



US005795782A

United States Patent [19]  
Church et al.

[11] Patent Number: 5,795,782  
[45] Date of Patent: Aug. 18, 1998

[54] CHARACTERIZATION OF INDIVIDUAL POLYMER MOLECULES BASED ON MONOMER-INTERFACE INTERACTIONS

[75] Inventors: George Church, Brookline, Mass.; David W. Deamer, Santa Cruz, Calif.; Daniel Branton, Lexington; Richard Baldarelli, Natick, both of Mass.; John Kasianowicz, Darnestown, Md.

[73] Assignees: President & Fellows of Harvard College, Cambridge, Mass.; The Regents of the University of California, Oakland, Calif.

[21] Appl. No.: 405,735

[22] Filed: Mar. 17, 1995

[51] Int. Cl.<sup>6</sup> ..... G01N 33/483

[52] U.S. Cl. .... 436/2; 436/151; 435/4

[58] Field of Search ..... 435/6, 4, 5; 436/2, 436/151

[56] References Cited  
PUBLICATIONS

Auld et al., "A Neutral Amino Acid Change in Segment IIS4 Dramatically Alters the Gating Properties of the Voltage-Dependent Sodium Channel", 1990, *Proc. Natl. Acad. Sci. USA*, 87:323-27.

Bensimon, A., et al., "Alignment and Sensitive Detection of DNA by a Moving Interface", 1994, *Science*, 265:2096-98.

Benz et al., "Pore Formation by LamB of *Escherichia Coli* in Lipid Bilayer Membranes", 1986, *J. Bacteriology*, 165(3):978-86.

Benz et al., "Mechanism of Sugar Transport through the Sugar-Specific LamB Channel of *Escherichia Coli* Outer Membrane", 1987, *J. Membrane Biol.*, 100:21-29.

Bezrukov et al., "Counting Polymers Moving Through a Single Ion Channel", 1994, *Nature*, 370:279-81.

Boulain et al., "Mutagenesis by Random Linker Insertion into the LamB Gene of *Escherichia Coli* K12", 1986, *Mol. Gen. Genet.*, 205:339-48.

Boulanger et al., "Characterization of Ion Channels Involved in the Penetration of Phage T4 DNA into *Escherichia Coli* Cells", 1988, *J. Biol. Chem.*, 263(20):9767-75.

Boulanger et al., "Ion Channels Are Likely to Be Involved in the Two Steps of Phage T5 DNA Penetration into *Escherichia Coli* Cells", 1992, *J. Biol. Chem.*, 267(5):3168-72.

Boyd et al., "Determinants of Membrane Protein Topology", 1987, *Proc. Natl. Acad. Sci. USA*, 84:8525-29.

Charbit et al., "Permissive Sites and Topology of an Outer Membrane Protein with a Reporter Epitope", 1991, *J. Bacteriology*, 173(1):262-75.

Dargent et al., "Selectivity for Maltose and Maltodextrins of Maltoporin, a Pore-Forming Protein of *E. Coli* Outer Membrane", 1987, *FEBS Letters*, 220(1):136-42.

Dargent et al., "Effect of Point Mutations on the in-Vitro Pore Properties of Maltoporin, a Protein of *Escherichia Coli* Outer Membrane", 1988, *J. Mol. Biol.*, 201:497-506.

DeBlois et al., "Electrokinetic Measurements with Submicron Particles and Pores by the Resistive Pulse Technique", 1977, *J. Colloid and Interface Science*, 61(2):323-35.

Ehrmann et al., "Genetic Analysis of Membrane Protein Topology by a Sandwich Gene Fusion Approach", 1990, *Proc. Natl. Acad. Sci. USA*, 87:7574-78.

Ferenci et al., "Channel Architecture in Maltoporin: Dominance Studies with LamB Mutations Influencing Maltodextrin Binding Provide Evidence for Independent Selectivity Filters in Each Subunit", 1989, *J. Bacteriology*, 171(2):855-61.

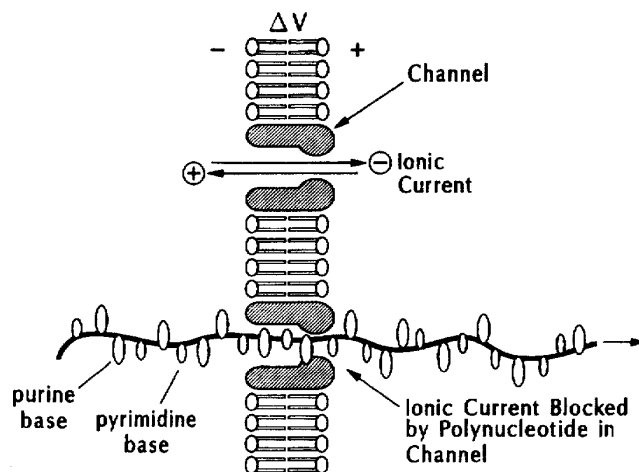
(List continued on next page.)

Primary Examiner—Charles L. Patterson, Jr.  
Attorney, Agent, or Firm—Fish & Richardson P.C.

[57] ABSTRACT

A method is disclosed for characterizing a linear polymer molecule by measuring physical changes across an interface between two pools of media as the linear polymer traverses the interface and monomers of the polymer interact with the interface, where the physical changes are suitable to identify characteristics of the polymer.

