BIOFLEXOELECTRICITY: A PHYSICAL MOTOR OF THE LIVING CELL

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30 slides

- Several types of motors driving living cell alive have so far been recognized:
- electric motors, light motors,
- chemical motors,
- thermal motors,
- mechanical motors, etc. etc.

Recently, the existence of a new type of electro-mechanical motor has been recognized in cell membranes: bioflexoelectricity.

SUMMARY

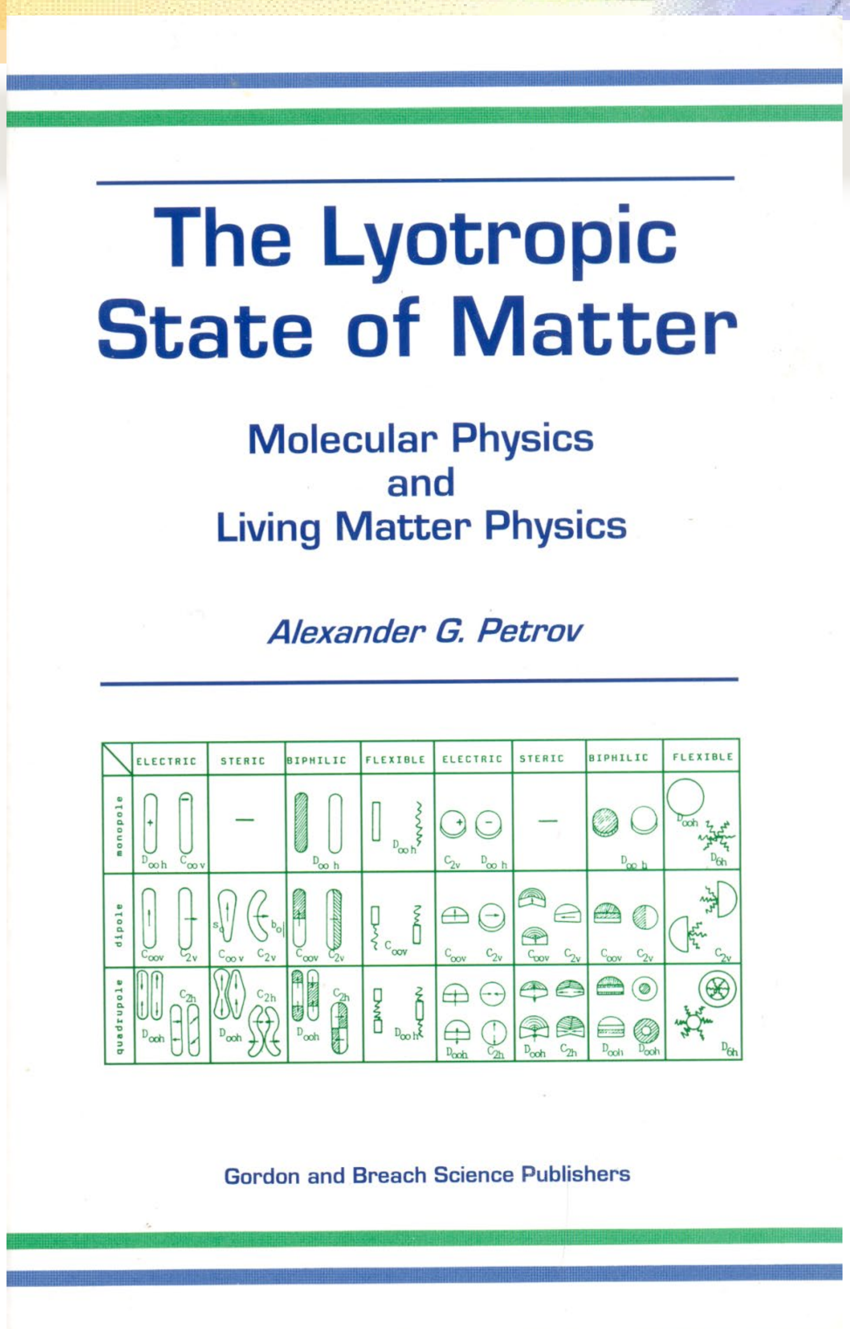
- Biological membrane as a liquid crystal mechanism. Flexoelectricity in living matter physics.
- Bioflexoelectricity: a reciprocal relationship between mechanical and electrical degree of freedom of a living membrane
- Direct flexoeffect (curvature $\rightarrow$ polarization)
- Converse flexoeffect (voltage $\rightarrow$ bending)
- Membrane machines. Outer hair cells and hearing. Protocells: giant lipid vesicles with 3 generalized degrees of freedom
- Conclusion

THE KNOWLEDGE OF BIOFLEXOELECTRICITY

From Soft Matter Physics to Living Matter Physics

- **The Lyotropic State of Matter: *Molecular Physics and Living Matter Physics***, by A.G. Petrov, Gordon & Breach Science Publishers, L.-N.Y. (1999), 549 pp.

  Lyotropic State of Matter (*eBook Edition*), Publish Date: 04/17/2007, Taylor & Francis, UK
- **Flexoelectricity of model and living membranes**, by A.G. Petrov, BBA - Rev.Biomembranes **1561**, 1-25 (2002)
- **Flexoelectricity in Lyotropics and in Living Liquid Crystals**, by A G. Petrov. In: Flexoelectricity, Chapter 6, N.Eber and A.Buka, Eds, Imperial Coll Press, Singapoure (2012), pp169-202.


The Lyotropic
State of Matter
Molecular Physics
and
Living Matter Physics
Alexander G. Petrov
ELECTRIC
STERIC
BIPHILIC
FLEXIBLE
monopole
dipole
quadrupole
Gordon and Breach Science Publishers

Living Matter Physics

starts with the book of

Erwin Schrödinger

What is Life?

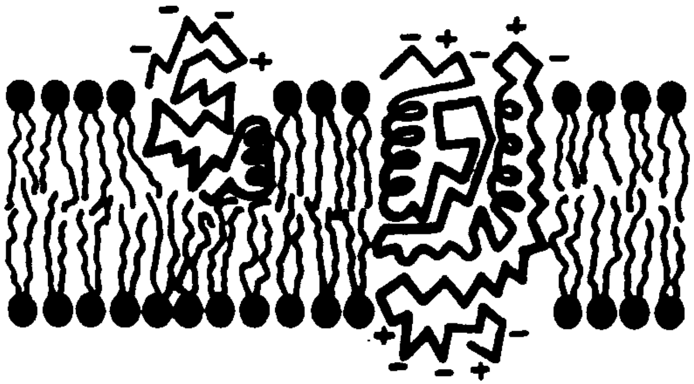
The Physical Aspect of the Living Cell (1943)

Schrödinger: Dynamical laws are most suitable for cells**.** A cell should function like a mechanism. Clock mechanism analogy. Solid state physics. Solid parts of cells: DNA molecules (1D aperiodic crystals)

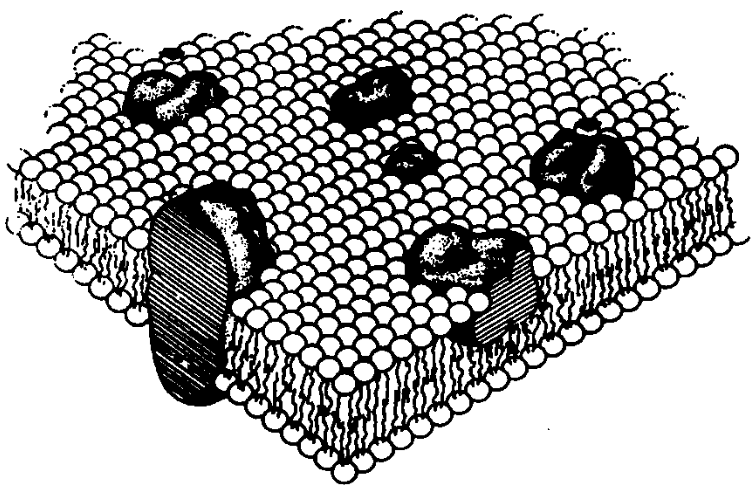
Next step: Liquid Crystal Physics: embracing lipids and proteins besides DNA. Cellular mechanisms: need the existence of a

