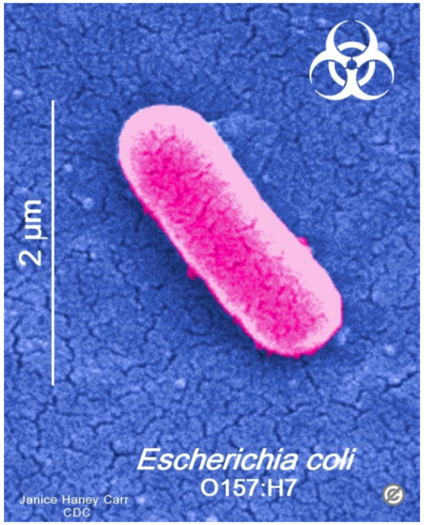
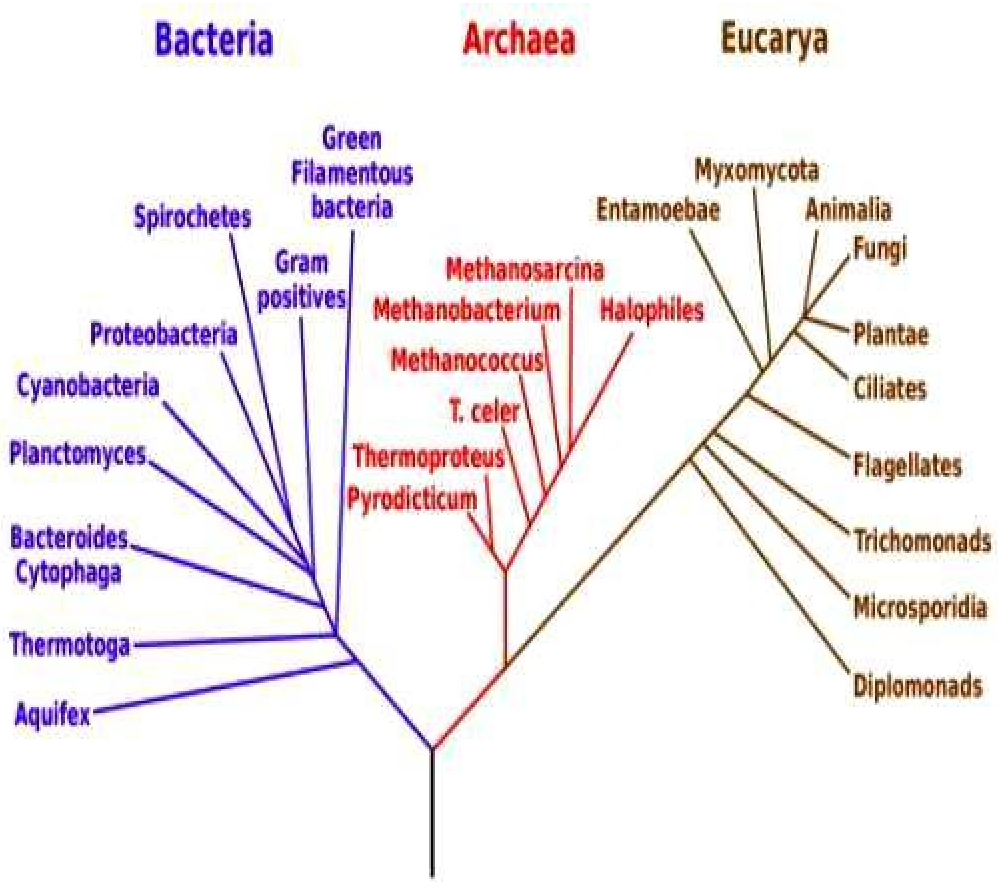
Microorganisms and Electricity:

Paradigms to Approach Interaction

Dominique Rauly



What are microorganisms ?



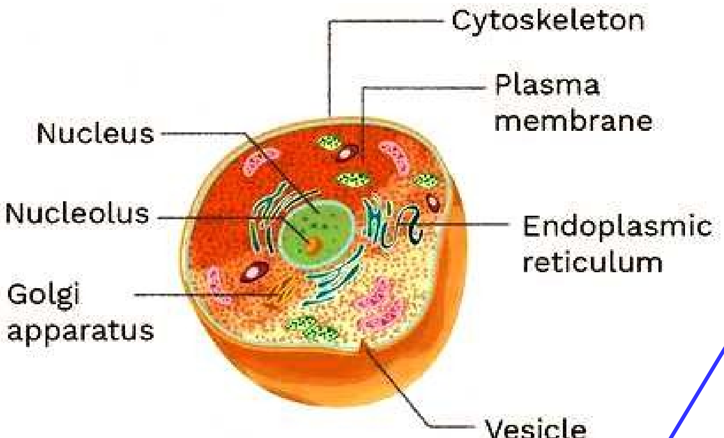
Phylogenetic Tree of Life *

* Credit: Carl A. Batt, Chapter in Book
Reference Module in Food Science , dec 2016

What are microorganisms ?

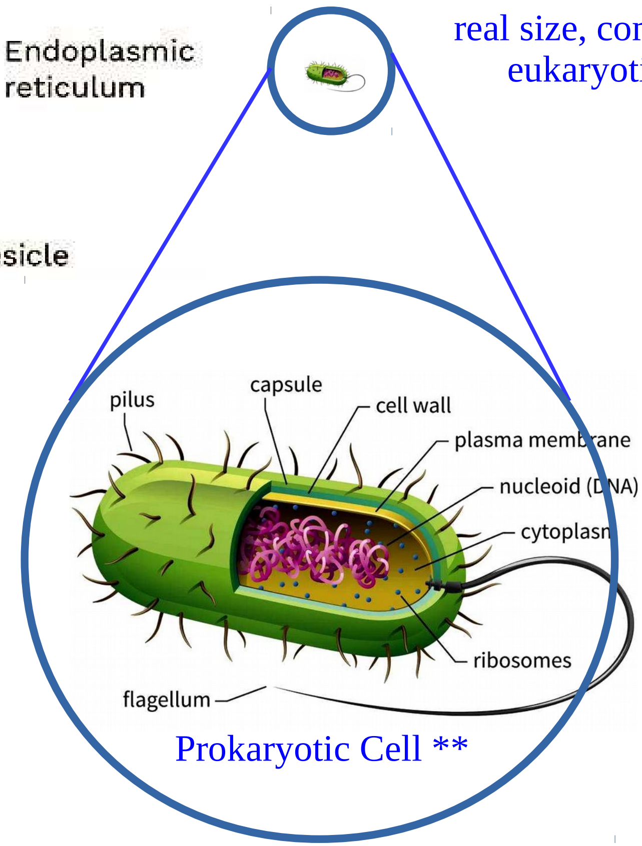
A distinction is made between:

Eukaryotes



Eukaryotic Cell *

Prokaryotes (Bacteria & Archaea)



Prokaryotic Cell **

* Laura Klappenbach, Jan 2020
** Credit: Bacterial Cell Anatomy and Internal Structure. Jack0m/Getty Images

What are microorganisms ?

Microorganisms:

- Unicellular, living in single-celled form, or in colony of cells
- Bacteria, yeast, fungi, and in some definition viruses
- Are included, according some classification:

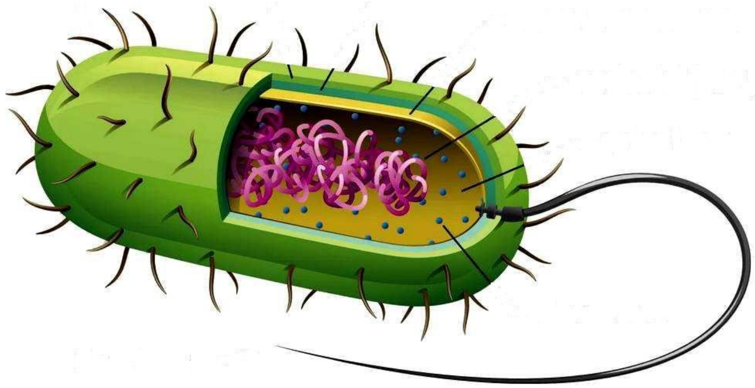
  Both microorganisms that are capable of replication outside of any host and those that require a host to survive
- Size from less than 100 nm to 750 µm

  (Thiomargarita *namibiensis**)

* Schulz and Jørgensen, 2001

Prokaryotes

- At this stage of evolution, prokaryotes already contain almost all the building blocks for the functionalities of life
  - ► Focus of present questioning on prokaryotes.



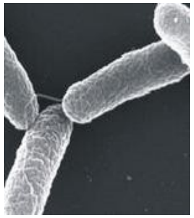
Prokaryotes

Various shapes

- Spherical (cocci)
  - Staphylococcus aureus
  - Streptococcus
  - etc..

Credit: Janice Carr/Centers for Disease Control and Prevention

- Rod shaped (bacilli)
  - Bacillus, Lactobacillus
  - Escherichia coli
  - etc..



Credit: Volker Brinkmann, Max Planck Institute for Infection Biology, Berlin, Germany

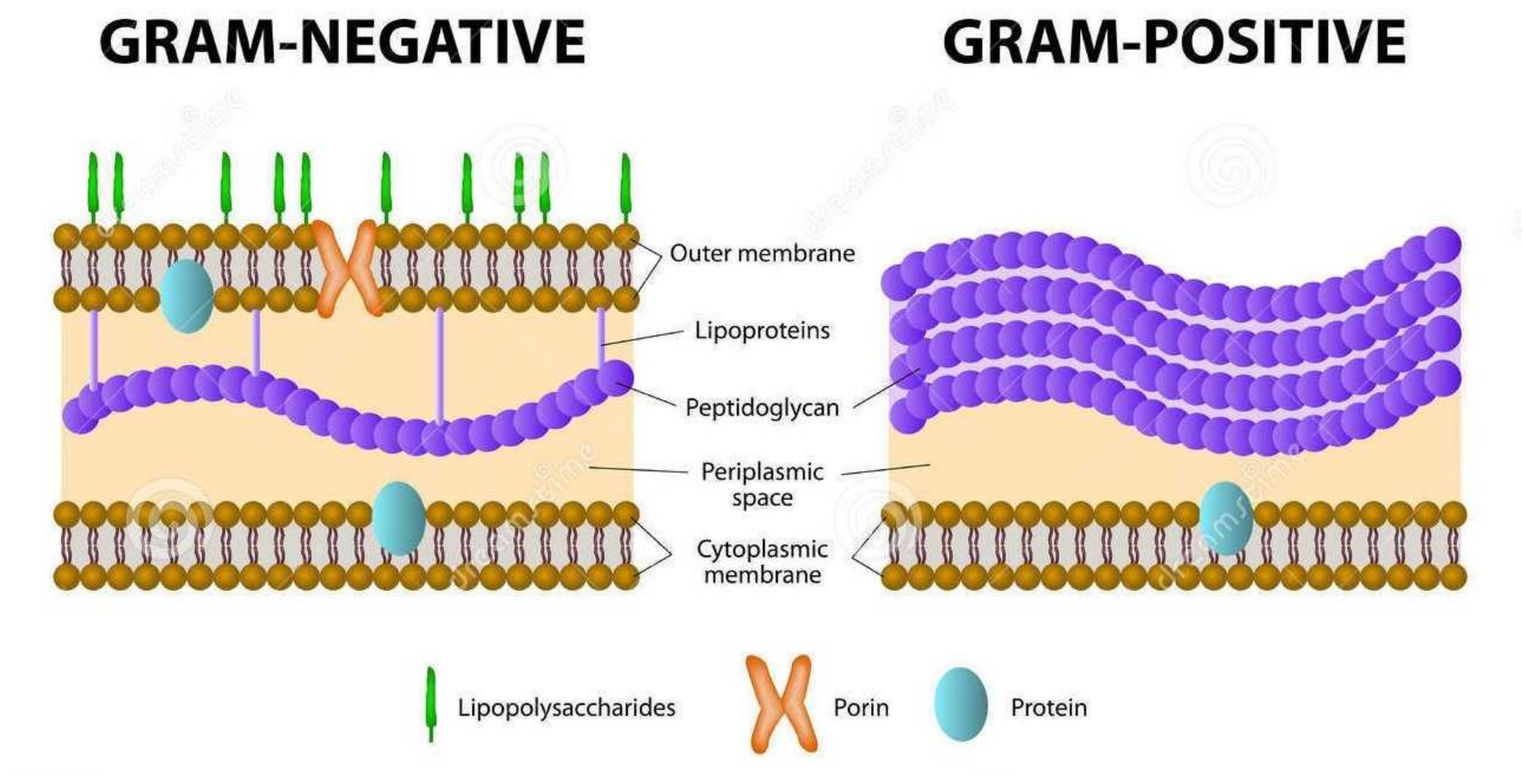
- Spiral-shaped (spirilli)
  - Spirillum volutans
  - Helicobacter
  - etc..

Credit: Phillip B Hylemon, Int. Jal of Systematic and Evolutionary Microbiology, 1973

Prokaryotes

Two variants of the cell envelope

Following the Gram stain test developed by Hans Christian Joachim Gram (1853-1938) , bacteria can be distinguished:



Credit: Dreamstime.com/Pinterest

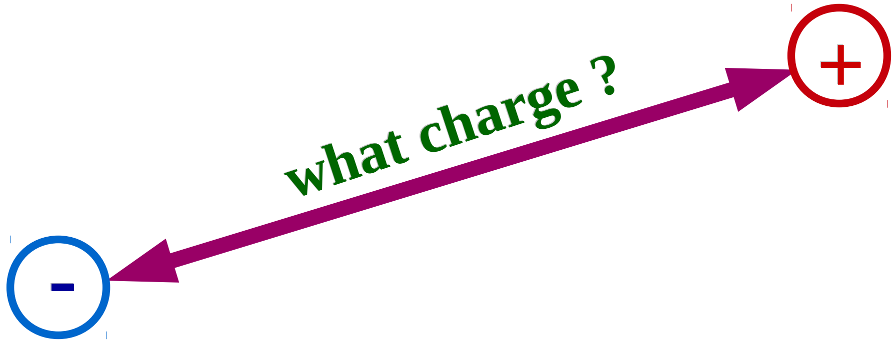
Possible axes for organizing the description

- Define a descriptive line of thought :

  - Choose a physical quantity or a concept
    (e.g., electrical charge, physical entity)

  - Check the existence of two opposite poles
    (e.g. positive charge vs negative charge, particle vs medium)

