A Vision of Science & Technology at the Biology-Electronics Interface

John J. Kasianowicz

Semiconductor Electronics Division, NIST, EEEL

SRC Bioelectronics Roundtable Workshop

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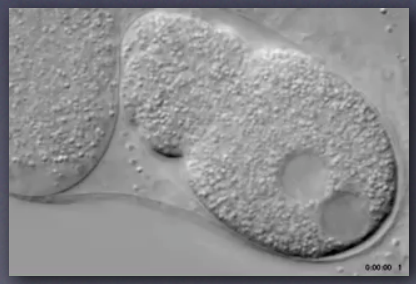
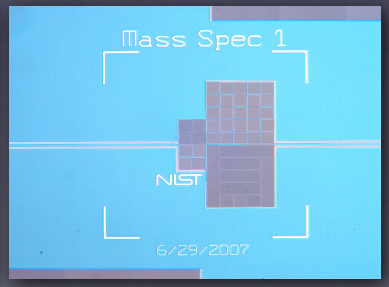
Electronics for Biology

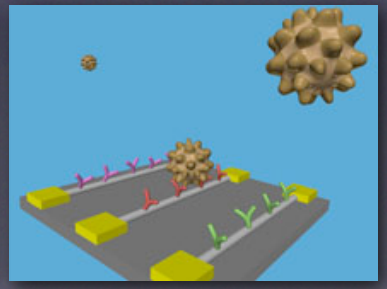
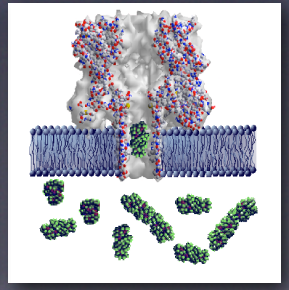
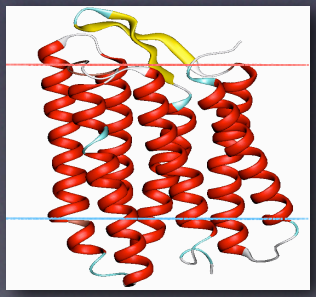
Systems Biology & Health Care: need new metrologies

Electronic measurements:

- revolutionized biological sciences in the past
- at a new frontier: genomics; protein detection & quantification; mass spectrometry; HLS apps; Structural Biology of membrane proteins, . . .

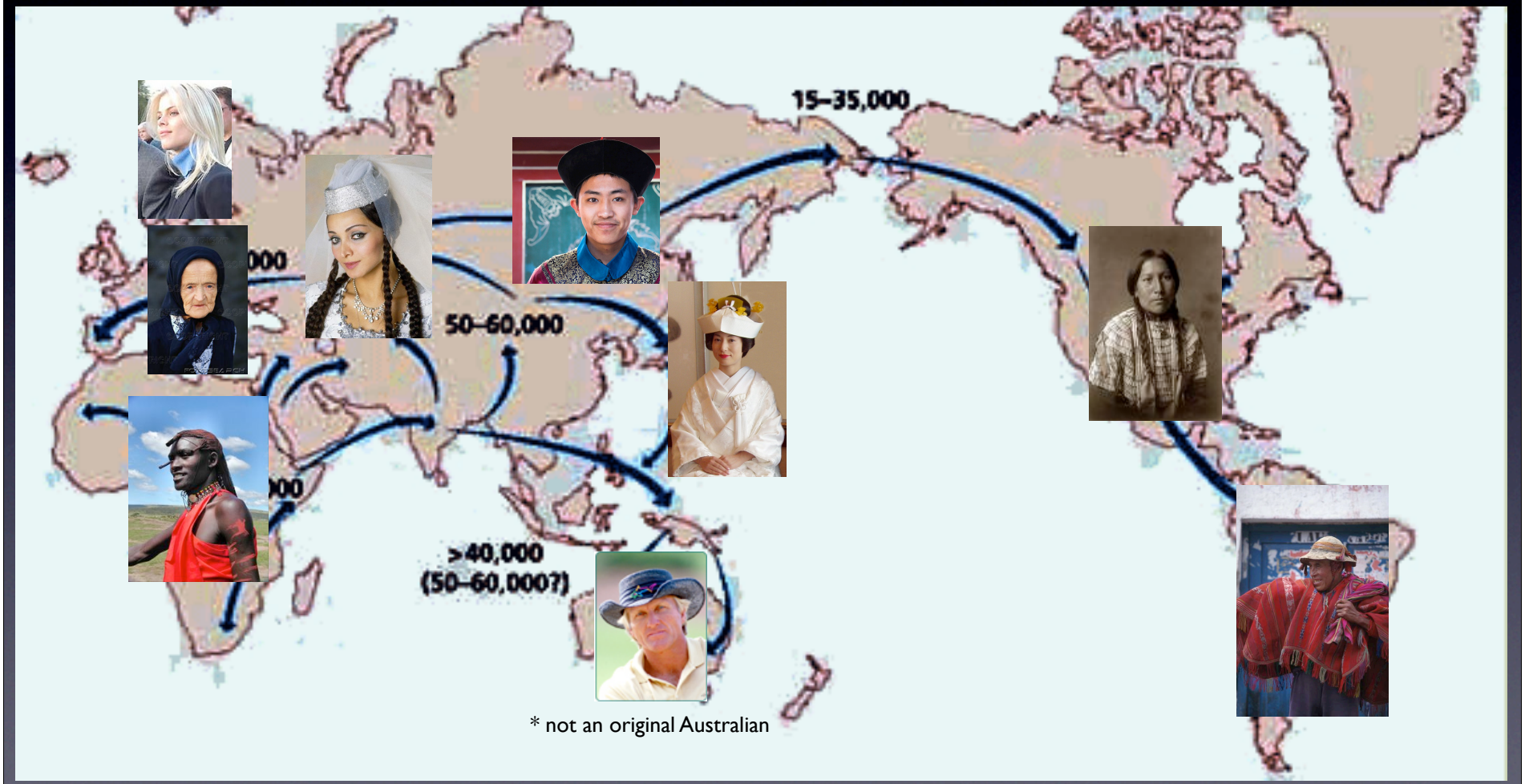
EM fields: Optical tweezers, dielectrophoresis, . . .

Some Inherited Traits are Obvious

but an individual's predisposition to disease is not



* not an original Australian

Biological Complexity

Cell Growth & Division

Cells: some are motile, sense & react to stimuli, divide, become tissue, communicate w/other cells via networks, . . .

Biomolecules: nanomachines, signaling agents, . . .

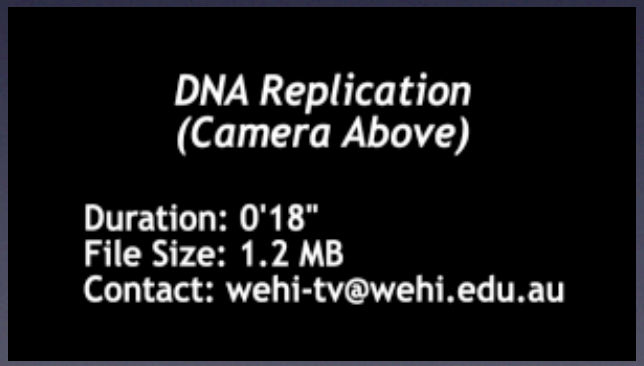
Need for new metrologies for Systems Biology
(Leroy Hood, Institute for Systems Biology)

The underlying physics & chemistry should be simple: *how smart can molecules be?*

Reductionist paradigm has been highly successful, but we still know relatively little about how even single cells work.

New metrologies for basic cell research will provide insight into disease.

Alberts, B. 2008. *Science* **319**, 1733



DNA Replication

www.wehi.edu.au

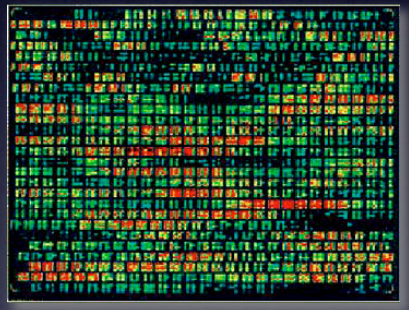
New Era of Personalized Medicine

Need

New measurement capabilities that will identify an individual's predisposition for disease.

