• What Physicists can do in Biology ?

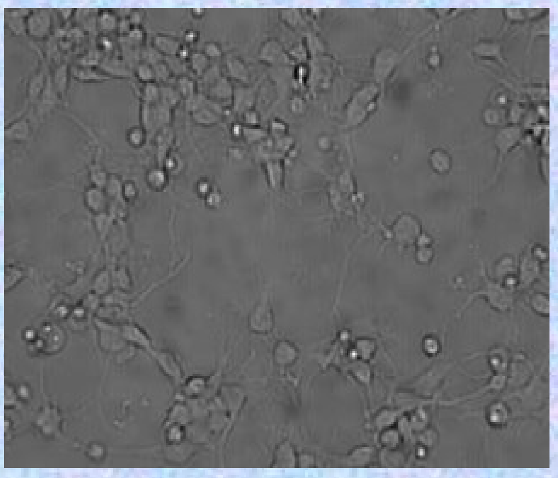
Pik-Yin Lai 黎璧賢

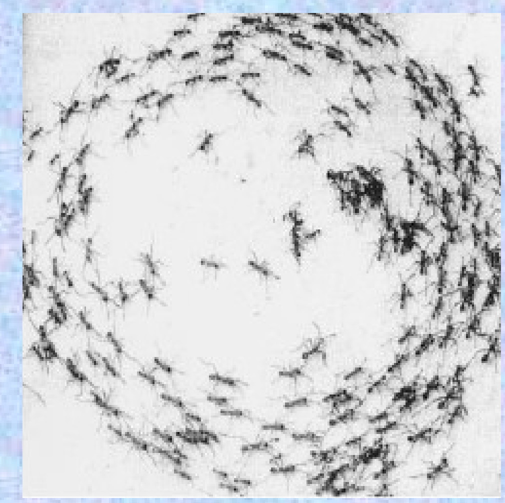
Dept. of Physics & Graduate Institute of BioPhysics

Center for Complex Systems,

National Central University, Chung-Li, Taiwan 320

Email: pylai@phy.ncu.edu.tw





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

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 (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)
- 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: Schroedinger, Cooper, Feigenbaum…

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

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.

Era of modern Biophysics

- **Length Scales:**

nm □ μm □ mm □ cm □ m □ km

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

- **Time Scales:**

fs □ ps □ μs □ 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

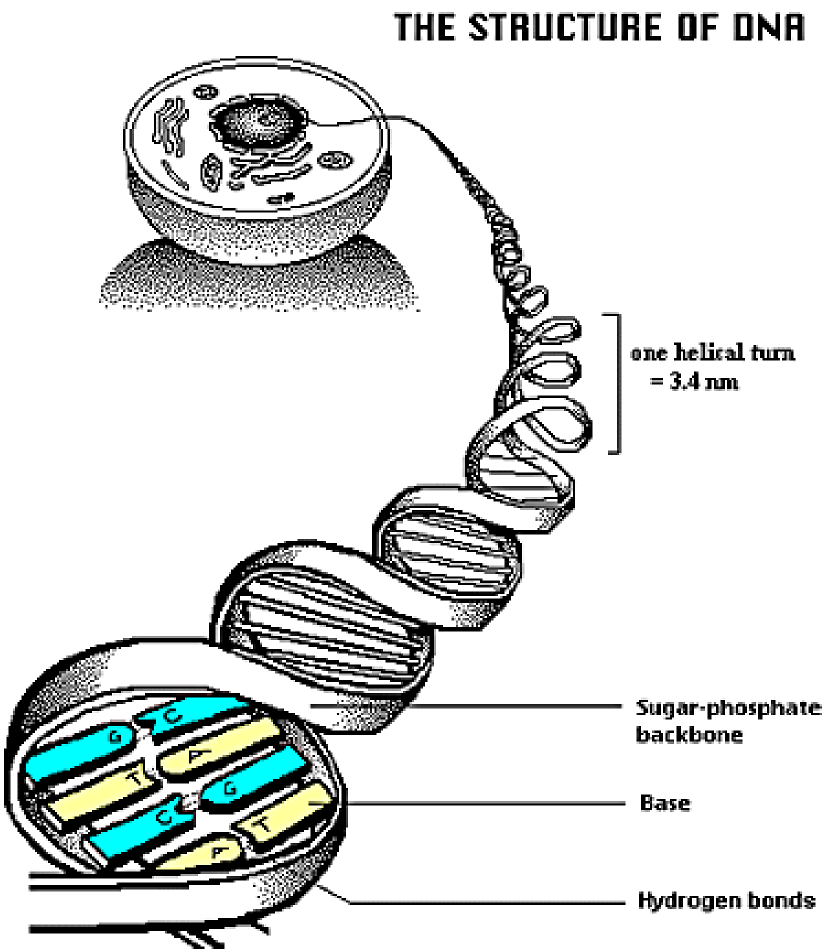
Mathematics□□Physics□□Chemistry□□Biology□□Medical

BioPhysics □□□ Biology + Physics

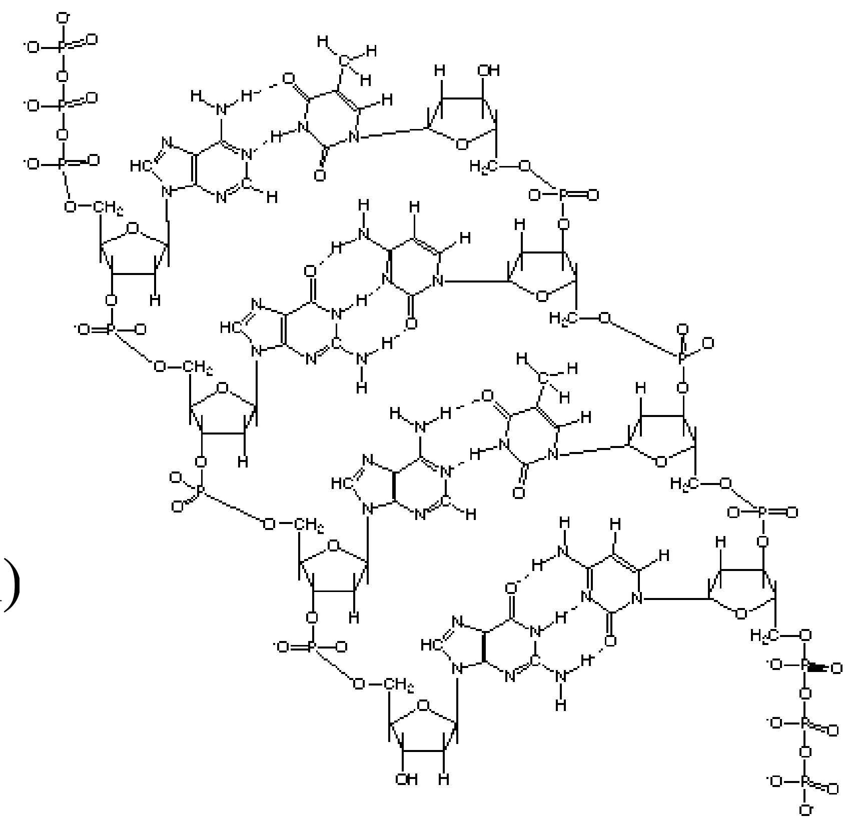
- Biophysicist is a TRUE Scientist ! Explore to the maximum freedom for doing science!
- 需要物理與非物理背景人材加入 ！

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 …….

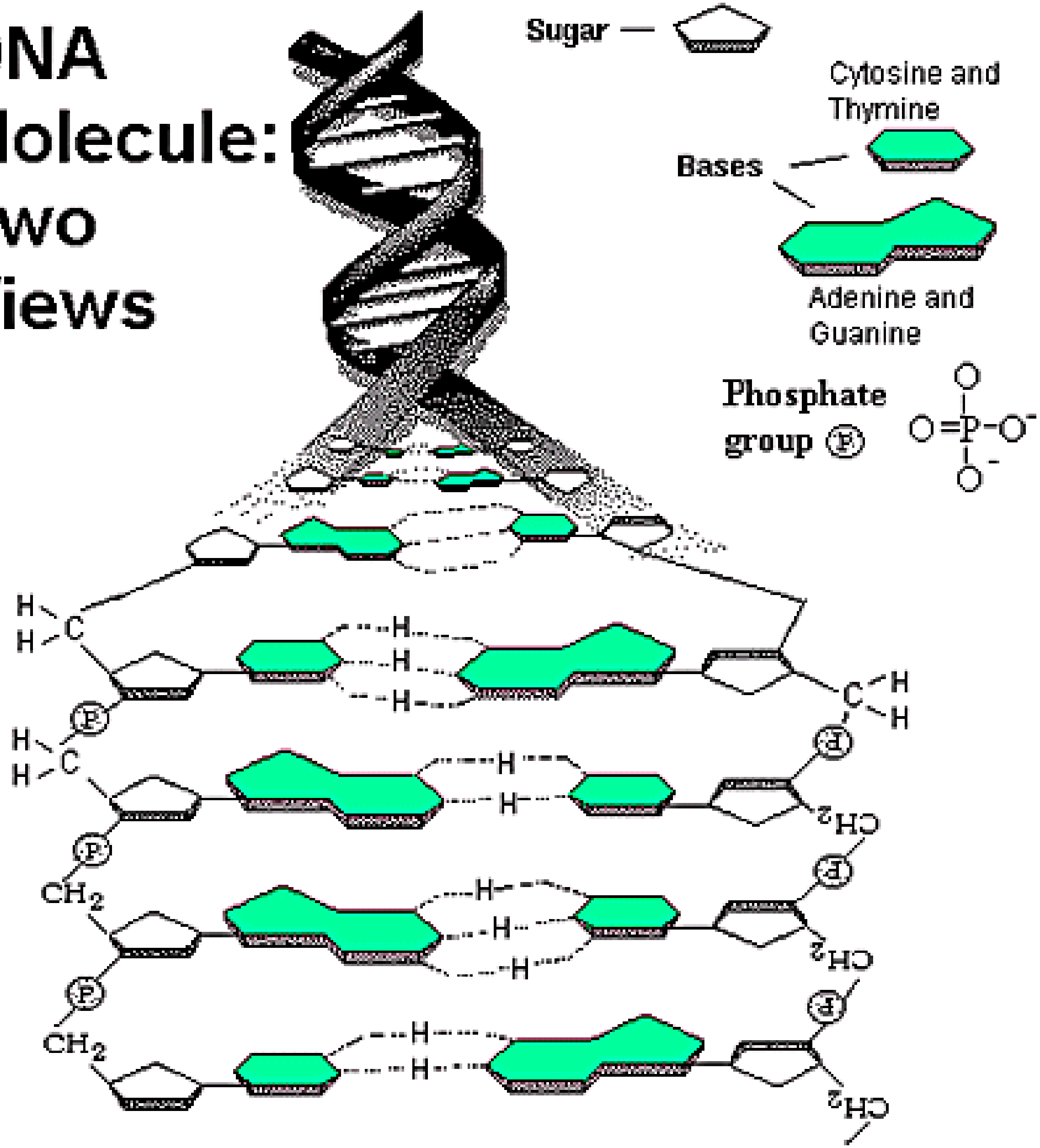


Cell
Nucleus
Chromosome
Chromatin



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**

Play (Torture) with DNA

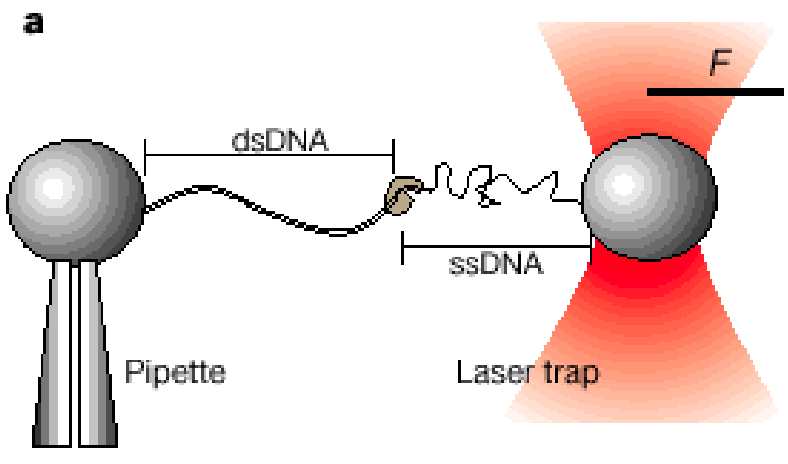
- DNA stretching, elasticity
- DNA drag reduction
- DNA thermo-phoresis
- DNA condensation
- DNA under external fields
- DNA photolysis
- DNA ratchet motion
- ……..

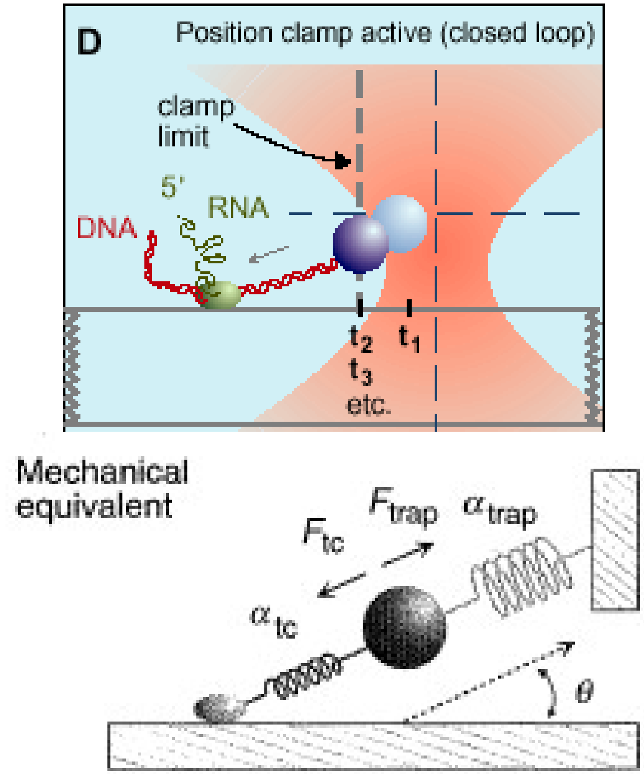
Mechanics/Elasticity of Single Bio-molecules

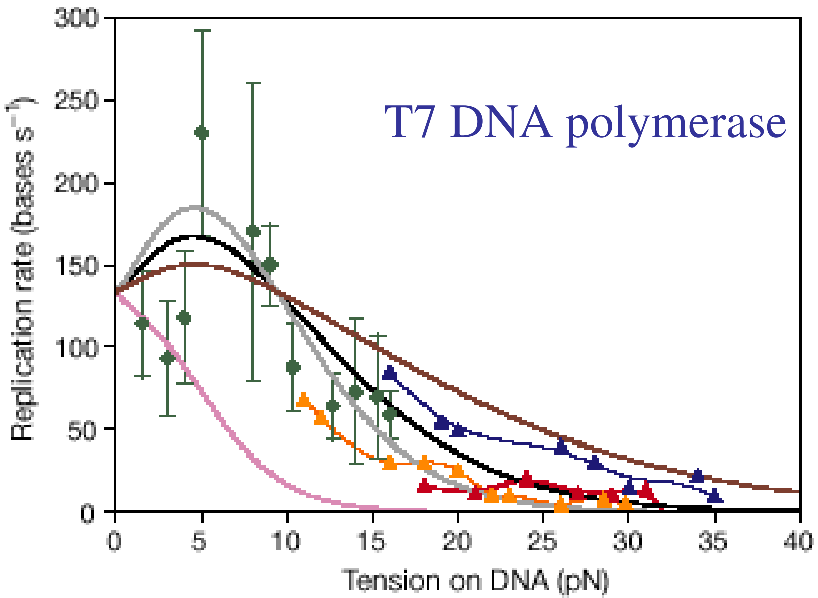
- To investigate the conformational changes in single bio-molecules, may provide significant insight into how the molecule functions.
- How forces at the molecular level of the order of pN underlie the varied chemistries and molecular biology of genetic materials?

