Microtubule I-V Characteristics Are Consistent with Memristor-like Behavior

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Edmonton, Alberta
(so close and so far away)

Edmonton, Alberta, Canada

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novocure™

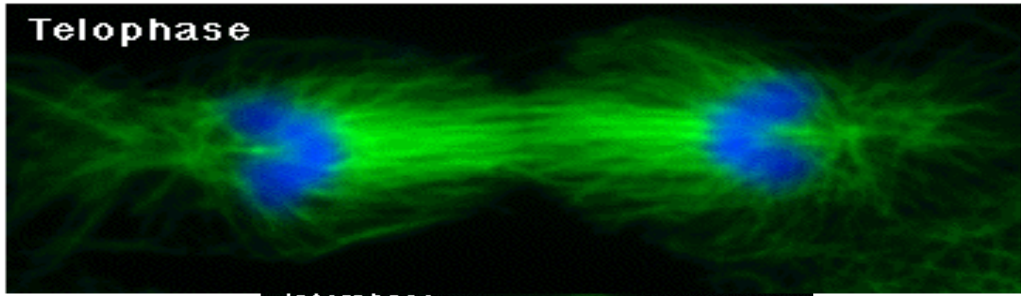
Government of Alberta

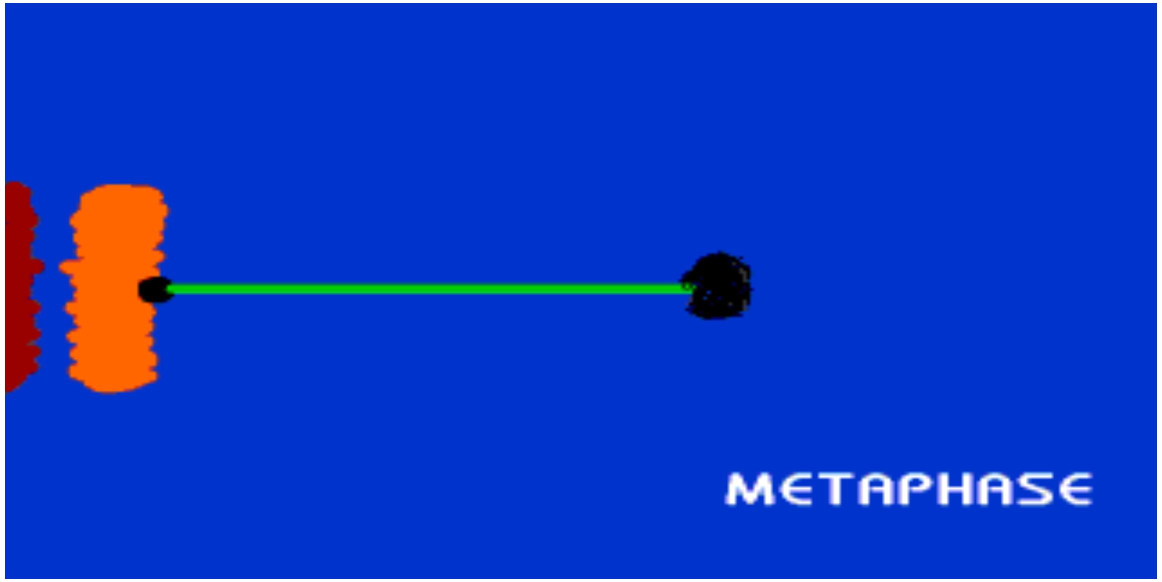
Alberta Cancer Foundation

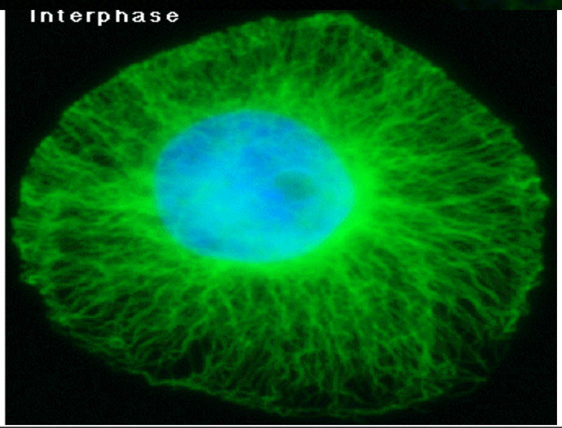
Allard Foundation
A LEGACY OF CARING

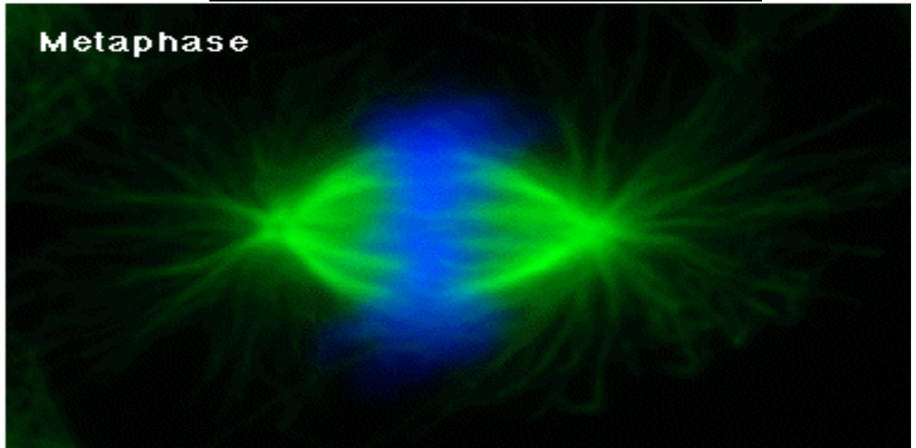
IBM®

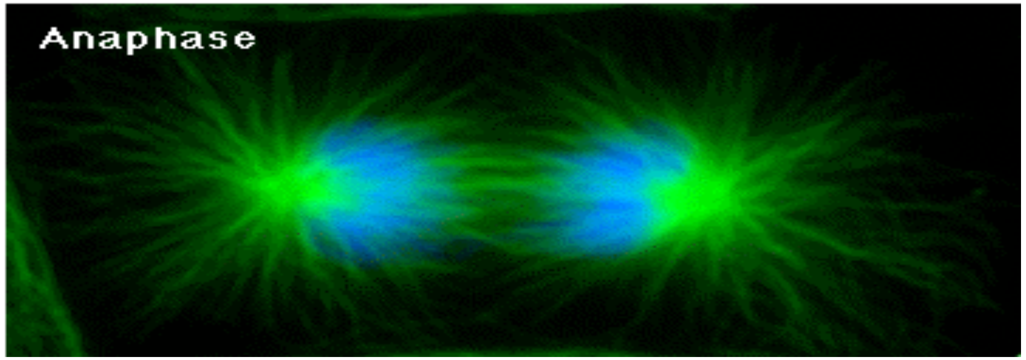
Microtubules in Mitosis



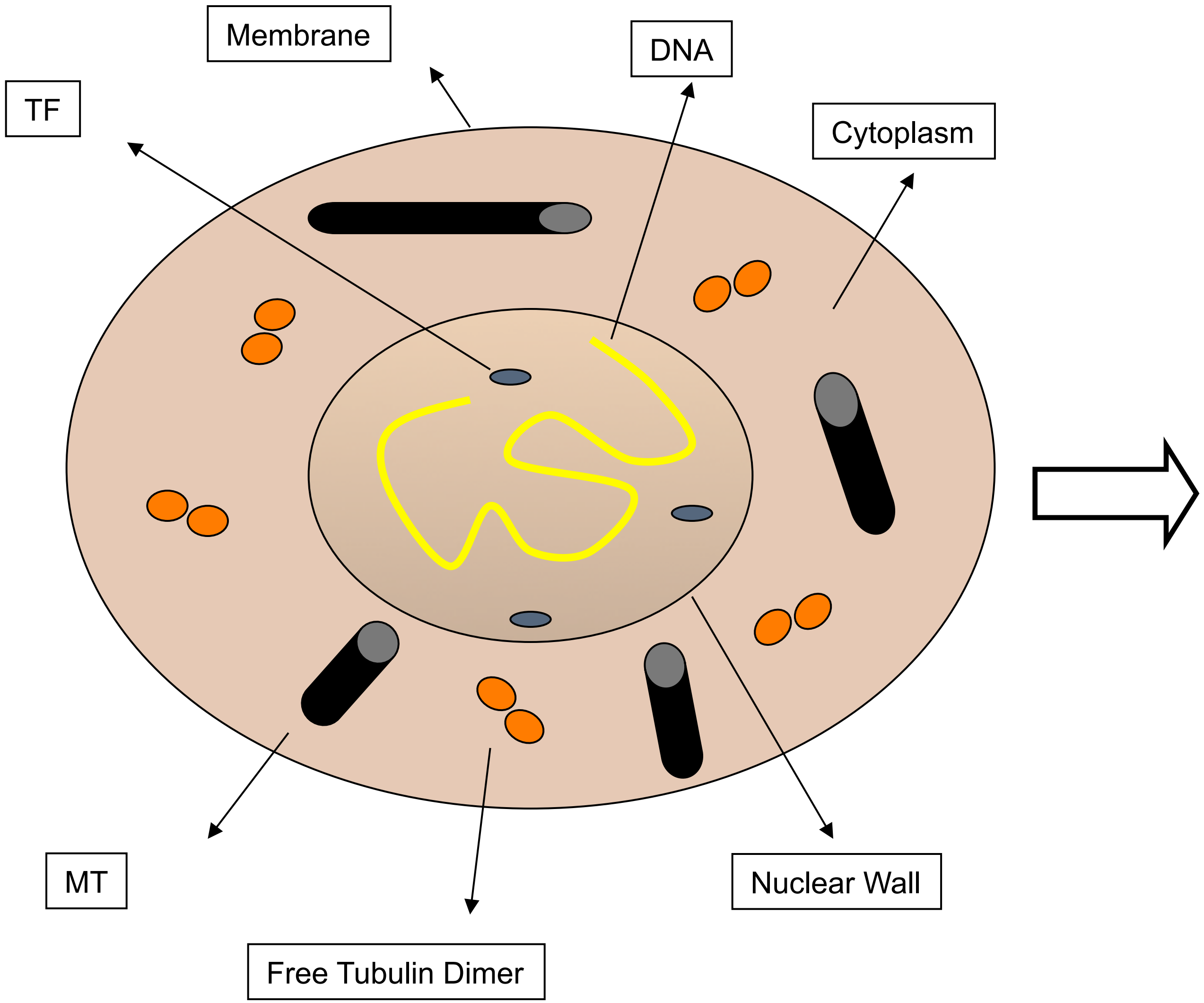


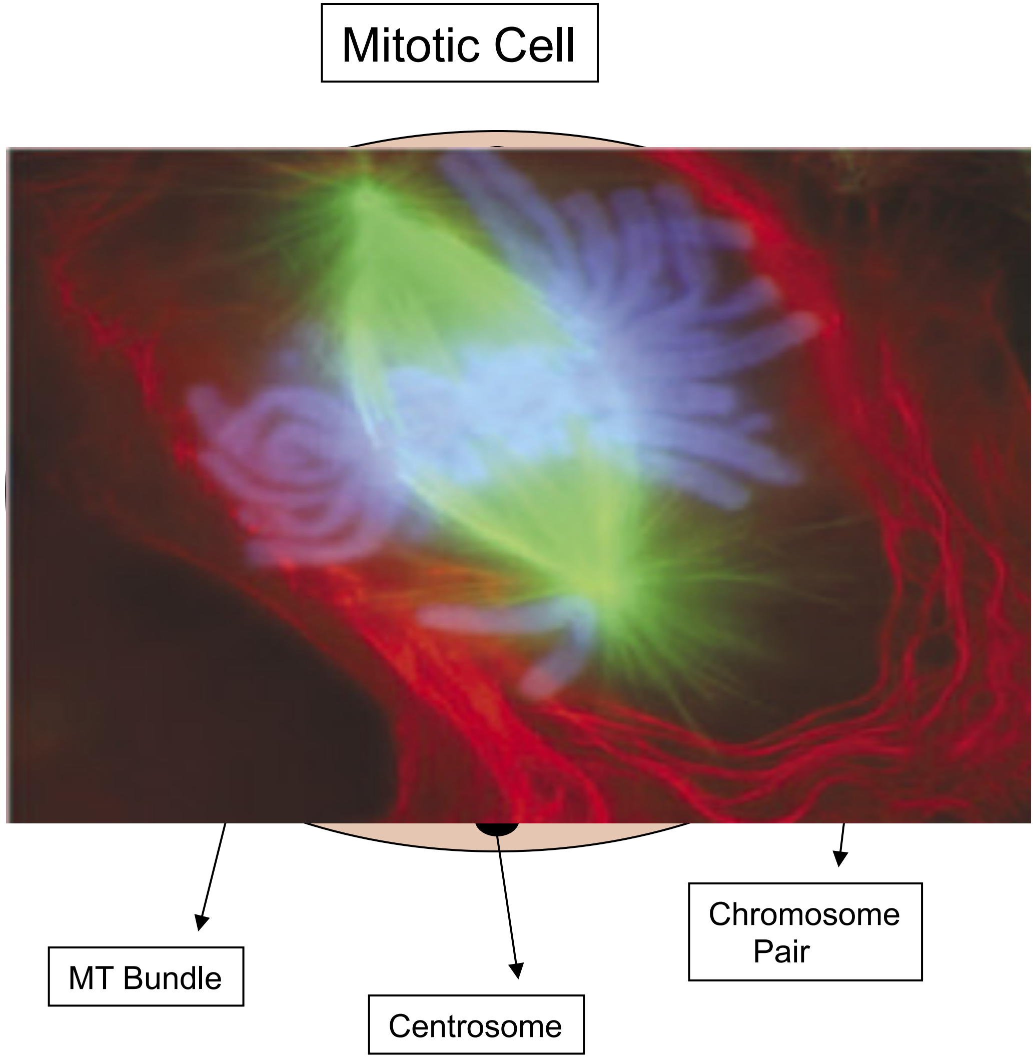




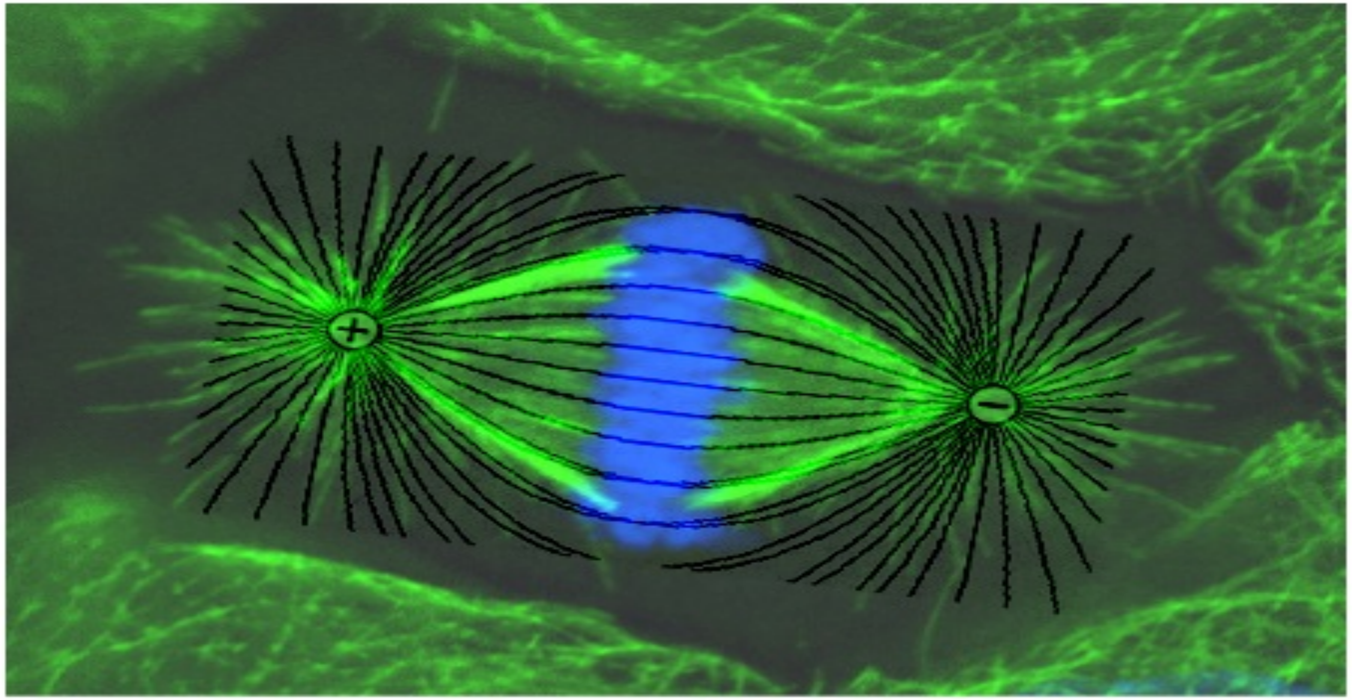


Microtubule: function

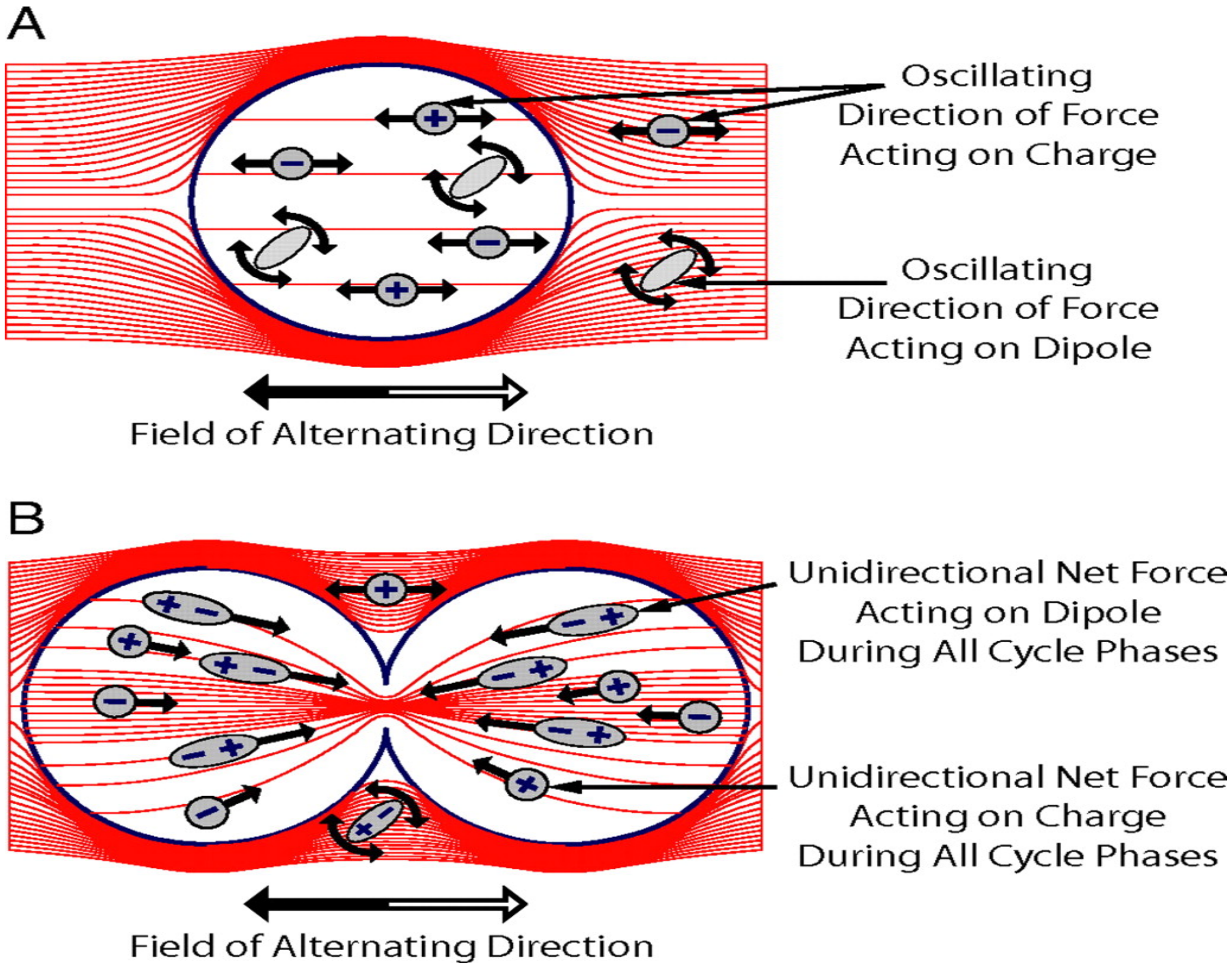




The mitotic spindle viewed as a dipole

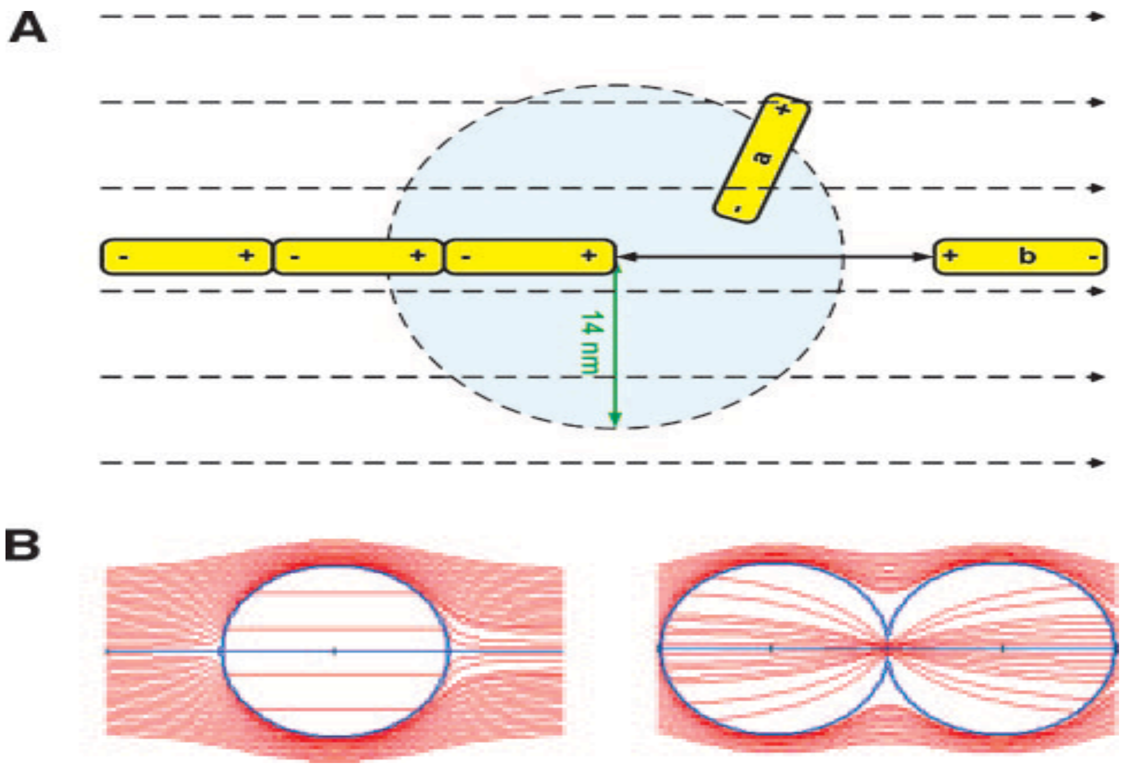


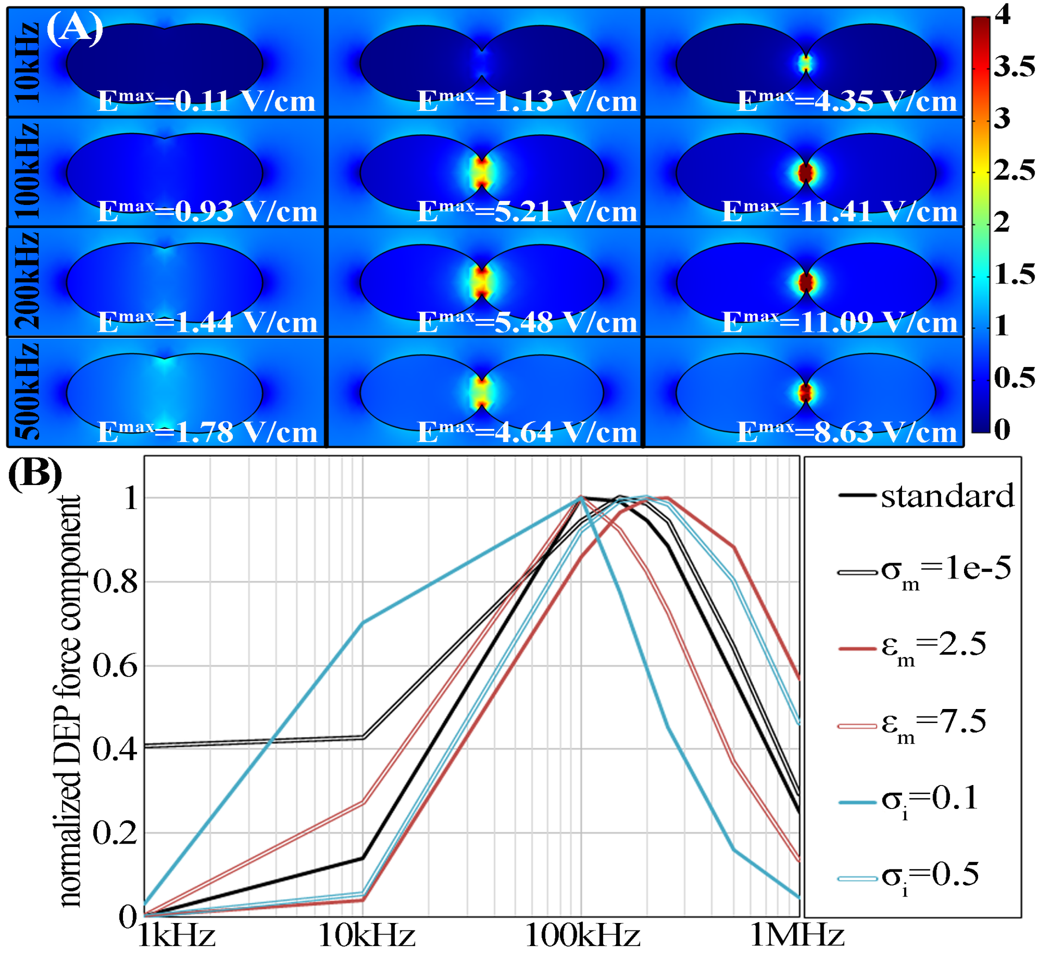
100-200 kHz AC field distribution in and around quiescent (A) and dividing (B) cells.



Kirson E D et al. PNAS 2007;104:10152-10157

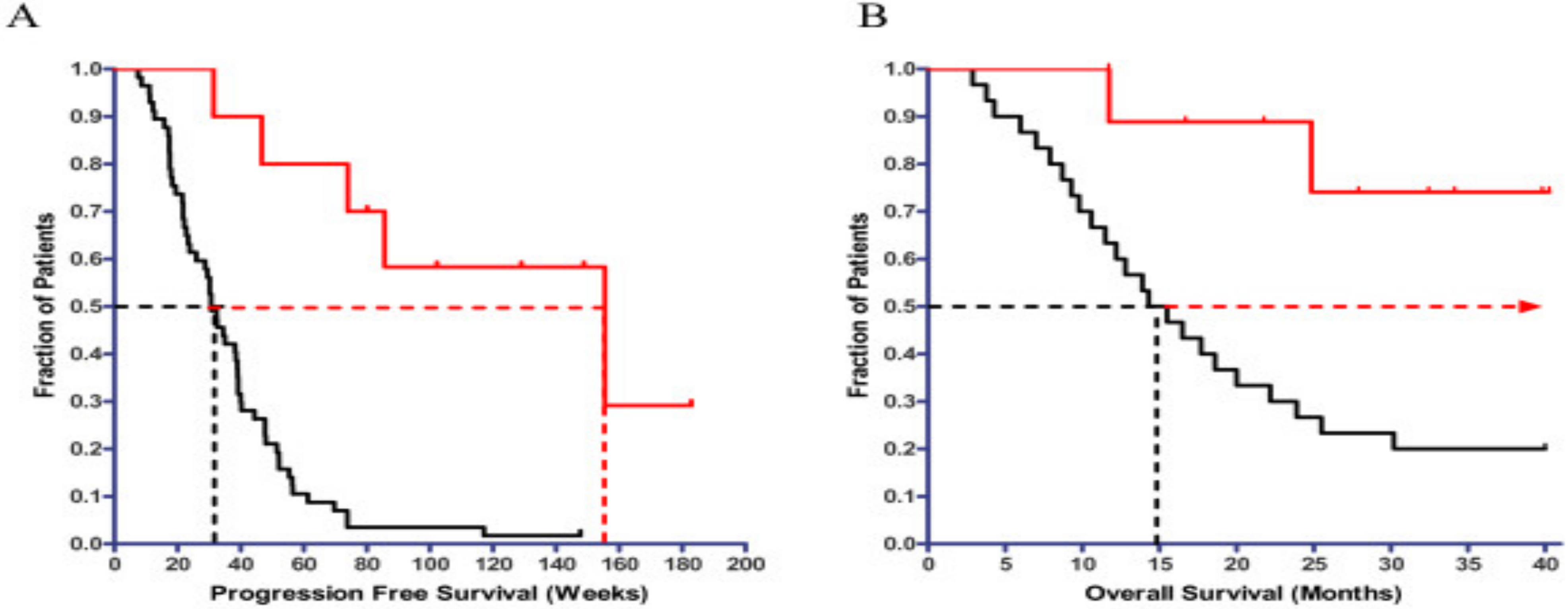
Tumor Treating Fields:
Adjuvant Alternating Electric Fields target MTs





COMSOL SIMULATIONS:
Wenger C, Giladi M, Bomzon Z, Salvador R, Basser PJ, Miranda PC (2015) Eng. Med. Biol. Soc. (EMBC), 2015 37th Annu. Int. Conf. IEEE. pp 6892–6895

Adjuvant Alternating Electric Fields for GBM
Novocure, Haifa, Israel



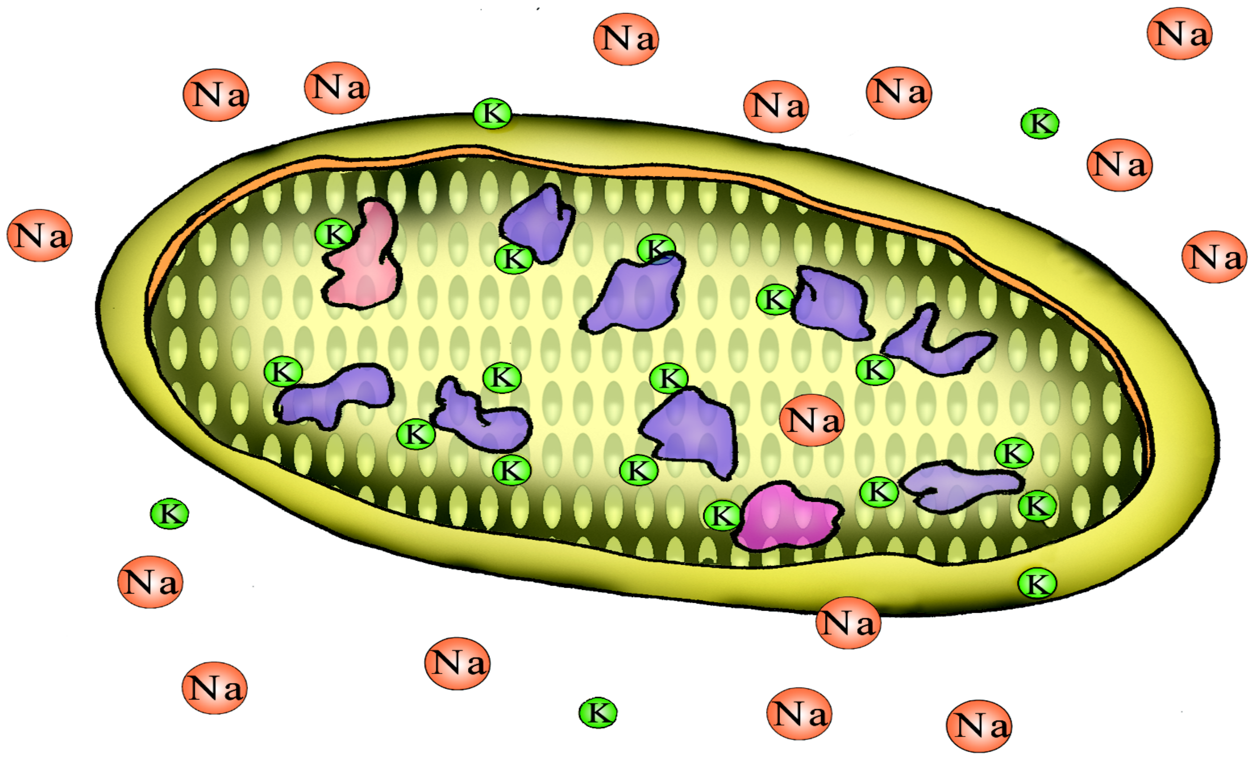
