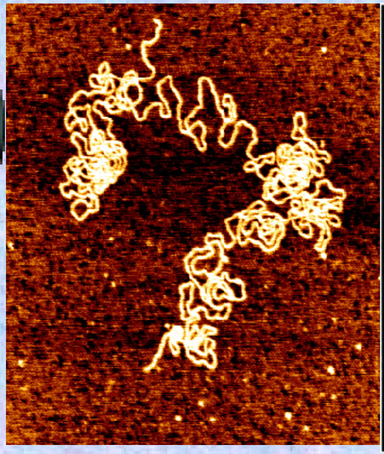
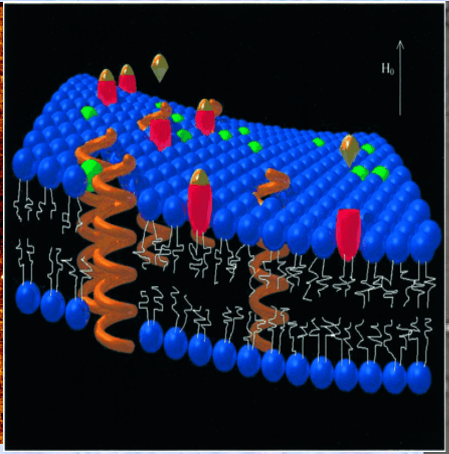
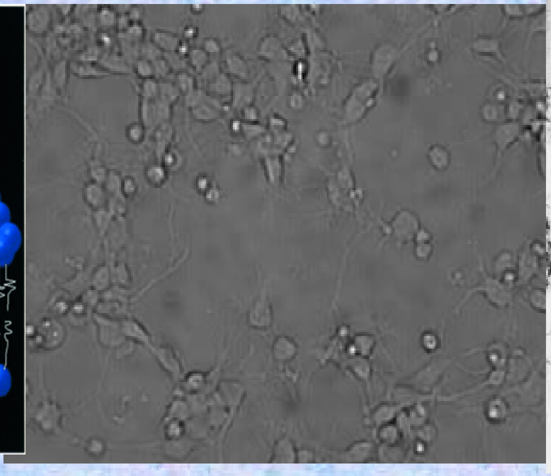
• BioPhysics--- impact on science & life

Pik-Yin Lai (黎璧賢)

Dept. of Physics & Graduate Institute of BioPhysics ,
National Central University, Chung-Li, Taiwan 320

Email: pylai@phy.ncu.edu.tw


http://www.phy.ncu.edu.tw/~ibp/

2008 Winter

What is Biophysics?

Biophysical Society defines as: "that branch of knowledge that applies the principles of physics and chemistry and the methods of mathematical analysis and computer modeling to understand how the mechanisms of biological systems work" .

- **Why BioPhysics ?**
- Material Nature of Bio-substances affect Biological properties. (Evolution made use of the physical properties of bio-materials)
- Physical principles & Laws holds from microscopic level → macroscopic level
- Traditional Biology is descriptive, non-quantitative

Physics is vital in breakthrough in life sciences

- Breakthrough in physical instrument: optical microscope (Hooke, 1665), amplifier, X-ray, electron microscope, MRI, SPM, mass spectrometer, Single molecule microscopy,….

Nobel laureates in physiology/medicine

that were physicists/had physics training:

- Georg von Békésy (physical mechanism of the cochlea, 1961)
- Francis Crick, Maurice Wilkins (DNA, 1962)
- Alan Hodgkin (nerve cell ,1963)
- Haldan Hartline (visual processes in the eye, 1967)
- Max Delbrück (bacteriophage ●, 1969)
- Rosalyn Yalow (radio-immunoassays of peptide hormones, 1977)
- Werner Arber (restriction enzymes , 1978)
- Alan Cormack (tomography, 1979)
- Erwin Neher (single ion channels in cells ,1991)
- Paul Greengard (signal transduction in nervous systems, 2000)
- Leland Hartwell (regulators of cell cycle, 2001)
- Peter Mansfield (NMR, 2003)……

Others: Helmholz, Schroedinger, Cooper, Feigenbaum…

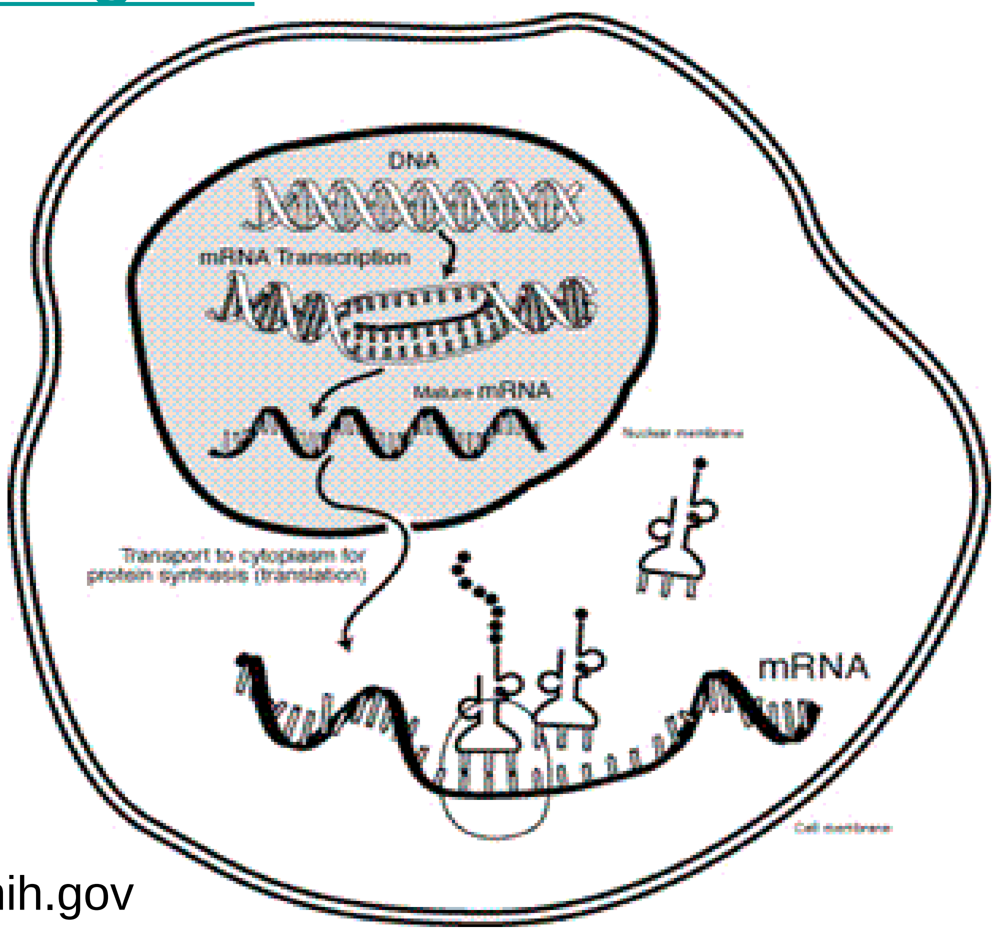
Why BioPhysics ?

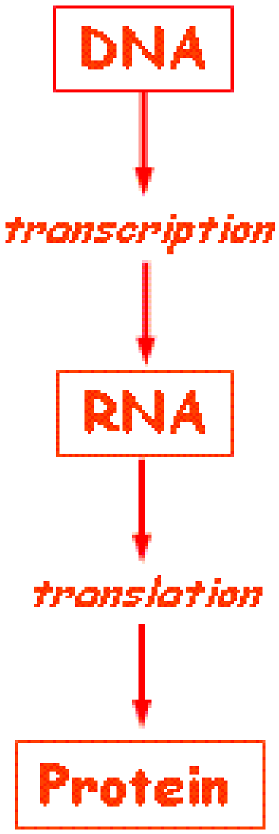
- Physics is universal.
- Rise of molecular biology: DNA, RNA, protein, ATP… are universal in all living matters
- Universality in Central Dogma: DNA→RNA→protein→Biological functions…
- New, interesting, exciting & useful.
- Lots of unsolved important problems.
- Techniques & Methodology in physics can probe the fundamental principles in bio-systems of a wide spectrum of scales in a quantitative way.

Brief Molecular biology

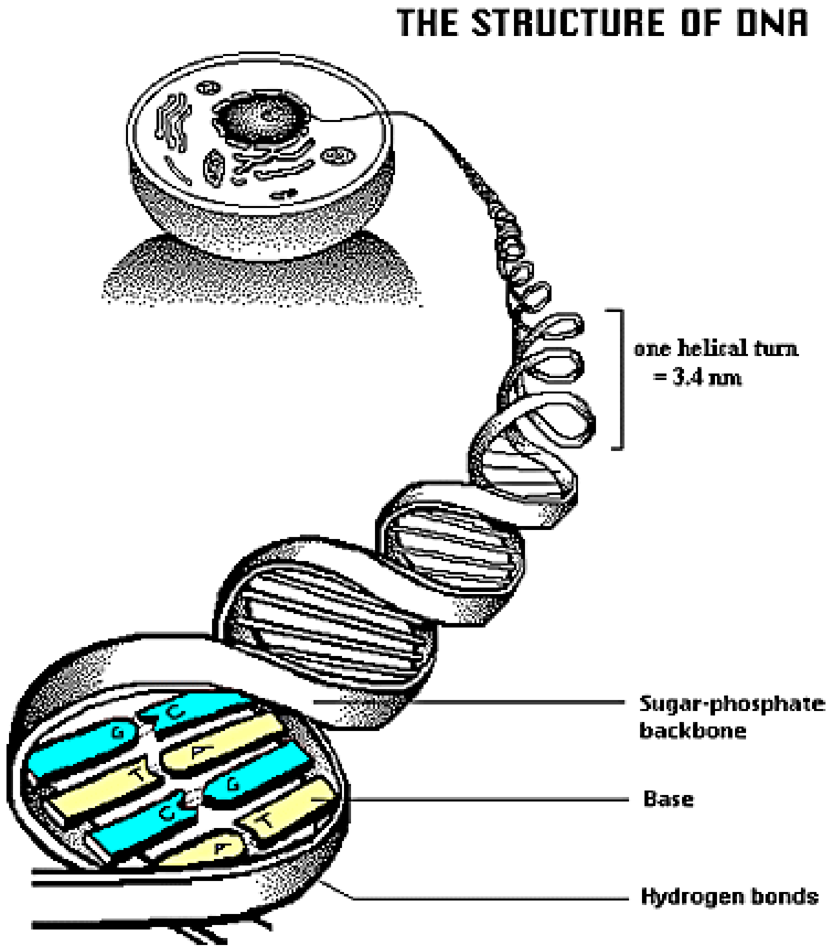
- [Molecular Biology of the Cell](#)

[Central Dogma](#)

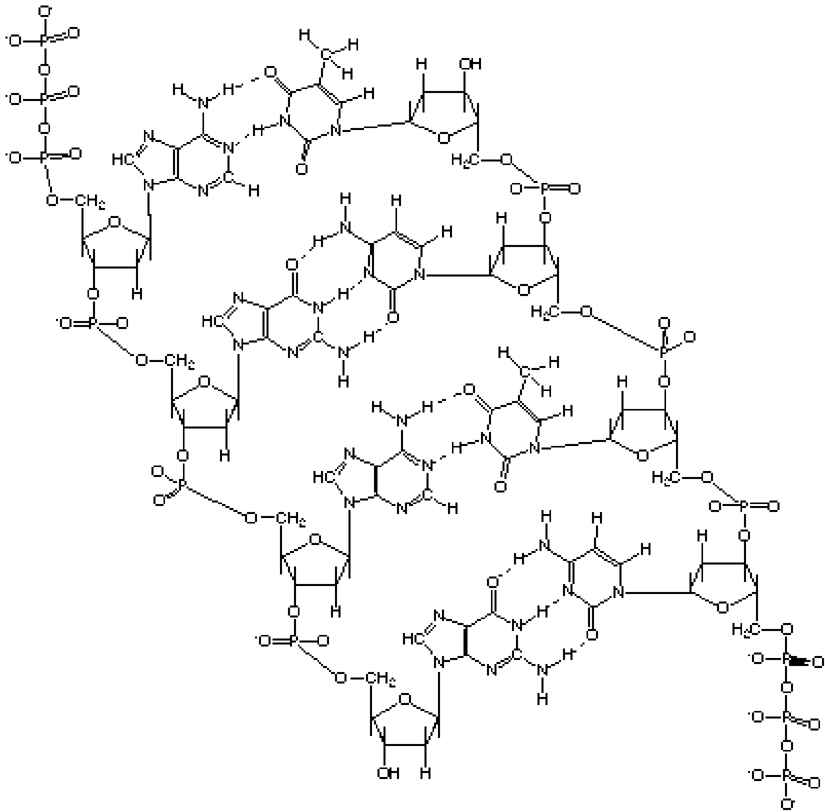




www.ncbi.nlm.nih.gov

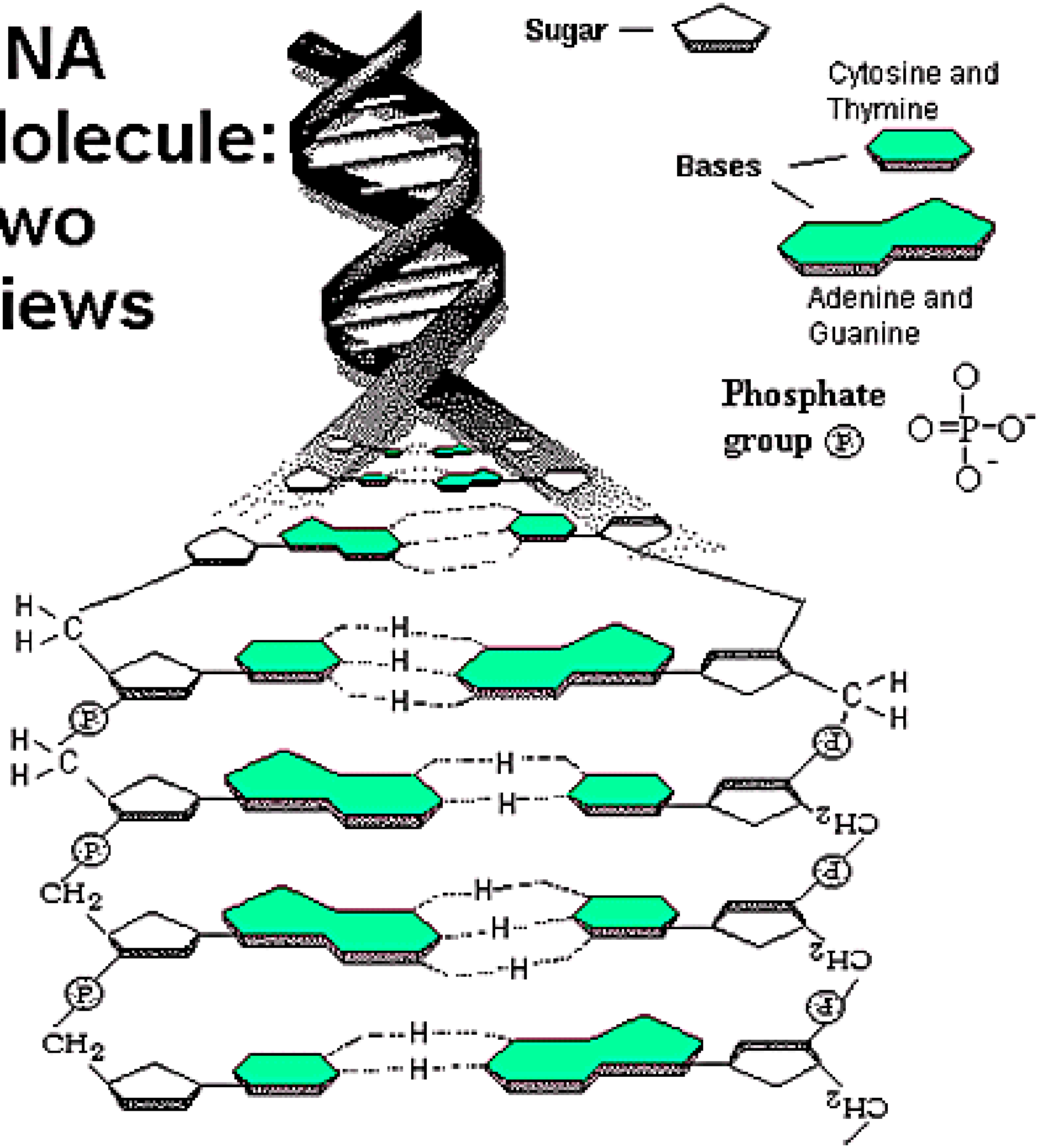


Cell
Nucleus
Chromosome
Chromatin



Double-stranded biopolymer, 2 sugar-phosphate chains (backbones) twisted around each other forming a RH (B-form) double helix.