DNA transcription by RNA polymerase

Bustemante et al, Nature 404, 103 (2000)

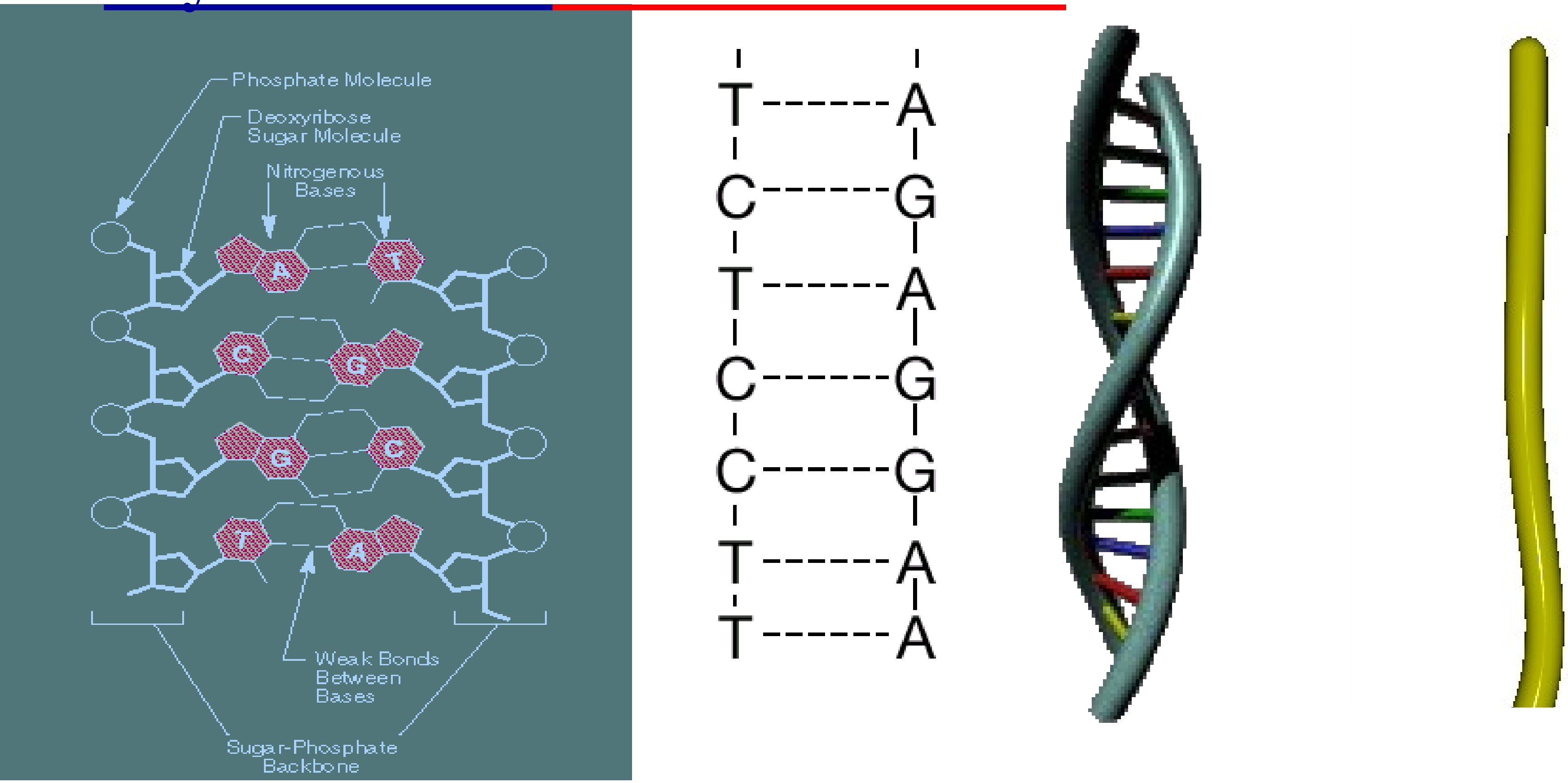






- effect of template tension polymerase activity
- Pausing & arrest during polymerase
- Mechanism of polymerization kinetics
- Tuning rate of DNA replication with external stresses

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

Worm-like chain model (stiff chain)

$$\frac{E_{el}}{k_B T} = \frac{A}{2} \int_0^L ds \left(\frac{d\mathbf{t}}{ds} \right)^2 \qquad \mathbf{t} = \frac{d\mathbf{r}(s)}{ds}$$

|t|=1 inextensible

single strand

Rod-like chain model (twisted stiff chain)

Marko et al., Science 256, 506, 1599 (94); L 80, 1556 (98)

$$\frac{E_{el}}{k_B T} = \frac{1}{2} \int_0^L ds \left[A \left(\frac{d\mathbf{t}}{ds} \right)^2 + C(\omega(s) - \omega_0)^2 \right]$$

Fitting from expts: A=53nm; $\frac{C}{A} = 1.64 \pm 0.04$ $\quad \omega_0 = 2\pi/(10.5 \cdot 0.34)\ \mathrm{nm}^{-1}$

Can account for some supercoiling properties of DNA
Phenomenological model, no description of underlying mechanism.

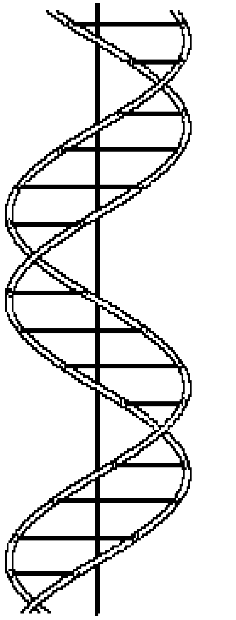
ZZO model for double-stranded DNA

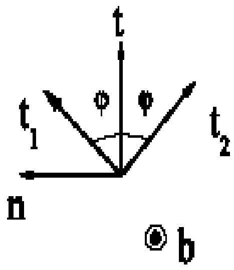
H. Zhou, Z. Yang, Z-.c. Ou-Yang, PRL **82**, 4560 (99)

$$E_{bs} = \int_0^L \left[\frac{1}{2}\kappa \dot{\mathbf{t}}_1^2 + \frac{1}{2}\kappa \dot{\mathbf{t}}_2^2 + \rho(\varphi)\right] ds$$

$$= \int_0^L \left[\kappa \dot{\mathbf{t}}^2 + \kappa \dot{\varphi}^2 + V(\varphi)\right] ds,$$

$$V(\varphi) = \frac{\kappa \sin^4 \varphi}{R^2} + \rho(\varphi),$$





$$\dot{\mathbf{r}} = \frac{1}{2}(\mathbf{t}_1 + \mathbf{t}_2) = \mathbf{t}\cos\varphi$$

φ=folding angle

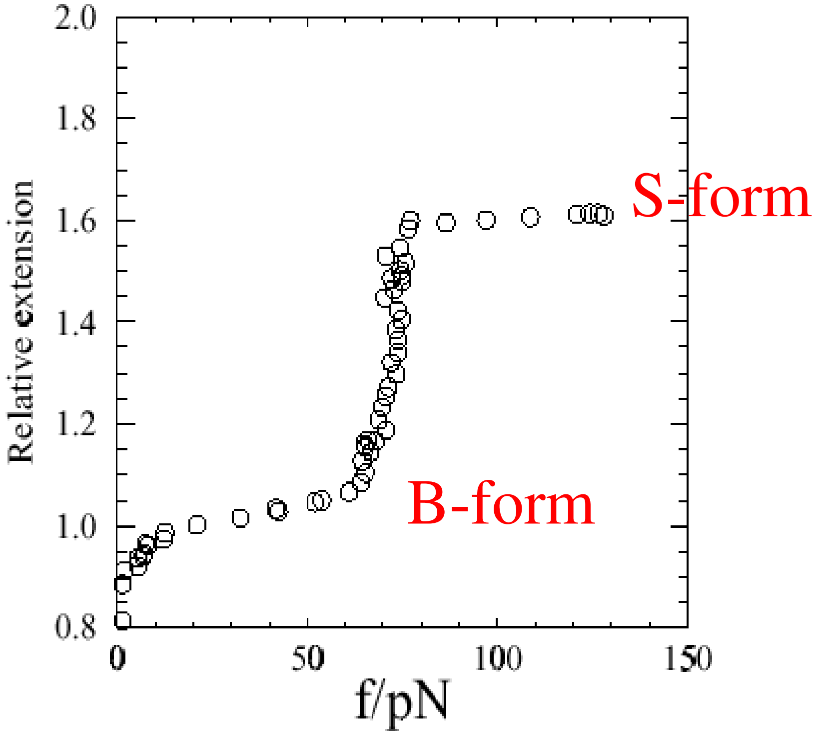
$$\rho(\varphi) = \begin{cases} \frac{\epsilon}{r_0}\left[\left(\frac{c_0}{\cos\varphi}\right)^{12} - 2\left(\frac{c_0}{\cos\varphi}\right)^{6}\right] & (\varphi > 0) \\ \frac{\epsilon}{r_0}\left(c_0^{12} - 2c_0^{6}\right) & (\varphi \le 0) \end{cases}$$

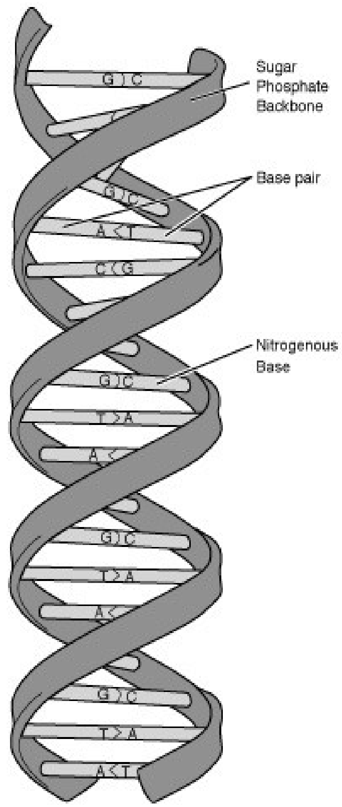
B-form to S-form Transition under a Stretching force

Lai & Zhou, J. Chem. Physics 118, 11189 (2003)

Force Experiments

Stretching a single end-grafted DNA

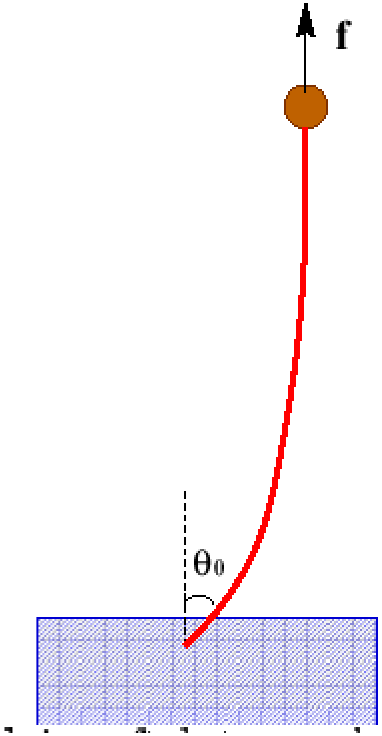


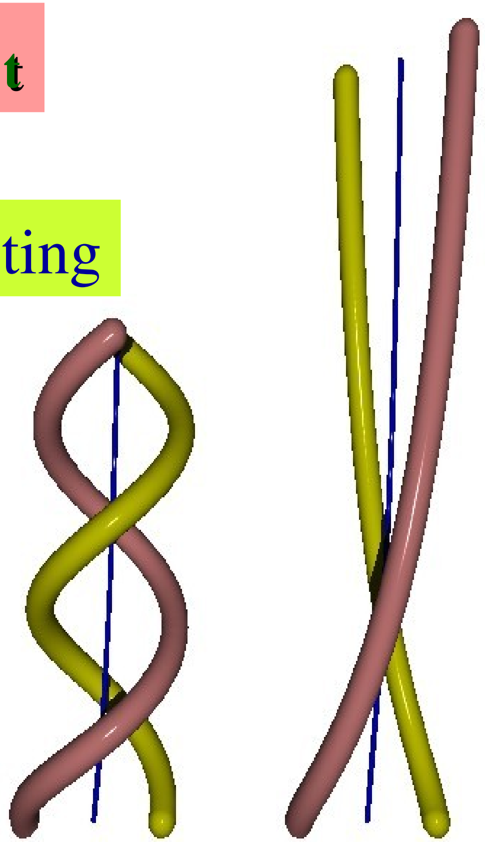


- **Abrupt increase of 1.7 times in contour length of dsDNA near 65pN.**
- **Thermal fluctuations unimportant near onset of transition.**

First order phase transition at β_t

First-order elongation:Stretch by untwisting





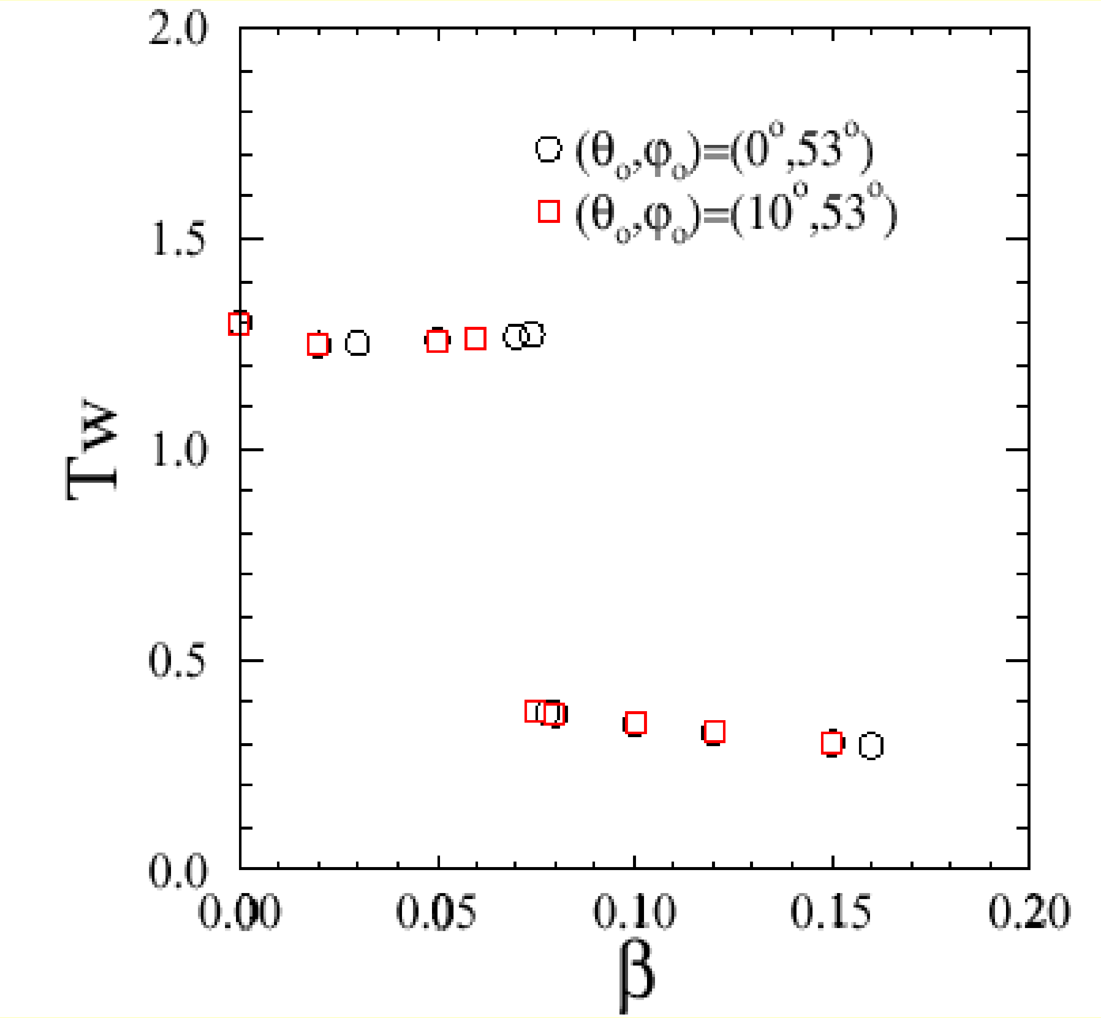
β=0.073 β=0.075

<u>Untwisting upon stretching</u>

Untwist per contour length from B□S, ΔTw/Lo~-100 deg. /nm;