Red line – patients receiving combined TTFields – Temozolomide treatment (n = 10). Black line – concurrent/historical control patients that received Temozolomide treatment alone.

Kirson *et al. BMC Medical Physics* 2009 **9**:1

Structured water and ions

Transmembrane potential:
120 mV normal cells, 70 mV cancer cells

Mitochondrial potential: 140 mV



Negative protein charge: 1.6 mol/kg
Positive protein charge: 1.01 mol/kg
Net protein charge: 0.6 mol/kg (-)
(Wiggins, 1990)

Potassium ion: 0.5 mol/kg (+)
Chloride ion: 0.2 mol/kg (-)
Net ion charge: 0.3 mol/kg (+)

NET CYTOPLASM CHARGE: 0.3 mol/kg (-)

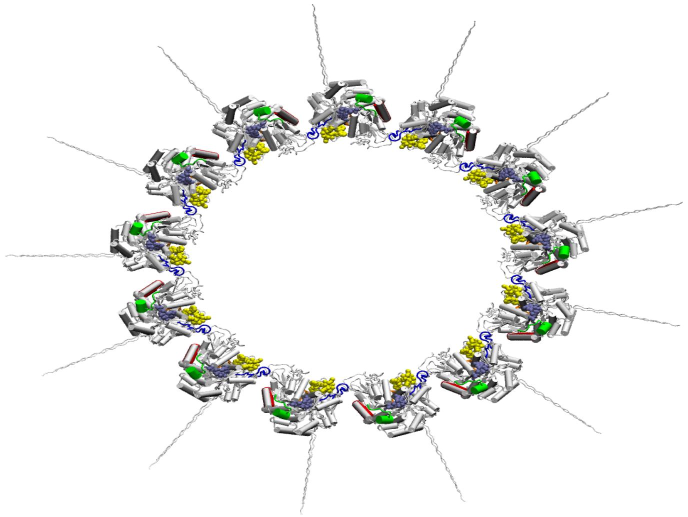
Gerald Pollack, Univ. of Washington

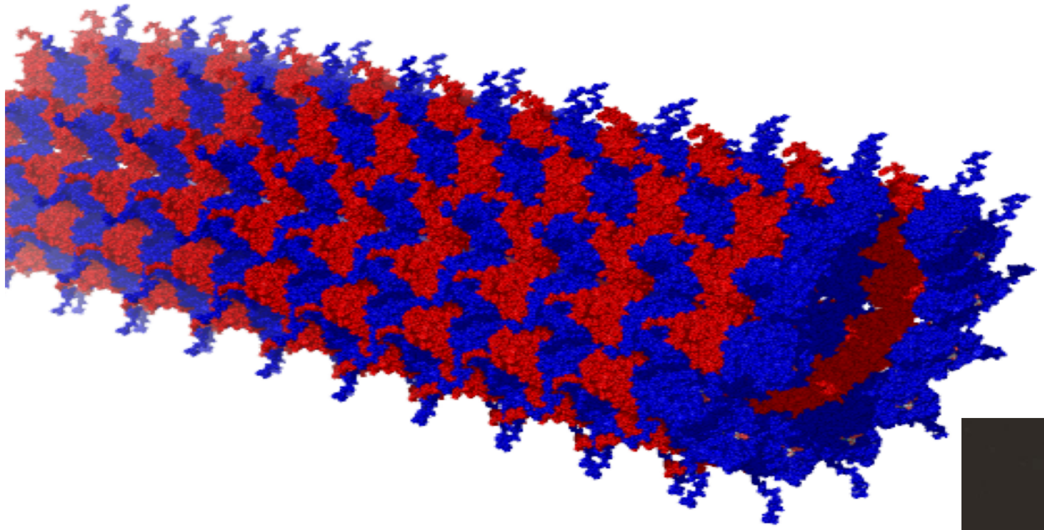
The Cytoplasm

Major Components of Cytoplasm in a Typical Mammalian Cell

Ions	Concentration mM	Non-ionic constituents	Concentration mg/mL
K^+	140	protein	200 - 300
Na^+	10	actin	2 - 8
Cl^-	10	tubulin (electrolyte)	4
Ca^{2+}	10^{-4}	pH	~ 7.2
Mg^{2+}	0.5	Specific tissues may differ	Specific tissues may differ

Microtubule Reconstruction



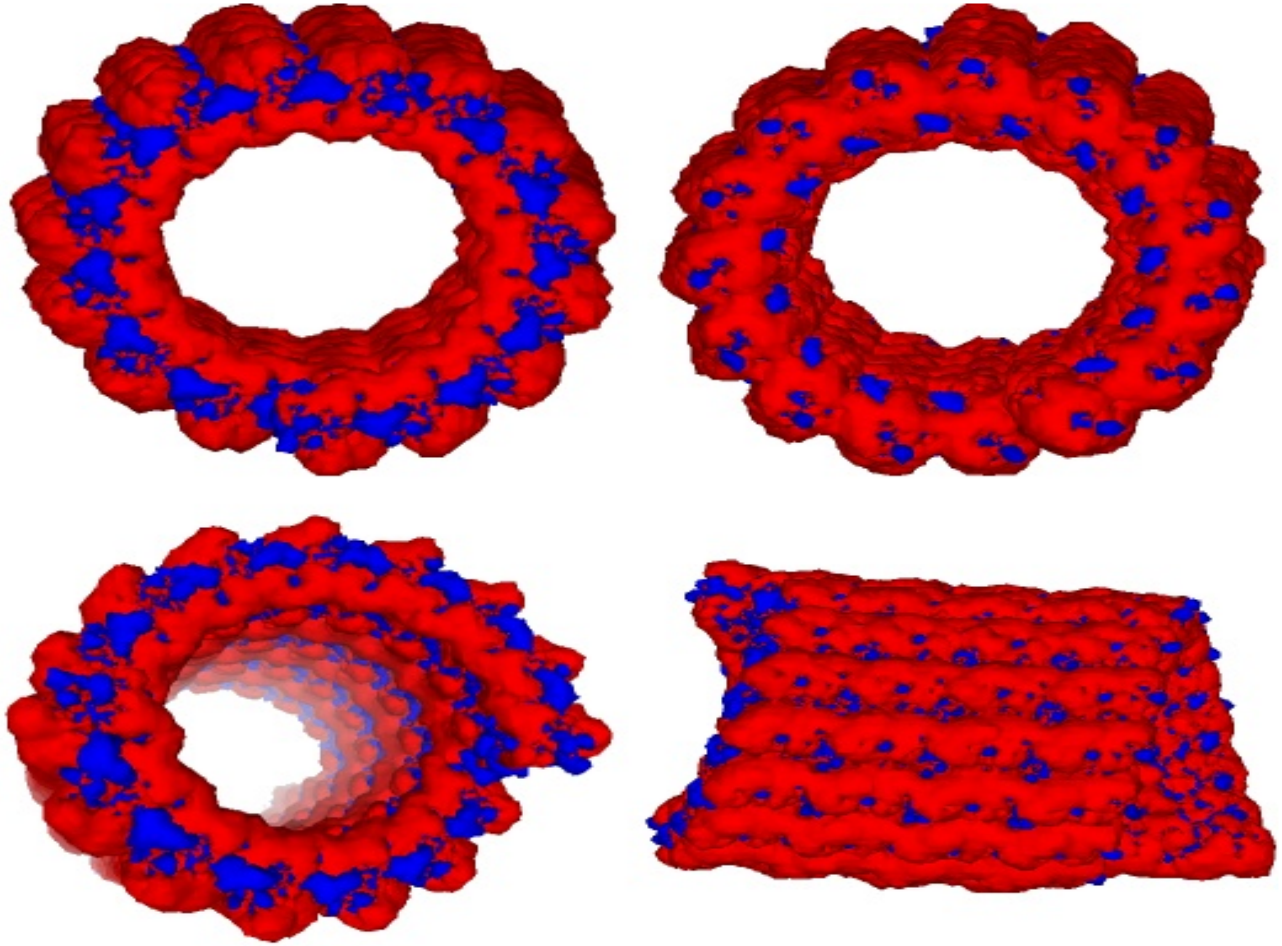


Microtubules move along electric field lines (E= 200 V/m):

R. Stracke, K.J. Boehm, L. Wollweber, E. Unger and J.A. Tuszynski,
Analysis of the migration behavior of single microtubules in electric fields,
Biochemistry and Biophysics Research Communications 293, 602-609 (2002)

Charge distribution on a microtubule.