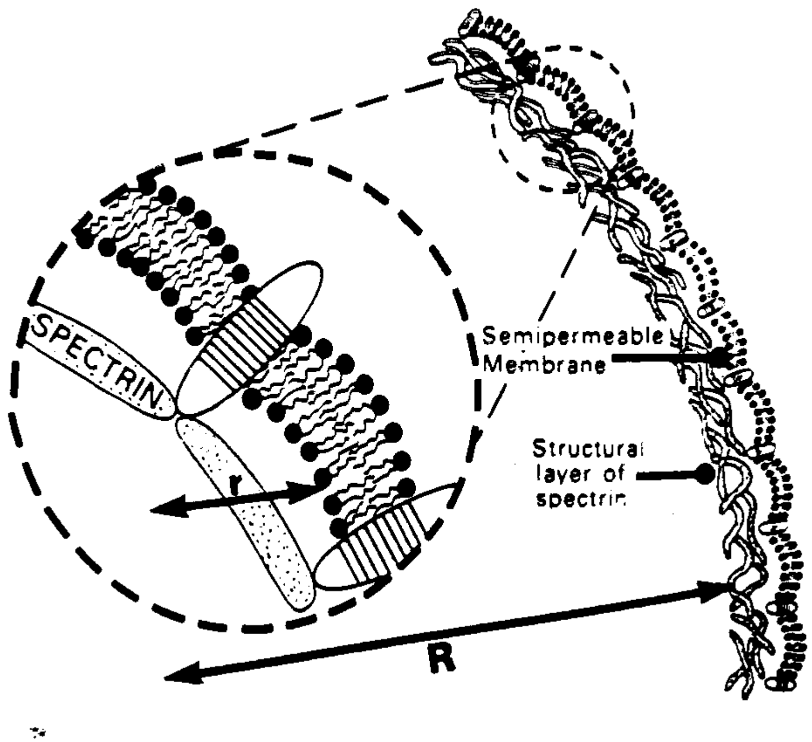
Mechanical degree of freedom

CELL MEMBRANE

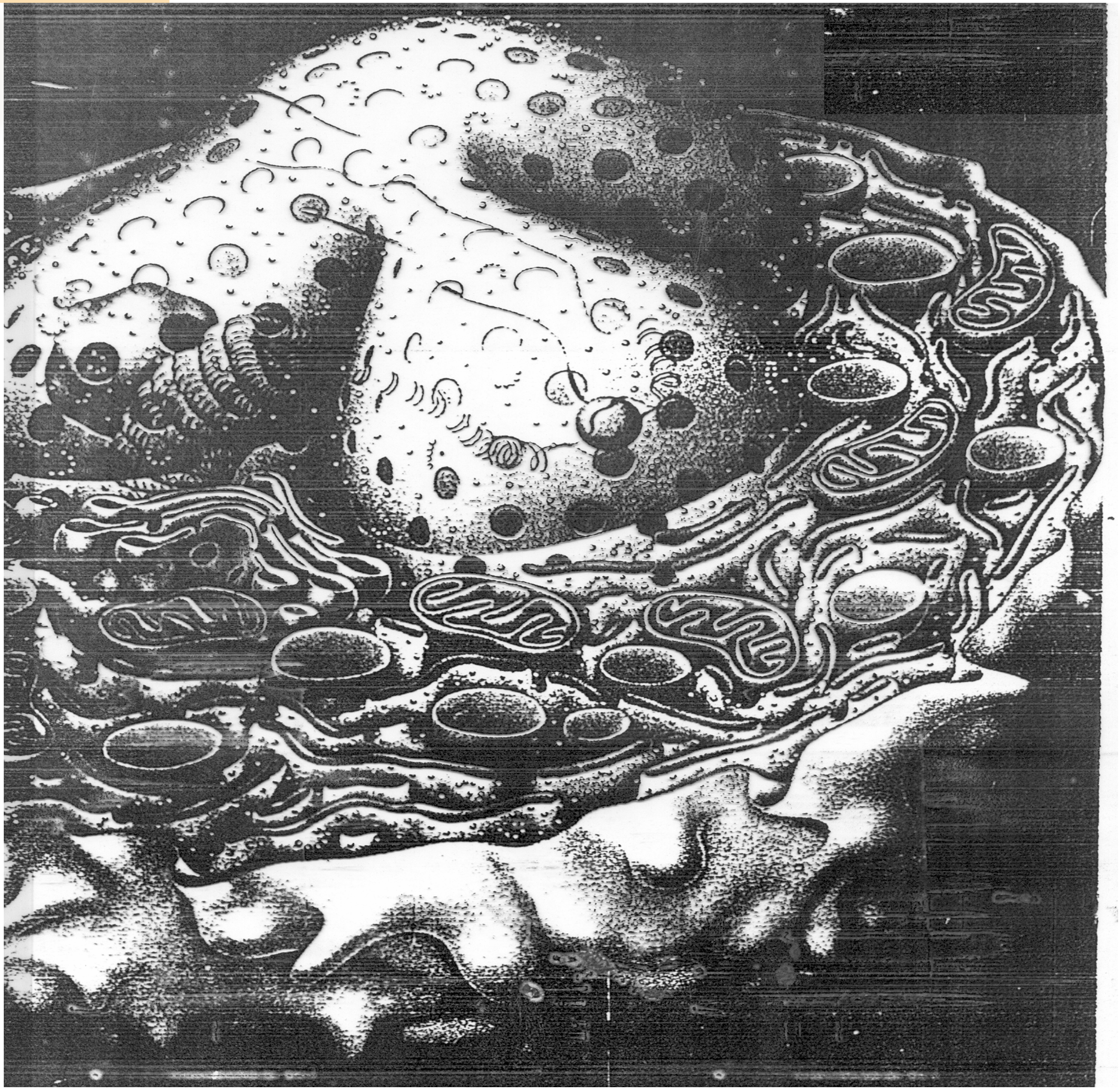


Singer and Nicolson (1972):
Fluid Lipid Globular Protein
Mosaic Model of Membranes





Membrane and cytoskeleton



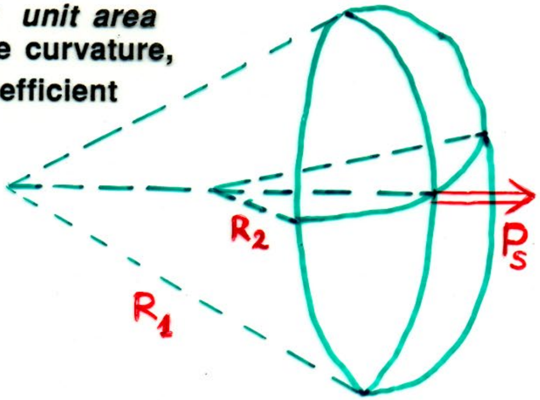
Curved membranes in a living cell

PHENOMENOLOGY (Petrov, 1975) :

$$P_S = f\,(1/R_1 + 1/R_2)$$

cf. R.B.Meyer (1969)

P_S is membrane polarization per *unit area*
R_1 , R_2 are the radii of membrane curvature,
f is membrane flexoelectric coefficient



Dimensions:

$$[P_S] = C\,.\,m^{-1}$$
$$[f] = C$$

f *ca.* $1\,.\,10^{-18}$ C

$f \approx e.d$

f = e.d

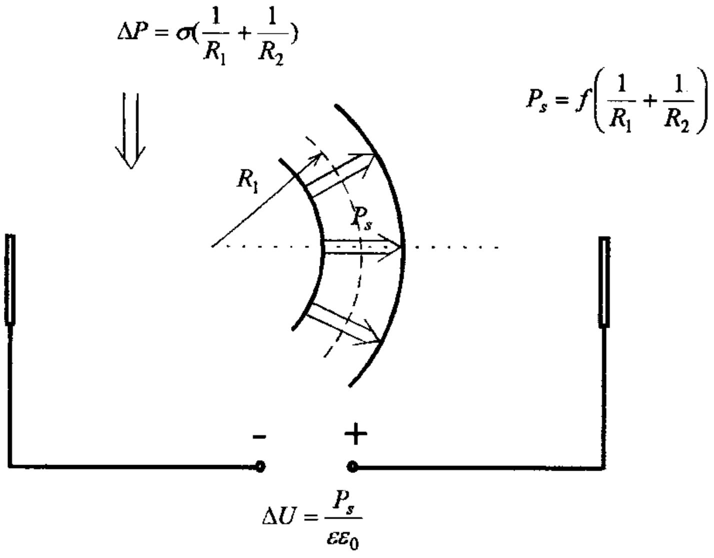
For spherical curvature of radius R the transmembrane flexoelectric voltage is :

$$\Delta U = P_S/\varepsilon_0 = (f/\varepsilon_0)(2/R)$$

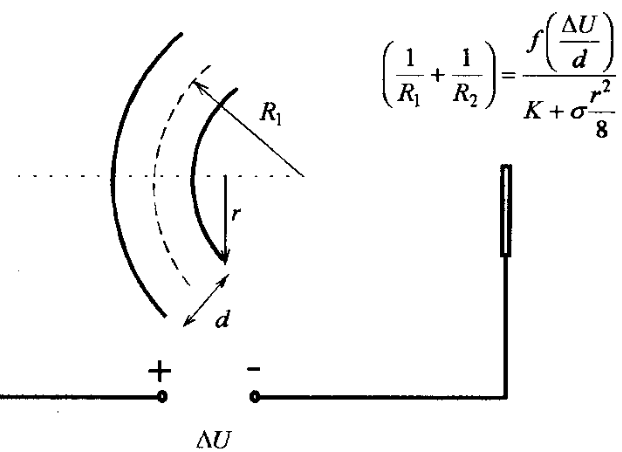
Helmholtz eqn.