- **Almost completely unwound ~ 34deg./bp**
- **Torque ~ 60 pN nm**

Untwisting upon stretching



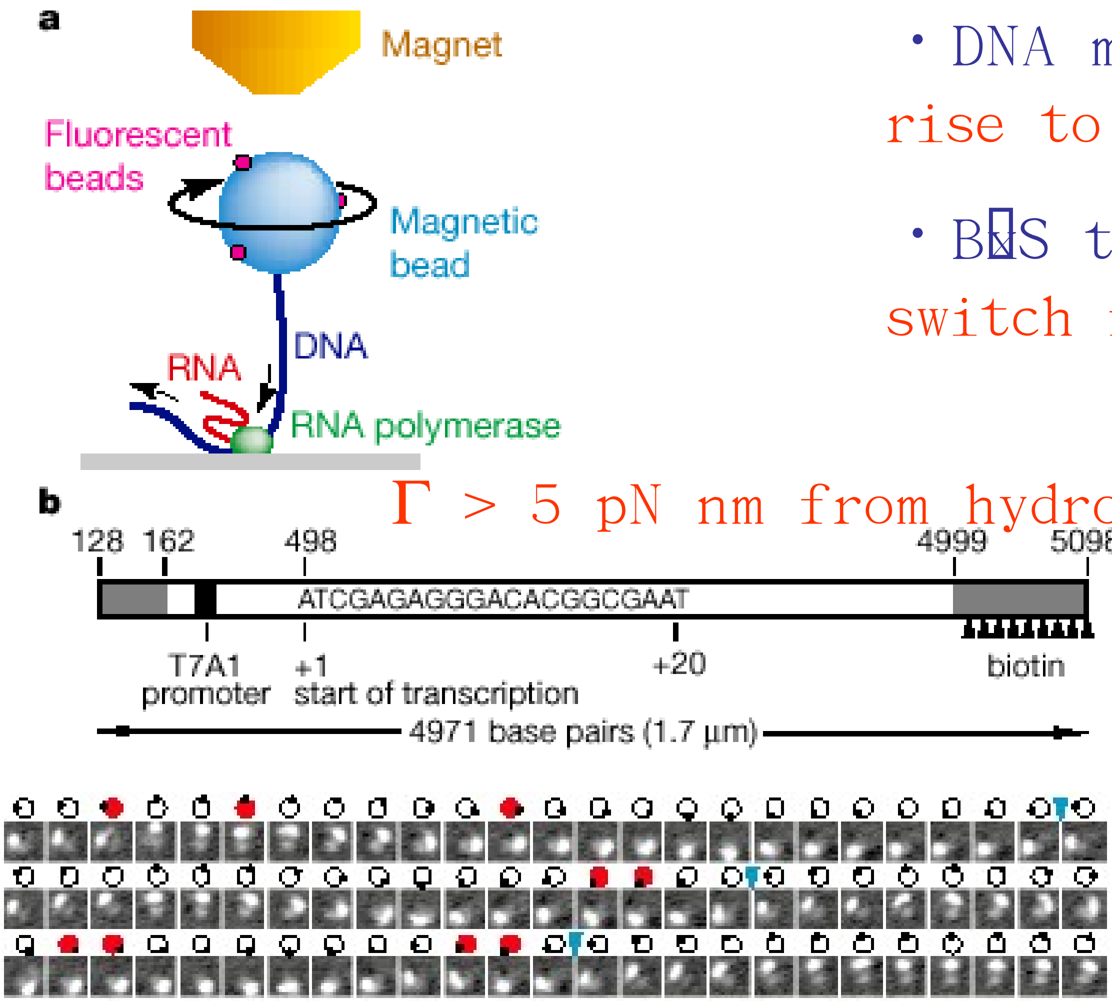
$$\mathrm{Tw} = \frac{1}{2\pi}\int_0^L \sin\varphi\, ds$$

- Untwist per contour length from B S, ΔTw/Lo~-100 deg. /nm;
- Almost completely unwound ~ 34deg./bp
- Torque ~ 60 pN nm

$$\Gamma = \frac{\kappa}{R}\csc\varphi_t\left(V(\varphi_t) + 2\beta_t(1-\cos\varphi_t)\right)$$

Direct observation of DNA rotation during transcription by Escherichia coli RNA polymerase

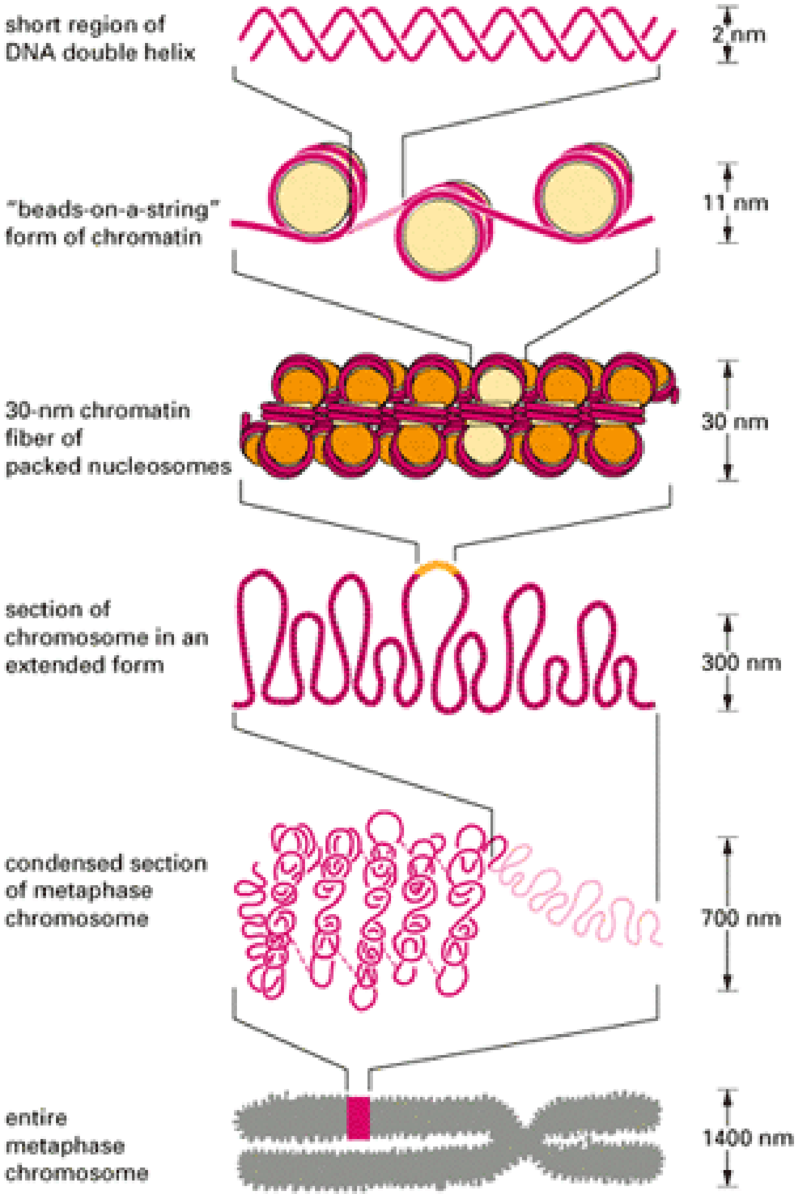
Harada et al., Nature **409**, 113 (2001)

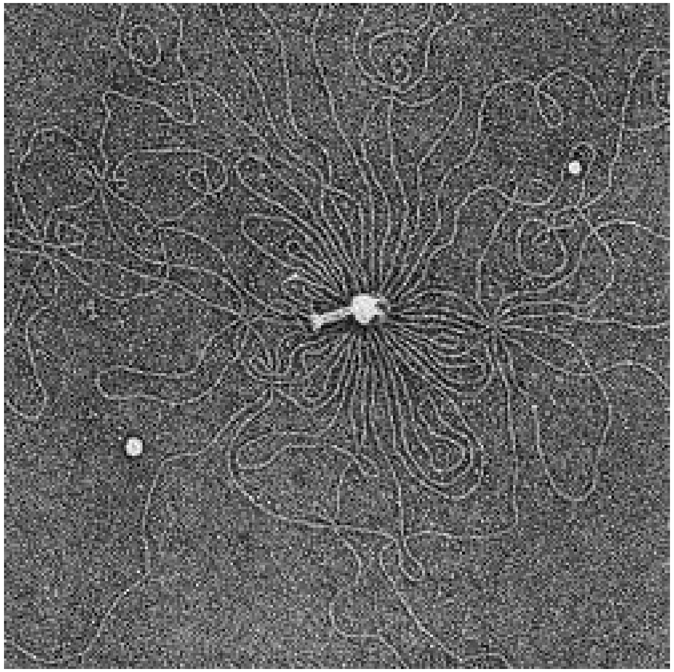


- DNA motor: untwisting gives rise to a torque
- B□S transition provides a switch for such a motor.

Γ > 5 pN nm from hydrodynamic drag estimate

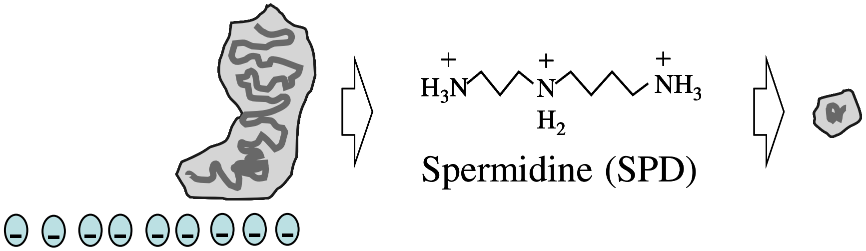
DNA condensation & packing

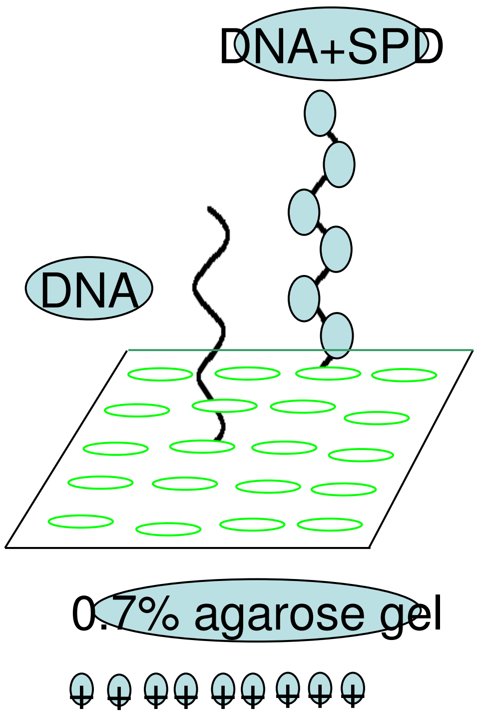


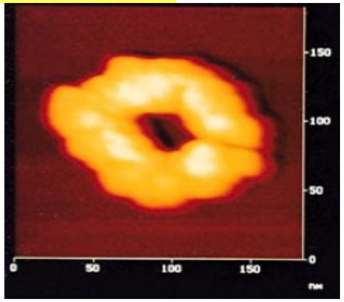


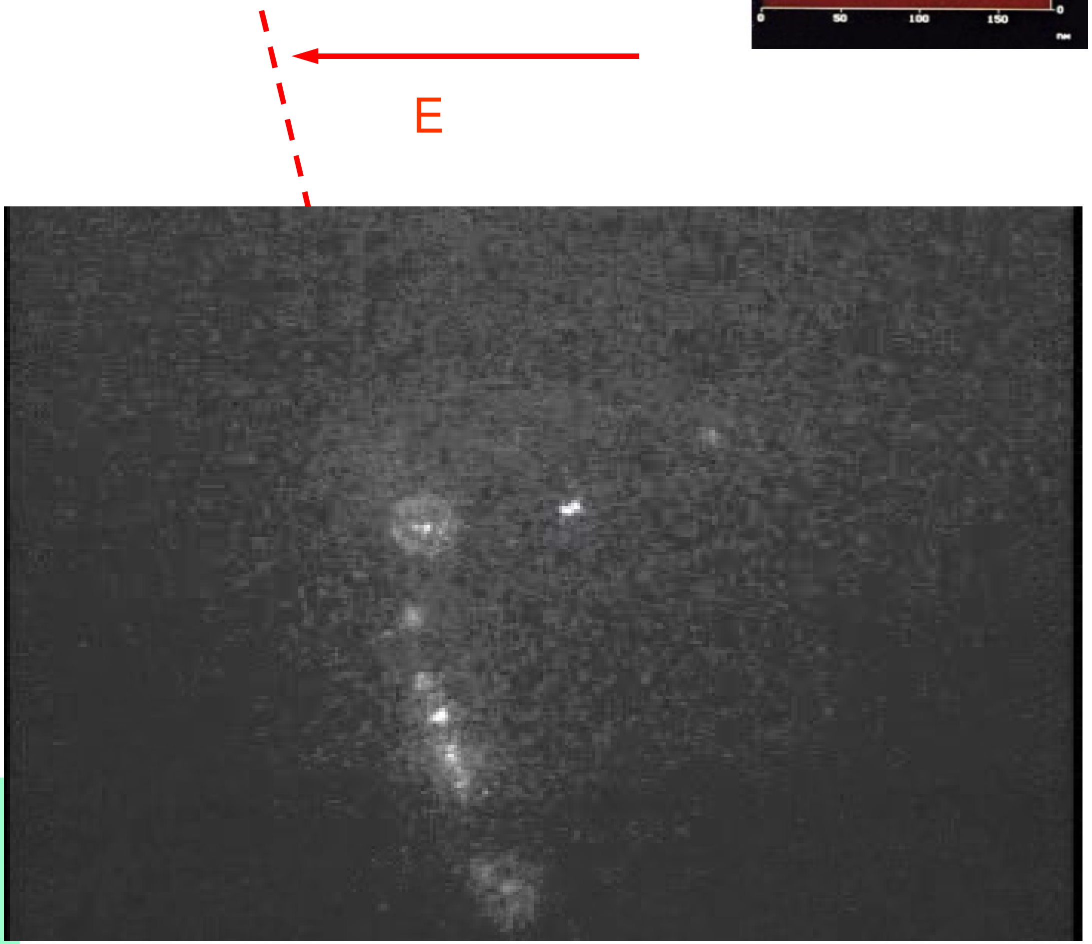
Complex competition of DNA elasticity, charge interactions, volume interactions, solvent effects…..