- *Heart diseases, diabetes, cancers, cystic fibrosis, Alzheimer's, . . .*

gene chips hold promise

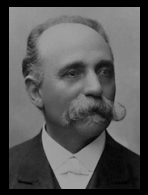


Potential for electronics

Can it provide precise & accurate measurements at low-cost?

- $1,000 USD genome
- simultaneous measurement of ~ 2,500 proteins in blood (L. Hood)
- structures of membrane proteins (> 50% of pharma targets)

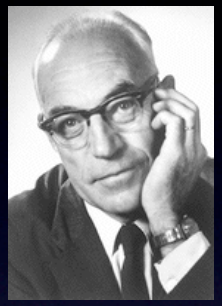
Electronics Revolutionized Biological Sciences

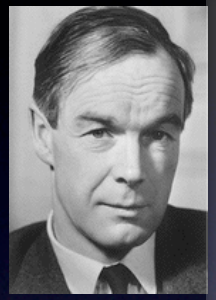


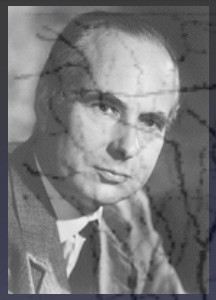
C. Golgi

molecular basis for nerve conduction & single molecule detection

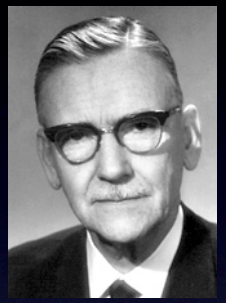
S. Ramon y Cajal

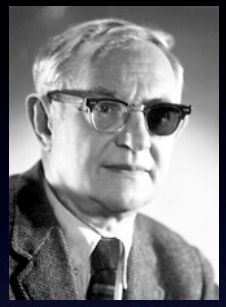




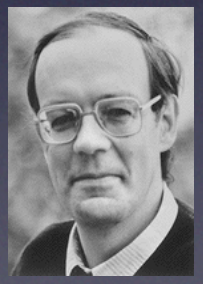


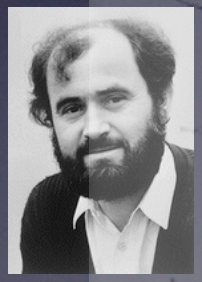
1963 Nobel Prize for Physiology or Medicine: J. Eccles, A. Hodgkin & A. Huxley for their discoveries concerning the ionic mechanisms involved in excitation and inhibition in the peripheral and central portions of the nerve cell membrane



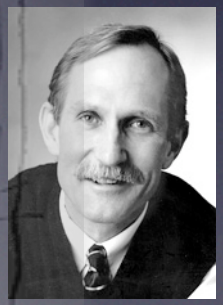


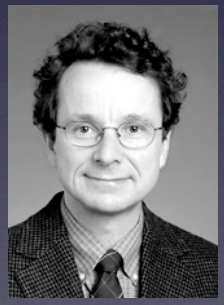
1970 Nobel Prize for Physiology or Medicine: B. Katz, U. Van Euler, & J. Axelrod, for their discoveries concerning the humoral transmitters in the nerve terminals & the mechanism for their storage, release & inactivation





1991 Nobel Prize for Physiology or Medicine: E. Neher, & B. Sakmann for their discoveries concerning the function of single ion channels in cells

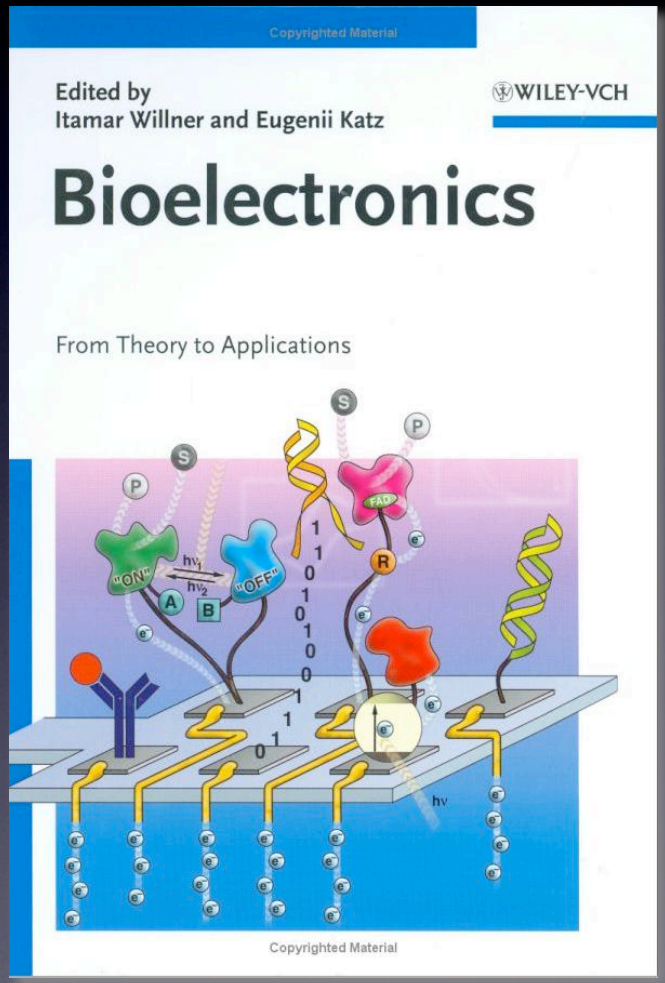




2003 Nobel Prize for Chemistry: P. Agre & R. MacKinnon for discoveries concerning channels in cell membranes

Next Era of Bioelectronics

will use more complex materials systems



2005

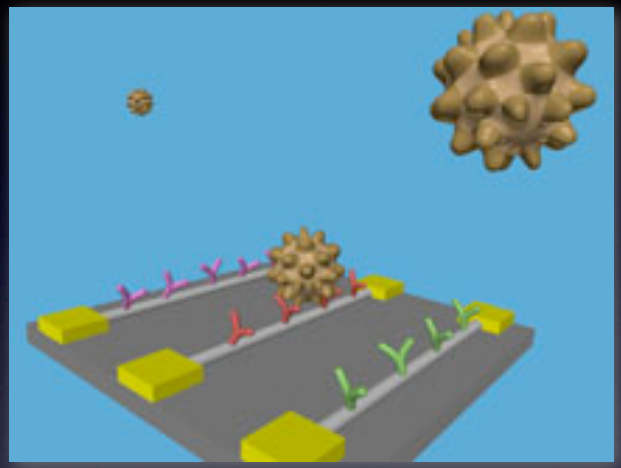
Electrochemical impedance spectroscopy

- e^- transfer through proteins
- biosensors
- biocomputing

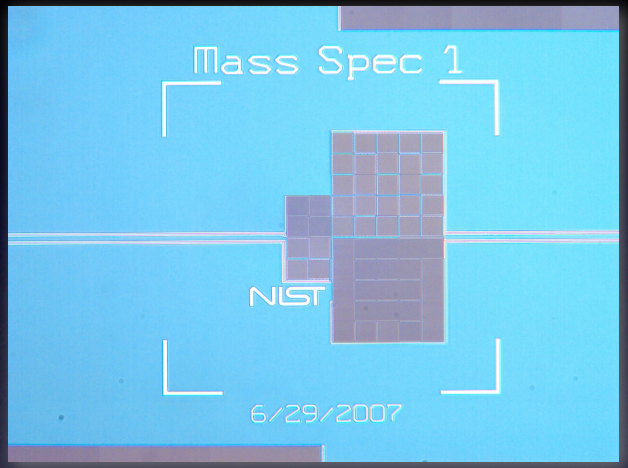
Enabled via conjugation of biomolecules to solid-state materials