ΔU *ca.* 200 μV for R = 1 mm
ΔU *ca.* 20 mV for R = 10 μm

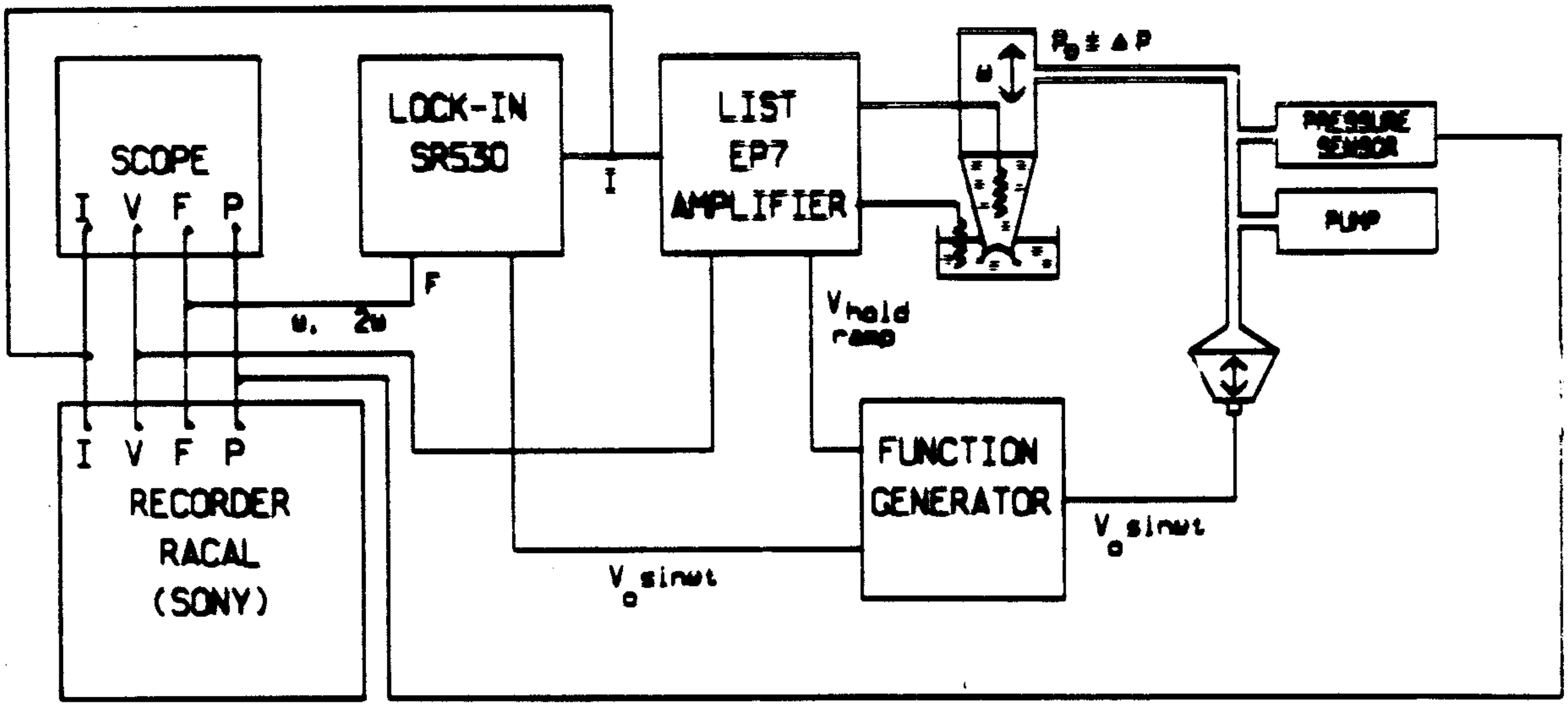
DIRECT FLEXOEFFECT



CONVERSE FLEXOEFFECT



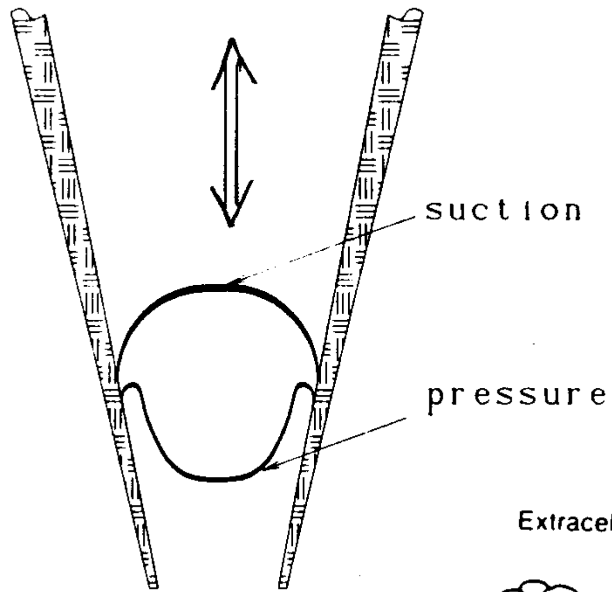
DIRECT FLEXOEFFECT IN NATIVE MEMBRANE PATCHES – EXPERIMENTAL SET-UP

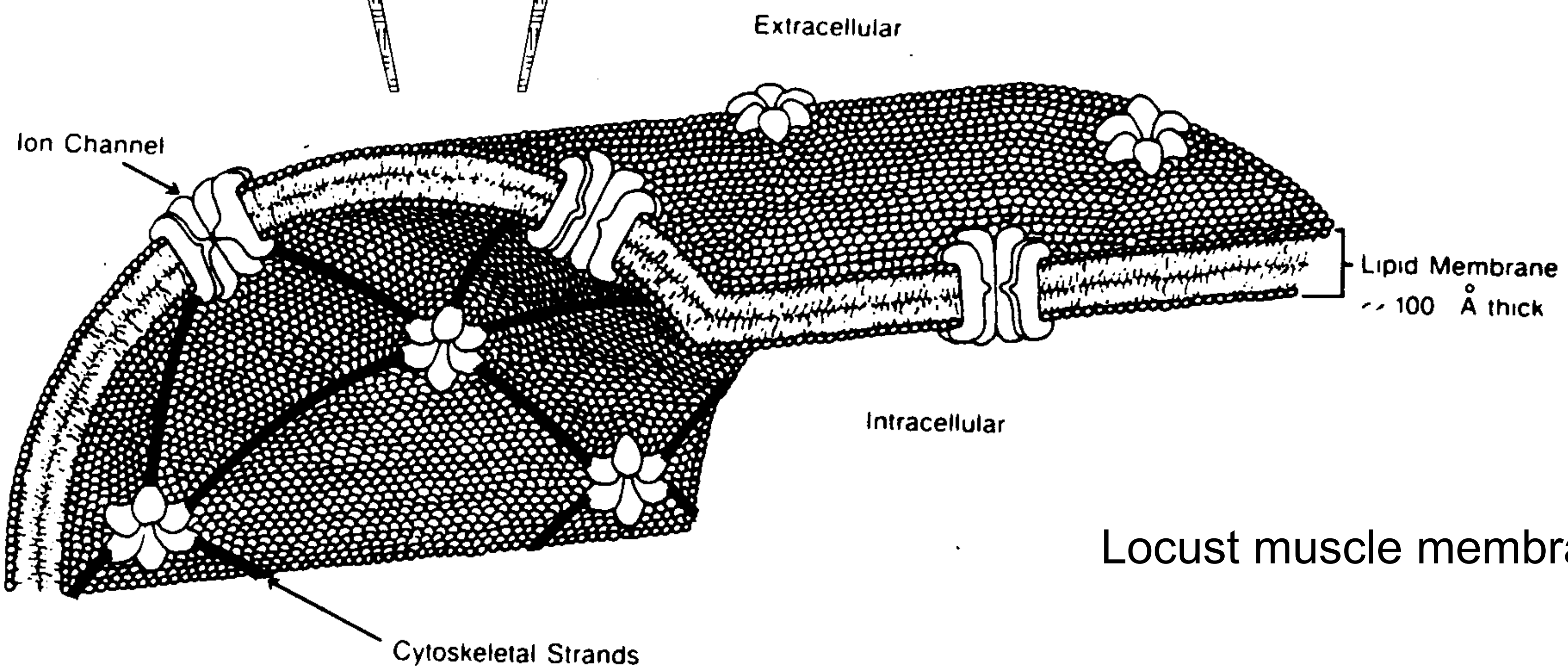


Patch clamp + oscillation pressure technique
Petrov, Usherwood et al., EBJ, 1990ies