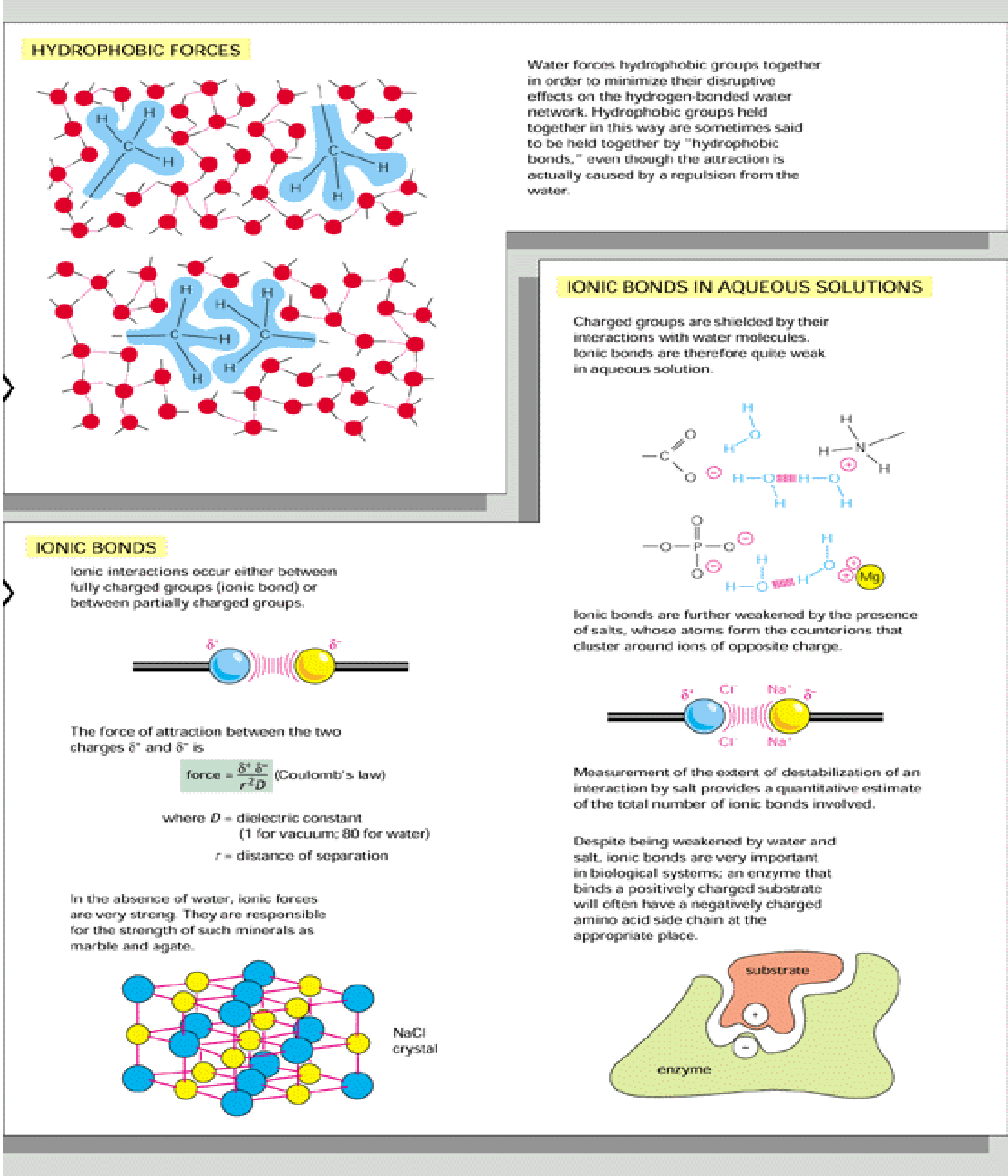
DNA Molecule: Two Views



base pairs: **A-T** & **C-G**

Bonding &
Forces in bio-systems

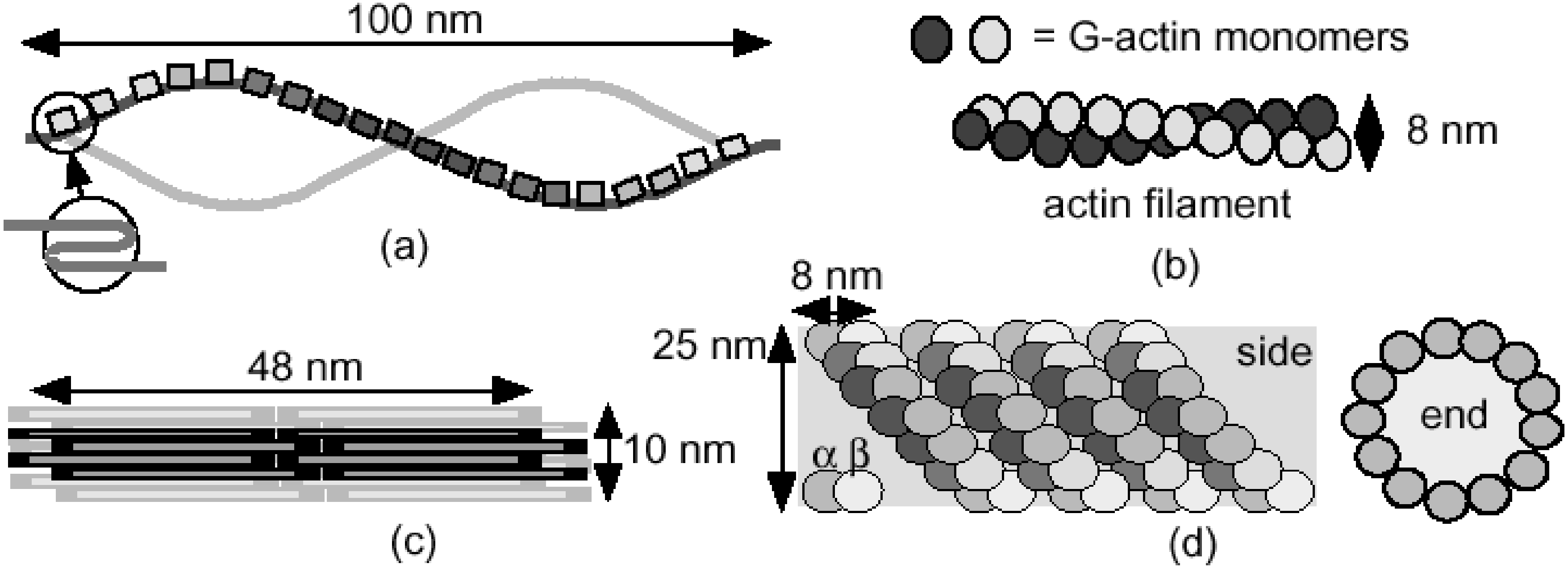
- •Van der Waals: ~2.5kT
- •Ionic: ~250kT
- •Covalent: ~100-300kT
- •H-bonds: ~5-10kT
- •Hydrophobic: ~few kT



Some common Biomolecular chains

Spectrin

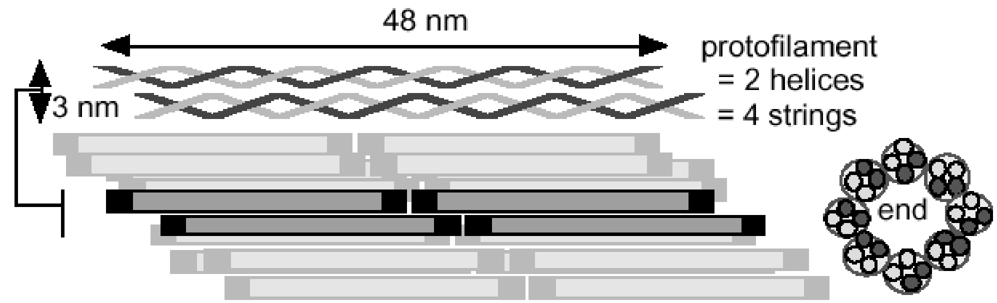
Globular actin



Intermediate filament

Microtubule

F-actin



Era of modern Biophysics

- **Length Scales:**

nm → ❍m → mm → cm → m → km

DNA,RNA,protein, intracellular, virus, bacteria, Intercellular, collective motion, insects, animals/plants, migration

- **Time Scales:**

fs → ps → ❍s → ms → s

e transfer,H-bonding,water DNA,RNA,protein rearrangement , protein folding DNA transcription

→ hr → day → year → Byr

cell division Earth organisms , animal migration evolution

- Knowledge: Interdisciplinary 跨越各學科領域

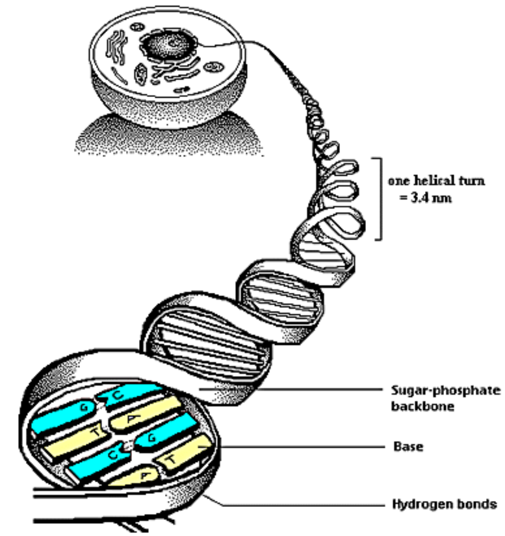
Mathematics←→Physics←→Chemistry←→Biology←→Medical

BioPhysics □ Biology + Physics

- **Biophysicist is a TRUE Scientist ! Explore to the maximum freedom for doing science!**
- **Need both physics & non-physics background people !**

Elementary particles of Life

- Universal molecules: DNA, RNA, protein, ATP
- Interactions giving rise to bio-process: Central Dogma: DNA→RNA→protein→Biological functions…
- Nanomachines: molecular motors, FoF1 ATPase..
- How physical and chemical interactions lead to complex functions in cells ?
- Gene networks, protein networks …….



Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 13999–14000, November 1998

Commentary

The rise of single-molecule DNA biochemistry

Alexander Rich

Department of Biology, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Room 68-233, Cambridge, MA 02139

We have known about conformational changes in DNA far longer than we have known its three-dimensional structure. Early experiments showing the irreversibility of acid-base titration of DNA and of changes in the ultraviolet absorption associated with heating suggested that changes in conformation were occurring. Experiments with DNA fibers that facilitated the derivation of the double helix by Watson and Crick started with the observation of a conformational change.

it is of considerable interest that r
involving the study of single I
adopted to study conformational
cant addition to this literature
Allemand *et al.* (3) in this issue o
show that stretching and overwir
results in a striking conformatior
Single-molecule DNA biochei

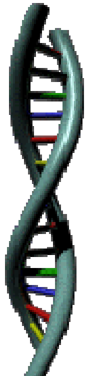
polymerase chain reaction (PCR)聚合酵素鏈鎖反應

Mullis Nobel Prize in Chemistry 1993

- is a technique widely used in molecular biology
- DNA copy machine using DNA polymerase under thermal cycles
- Amplify(> million times) specific regions of a DNA strand, for gel electrophoresis
- Breakthrough technique for forensic science, DNA fingerprint
- DNA sequencing, genetic diseases…..

Play (Torture) with DNA

- DNA stretching, elasticity
- DNA drag reduction
- DNA thermo-phoresis
- DNA condensation
- DNA under external fields
- DNA photolysis
- DNA ratchet motion
- DNA electronics……..



Mechanics/Elasticity of Single Bio-molecules

- To investigate the conformational changes in single bio-molecules, may provide significant insight into how the molecule functions.
- How forces at the molecular level of the order of pN underlie the varied chemistries and molecular biology of genetic materials?

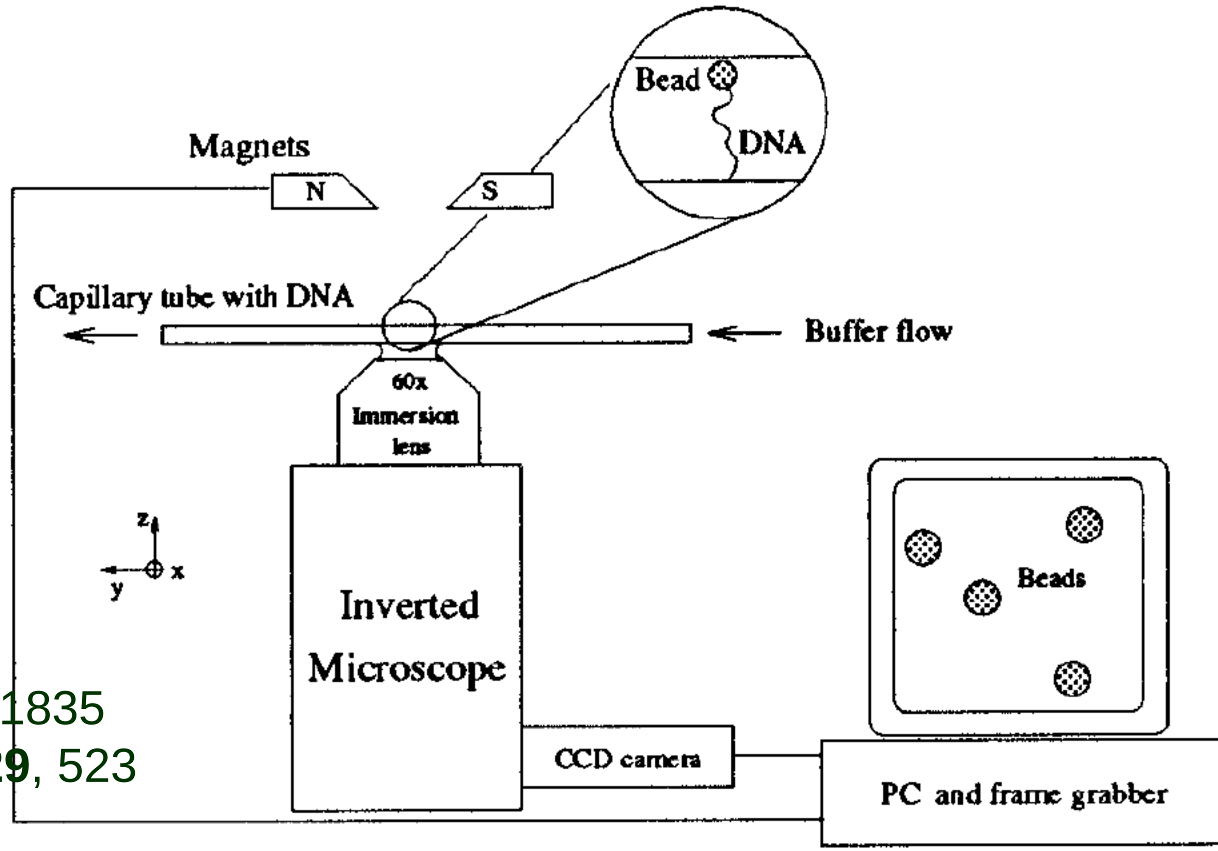
Force scales

- Size of bead/cell, d~2 micron
- thermal agitation sets the lower limit to force measurements $k_B T = 4\ 10^{-21}\ \mathrm{J} = 0.6\ \mathrm{kcal/mol}$-
- Langevin force~10fN/Hz$^{1/2}$
- Weight of a cell ~ 10fN
- Entropic forces ~kT/nm ~ 4 pN
- Non-covalent bond~eV; elastic forces~eV/nm=160pN
- Force to break covalent bond~ eV/A~1600pN

Experimental Tools in Force expts.

- Micro-mechanical springs (fibers,mciro-pipette,cantilevers),
- Hydrodynamic drag
- Optical or magnetic tweezers
- Scanning force/Atomic force microscopy

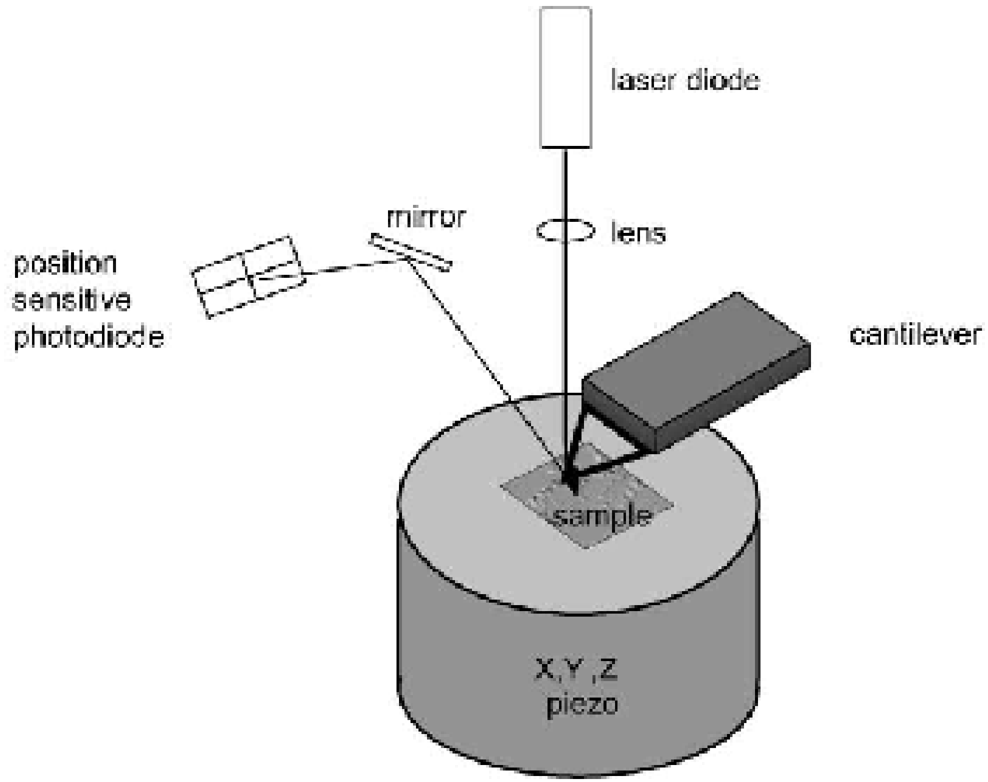
- Imaging techniques and Fluorescence microscopy



Strick et al., Science **271**, 1835 (96); Ann. Rev. Biophys. **29**, 523 (00)

Scanning force microscopy

- Commercial SFM tips can have stiffness low as ~10mN/m;
  can measure forces as low as 10pN.
- Etched optical fibre/glass microneedles are ultra-soft, ~1.7 ❍N/m;
  force precision of ~1 pN



Magnetic tweezers

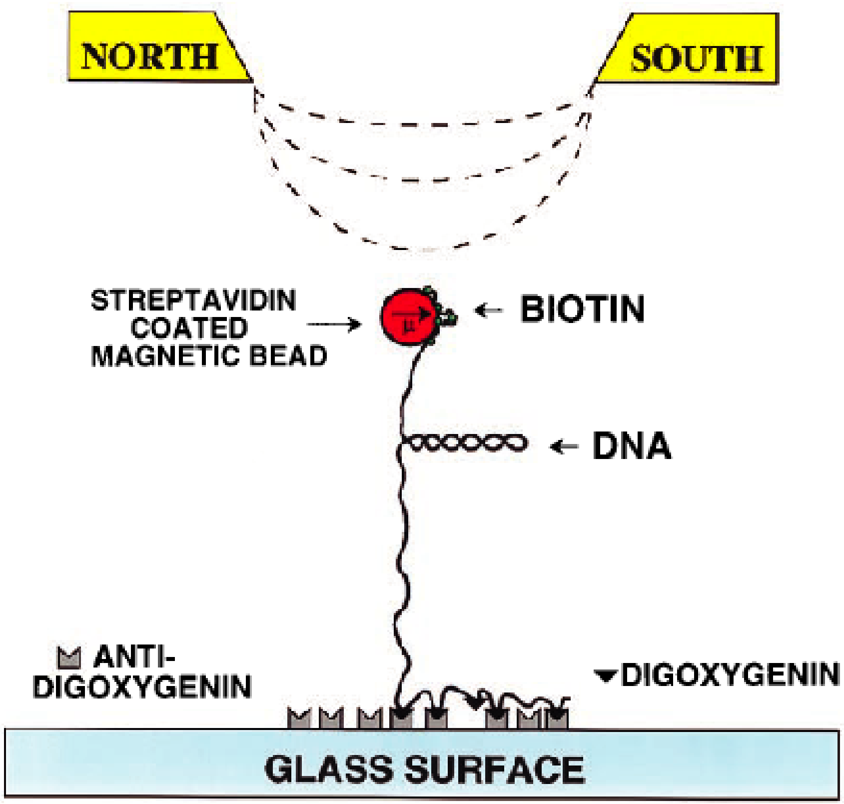


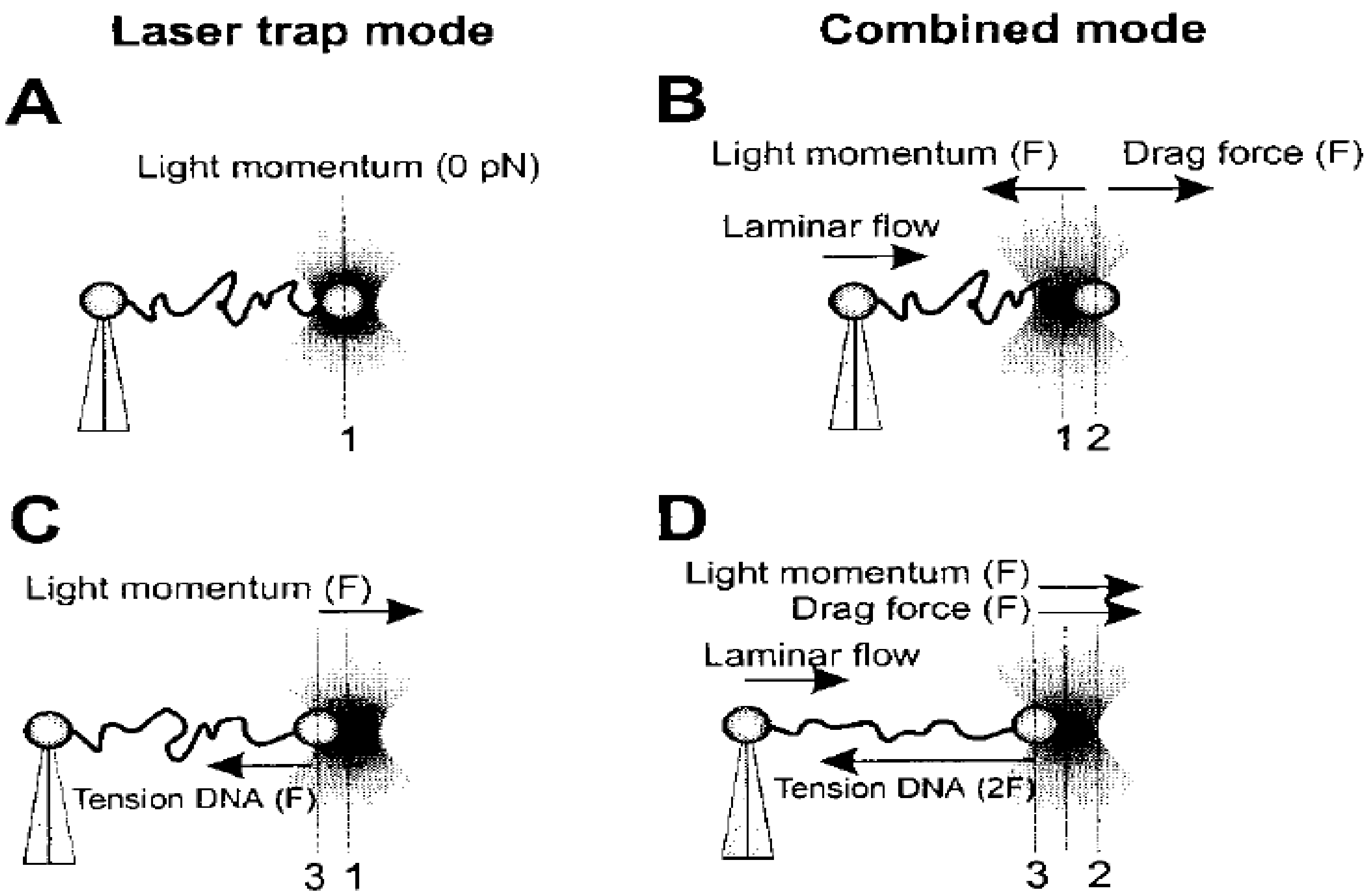
FIG. 1. A streptavidin-coated magnetic bead attached to DNA can be rotated or moved vertically in the magnetic field. This can stretch or twist the DNA attached to a glass slide (adapted from ref. 3).