-end +end asymmetry



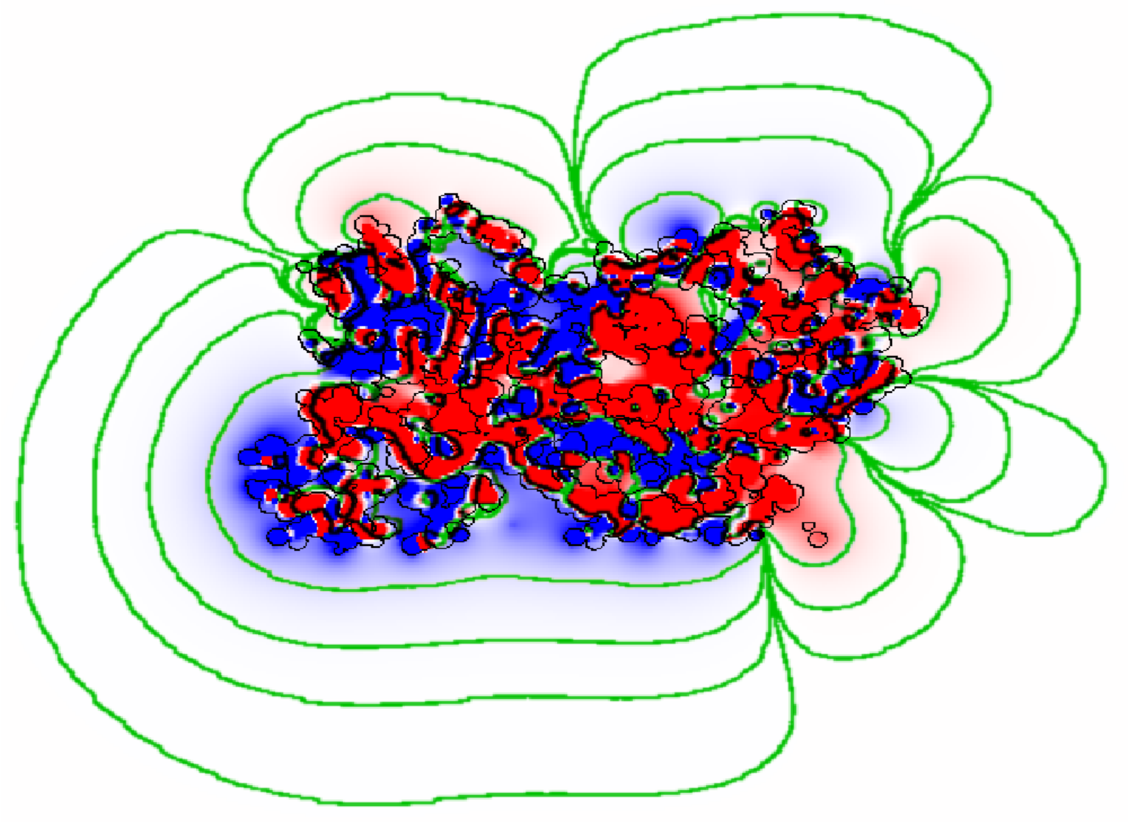
may explain different kinetics

Outer diameter: 25 nm
Inner diameter: 15 nm

not all charge compensated in solution

NA Baker, D Sept, S Joseph, MJ Holst, JA McCammon
PNAS 98 (18), 10037

Electric Potential

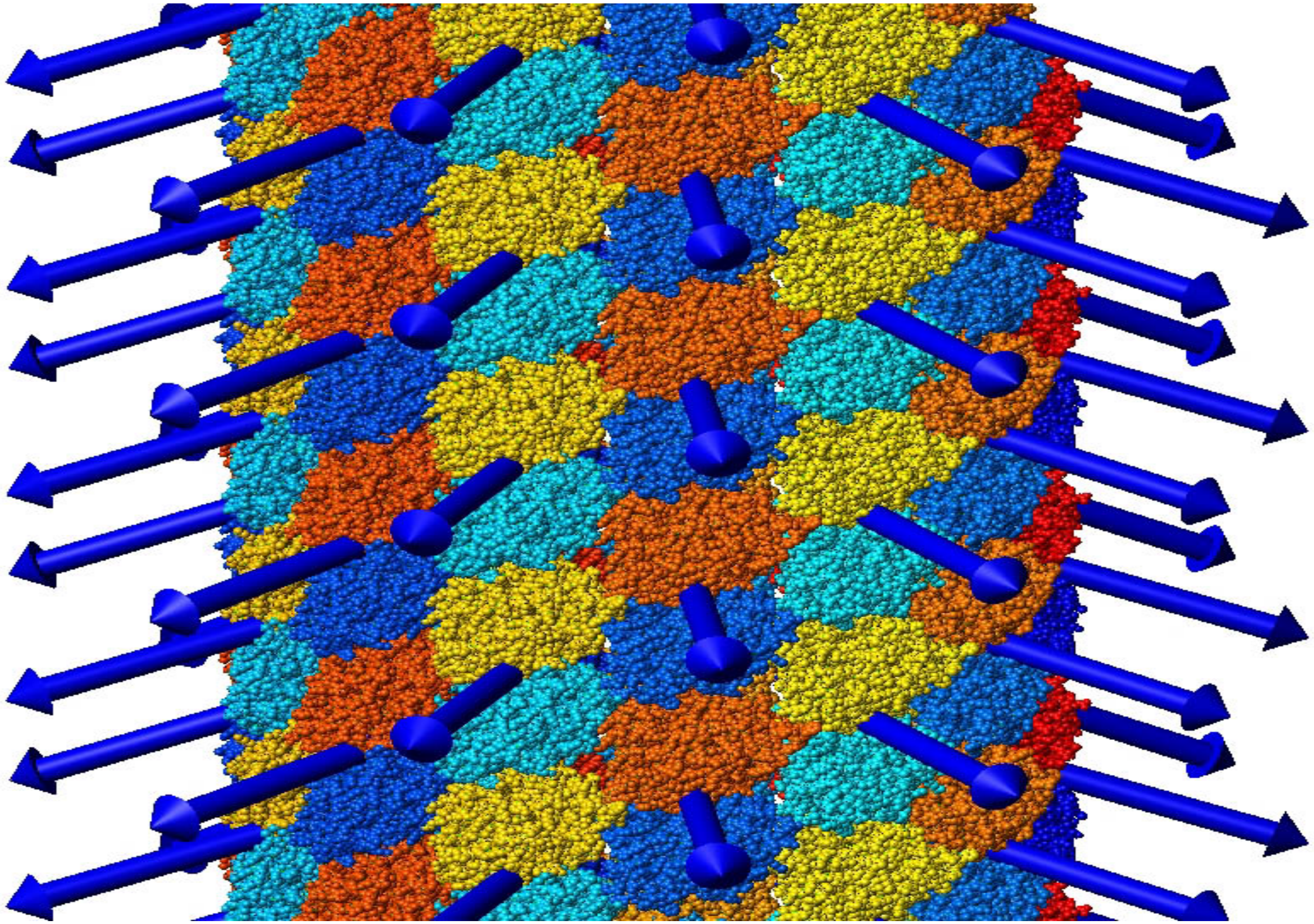


- slice through dimer
- red/blue= negative/positive
- green=isopotential lines

Poisson-Boltzmann equation:

$$\nabla \cdot (\epsilon(\vec{x}) \nabla \phi(\vec{x})) - \kappa(\vec{x})^2 \sinh(\phi(\vec{x})) = -4\pi\rho(\vec{x})$$

Dipole moments of tubulin dimers in a microtubule



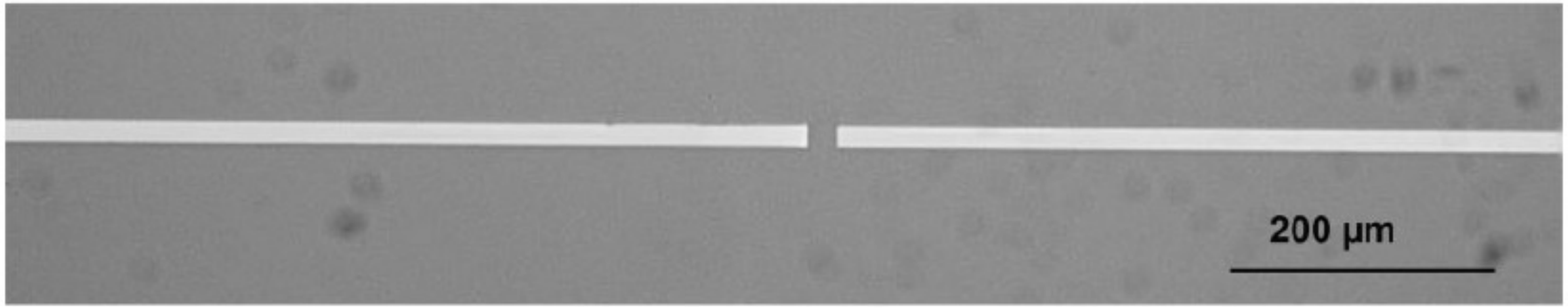
Section of a microtubule with the dipole moments of each tubulin dimer shown with blue arrows.

A Tuszynski, EJ Carpenter, JT Huzil, W Malinski, T Luchko, RF Luduena
International Journal of Developmental Biology 50 (2-3), 341-358

Experiments on Microtubules in AC Electric Fields

- GOAL : To manipulate microtubules using AC electrokinetics and organize microtubules into aligned bundles on surfaces.

A pair of electrodes was fabricated on a glass surface to observe response to AC voltage.

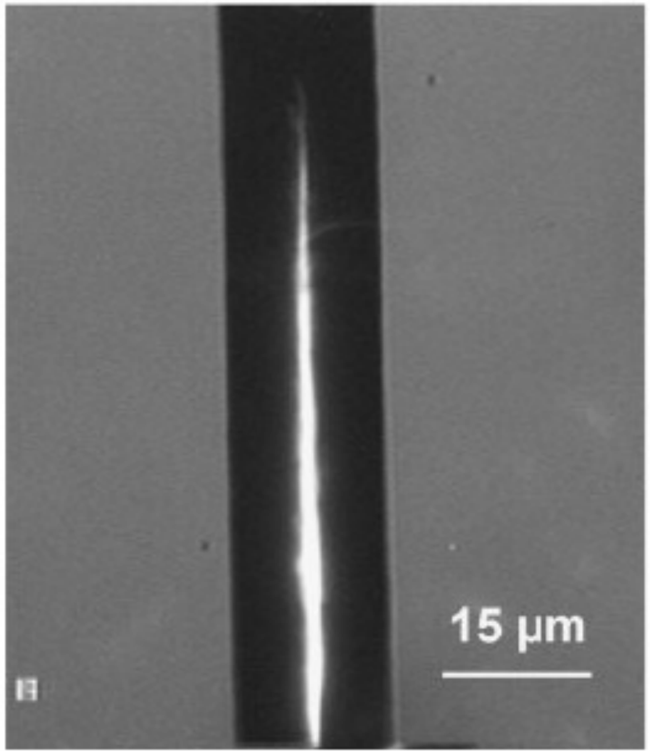


Electrode dimensions: 15µm x 12mm Gap: **20µm**, **40 V** Voltage bias

Uppalapati, M., Huang, Y.-M., Jackson, T.N., Hancock, W.O., 2008. Microtubule alignment and manipulation using AC electrokinetics. Small 4, 1371-1381.

Microtubule Alignment and Manipulation using AC electrokinetics

500 kHz MT accumulation:

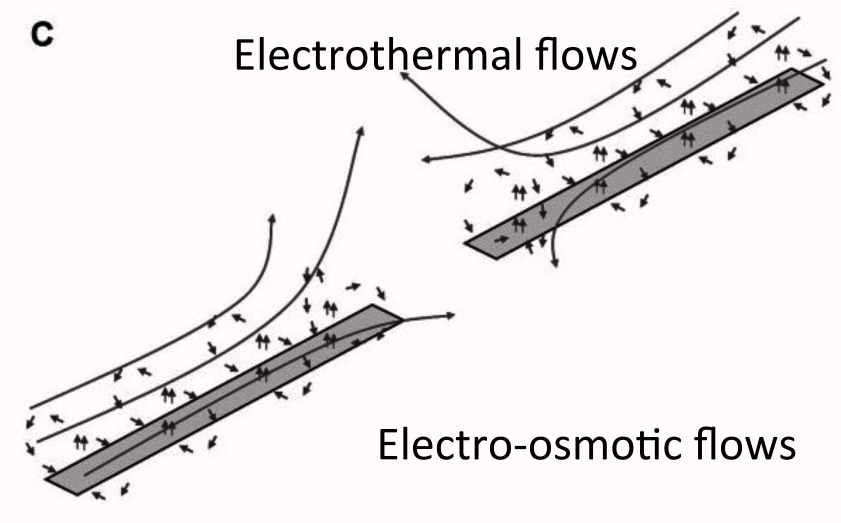


Below 500kHz: Microtubules flow **toward centerline** of electrodes.

Above 500kHz: Microtubules flow **toward gap** between electrodes .

Below 500kHz:

1. Vortex-like, with microtubules circling electrodes.
2. Along the length of electrodes, directed at gap between electrodes.



Maruti Uppalapati, Yin-Ming Huang, Thomas N. Jackson, and William O. Hancock

Below 500kHz:

The *Electro-osmotic force* causes the movement of the fluid in a vortex-like manner. This represents the Coulomb-force experienced by the <u>fluid</u> due to the applied voltage.

❖Observed within 7µm from above the electrode surface.

Flow velocity given by:

$$v = \frac{E_t \sigma_q}{\kappa \eta}$$

Verification :

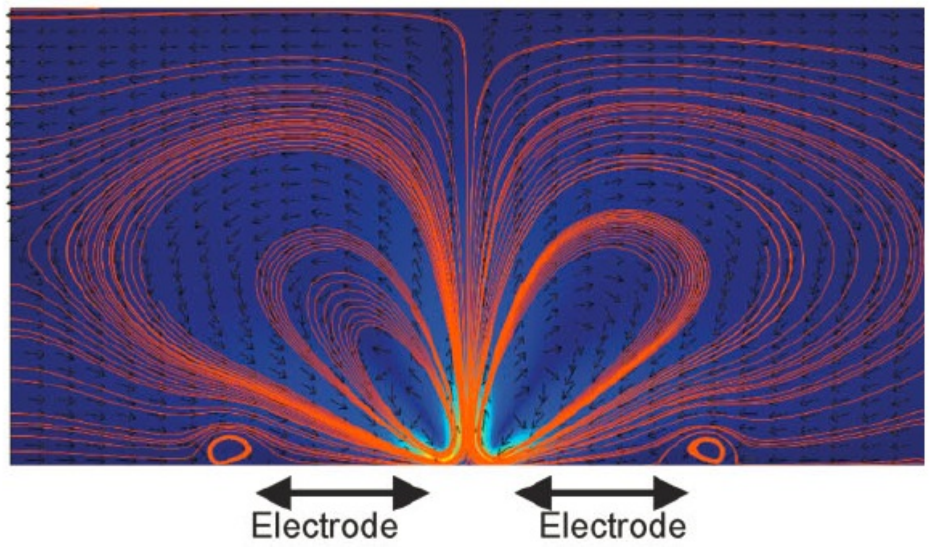
1. Flow velocity proportional to the electric field, as predicted by equation.
2. At lower frequencies, flow velocity is larger(predicted).

Below 500kHz:

The ***Electro-thermal force*** causes motion of microtubules along the length of the electrodes. This represents the force experienced by the fluid due **to heating-up of the electrodes.**

❖Observed beyond 7μm from the electrode surface.

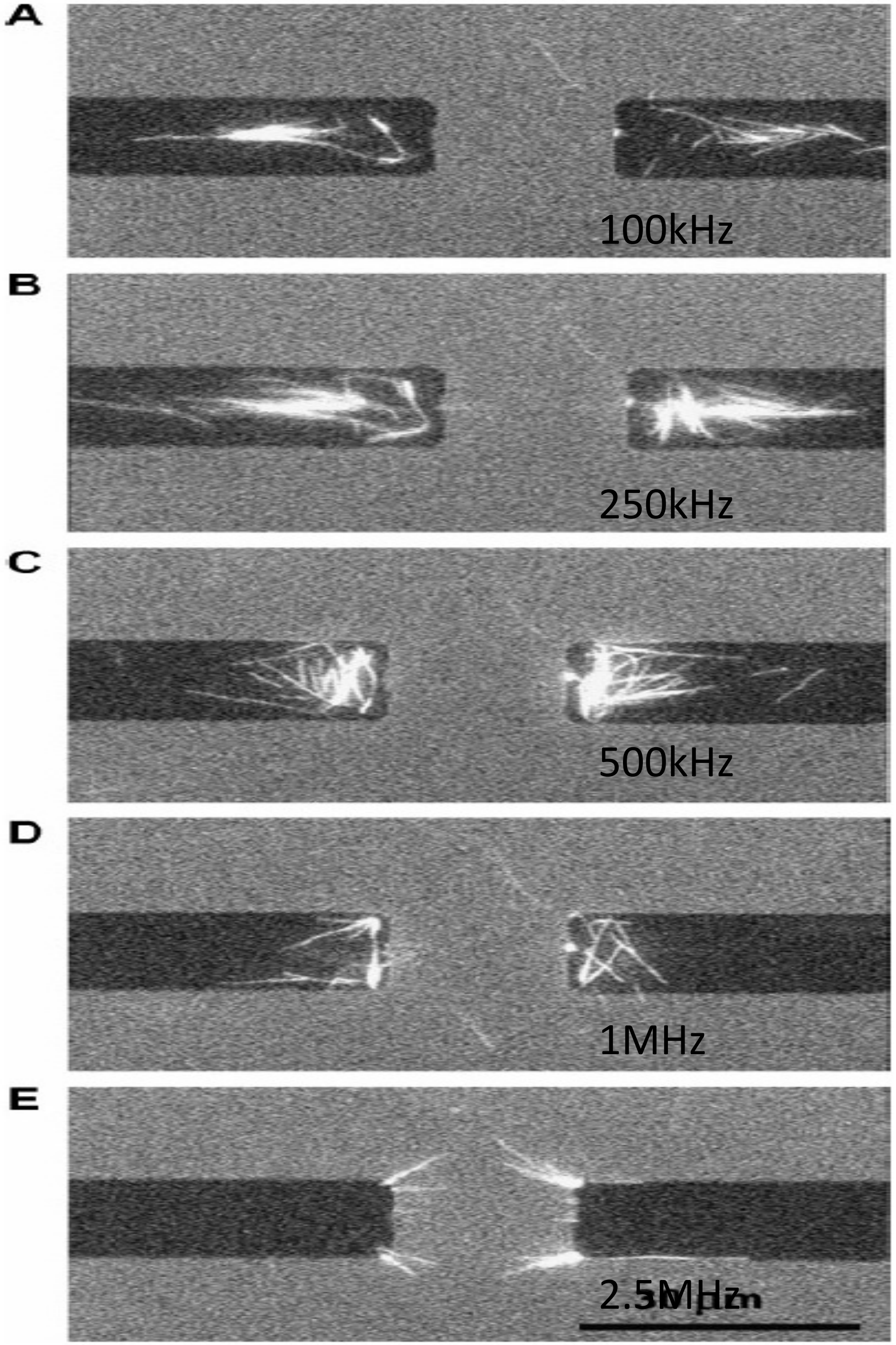
The electro-thermal force was found to arise due to the heating effect of the light-source of the microscope.



Above 500kHz:

Movement of microtubules *toward the gap* between electrodes was observed.

Dielectrophoresis was purported to be responsible for this flow. This is the force experienced by <u>microtubules</u> in a non-uniform electric field.



Conductivity measurement

Force due to Dielectrophoresis: $\langle F_{DEP} \rangle = \frac{1}{4} v\varepsilon_{\mathrm{m}} \left[\frac{\omega^2 \varepsilon_{\mathrm{m}} \left(\varepsilon_{\mathrm{p}} - \varepsilon_{\mathrm{m}} \right) + \sigma_{\mathrm{m}} \left(\sigma_{\mathrm{p}} - \sigma_{\mathrm{m}} \right)}{\omega^2 \varepsilon_{\mathrm{m}}^2 + \sigma_{\mathrm{m}}^2} \right] \nabla |E|^2$

- Force is dominated by $(\sigma_p - \sigma_m)$

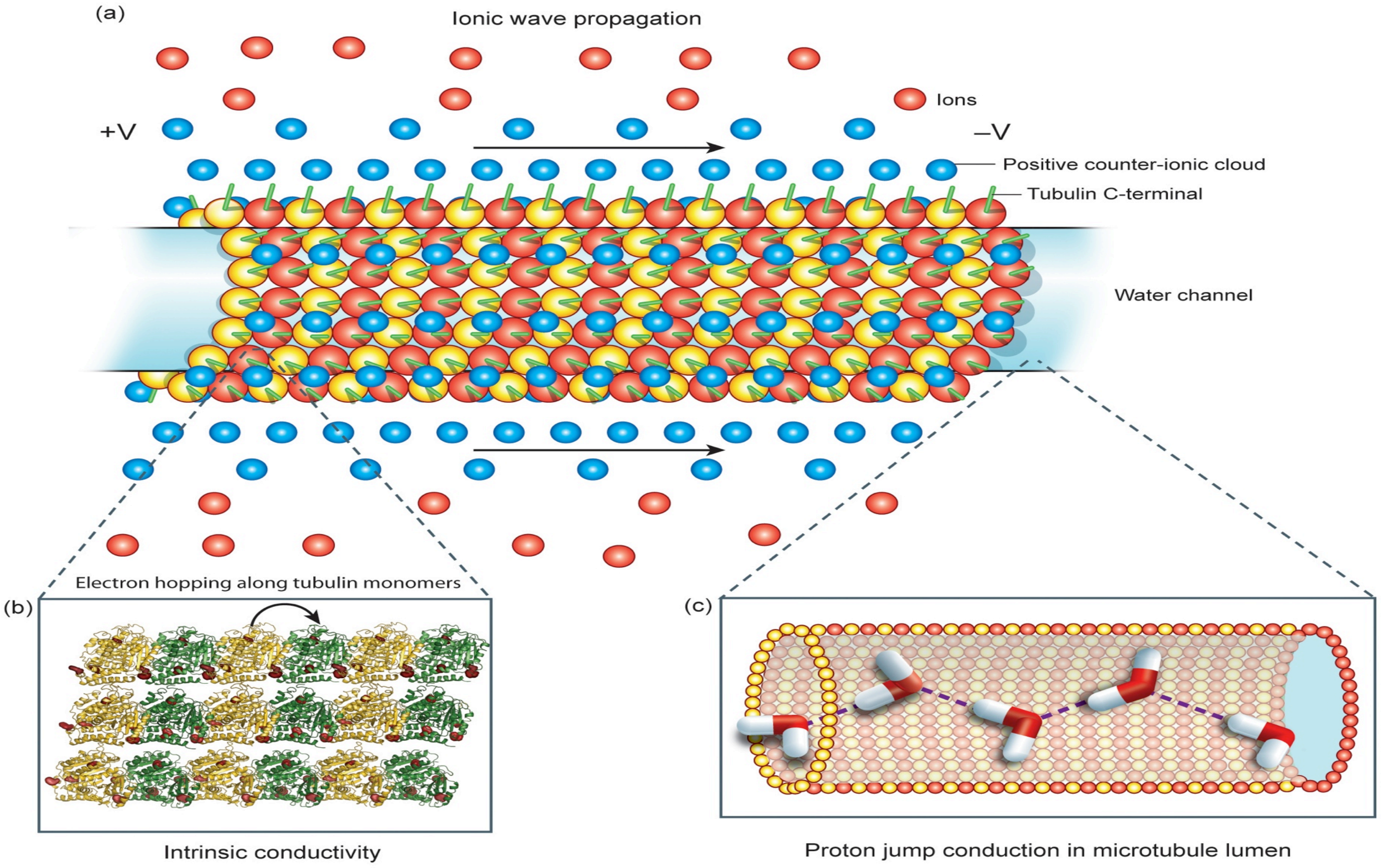
p=particle (MT), m=medium

At ~**5MHz, the electro-osmotic and electro-thermal flow balance each other out, the flow of microtubules is now solely due to dielectrophoresis.**

No accumulation $\longrightarrow$ No DEP $\longrightarrow$ $(\sigma_p - \sigma_m) = 0$ $\longrightarrow$ $\sigma_p = \sigma_m$

Measured σ_m independently ~250 mS m^{-1}

Microtubule modes of conductivity: ionic, protonic, electronic (?)