Bioelectronics

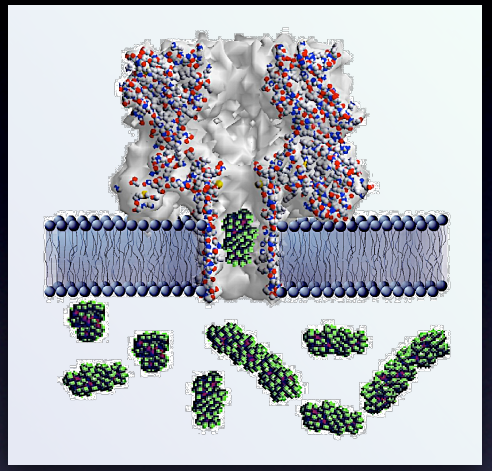
detection & characterization



Nanowire transistor arrays
detection of viral particles
(C. Leiber, Harvard University)



Extension of Mass Spectrometry for very large nanoparticles (proteins, viruses, & pathogens)
(R. Schwall, NIST, EEEL & M. Ohkubo, Japan)



Nanopore-based detection, identication & quantification of ions, toxins & molecules
(J.J. Kasianowicz, NIST, SED, EEEL)

Grace, D. 2008. *Medical Product Manufacturing News* **12**, 22-23

Cavalcanti, A., B. Shirinzadeh, R.A. Freitas Jr, & T. Hogg. 2008. *Nanotechnology* **19**, 015103

Cheng, M., G. Cuda, Y.L. Bunimovich, M. Gaspari, J.R. Heath, H.D. Hill, C.A. Mirkin, A. Nijdam, T. Jasper, R. Terracciano, T. Thundat, & M. Ferrari. 2006. *Current Opinion in Chemical Biology* **10**,11-19

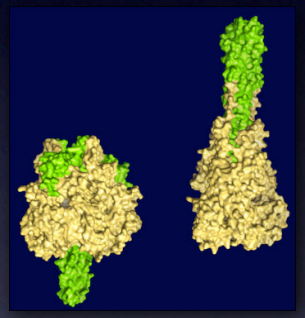
K. Suzuki, S. Miki, S. Shiki, Z. Wang, & M. Ohkubo. 2008. *Appl. Physics Express* **1**, 031702

Patolsky, F.; B.P. Timko, G. Yu, Y. Fang, A.B. Greytak, G. Zheng, & C.M. Lieber. 2006. *Science* **313**,1100-1104

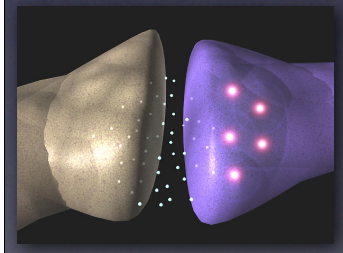
Kasianowicz, J.J., J.W.F, Robertson, J.E. Reiner, E.R. Chan, & V.M. Stanford, 2008. *Annual Review Analytical Chemistry* 1

Short List of Targets

DNA, RNA



Proteins



Cell-signaling agents

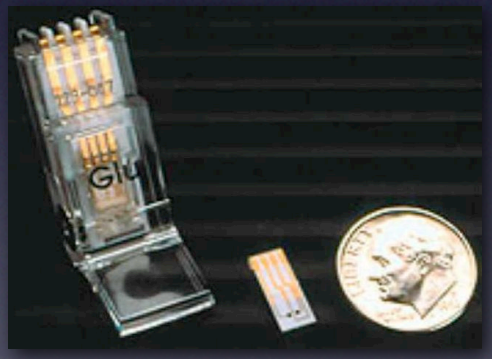
New Metrology Paradigm

for Health Care & Systems Biology

Real-time electronic detection, identification, quantification of DNA, proteins, & other biomolecules (label-free, etc.)

- Electronics: systems integration, miniaturization, . . .
- *Potential impact*: early cancer detection, other health care measurement needs, Systems Biology, Homeland Security, . . .

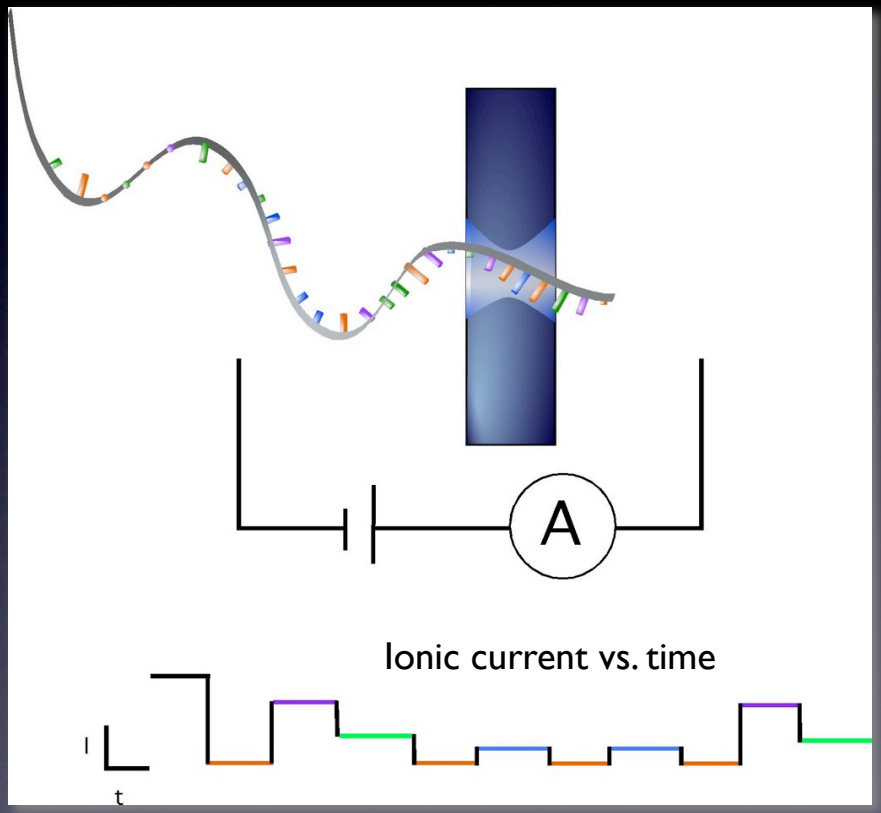
Star Trek Tricorder: Hollywood's concept of future biomedical instrumentation.



Actual devices in the near term will be based on measurements of molecules (e.g., cell-signaling agents, proteins, nucleic acids, metabolites) in cytoplasm, extracellular fluid, urine & blood (e.g., like glucose sensors).

Nanopore-Based Rapid DNA Sequencing?

linear transport, 1-10 microsec/base



Could be achieved if each of the 4 different DNA bases blocked single nanopore ionic current to different degree . . .