DNA condensed by spermidine





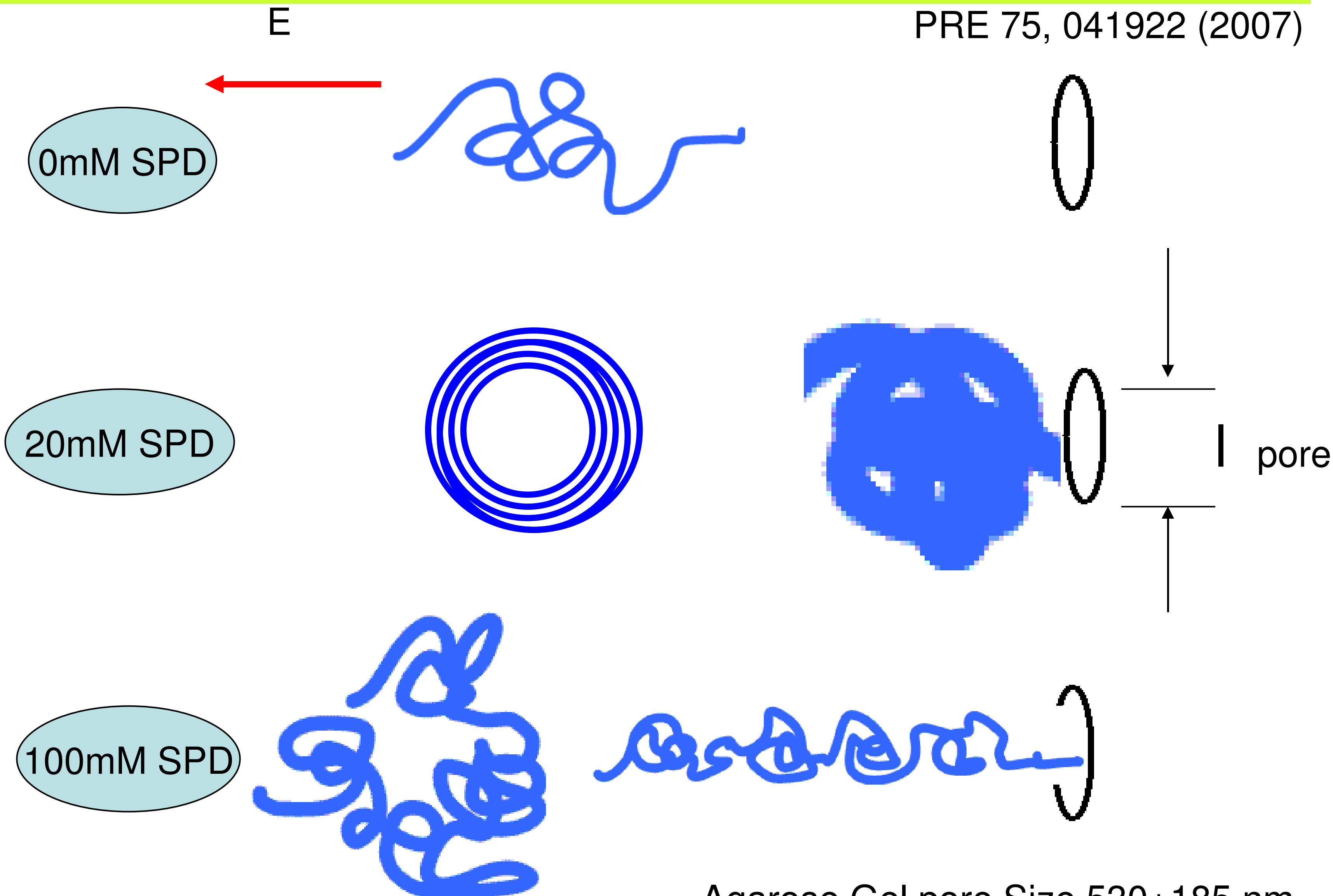




Single-λ DNA with [SPD]=20mM jamming when entering in 0.7% gel

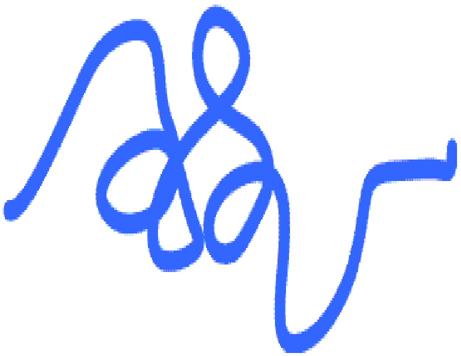
Jamming: due to conformation changes in DNA

PRE 75, 041922 (2007)

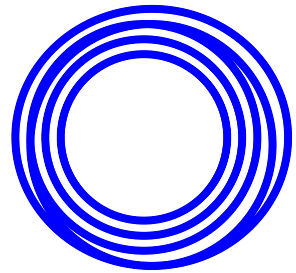


Agarose Gel pore Size 530±185 nm from AFM

De-Jamming: due to re-entrant condensation of DNA



Flexible coil (reptate thro' gel)



rigid condensed globule
(size>minimal pore size)

flexible again

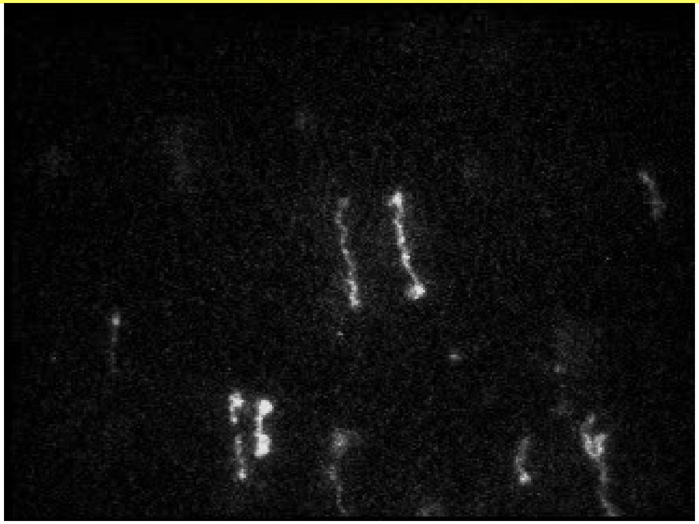
[SPD]

Re-entrant due to charge inversion (over-charge of DNA) ?

–

Controlled motion of DNA: external drives

In gel
under DC E field

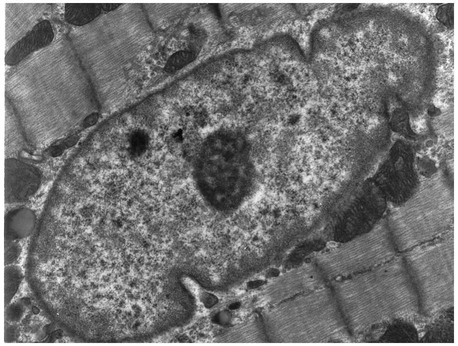


DNA ratchet motion under AC electric field

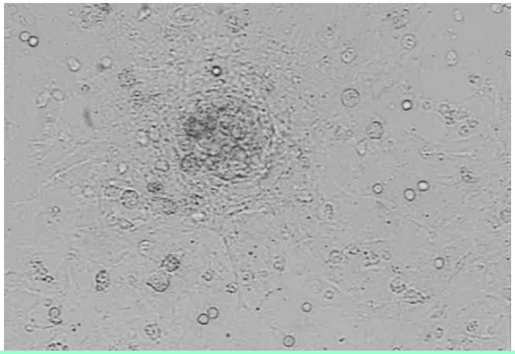
Simple to Complex: emerging properties in bio-systems

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

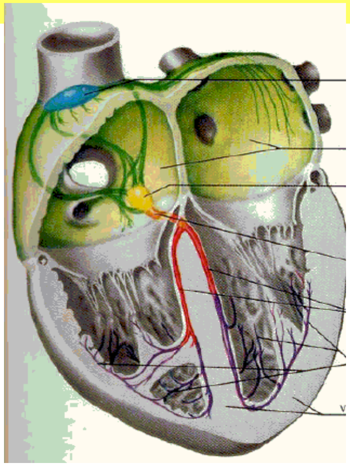
(I) cardiac cells → Heart



Cardiac myocyte



Synchronized beating of myocytes



spiral waves:

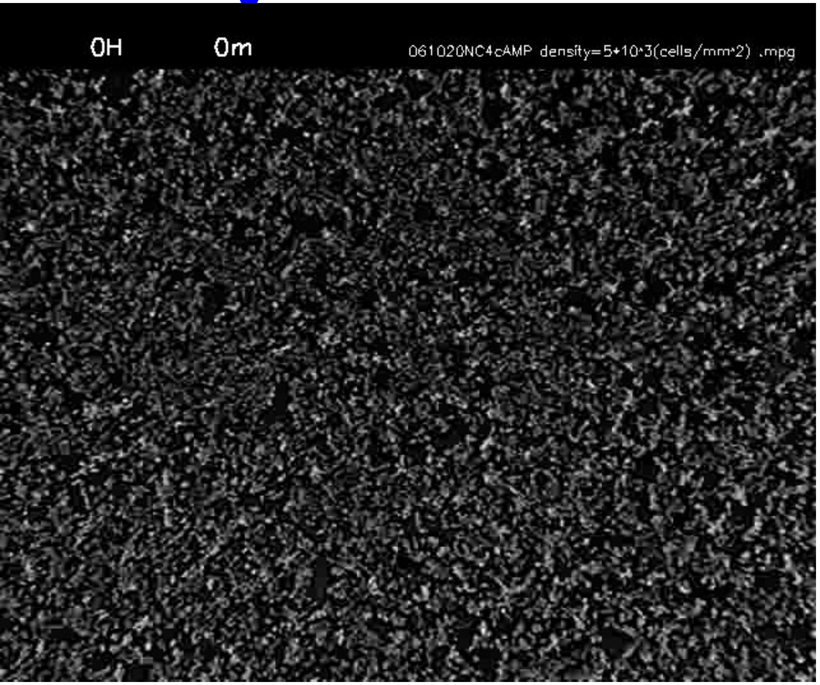
Coupled oscillator networks of Cardic cells:

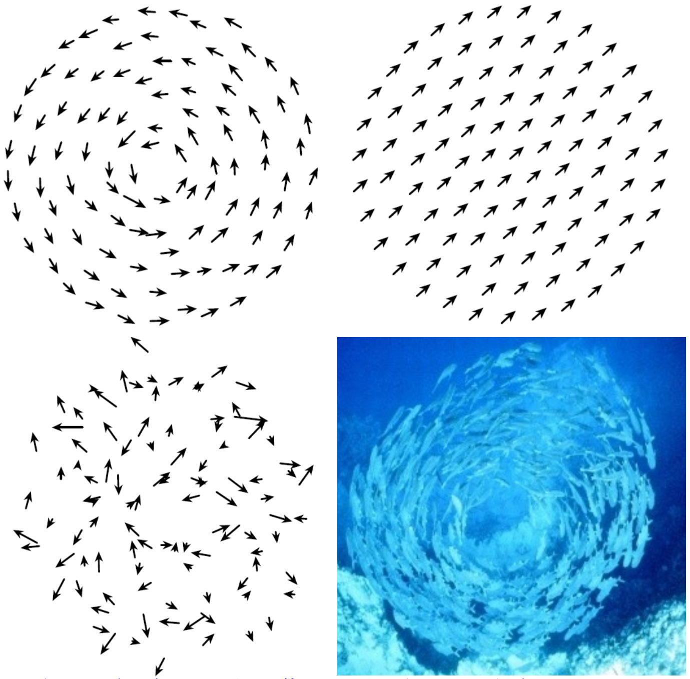
nonlinear dynamics, spiral waves, spatio-temporal patterns…

Simple to Complex: emerging properties in bio-systems

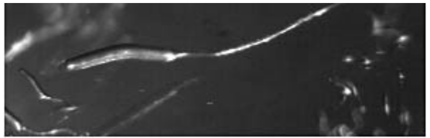
(II) Single cell/organismcollective motion

Dictyostelium discodium



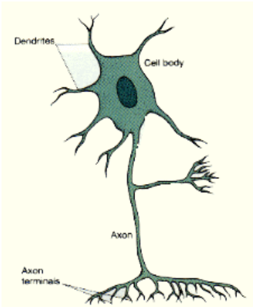


物種之群體運動理論：
魚群、昆蟲、細菌之習
体運動模式

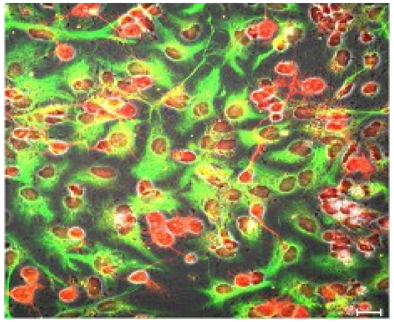


emerging properties in bio-systems

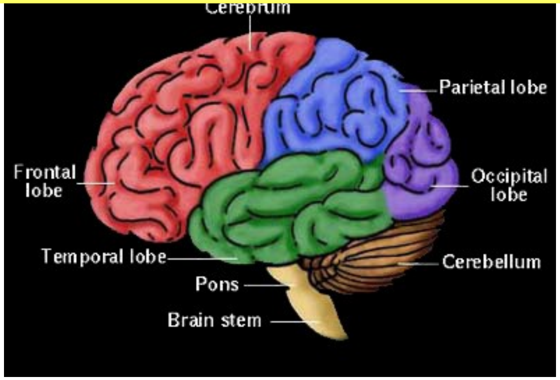
(III) Neurons Network Brain Behavior



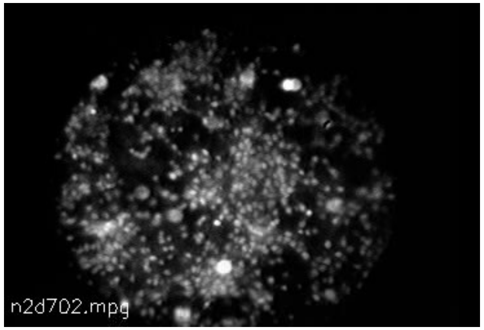
Hodgkin-Huxley Model (1952)



Network connection:synapses



Neuro/cognitive science



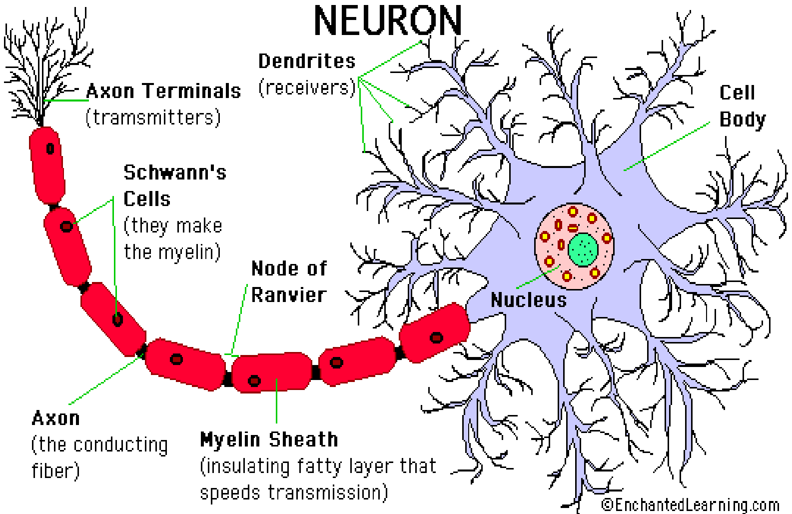
Synchronized Firing

Complex behavior/function determined by neuron connections.

Complex neuronal Network:

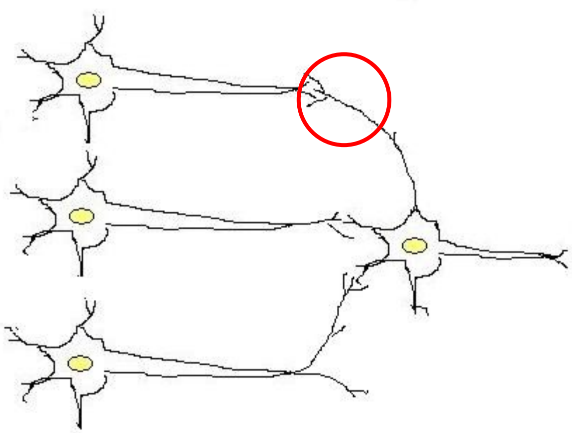
- A single neuron in vertebrate cortex connects ~10000 neurons
- Mammalian brain contains > 10**11 interconnected neurons
- Signal & information convey via neuronal connections—coding

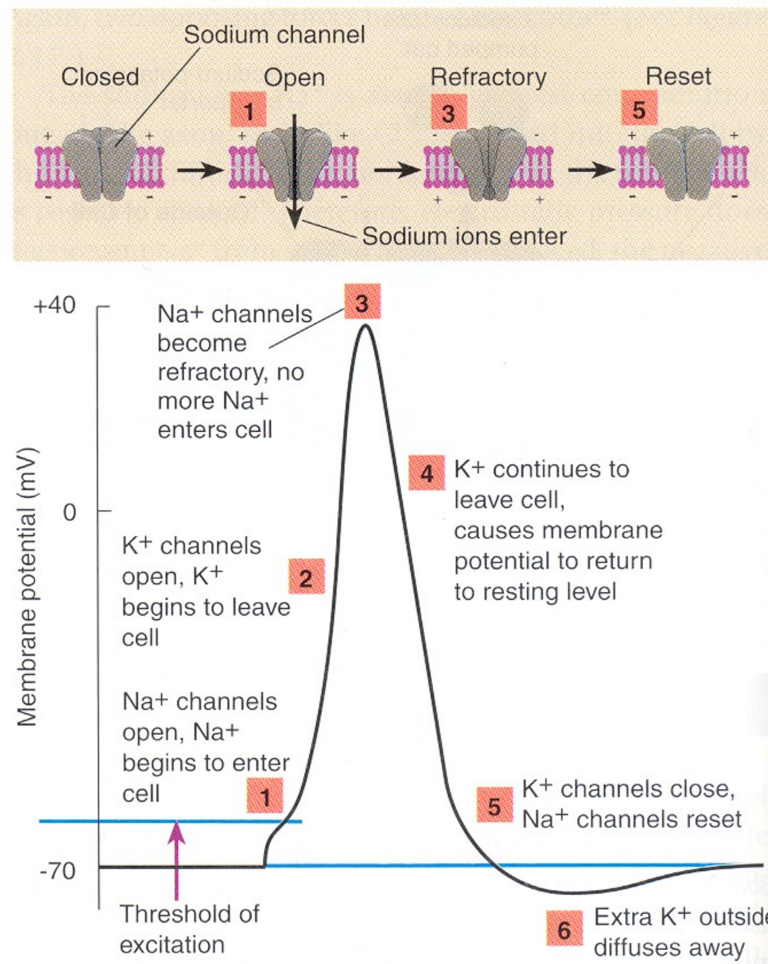
Neuron & Action Potential



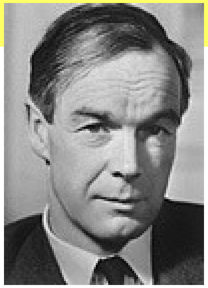
Spike: ~ 1 ms, 100mV

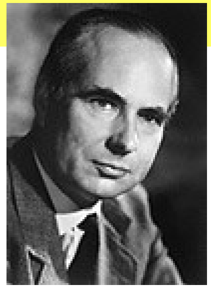
Propagates along the axon to the junction of another neuron ---synapse