$P_0 \sin \omega t$

$I = S\, dP/dt = \omega S\, (P_0 / d) \cos\omega t$





Locust muscle membrane

Locust

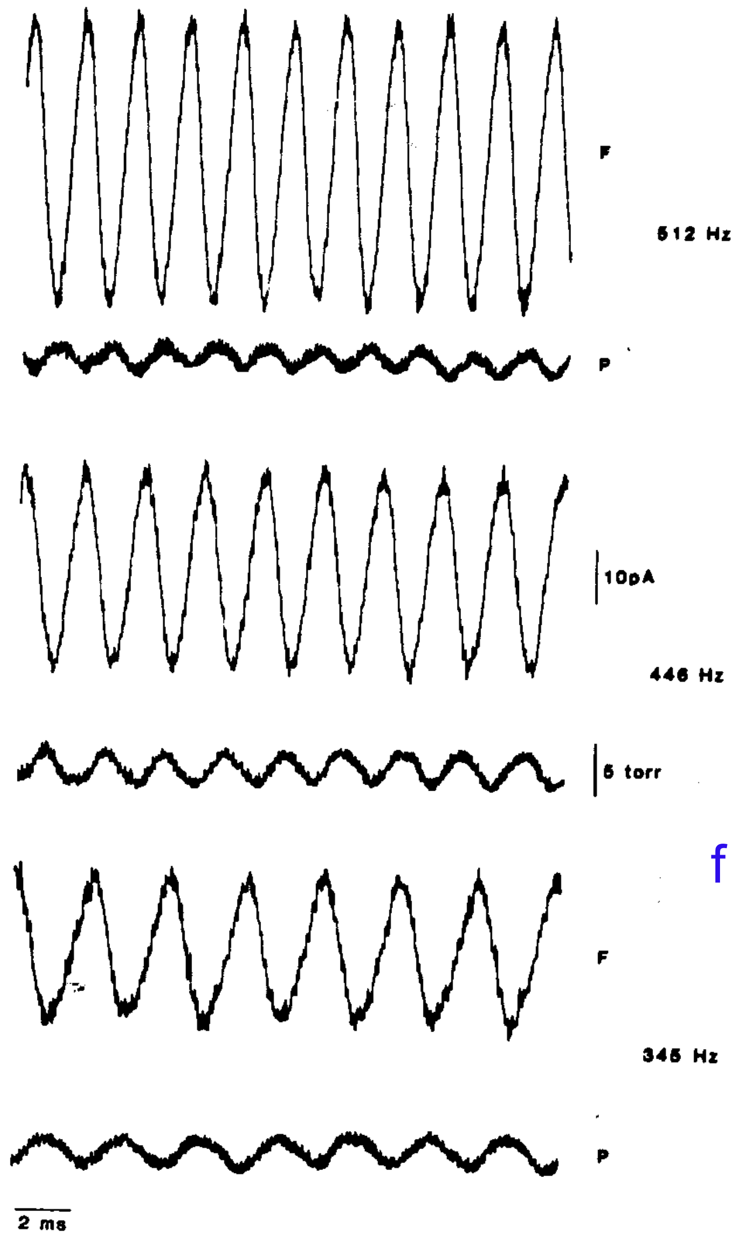
muscle

flexo-

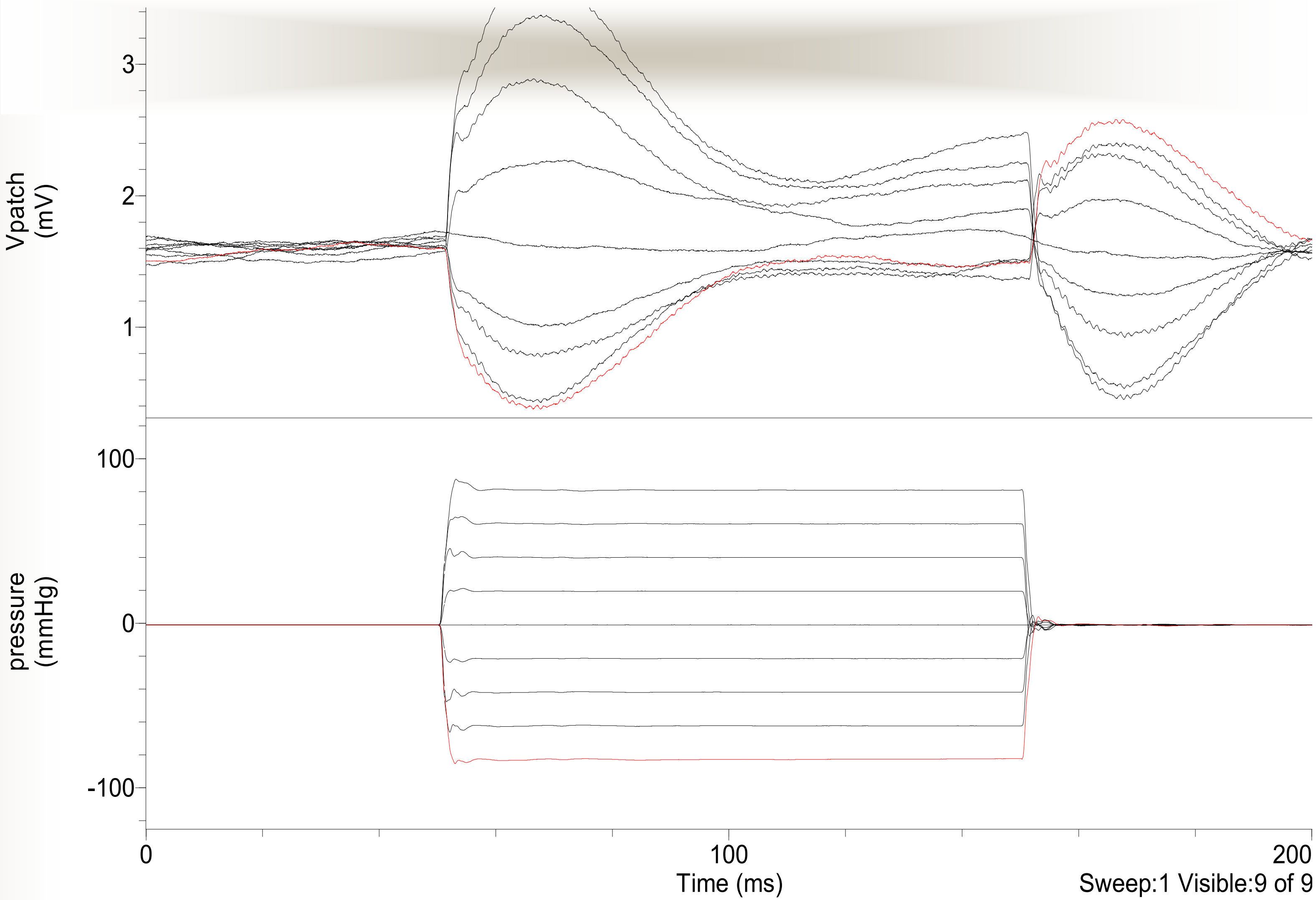
response

at higher

frequency



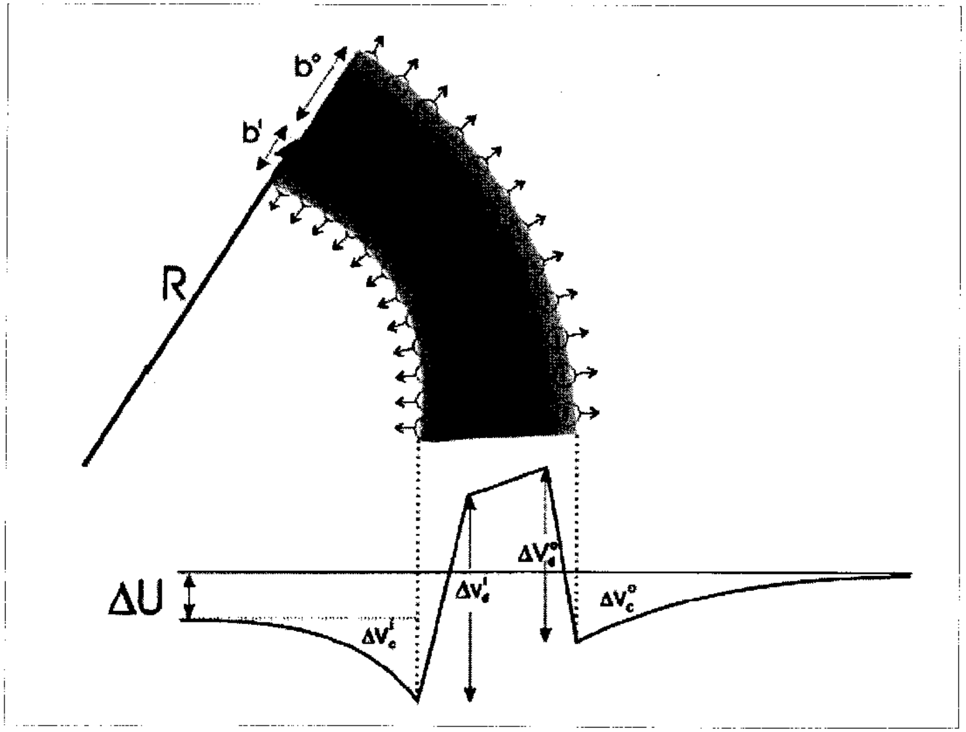
f = 2,5 (±50%)·10^{-18}C



Astro I/O p3, 0 holding current, NaCl-Bath, NaCl-Pip

The converse flexoelectric effect

Includes a differential change in surface tension at the two membrane interfaces



Petrov & Sachs, PhysRevE, 2002

CONVERSE FLEXOEFFECT: EXPERIMENTS

Voltage induced curvature:

Tension-free membrane :

$$(2/R) = (f/K)(U/d)$$

R - radius of curvature
f - flexocoefficient
K - curvature elasticity modulus
U - transmembrane voltage
d - membrane thickness

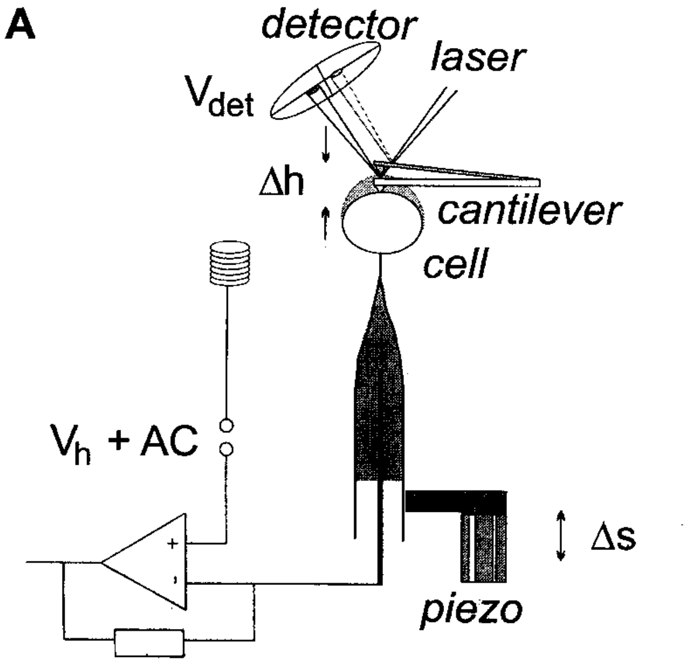
Membrane under lateral tension :

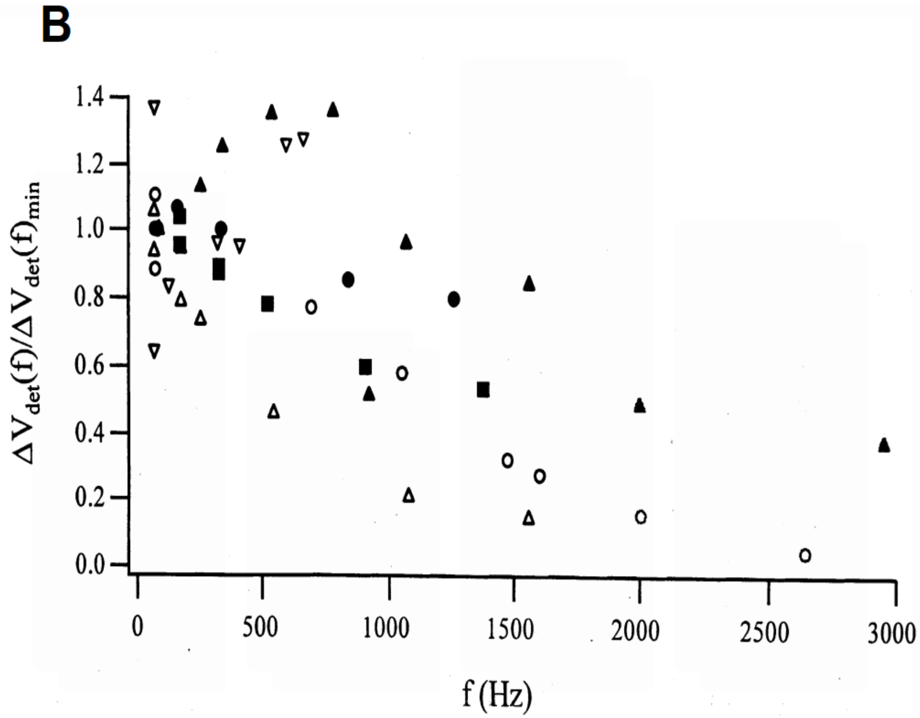
$$(2/R) = (4\,fU/d + p\,r^2/2)\;/(4K + \sigma\,r^2/2)$$