Force & torque exert on paramagnetic bead

$$\boldsymbol{F} = (\boldsymbol{M} \cdot \nabla)\boldsymbol{H}, \qquad \boldsymbol{T} = \boldsymbol{M} \otimes \boldsymbol{H} ,$$

Wide range of forces

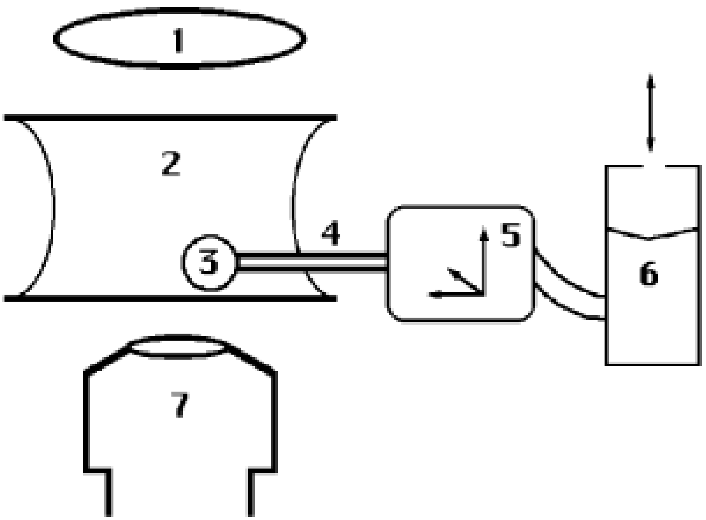
Optical tweezers



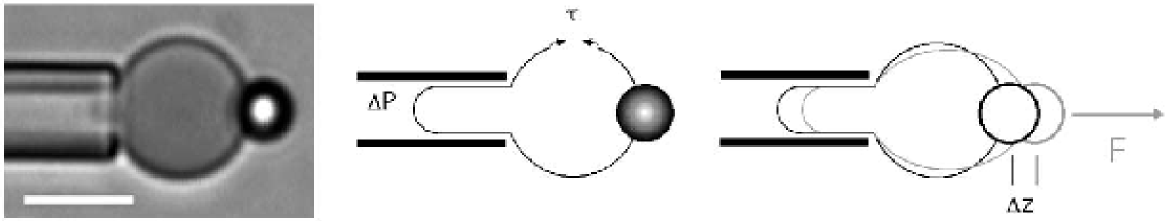
Bustamante et al., Science **258**, 1122 (92);
Biophys. J. **79**, 1155 (00

Micropipette aspiration

Pipette diameter~1~10❍m
Suction P~1 Pa to 50kPa; f~1 pN to 1❍N

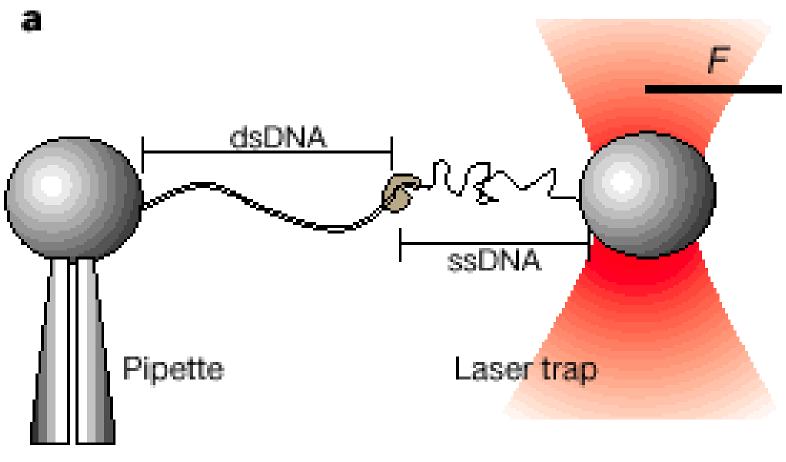


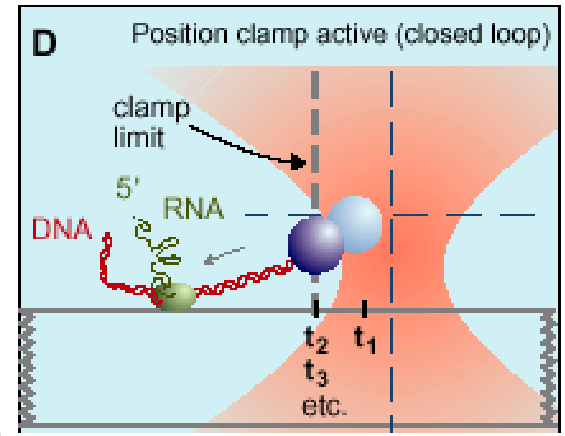
Biointerface force probe

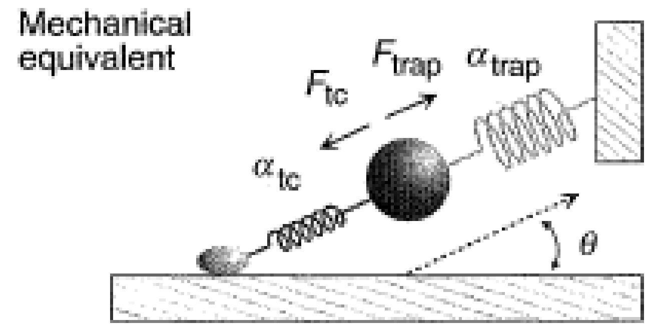


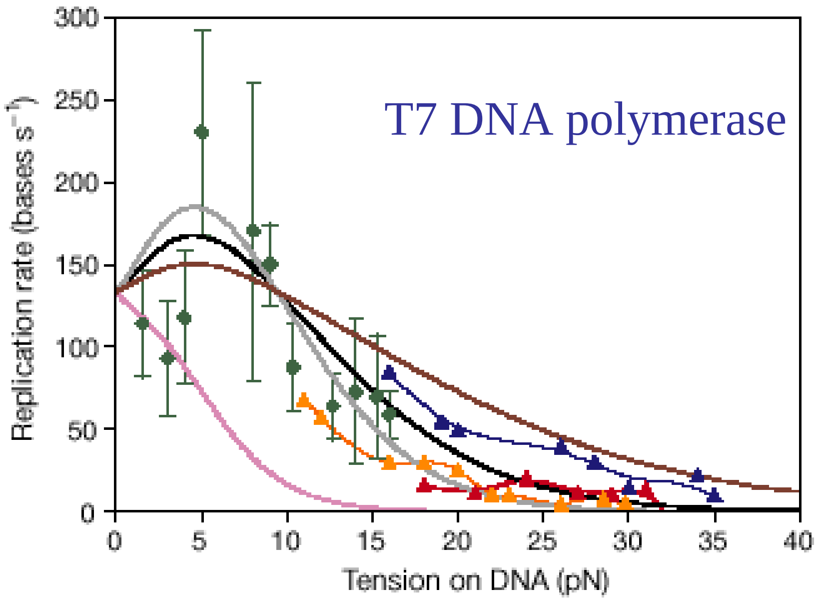
DNA transcription by RNA polymerase

Bustemante et al, Nature 404, 103 (2000)



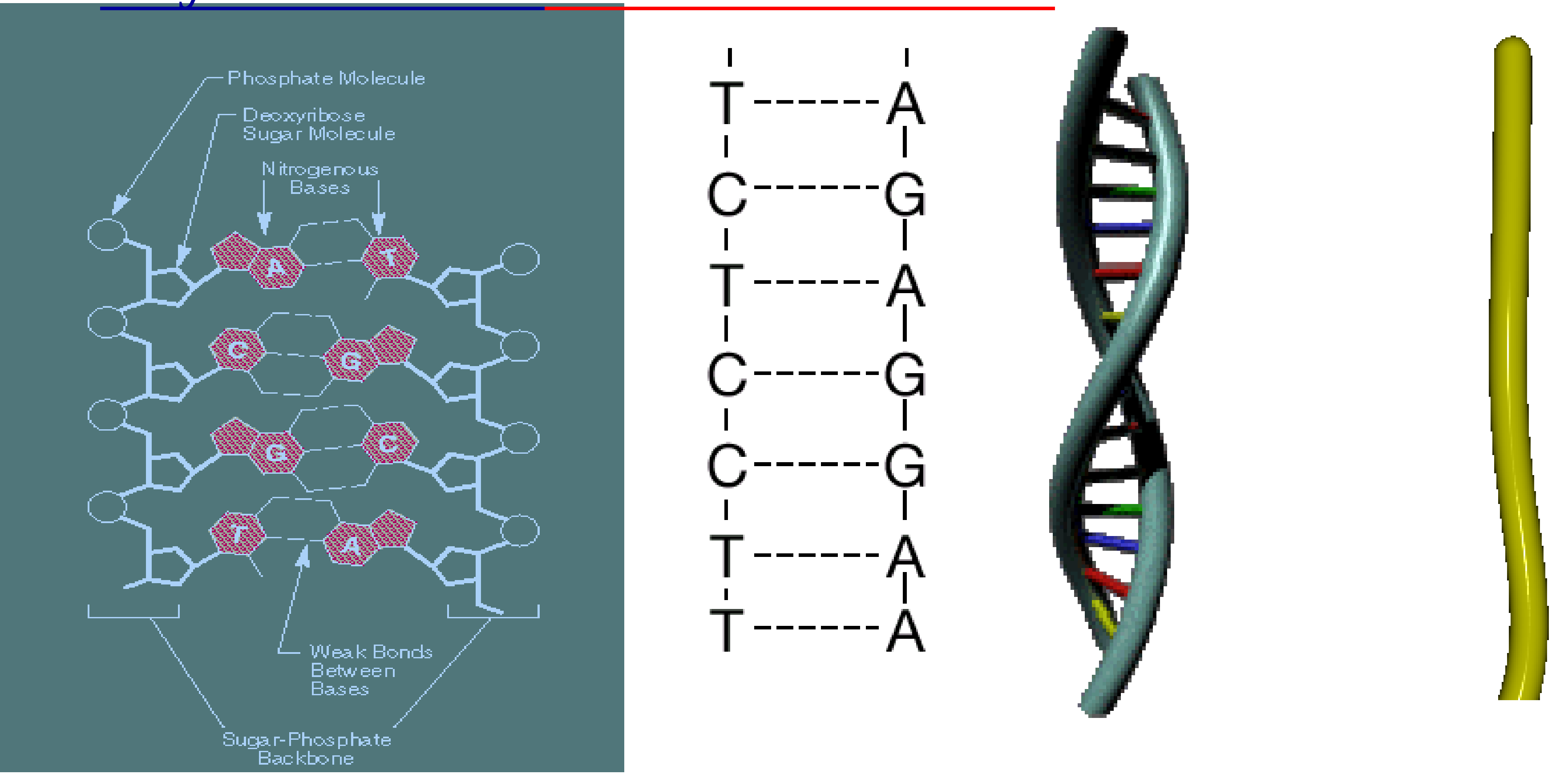






- •effect of template tension polymerase activity
- •Pausing & arrest during polymerase
- •Mechanism of polymerization kinetics
- •Tuning rate of DNA replication with external stresses

Physicist's view of the DNA chain

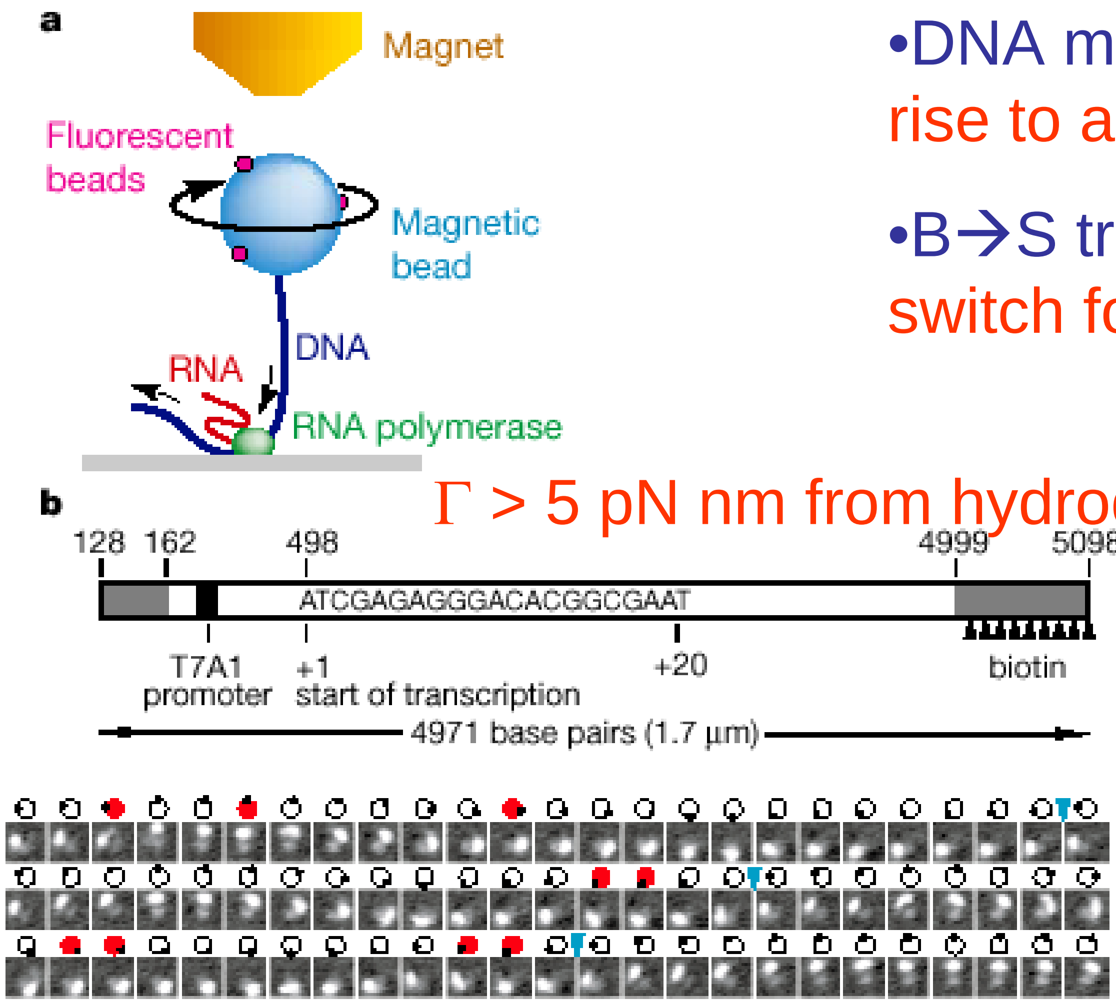


Double helix stabilized by H-bonds (bp interactions)

Polymer of persistence length ~50nm under low force (<10pN):Entropic elasticity. Complicated at high forces: cooperative behavior

Elasticity of dsDNA affect its structure and can influence the biological functions

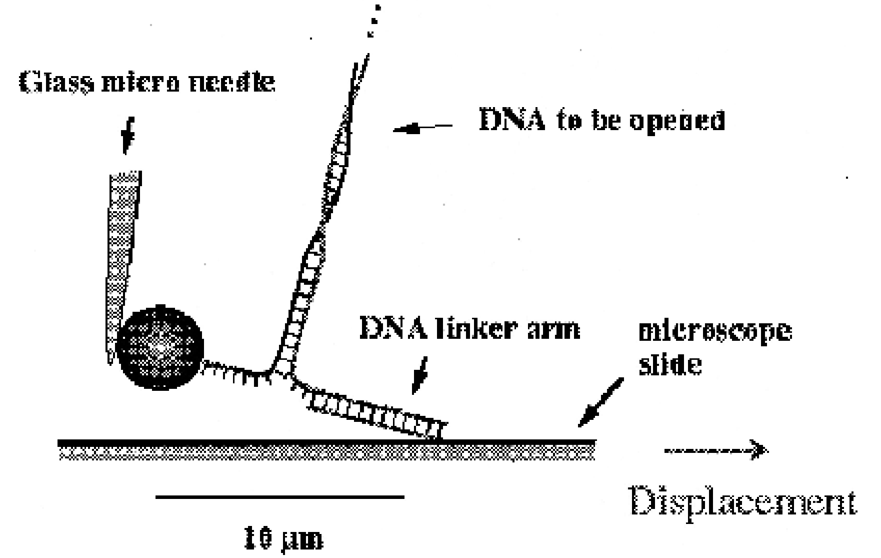
Direct observation of DNA rotation during transcription by Escherichia coli RNA polymerase Harada et al., Nature **409** , 113 (2001)



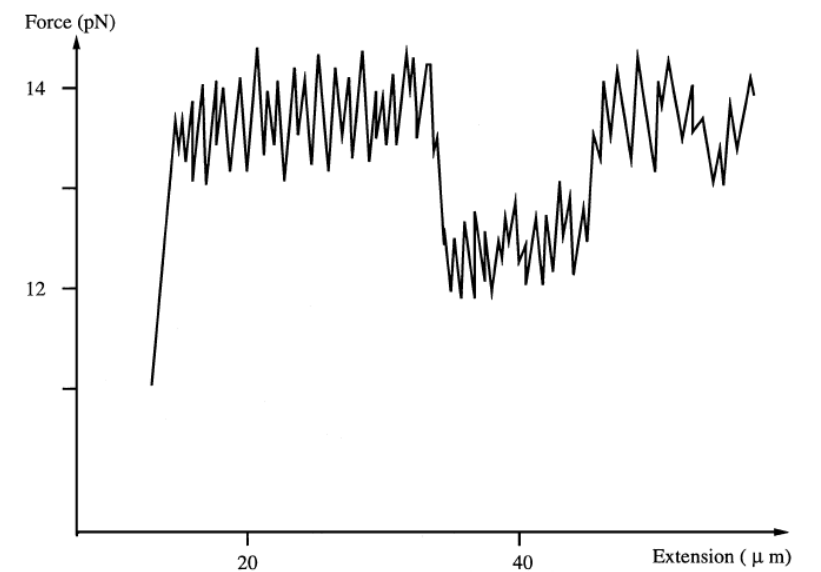
- DNA motor: untwisting gives rise to a torque
- B→S transition provides a switch for such a motor.