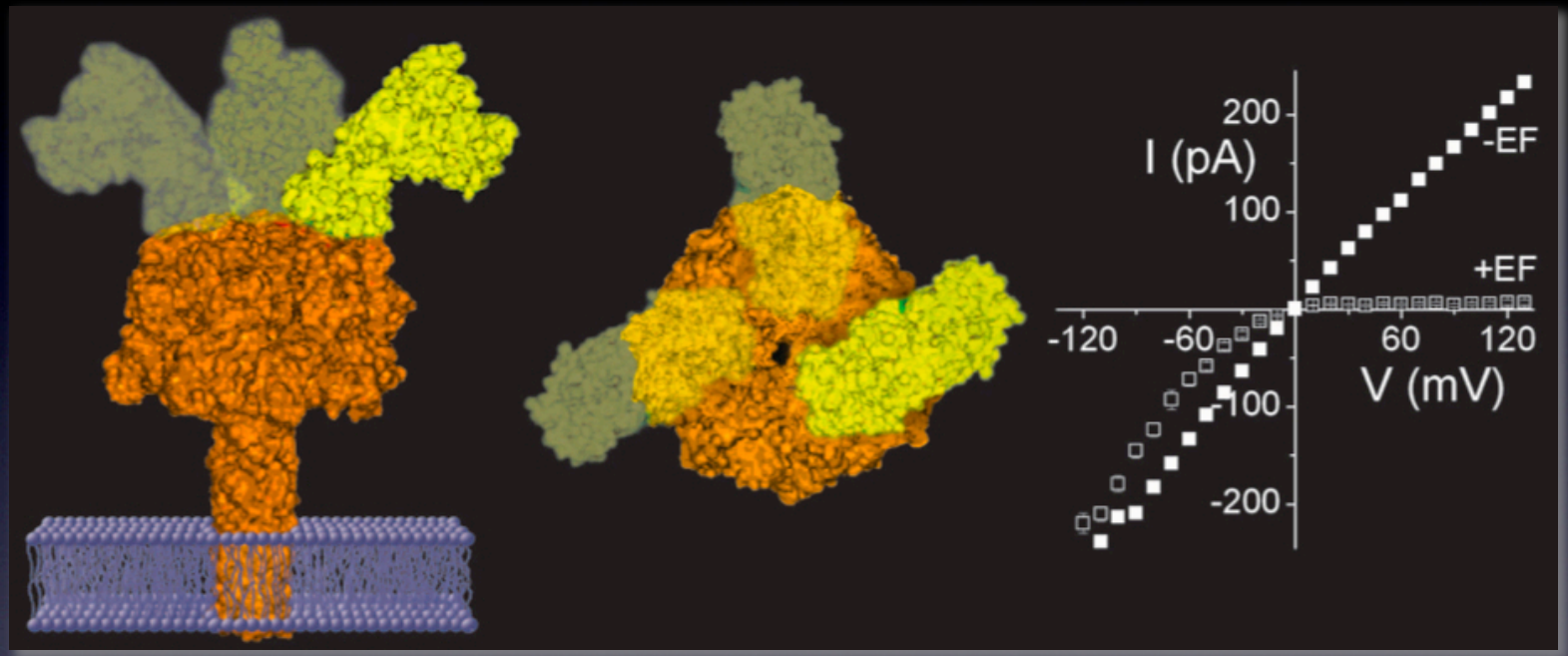
Not yet reduced to practice.

Variations of this method might work.

If made practical, could a similar approach be used to discriminate between different proteins?
(existing methods: Edman degradation, 2D gel electrophoresis, antibody-based detection)

Kasianowicz, Brantin, Branton & Deamer. 1996. *PNAS* **93,** 13770
Church, Deamer, Branton, Baldarelli & Kasianowicz. 1998. USP 5,795,782

Electronic Detection of Anthrax Toxins



Anthrax EF or LF (yellow)
PA_{63} nanopore model (orange) from Nguyen, 2004

Binding of Anthrax Edema Factor (EF) or Lethal Factor (LF) to the Anthrax nanopore changes the latter's I-V characteristic.

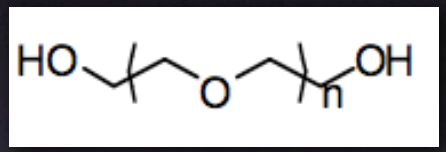
Add'l significance: proof-of-concept for the use of this technique to *rapidly* screen for therapeutic agents against anthrax infection (i.e., 2s). Conventional assays take *hours* and some do not probe function.

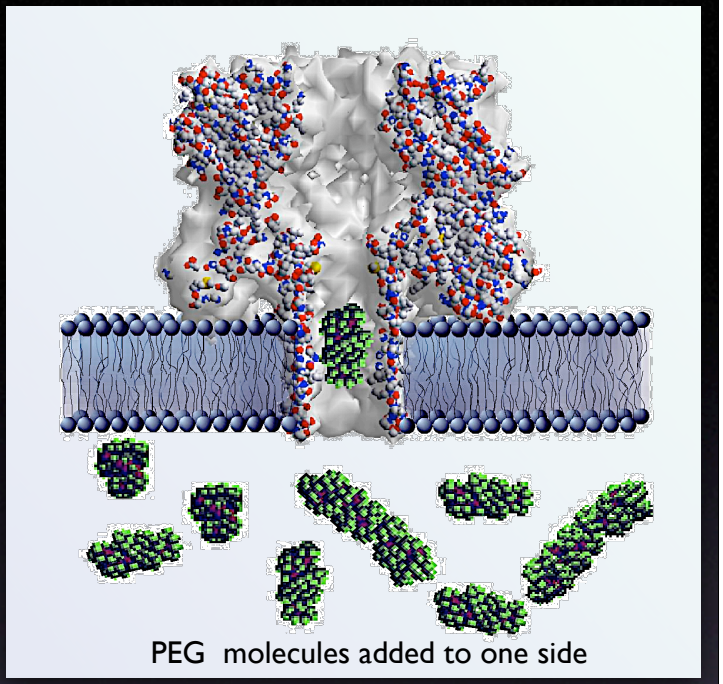
Halverson, Panchal, Nguyen, Gussio, Little, Misakian, Bavari & Kasianowicz. *J. Biol. Chem.* 2005.

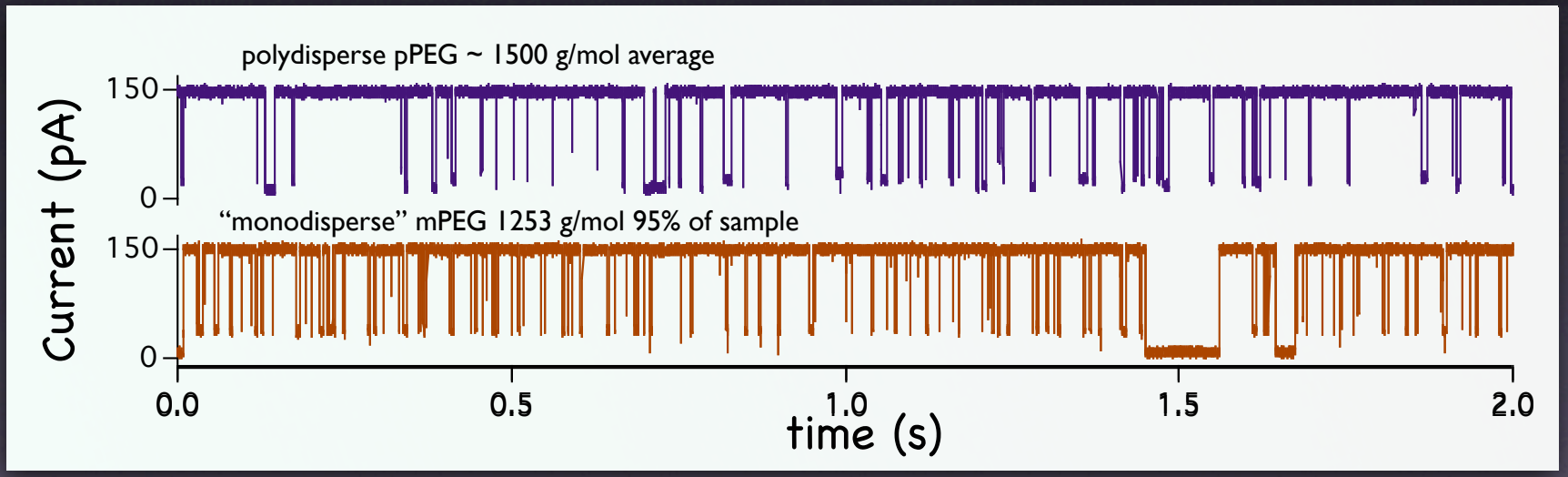
Single-Molecule Mass Spectrometry in Solution

Ionic current blockade depth & residence time depends precisely on polymer size

model polymer: PEG
poly(ethylene glycol)







Robertson, Rodrigues, Stanford, Rubinson, Krasilnikov, & Kasianowicz. 2007. *PNAS* **104**, 8207.
NIST Invention Disclosure submitted 2/2007. *Patent app. submitted.*