10^{-19} J vs. 10^{-9} J

p - transmembrane hydrostatic pressure difference
σ – lateral membrane tension
r - membrane radius

AFM and converse flexoeffect

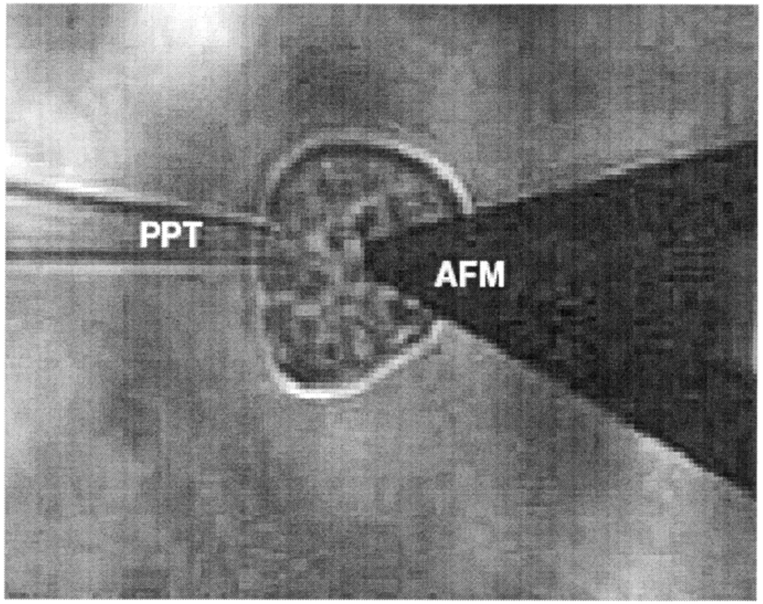


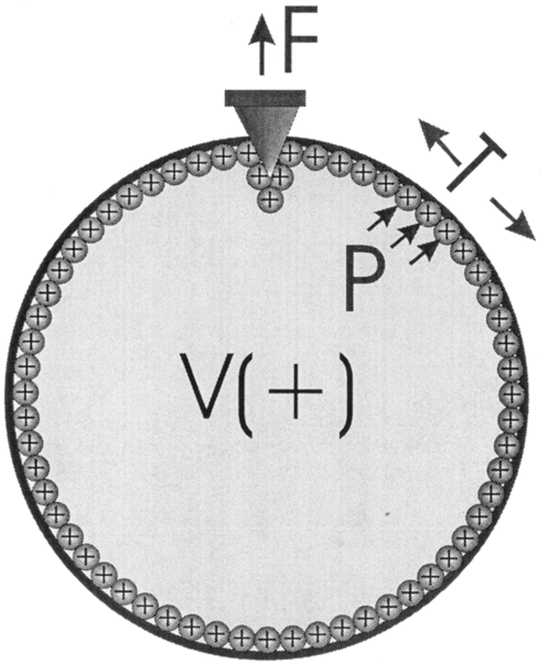


J. Mosbacher, M. Langer, J.K.H. Horber, F. Sachs, J. Gen. Physiol. 111 (1998) 65-74.

HEK electromotility

measured under voltage clamp with AFM





P.-Ch. Zhang, A.M. Keleshian, F. Sachs,

Nature, 413(2001)428.

Zhang et al., 2001
Mosbacher et al., 1998

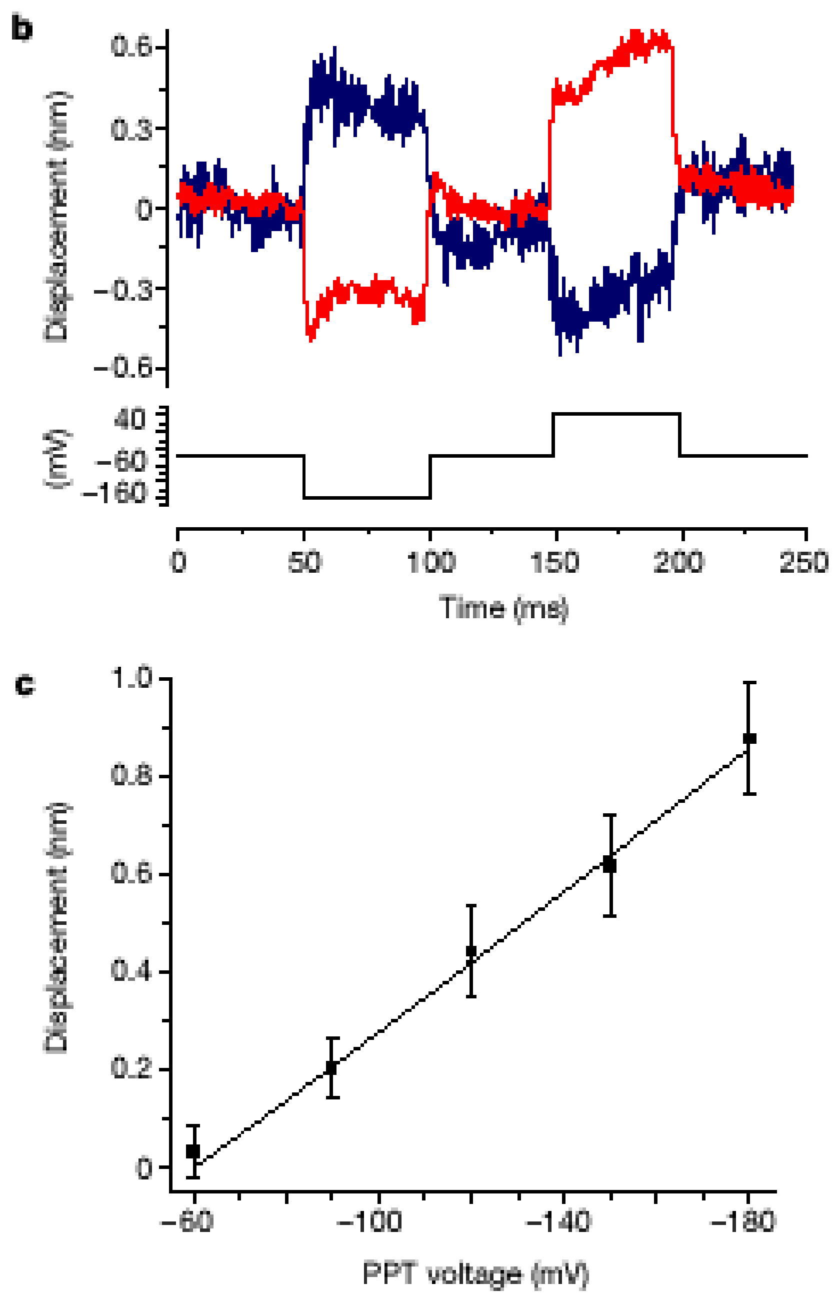


Figure 1 Voltage-induced membrane movement in HEK293 cells. **a**, Micrograph of a voltage-clamped cell with the cantilever (AFM) in place. The pipette (PPT) can be seen on the left side. **b**, Representative movements driven by ± 100-mV voltage pulses from a holding potential of −60 mV at normal (blue trace) and nominal 0 mM (red trace) ionic strengths. A positive displacement corresponds to the cantilever moving into the cell. **c**, The movement is linear with voltage (normal ionic strength). Solid line is the fit from the model (see text). **d**, Movement increases with indentation force. Solid line is the fit from the model, assuming hertzian mechanics (see text). Error bars represent the s.d.

PHYSICAL REVIEW E, VOLUME 65, 021905 - 10 (2002)

Flexoelectricity and elasticity of asymmetric biomembranes

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(Received 6 July 2001)

In view of the well-established charge and dipolar asymmetry of the two leaflets of a native membrane, the theory of flexoelectricity (and curvature elasticity) is extended to take into account this asymmetry using linear and nonlinear forms of the Poisson-Boltzmann equation. The results are discussed with respect to data from atomic force microscopy studies of electromotility in biomembranes.

DOI: 10.1103/PhysRevE.65.0119XX PACS number(s): 87.16.Dg, 87.10.+e

INTRODUCTION

The theory of flexoelectricity has been developed and tested with symmetric lipid bilayers [1,2]. Recent flexoelectric models of electromotility in outer hair cells of the cochlea [3] and atomic force microscopy (AFM) measurements in native membranes [4,5] call for an extension of this theory to the asymmetric situation. It is well established that the composition of outer and inner leaflets are asymmetric [6]. To more fully understand the mechanics, the curvature elasticity also needs to be generalized.