$\Gamma > 5$ pN nm from hydrodynamic drag estimate

Unzipping DNA

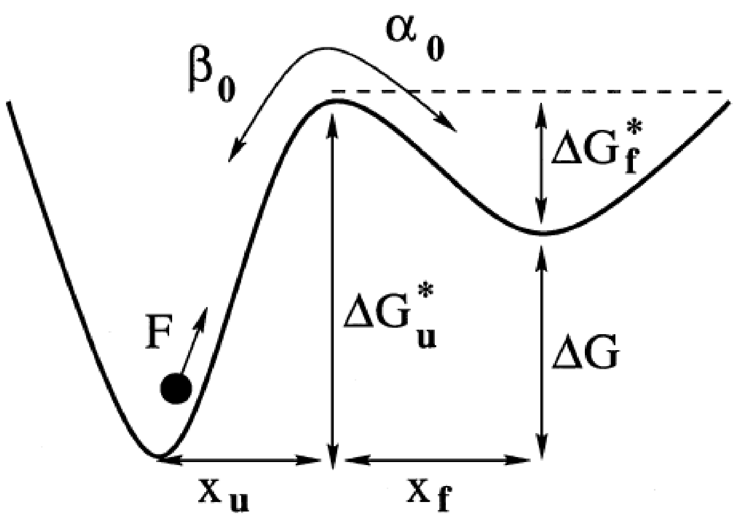


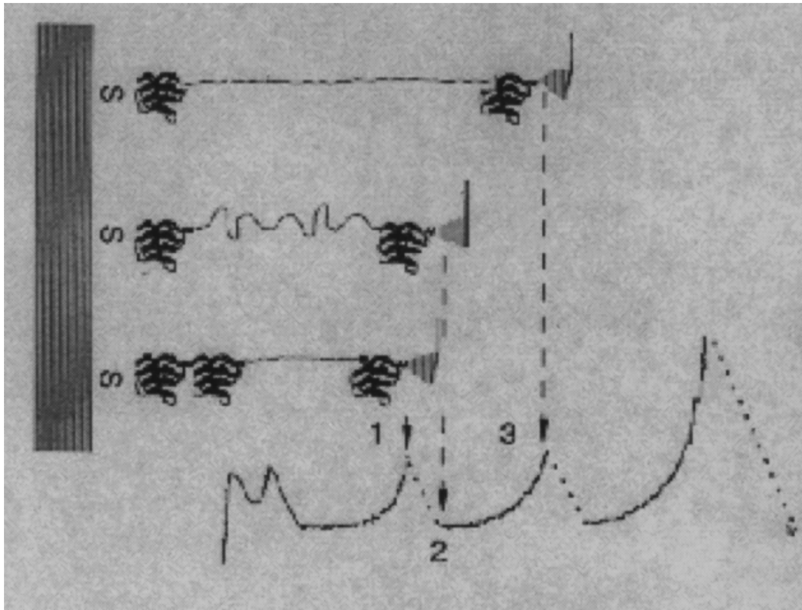
- Measure force to unpair two bases
- Stick-slip response
- Prototype for DNA sequencing, need higher resoultion
- Complexed with protein can make filament stiffer→higher sensitivity



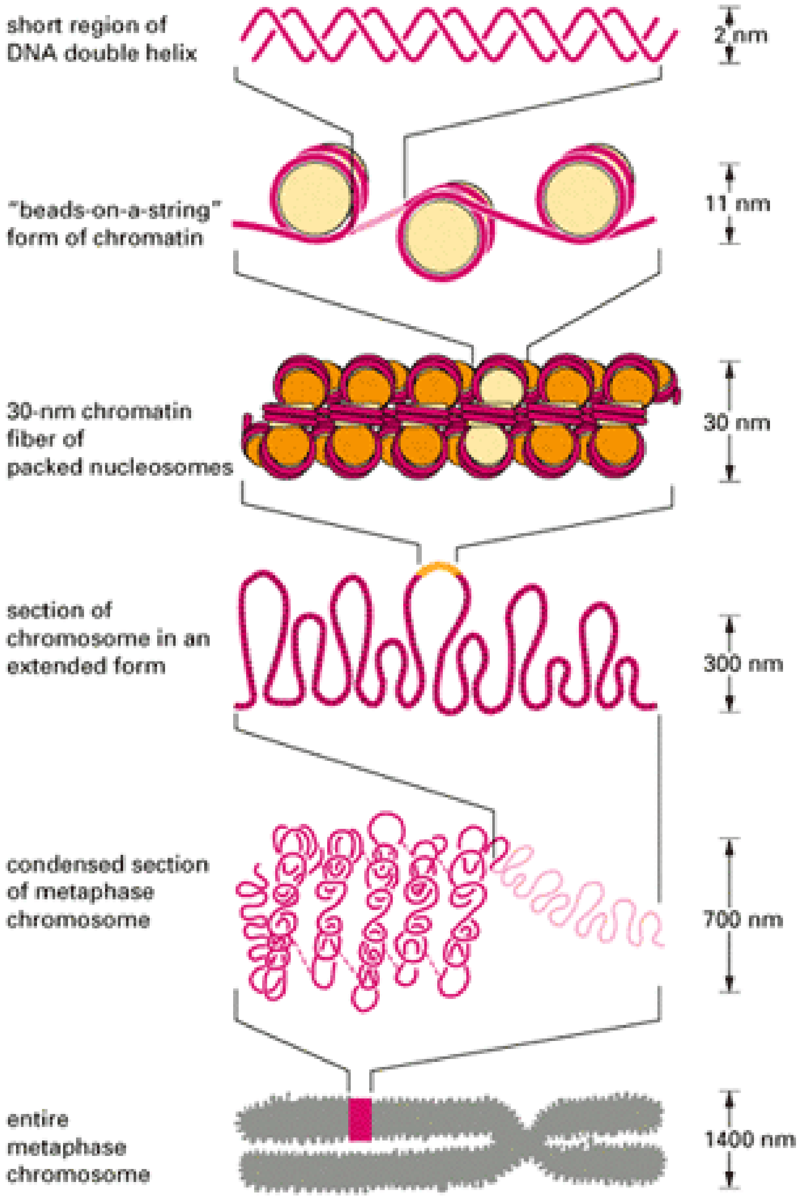
Stretching Proteins

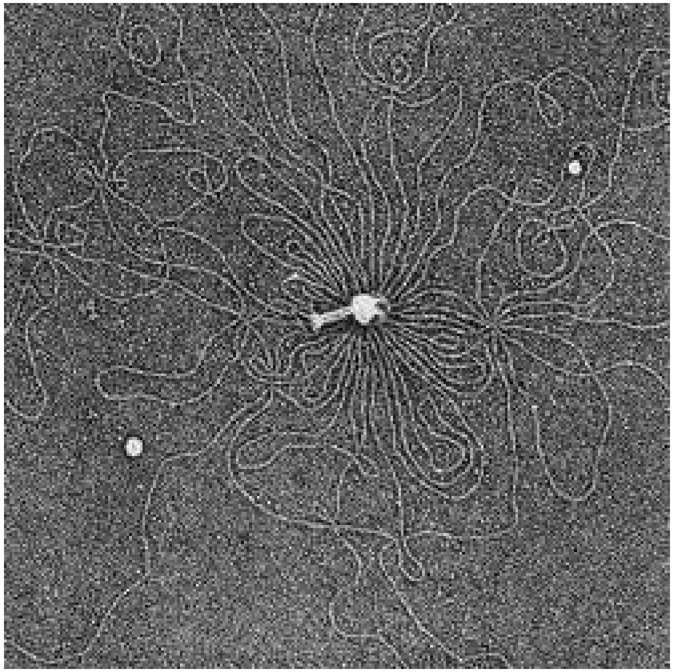
•undergo independent folding/unfolding transitions as the polypeptide is stretched.
•display a typical sawtooth pattern, due to the coexistence in the stretched protein of folded and unfolded domains.
•Pulling rate dependent: ~20pN to unfolding titin at 60nm/s (optical tweezers); ~150pN at 1000nm/s (AFM)
•Two-level model.





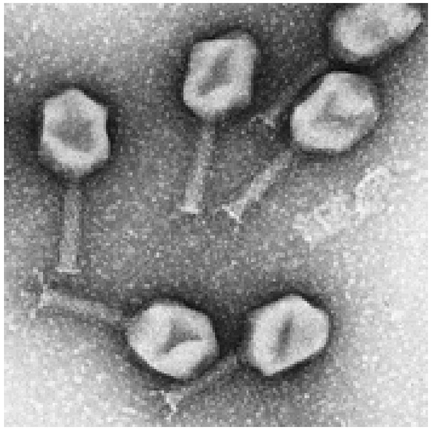
DNA condensation & packing

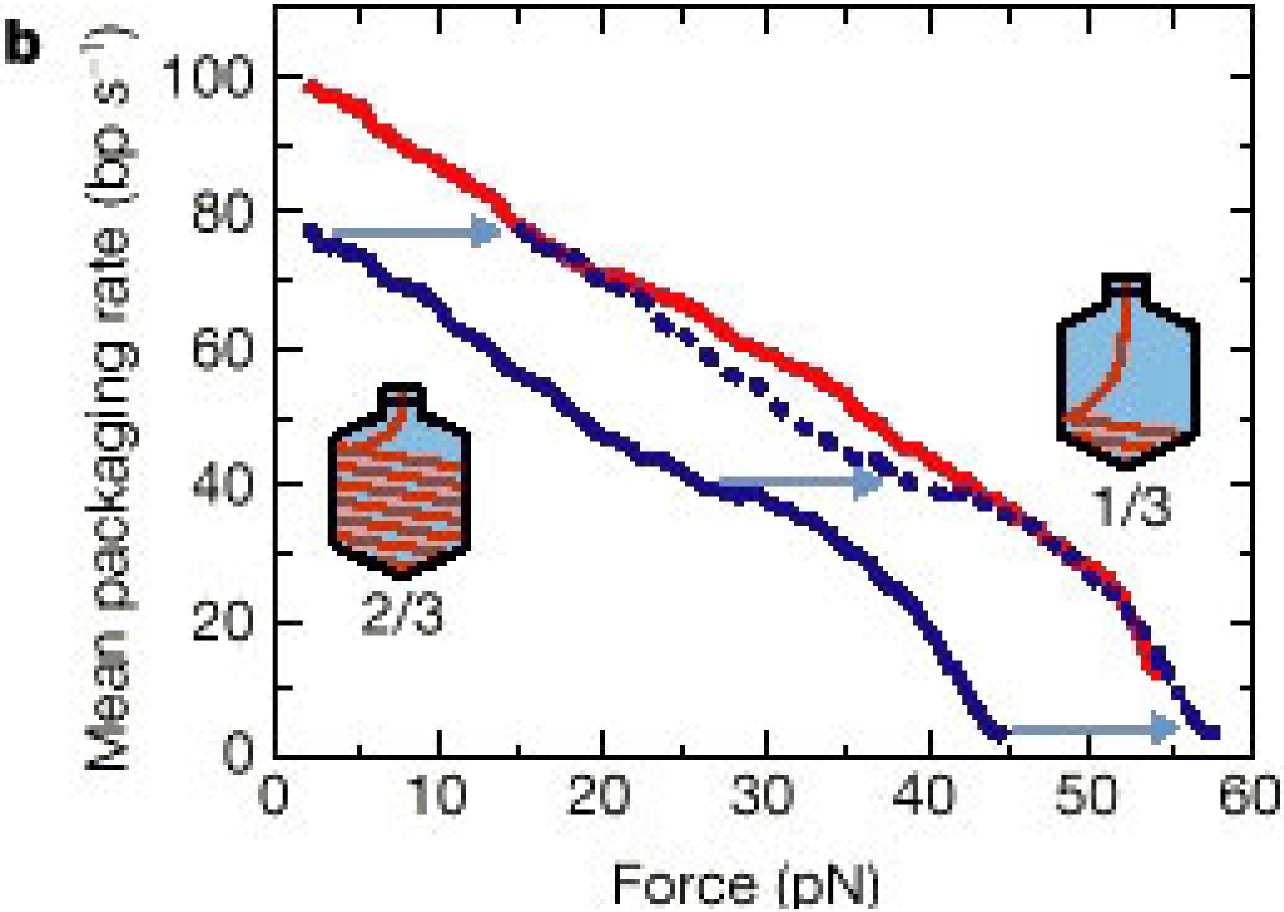




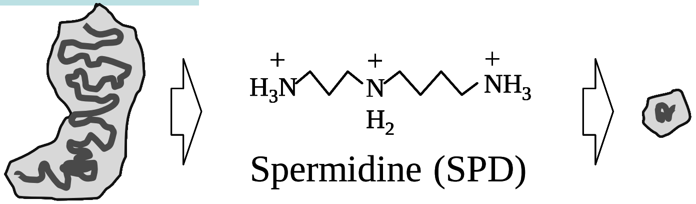
Complex competition of DNA elasticity, charge interactions, volume interactions, solvent effects…..

Packing of DNA/RNA in virus

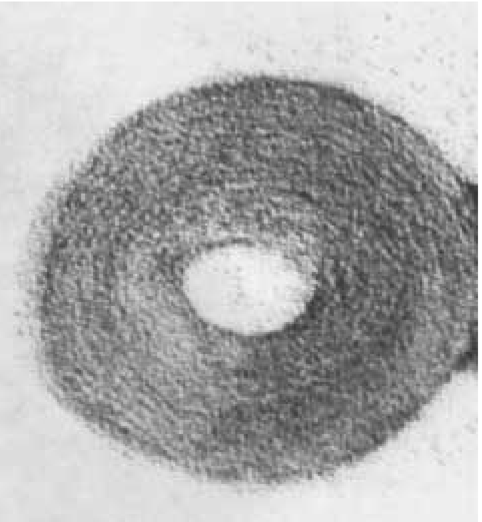




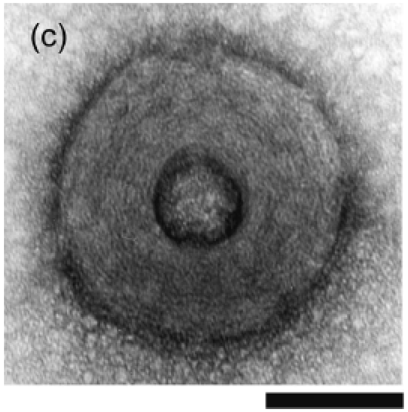
DNA condensation



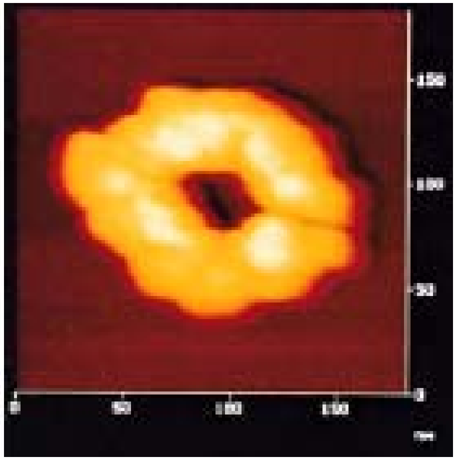
- DNA condensed by multi-valent ions:spermidine[3+], spermine [4+]
- Condensed structures: toroidal, racquet,..

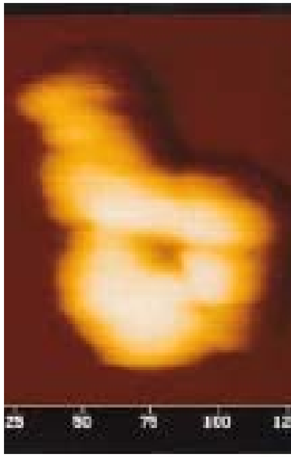


Electron micrograph of condensed ●-DNA Phys. Today (Sept. 2000))



Electron micrograph of condensed T4-DNA in 6mM SPD (Langmuir 15, 4085 (99))





AFM image of condensed ●-DNA in SPD (Nucl. Acid. Res. 26, 3228 (98)))

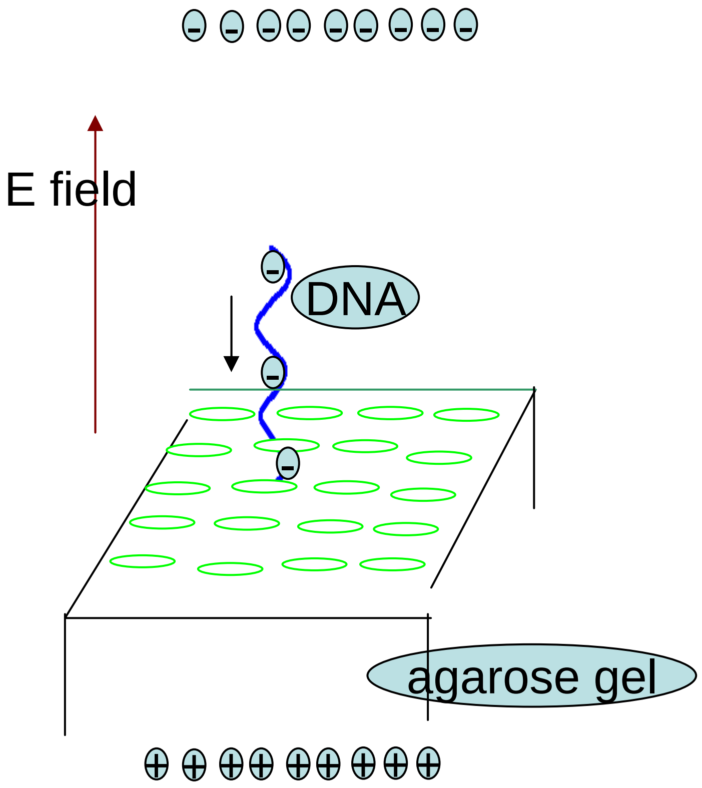
DNA is charged –ve

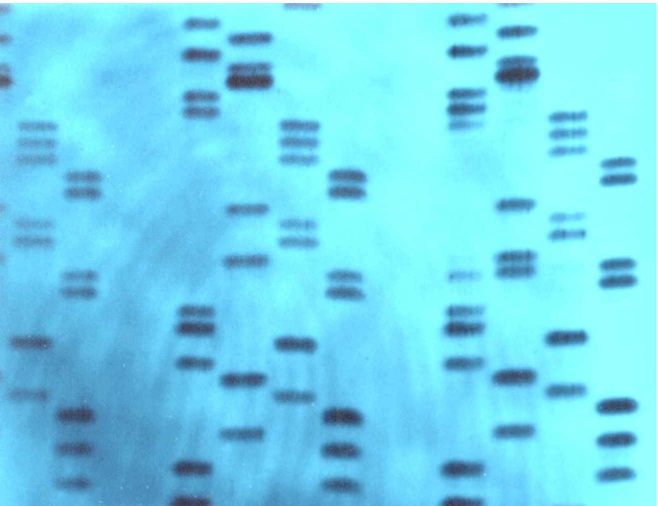
motion under external Electric field

Gel electrophoresis under DC E field

smaller pieces of DNA fragment move faster, different DNAs will be separated by size