Single-molecule mass spectrometry in solution

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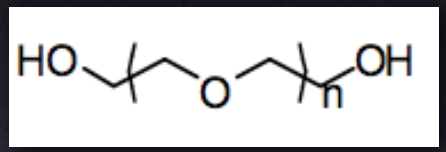
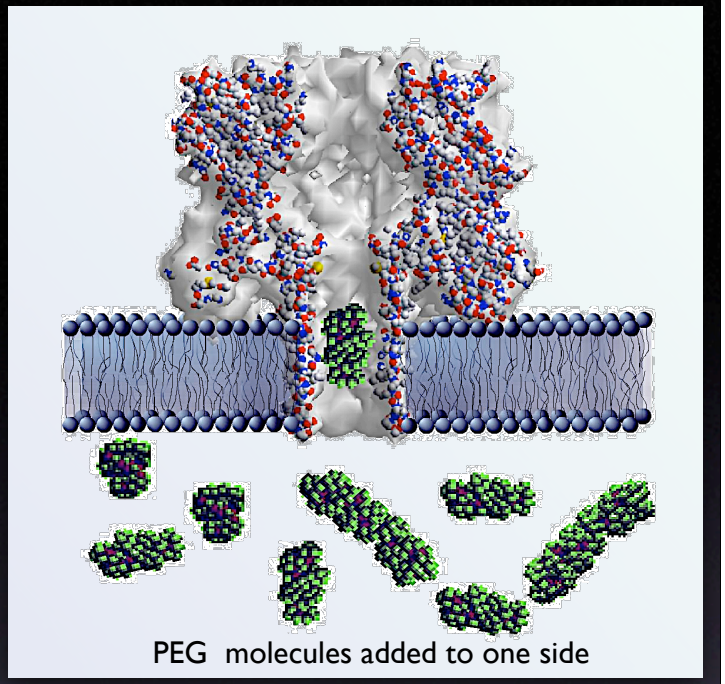
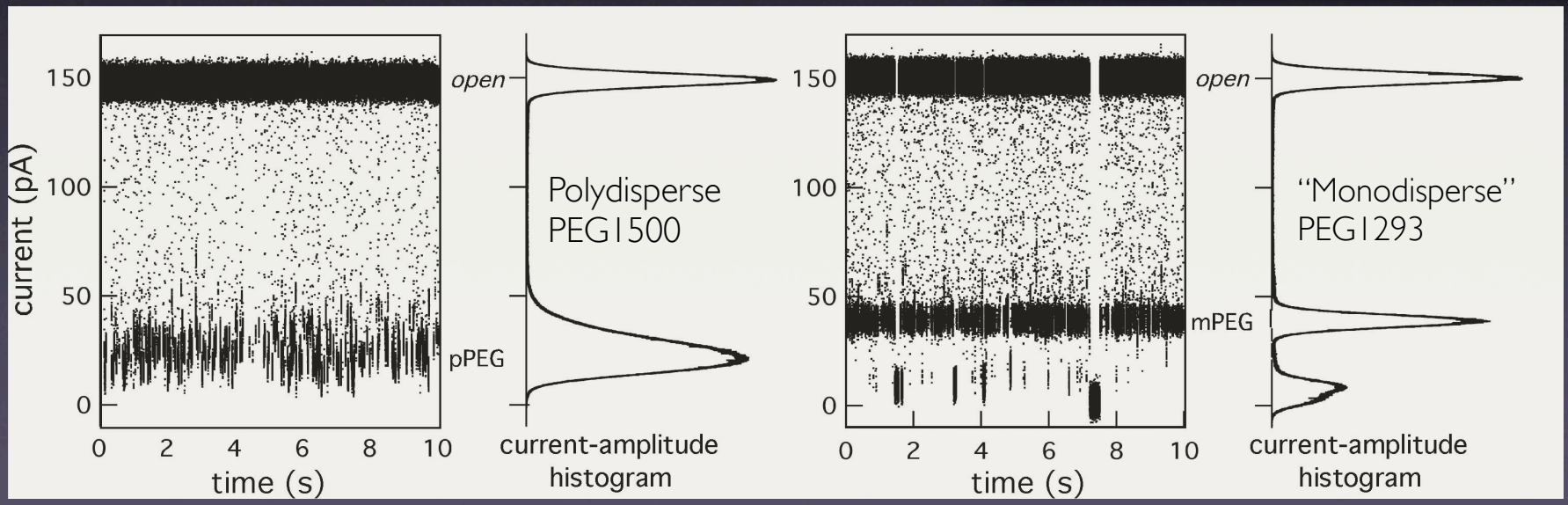
model polymer: PEG
poly(ethylene glycol)





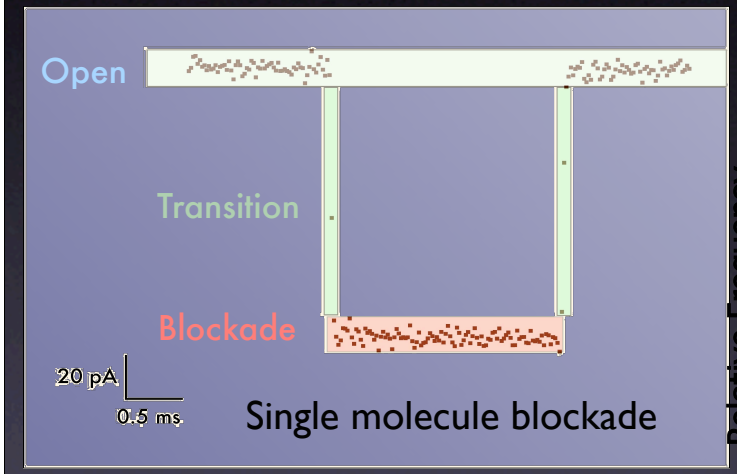


Robertson, Rodrigues, Stanford, Rubinson, Krasilnikov, & Kasianowicz. 2007. *PNAS* **104**, 8207.
NIST Invention Disclosure submitted 2/2007. *Patent app. pending.*

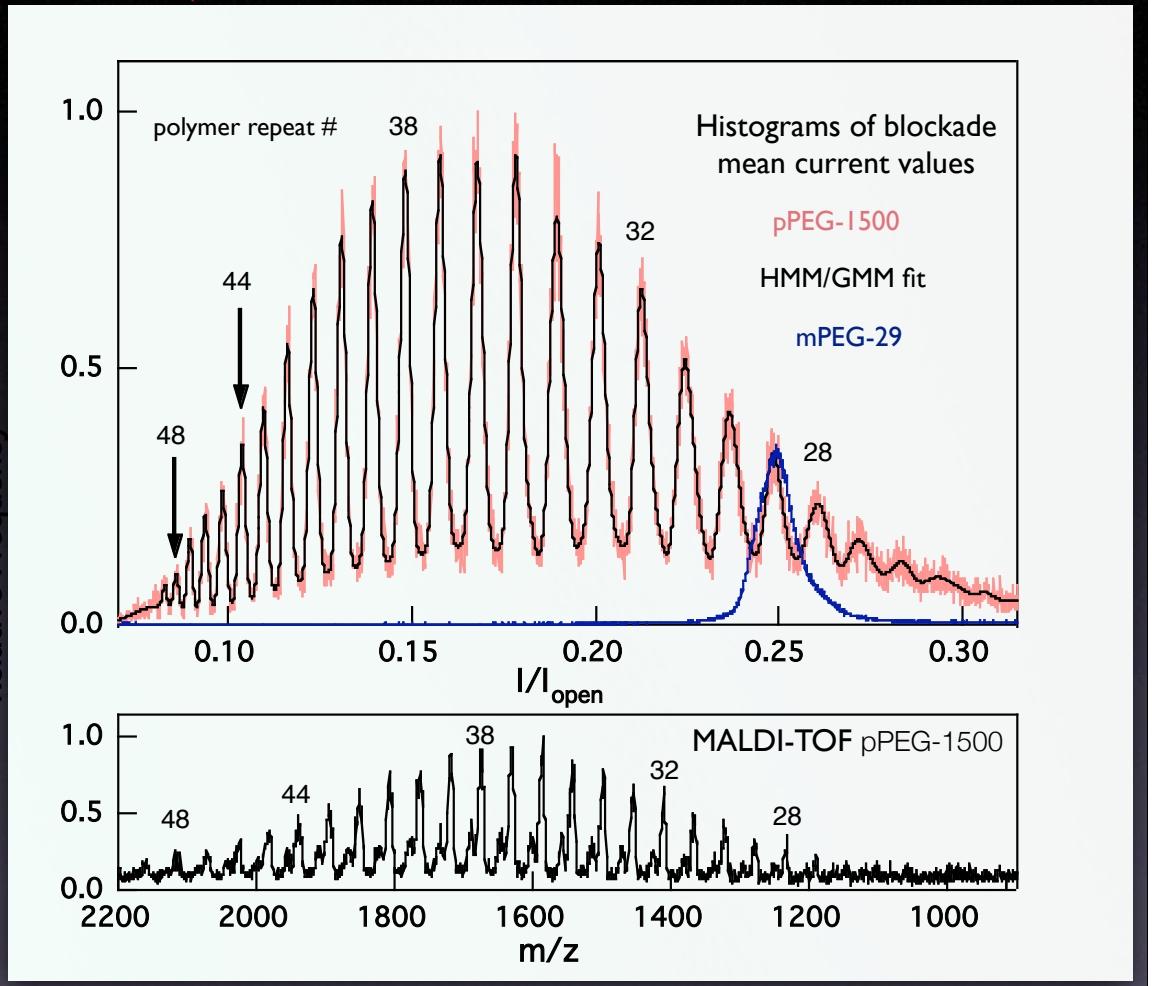
Nanopore Conductance-Based Mass Spectrum