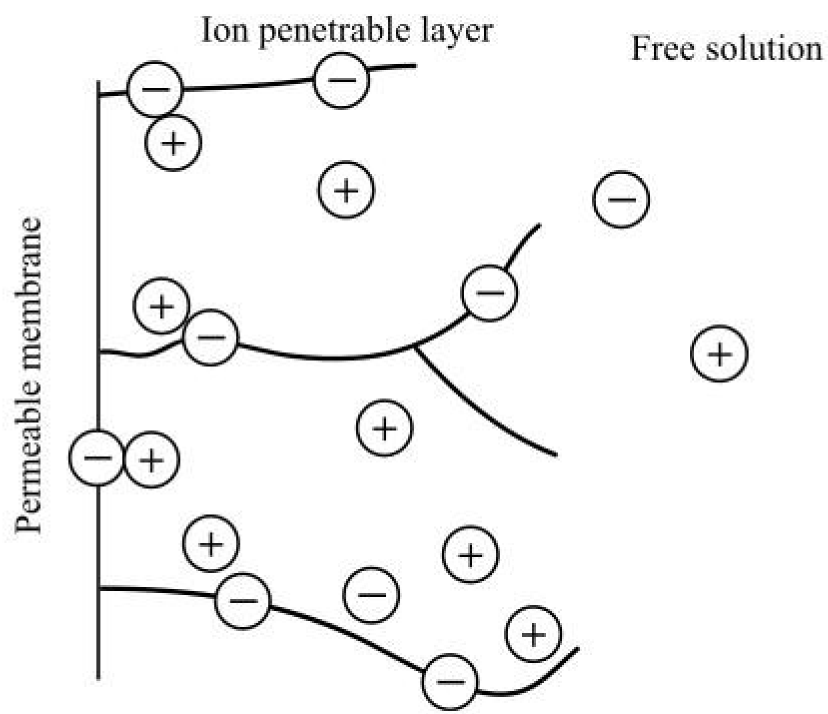
  - Check if you can describe your system along this line



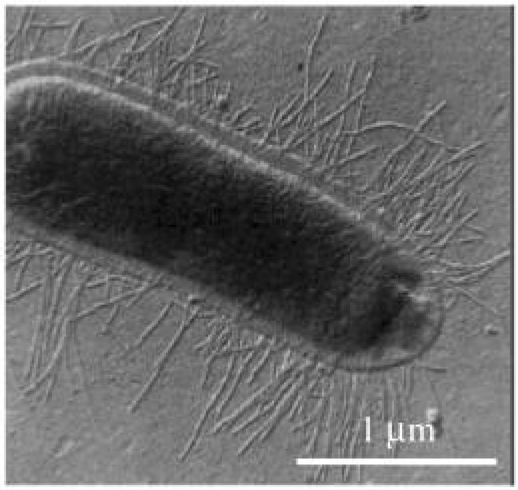
Possible axes for organizing the description

- Microorganism ⟷ Surrounding Medium
  Always must be considered together,
  whether you want to discuss… :

- Electrical charges:



E. coli cell



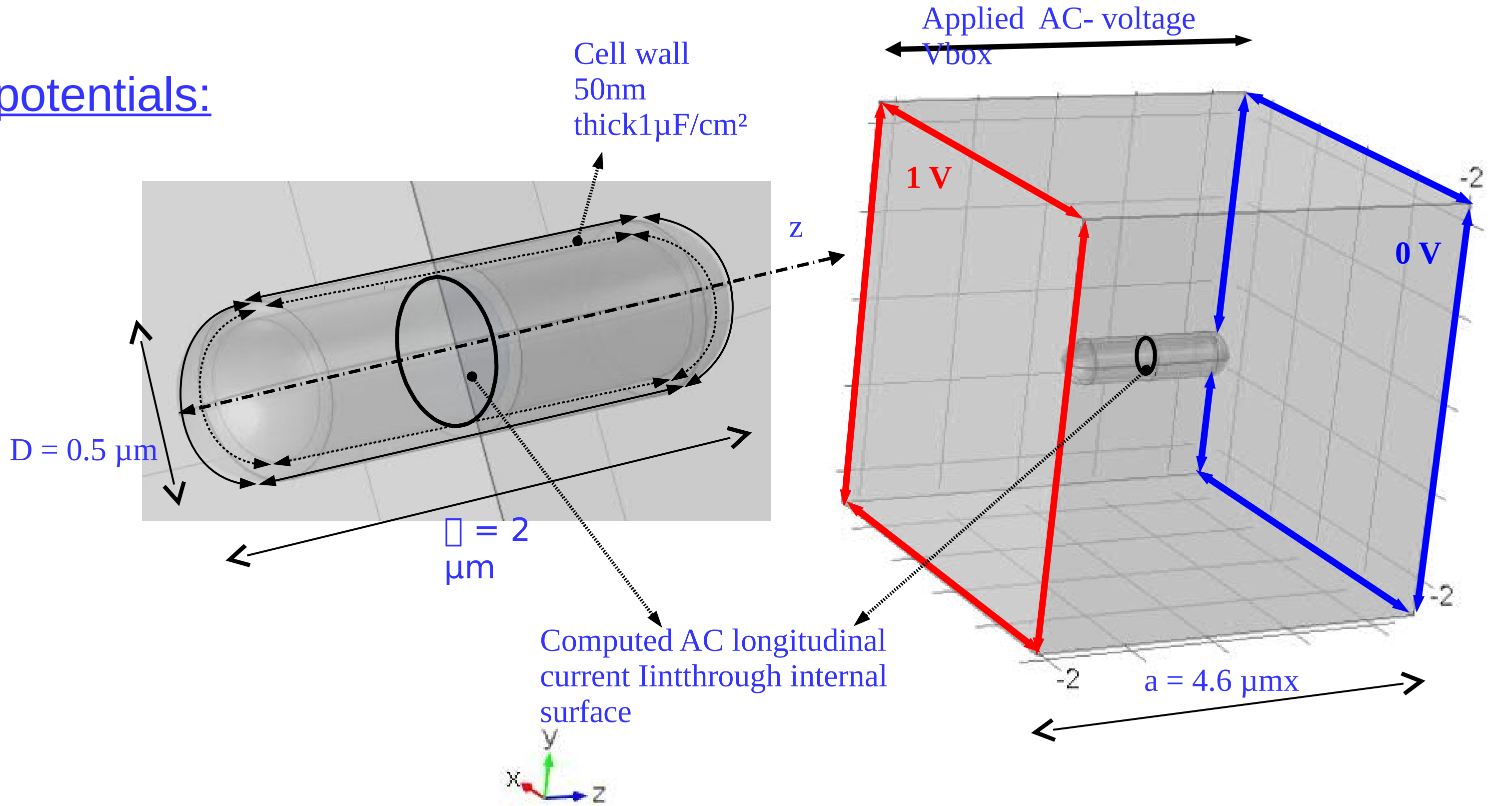
Revil et al. , WATER RESOURCES RESEARCH, VOL. 48, W09545, 2012

Possible axes for organizing the description

- Microorganism ⟷ Surrounding Medium

or:

- Electrical potentials:



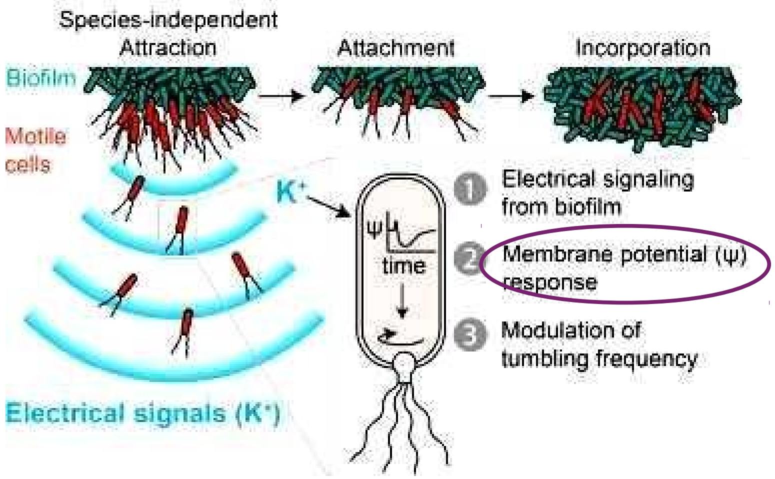
Xavier et al. , Biolectromagnetics, 38:213-219, 2017

Possible axes for organizing the description

- Source ⟷ Receiver

“**Do bacteria sing**?
Sonic intercellular communication
between bacteria may reflect electromagnetic
intracellular communication involving coherent
collective vibrational modes that could integrate
enzyme activities and gene expression”

Norris V. et al. Molecular Microbiology (1997) 24(4), 879–883

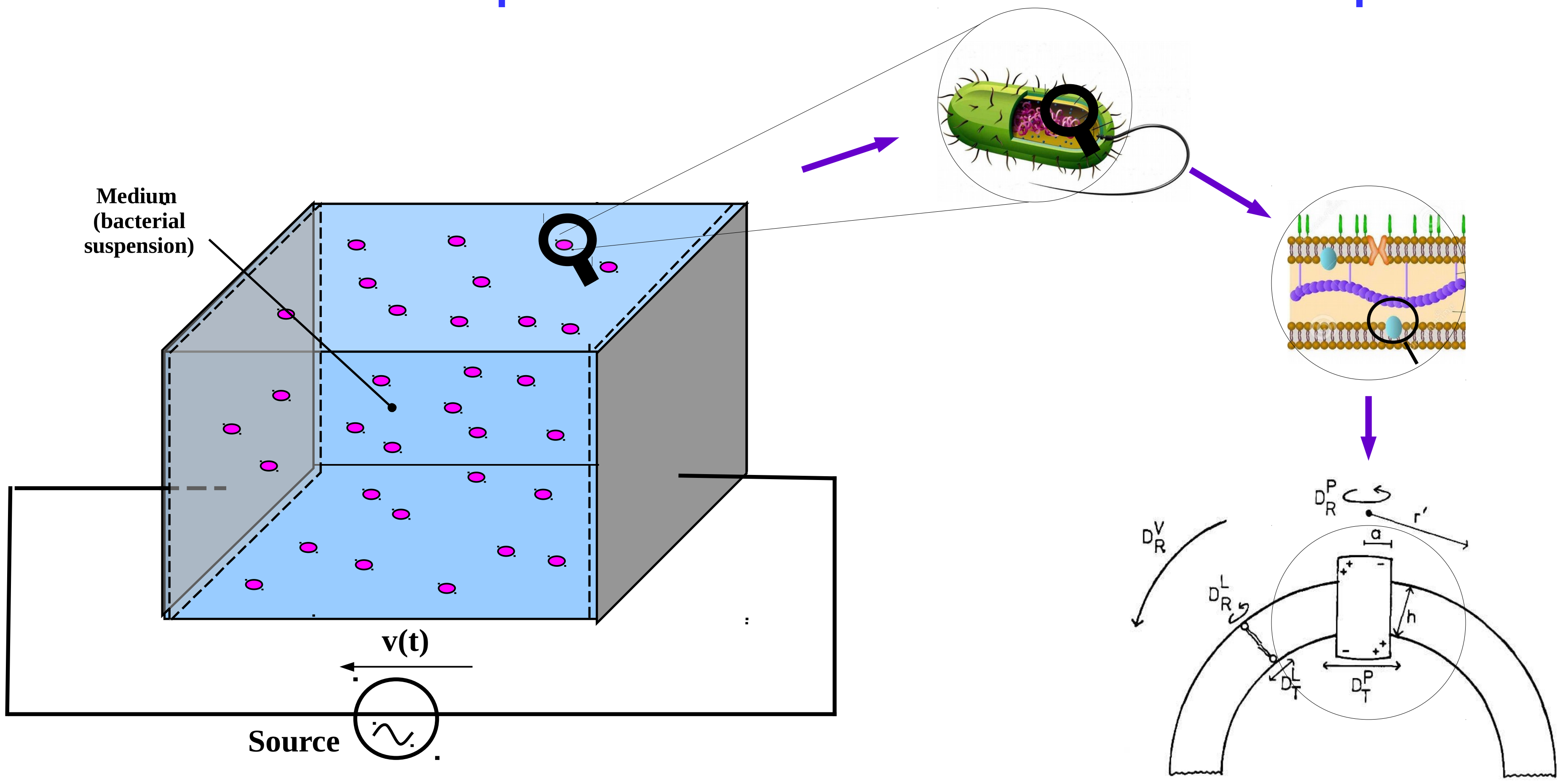


Humphries J. et al., 2017, Cell 168, 200–209

Attraction is caused by
Membrane-potential-dependent
modulation of tumbling frequency
(“run and tumble” swimming mode)

Possible axes for organizing the description

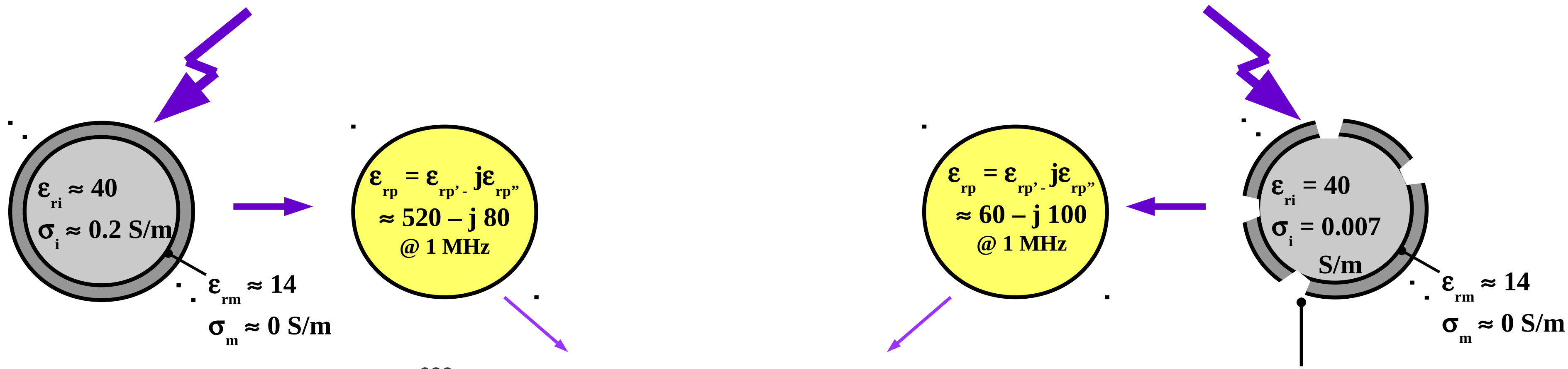
- Macroscopic ⟷ Microscopic



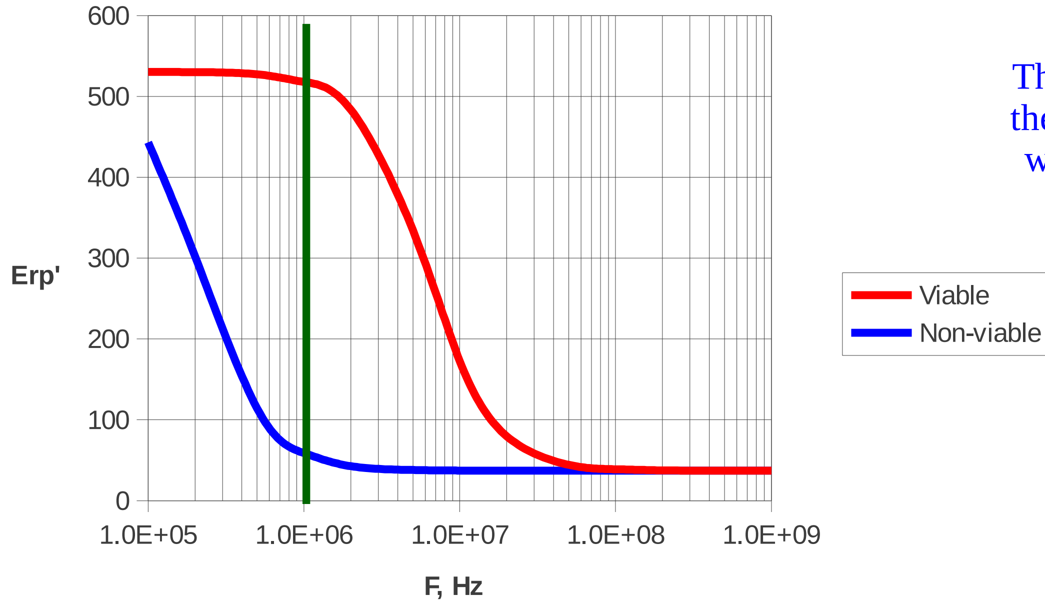
Kell D.B. Jal of Bioelectricity, 4(2) , 317-348 (1985)