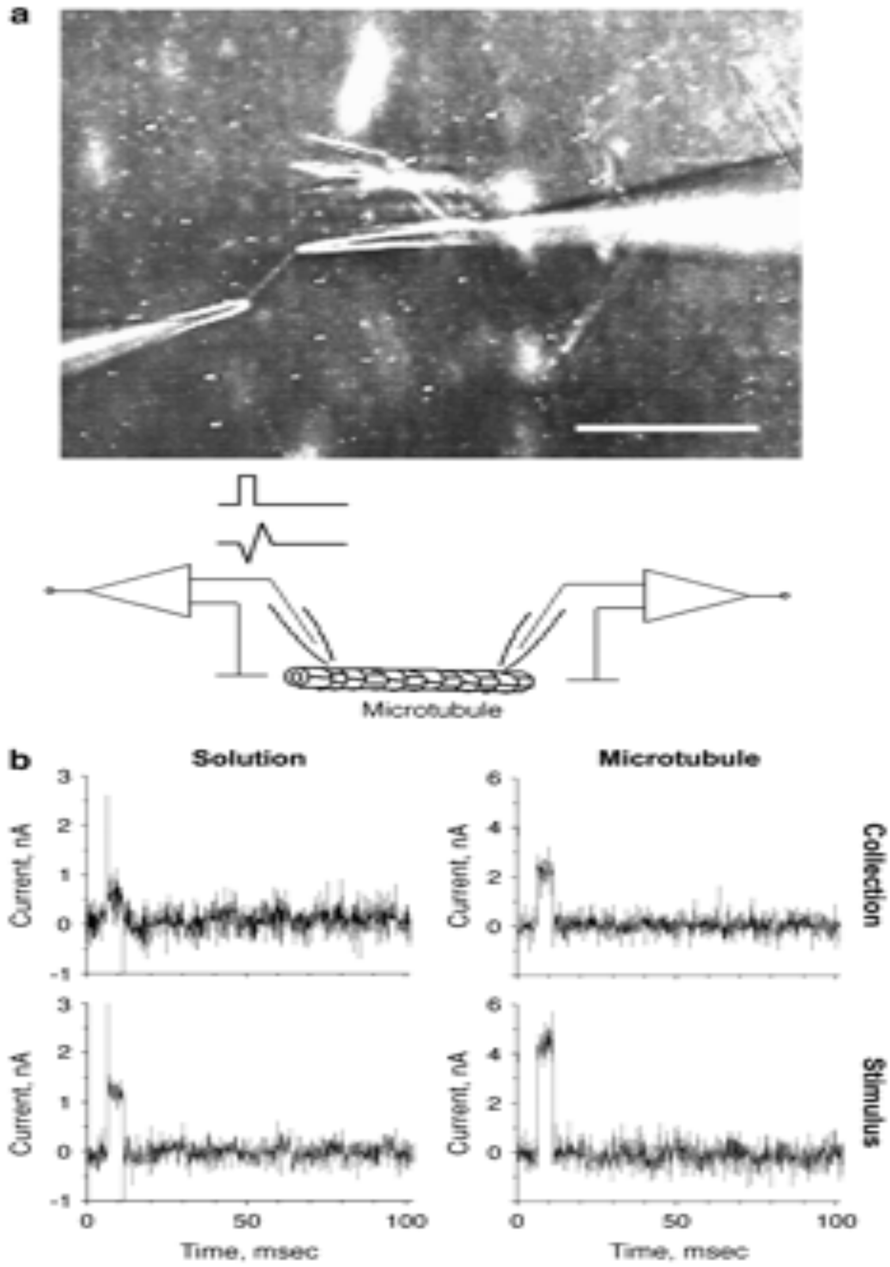
Friesen et al. BioSystems 127:14-27, 2015

Table 1. Conductivity data for microtubules and reference solutions

Paper	Type of experiment	Conductivity type	Conductivity (S/m)
(Minoura and Muto, 2006)	Electro-orientation	Ionic	0.15
(Uppalapati et al., 2008)	AC electrokinetics	Ionic	0.25
(Foster et al., 1976)	Cytoplasm	Ionic	2.5
(Fritzsche et al., 1999)	Dry MTs	Intrinsic	Less than 3
(Cole, 1975)	Axoplasm	Ionic	3.4
(Shang et al., 2004)	Sea water	Ionic	4.8
(Wada et al., 2009)	TMR-coated, 4-point probe	Ionic	~40
(Sahu et al., 2013b)	DC MTs	Intrinsic	0.6 to 136
(Umnov et al., 2006)	Gold electrodes, channel	Ionic/Intrinsic	Less than 80
(Priel et al., 2006)	Patch-pipette	Ionic	367
(Sahu et al., 2013a)	AC MTs. Water inside.	Intrinsic/Water channel	$10^3 – 10^5$
(Goddard and Whittier, 2006)	RF reflectance spectroscopy	Ionic/Intrinsic	1-5 x 10^6 (they reported same as lead or stainless steal)

Transistor-like amplification of ion flow along Microtubules

- Priel *et al.* (2006) have shown that microtubules can amplify an electrical signal
- To understand how, we are studying flow of ions through pores and around microtubules
- Apply computational /theoretical methods developed for ion channels

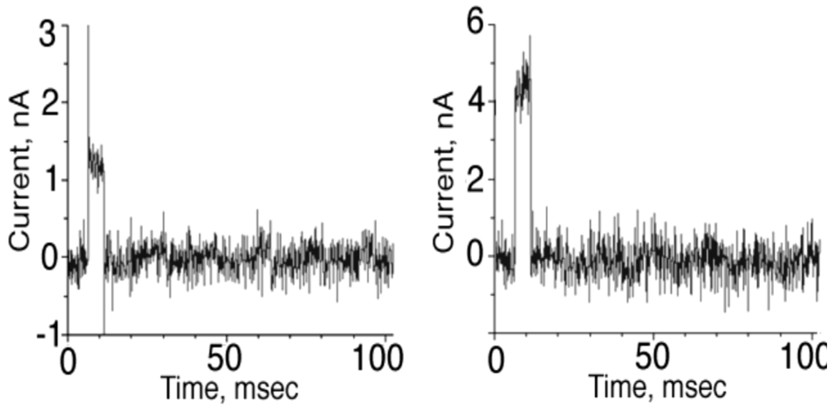


Priel et al. (2006) Biophys. J. 90, 4639-4643

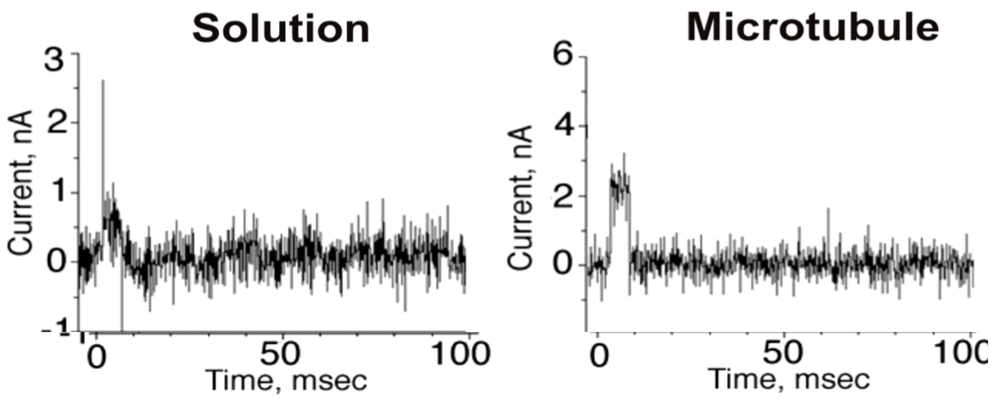
Typical Signals:
Collection vs. stimulus

Stimulus

Solution **Microtubule**



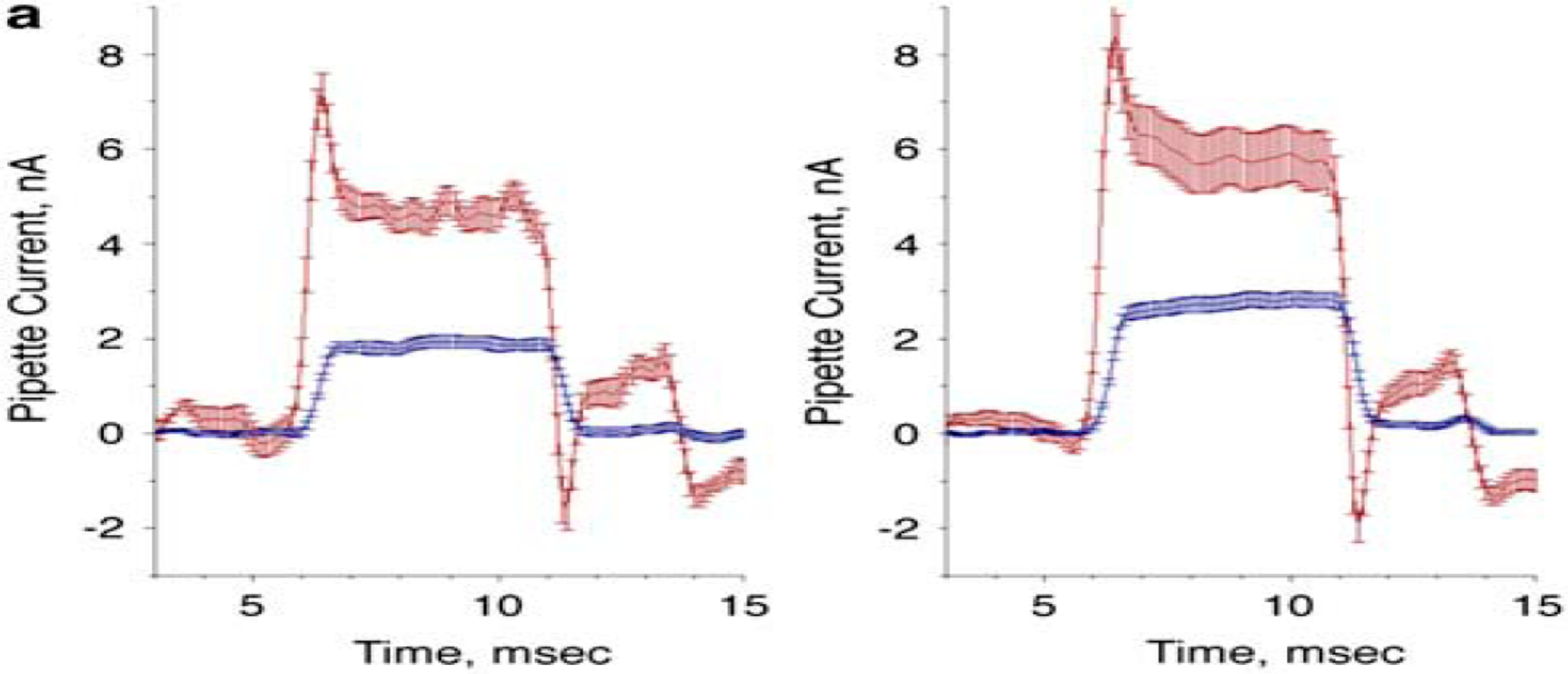
Collection



- **A connected MT increased the conductance of the stimulus pipette more than <u>4</u> times !!**

- **Amplification transfer factor Acoll / Astim was up to 50%, indicating a true amplification phenomenon.**

Current amplification by microtubule



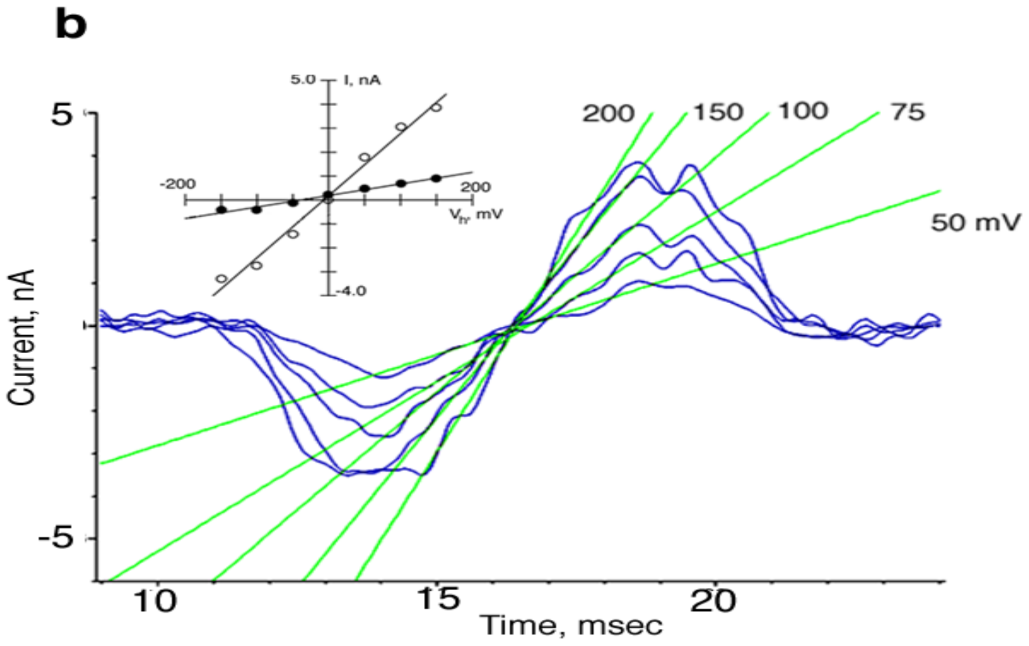
Priel et al. (2006)

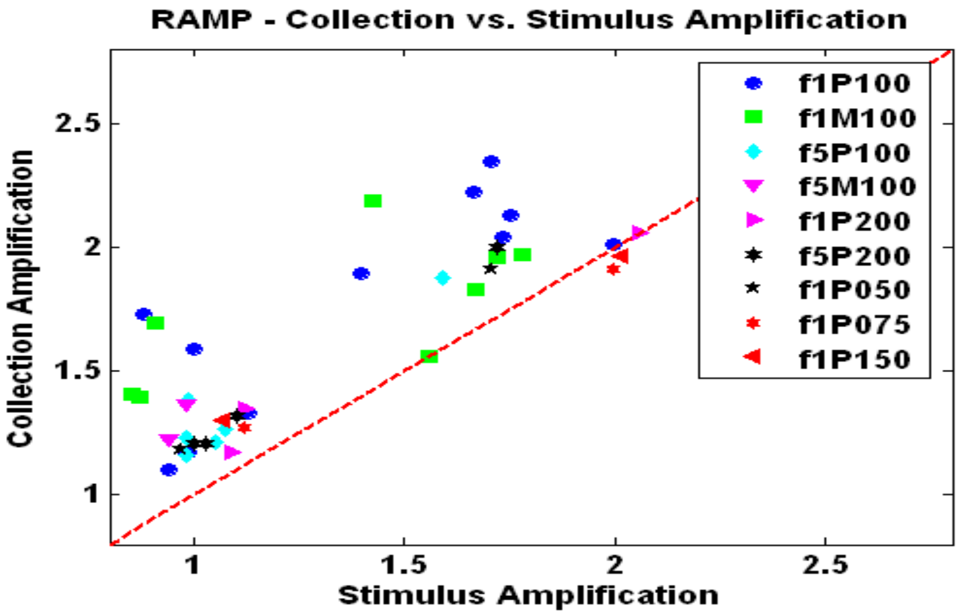
Blue: the input voltage with amplitude of ~ 200 mV applied to the one side of MT

Red: the resulting electrical signal at other end of MT (20 -50 μm away) increased by > 300%

Main Observations

- **Ramp signals were used to establish the linear conductance property (I/V curve) in the stimulus voltage range tested, ± 200mV .**





Main Observations

- **The coupling ratio between pipettes in solution was ~40% before attachment to an MT.**
- **A connected MT increased the conductance of the stimulus pipette more than 4 times !!**
- **The evoked current increased after attachment from ~1.9(± 0.13) nA to ~2.8(± 0.17) nA.**
- **Amplification transfer factor A_{coll} / A_{stim} was up to 50%, indicating a true amplification phenomena.**

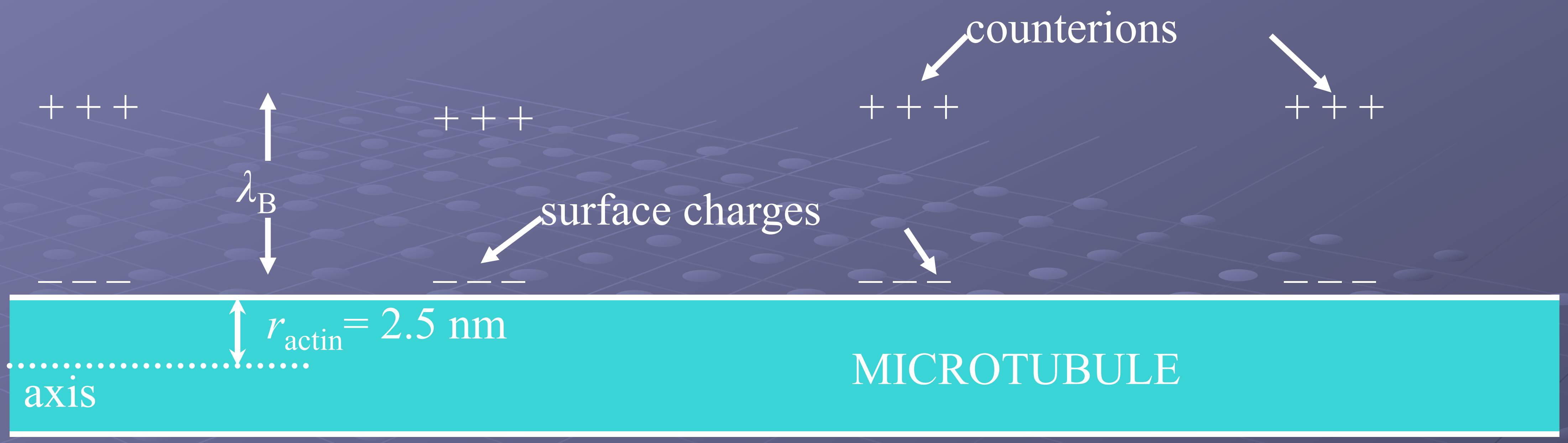
Ionic conductivity

- **Actin filaments provide a conduit for near lossless ionic wave propagation**

J.A. Tuszynski, S. Portet, J.M. Dixon, C. Luxford and H.F. Cantiello, Ionic Wave Propagation Along Actin Filaments, Biophysical Journal 86, 1890-1903 (2004)

- **Microtubules amplify ionic wave propagation in a transistor-like manner**

Priel, A.J. Ramos, J.A. Tuszynski and H.F. Cantiello, A Bio-Polymer Transistor: Electrical Amplification by Microtubules, Biophysical Journal vol. 90 issue 12: 4639-4643 (2006).



At $T = 293$ K λ_B is typically ~ 7.13×10^{-10} m

Counterions not uniform along polymer's length (due to highly non-linear interactions between the hydrated molecule and its surrounding counterions).

Expect spatially dependent electric fields along the actin fibre

≈ 99 % of counterions within 8 nm of the polymer's radial axis

Ionic Cable Model: Electrical Model Components

- **Inductive -** The solenoidal element stems from the intrinsic helical structure observed in actin and MTs, resulting from the lateral translocation between adjacent protofilaments.

- **Resistive -** The resistive element is composed of two contributions: parallel and perpendicular to the MT's main axis. The perpendicular contribution arises from the resistivity between the two concentric cylinders.

- **Capacitive -** The capacitance in our model represents the charge distribution in the region spanning from the surface of the MT and approximately one Bjerrum length away perpendicularly to it. Since the ions in this layer are assumed to be condensed, the distribution of charges would be non-

  linear.

A Priel, JA Tuszyński, EPL (Europhysics Letters) 83 (6), 68004

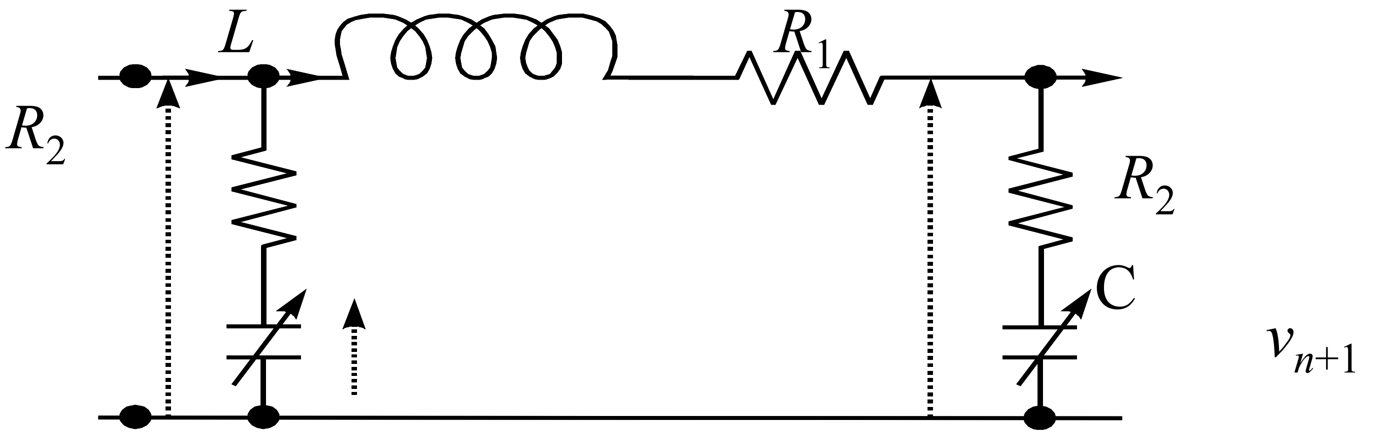
A UNIT CIRCUIT ELEMENT OF THE MT CABLE

L: Bjerrum ions generate time dependent current by movement along helical paths.