FLEXOELECTRICITY

Flexoelectricity is a mechanoelectric phenomenon known from liquid crystal physics. In the case of a membrane, flexoelectricity refers to the curvature-dependent membrane polarization [1,2],

$$P_S = f(c_1 + c_2), \quad (1)$$

where P_S is the electric polarization per unit area in C/m, c_1 and c_2 are the two principal radii of membrane curvature in m^{-1}, and f is the area flexoelectric coefficient in C (coulombs), typically a few units of electron charge. This effect is manifested in membrane structures where an overall curvature is related to splay deformation of the membrane molecules (lipids, proteins) (cf. [2]). Across a polarized membrane, a potential difference develops according to the Helmholtz equation. Its curvature-dependent part is

$$\Delta U = P_S/\varepsilon_0 = (f/\varepsilon_0)(c_1 + c_2). \quad (2)$$

By measuring simultaneously this potential difference and the curvature, one can determine the flexoelectric coefficient of a given membrane.

A general expression for the flexoelectric coefficient [7] expresses it as an integral of the curvature derivative of the distribution of membrane polarization along the membrane normal ($c_+ = c_1 + c_2$ is the total membrane curvature). A Taylor expansion of the total polarization with respect to total curvature is

$$P_S = \int P(z,c_+)dz = \int \left[P^0(z) + \left.\frac{\partial P}{\partial c_+}\right|_0 c_+ + \cdots \right] dz$$

$$= P_S^0 + \left[\int \left.\frac{\partial P(z,c_+)}{\partial c_+}\right|_0 dz \right] c_+ + \cdots$$

i.e., in view of definition (1),

$$f = \int_{-\infty}^{\infty} \left.\frac{dP(z,c_+)}{dc_+}\right|_0 dz. \quad (3)$$

Now, consider a membrane (Fig. 1) with an average surface charge density of the outer (inner) monolayer of $\sigma^o(\sigma^i)$, equivalent to a mean degree of ionization per lipid head of β^o (β^i, the sign of β being determined by the sign of σ). $\sigma^i = \beta^i e/A_0^i$, e is the proton charge, A_0^i is the area per lipid head in the *flat* state of the outer monolayer. For convenience, all surface charges are lumped in σ^o. The two membrane surfaces can be bathed by different ionic strength electrolytes, with corresponding Debye lengths λ_D^i (λ_D^o). Let the

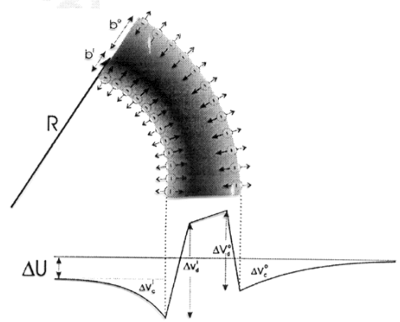
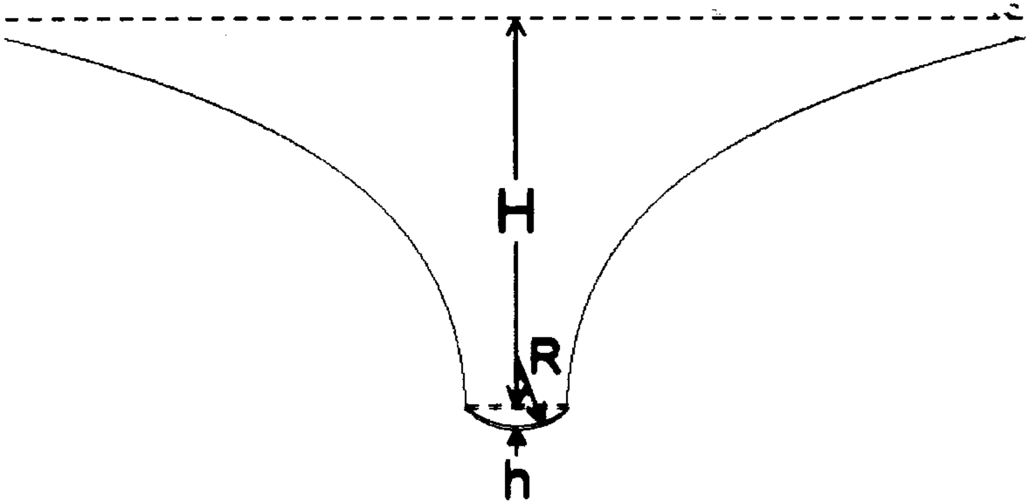
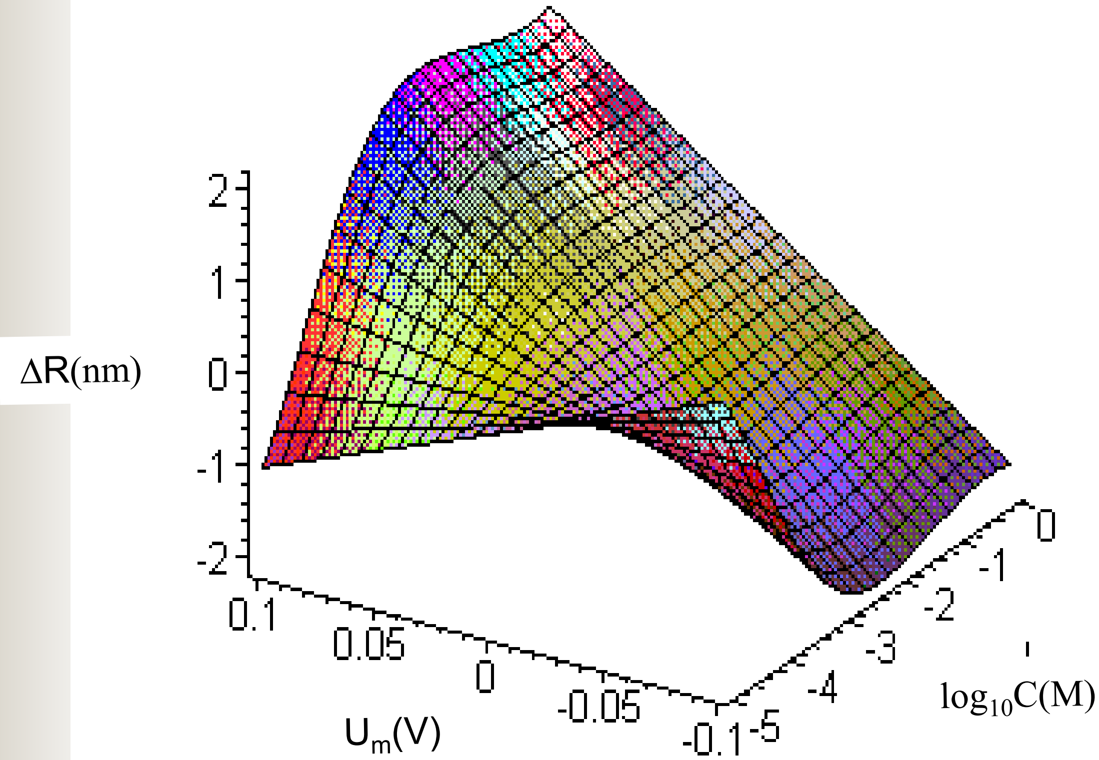


FIG. 1. Cartoon of a curved asymmetric bilayer membrane and its electric potential distribution. Double layer (i.e., charge, $\Delta V_c^{o,i}$) and dipole ($\Delta V_d^{o,i}$) components of the surface potential of each monolayer are indicated. ΔU is flexoelectric voltage, which is proportional to the membrane curvature $(2R)$ (see text) b^o and b^i are the distances between the mechanically neutral surface of the bilayer and the corresponding aqueous interface.

*On leave from Institute of Solid State Physics, Bulgarian Academy of Sciences, 72 Tzarigradsko chaussee, 1784 Sofia, Bulgaria.

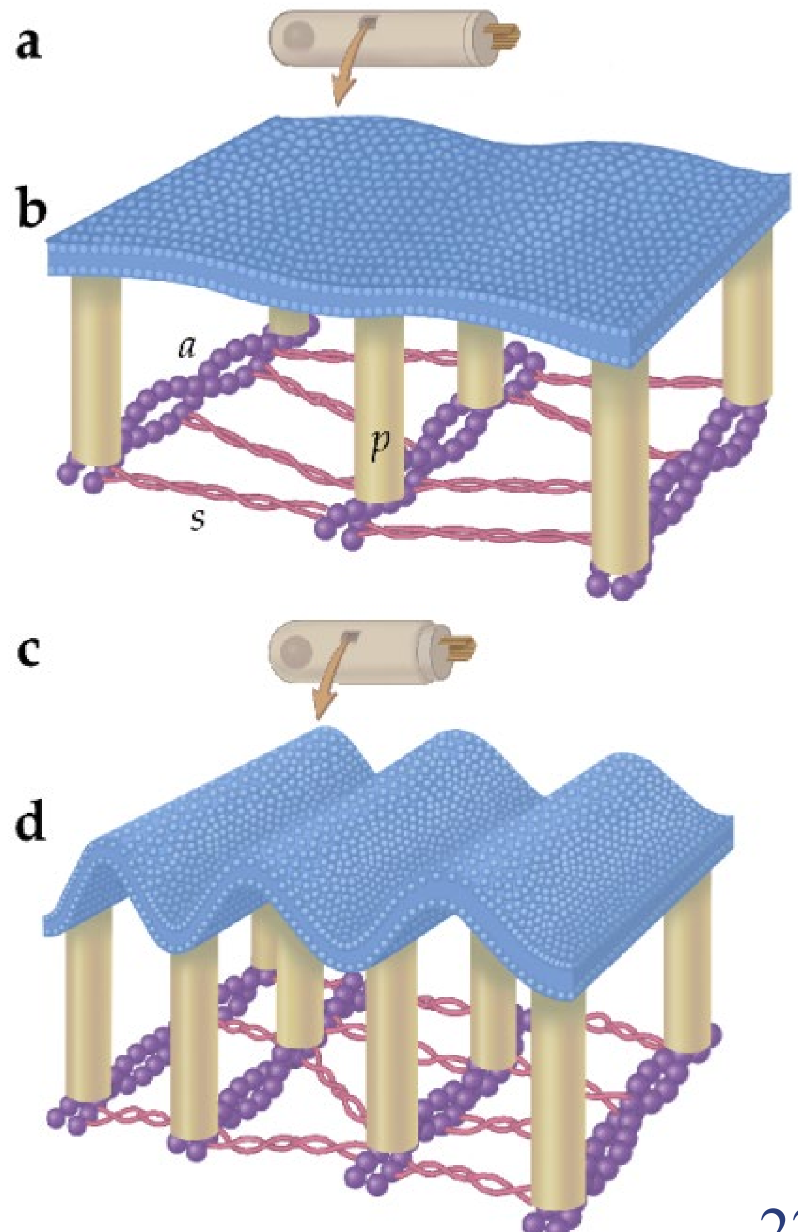

H
R
h


ΔR(nm)
2
1
0
-1
-2
0.1
0.05
0
-0.05
-0.1
Um(V)
-5
-4
-3
-2
-1
0
log10C(M)

