How Physical Systems Can Help in Studying Function of Biological Systems?

Zuzanna S. Siwy

University of California, Irvine, CA
zsiwy@uci.edu
Biological Scales

Microns to Nanometer -- Biological/Chemical/Atomic

- Plant, Animal Cell
- Bacteria
- Virus
- Simple Molecules
- Atoms

http://www.ece.purdue.edu/~janes/whats_nano.htm
Scales of Life

Cells – units from which an organisms is built

Scales of Life
Complexity of Biological Objects

“The closer one looks at [the] performances of matter in living organisms the more impressive the show becomes. The meanest living cell becomes a magic puzzle box full of elaborate and changing molecules, and far outstrips all chemical laboratories of man in the skill of organic synthesis...."

[Max Delbruck]

Although biological objects are complex, and a living organism cannot be described by a collective behavior of individual components, yet the components of a living cell obey the same laws of physics as all other systems”. Studying individual components is crucial in understanding functions of a living organism.
Importance of Wiring - Connections
Importance of Wiring - Connections

We are filled 70% with water

Most important ions: $\text{Na}^+$, $\text{K}^+$, $\text{Cl}^-$, $\text{Ca}^{2+}$

Quantitative description of flow of ions: ion current

Electrode reactions
Signal in Neurons

Structure of a Typical Neuron

Sodium and potassium ions play a crucial role in nerve signal transduction.

- Sodium ions pass inside of the cell
- Potassium ions flow out of the cell
- Action potential moves with speed between 0.1 to 100 m/s.
Lessons from Nature
Transport Proteins are Nature’s Nanotubes

Impermeable lipid bilayer membrane

Membrane-Bound Transport Proteins
Allow for highly selective transport of ions, sugars, amino acids, etc. across the lipid bilayer membrane
Ion Channels as NanoDevices and Smart Holes

- Voltage-gated channels
- Ligand-gated channels
- Mechano-sensitive channels

“Smart hole”

Symbiosis of Physical and Biological Approaches to Study Transport Phenomena in Biological Systems
How People Saw/Treated Ion Channels at the beginning of XX Century

Almost Everything We Know about Ion Channels Came from Electrical Measurements

1909 Nobel Prize in Chemistry

www.emcesd.com/seminars/Ellsem21.jpg
What Did We Know about Channels and Membranes in 40’/50’ties?

Not much..

Excitability of the Membrane

\[ I(t) = [g(t)](V - V_0) \]
Information Was Obtained from Studies with Blockers of Ion Channels

Chemical agents selectively block sodium (potassium) current indicating existence of separate paths for sodium and potassium transport through the pore.

Pathways for sodium and potassium, most probably separate

LACK OF DIRECT EVIDENCE
Hodgkin-Huxley Model for Signal Transduction

The Nobel Prize in Physiology and Medicine 1963

\[ \frac{a}{2R_i \theta^2} \frac{d^2V}{dt^2} = C_m \frac{dV}{dt} + G_K n^4 (V - V_K) + G_{Na} m^3 h (V - V_{Na}) + G_L (V - V_L) \]

Picture of nerve axon as an excitable medium, capable of transmitting nonlinear waves of excitation over long distances without loss of signal strength or character of that wave.

This is all based on Hodgkin-Huxley biophysical measurements that contained no direct evidence for individual ion channels or structure of membrane!
Patch Clamp Setup

Recordings from One Molecule

E. Neher & B. Sakmann
1991
More Complete Picture of Membrane and Channels


Still no direct picture of channels, channels were recognized by conductance and chemical response.
Now We Can See the Channels

Relation Between Structure and Function

**Electrical Measurements**

**Function**

**STRUCTURE**

Which parts of the complex structures are responsible for various features like voltage gating, selectivity?
Focusing on “Important” Part of the System


Selectivity of L-Type Calcium Channels

There are two binding sites for calcium ions


Calcium channel is selective for calcium ions although concentration of $\text{Ca}^{2+} \ll$ than concentration of $\text{Na}^+$ and $\text{K}^+$
Which Part of the Channel is Responsible for Ca Selectivity? – Importance of Electrostatics

OmpF porin functions to regulate osmotic pressure between the cell and its surroundings. It has one e, it is basically not selective.


Protein data bank:
http://www.rcsb.org/pdb/cgi/explore.cgi?job=chains&pblId=2OMF&page=
Introduction of 2 additional COOH groups turns non-selective OmpF channel into Calcium Selective Channel.

The mutant is calcium selective!

Ion current switches between discrete levels in a voltage-dependent manner


BK channel (P.N.R. Usherwood)
Synthetic NOT-PROTEIN PORES


Gating with Non-Protein Nanopores


Physicists Can Think About Even a More Crazy Approach

Grafting a feature that we believe plays a crucial role in a given function into an entirely synthetic system.

A synthetic “hole”

Focusing on basic physical and chemical effects underlying transport properties of biological channel.
Heavy Ions as a Working Tool

e.g. Xe, Au, U
(~2.2 GeV i.e. ~ 15% c)

Chemical etching

1 ion → 1 latent track → 1 pore!