R_1: Due to viscosity we expect a series resistance

R_2: Resistance between the Bjerrum ions and surface of the filament – denoted by R_2 (in parallel)

C_0: In series with R_2 a capacitance C_0,

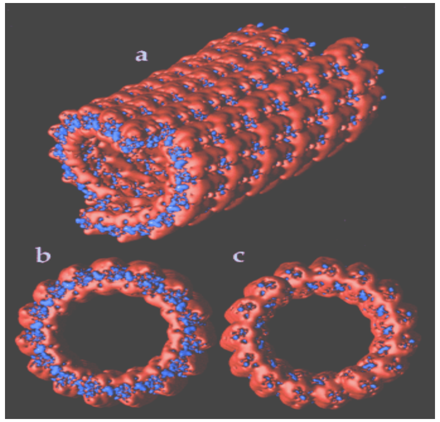


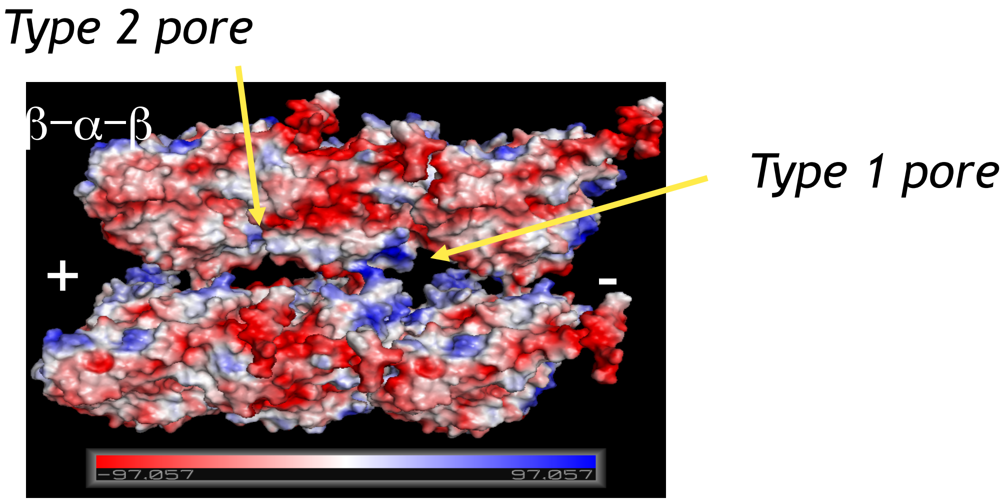
For each dimer of tubulin one finds:

C_0=6x10^{-16} F R_1= 6 MW R_2=1 MW L=2 pH

Additional Ion flow through pores in microtubules

Baker, Nathan A. et al. (2001) Proc. Natl. Acad. Sci. USA 98, 10037-10041

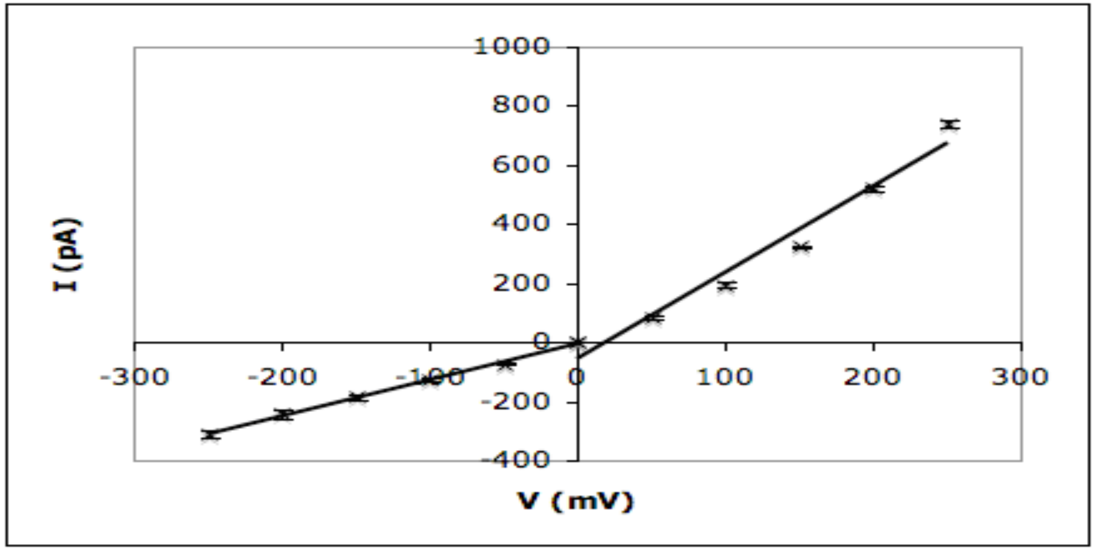




- Unique channel-like pore occurring in cellular polymer
- Potential isocontours are shown at +1 kT/e (blue) and -1 kT/e (red)
  b) View of '-' end (α tubulin).
  c) View of '+' end of (β tubulin)
- Note inner shell of positive charge
- Most negative on outer surface

Simulations of Microtubule's I-V characteristics

Current-voltage relation from Molecular Dynamics simulation for conductance of cations through a nano pore.

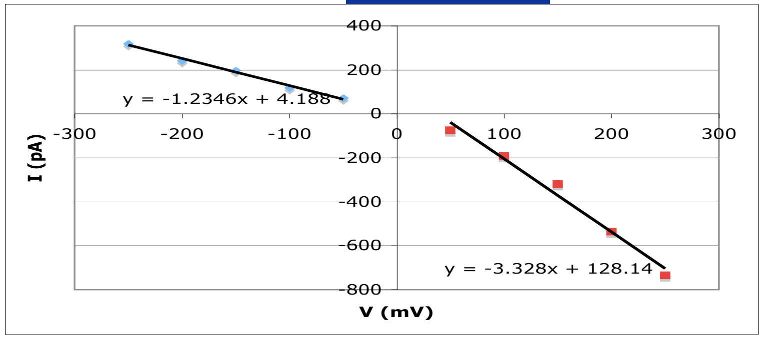


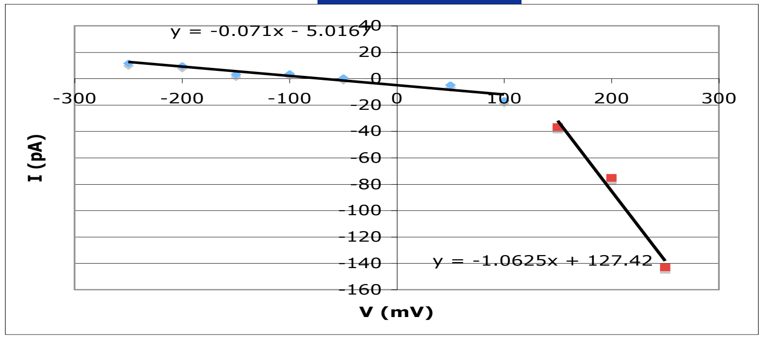
amplification of the lumen's current due to:
(1) thermal fluctuation of C-terminal tails
(2) asymmetric nanopores
(3) axial electric field along an MT

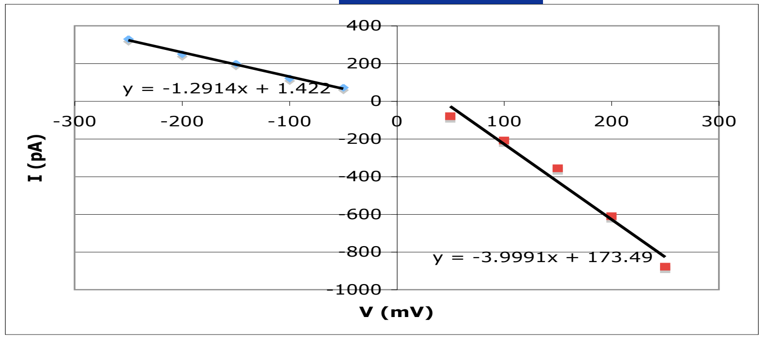
H Freedman, V Rezania, A Priel, E Carpenter, SY Noskov, JA Tuszynski, Physical Review E 81 (5), 051912

Type 1 pore

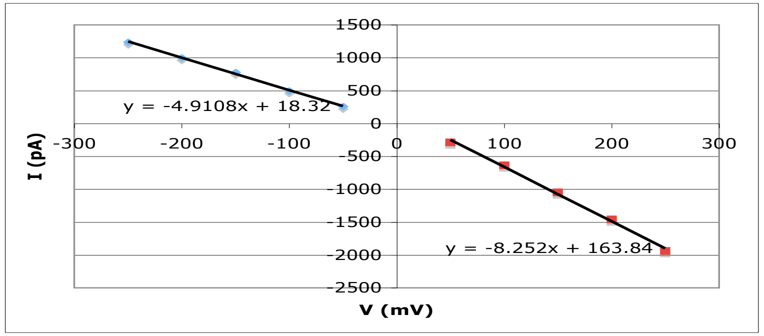
cation anion total

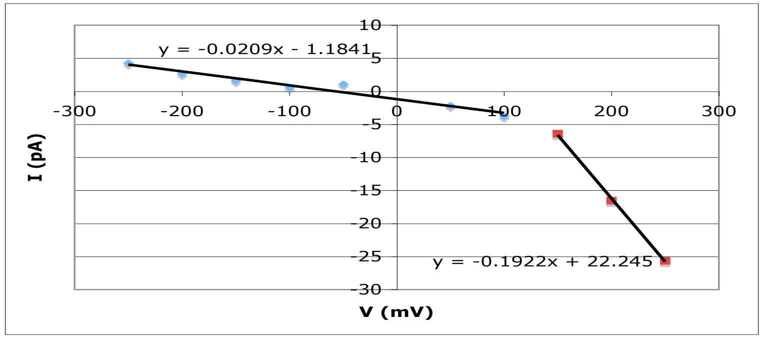


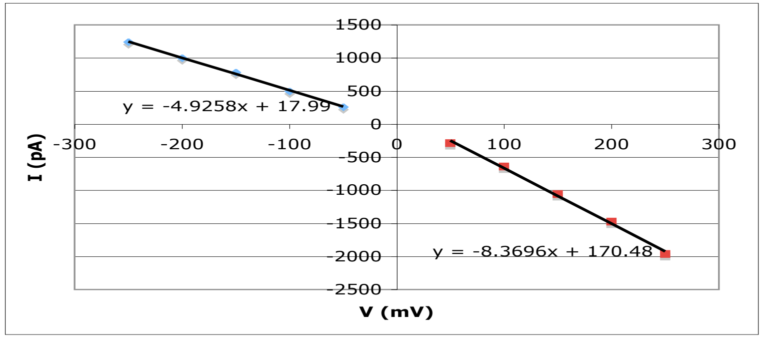




Type 2 pore



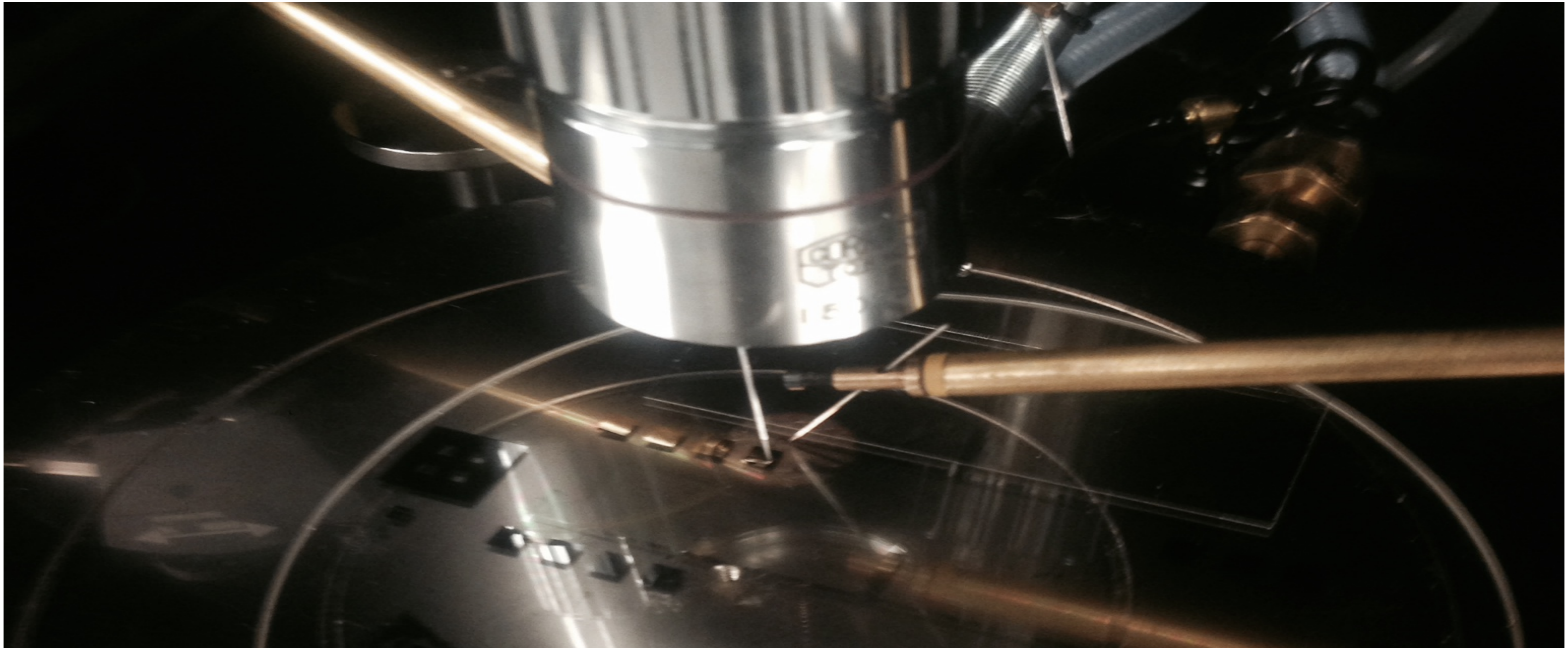




- Slopes give conductances in **nS** - very high total values!
  - Almost completely impermeable to anions at negative and small positive voltages
  - Conductance of cation greater for pore 2, anion for pore 1
  - Both pores show asymmetric conductances

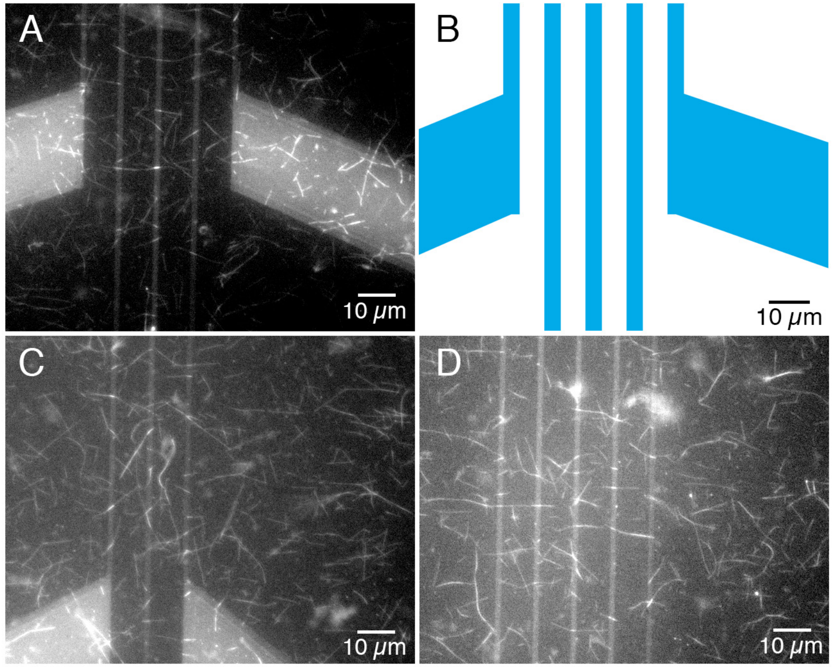
  - **Because of more negative outer surface potential, cations prefer to flow into MT and anions out, both occurring at positive potentials**

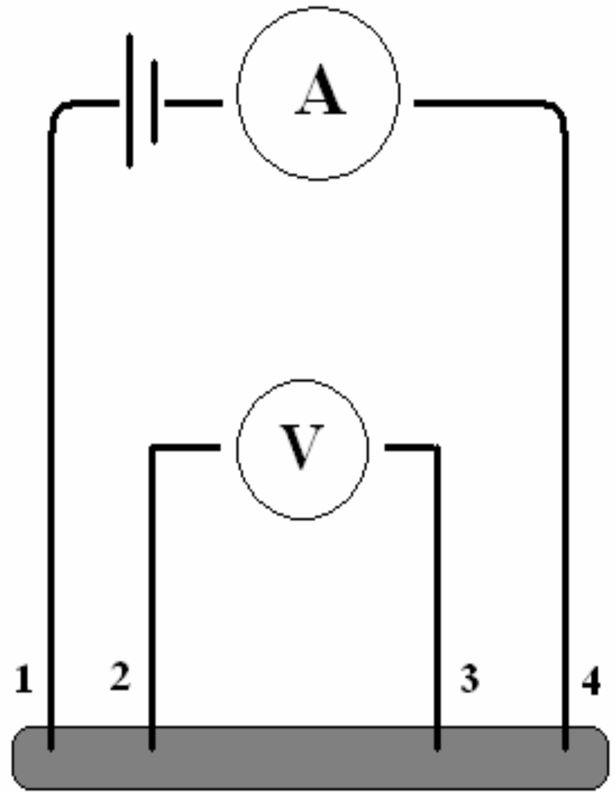
Tuszynski/Shankar Lab Nanofluidic Experiments

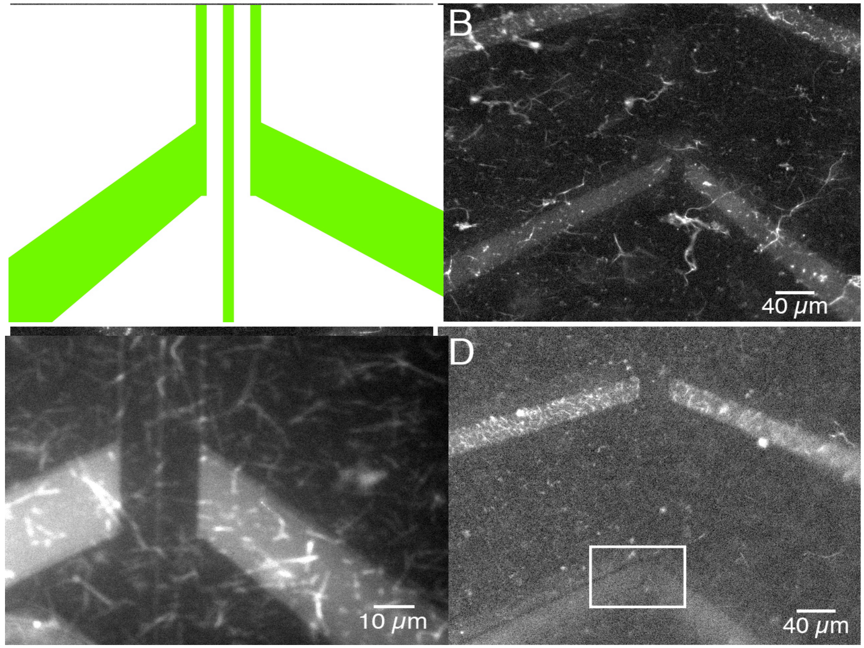


Nanofab Keithley 4200-SCS Sourcemeter to characterize conductivity

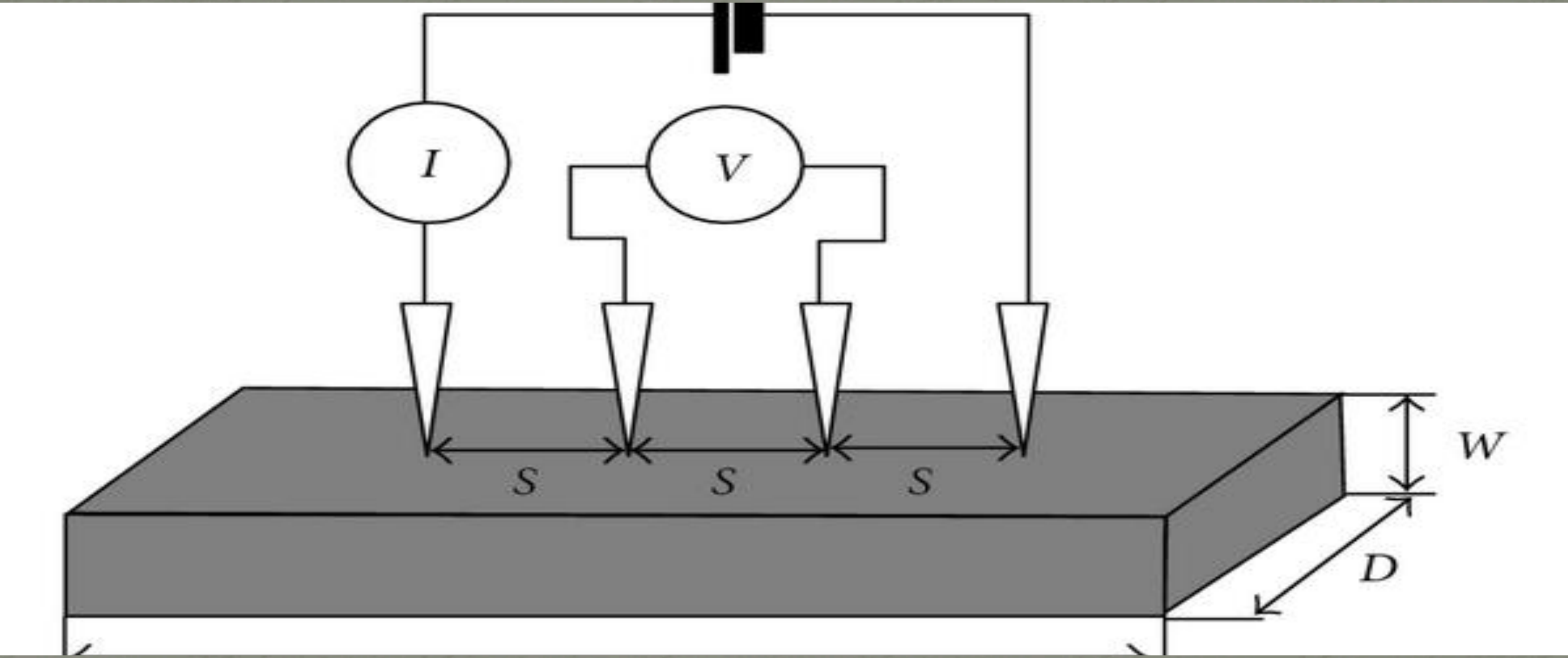
Four-probe and two-probe I-V measurements