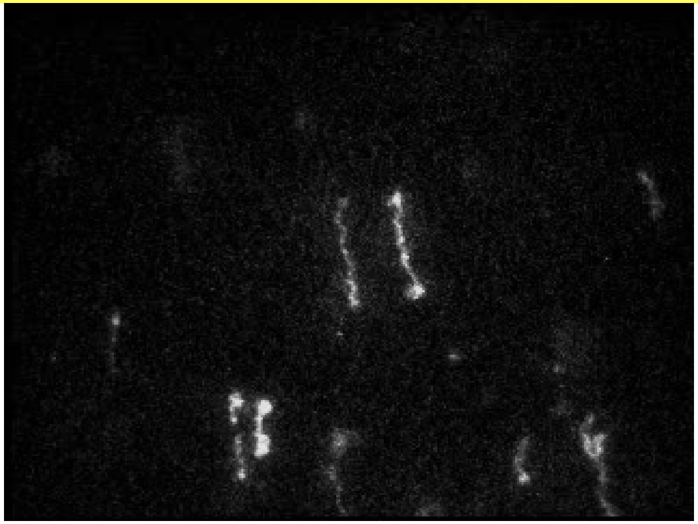
DNA fingerprint: **DNA can be cut into shorter pieces by enzymes "restriction endonucleases".**
DNA pieces then separated by gel electrophoresis.
Each piece of DNA forms a band in gel.
The number, positions, sizes of bands→fingerprint





Controlled motion of DNA: external drives

In gel
under DC E field



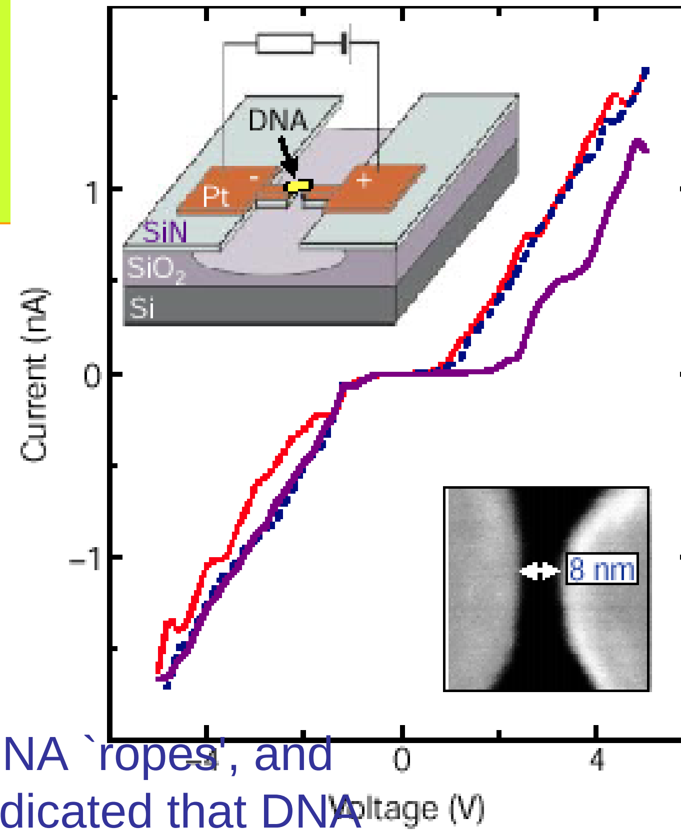
DNA ratchet motion under AC electric field

Electrical properties of Single Bio-molecules

- Electronic excitations and motion of electric charges are well known to play a significant role in a wide range of bio-macromolecules
- Electron transfer involving the DNA double helix is thought to be important in radiation damage and repair and in biosynthesis
- the double helix may mediate charge transfer between different metal complexes
- DNA can be viewed as a one dimensional well conducting molecular wire
- Molecular electronics /devices

Direct measurement of electrical transport through DNA molecules

Porath et al. Nature 403, 635 (2000)



•Electrical transport measurements on micrometer-long DNA `ropes', and also on large numbers of DNA molecules in films, have indicated that DNA behaves as a good linear conductor.

•10.4nm-long, (30bps) double-stranded poly(G)-poly(C) DNA molecules connected to two metal nano-electrodes

•After a DNA molecule was trapped from the solution, the device was dried in a flow of nitrogen and electrical transport was measured. No current was measured between the bare electrodes before trapping

DNA as nano-materials

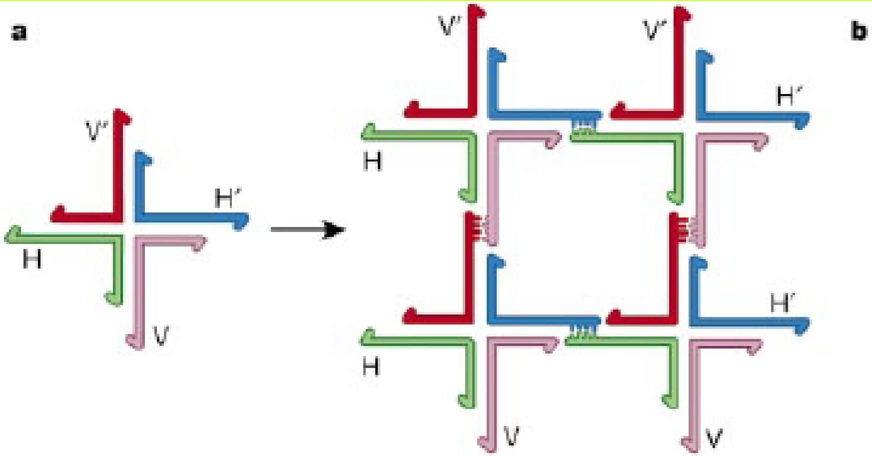
- *“The nucleic-acid ‘system’ that operates in terrestrial life is optimized (through evolution) chemistry incarnate. Why not use it ... to allow human beings to sculpt something new, perhaps beautiful, perhaps useful, certainly unnatural.”* Roald Hoffmann, writing in *American Scientist*, 1994
- powerful molecular recognition system can be used in nanotechnology to direct the assembly of highly structured materials with specific nanoscale features,

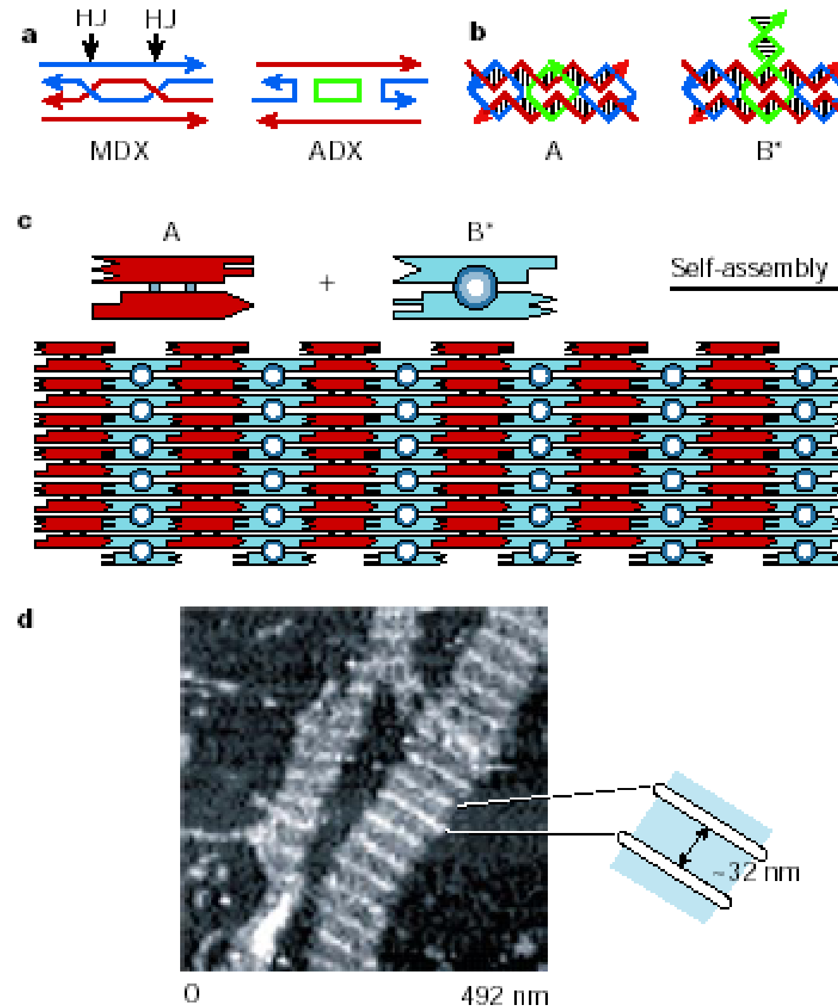
Branched DNA

- To produce interesting materials from DNA, synthesis is required in multiple dimensions branched DNA is required. Branched DNA occurs naturally in living systems, as ephemeral intermediates formed when chromosomes exchange information during meiosis, the type of cell division that generates the sex cells (eggs and sperm). Prior to cell division, homologous chromosomes pair, and the aligned strands of DNA break and literally cross over one another, forming structures called Holliday junctions. This exchange of adjacent sequences by homologous chromosomes — a process called recombination — during the formation of sex cells passes genetic diversity onto the next generation.
- The Holliday junction contains four DNA strands (each member of a pair of aligned homologous chromosomes is composed of two DNA strands) bound together to form four double-helical arms flanking a branch point. The branch point can relocate throughout the molecule, by virtue of the homologous sequences. In contrast, synthetic DNA complexes can be designed to have fixed branch points containing between three and at least eight arms.
- Other modes of nucleic acid interaction aside from sticky ends available. For example, Tecto-RNA molecules, held together loop–loop interactions, or paranemic crossover (PX) DNA, cohesion derives from pairing of alternate half turns in inter-wrapped double helices. These new binding modes represent programmable cohesive interactions between cyclic single-stranded molecules do not require cleavage to expose bases to pair molecules together.

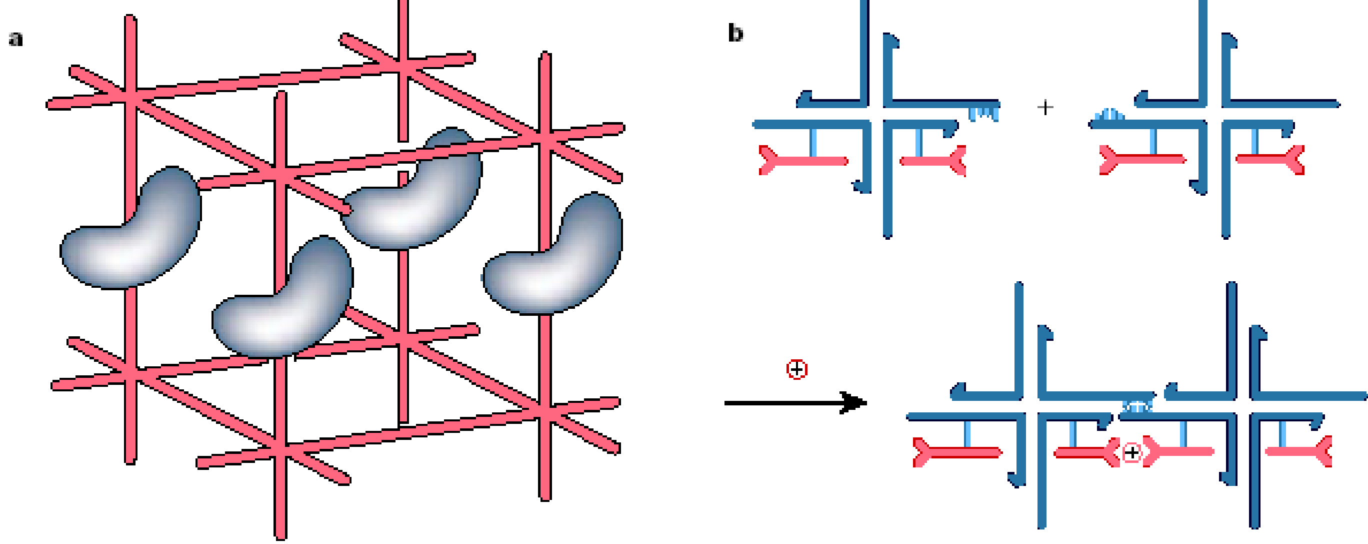
Branched DNA

In DNA replication: Holliday junction





Assembly of branched DNA molecules.
Self-assembly of branched DNA molecules into a two-dimensional crystal.



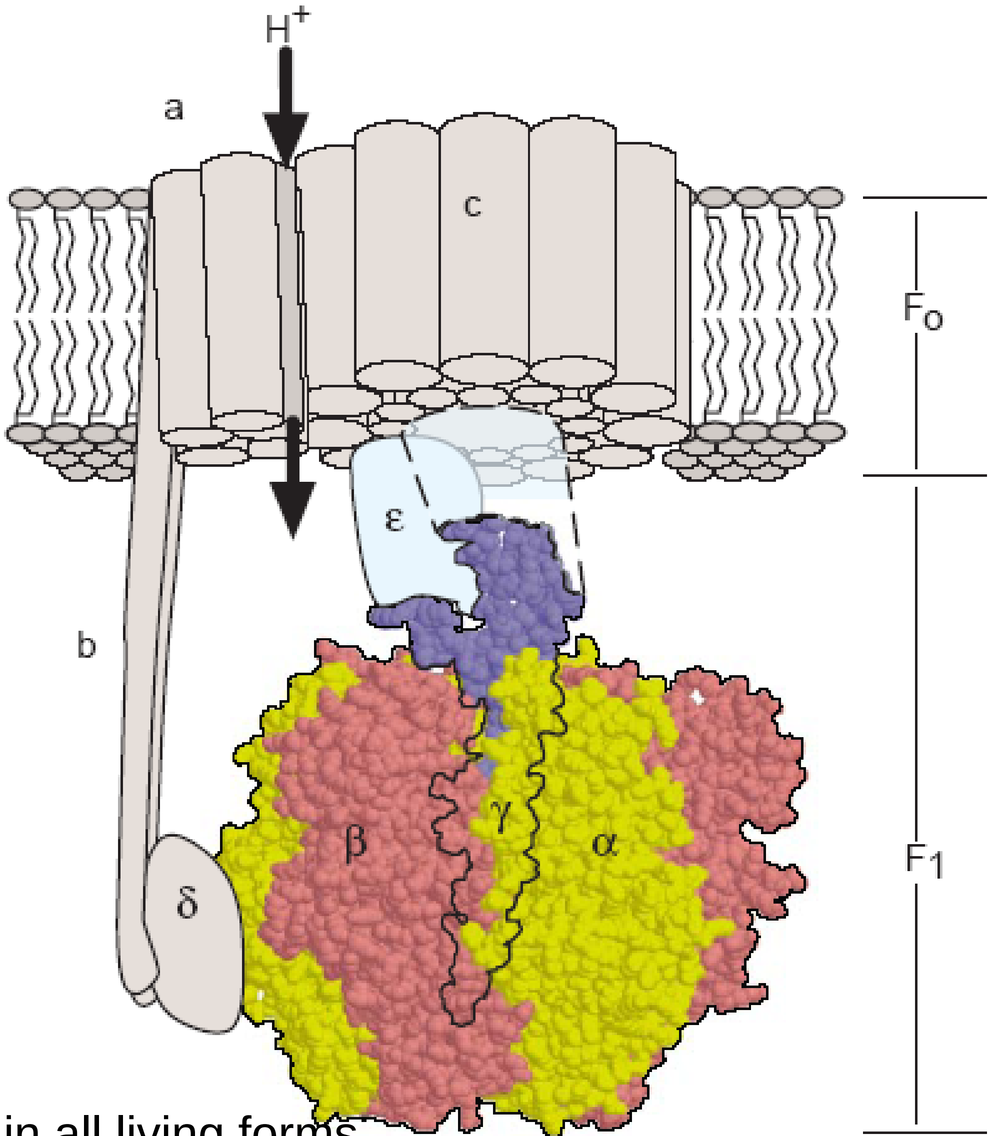
•Applications of 3D DNA scaffolds.

•**a**, Scaffolding of biological macromolecules. A DNA box (red) is shown with protruding sticky ends that are used to organize boxes into crystals. Macromolecules are organized parallel to each other within the box, rendering them amenable to structure determination by X-ray crystallography.

•**b**, DNA scaffolds to direct the assembly of nanoscale electrical circuits. Branched DNA junctions (blue) direct the assembly of attached nanoelectronic components (red), which are stabilized by the addition of a positively charged ion.

