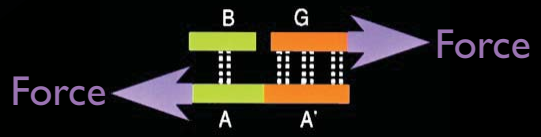
Mechanical unfolding of a single protein with atomic force microscope

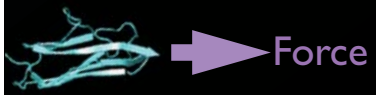


# Titin's Ig domains are mechanically stable



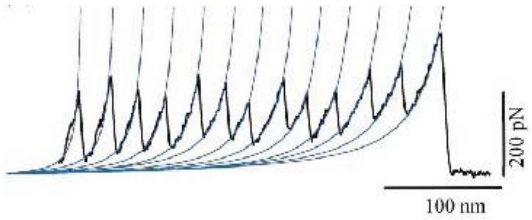




# Basis of mechanical stability: parallel coupling of H-bonds

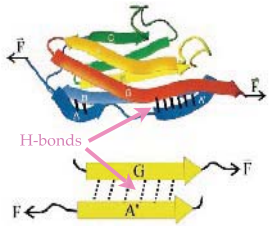
Mechanical stability provided by shear pattern of H-bond patch

Force spectrum




Domain unfolding force > 200 pN

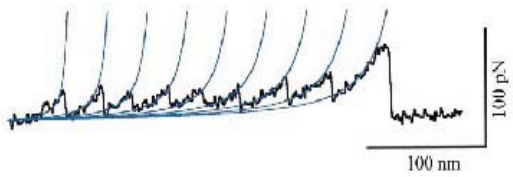
Structure-stabilizing H-bonds:




# Low mechanical stability: H-bonds are coupled in series

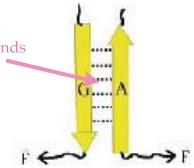
Low mechanical stability due to zipper pattern of H-bond patch

Force spectrum of $C2A_9$



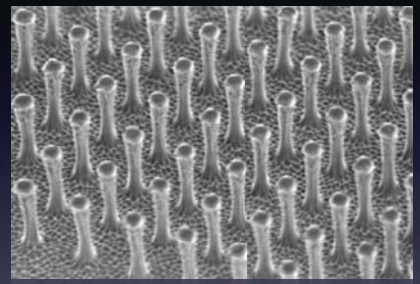

Domain unfolding forces ~ 20 pN

Structure-stabilizing H-bonds:




# Macroscopic mechanical stability

Highly efficient glue based on the principle or parallel coupling



Artificial gecko foot
Nanotechnology

Surface attachment of the gecko foot:
Numerous Van der Waals interactions - between bristles and surface - coupled in parallel