4 Point Probe Theory



σ = conductivity (S/m)

$$\sigma = \left(\frac{\pi}{\ln(2)} \times \frac{V}{I} \times t \right)^{-1}$$

t = thickness (55 nm)

2- Electrode Conductivity Results

	Resistance (Ω)	Resistivity (Ω•m)	Conductivity (S/m)
PLL	9.77E+05	0.115	8.7
PEM	4.98E+05	0.059	17.1
MT 37nM	1.08E+06	0.127	7.9
MT 74nM	2.39E+06	0.282	3.5

$L = 14\ \mu m$

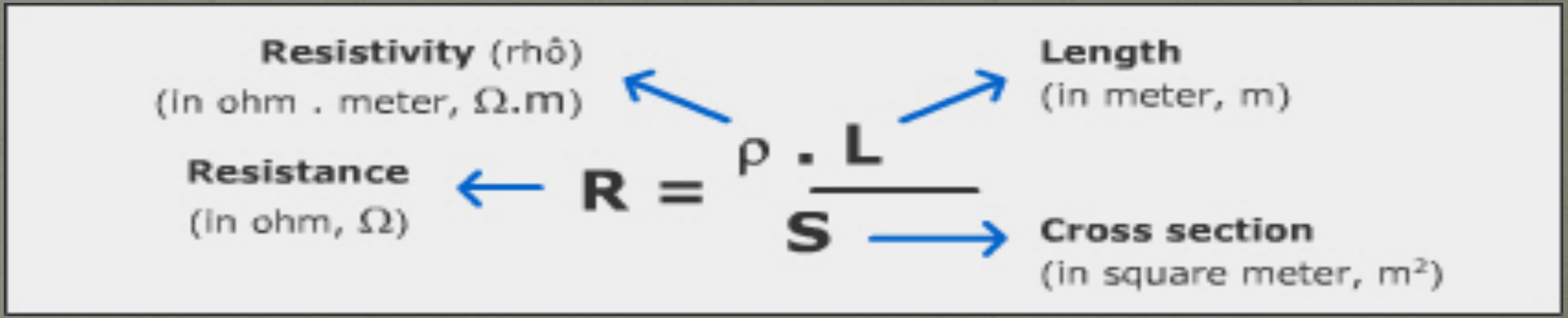
$w = 30\ \mu m$

$h = 55\ nm$

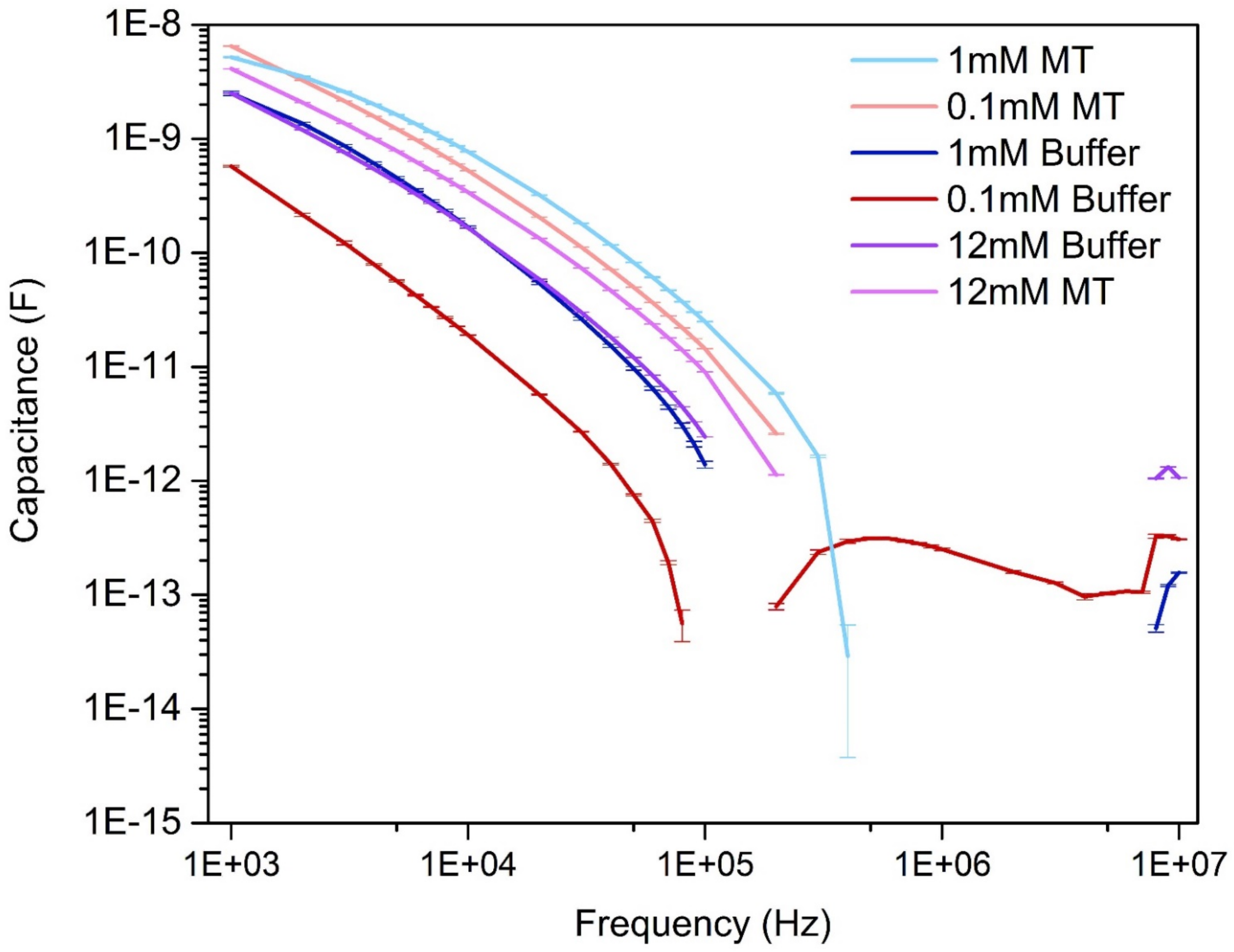
$S = w \bullet h$

$\rho = 1/\sigma$

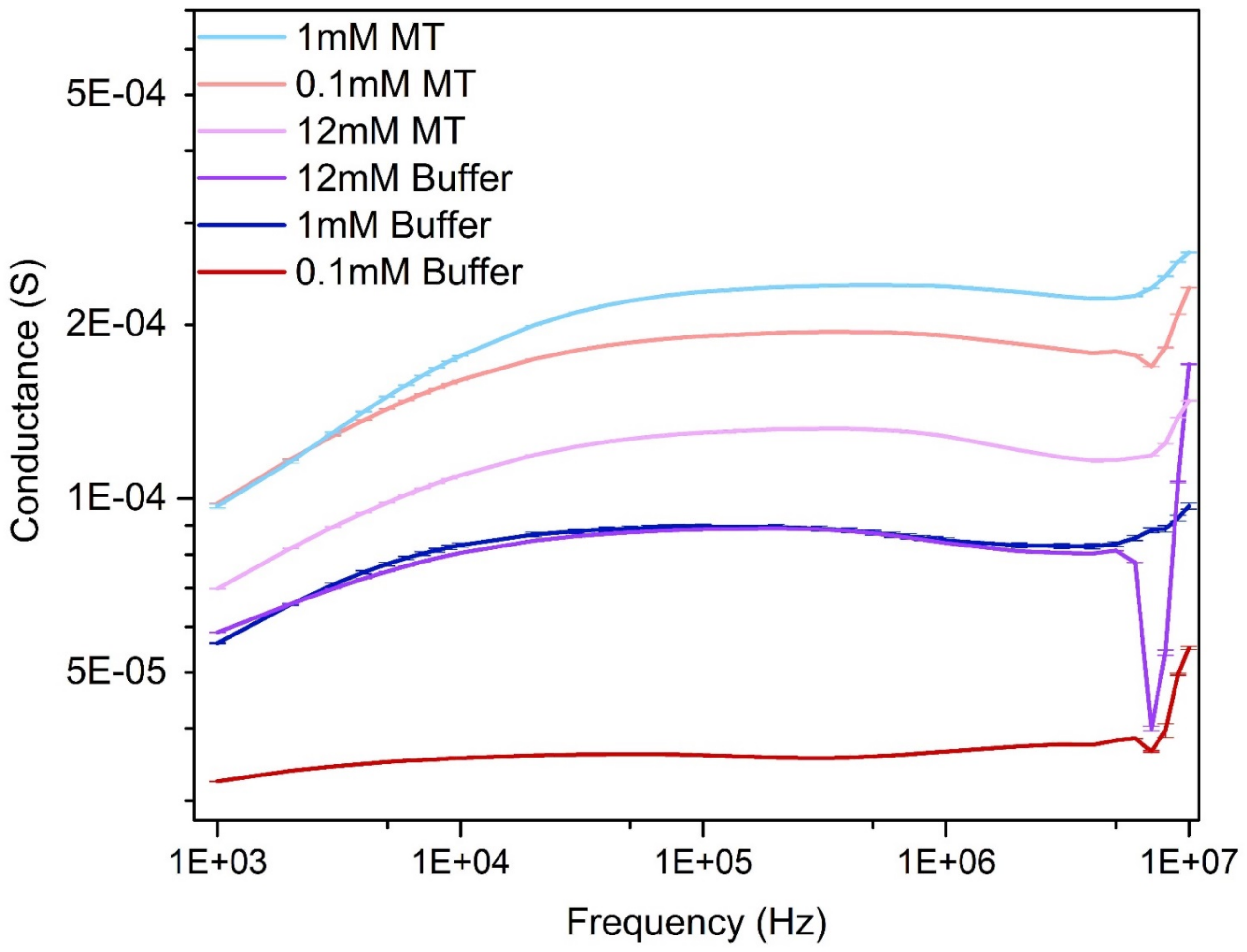
ρ is conductivity (S/m)



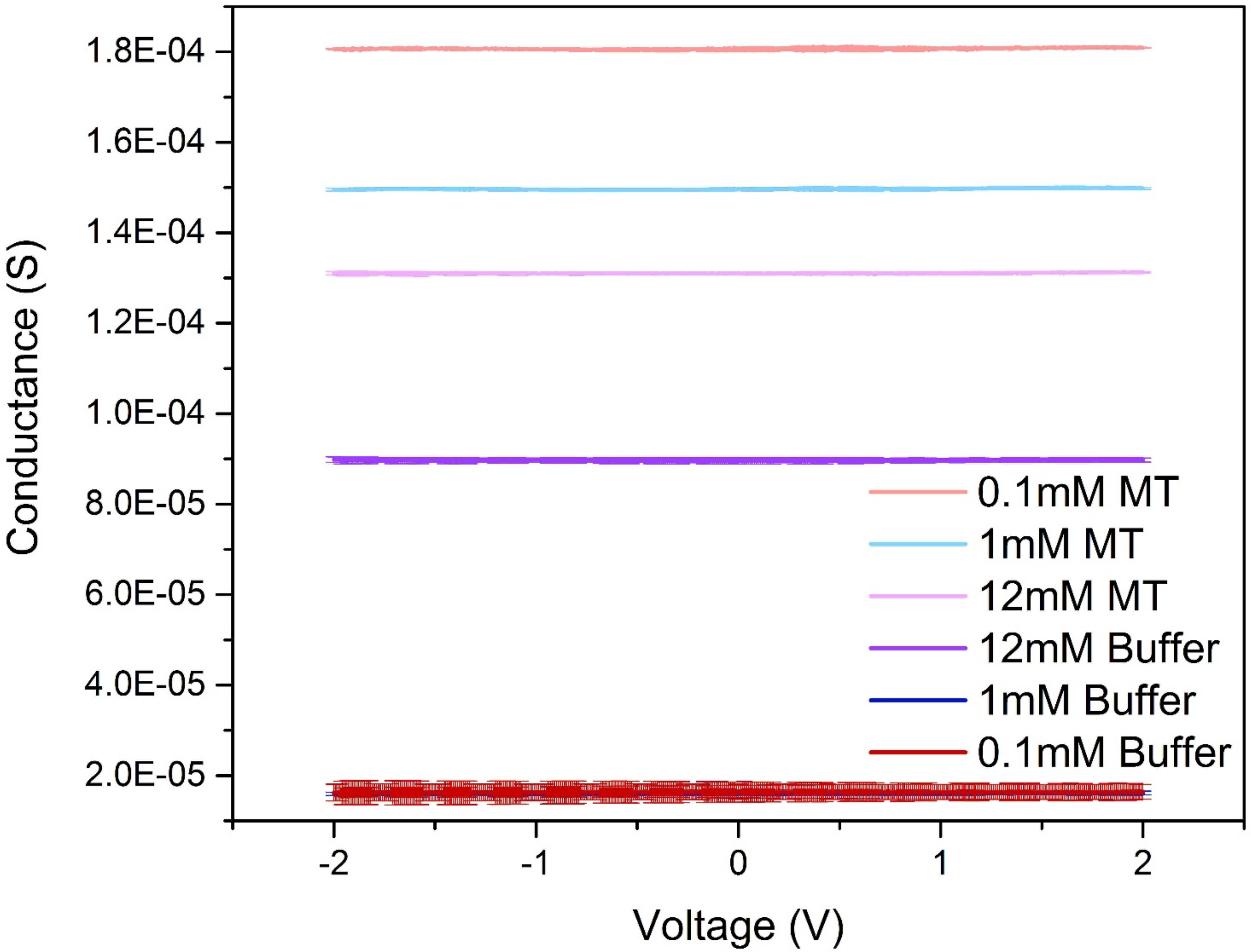
Capacitance vs Frequency for Buffer, MTs and Tubulin.



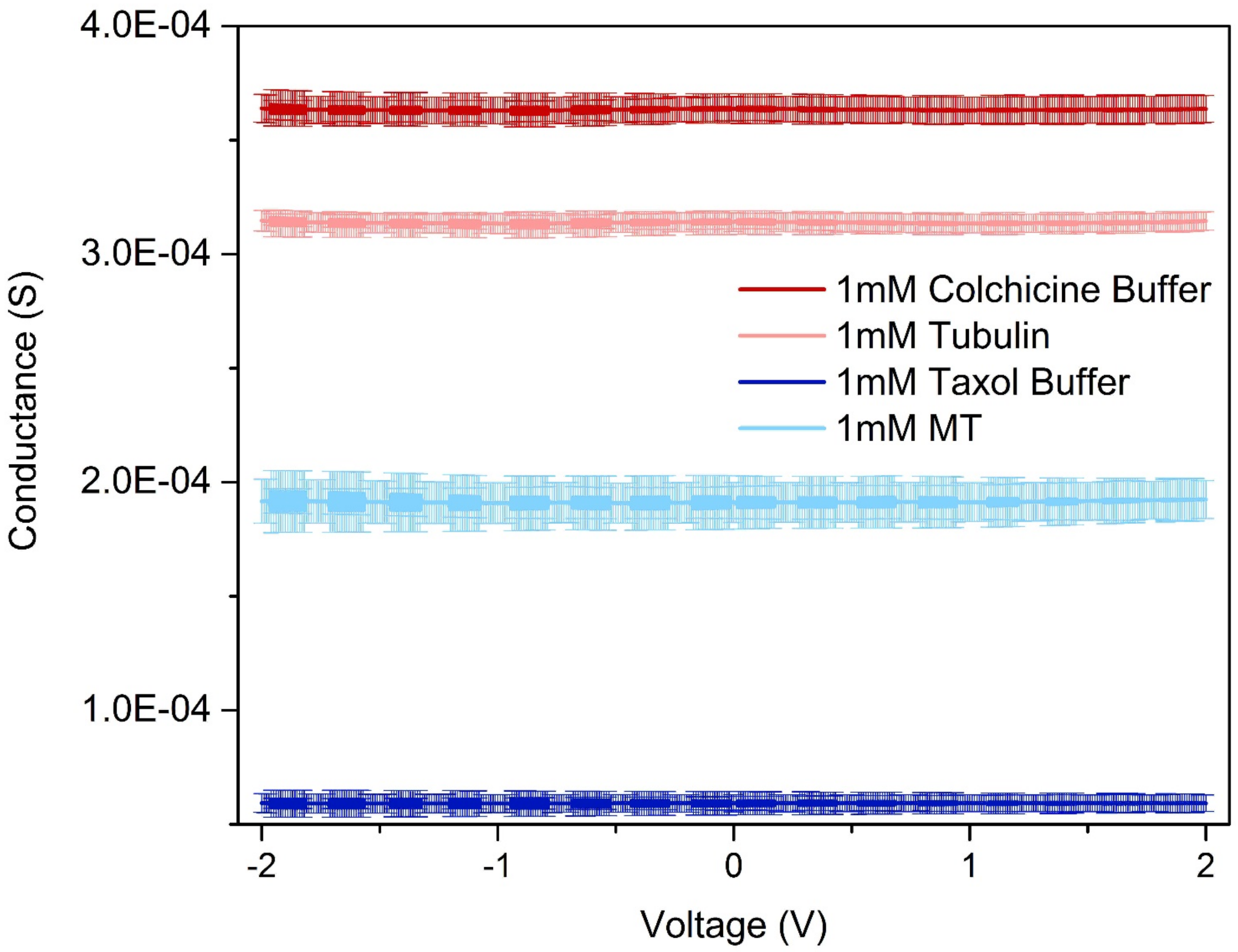
Conductance vs Frequency for Buffer, Tubulin and MTs.



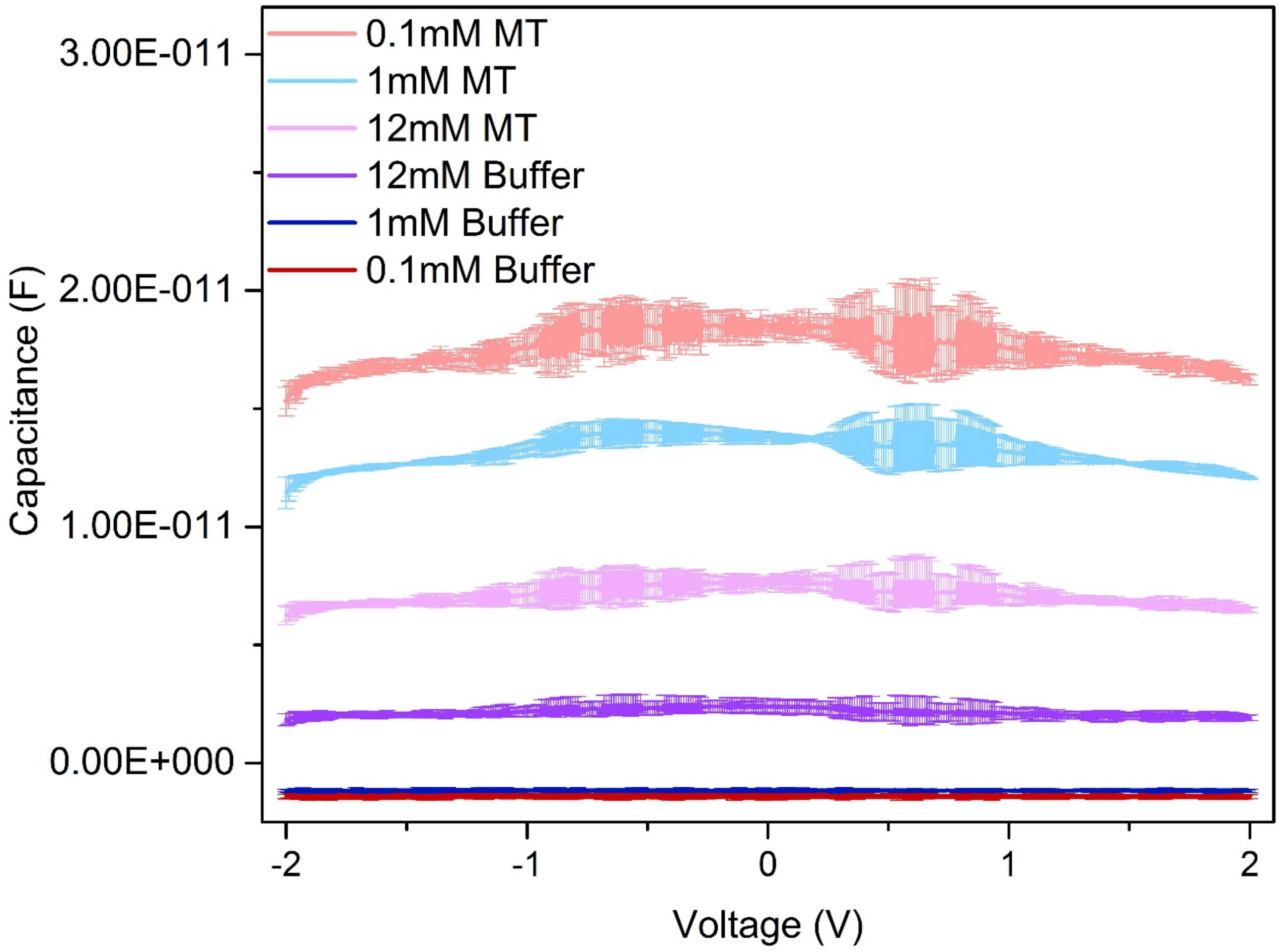
Conductance vs Bias Voltage at 100kHz for MTs at different ionic concentrations.