increasing polymer mass

High mass resolving power achieved by averaging each event



Larger polymers block the nanopore conductance more & spend more time in the pore

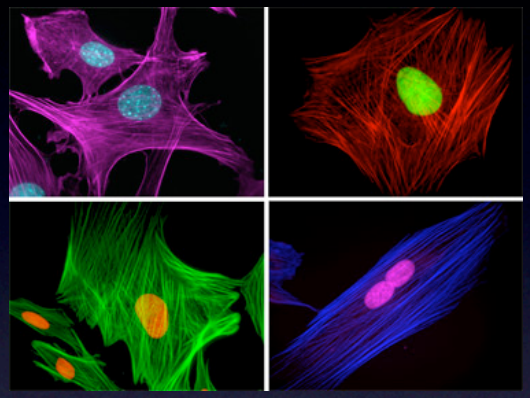


Each peak in the mean blockade current corresponds a slightly different size polymer

Robertson, Rodrigues, Stanford, Rubinson, Krasilnikov, & Kasianowicz. 2007. *PNAS* **104**, 8207.

Understanding How Cells "Think"

Fluorescent probes: identify cell ultra-architecture ...

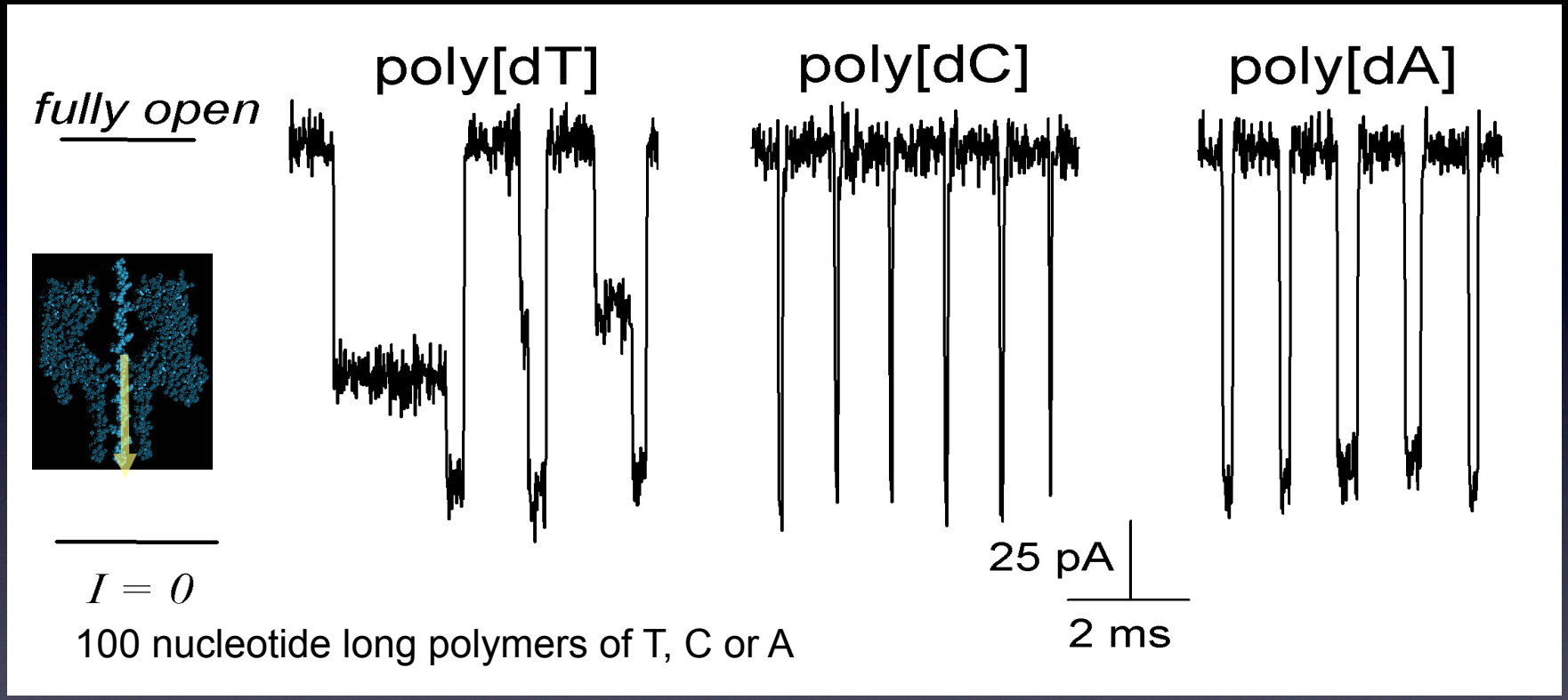


... and how cells respond to stimuli ~ one protein at a time ...

Can nanbioelectronic systems address this problem?