Nano-machine: molecular motor

FoF1 ATPase



ATP: universal energy currency in all living forms

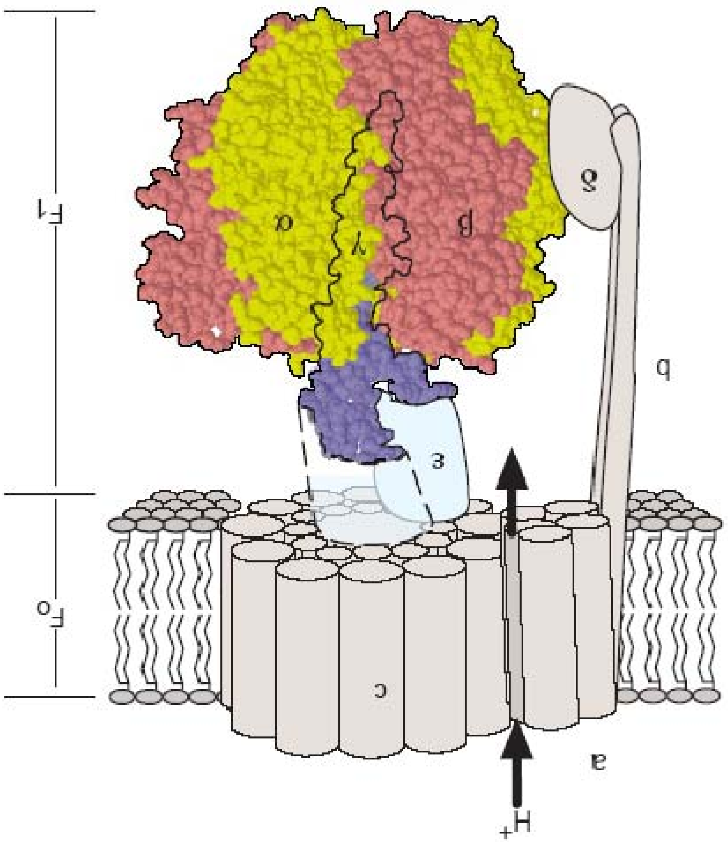
History of ATP/ATPase

- **In 1929, The Karl Lohmann discovered ATP ;**
- **In 1939-41, Fritz Lipmann showed that ATP is the universal carrier of chemical energy in the cell and coined the expression "energy-rich phosphate bonds". *1953;**
- **In 1948, Alexander Todd synthesized ATP chemically. *1957;**
- **1940s - 1950s, it was clarified that the bulk of ATP is formed in cell respiration in the mitochondria and photosynthesis in the chloroplasts of plants.**
- **In 1960, Efraim Racker isolated, from mitochondria, the enzyme "FoF1ATPase" .**
- **In 1961 Peter Mitchell presented the “chemiosmotic hypothesis”. *1978.**
- **60’s-70’s Boyer’s binding change mechanism for ATP-sythase. *1997**
- **1994 Walker determined the 3D structure of AP-synthase. *1997**

Synthesis of ATP

$$\mathrm{AMP} + \mathrm{ATP} \xrightarrow{\text{kinase}} 2\mathrm{ADP}$$

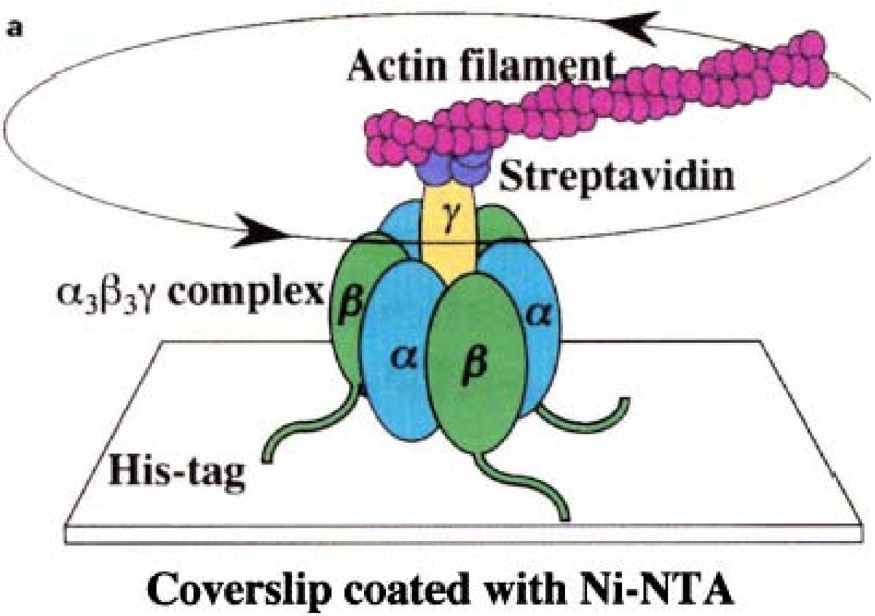
$$\mathrm{ADP} + \mathrm{P_i} \underset{}{\overset{F_1}{\rightleftharpoons}} \mathrm{ATP} + \mathrm{H_2O}$$

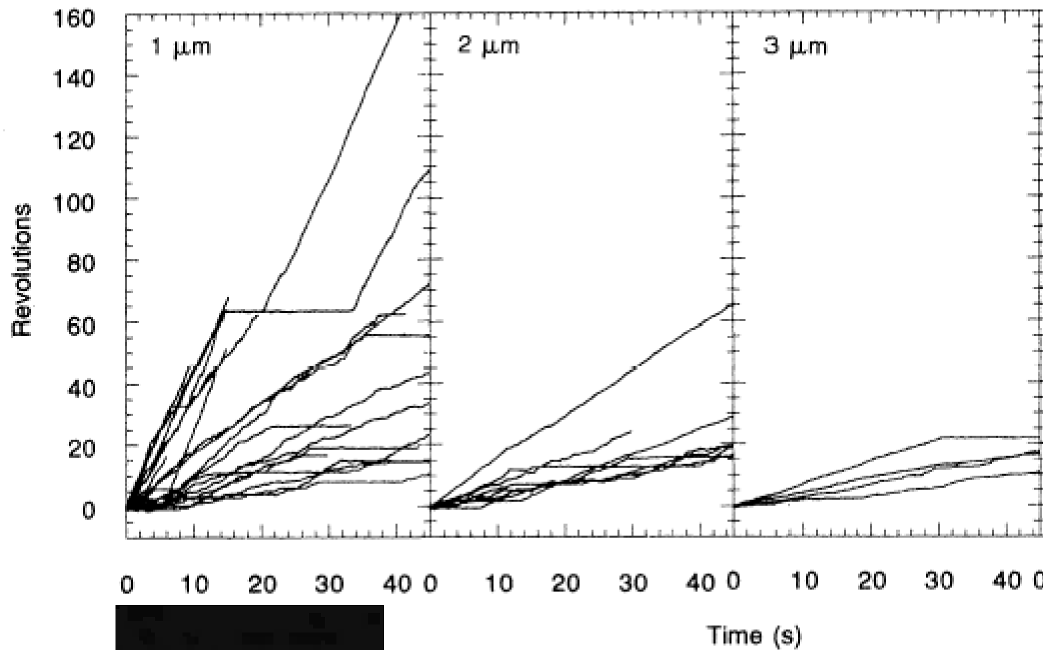


[Cartoon movie of ATP synthesis by F0F1-ATPase](#)

Junge's group

Single molecule experiments 1

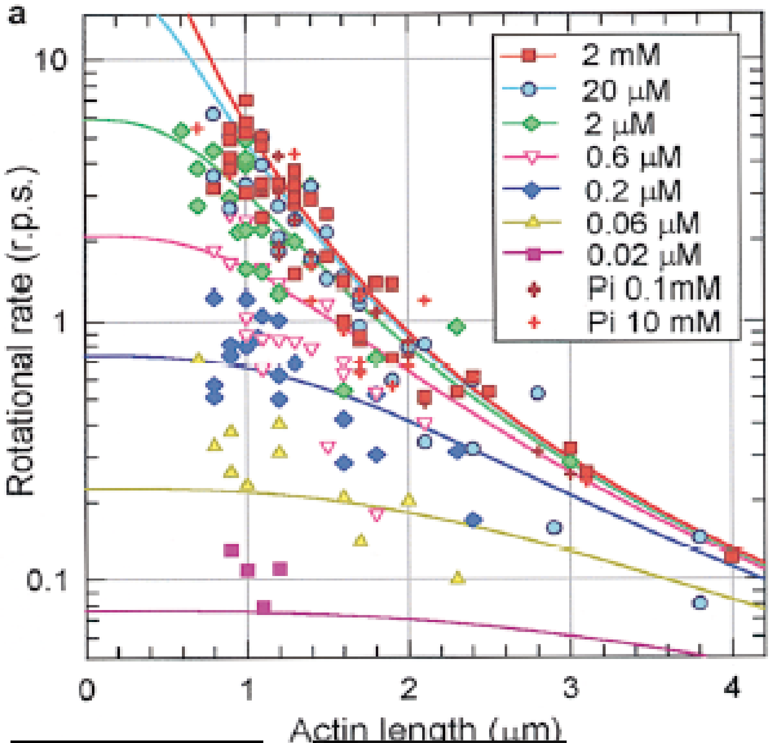


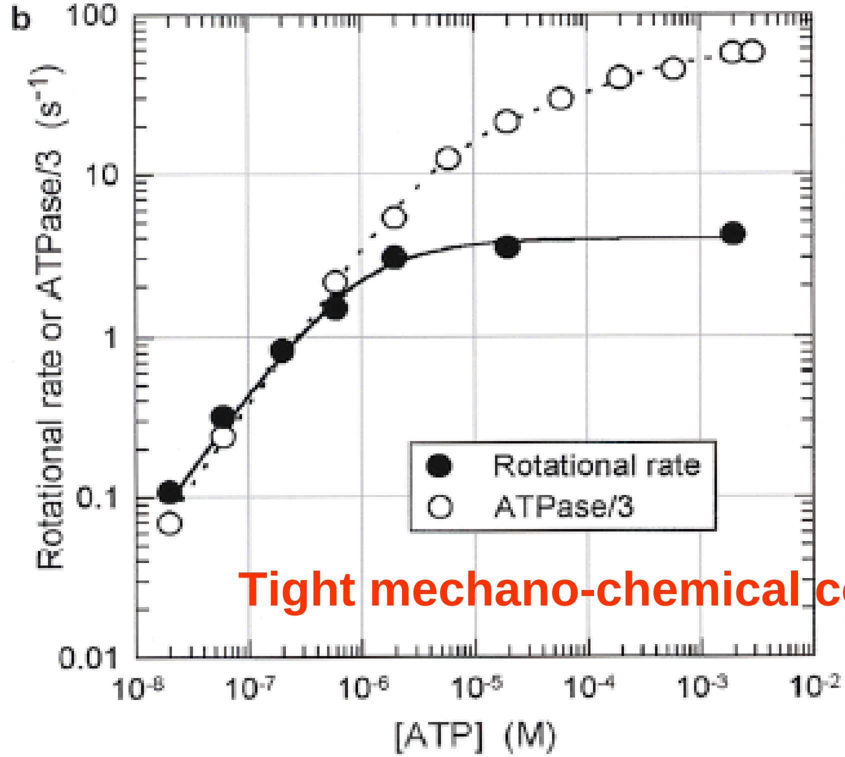


F1 ATP hydrolysis

Kinosita's group, Nature, 386, 299 (1997)

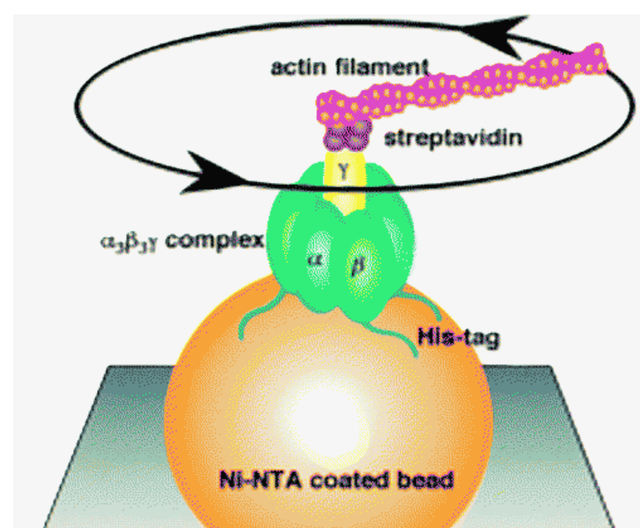
Single molecule experiments 2





Tight mechano-chemical coupling

F1 ATP hydrolysis

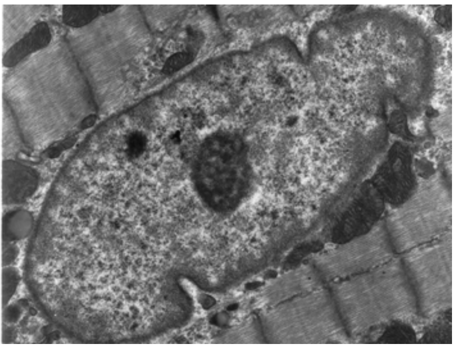


Cell 93,1117 (1998)

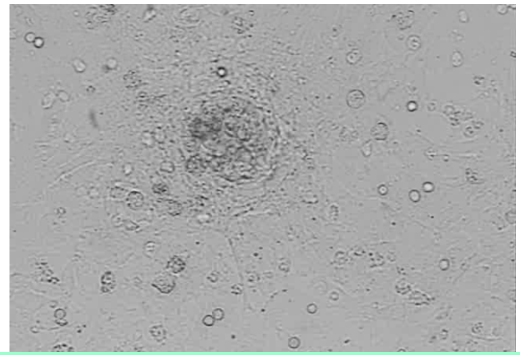
Simple to Complex: emerging properties in bio-systems

Couplings, interactions, nonlinearity, feedback…→ collective behavior, bio-functions

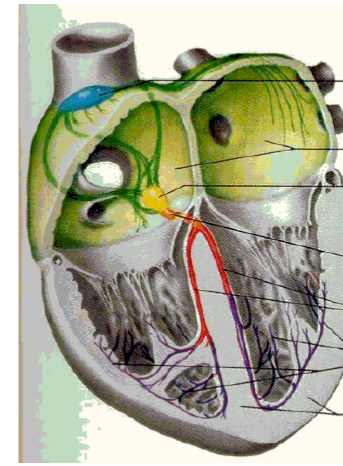
(I) cardiac cells → Heart



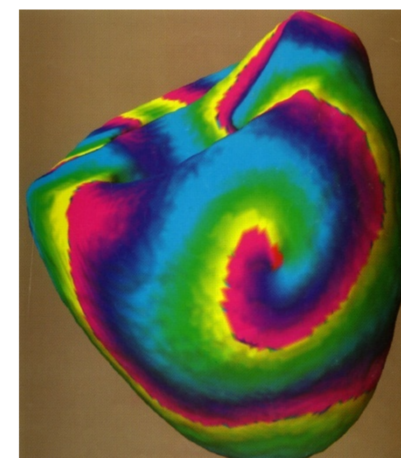
Cardiac myocyte



Synchronized beating of myocytes



spiral waves:



Coupled oscillator networks of Cardic cells:

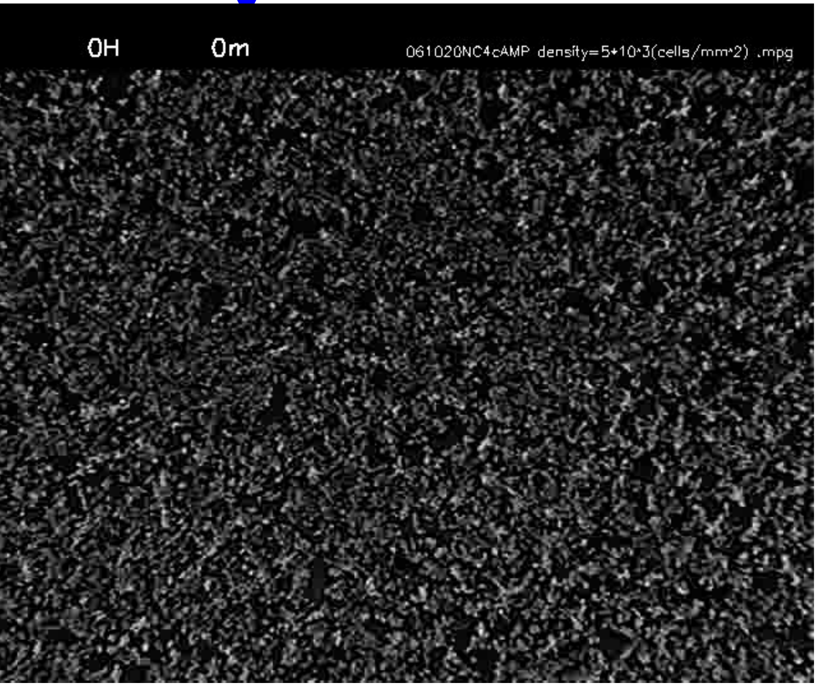
nonlinear dynamics, spiral waves, spatio-temporal patterns…

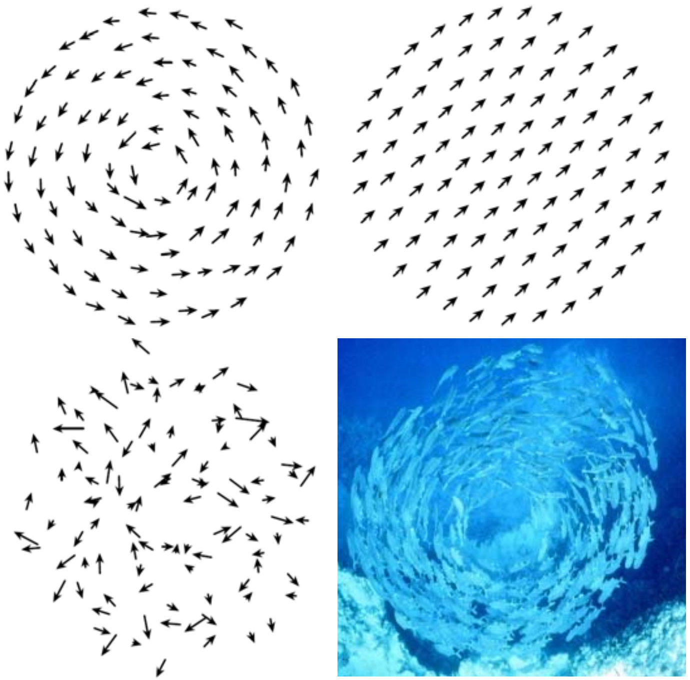
Simple to Complex: emerging properties in bio-systems

Couplings, interactions, nonlinearity, feedback…→ collective behavior

(II) Single cell/organism→collective motion

Dictyostelium discodium



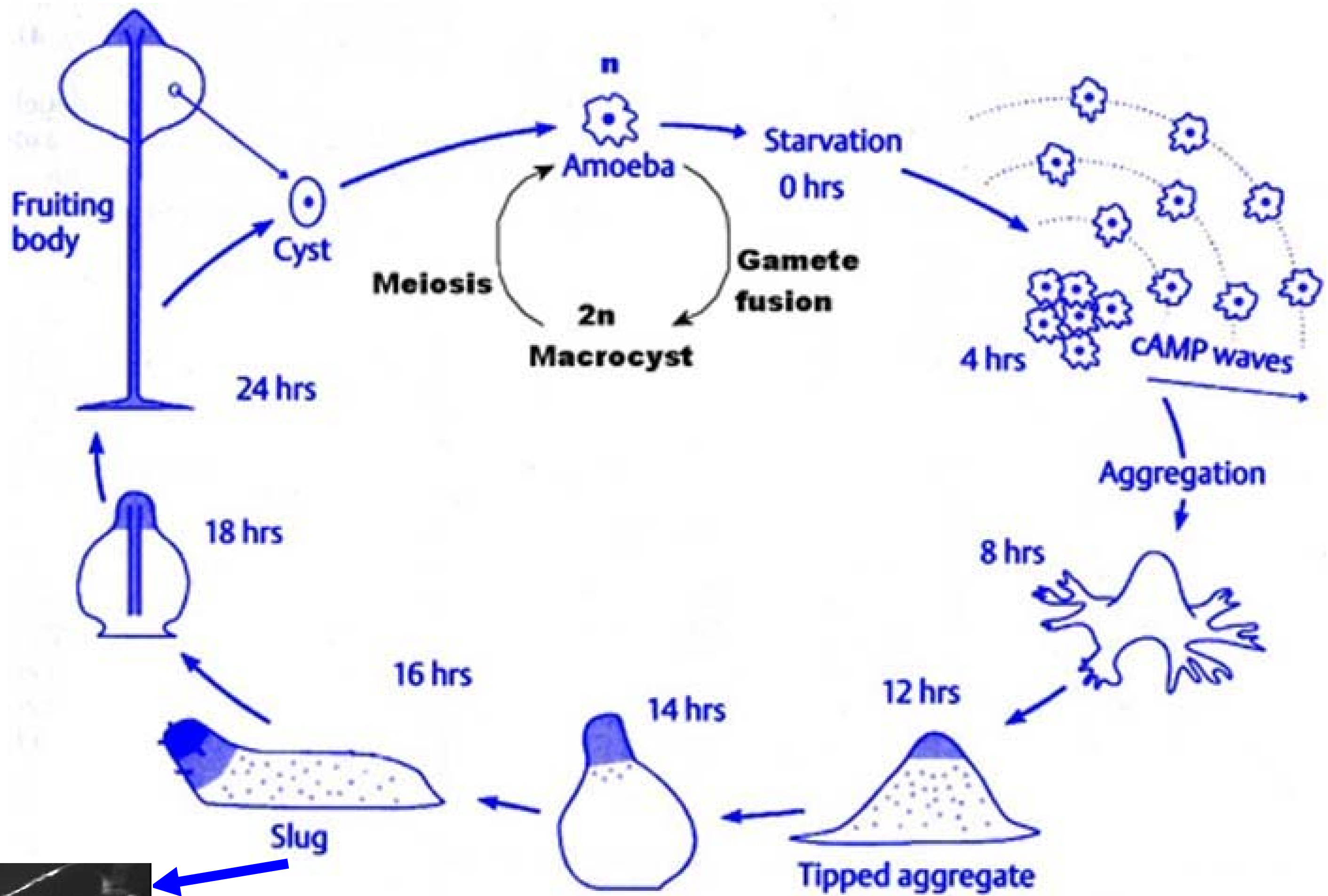


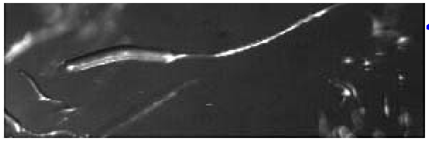
物種之群體運動理論：魚群、昆蟲、細菌之習体運動模式

Simple to Complex: emerging properties in bio-systems

Dictyostelium discodium (slime mold)