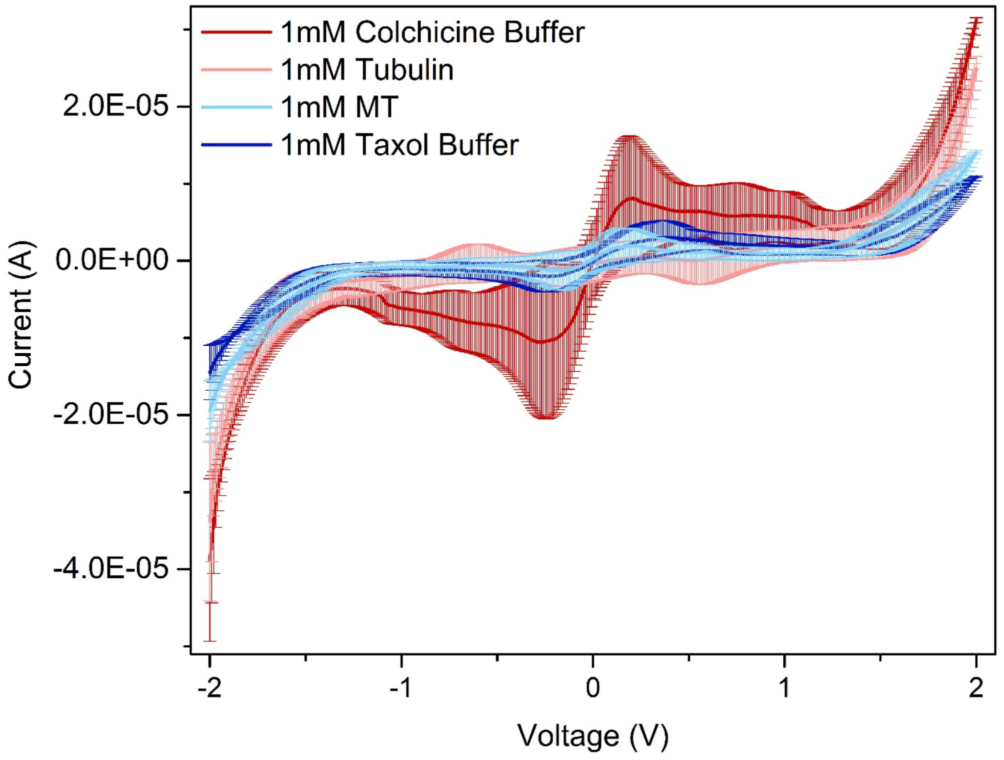
Conductance vs Voltage at 100kHz for low ionic MTs and Tubulin.

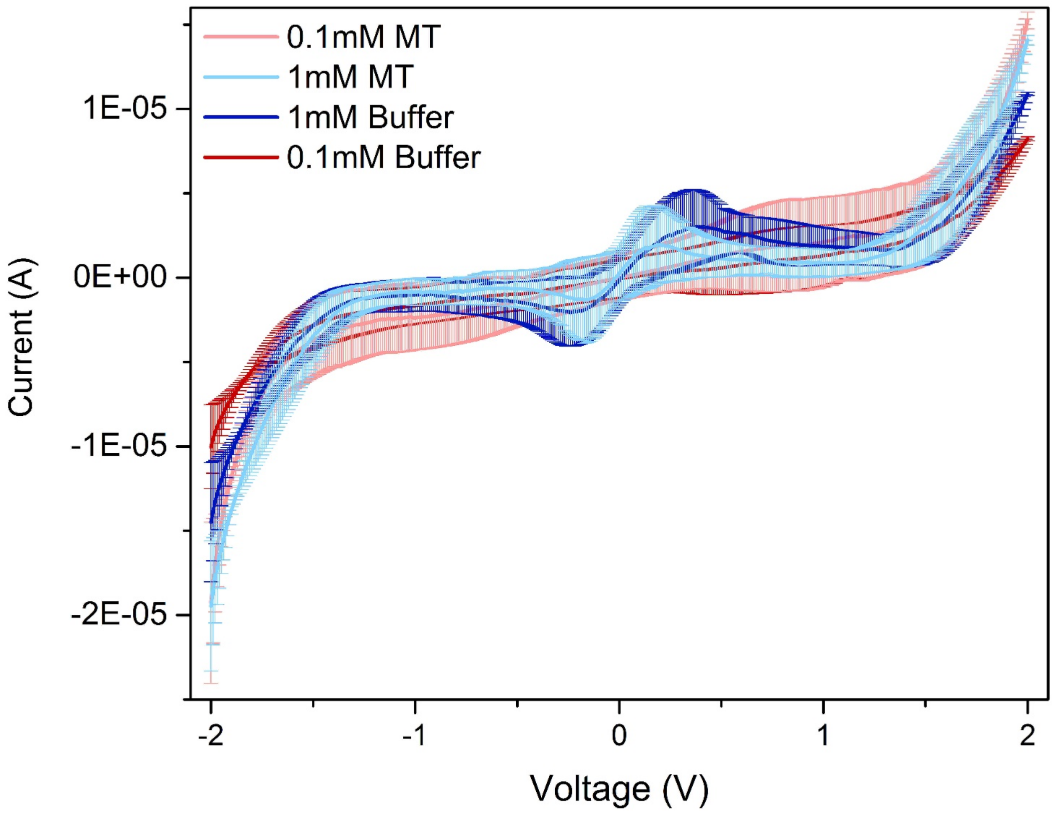


Capacitance vs Bias Voltage at 100kHz for MTs at different ionic concentrations.

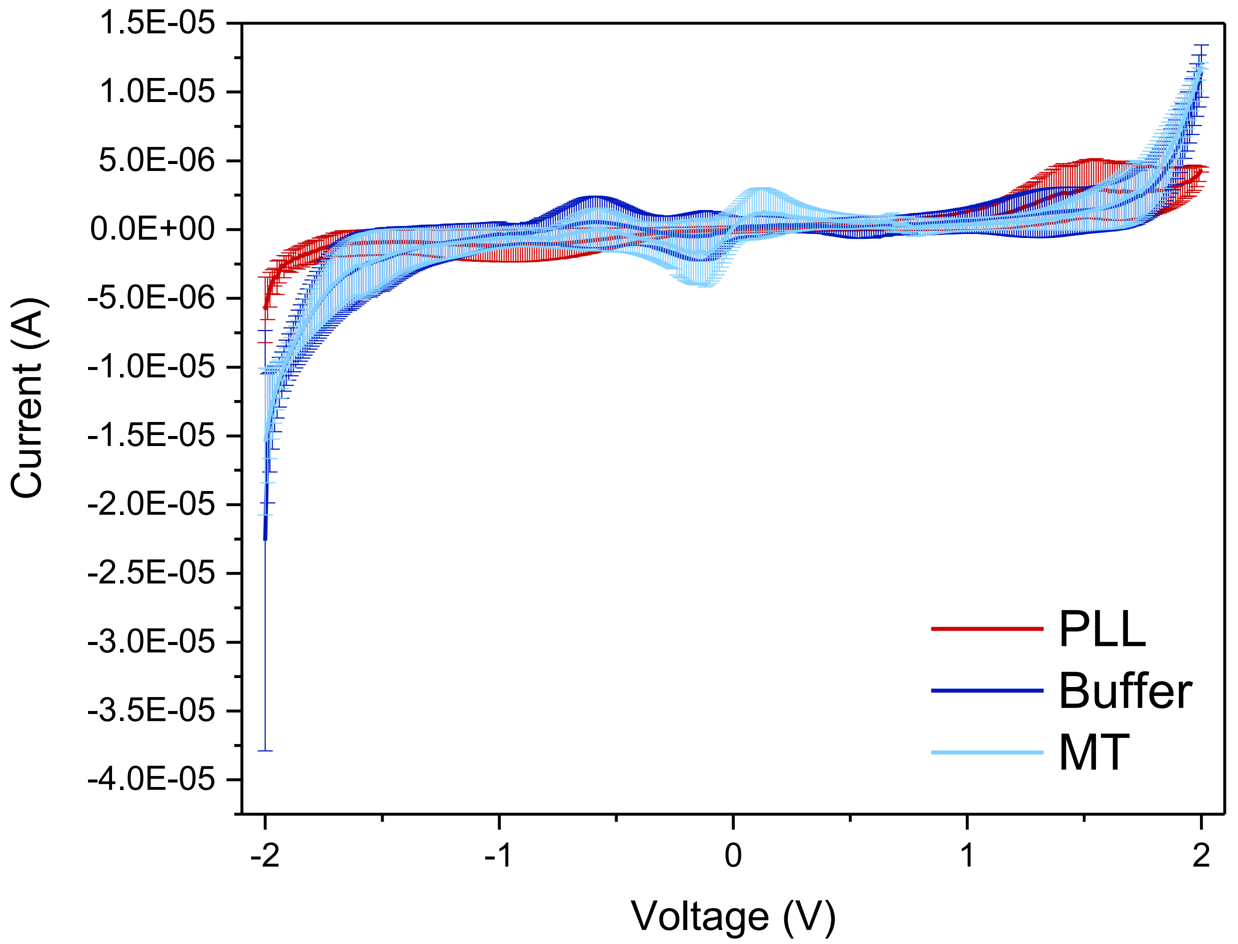


Current vs Voltage for low ionic Buffer, MTs and Tubulin.

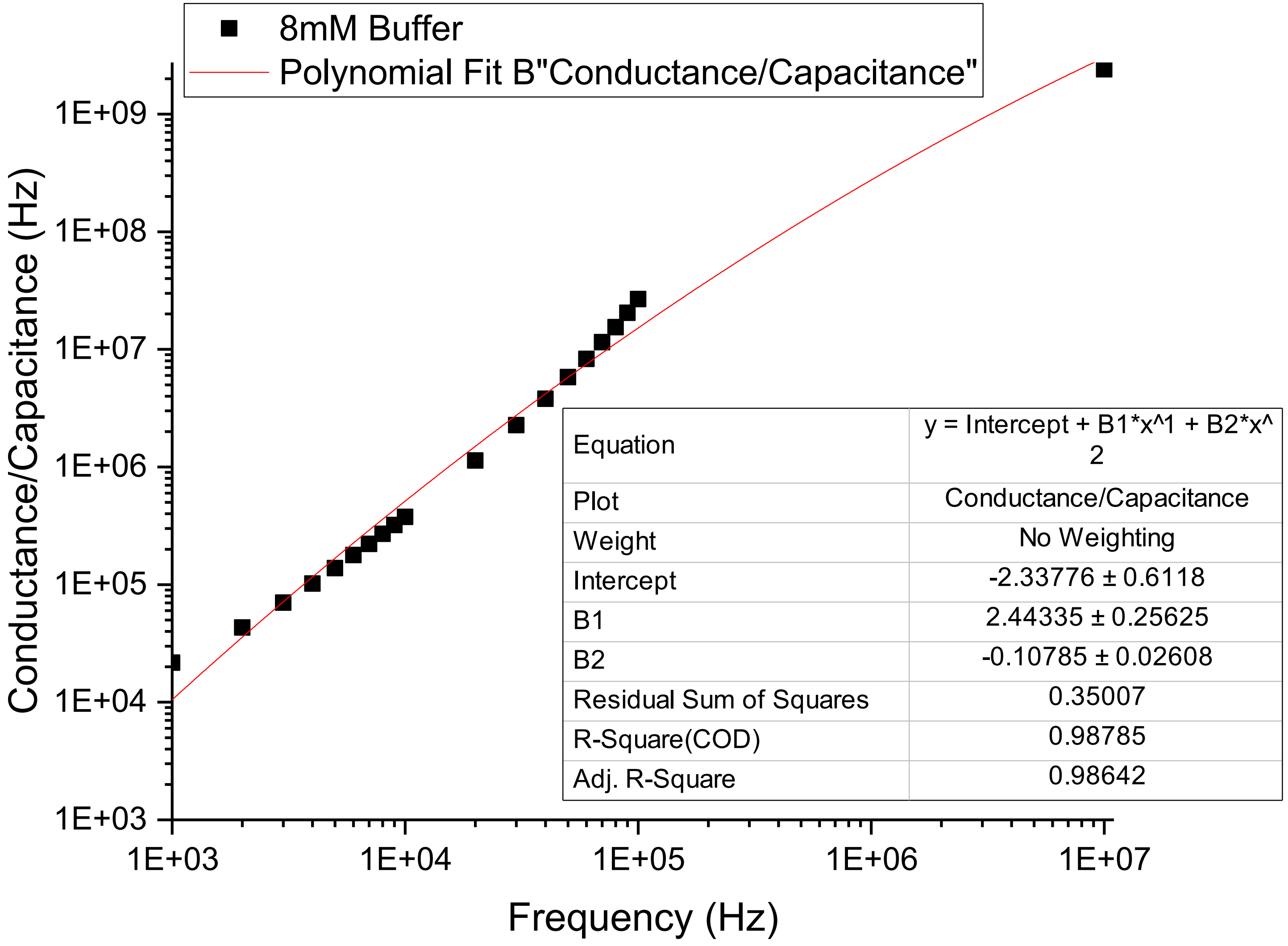




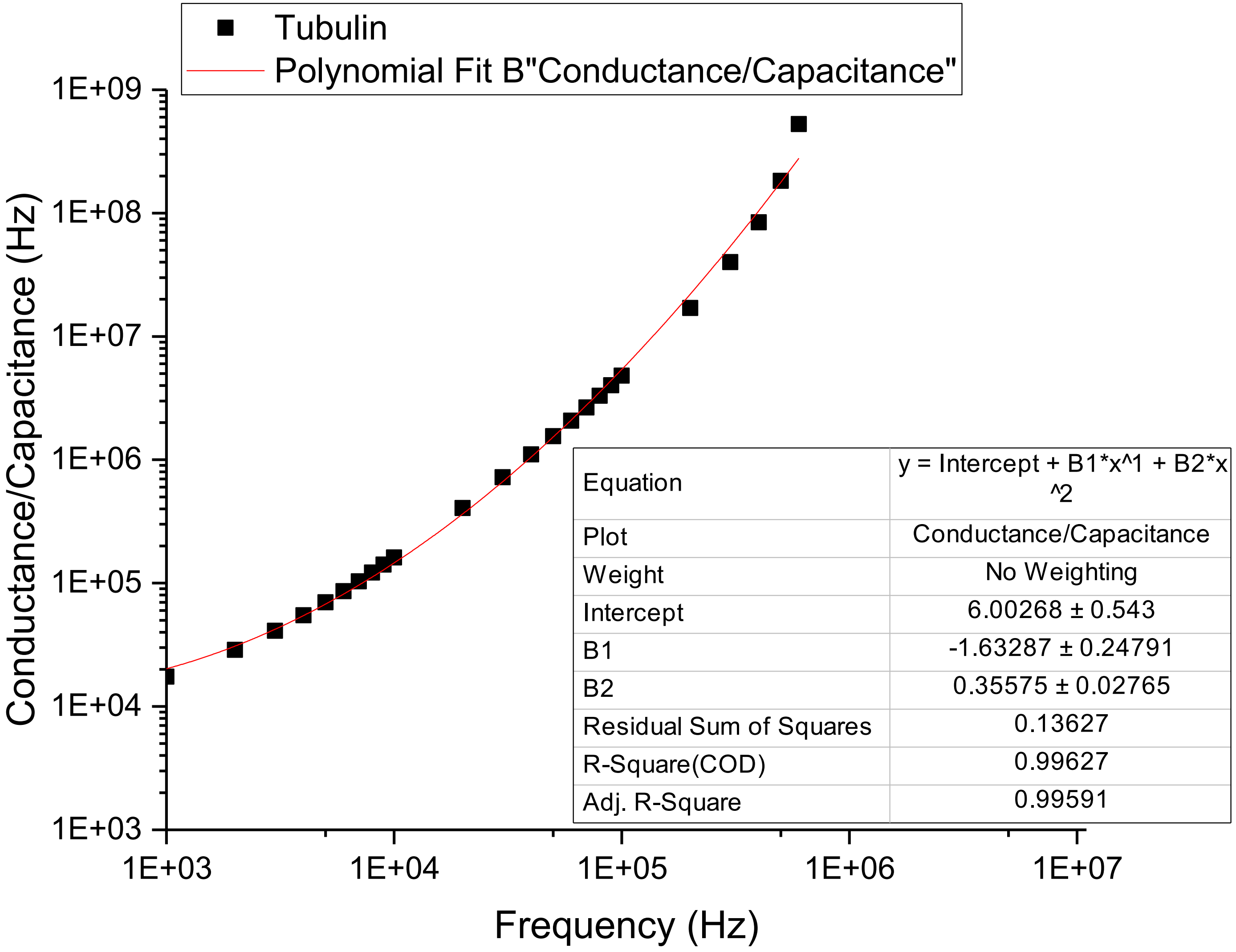
Current vs Voltage



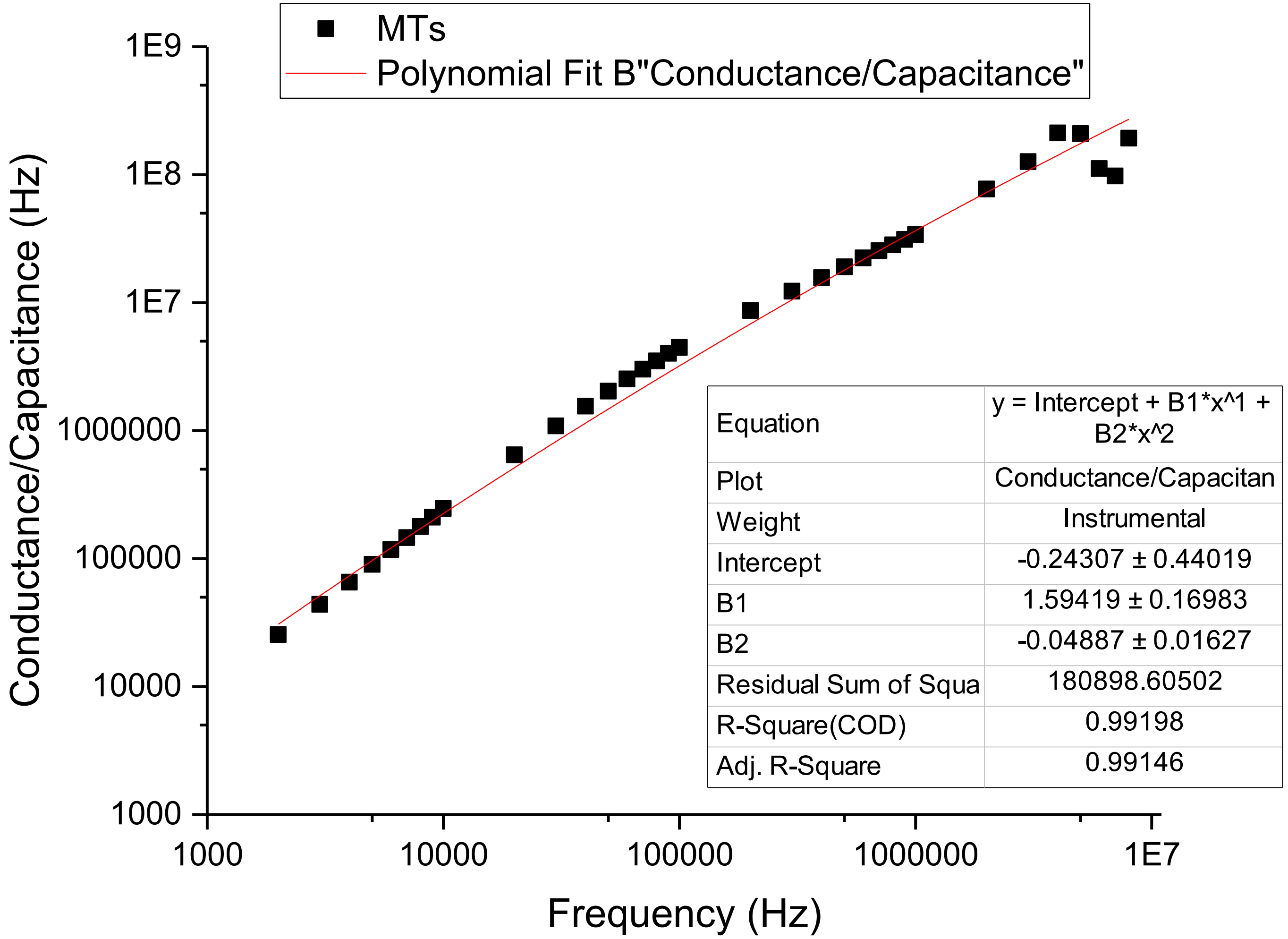
1/RC vs Frequency for 8mM Buffer



1/RC vs Frequency for Tubulin



1/RC vs Frequency for MTs



Potential Effects of MTs in solution

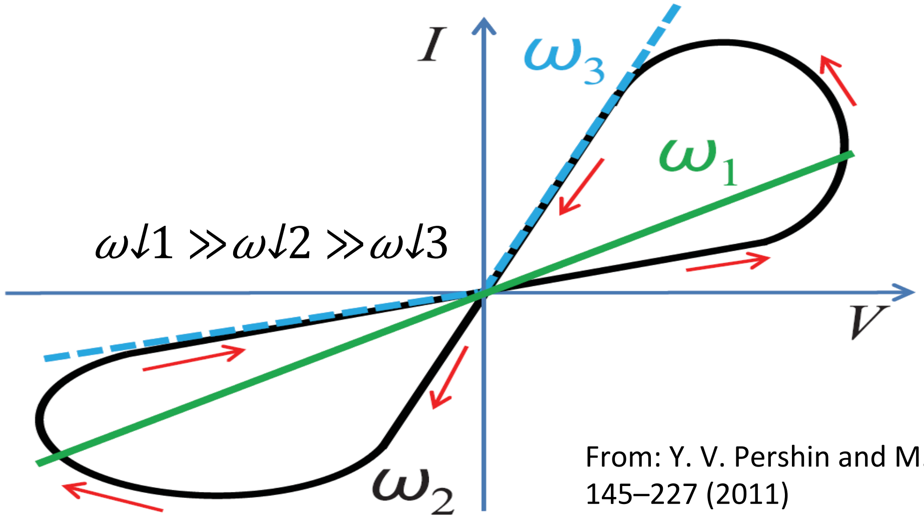
Hypotheses to include in building a mathematical model

1. MTs form a physical barrier that impedes ionic conductance between the electrodes
2. MTs attract and accelerate ions in a favorable direction along MTs (and vice versa) leading to increased (decreased) conductance
3. MTs condense ions along MTs leading to less overall mobile charge carriers in the medium
4. MTs use C-termini for ion fluxes (asymmetrically) in and out of the lumen

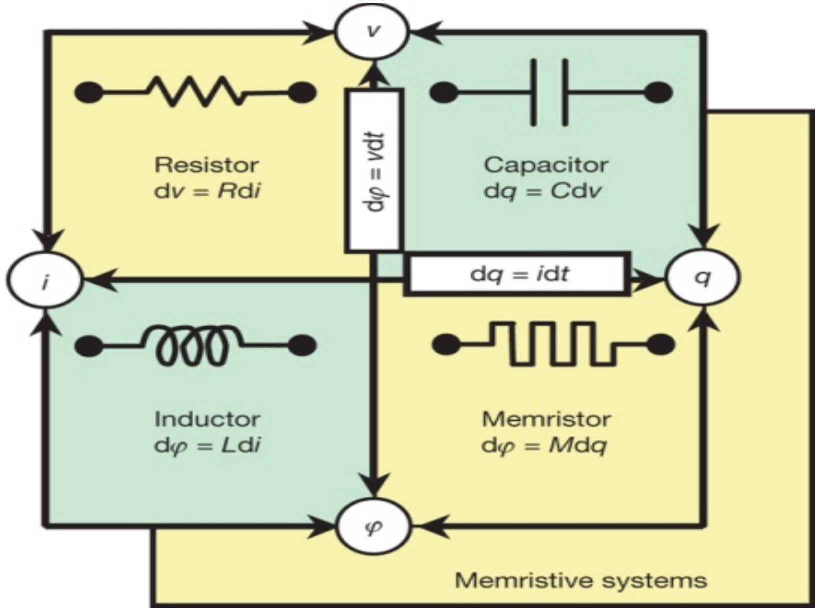
The Memristor

Theorized in 1971[1], physically achieved in 2008[2]:

- Two-terminal passive circuit element;
- Resistance depends on the history of applied voltage or current;
- Self-crossing, pinched hysteretic I-V loop, frequency dependent.