Nanopore-Based Electronic Signals

obtained with one pore type are characteristic of polymer identity

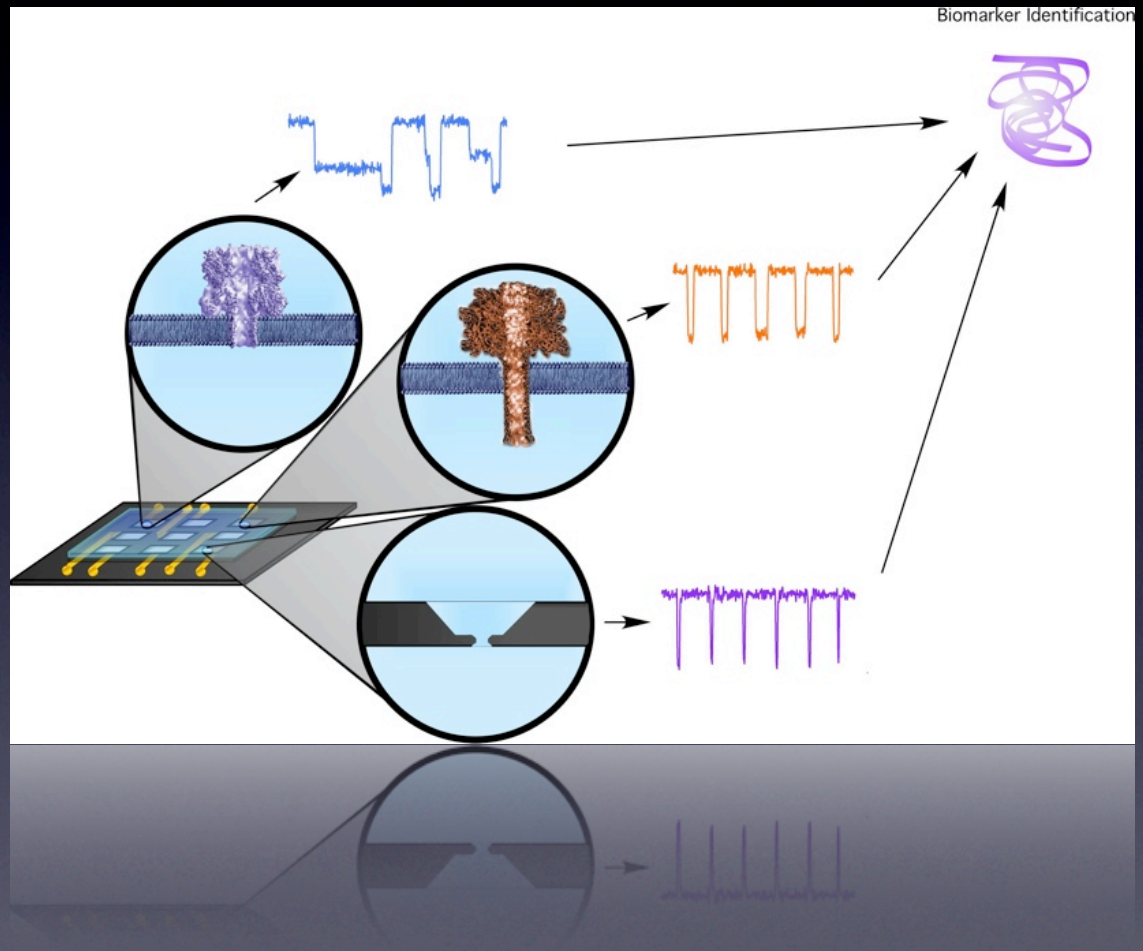


Different nucleic acids can be easily distinguished from each other using advanced statistical signal processing.

Kasianowicz, Henrickson, Weetall & Robertson. 2001. *Analytical Chemistry*
Stanford & Kasianowicz. 2004. GENSIPS
Kasianowicz, Robertson, Reiner, Chan & Stanford. 2008. *Annual Review Analytical Chemistry*, ❙ (*in the press*)

Determining How Cells "Think"

hybrid nanopore array on a chip, pores with imperfect selectivity still ok



Array of single nanopores

- Calibrated w/biomarkers
- Each biomarker makes unique signal pattern w/each nanopore; pattern decoded by advanced statistical signal processing
- Challenge array with biological sample from blood, single cells, or cell networks.
- Directly measure presence of biomarkers with electronics.

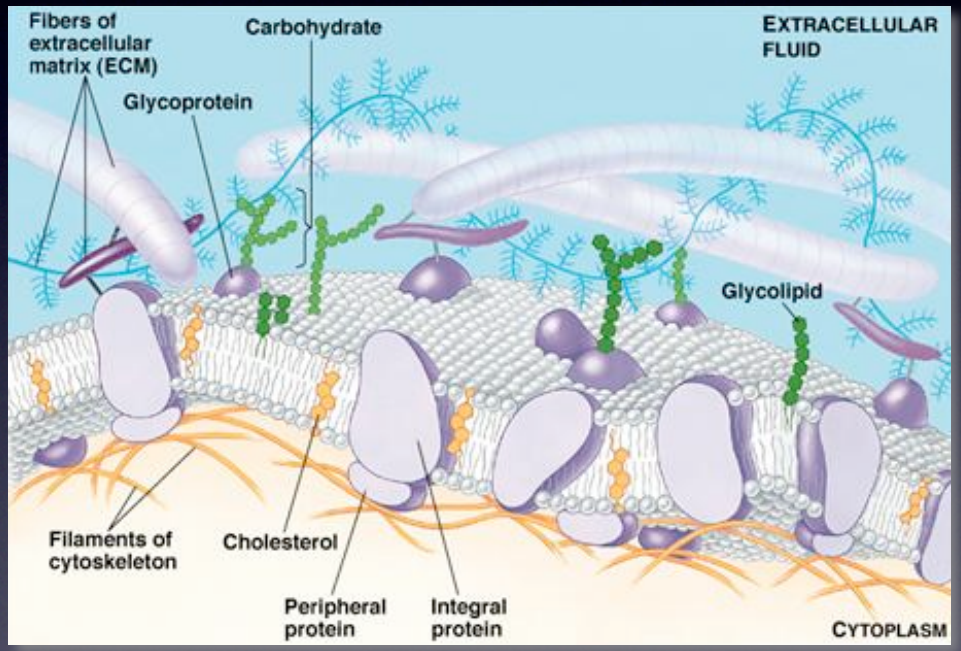
Kasianowicz, J.J., J.W.F. Robertson, J. E Reiner, V.M. Stanford, & M. Kim. *In progress.*

Membrane Proteins

Drug discovery by rational design is needed

Integral Membrane Proteins (IMPs)

- Control transport in and out of cells
- Interact with outside agents
- Directly involved in cell signal transduction
- Predominant targets for drug discovery
- Weaponized biotoxins

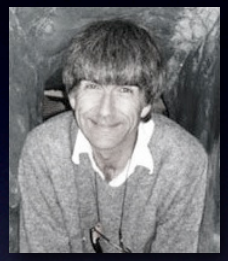


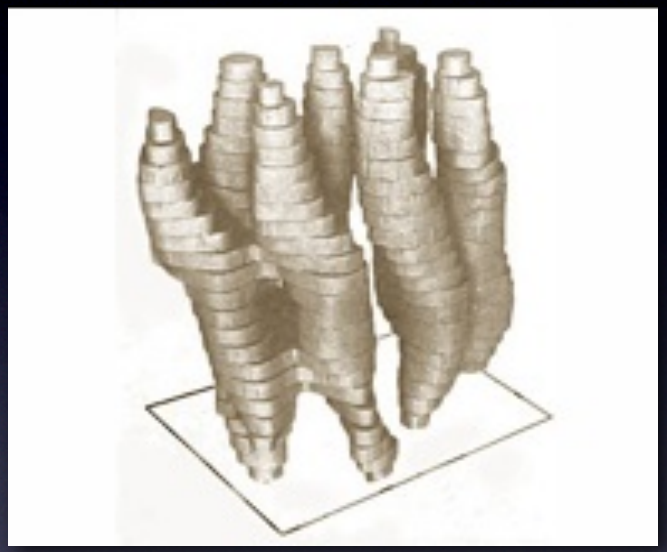
Need protein structure-function correlation

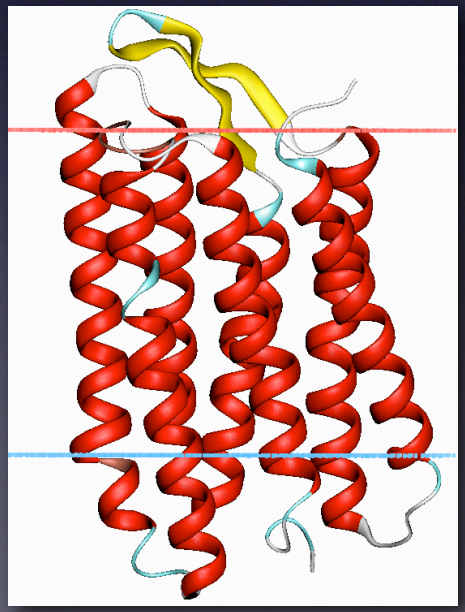
Simultaneous Determination of Structure & Function Will Define New Research & Applications

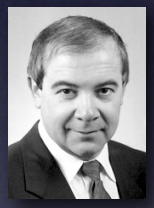
Pioneering efforts

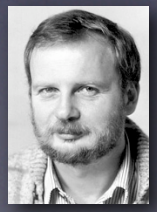
- Electron microscopy
- Bacteriorhodopsin
- Henderson & Unwin. Nature 1975