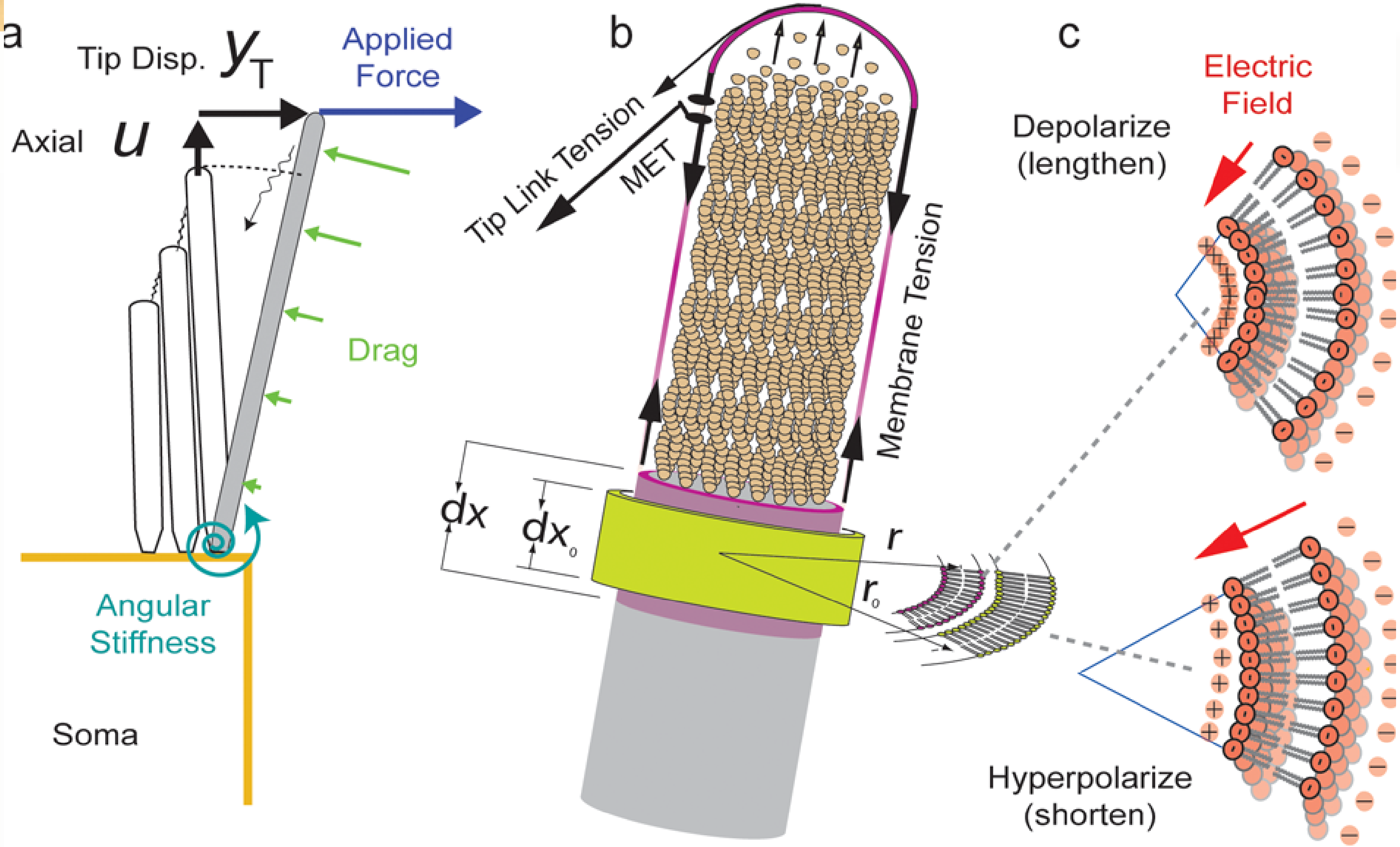
Flexoelectricity and hearing.

Bending Model of Outer Hair Cell Electromotility

Raphael, Popel, Brownell Biophys J, Vol 78, 2844–2862 (2000)



Circumferential ripples during length changes

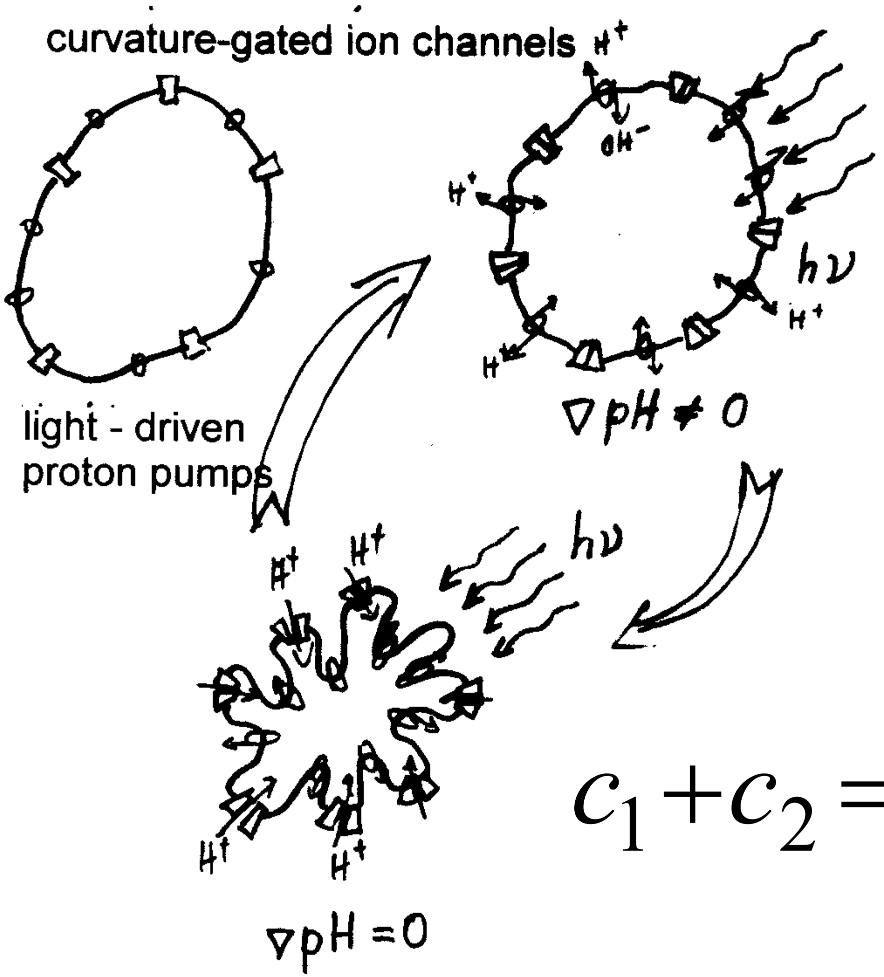


Hair Cell Bundles: Flexoelectric Motors of the Inner Ear.

Breneman KD, Brownell WE, Rabbitt RD, PLoS ONE 4(4): e5201 (2009) .

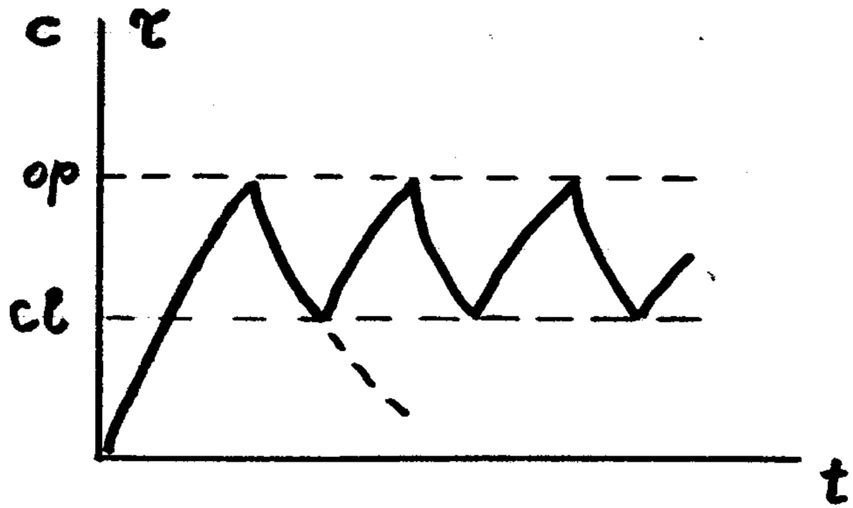
a) As an excitatory force is applied the bundle deflects towards the tallest stereocilia and the tip link tension increases. Tip displacement causes the MET to open, current (IT) to enter the stereocilia, thus leading to cable-like membrane depolarization. b–c) Through the membrane flexoelectric effect, depolarization compels a decrease in radius ($r_0 \rightarrow r$) and increase in height ($dx_0 \rightarrow dx$) under constant volume. Changes in length are accompanied by transverse motion due to the staircase gradient in stereocilia lengths and diagonal tip links.