From: Y. V. Pershin and M. Di Ventra, Advances in Physics 60, 145–227 (2011)



From [2]: D. B. Strukov, G. S. Snider, D. R. Stewart, and R. S. Williams, Nature 453, 80 (2008).

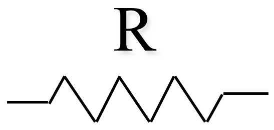
[1] Chua, L. Memristor - The Missing Circuit Element. *IEEE Transactions On Circuit Theory* **CT-18**, 507–519 (1971).

Four basic passive elements

Nonlinear Linear Local value

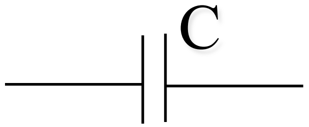
Resistor

$$v = f(i) \quad \Rightarrow \quad v = Ri \quad \Rightarrow \quad dv = R\,di$$



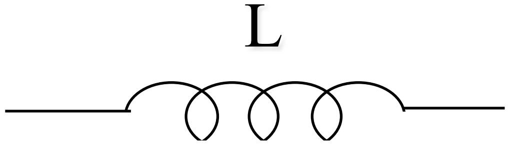
Capacitor

$$q = f(v) \quad \Rightarrow \quad q = Cv \quad \Rightarrow \quad dq = C\,dv$$



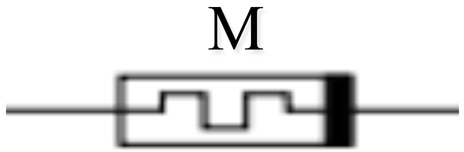
Inductor

$$\phi = f(i) \quad \Rightarrow \quad \phi = Li \quad \Rightarrow \quad d\phi = L\,di$$



Memristor

$$\phi = f(q) \quad \Rightarrow \quad \phi = Mq \quad \Rightarrow \quad d\phi = M\,dq$$



How memristance works?

- Memristor is defined as an element that relates flux and charge

$$\phi = f(q)$$

- Memristance value is computed as

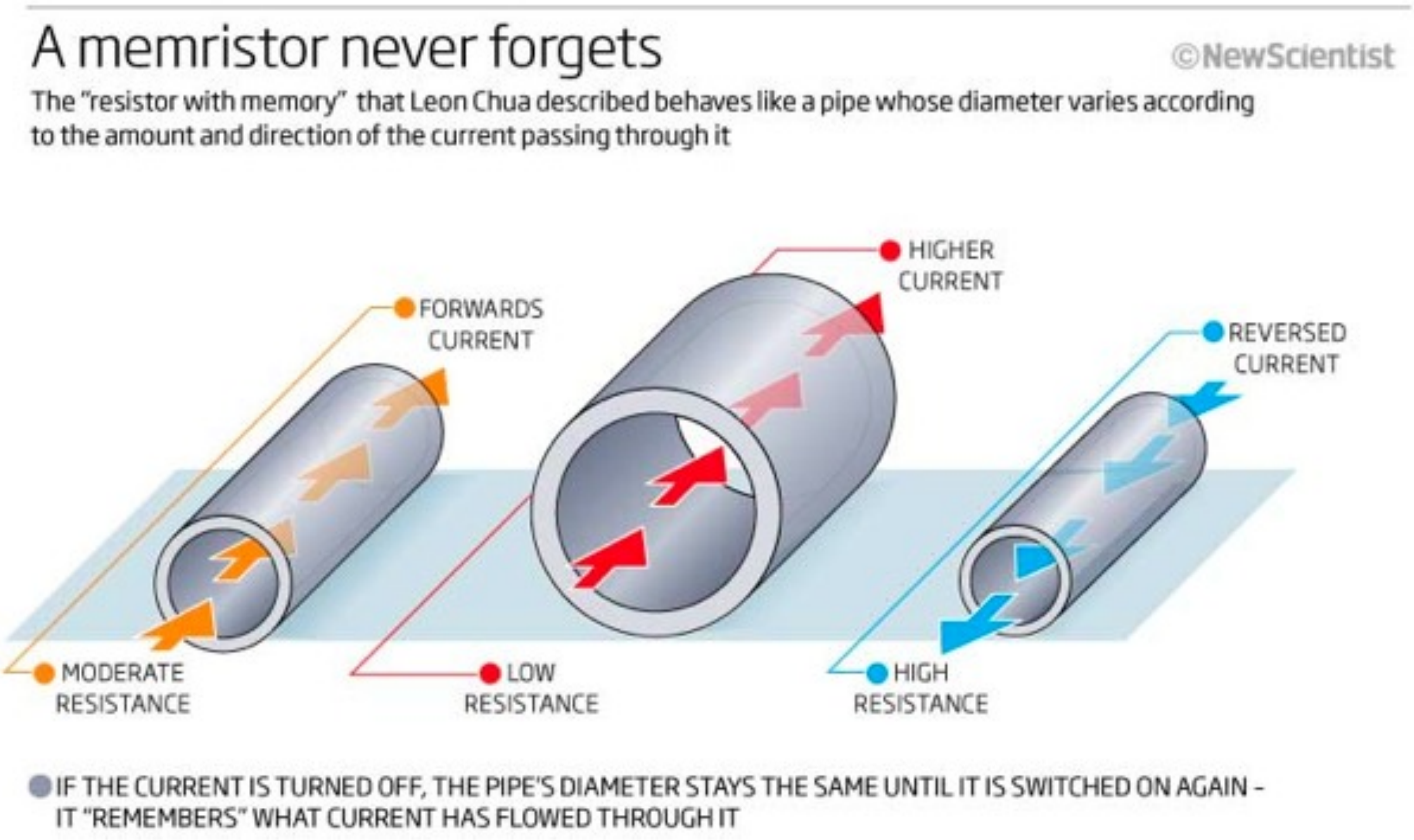
$$M(q) = \frac{d\phi}{dq}$$

- and can be related to voltage – current relation as follows

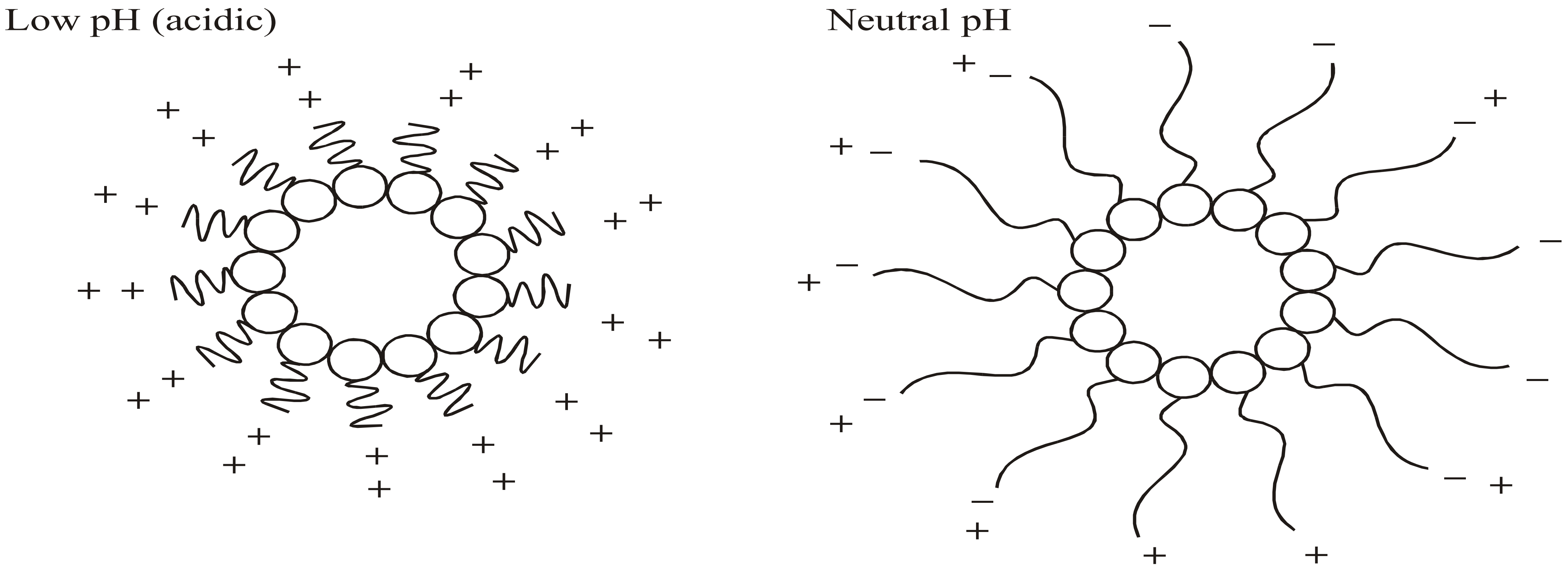
$$M(q(t)) = \frac{d\phi / dt}{dq / dt} = \frac{v(t)}{i(t)}$$

- Thus effectively it is a charge dependent resistance

A memristor is a pipe that changes diameter with the amount and direction of water that flows through it. If water flows through this pipe in one direction, it expands (becoming less resistive). But send the water in the opposite direction and the pipe shrinks (becoming more resistive). Further, the memristor remembers its diameter when water last went through. Turn off the flow and the diameter of the pipe "freezes" until the water is turned back on. That freezing property suits memristors brilliantly for computer memory. The ability to indefinitely store resistance values means that a memristor can be used as a nonvolatile memory.



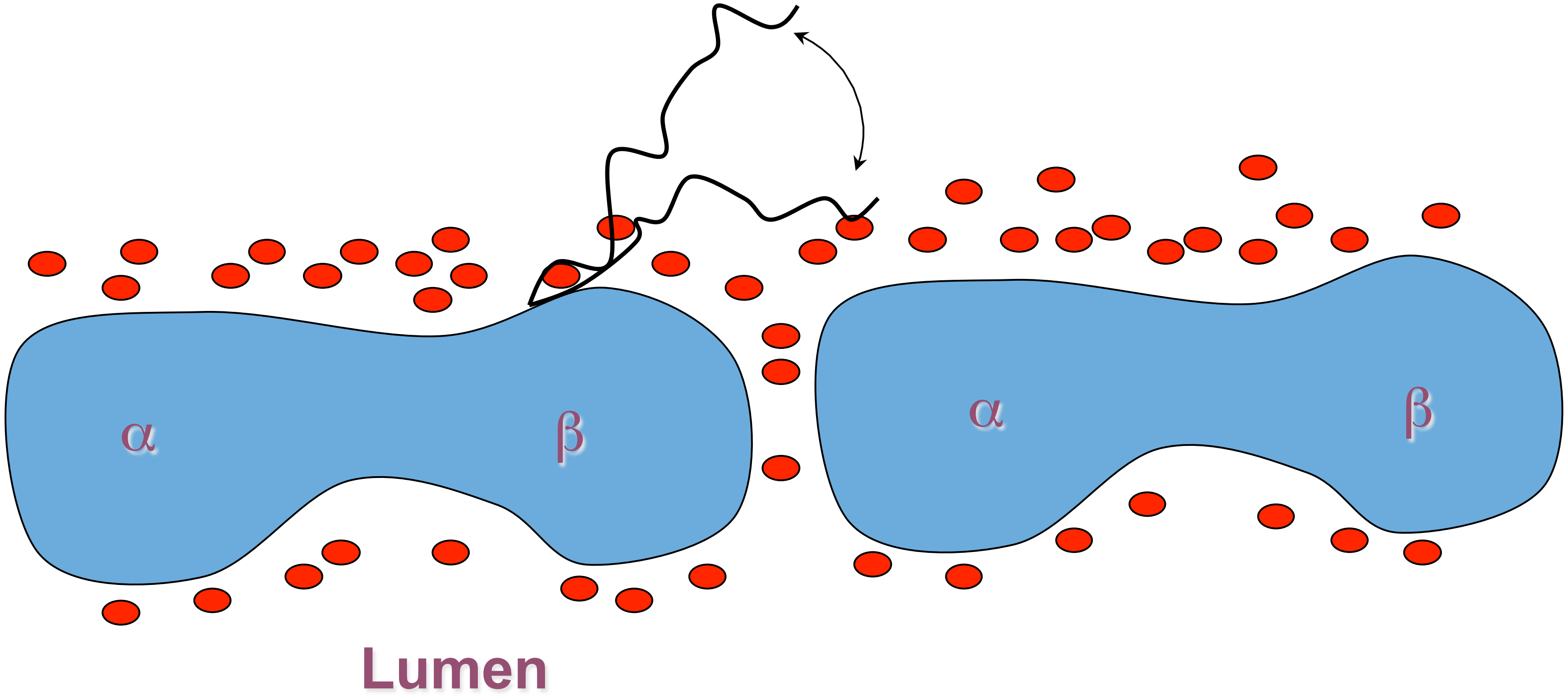
Is it plausible that a microtubule is a biological memristor?



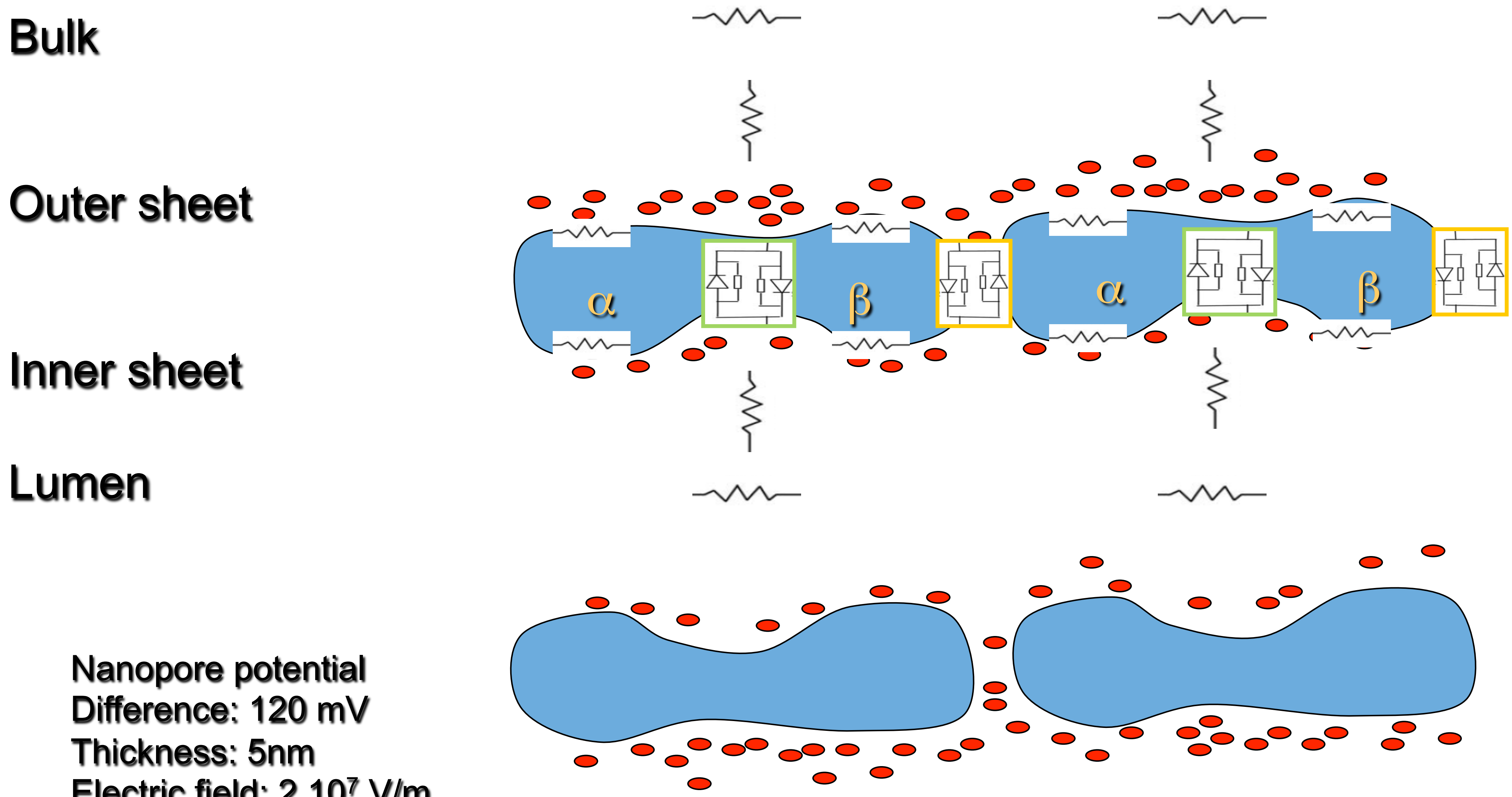
C-termini are antenna-like charged parts of MTs

C-terminal tails dynamics

- C-terminal tails exist in multiple states that **dynamically oscillate** on the order of ~GHz

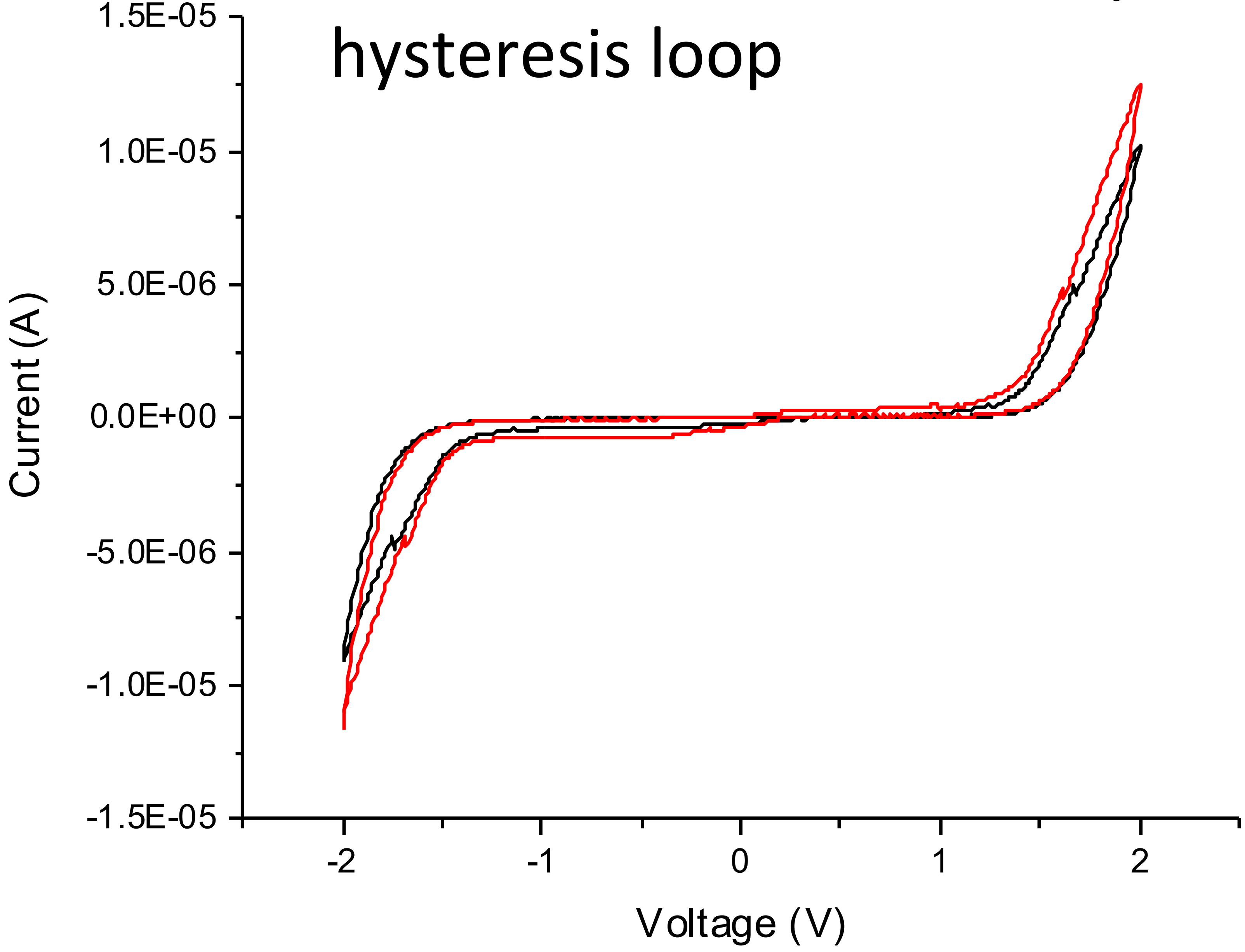


The electrical circuit model for the luminal flows



Nanopore potential
Difference: 120 mV
Thickness: 5nm
Electric field: $2\ 10^{7}$ V/m

IV Characteristic of MT: a pinched hysteresis loop



To be continued…

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