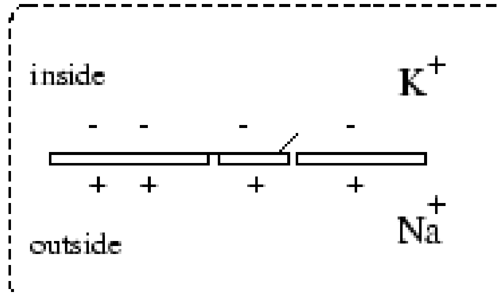


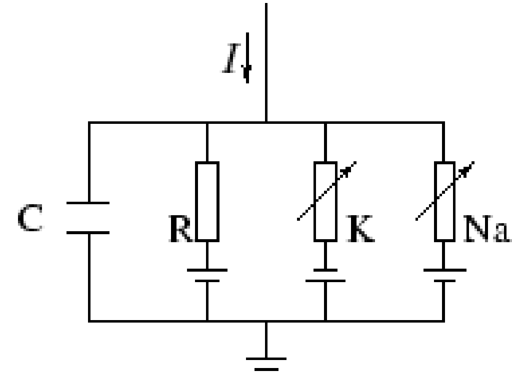
Hodgkin-Huxley model (1952)





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





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

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

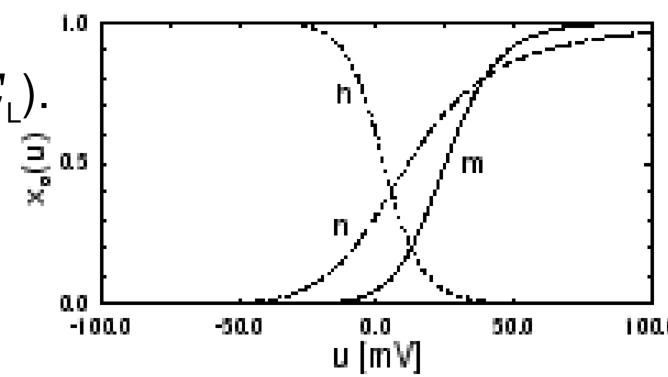
gating variables:

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

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

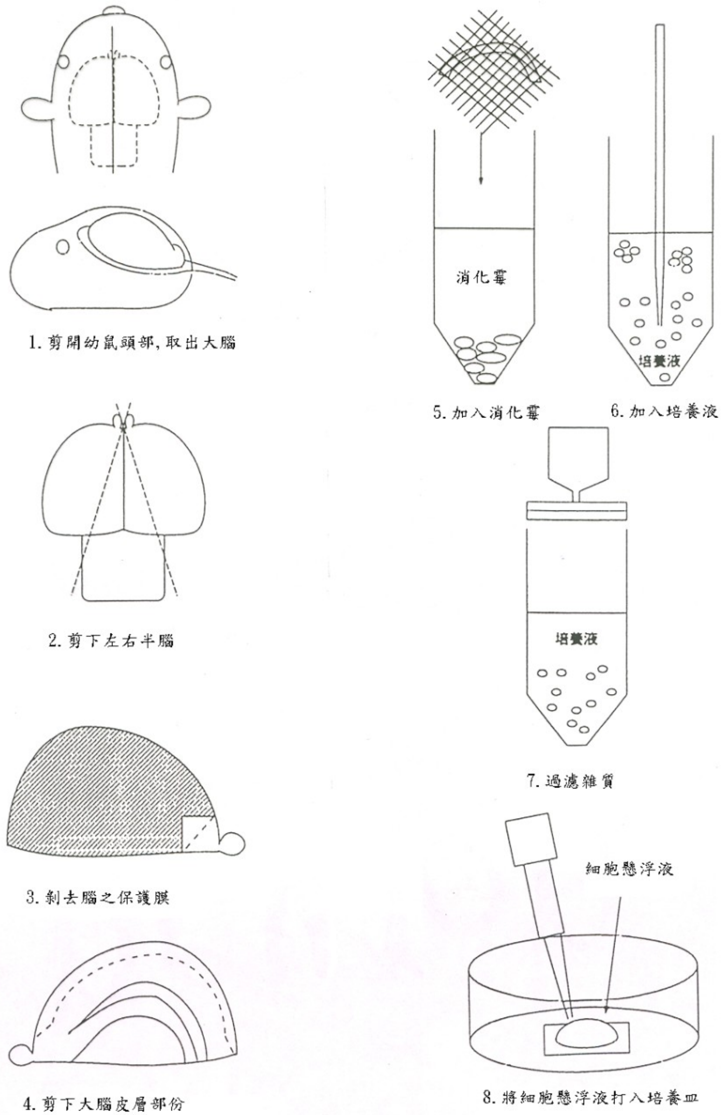
$$\dot{h} = \alpha_h(u)\ (1 - h) - \beta_h(u)\ h$$

empirical functions



Experiments

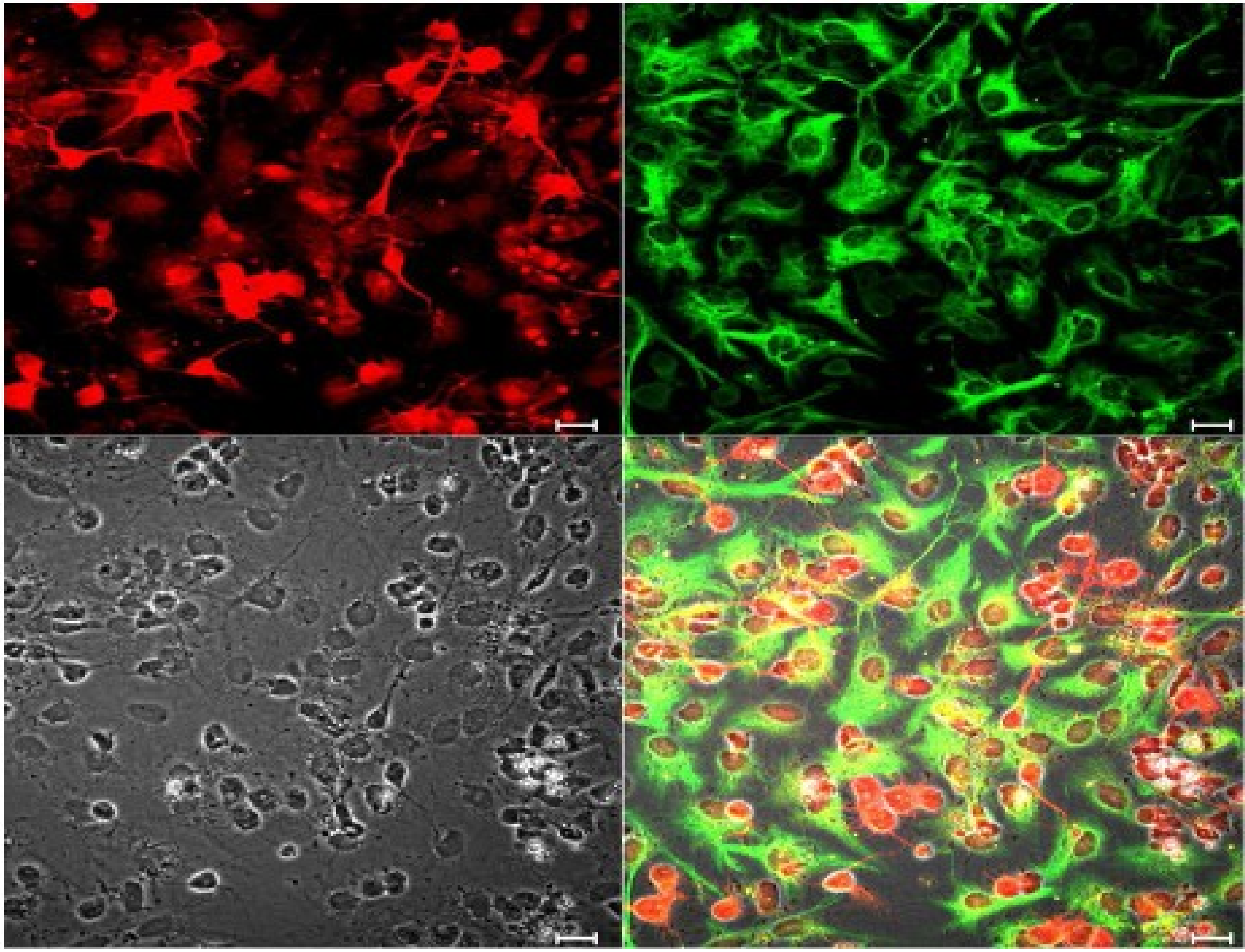
Schematic procedures in preparing the sample of neuron cells from celebral cortex embryonic rats



Embryos of Wistar rats
E17~E18 breeding days

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

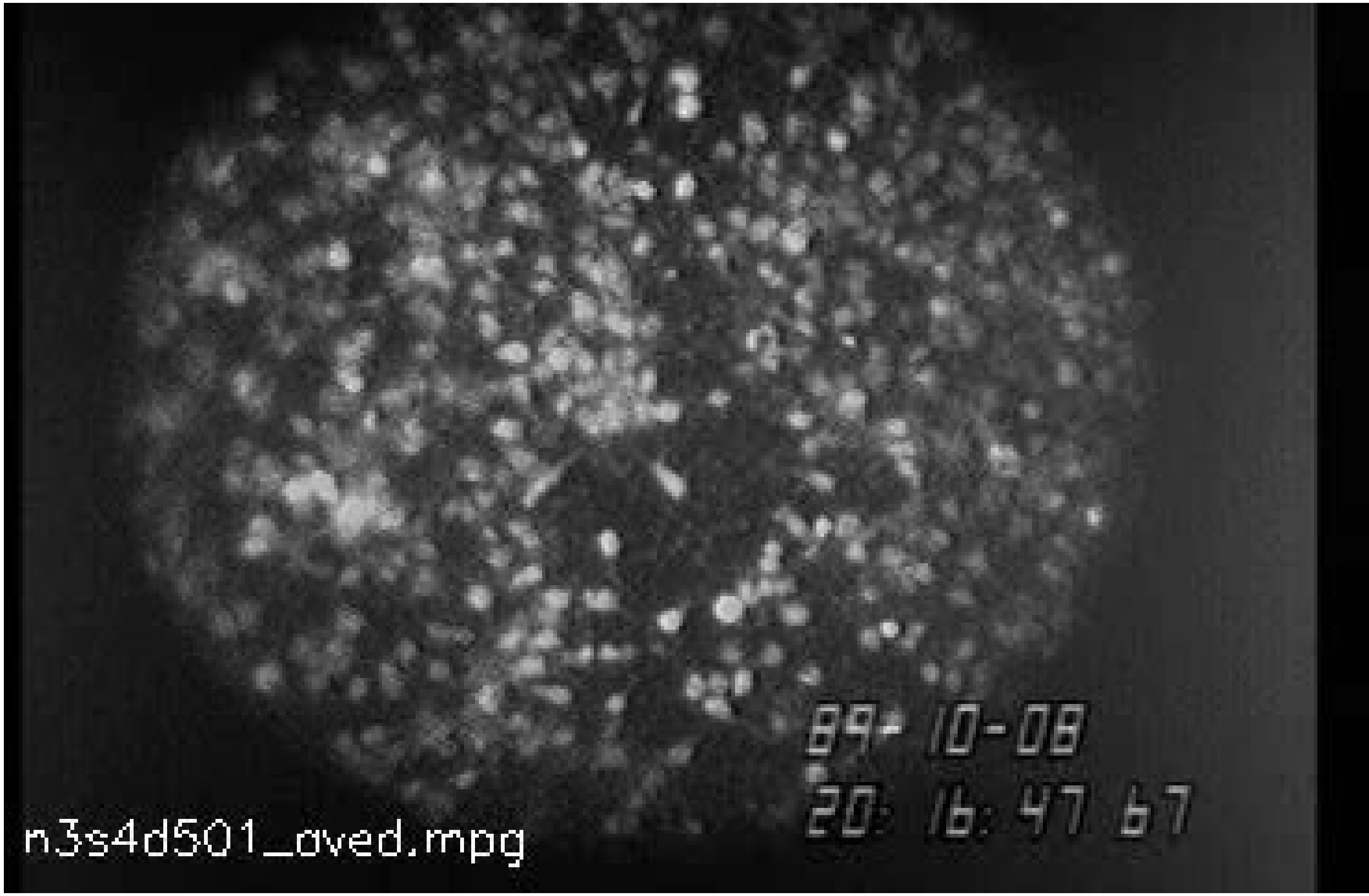
Growth of axon connection to form a network



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).