Possible axes for organizing the description

- Alive ⟷ Dead



Perforated membrane:
The cell can no longer regulate
the parameters of its cytoplasm
whose conductivity collapses



Electrical Charges: WWW paradigm

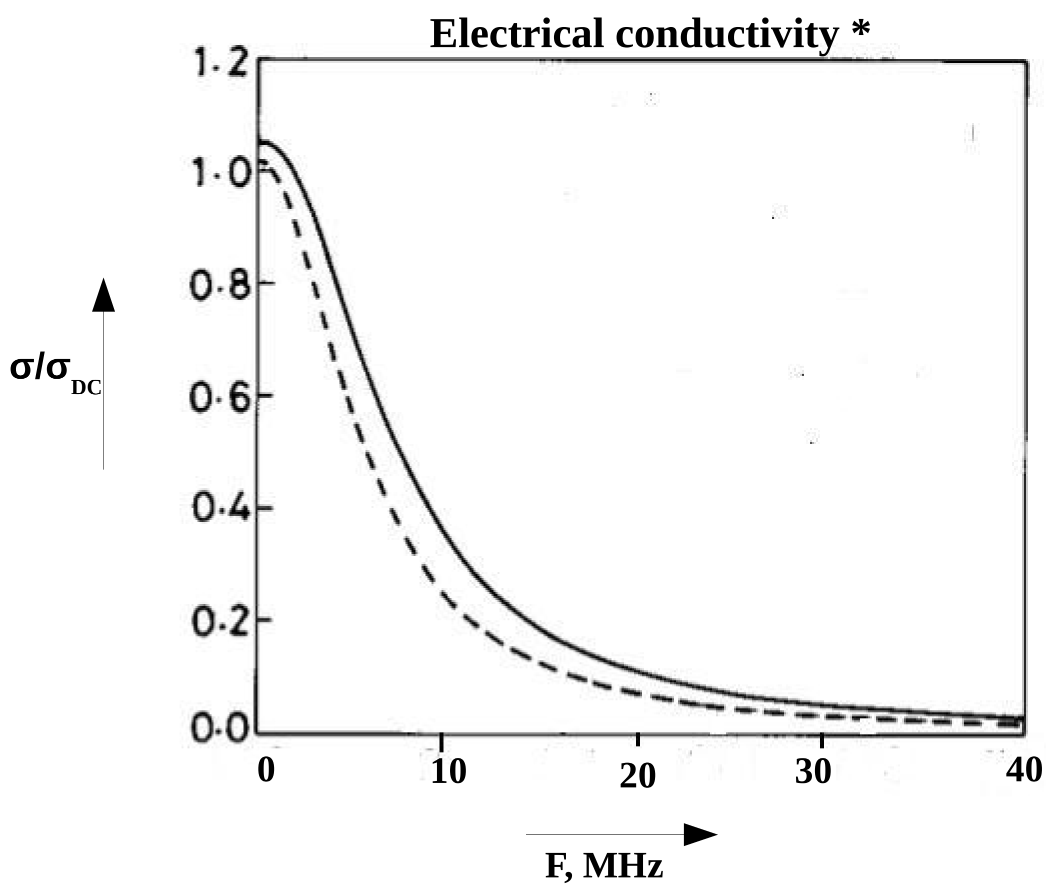
- WHAT ? →
  - sign
  - nature (ion, proton, electron, ..)
  - state (free, bound, semi-free)
  - quantity [C]

- WHERE ? →
  - medium (electrolyte)
  - membrane (proteins, transporters,..)
  - periplasm space
  - cytoplasm, organelles

- WHEN ? →
  - relaxation times of polarization (dispersion)
  - diffusion velocity (transporters, pores and membrane channels)
  - tunnel transports

Electrical Charges: WWW paradigm

- Ions in Aqueous medium: dynamics



Conditions *:

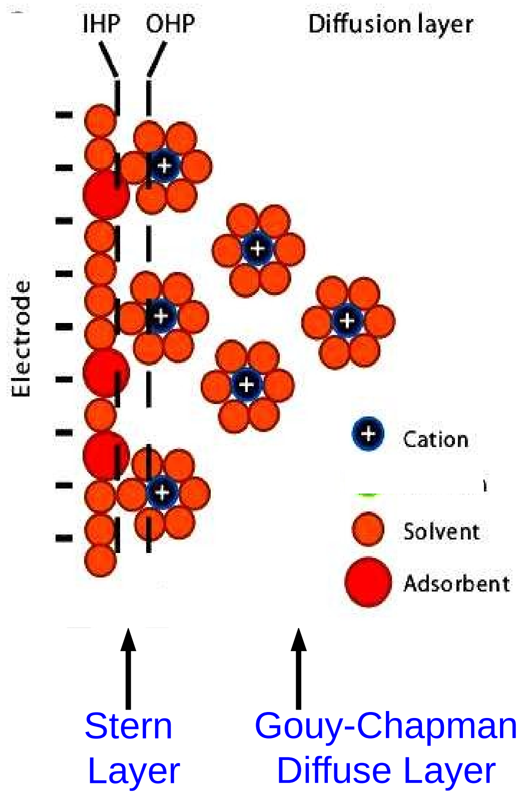
- Electrolyte 1:1   (ions of valence ± 1 ex. NaCl)
- Diffusion coefficient: 2 x 10^{-9} $m^2.s^{-1}$
- Dynamic viscosity: 8.95 x10^{-4} $kg.m^{-1}.s^{-1}$
- Masse: 6,64 x10^{-26} kg
- Concentration:
  - ———— : 0.1 M (M = mol/liter)
  - ----------- : 0.01 M

* Chandra A. et al. Chinese Journal of Chemical Physics · Jan 2000

Electrical Charges: WWW paradigm

- ## Electrical Double Layer (EDL)

- On Electrolyte/Electrode Interface:



Stern Layer (Helmoltz):

- Contains counter-ions (here cations +)

Gouy-Chapman Diffuse Layer:

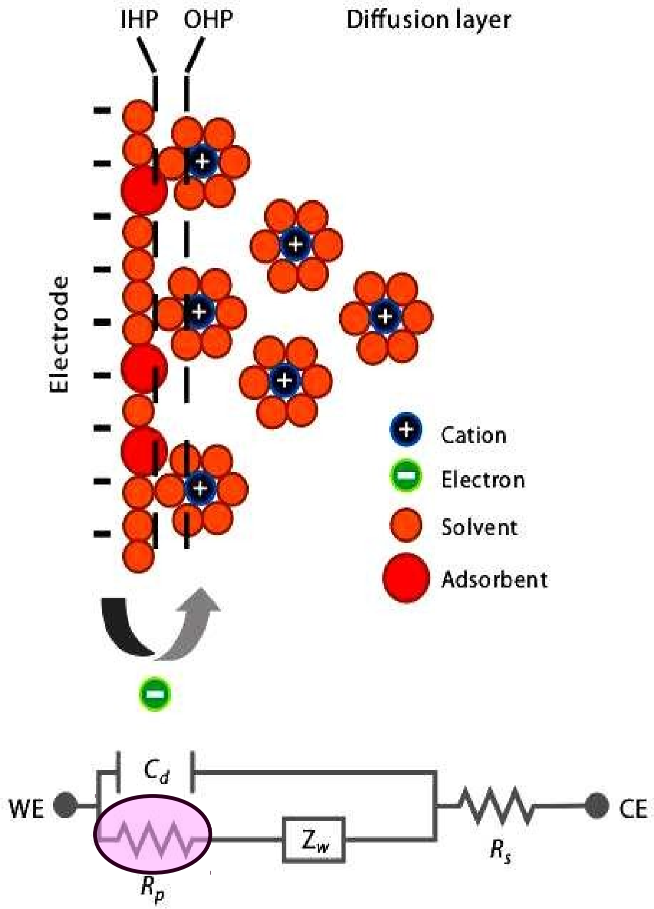
- Contains both counter-ions (here cations +)
  and co-ions (here anions -)

Note: Ions are circled by solvent molecules (e.g. H_2O).

Electrical Charges: WWW paradigm

- Electrical Double Layer (EDL)

- Equivalent electrical circuit *



Polarization Resistance R_p:

- Models electron exchange during reduction of Cations at the electrode (they acquire electrons)

- Exchanged energy q.ΔV is proportional to the flow of electrons ($\Delta V = R_p I_e$)

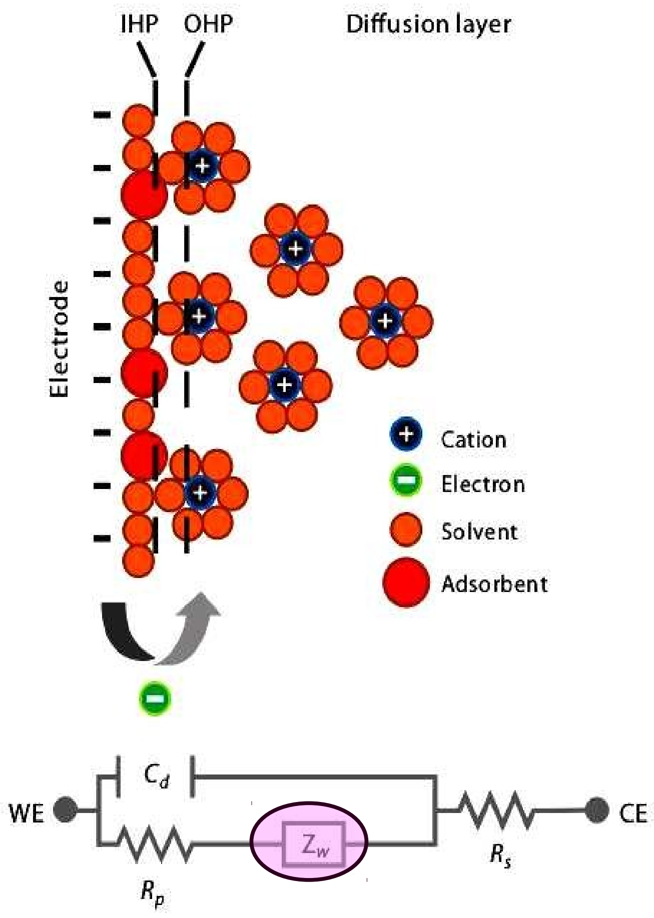
- Contributes to the global model only at low frequencies. (typ. < 10kHz)

** Randles JEB. Discuss. Faraday Soc. 1:11–19, 1947*

Electrical Charges: WWW paradigm

- Electrical Double Layer (EDL)

- Equivalent electrical circuit *



Warburg Impedance Z_W:

- Models diffusive ionic exchange in harmonic regime (electro-diffusion and conservations Fick's Laws)

- Contributes to the global model only at low frequencies. (typ. < 10 kHz)

$$\bar{Z}_W = \frac{K(Di, Ci)}{\sqrt{2\omega}} \cdot (1 - j)$$ *

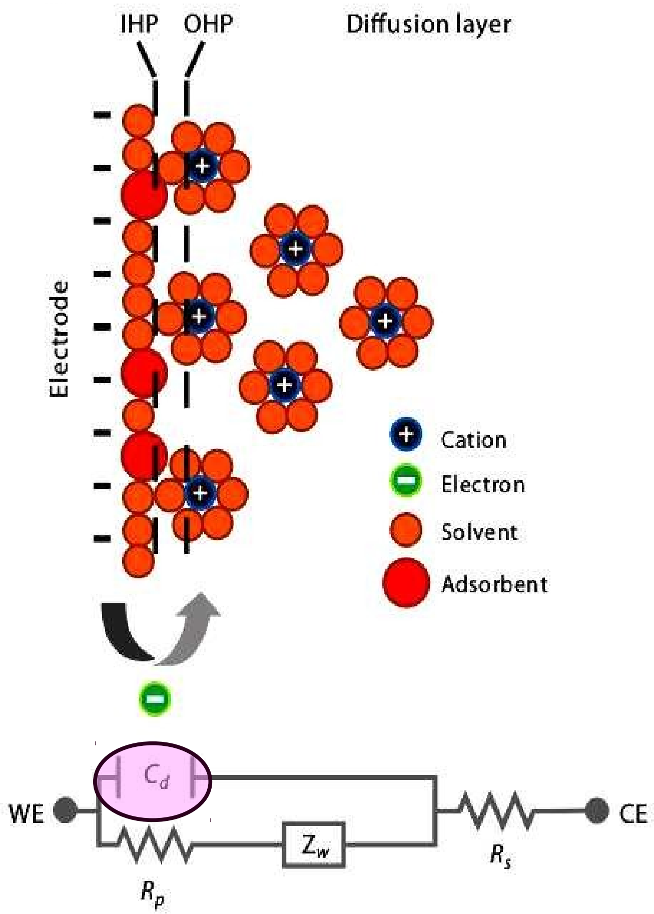
Di and Ci are respectively diffusion coefficients and concentrations of species I.