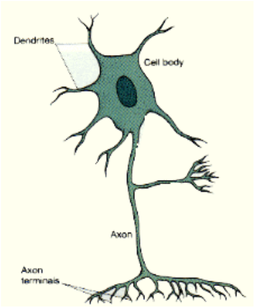
Single cell organism→ multi-cellular



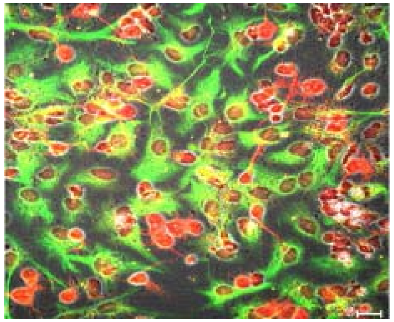


emerging properties in bio-systems

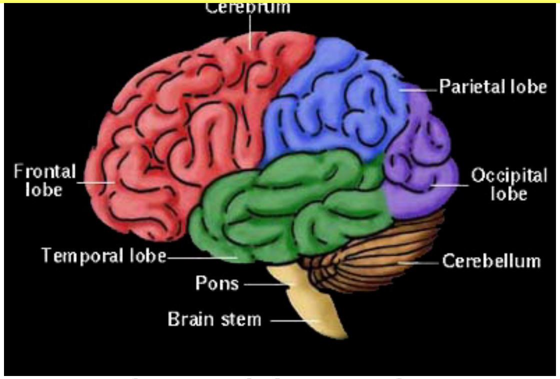
(III) Neurons → Network → Brain → Behavior



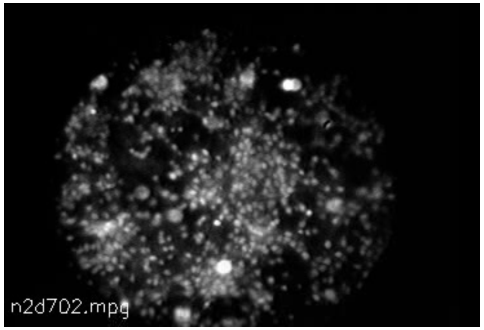
Hodgkin-Huxley Model
(1952)



Network connection: **synapses**



Neuro/cognitive science



Synchronized Firing

Complex behavior/function determined by neuron connections.

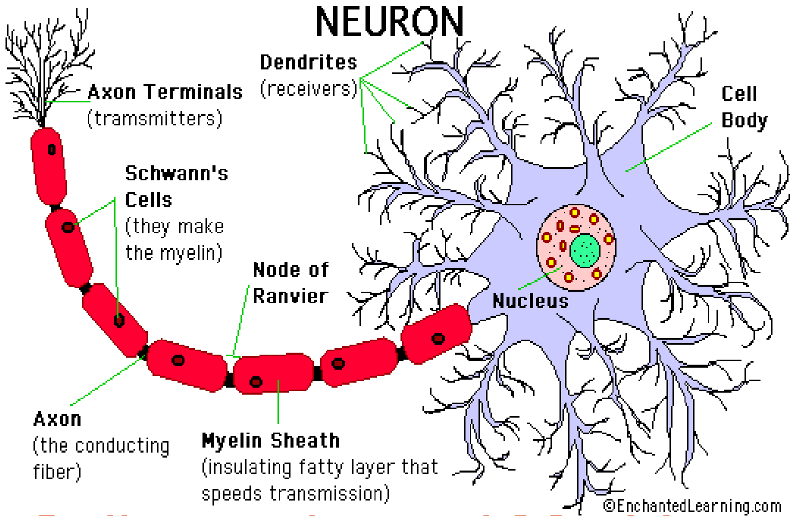
Complex neuronal Network:

•A single neuron in vertebrate cortex connects ~10000 neurons

•Mammalian brain contains > 10**11 interconnected neurons

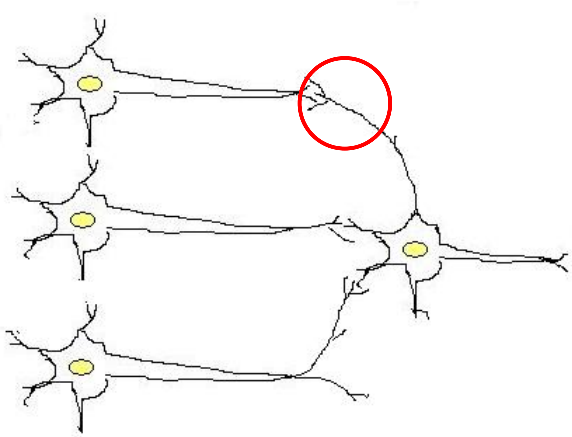
•Signal & information convey via neuronal connections—coding

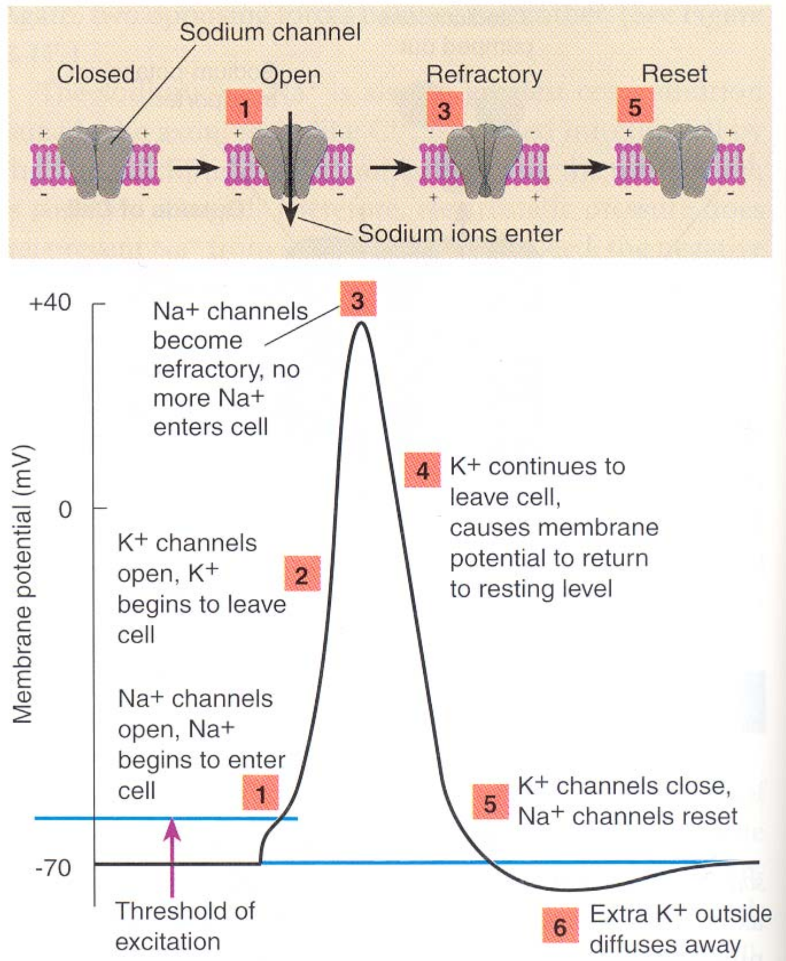
Neuron & Action Potential



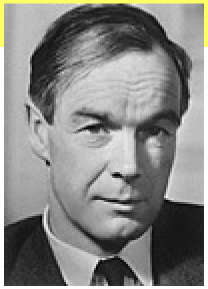
Spike: ~ 1 ms, 100mV

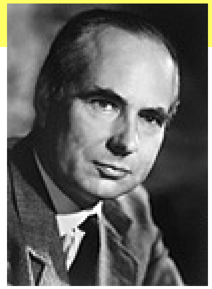
Propagates along the axon to the junction of another neuron ---synapse



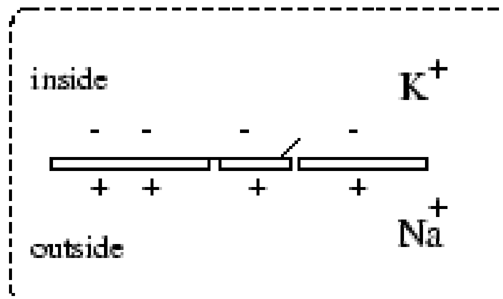


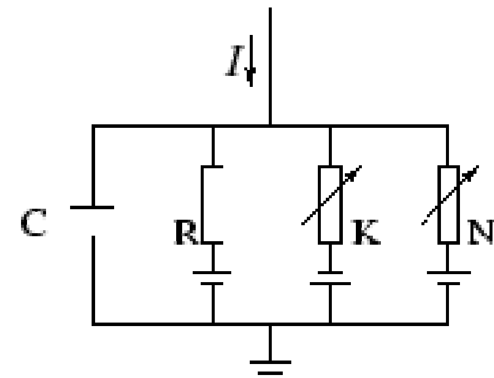
Hodgkin-Huxley model (1952)



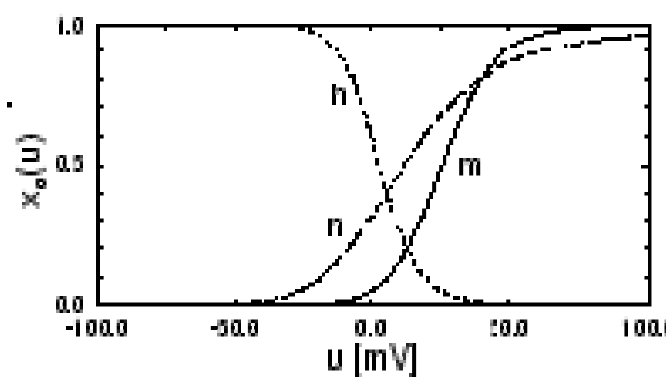


Expts. On giant axon of squid:
time & voltage dependent Na, K ion channels
+ leakage current





$$I(t) = I_C(t) + \sum_k I_k(t)$$



$$\sum_k I_k = g_{Na}\, m^3 h\, (u - E_{Na}) + g_K\, n^4\, (u - E_K) + g_L\, (u - E_L).$$

gating variables:

$$\dot{m} = \alpha_m(u)\,(1 - m) - \beta_m(u)\, m$$

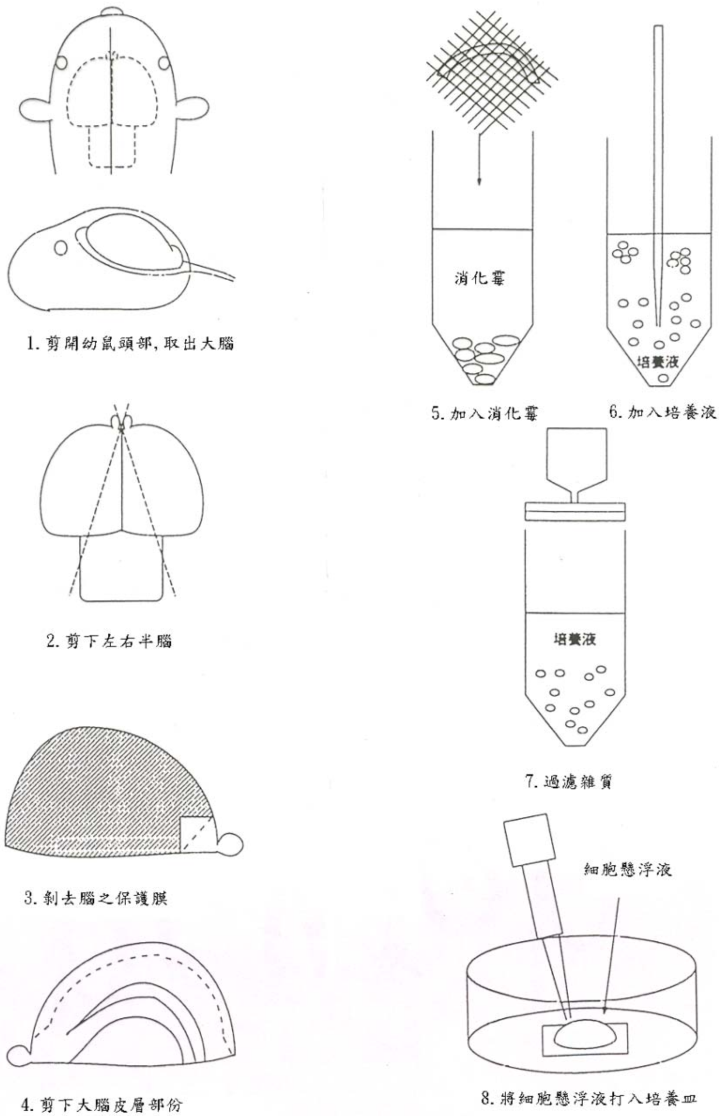
$$\dot{n} = \alpha_n(u)\,(1 - n) - \beta_n(u)\, n$$

$$\dot{h} = \alpha_h(u)\,(1 - h) - \beta_h(u)\, h$$

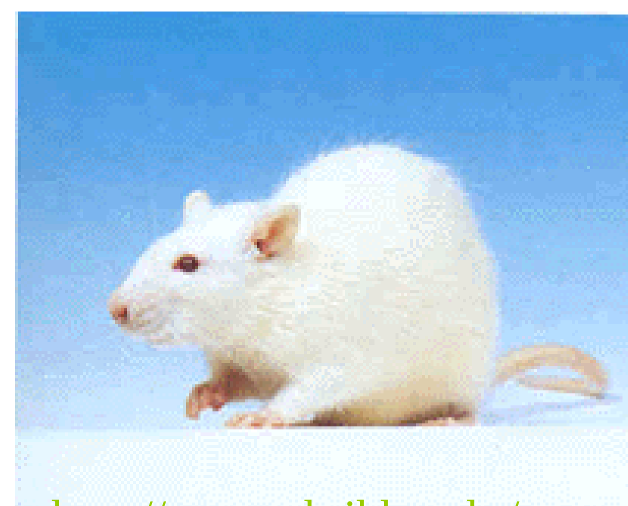
empirical functions

Experiments

Schematic procedures in preparing the sample of neuron cells from celebral cortex embryonic rats