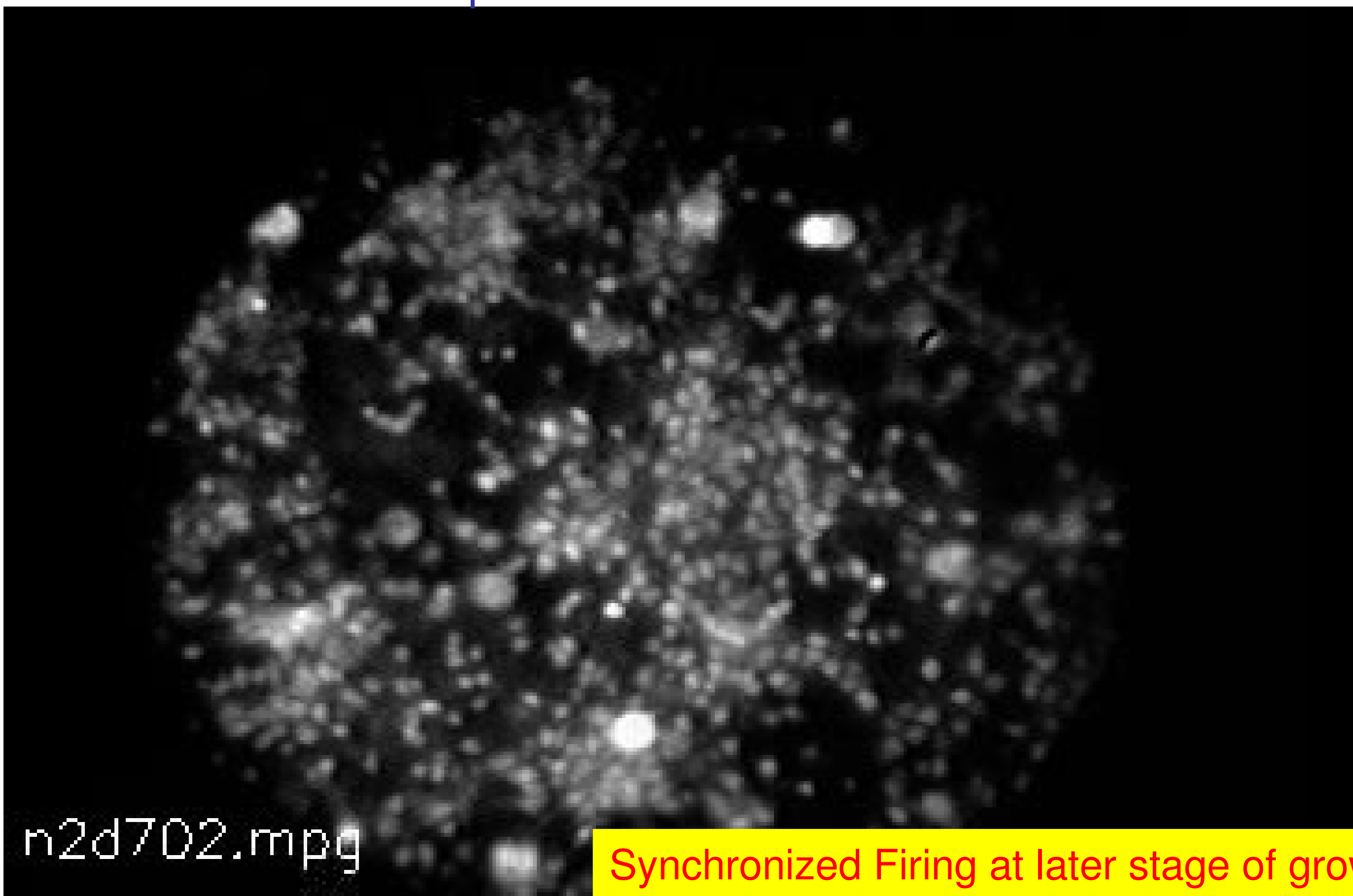


Non-synchronous Firing in early stage of growth

Synchronized Firing of Neuronal Network Culture

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

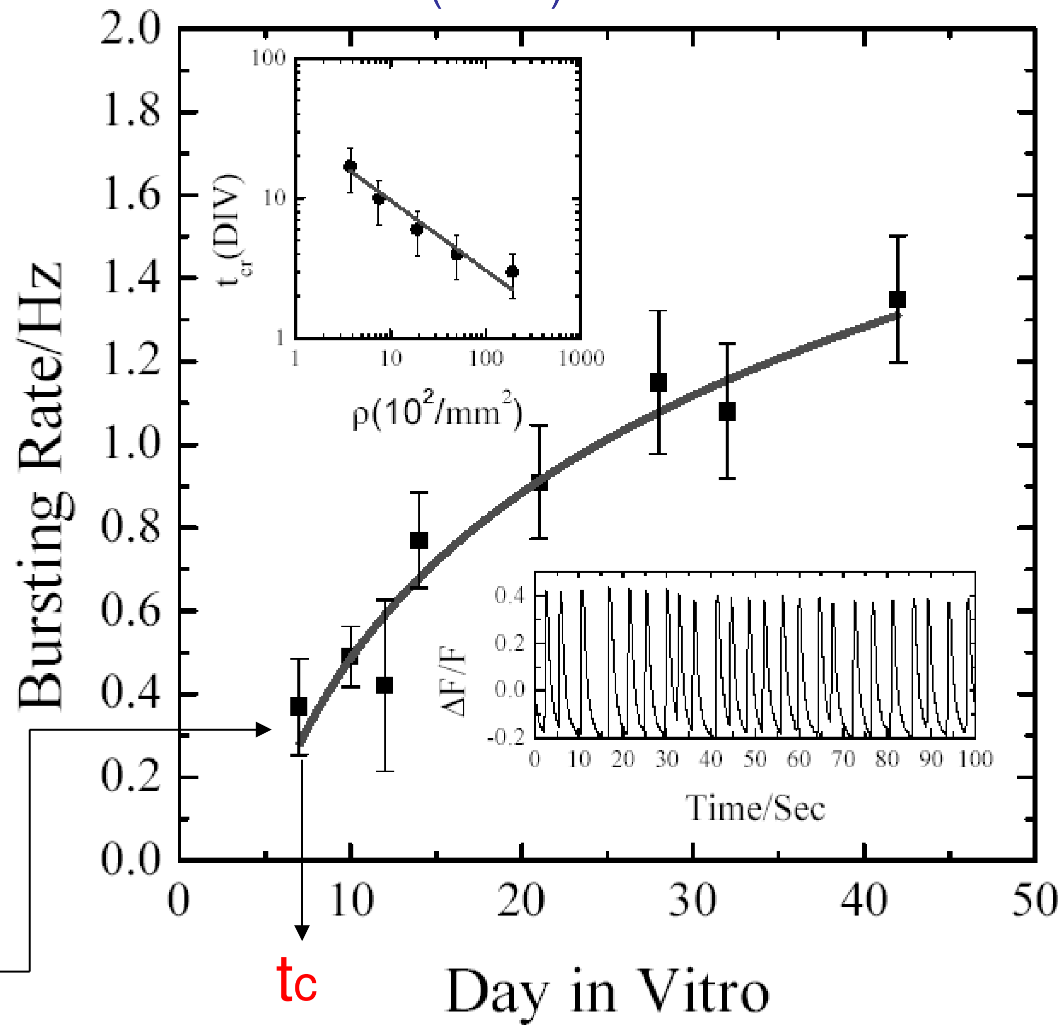
Firing → the changes in intracellular [Ca 2+] indicated by the fluorescence probe.



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)



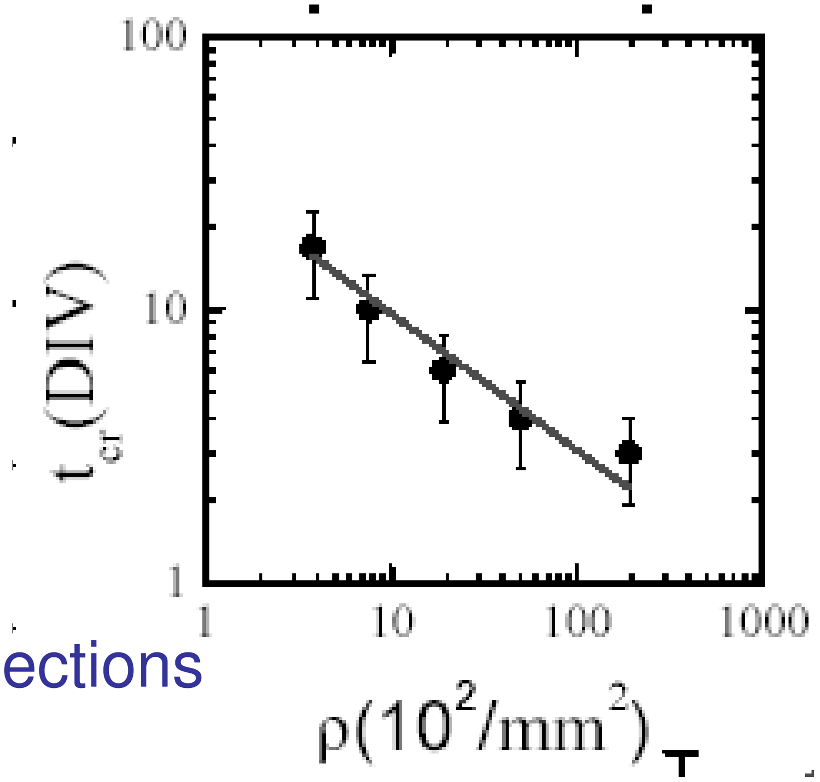
- Critical age for SF, tc
- SF freq. grows with time

  $f=fc+fo \log(t/tc)$

Onset time for SF as a function of cell density

- Critical age for SF

$$t_c \propto \frac{1}{\sqrt{\rho}}$$

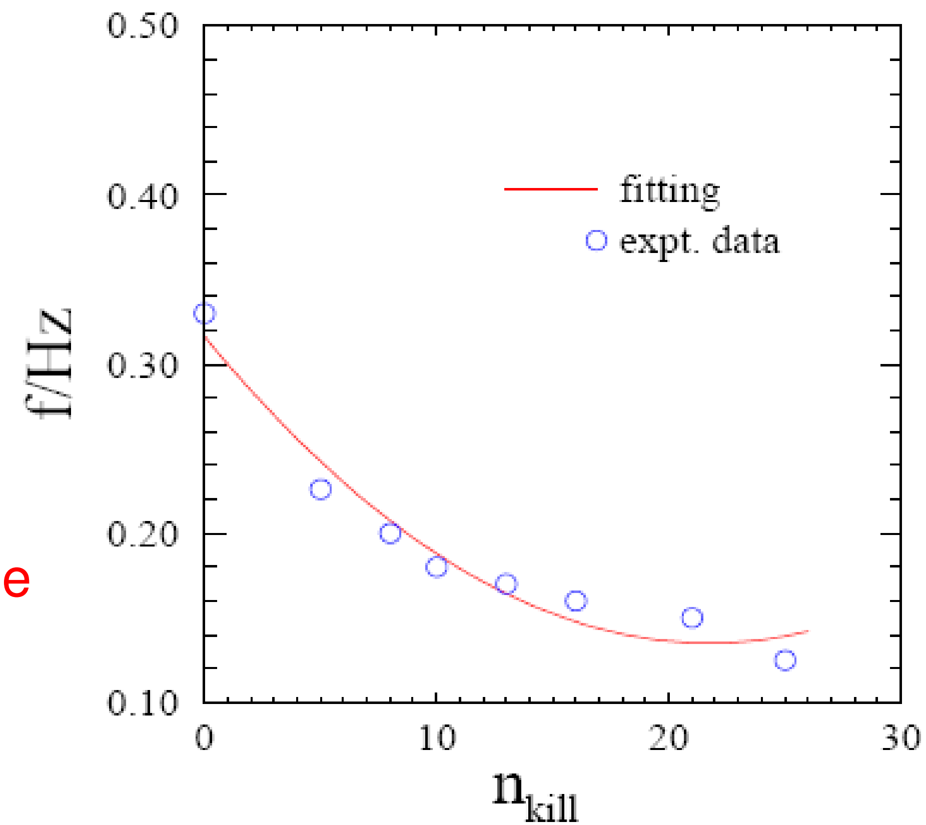


- f=fc+fo log(t/tc)
- f increases with the effective connections
- fc is indep. of □

Synchronous firing frequency *f* ~ mean connectivity *k*

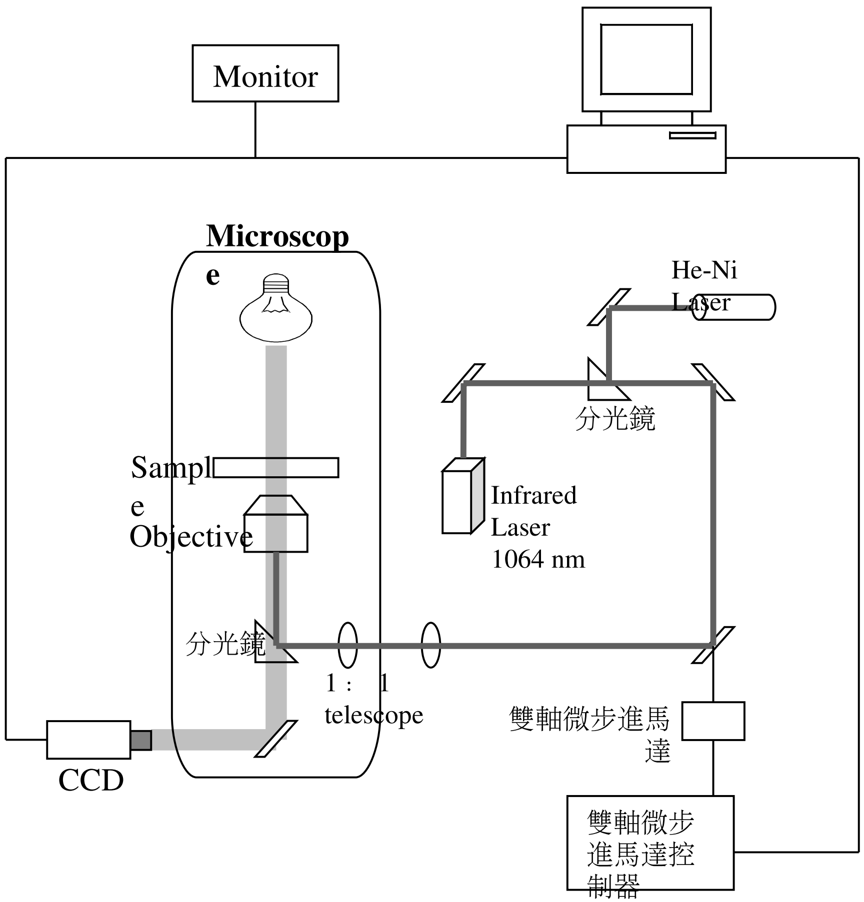
$$k \simeq \frac{1}{N}(N - n_{kill})(N - n_{kill} - 1)P_o,$$

- Well fitted by taking f ~ a + b k, with a small.
- f ~ k



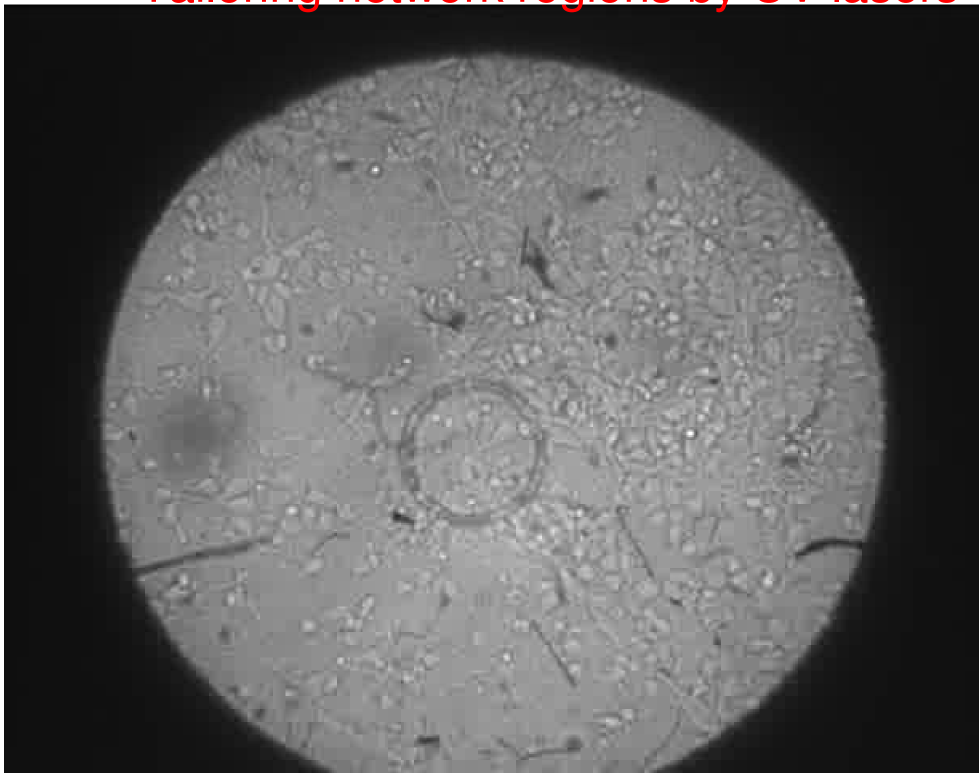
Use synchronized firing freq. to probe the Growth behavior of the network

Manipulating/attacking the neuronal network



Optical Tweezers

Tailoring network regions by UV lasers

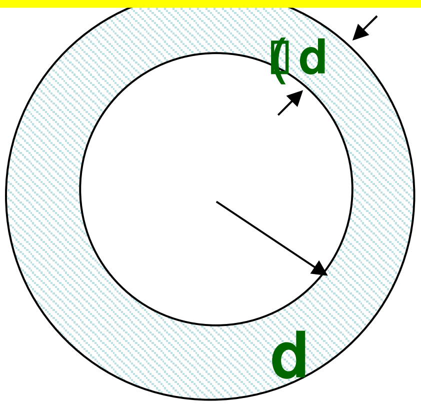


Network attack: random or target attack
Network robustness
Regenerative & Re-routing behavior

Model for Neuronal Network Growth

Phys.Rev. E 2006

k=mean connectivity in domain of radius d
P(k)= prob. of connecting 2 neurons
□=mean cell density



Synchronized Cluster occurs at t=tc with f=fc

Increase in connectivity: **□k =2P□d □d P(k)**

$$d = ut \qquad t < t_c$$

•**Enhanced growth towards synchronized cluster** (active search) for t<tc

•Experimentally: SF occurs at t=tc with f=fc

fc is indep. of P: □□

Assume fc ~ kc, □ SF occurs when sufficient connections are made:

kc~ □ tc^2 □

$$t_c \propto \frac{1}{\sqrt{\rho}}$$

Retarded growth for t>tc

Empirical result : (f~k) $k = k_c + k_o \ell n(\frac{t}{t_c}) \quad t > t_c,$

At t ~ tc (*k ~ kc*), the neurons have made enough connections among themselves and cooperativity begins:- a neuron gets enough signals from other neurons so that it surmounts its threshold for further fast growth.