* *Randles JEB. Discuss. Faraday Soc. 1:11–19, 1947*

Electrical Charges: WWW paradigm

- Electrical Double Layer (EDL)

- Equivalent electrical circuit



Capacitance of the Electric Double Layer C_D:

- Models the accumulation of charges of opposite signs upstream/downstream of EDL

- Bypass R_p and Z_W in high frequency (> 10kHz)

- Examples found in literature * :
  - Equal concentrations and diffusion coefficients of oxidizing and reducing species, i.e. respectively : C = 0.1 M (mol/L) and D = 10^{-10} $m^2.s^{-1}$

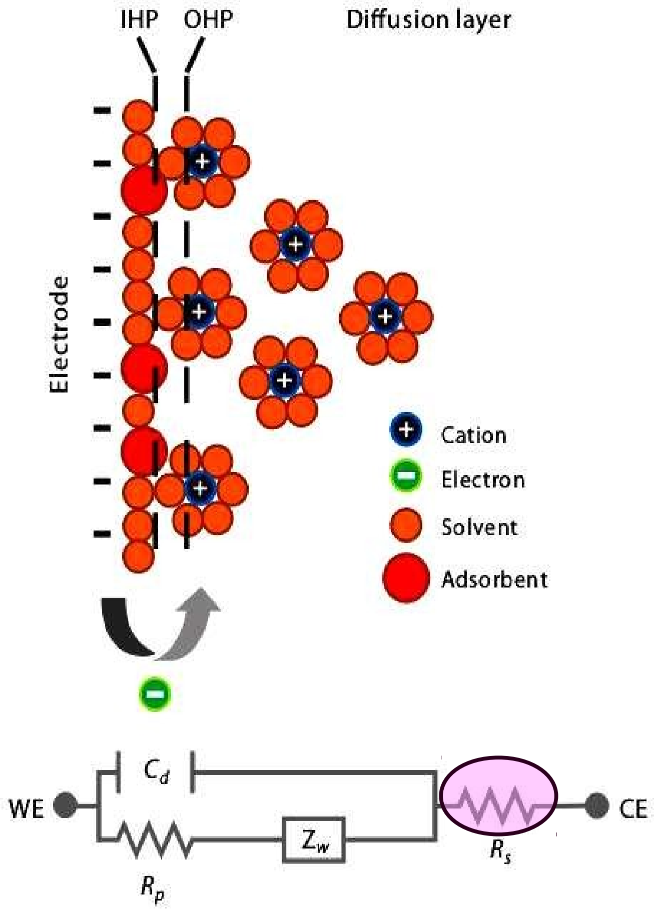
► C'_D = 10 µF/cm^2

** Chang, Anal chem., 2006 ; Dhillon, J. Chem.Sci., 2017*

Electrical Charges: WWW paradigm

- Electrical Double Layer (EDL)

- Equivalent electrical circuit



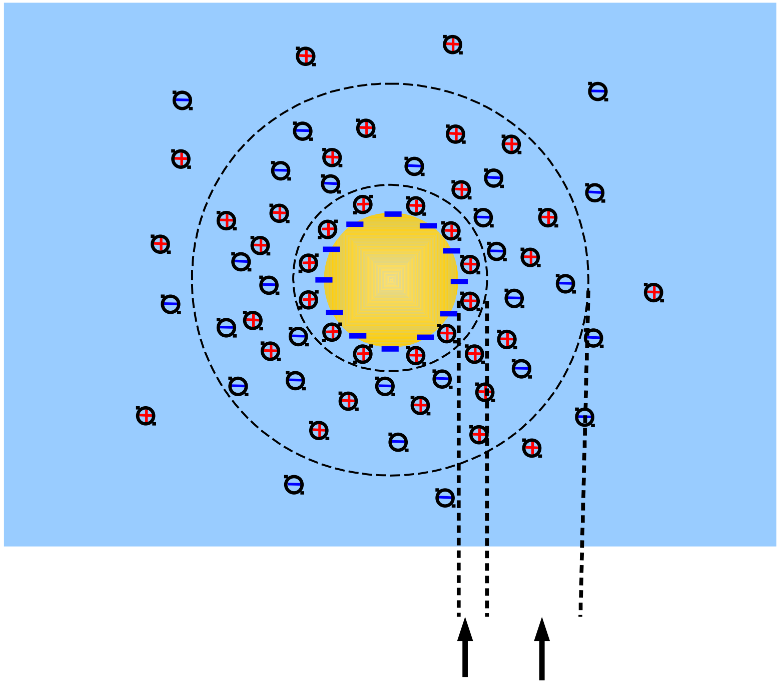
Series resistor Rs:

- Models static ionic conduction

- Away from interface effects properly speaking

Electrical Charges: WWW paradigm

- Electrical Double Layer (EDL)
  - On bacterium' scale, in the context of electrolyte medium



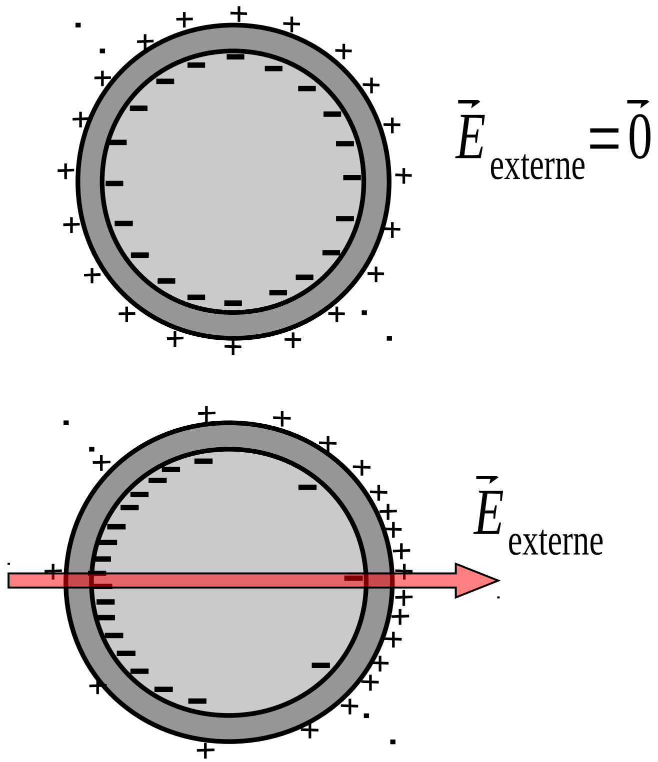
EDL may also exist around bacterium:

- Provided the bacterium carries a non-zero net electrical charge

- Conditions of occurrence and experimental methods will be discussed below.

Electrical Charges: WWW paradigm

- Electrical Double Layer (EDL)
- Dynamics of electrical charges in EDL around the bacterium*



When submitted to a variable electric field:

- They can diffuse, but remain on the surface (they are semi-free).

► "giant" polarization induced

- Effect no longer viable when the frequency becomes too high.

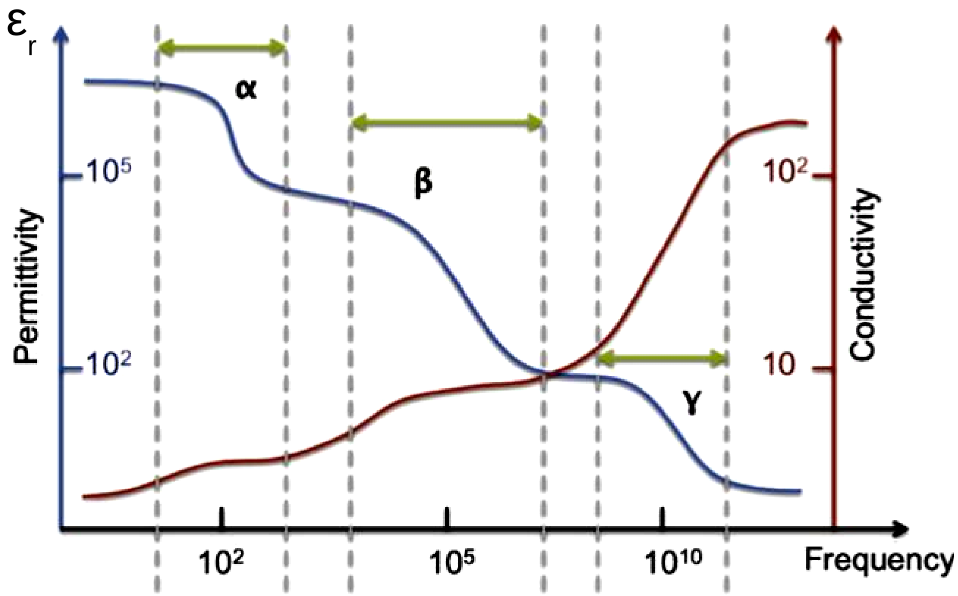
► known as “alpha” dispersion.

* Detailed namely in *Prodan et al, Biophysical Journal, 2008*

Electrical Charges: WWW paradigm

- Electrical Double Layer (EDL)

- Back to macroscopic scale: Bacterial suspension *



Relative Permittivity of Electrolyte + Bacteria:

- Alpha dispersion:
  - Due to EDL
  - F < a few 10 kHz
  - ε_r up to 10^6 (!)

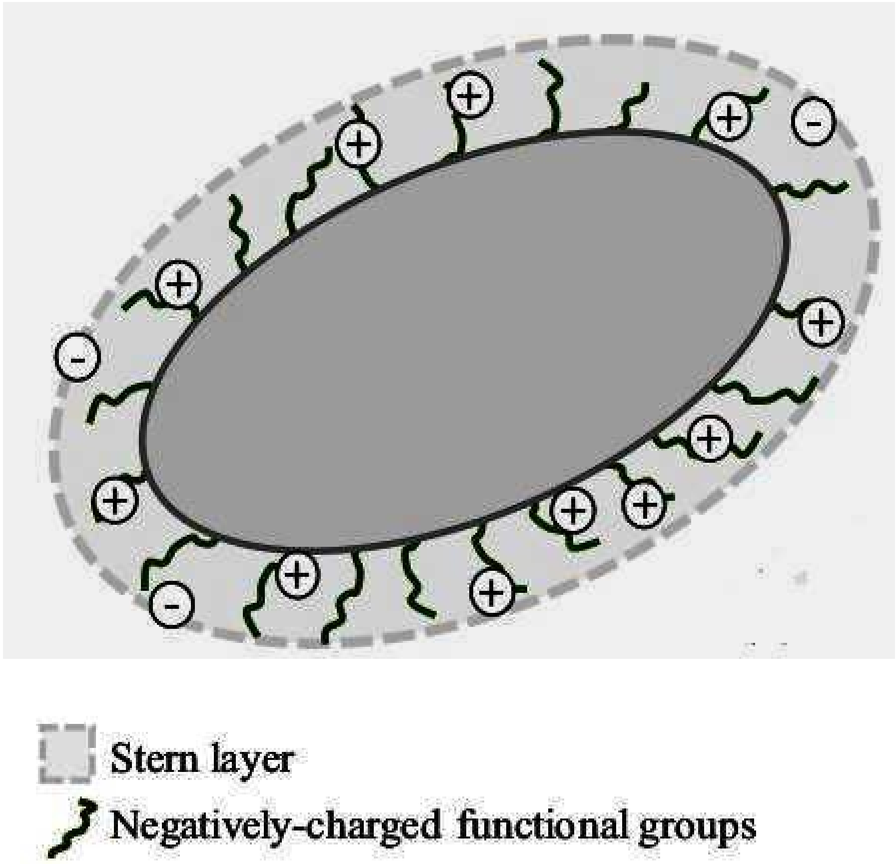
- Beta dispersion (Maxwell-Wagner):
  - Due to dielectric polarization of materials in the cell wall and interfacial polarization.
  - a few 10 kHz < F < a few 10 MHz

- Gamma dispersion:
  - Due to polarization of constituents in the cell: organelles, proteins, molecules, etc...
  - F > a few 10 MHz)

* Heileman et al, Biosensors & Biolelectronics, 2013

Electrical Charges: WWW paradigm

- Protonation of Bacterium's Outer Membrane
- (De/)Protonation of terminal groups depends on medium pH



- Terminal functional groups:

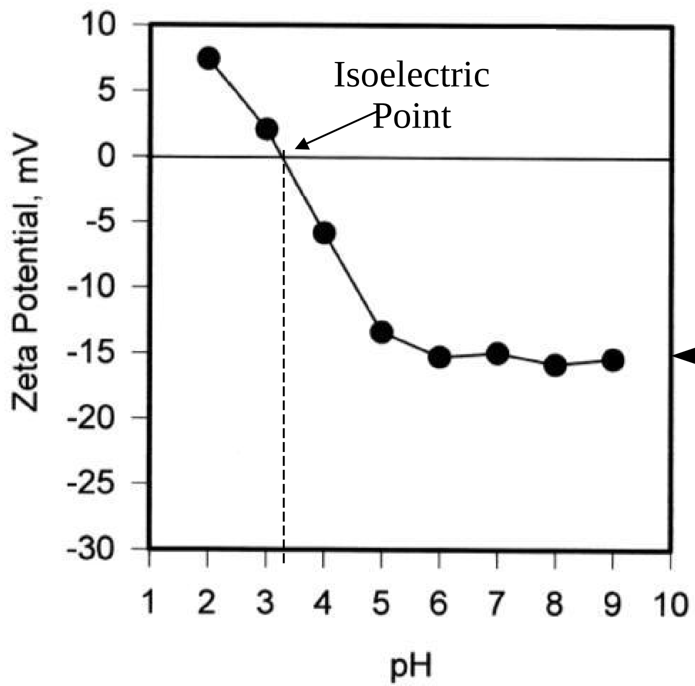
- Amino group
  $NH_2 + H^+ \leftrightarrow NH_3^+$
- Carboxyl group
  $COOH \leftrightarrow COO^- + H^+$

- Net bacterial charge (excluding Stern Layer) depends on pH
  (often negative under standard pH conditions)
- Stern Layer collects the counter-ions from the surrounding medium

Electrical Charges: WWW paradigm

- Protonation of Bacterium's Outer Membrane

- Isolectric Point pI



Suspension of *P . Multocida*
10^8/ml colony-forming units *

- pI : pH value at which the bacterium net electrical charge is zero.
- Zeta potential (more on this concept later) proportional to net charge

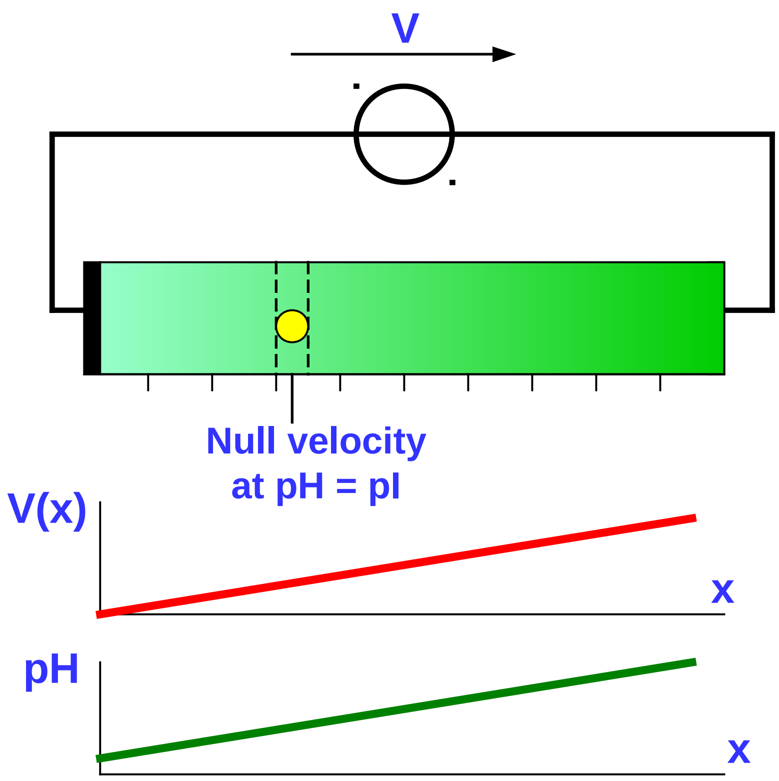
Protonation of terminal groups saturates when pH > 5 (deficiency of protons)