FLEXO-ELECTRO-OPTIC MEMBRANE MACHINE

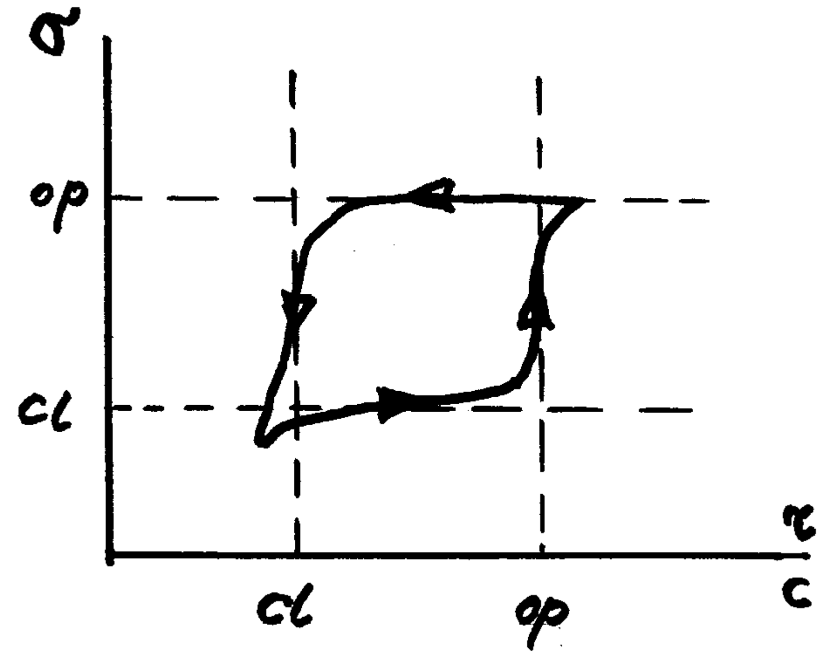


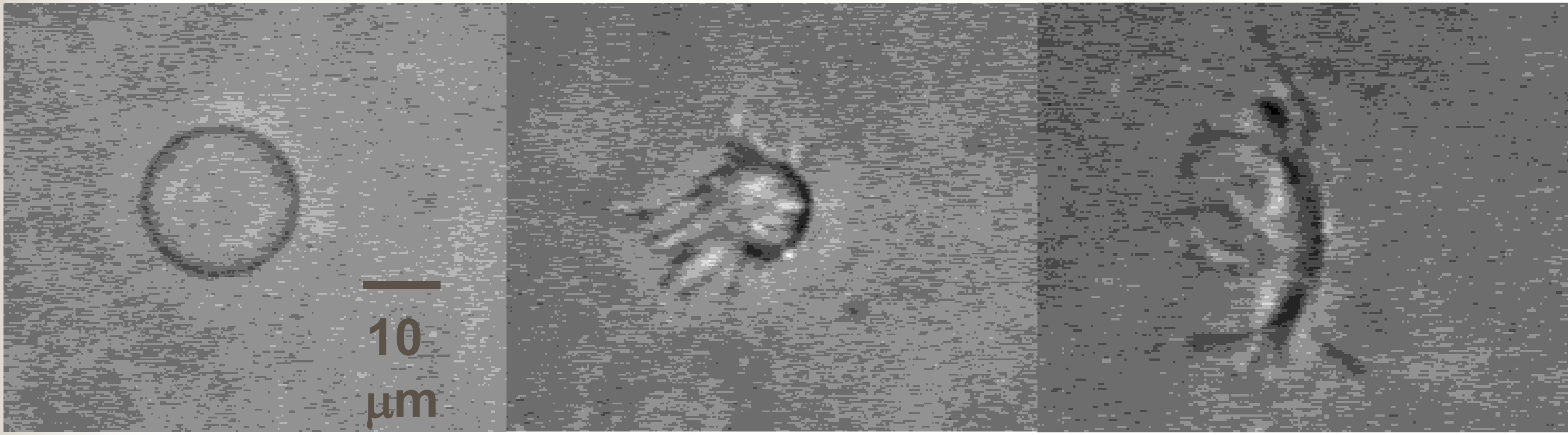
$$c_1 + c_2 = \frac{f}{K}\frac{\Delta U}{d}$$

Curvature vs. time



Conductance vs. curvature





0 sec **15 sec** **30 sec**

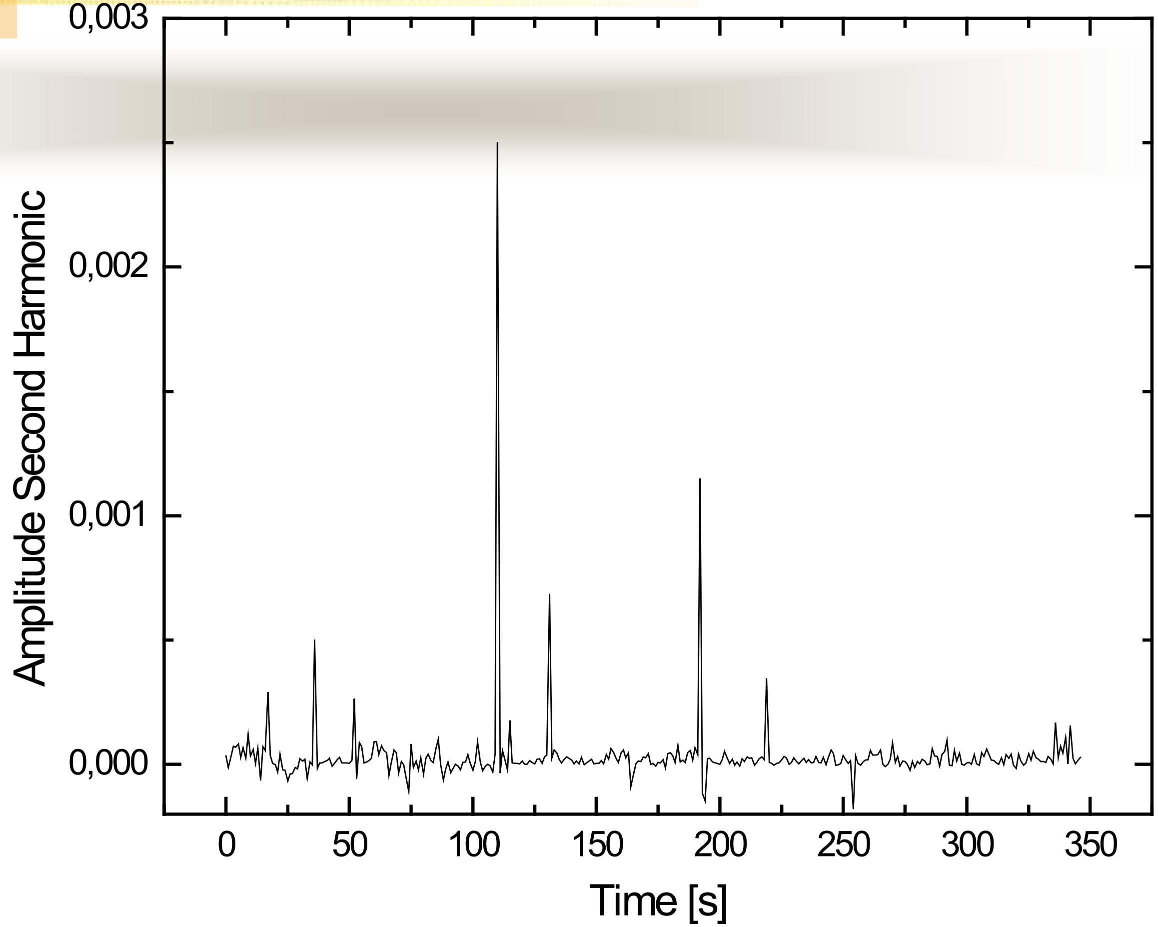
DPhPC giant vesicle in $K_3Fe(CN)_6$ solution, green illumination

Courtesy: Dr. V.Vitkova, ISSP- BAS

$$Fe(CN)_6^{3-} + H_2O \leftrightarrow h\nu \leftrightarrow Fe(CN)_5.H_2O^{2-} + CN^-$$

(after Petrov, Lee, Doebereiner, *Europhys. Lett.* 48, 435 (1999))

A G. Petrov. In: Flexoelectricity, Chapter 6, N.Eber and A.Buka, Eds, Imperial Coll Press, Singapoure (2012), pp169-202.



Time dependence of curvature fluctuations of a giant lipid vesicle with stress sensitive alamethicin channels in its membrane. Inside the vesicle there is a ferricyanide solution undergoing a photochemical reaction under illumination, which produces a pH gradient and a photopotential across the membrane. The graph shows the second Legendre polynomial amplitude of the angular autocorrelation function of the vesicle radius as a function of time. Brief episodes (peaks) of extensive curvature fluctuations in a tension-free membrane are separated by long periods of a tensed, non-fluctuating vesicle membrane.

- **CONCLUSION**

The knowledge of bioflexoelectricity has been established. Flexoelectricity provides a reciprocal relationship between electrical and mechanical properties of liquid crystals and biomembranes. A specific mechanical degree of freedom, membrane curvature, is involved in the case of bioflexoelectricity.

Biolexoelectricity enables the membranes to function like soft machines that are fast enough (kHz) and can operate in the process of hearing, and many other electrophysiological processes. Bioflexoelectric concepts could be of major importance in preparation of artificial membrane machines ("protocells").