It knows that there are enough connections for cooperativity and there is no need for further increase of connection. Thus the rate of growth *R* starts to decay.

$$\mathcal{R} \equiv dk/dt = \frac{k_o}{t_c} \exp[-\frac{k - k_c}{k_o}] \quad \text{for} \quad k \geq k_c.$$

Slowing down for t>tc

$$k = k_c + k_o \ell n(\frac{t}{t_c}) \quad t > t_c,$$

Using diffusive search model: $$d^2 = Dt$$

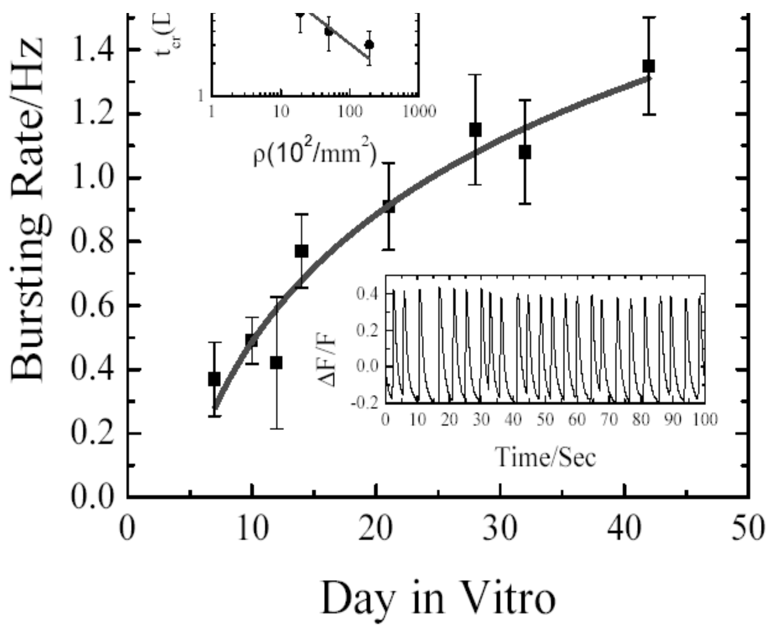
→**P(k)=Pc exp[-(k-kc)/ko]   k>kc**

ðk =2 d d P(k)

Retarded growth for t>tc:

$$\mathcal{R} \equiv dk/dt = \frac{k_o}{t_c} \exp[-\frac{k-k_c}{k_o}] \quad \text{for} \quad k \geq k_c.$$

$$\frac{d\mathcal{R}}{dk} = -\frac{\mathcal{R}}{k_o} \qquad k > k_c.$$



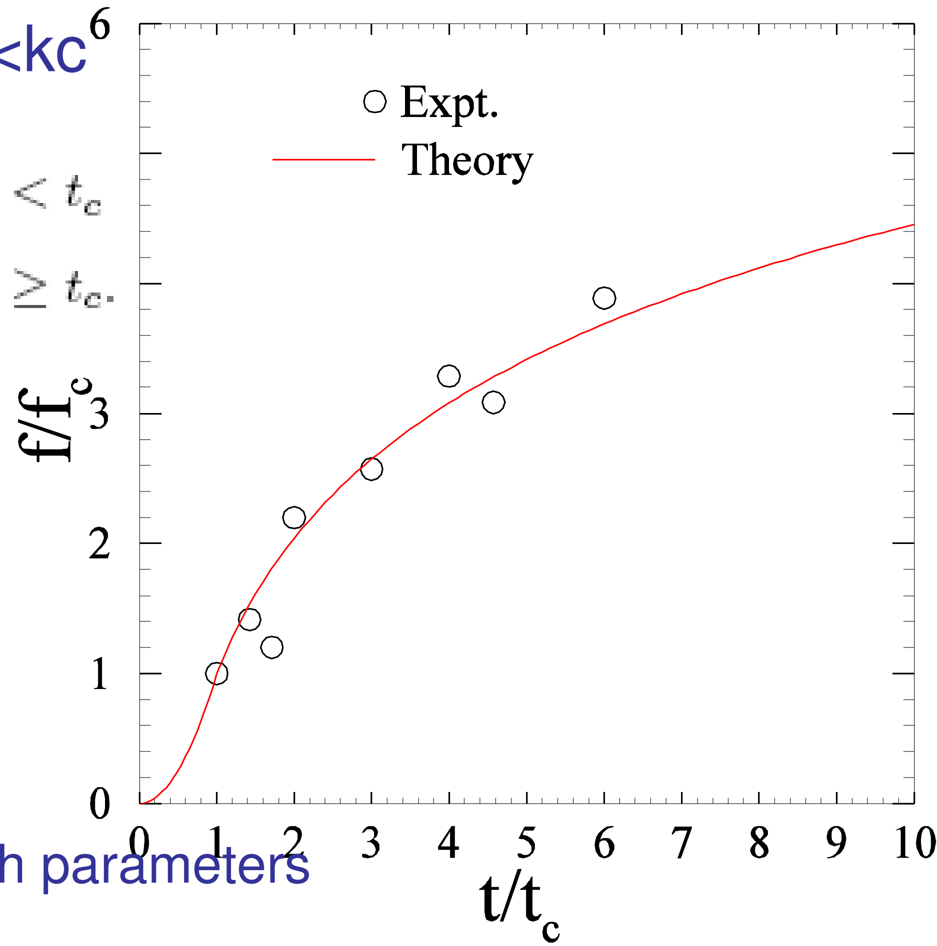
Expt: f=fc+fo log(t/tc)
Assume k/kc=f/fc:
Assume P(k)~1 for k<kc

$$\frac{f}{f_c} = \frac{k}{k_c} = \begin{cases} \left(\frac{t}{t_c}\right)^2 & t < t_c \\ 1 + \frac{D}{u^2 t_c} \ell n \left(\frac{t}{t_c}\right) & t \geq t_c. \end{cases}$$

In general with $P(k) = \tilde{P}(k/k_c)$ for k<kc

$$\frac{f}{f_c} = \frac{k}{k_c} = \begin{cases} \Phi^{-1}[\Phi(1)\left(\frac{t}{t_c}\right)^2] & t < t_c \\ 1 + \frac{D\Phi(1)P_c}{u^2 t_c} \ell n \left(\frac{t}{t_c}\right) & t \geq t_c. \end{cases}$$

$$\Phi(x) \equiv \int_0^x \frac{dx}{\tilde{P}(x)}$$



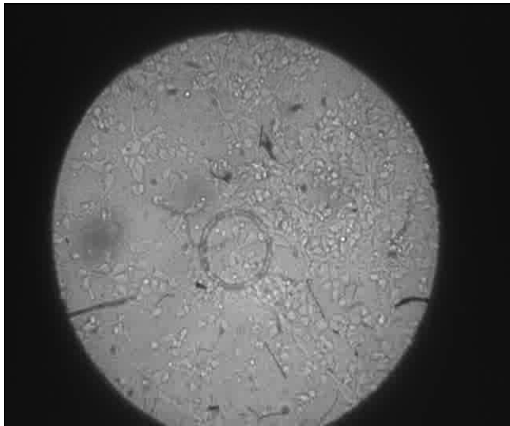
Fitting: fo/fc=ko/kc=D/(u^2 tc)~1.5
relation between the microscopic growth parameters

Estimates of *u* and *D*

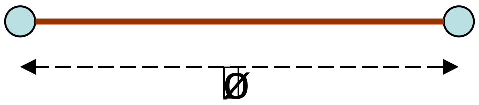
- minimal radius, r_c, of an isolated domain such that SF still occur determined by UV laser tailoring
- For ρ =10^4/mm^2, we found r_c~0.15mm

$$(ut_c)^2\rho \sim N_c \sim r_c^2\rho. \qquad D \sim u^2 t_c f_o / f_c$$

- *u* ~ 25μm/day; D~0.0056mm^2/day



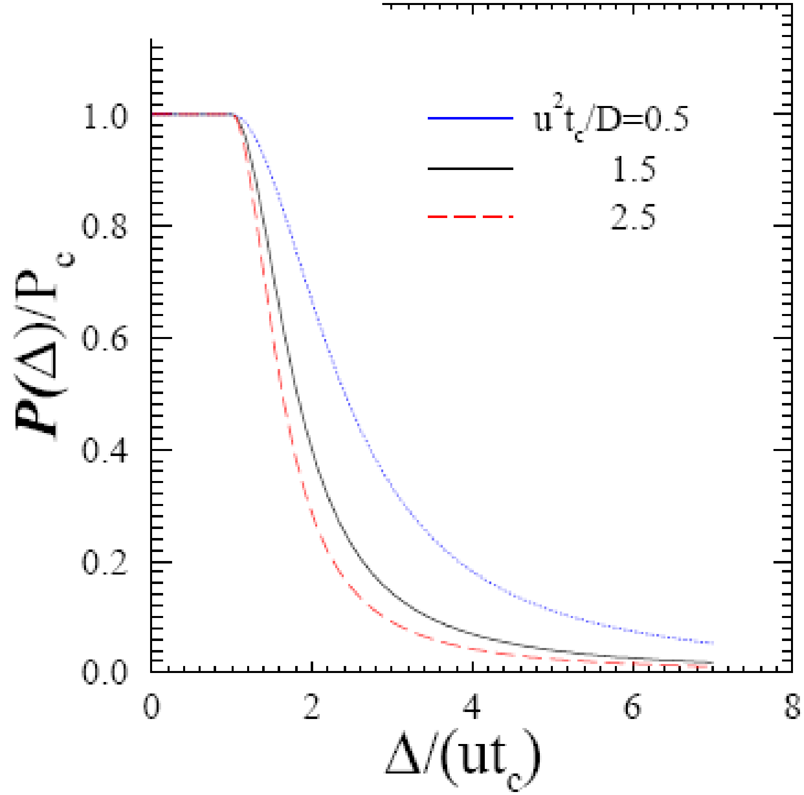
Coupling between neurons



- ***P***(Δ)=mean prob. that 2 neurons initially separated by Δ will be connected

$$\mathcal{P}(\Delta) = \tilde{P}\left(\Phi^{-1}\left(\Phi(1)(\frac{\Delta}{ut_c})^2\right)\right) = \Phi^{-1\prime}\left(\frac{\pi\rho\Delta^2}{k_c}\right) \qquad \text{for} \qquad \Delta < ut_c$$

$$\mathcal{P}(\Delta) = \frac{P_c}{1 + \frac{(\Delta - ut_c)^2}{Dt_c}} \qquad \text{for} \qquad \Delta > ut_c.$$

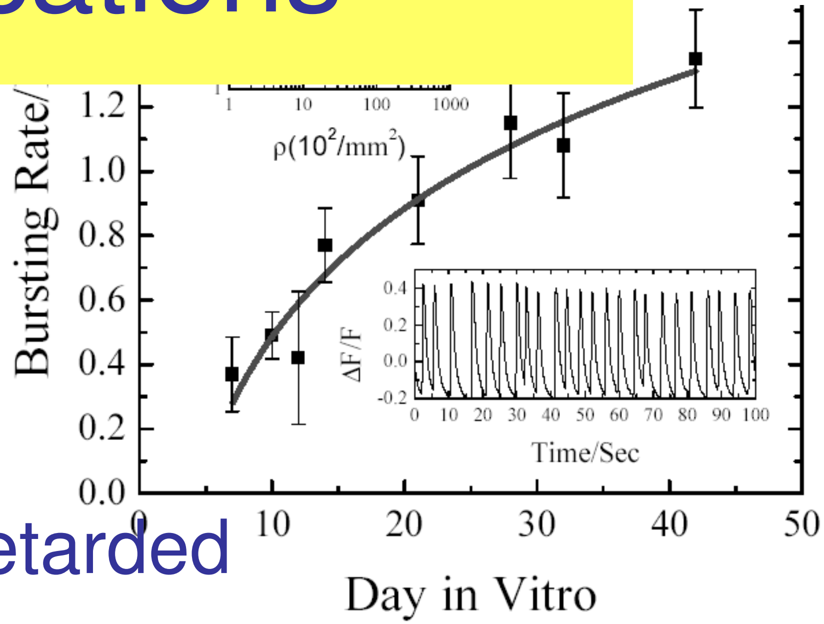


- Characteristic coupling length

$$\Delta_c = ut_c + \sqrt{Dt_c}.$$

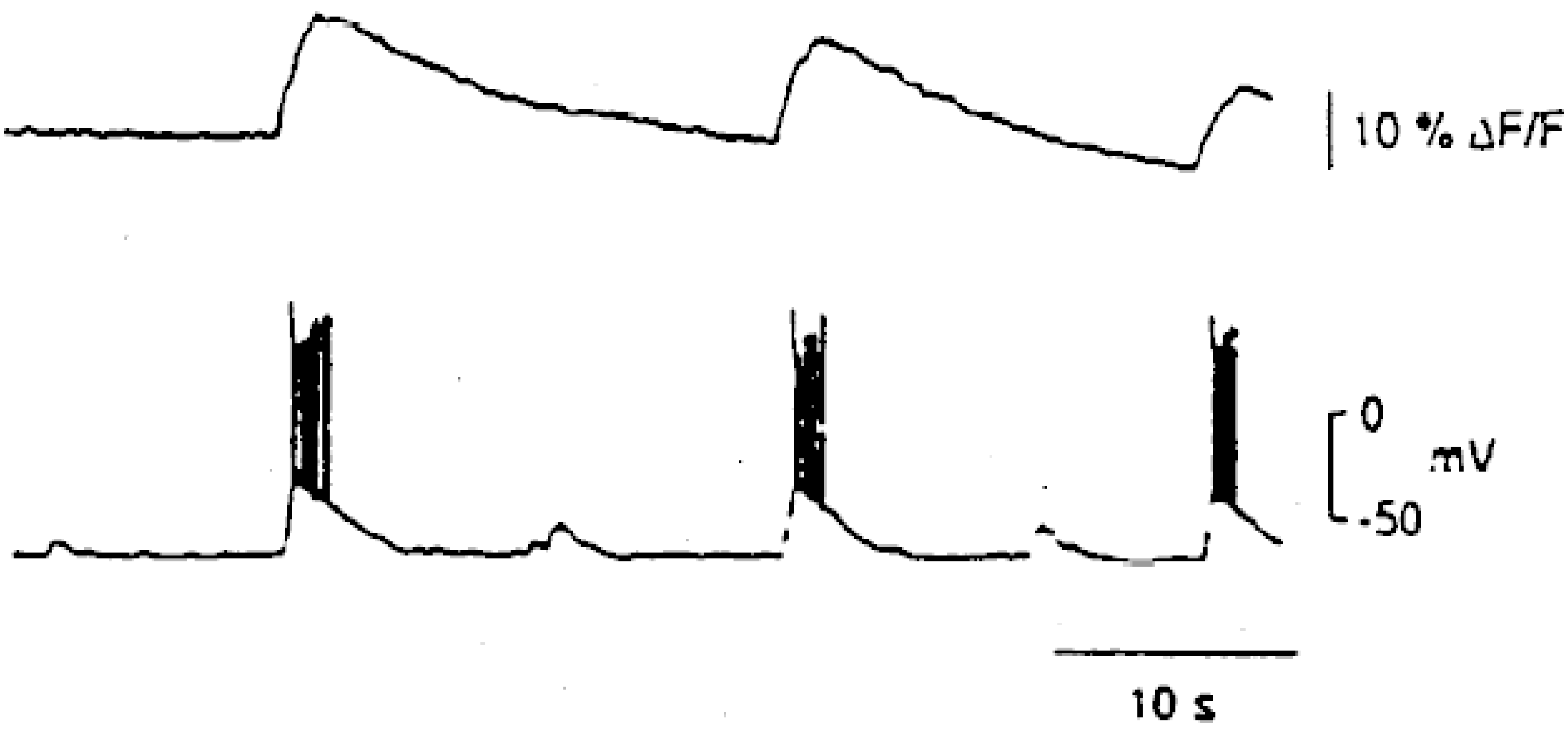
- Δc ~ 0.33mm ~ 2 rc

Biological implications

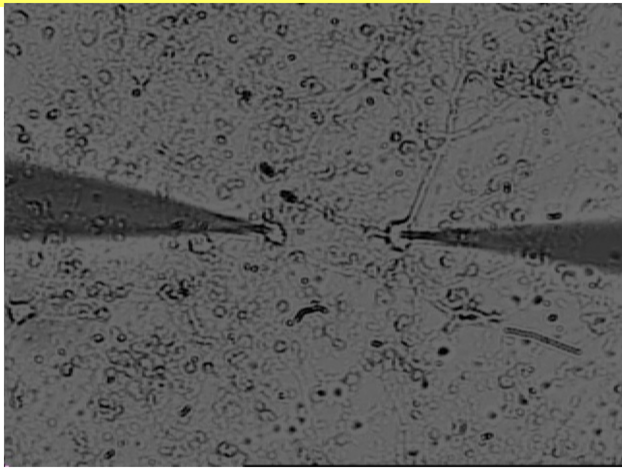


- Active growth in early stage, retarded once goal is achieved.
- Slowing down to maintain a long time span for function: homeostasis
- Continuing fast growth used up energy
- Too much connections may exceed information capacity for a single neuron