E. Loriot

Linear accelerator
UNILAC, GSI
Darmstadt, Germany
A Short Glimpse at the “Product” of Track Etching Technique

http://www.Iontracktechnology.de
Why Do We Want to Work with Asymmetric Pores?

Cylindrical pore: 

\[ R_1 = \frac{4L}{\kappa\pi d^2} \]

Tapered cone: 

\[ R_2 = \frac{4L}{\kappa\pi dD} \]
Focusing of Resistance in a Conical Nanopore

\[ R = \frac{4L}{\kappa \pi \delta \Delta} \]

- \( D = 1 \ \mu m \)
- \( d = 3 \ \text{nm} \)
- \( L = 12 \ \mu m \)

50% of total resistance is focused over 36 nm

80% of total resistance is focused over 140 nm
Nature Likes Asymmetry Very Much


Conical Pores are Obtained by Putting Etch Solution on One Side of Membrane and Stop Solution of the Other

Polyethylene Terephthalate (PET) (RN 12 Hoechst)

Gold Replica of a Single Conical Pore

Dept. of Chemistry, Univ. of Florida, Gainesville
Gold Replica of a Single Conical Pore

Dept. of Chemistry, Univ. of Florida, Gainesville
Hydrolysis of Ester Bonds with NaOH in PET Causes Formation of COOH Groups

The surface density of COOH groups was estimated to be ~ 1.5 per nm²
Experimental Set-up

$U$

$I$

KCl

Ag/AgCl electrode

Ag/AgCl electrode

KCl
Current-Voltage Characteristics of Single Conical Pores

$U$ (V) vs $I$ (nA) graph showing the behavior of COOH and COO$^-$ groups at different pH levels: pH 7, pH 5, and pH 3.

Are the Nanopores Ion Selective?

PET and Kapton pores are cation selective \( t^+ \sim 0.90 \)

Which features are crucial for rectification?

Asymmetric shape of the pore

The pore has to be charged

The diameter of the pore has to be very small!
Why do Asymmetric Nanopores Rectify?

The profile of electrostatic potential $V(z)$ inside an asymmetric pore

Existence of Electrostatic Trap

Shape of electrostatic potential inside a conical pore with negative surface charge

External voltage

Cation trap

Model of ion current rectification in KCl assumes therefore a simple superposition of internal potential with externally applied voltage
Importance of COOH in Ca Sensitivity

K_d = 10^{-8} \text{ M}

Concentration of Ca^{2+} \ll Concentration of K^+
Current-Voltage Curves at Presence of Calcium Ions

Negative incremental resistance

Small opening: 5 nm

Current-Voltage Curves at Presence of Calcium Ions

Small opening: 9 nm
Importance of Surface Charge

Current (nA) vs Voltage (V) for pH 6 and pH 8 solutions in 0.1 M KCl + 1 mM CaCl₂ solution.

Negative incremental resistance!
What About Other Divalent Cations?

- pH 8
  - 0.1 M KCl
  - 0.1 M KCl + 1 mM MgCl₂
  - 0.1 M KCl + 1 mM CaCl₂
  - 0.1 M KCl + 0.1 mM ZnSO₄
Interactions of Ions Passing Through a Nanopore with Pore Walls as a Basis for Biosensors

Detection signal: current-voltage characteristic

Sensing Biowarfare Ricin

Au tube with anti-ricin

Au tube with anti-ricin + 100nM BSA

Au tube with anti-ricin + ricin (~100 nM)

Pore diameter ~ 13 nm
Buffer: 1 M KCl, pH 4.3
Detecting Single Molecules with Nanopores

http://www.mcb.harvard.edu/branton/

Voltage-Dependent Fluctuations in Time

- 60 mV
- 125 mV
- 175 mV
Fluctuations of ion current are self-similar in time

The closer we look the more we see!


POWER SPECTRA

Studies of the origin of $1/f^\alpha$ noise in membrane channels currents

The spectral density through a single ion channel; S.M. Bezrukov, in *Proc. First Int. Conf. on Unsolved Problems of Noise, Szeged 1996*, edited by C. R. Doering, L. B. Kiss, and M. F. Schlesinger.

---

The figure shows a power spectrum analysis of a BK channel at 60 mV, showing a power law decay predicted by the 1/f noise theory. The graph compares the power spectra of PET and Biol. Kapton, with different decay rates.

---

1/f noise !!  
No 1/f noise !!  

1. Combining Physical, Biological and Chemical Approaches enables one to answer intriguing scientific questions.

2. Experiments and modeling working together bring new insights into studied phenomena.

3. Synthetic systems give new opportunities to focus on basic physical and biological phenomena underlying a given biological function.
Acknowledgments

Matt Powell

Eric Kalman

- Dr. Christina Trautmann, GSI, Germany
- Prof. Charles R. Martin, Univ. of Florida, Gainesville
- Prof. Martin’s group, Univ. of Florida, Gainesville
- Birgitta Schiedt, GSI, Germany
- Prof. Clare Yu, University of California, Irvine
- Prof. Bob Eisenberg, Rush Medical Center, Chicago
- Prof. Andrzej Fulinski, Jagellonian University, Poland