 * *Wilson W.W. et al, Jal of Microbiological Methods, 2001*

Electrical Charges: WWW paradigm

- Protonation of Bacterium’s Outer Membrane

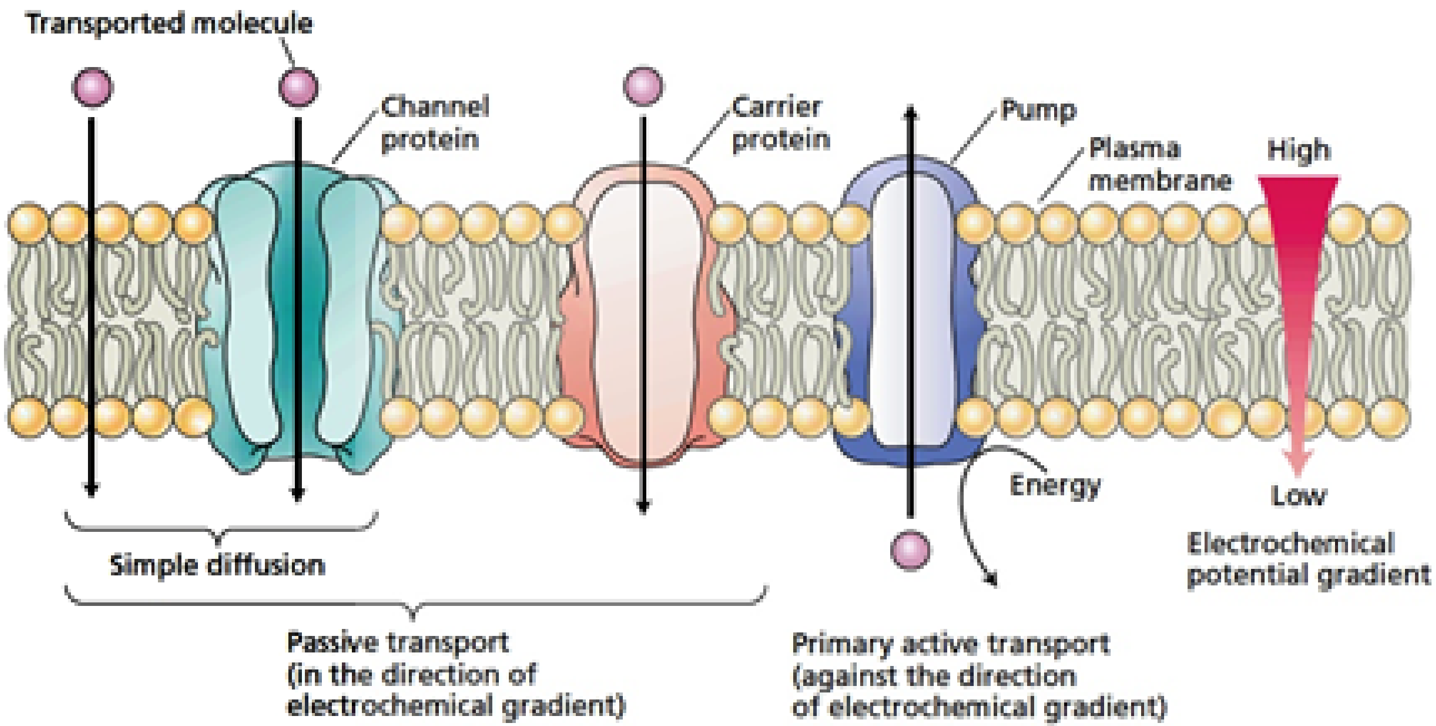
- Measurement of Isolectric Point pI



- Column with uniform electric field in the x-direction.
- Electrophoretic Force proportional to net charge

Electrical Charges: WWW paradigm

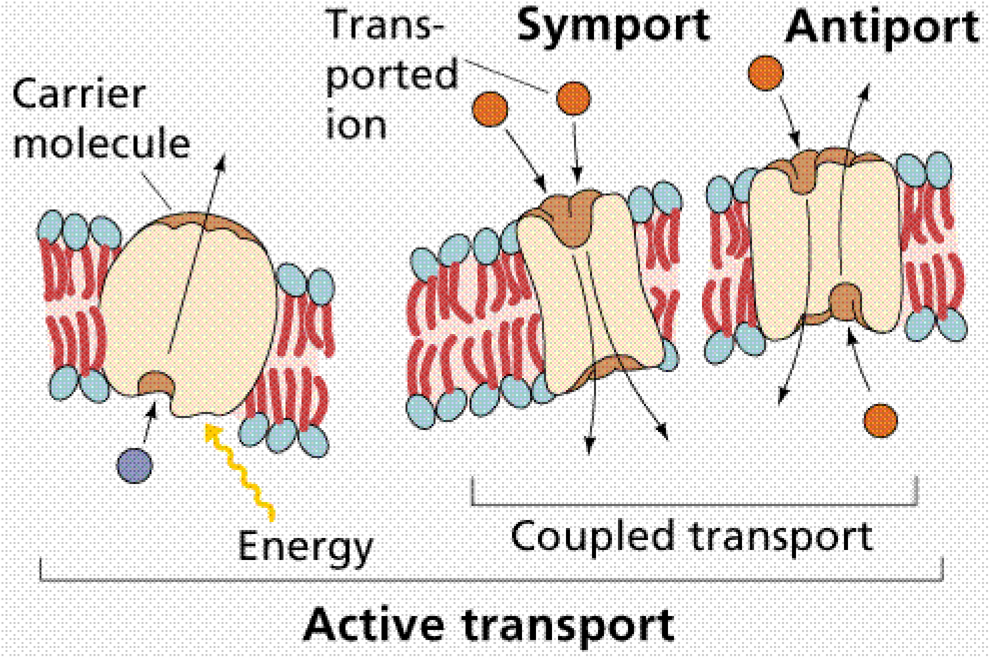
- Ion/molecule transport through the cell wall
- Active vs Passive Transport



Electrical Charges: WWW paradigm

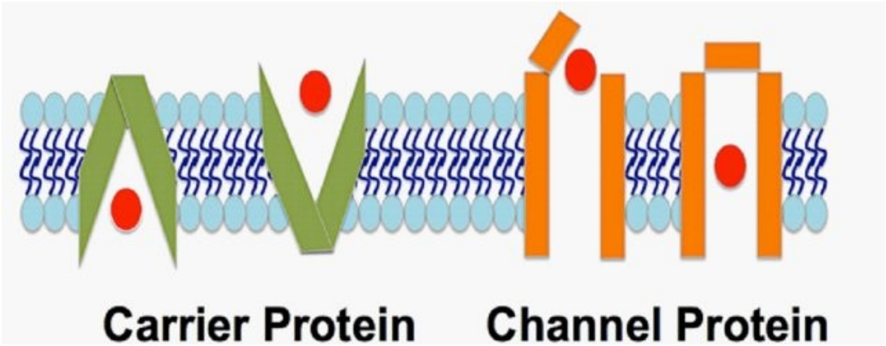
- Ion/molecule transport through the cell wall
  - Various forms of active transporters

Uniport



Electrical Charges: WWW paradigm

- Ion/molecule transport through the cell wall
- Biology semantics: carrier vs. channel



Credit *

- **Carrier:**

  Mechanism involving a conformational cycle

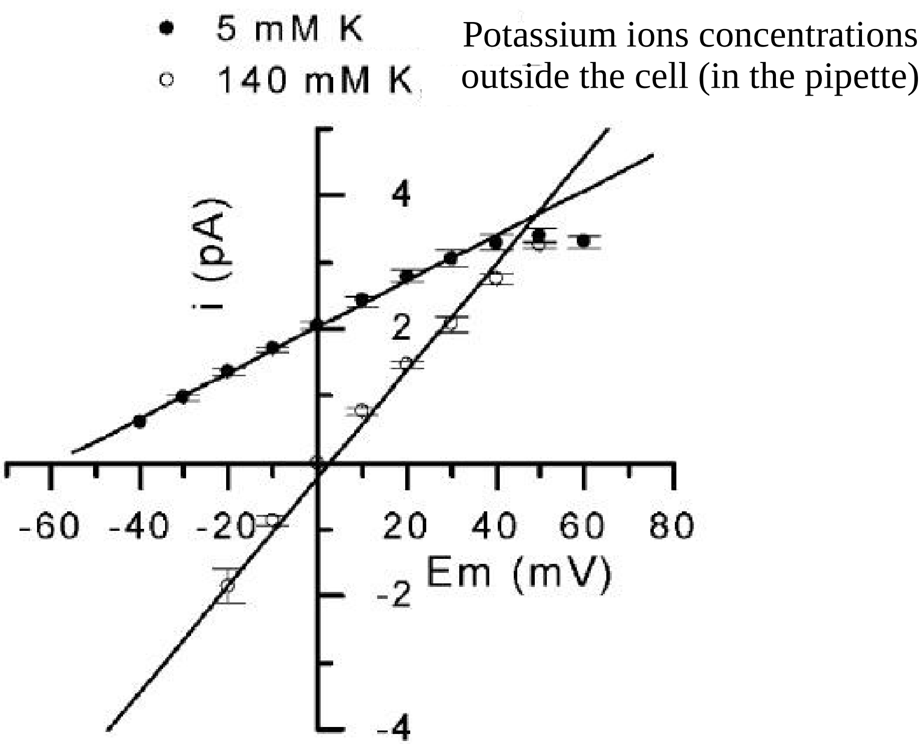
- **Channel:**

  The protein alters between either open or closed

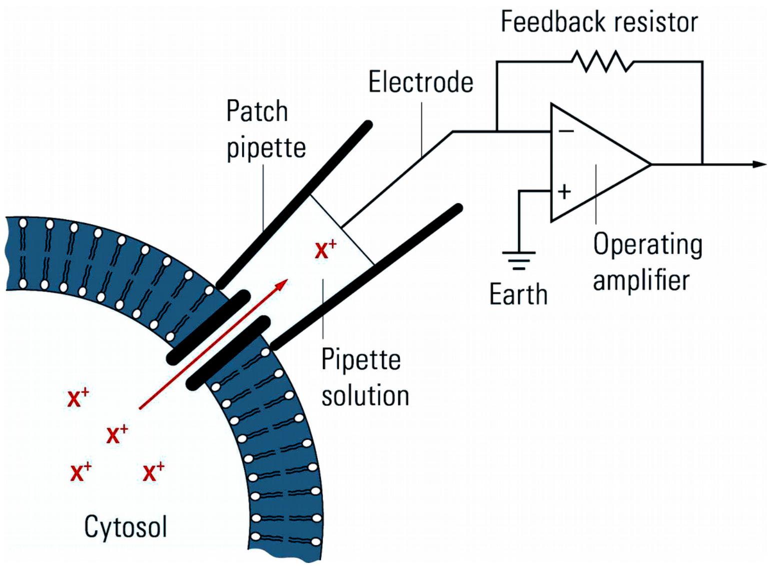
* Stephana Cherak et al chapt. .« Membrane Transport » in book « Basic Biochemistry », Austin Publishing Group, feb 2016

Electrical Charges: WWW paradigm

- Ion/molecule transport through the cell wall
- Current-Voltage characteristics of channels



K^+ channel in C. elegans muscle
Single-channel current recorded in
cell-attached patches (in-situ) *

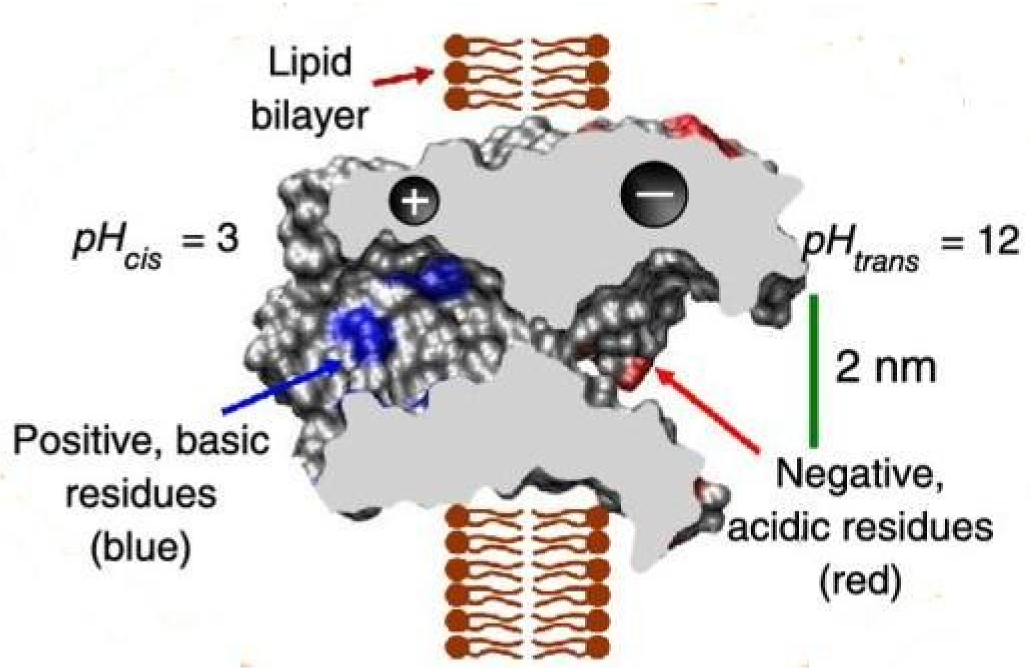


Cell-attached patch clamp
Image credit: Leica Micro-systems

* Maëlle Jospin et al. Journal of Physiology (2002), 544.2, pp. 373–384

Electrical Charges: WWW paradigm

- Ion/molecule transport through the cell wall
- Example : The outer membrane porin OmpF in *Escherichia coli*



Credit *

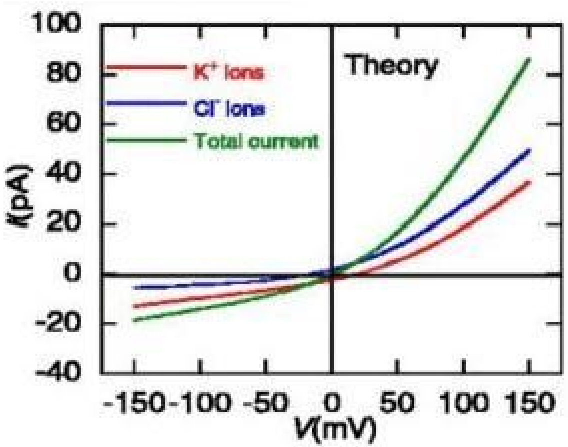
- Classified as ion channel
- Ability of uphill transport of K^+ ions
  (against concentration gradient)

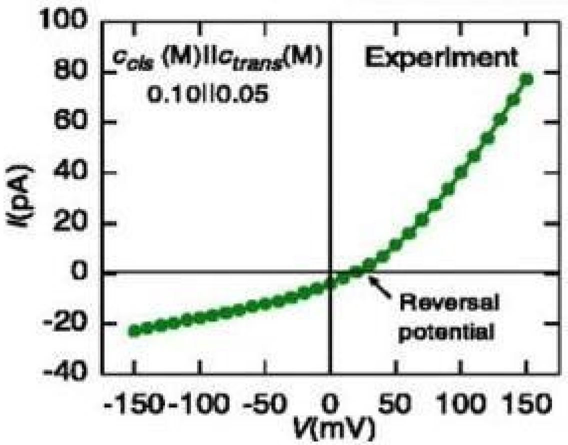
* Marıa Queralt-Martın et al. APPLIED PHYSICS LETTERS 103, 043707 (2013)