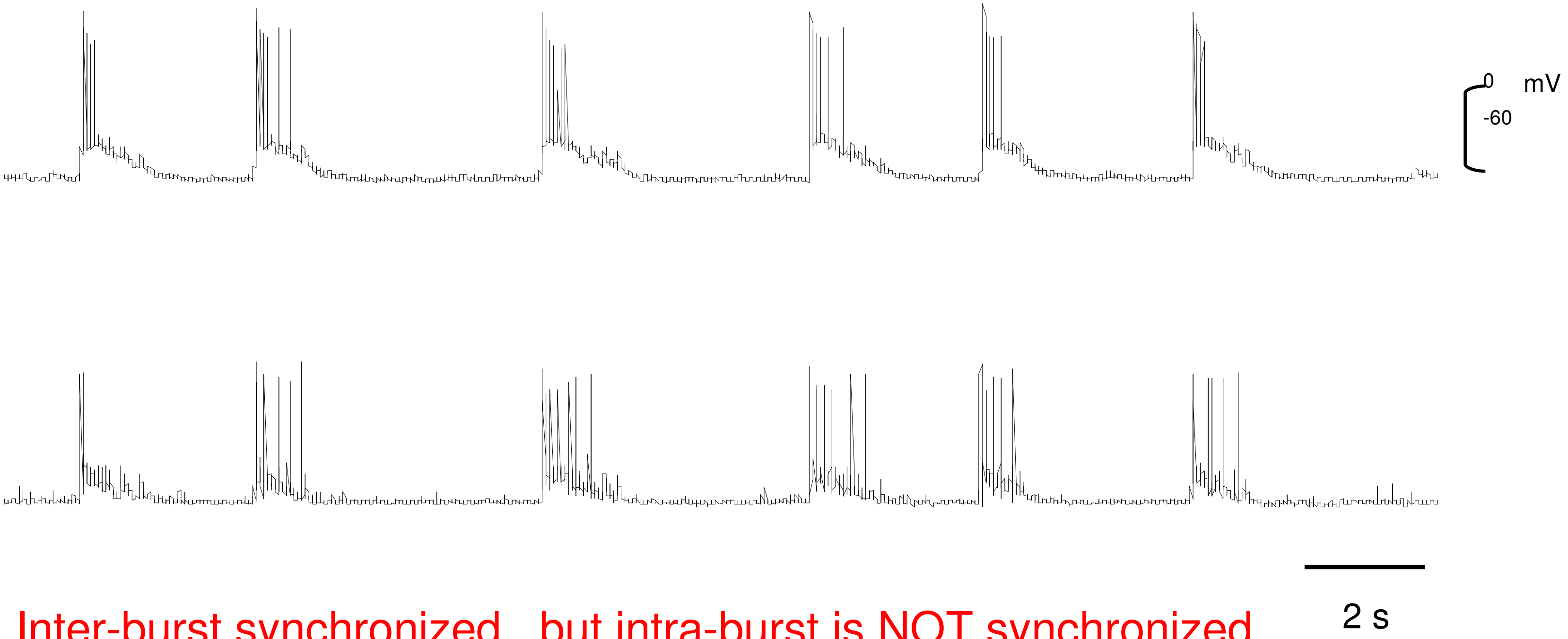
Many Spikes in one pulse: Bursting



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



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



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

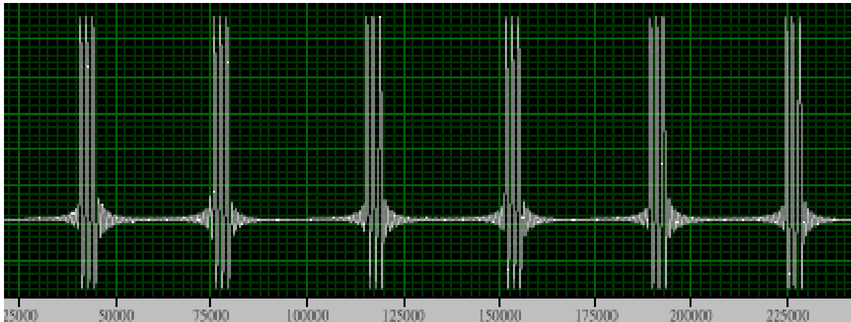
Bursting: role of inhibitory element

- Continuous firing (over excited) is harmful--excitotoxicity
- Inhibitors (Glia) suppress over-excited neurons
- g=inhibitory field
- Mean-field model
- z=mean connectivity of a neuron to inhibitors

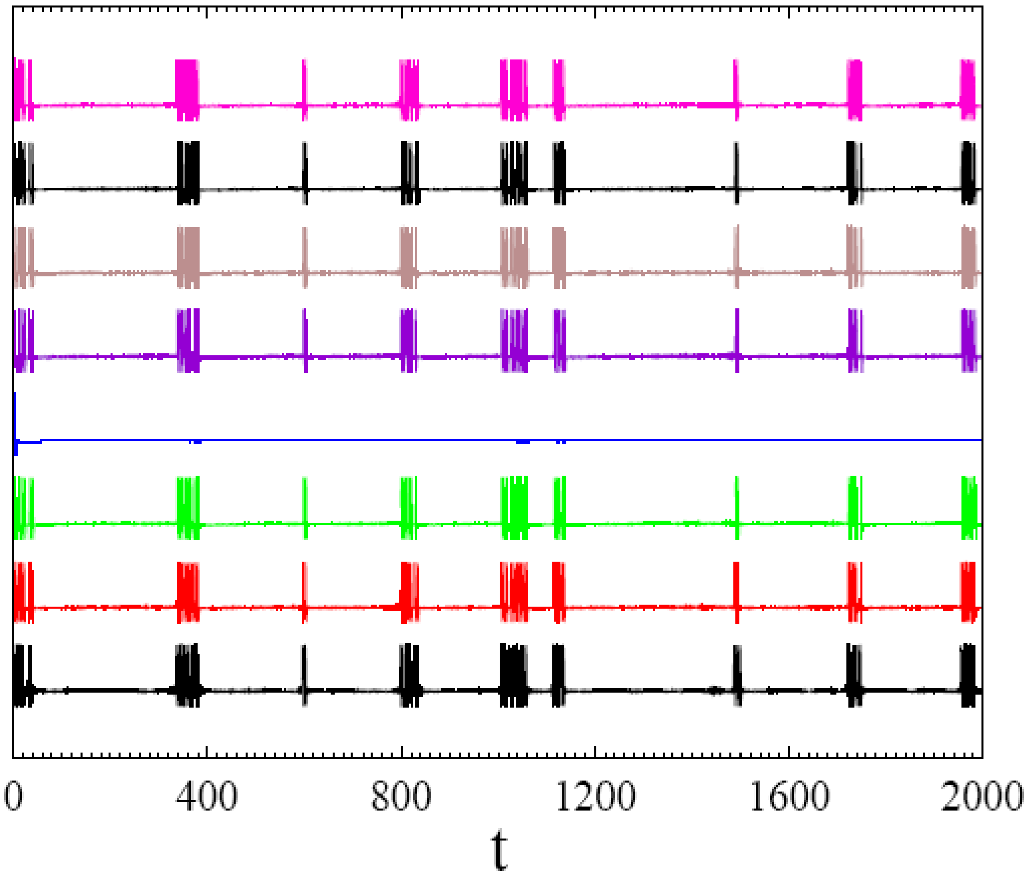
$$
\begin{aligned}
\epsilon \frac{dx}{dt} &= x - \frac{x^3}{3} - y - zg + \xi(t) \\
\frac{dy}{dt} &= a + bx - y \\
\frac{dg}{dt} &= -\frac{g}{\tau} + \gamma\, \Theta\left(rzx - \theta\right)
\end{aligned}
$$

FitzHugh-Nagumo model for neurons

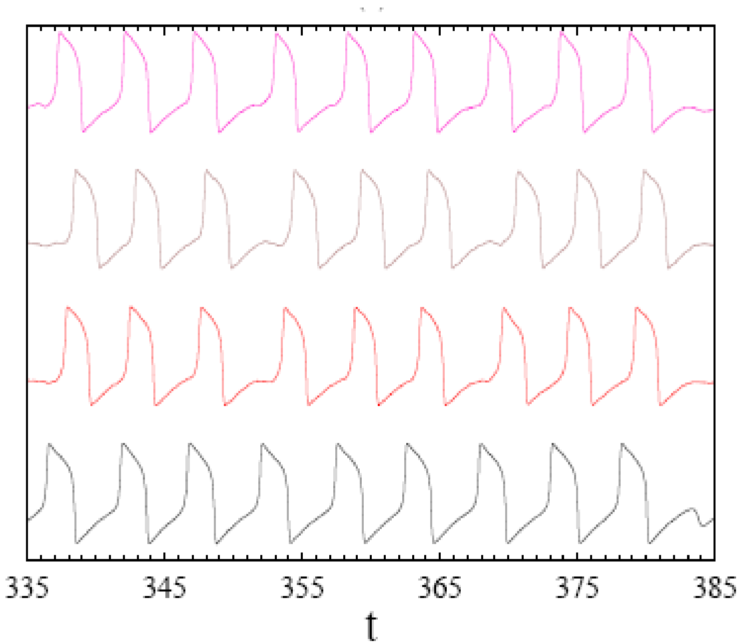
$$\Theta(u) = \frac{1}{2}\{1 + \tanh[\alpha u]\}$$



Network of neurons & inhibitors



Synchronized Bursting

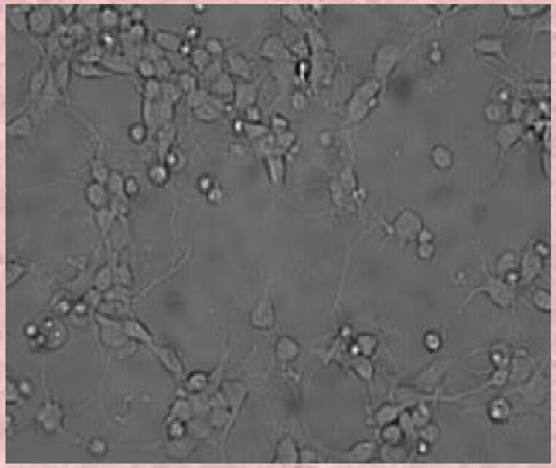


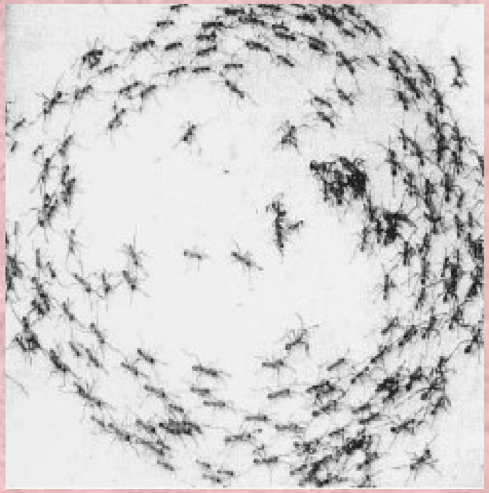
Intra-burst NOT synchronized

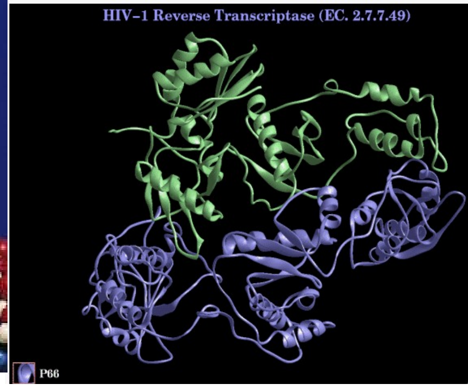
- **What Physicists can do in Biology ?**

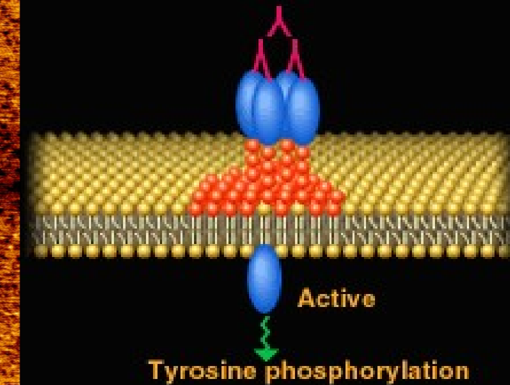
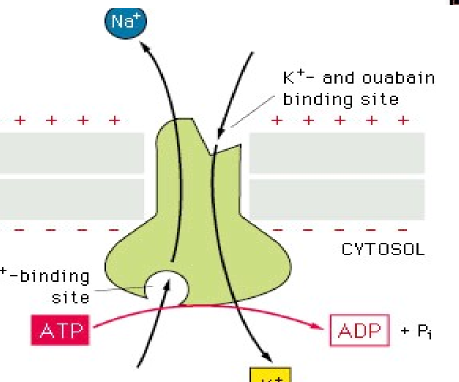
a lot of interesting and unexplored science

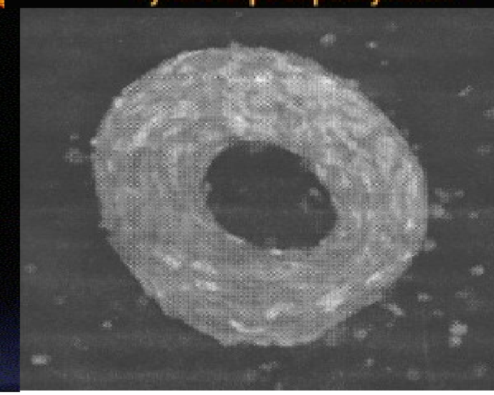
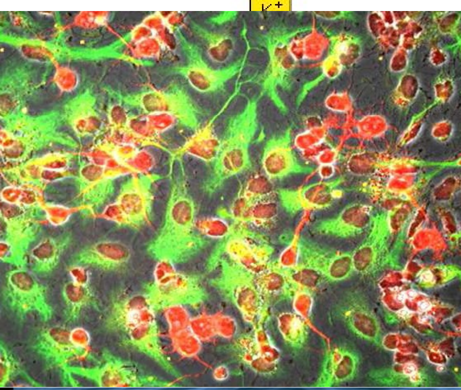
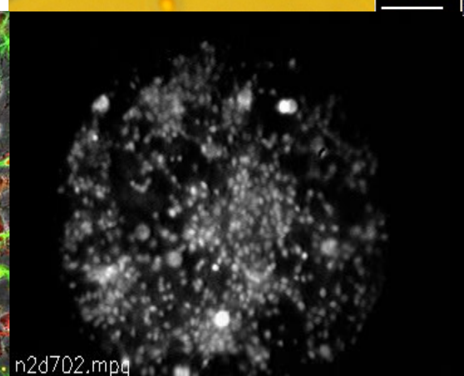
from molecules to collective behavior of organisms







HIV-1 Reverse Transcriptase (EC. 2.7.7.49)
P66


Active
Tyrosine phosphorylation

Na+
K+- and ouabain binding site
CYTOSOL
+-binding site
ATP
ADP + Pi
K+





n2d702.mpg




E

Acknowlegements

Collaborators

- C.K. Chan (陳志强) (Academa Sinica)
- L.C. Jia 賈魯强 (Yuanpei Univ.)
- Z.C. Zhou 周子聰 (Tamkang U.)
- Students: Y.S. Chou, H. H. Chang, C. R. Han, S.F. Hsu
- Postdocs: E. Avalos, J. Benoit

Support

National Science Council, Taiwan
Brain Research Center, U. Systems of Taiwan
Academia Sinica, Taiwan

The End