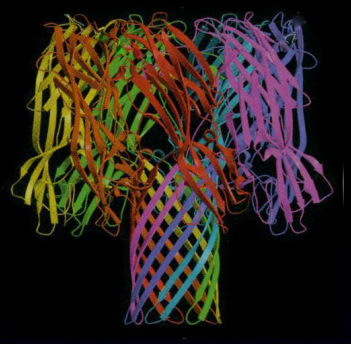




- X-ray crystallography
- 3-D structure of photosynthetic reaction center
- Deisenhoffer & Michel: Nobel Prize (1988)

How is it Solved Today?

to a great extent it is not



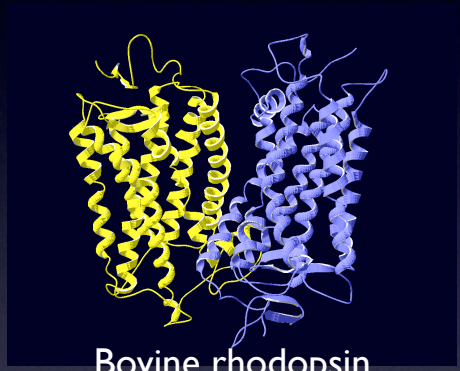
α-Hemolysin

Structure via x-ray crystallography can provide a starting point

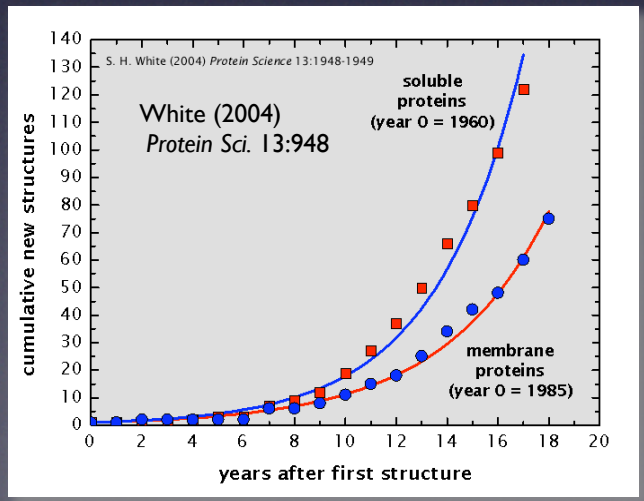
NMR data can show limited interactions of proteins with water & membranes limited to relatively small proteins (30-40 kDa.)

Function is ASSUMED from shapes ------ NOT measured

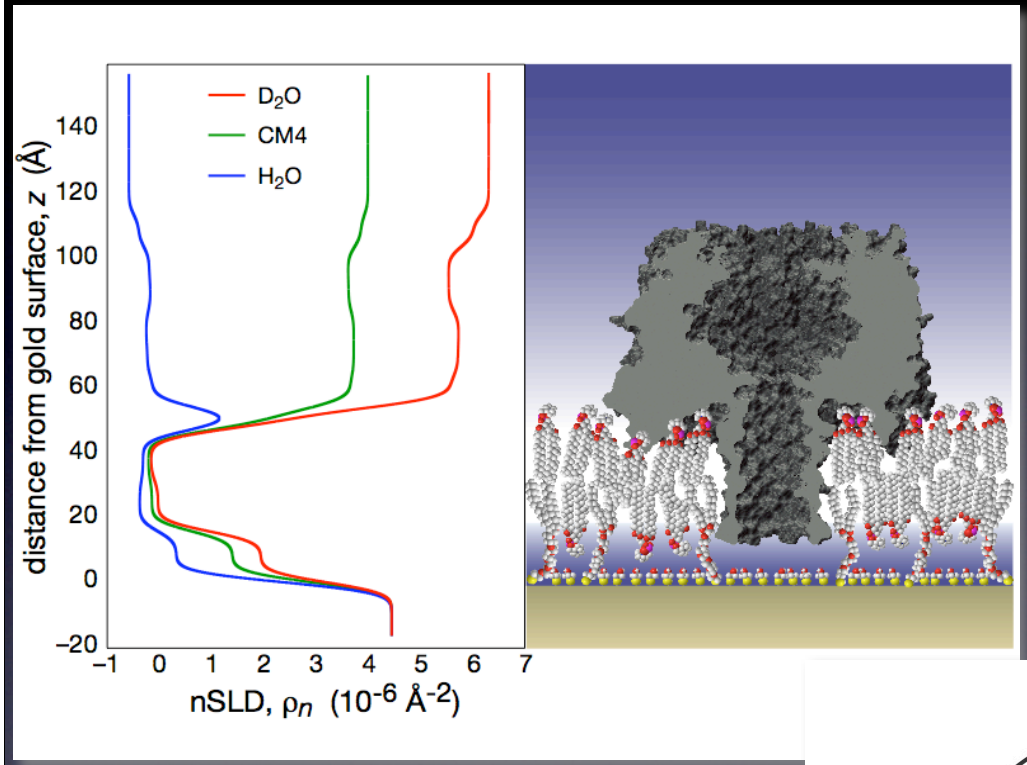
Structures of membrane proteins rare



Bovine rhodopsin



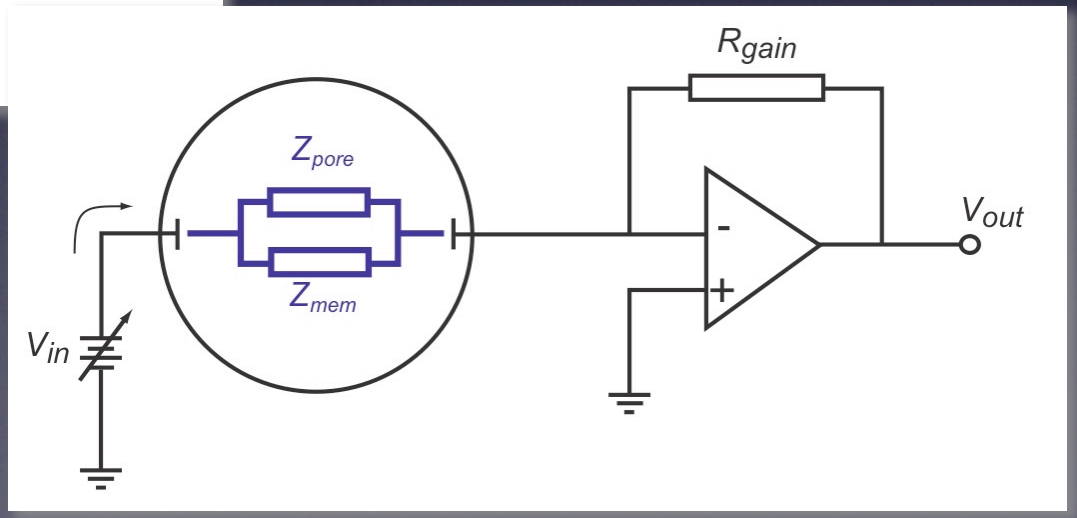
Simultaneous Structure & Function Measurements



Structure

Determined via

Neutron Reflectivity



Function

Determined via Impedance

MacGillvray, et al. *Submitted*

Summary

Electronic measurements *revolutionized* biological research *several* times over the past 70 years.

They can now be used to detect, quantify, and identify DNA, RNA, proteins, ions, and polymers (mass spec application)

Need better "marriage" of biomolecule transducers to solid-state materials, on-chip electronics & microfluics integration, miniaturization, . . .

When directed for the measurement of biological molecules, will such platforms be truly useful for real time Total Cell Analysis (i.e., for Systems Biology), Structural Biology, Cell Research, & future medical applications?

A Vision of Science & Technology at the Biology-Electronics Interface

John J. Kasianowicz

Semiconductor Electronics Division, NIST, EEEL

NIST
J.W.F. Robertson, J.E. Reiner
B.J. Nablo, & V.M. Stanford

UFPE (Brazil)
O.V. Krasilnikov
C.M. Rodrigues

DoD USAMRIID
K.M. Halverson, S. Bavari

NCI
T.L. Nguyen, R. Gussio,
R.G. Panchal

NIST Single Molecule Manipulation & Measurement (SM^3)
NIST Advanced Technology Program, NSF, & NIST Office of Law Enforcement Standards