Electrical Charges: WWW paradigm

- Ion/molecule transport through the cell wall

- Example : The outer membrane porin OmpF in *Escherichia coli*





DC I-V characteristics *

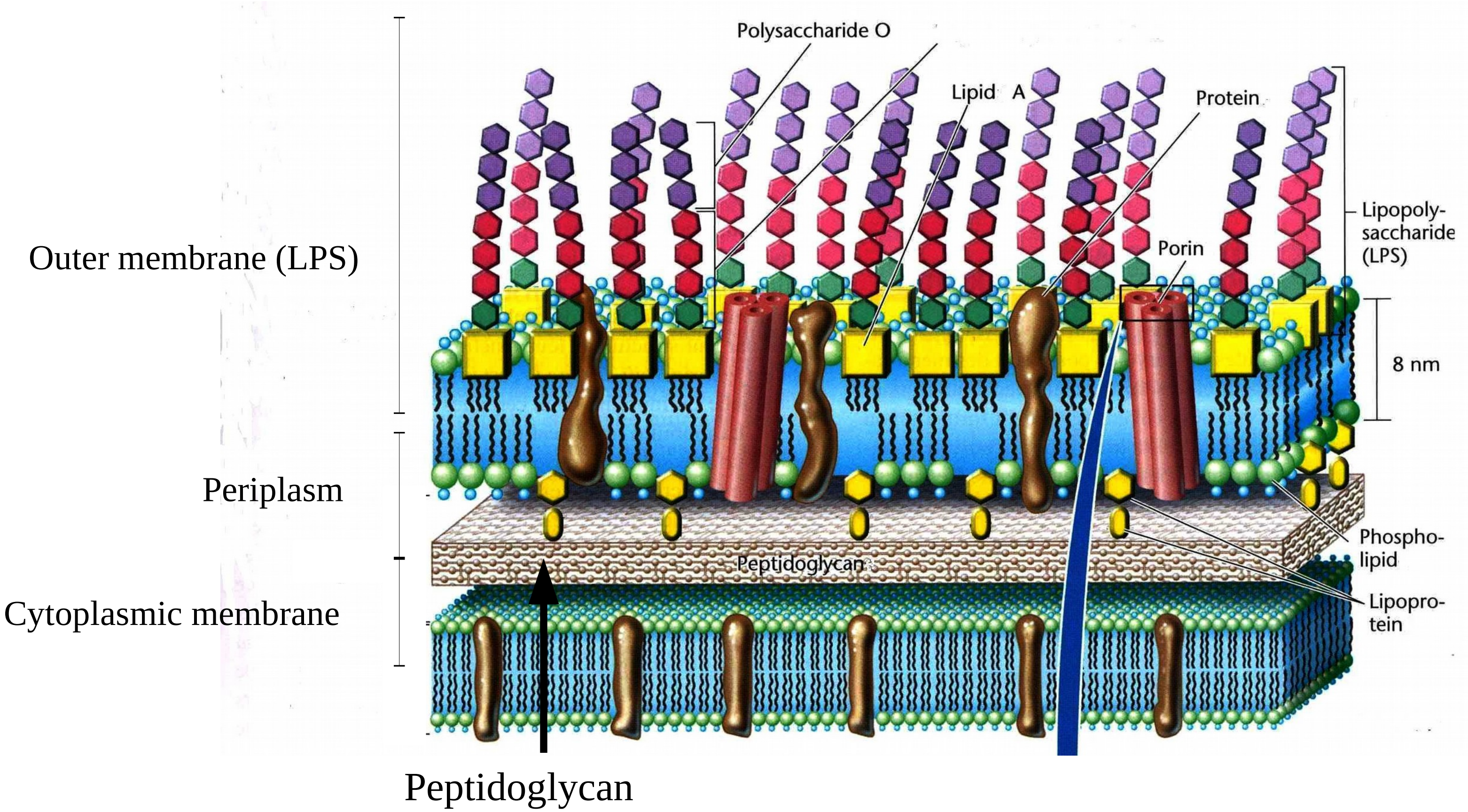
- **Rectifying I-V property**
  With asymetric outer/inner pH condition

- **Selective K+ ions transport property**
  (2003 Nobel Prize in Chemistry co-awarded to MacKinnon R., for his work on three-dimensional visualization of the channel selectivity filter by X-ray diffraction)

* Marıa Queralt-Martın et al. APPLIED PHYSICS LETTERS 103, 043707 (2013)

Electrical Charges: WWW paradigm

- Charges in peptidoglycan
- Location in cell envelope

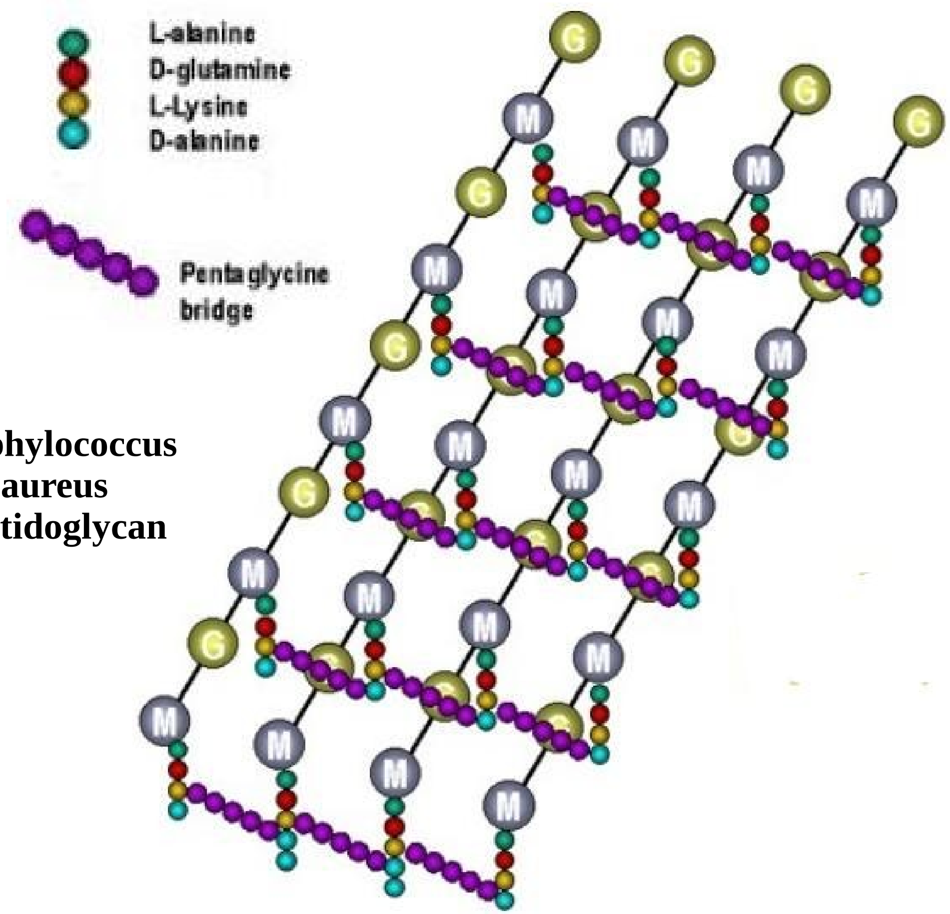


Gram-negative Cell Envelope *

* Image credit: Madigan et al, Brock 11th edition 2007

Electrical Charges: WWW paradigm

- Charges in peptidoglycan
- Structure of peptidoglycan

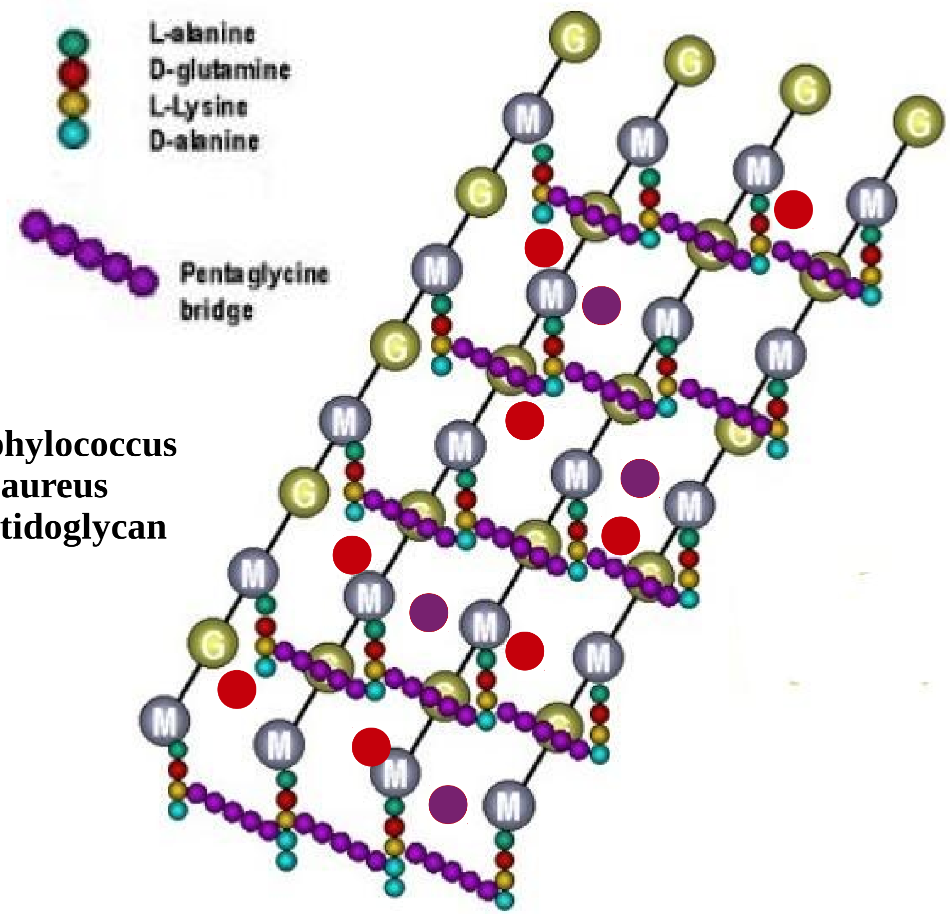


Staphylococcus aureus peptidoglycan

- Composed of repeating units of two amino sugar derivatives:
  - NAG (N-acetylglucosamine)
  - NAM (N-acetylmuramic acid)
- NAG/NAM sheets are linked by bridges (see figure)

Electrical Charges: WWW paradigm

- Charges in peptidoglycan
- Conductive model *



Staphylococcus aureus peptidoglycan

- Peptidoglycan acts like a ionic exchanged resin:
  - Polyanions attract cations of aqueous environment.
  - Carboxyl groups bind metal ions

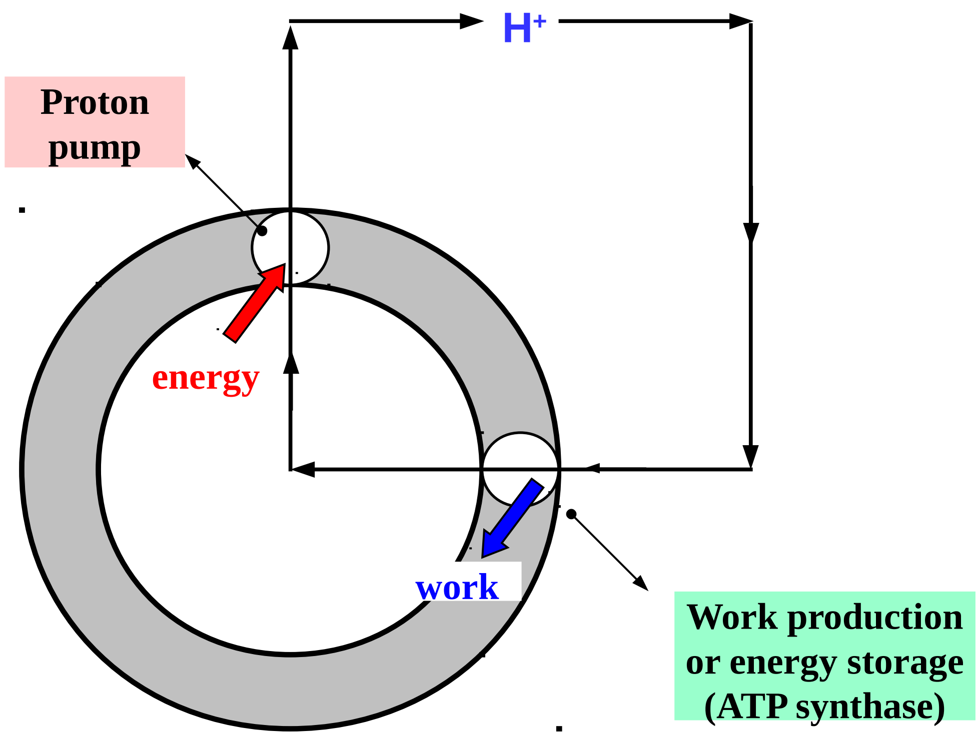
- Adsorption of many counterions
  - available for electrical conduction

- High electrical conductivity
  - about ten times than that of the cytoplasm

* Bai W et al. Colloids and Surfaces B: Biointerfaces 58 (2007) 105–115

Electrical Charges: WWW paradigm

- Charges in/around the cytoplasmic membrane
  - Chemiosmotic theory

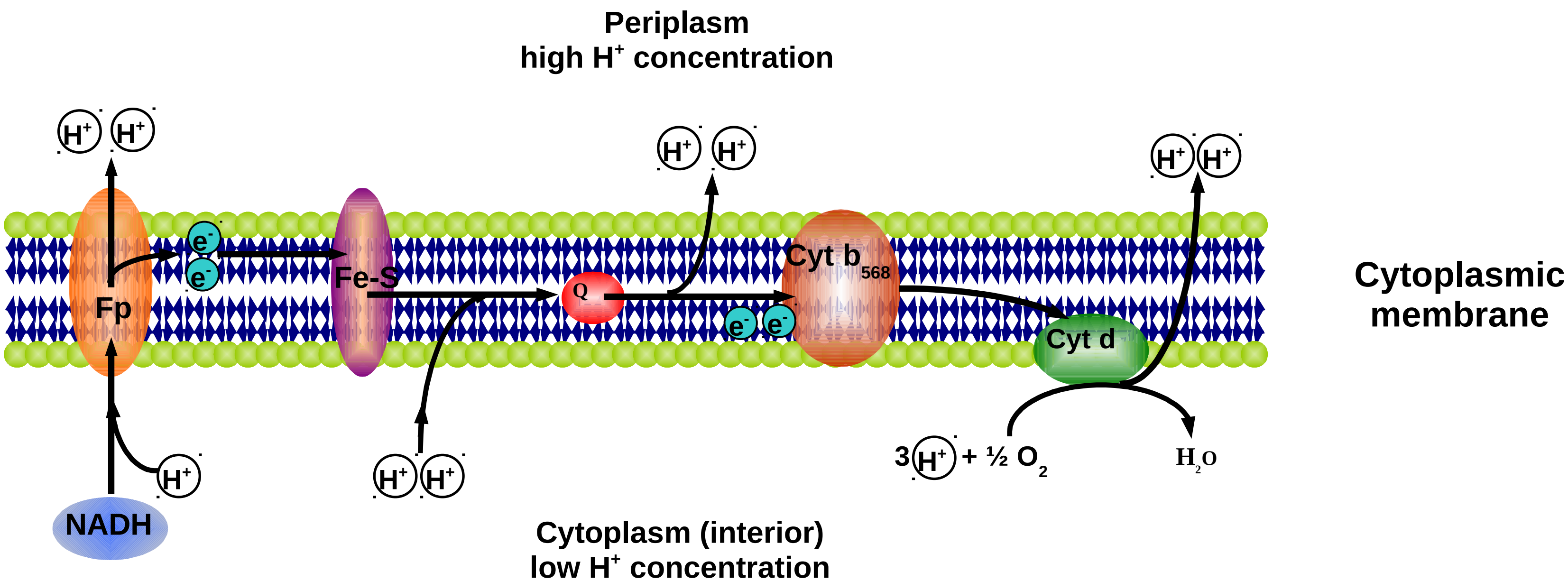


White D. « *The Physiology and Biochemistry of Prokaryotes* »
Third edition Oxford University Press, 2007