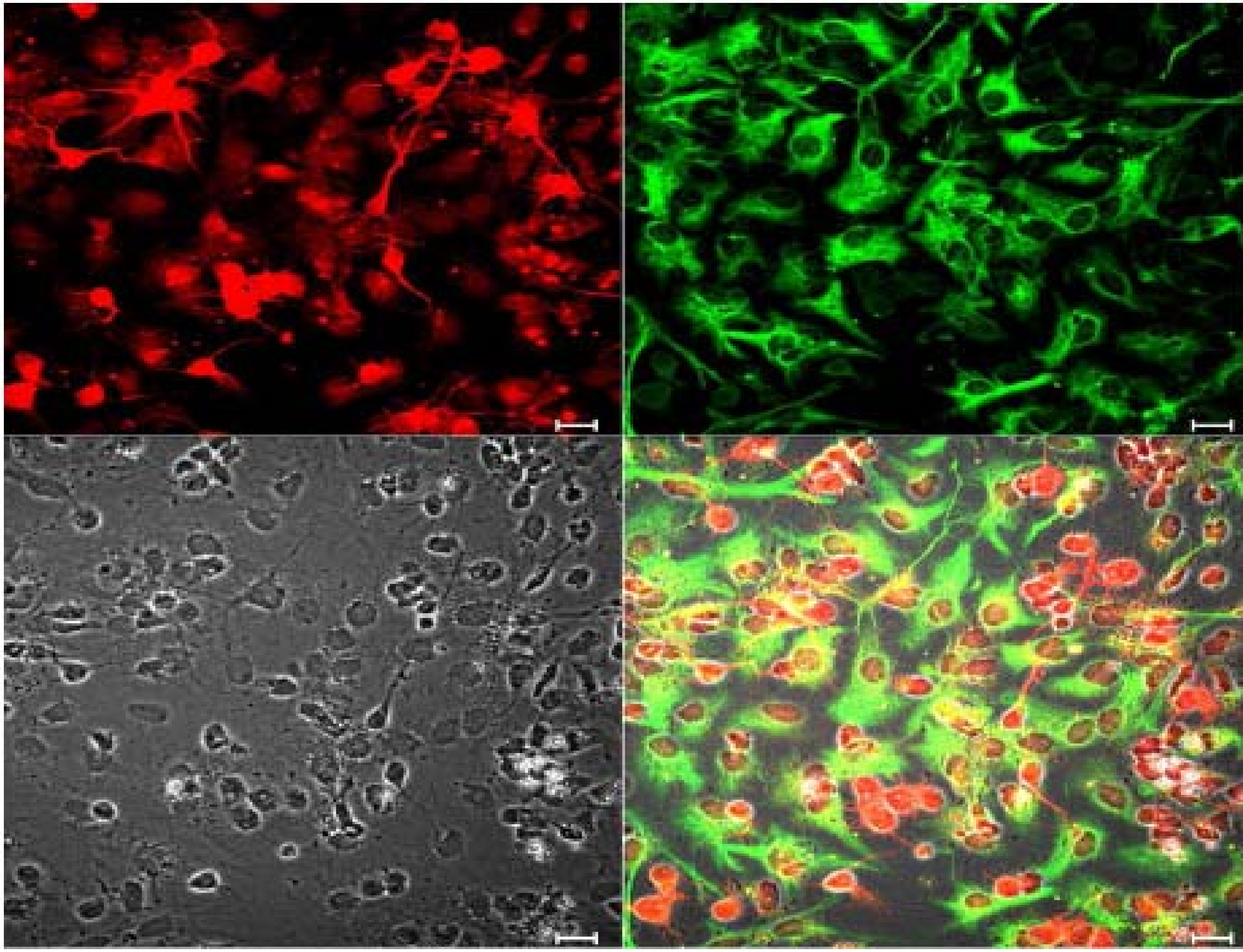


Embryos of Wistar rats
E17~E18 breeding days



http://mouse.kribb.re.kr/mousehtml/kistwistar.htm

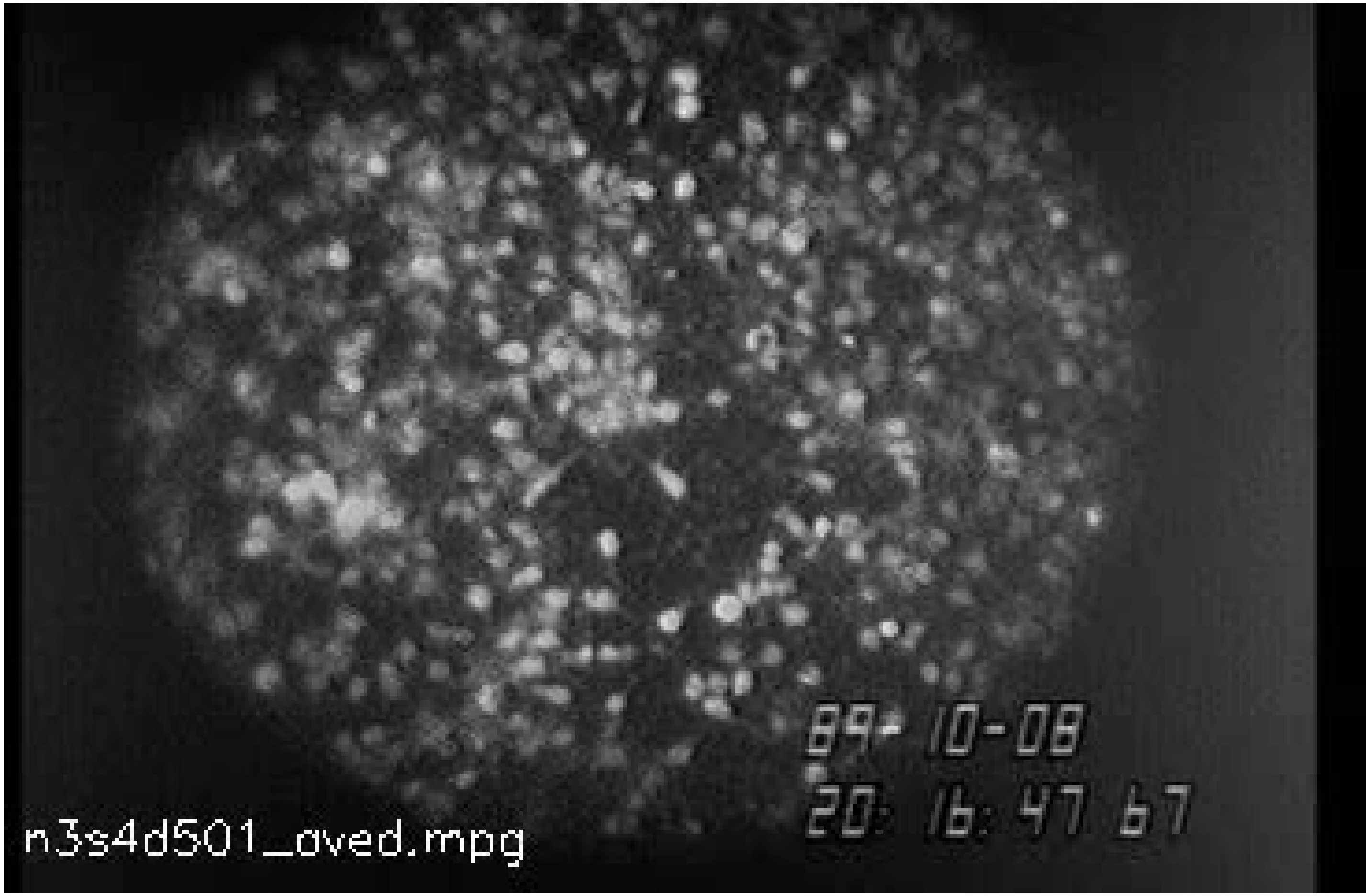
Growth of axon connection to form a network



Typical confocal microscope pictures of cultures used in our experiments.
Red: anti-MAP2 (neuronal marker); Green, anti-GFAP (glia marker). Black &white: phase contrast image; Merge of the three images above.

Optical recording of fluorescence signals from firing network

Firing of the network is monitored by the changes in intracellular [Ca 2+] which is indicated by the fluorescence probe (Oregon Green).

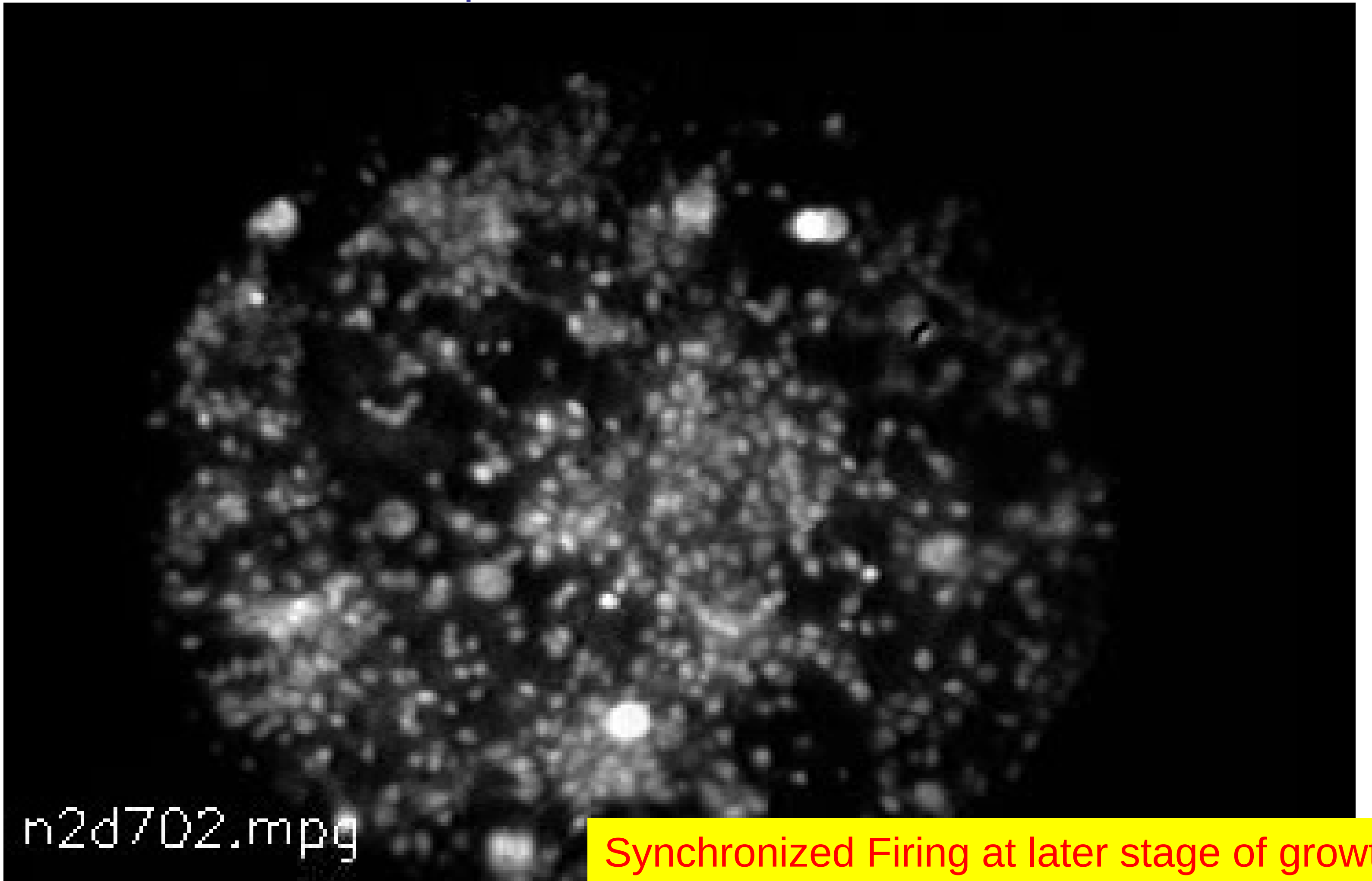


Non-synchronous Firing in early stage of growth

Synchronized Firing of Neuronal Network Culture

Spontaneous firing of the cultures are induced
by reducing [Mg2+] in the Buffered salt solution

Firing → the changes in intracellular [Ca 2+] indicated by the fluorescence probe.

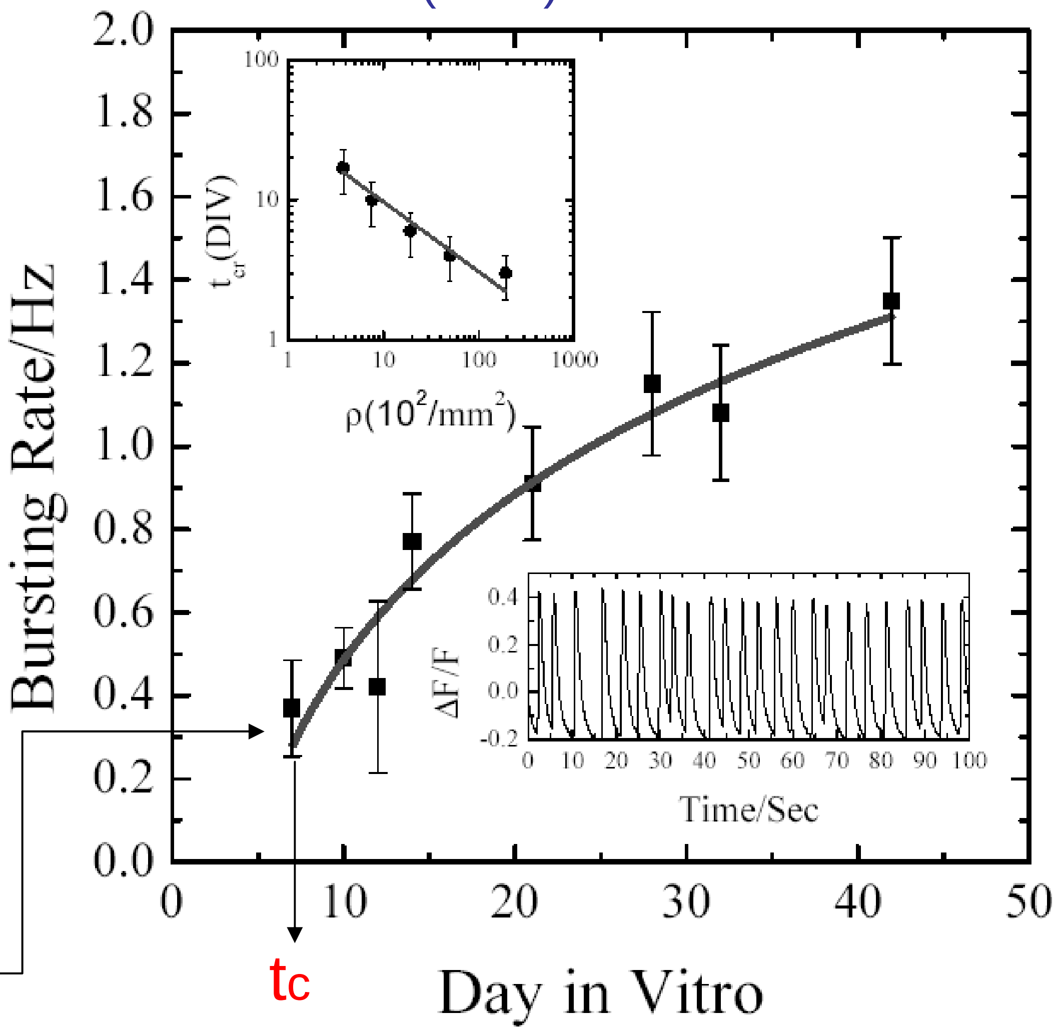


Synchronized Firing at later stage of growth

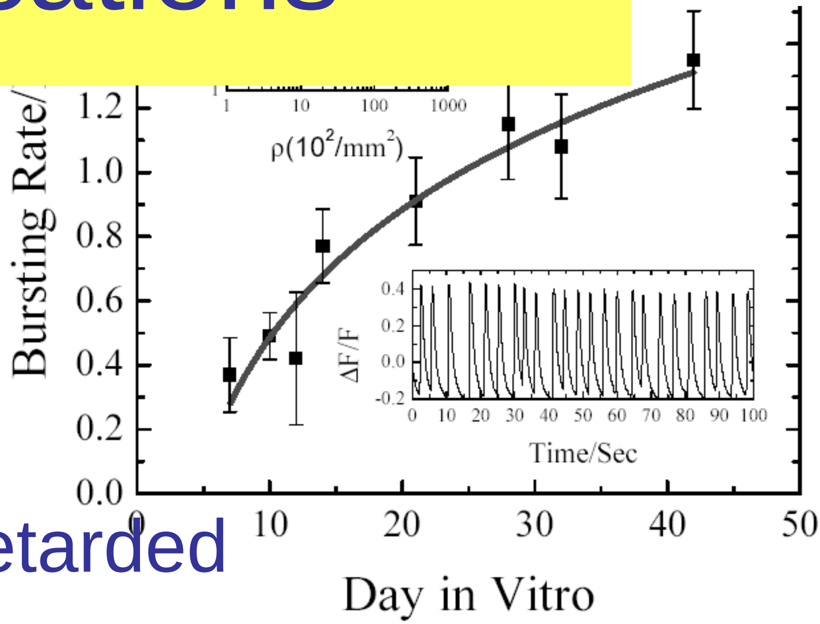
Time dependence of the SF frequency for a growing network

Phys. Rev. Lett. *93* 088101 (2004)
PRE (2006)

- Critical age for SF, tc
- SF freq. grows with time
  f=fc+fo log(t/tc)

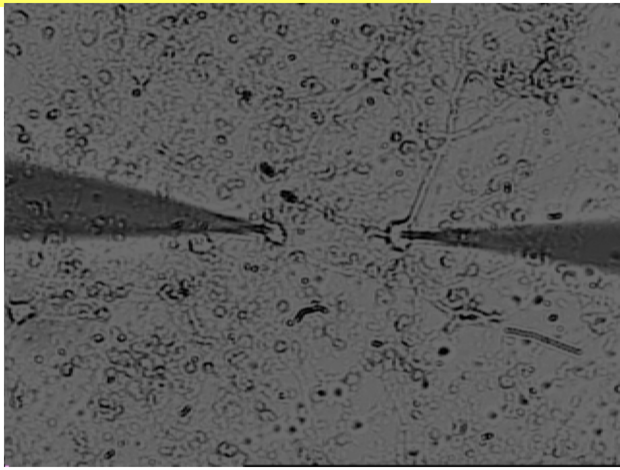


Biological implications

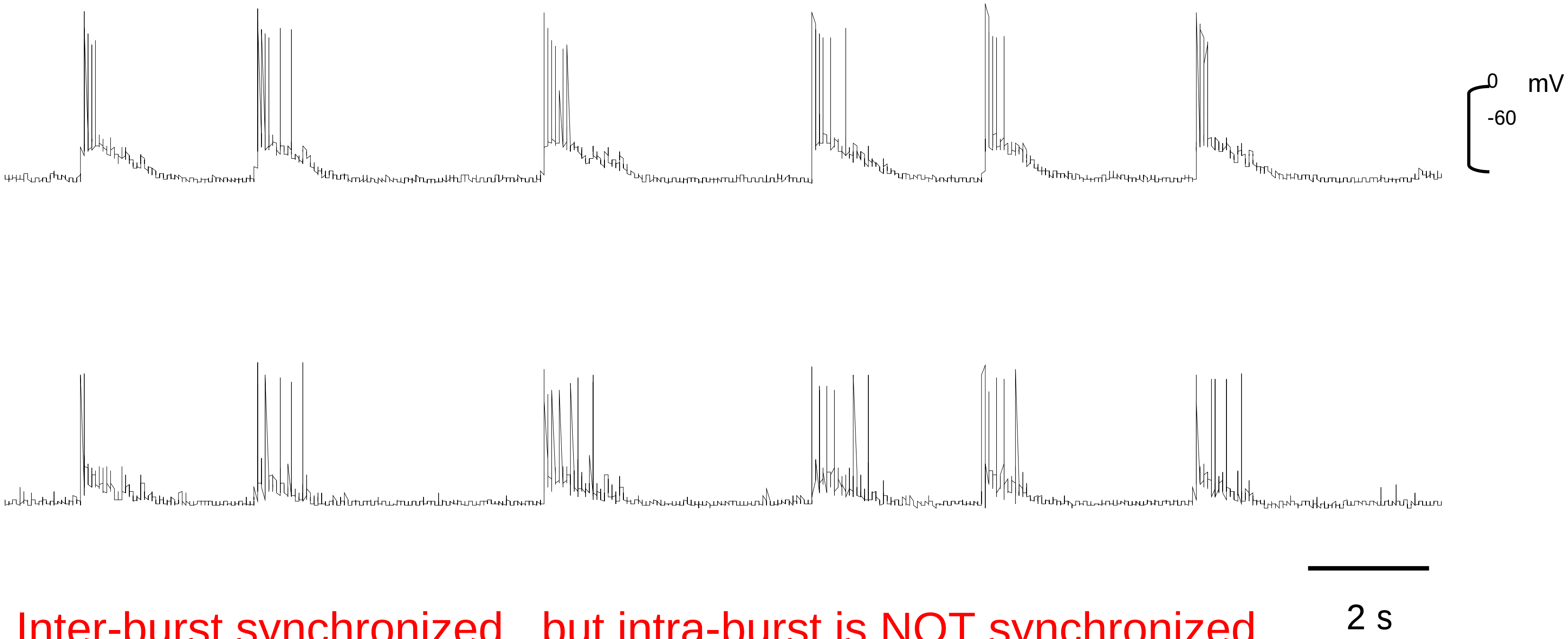


- Active growth in early stage, retarded once goal is achieved.
- Slowing down to maintain a long time span for function: homeostasis
- Continuing fast growth used up energy
- Too much connections may exceed information capacity for a single neuron

Electrophysiology measurement (whole-cell recording, current-clamp)



Glia and neuron mixed culture (8DIV, 5X10^5)

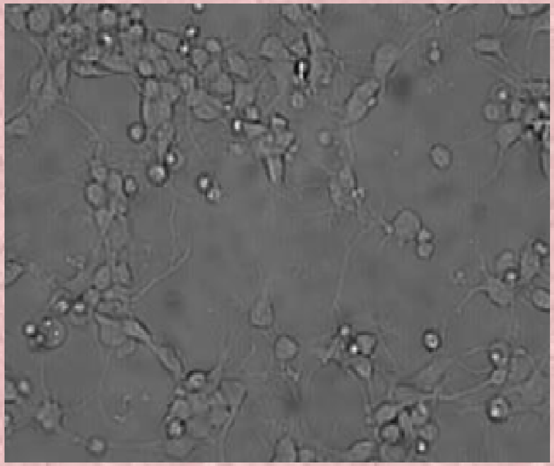


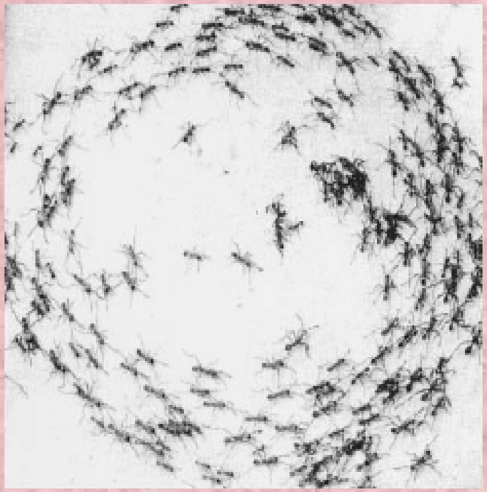
Inter-burst synchronized , but intra-burst is NOT synchronized

- **Modern BioPhysics**

a lot of interesting and unexplored science

from molecules to collective behavior in organisms





The End

Thank you for attention