Electrical Charges: WWW paradigm

- Charges in/around the cytoplasmic membrane

- Electron Transport Chain (ETC) & Cell Respiration



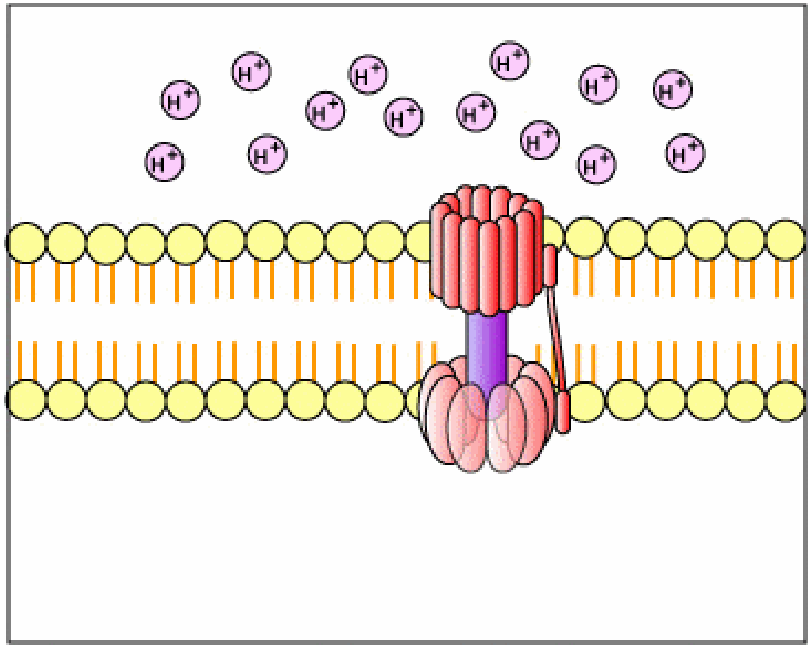
- Sequence of redox operations transports electrons along ETC, down to the electron terminal acceptor (oxygen in case of aerobic respiration)

- At each step the potential energy is diminished and protons are pushed up into the periplasm.

Electrical Charges: WWW paradigm

- Charges in/around the cytoplasmic membrane

- ATP Synthase



Credit https://gifer.com/en/7fP3

- Uses the electrochemical gradient of protons to complete phosphorylation of ADP to ATP
- Involves a rotational motor mechanism, proton-driven.
  - Rotation is used to sequence the synthesis of ATP by successively deforming subunits of the enzyme
- Similar process is used to rotate the bacterial flagellum

Electrical Charges: WWW paradigm

- Charges in the cytoplasm

- Constituents:

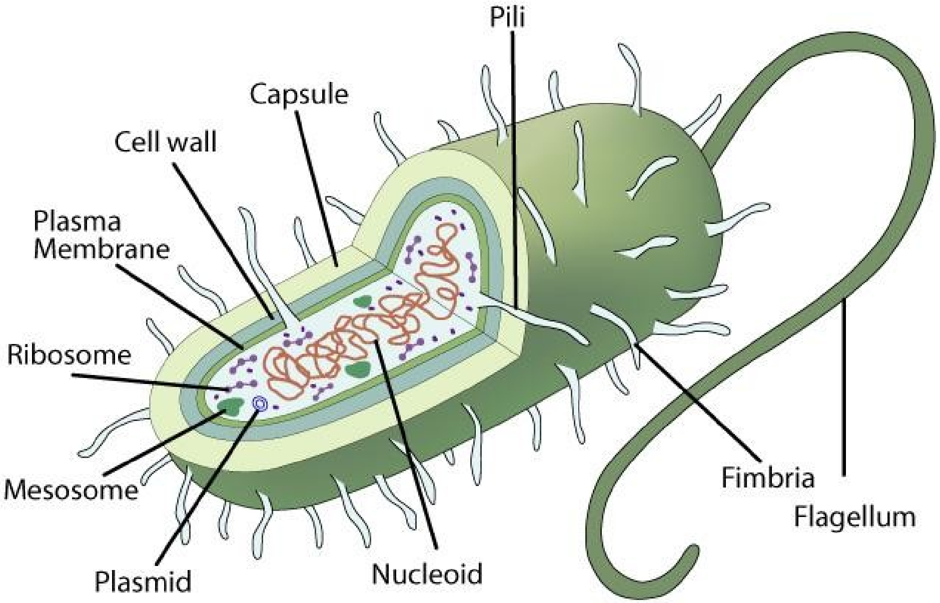


Image credit ScienceTopia

- Nearly 80% of water

- Water soluble protein, carbohydrate, inorganic salts, lipids, etc.

- Cell organelles:
  - Nucleoid
  - Plasmid
  - Ribosomes (→translate RNA into proteins)

▶ Overview *
- 2 millions protein molecules
- 55,000 ribosomes

* See references in Mullineaux. ELife 2017

Electrical Charges: WWW paradigm

- Charges in the cytoplasm

- Overall cytoplasm charge is negative

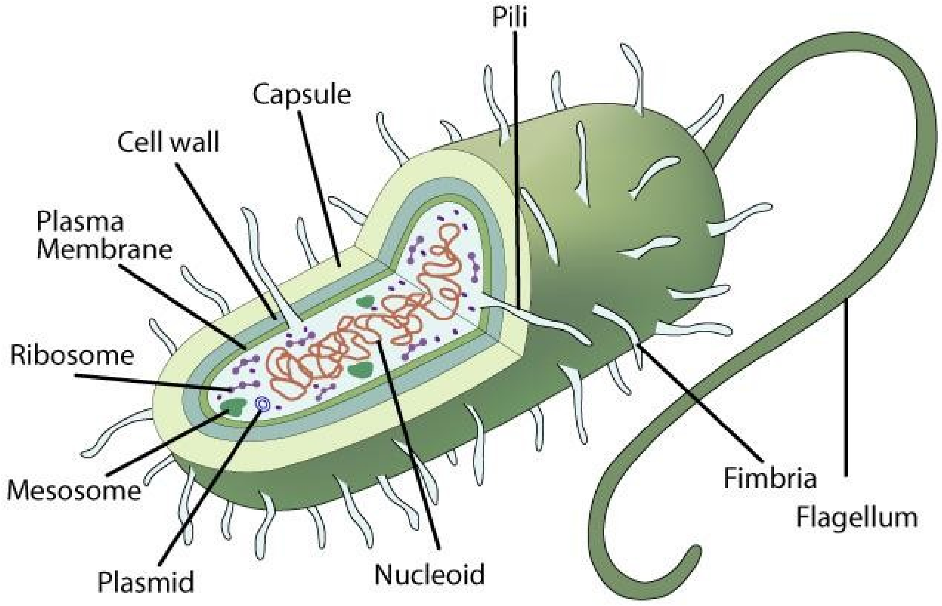


Image credit ScienceTopia

- For a large majority of prokaryotes:
  (at physiological pH)

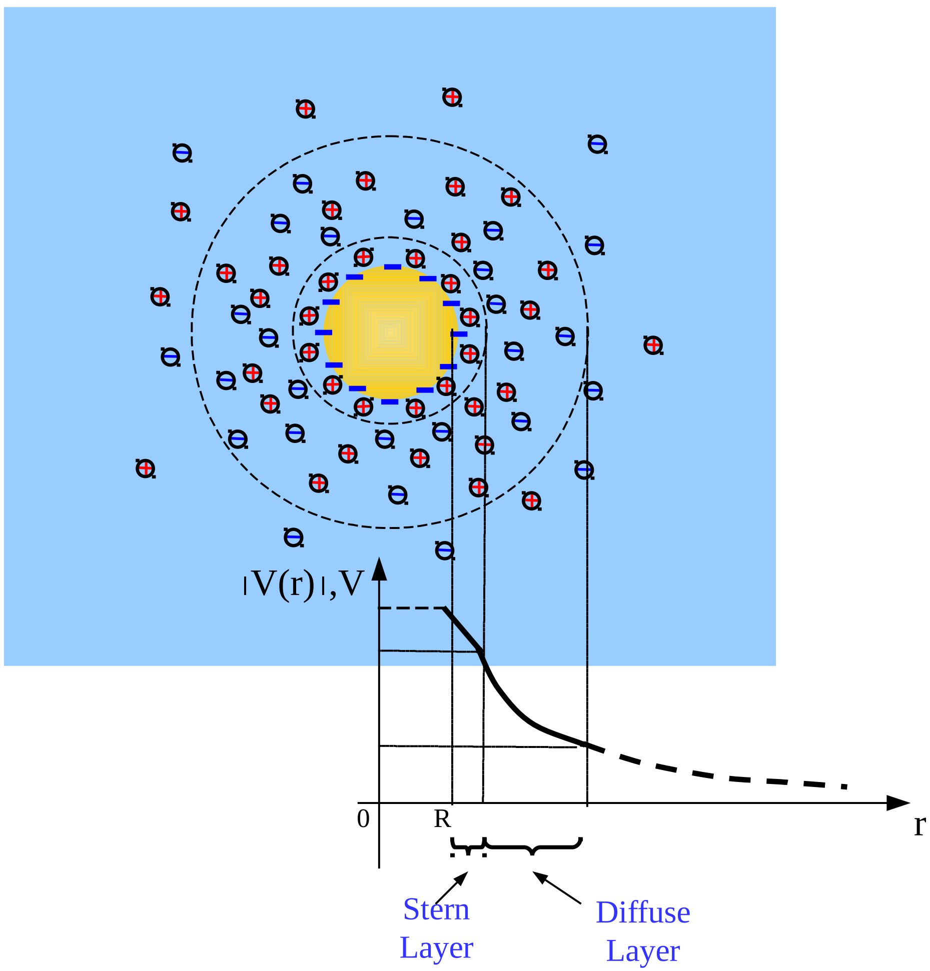
- Ribosomes carry a negative surface charge
  (mainly comes from their RNA)

- Majority of cytoplasm proteins have net negative charge *
  Except a few binding proteins

*Schwartz R. et al Genome Res. 2001 May; 11(5):703-9.

Derived Physical Quantities

- Electrical potential



- Context:
  - Particle (here the bacterium)
  - Carries a non-zero net charge
  - Suspended in an electrolyte:
    m ionic species, concentration n_i, valence z_i
    Relative permittivity ε_r

- Equation to be solved

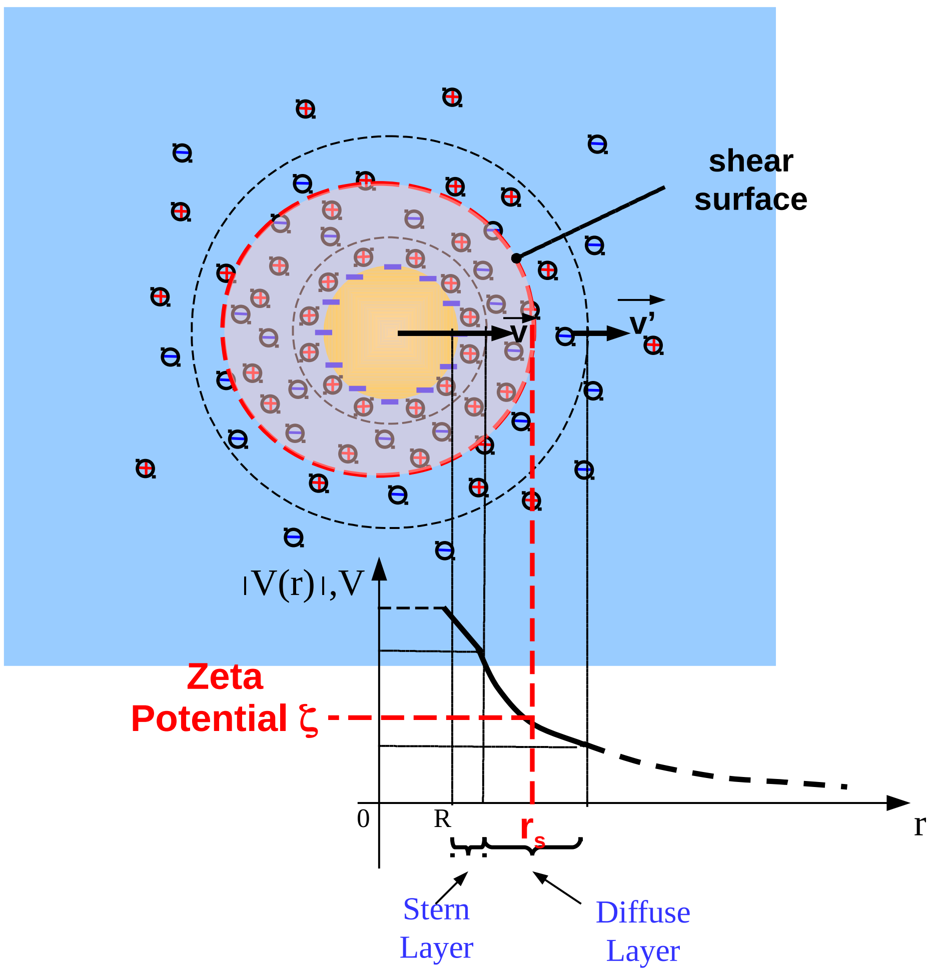
$$\Delta V = -\frac{e}{\varepsilon_o \varepsilon_r} \sum_i^m n_i \, z_i \cdot \exp\left[-\frac{z_i \cdot e \cdot V}{k_B \cdot T}\right]$$

- Debye-Hückel approximation
  z_i e V << k_BT

$$\Delta V = +\frac{1}{\varepsilon_o \varepsilon_r} \sum_i^m \frac{n_i \, z_i^2 e^2 V}{k_B T} = \kappa^2 V$$

Derived Physical Quantities

- Zeta potential



Context:

- Particle (here the bacterium)
- Carries a non-zero net charge
- In motion in an electrolyte

Definition:

ζ: Electrical potential at the abscissa r_s of the shear surface

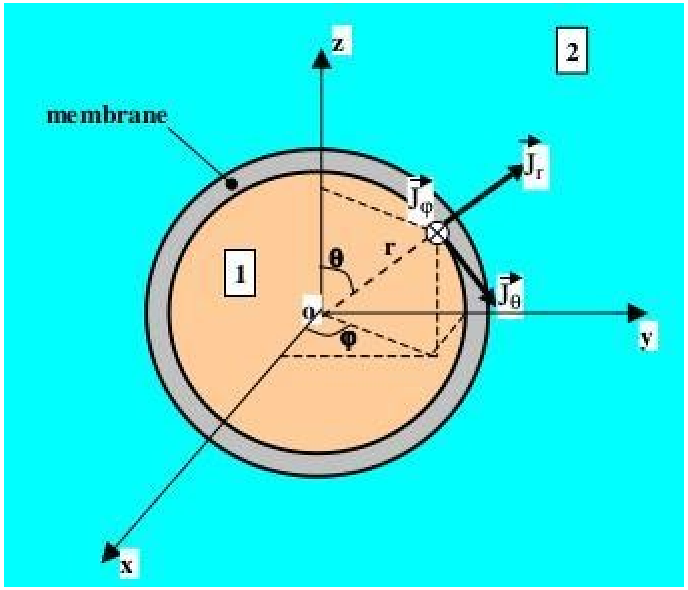
Measurement:

ζ extracted from measurement of electrophoretic mobility μ_e and knowledge of dynamic viscosity η (Smoluchowsky Eq.)

$$\mu_e = \frac{\varepsilon_o \varepsilon_r}{\eta} \xi$$

Derived Physical Quantities

- Transmembrane potential
- Electrodiffusion of a permeant ionic species S



- Electrodiffusion current density

Faraday constant; concentration; diffusion coefficient

$$\vec{J}_S = -z\,F\,D\left(\vec{\nabla}[S] + \frac{F\cdot z}{R\,T}\cdot[S]\cdot\vec{\nabla}V\right)$$

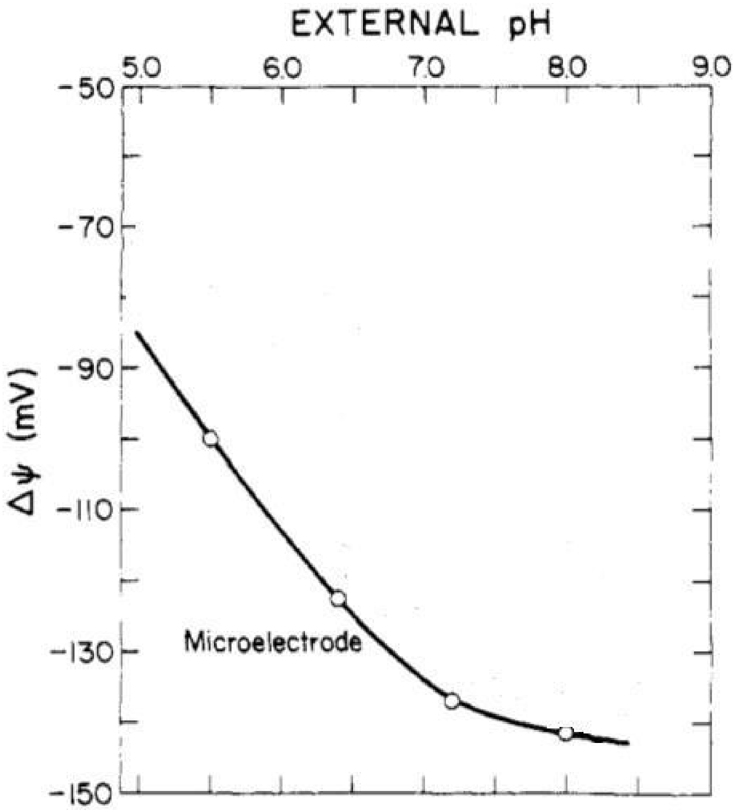
- Transmembrane voltage at Equilibrium

(valid for cations + ; reverse sign for anions -)

$$V_{int} - V_{ext} = \frac{R\,T}{F\cdot z}\cdot\ln\left(\frac{[S_+]_{ext}}{[S_+]_{int}}\right)$$

Derived Physical Quantities

- Transmembrane potential
- Case of protons H^+



- Hydrogen potential pH

$$pH \approx -\log_{10}([H_+])$$

- Transmembrane voltage at Equilibrium (@ 20°C)

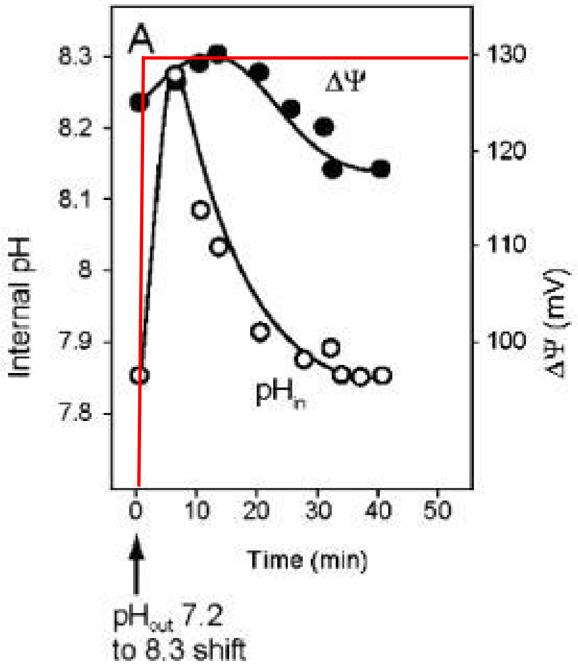
$$V_{int} - V_{ext} = -58 \cdot (pH_{ext} - pH_{int})$$

Trans-membrane potential measured by microelectrode on a giant mutant version of E. coli-K12 *

*Felle H. et al., Biochemistry Vol. 19, 1980, pp 3585-3590

Derived Physical Quantities

- Homeostasis of pH
- External pH step response



Ziberstein experiment on *Escherichia coli*
The external pH is subjected to an abrupt change from 7.2 to 8.3 in 30 seconds*

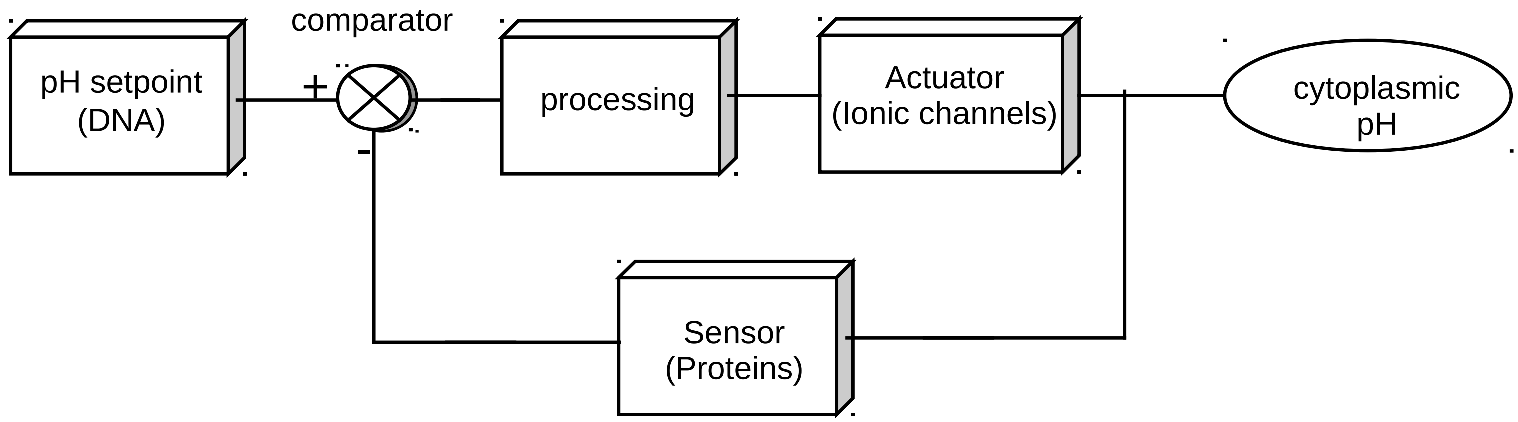
- Internal pH is returned to its nominal value
- Trans-membrane voltage is a control variable for regulation

*Zilberstein et al, J. Bacteriol, 1984

Derived Physical Quantities

- Homeostasis of pH

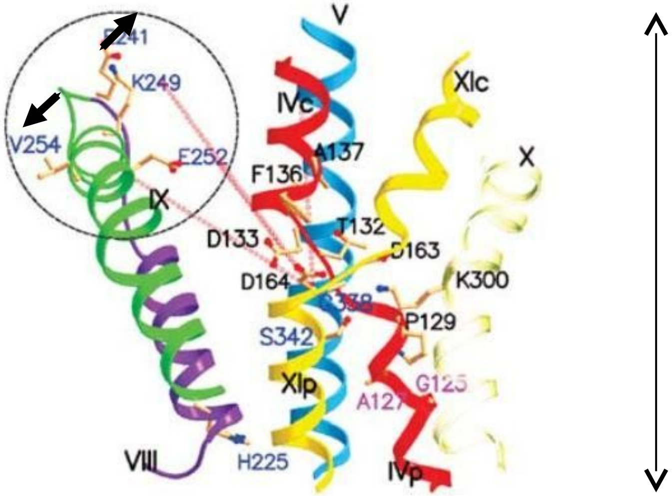
- Control loop model



Derived Physical Quantities

- # Homeostasis of pH

- ## Sensor/Actuator protein



Anti-port carrier
NhaA Na+/H+ in *E. coli* *

- **Terminal groups emerging into the cytoplasm:**
  - protonate to a greater or lesser degree, depending on the cytoplasmic pH
  - subjected to coulombian forces, depending on their degree of protonation.

- **Subsequent protein conformational change:**
  - allows an ion exchange between outside and Inside
  - appropriate ionic concentrations to obtain the nominal pH in the cytoplasm

 * Hunte et al, Nature, 2005

Indicators for biologists

- Transmembrane electrochemical potential

- Gibbs free Energy [J]

$$\underbrace{\Delta\mu_{H^+}}_{\text{Total energy}} = \underbrace{F \cdot \Delta\Psi}_{\text{Electrical component}} + \underbrace{R \cdot T \cdot \ln\left(\frac{[H_+]_{int}}{[H_+]_{ext}}\right)}_{\text{Chemical component}}$$

▸ Energy required to push one mole of protons from outside to inside:

- more appropriate parameter for ion exchanges assessment

- Proton-Motive Force (PMF) [V]

$$\underbrace{\Delta p}_{\text{PMF}} = \underbrace{\Delta\Psi}_{\text{Electrical component}} + \underbrace{\frac{R \cdot T}{F} \cdot \ln\left(\frac{[H_+]_{int}}{[H_+]_{ext}}\right)}_{\text{Chemical component}}$$

▸ Divide by Faraday constant F [C]:

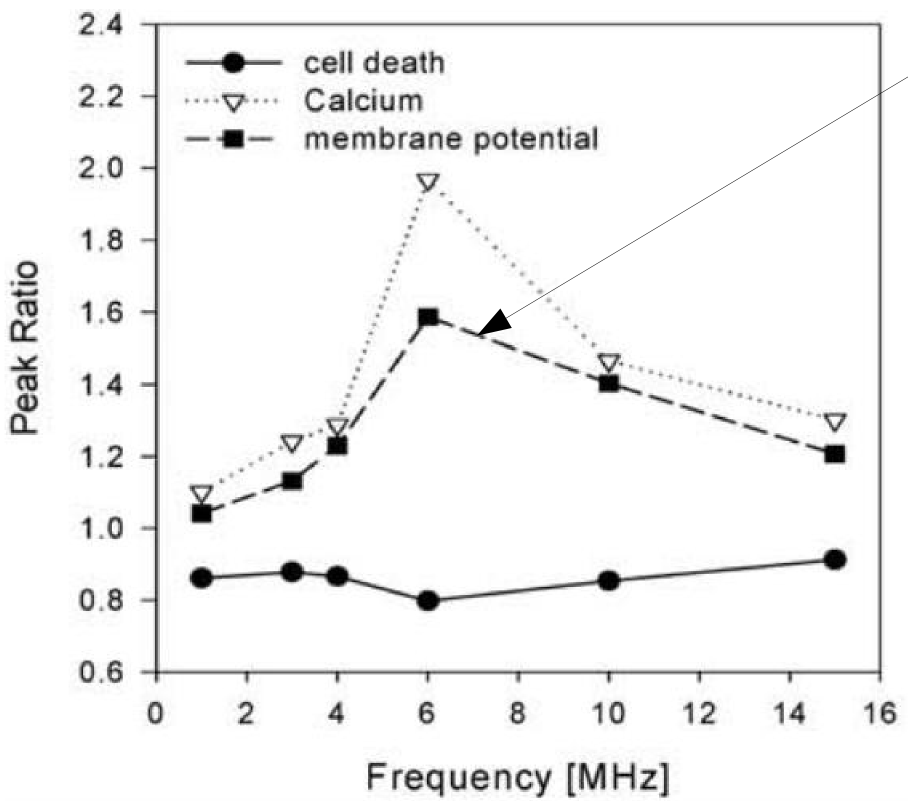
- easy-to-use physical quantity
- quantified in Volts

- **but not directly measurable with an electronic device**

Indicators for biologists

- Transmembrane electrochemical potential

- Ion-Selective Method



■ Addition of a depolarizing agent:

- allows passive diffusion of a species S through the membrane
  (ex . Valinomycin fo K^+ passive diffusion)

- Consequently cancels the total energy $\Delta\mu_S$, relating to this species

▸ Electrical potential is obtained:

- only in relation to the species S
- indirectly measured (several options)

$$\Delta\Psi_S = -\frac{R \cdot T}{F} \ln\left(\frac{[S]_{int}}{[S]_{ext}}\right)$$

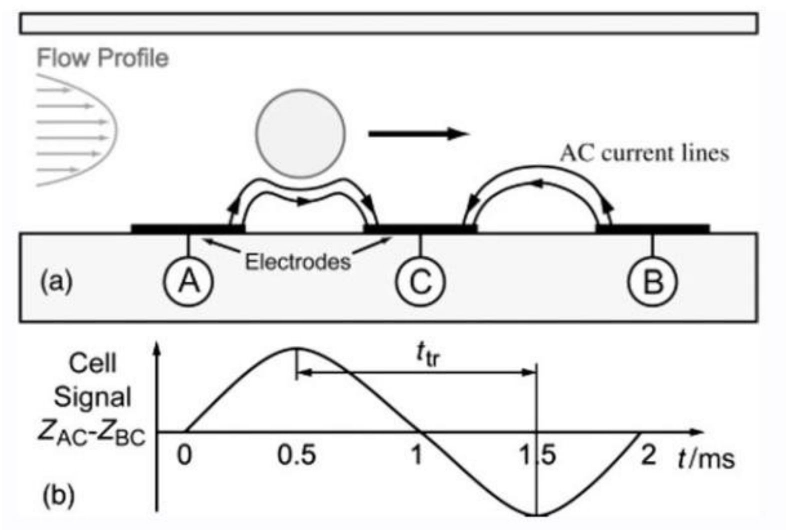
can be measured by fluorometry

image indicator can be built up from experiments (see next slide)

Indicators for biologists

- Microfluidic Impedance Flow Cytometry

- Experimental set-up



- Relative Phase PO indicator *

$$PO = Arg\left(\overline{Z}_{(F_{max})}\right) - Arg\left(\overline{Z}_{(F_o)}\right)$$

- Events counting in cytometry:
  - The frequency of appearance of a PO value is recorded.
  - PO value corresponding to a peak of apparitions is collected under various configurations
  - Final indicator constructed by combining various peak values → optimize contrast on the studied phenomenon. (see prev. slide)

* Cheung K.C. et al, Cytometry Part A Vol.77A, 2010, pp 648-666

Conclusion

- Living Cell - Electricity interaction can be seen according to several lines of thought.
- What-Where-When paradigm relating to electrical charges could be an interesting approach.
- Access to data through direct experimentation is still incomplete at present.
- Biologists use indicators, often indirectly related to electrical quantities, but effective in indicating the physiological state of their study subjects.
- Quantification of purely electrical quantities relating to living world is a means of promoting cross-disciplinary cooperation between scientific communities.

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Any questions ?