15 Claims, 6 Drawing Sheets



## PUBLICATIONS

- Ghadiri et al., "Artificial Transmembrane Ion Channels From Self-Assembling Peptide Nanotubes", 1994, *Nature*, 369:301-304.
- Hall et al., "Alamethicin: A Rich Model for Channel Behavior", 1984, *J. Biophys.*, 45:233-47.
- Hamill et al., "Improved Patch-Clamp Techniques for High-Resolution Current Recording from Cells and Cell-Free Membrane Patches", 1981, *Pflügers Archiv. Eur. J. Physiology*, 391(2):85-100.
- Harrington et al., "The F Pilus of *Escherichia Coli* Appears to Support Stable DNA Transfer in the Absence of Wall-to-Wall Contact Between Cells", 1990, *J. Bacteriology*, 172(12):7263-64.
- Heinemann et al., "Open Channel Noise IV: Estimation of Rapid Kinetics of Formamide Block in Gramicidin A Channels", 1988, *J. Biophys.*, 54:757-64.
- Heinemann et al., "Open Channel Noise V: Fluctuating Barriers to Ion Entry in Gramicidin A Channels", 1990, *J. Biophys.*, 57:499-514.
- Henry et al., "Blockade of a Mitochondrial Cationic Channel by an Addressing Peptide: An Electrophysiological Study", 1989, *J. Membrane Biol.*, 112:139-47.
- Hoshi et al., "Biophysical and Molecular Mechanisms of Shaker Potassium Channel Inactivation", 1990, *Science*, 250:533-38.
- Hoshi et al., "Two Types of Inactivation in Shaker K<sup>+</sup> Channels: Effects of Alterations in the Carboxy-Terminal Region", 1991, *Neuron*, 7:547-56.
- Kubitschek, "Electronic Counting and Sizing of Bacteria", *Nature*, 1958, 182:234-35.
- Lakey et al., "The Voltage-Dependent Activity of *Escherichia Coli* Porins in Different Planar Bilayer Reconstitutions", 1989, *Eur. J. Biochem.*, 186:303-308.
- Lopez et al., "Hydrophobic Substitution Mutations in the S4 Sequence Alter Voltage-Dependent Gating in Shaker K<sup>+</sup> Channels", 1991, *Neuron*, 7:327-36.
- Moellerfeld et al., "Improved Stability of Black Lipid Membranes by Coating with Polysaccharide Derivatives Bearing Hydrophobic Anchor Groups", 1986, *Biochimica et Biophysica Acta*, 857:265-70.
- Nath et al., "Transcription by T7 RNA Polymerase Using benzo[a]pyrene-modified templates", 1991, *Carcinogenesis*, 12(6):973-76.
- Neher et al., "Single-Channel Currents Recorded from Membrane of Denervated Frog Muscle Fibres", 1976, *Nature*, 260:799-801.
- Novick et al., "Fluorescence Measurement of the Kinetics of DNA Injection by Bacteriophage I into Liposomes", 1988, *Biochemistry*, 27:7919-24.
- Ollis et al., "Domain of *E. Coli* DNA Polymerase I Showing Sequence Homology to T7 DNA Polymerase", 1985, *Nature*, 313:818-19.
- Ollis et al., "Structure of Large Fragment of *Escherichia Coli* DNA Polymerase I Complexed with dTMP", 1985, *Nature*, 313:762-66.
- Ovchinnikov et al., 3. The Cyclic Peptides: Structure, Conformation, and Function: P. Gramicidin S. (851), Its Analogs and Tyrocidines A-C (904-906), 1982, *The Proteins, Third Edition*, 5:547-55.
- Ovchinnikov et al., 3. The Cyclic Peptides: Structure, Conformation, and Function: T. Valinomycin (913), 1982, *The Proteins, Third Edition*, 5:563-73.
- Patton et al., "Amino Acid Residues Required for Fast Na<sup>+</sup>-channel Inactivation: Charge Neutralizations and Deletions in the III-IV Linker", 1992, *Proc. Natl. Acad. Sci. USA*, 89:10905-909.
- Shiver et al., "On the Explanation of the Acidic pH Requirement for In Vitro Activity of Colicin E1", 1987, *J. Biological Chem.*, 262(29):14273-281.
- Sigworth et al., "Open Channel Noise: III. High Resolution Recordings Show Rapid Current Fluctuations in Gramicidin A and Four Chemical Analogues", 1987, *J. Biophys.*, 52:1055-64.
- Simon et al., "A Protein Conducting Channel in the Endoplasmic Reticulum", *Cell*, 65:371-80, (1991).
- Taylor et al., "'Reversed' Alamethicin Conductance in Lipid Bilayers", 1991, *J. Biophys.*, 59:873-79.
- Weiss et al., "Molecular Architecture and Electrostatic Properties of a Bacterial Porin", 1991, *Science*, 254:1627-30.
- West et al., "A Cluster of Hydrophobic Amino Acid Residues Required for Fast Na<sup>+</sup>-channel Inactivation", 1992, *Proc. Natl. Acad. Sci. USA*, 89:10910-14.
- Wonderlin et al., "Optimizing Planar Lipid Bilayer Single-Channel Recordings for High Resolution with Rapid Voltage Steps", 1990, *J. Biophys.*, 58:289-97.

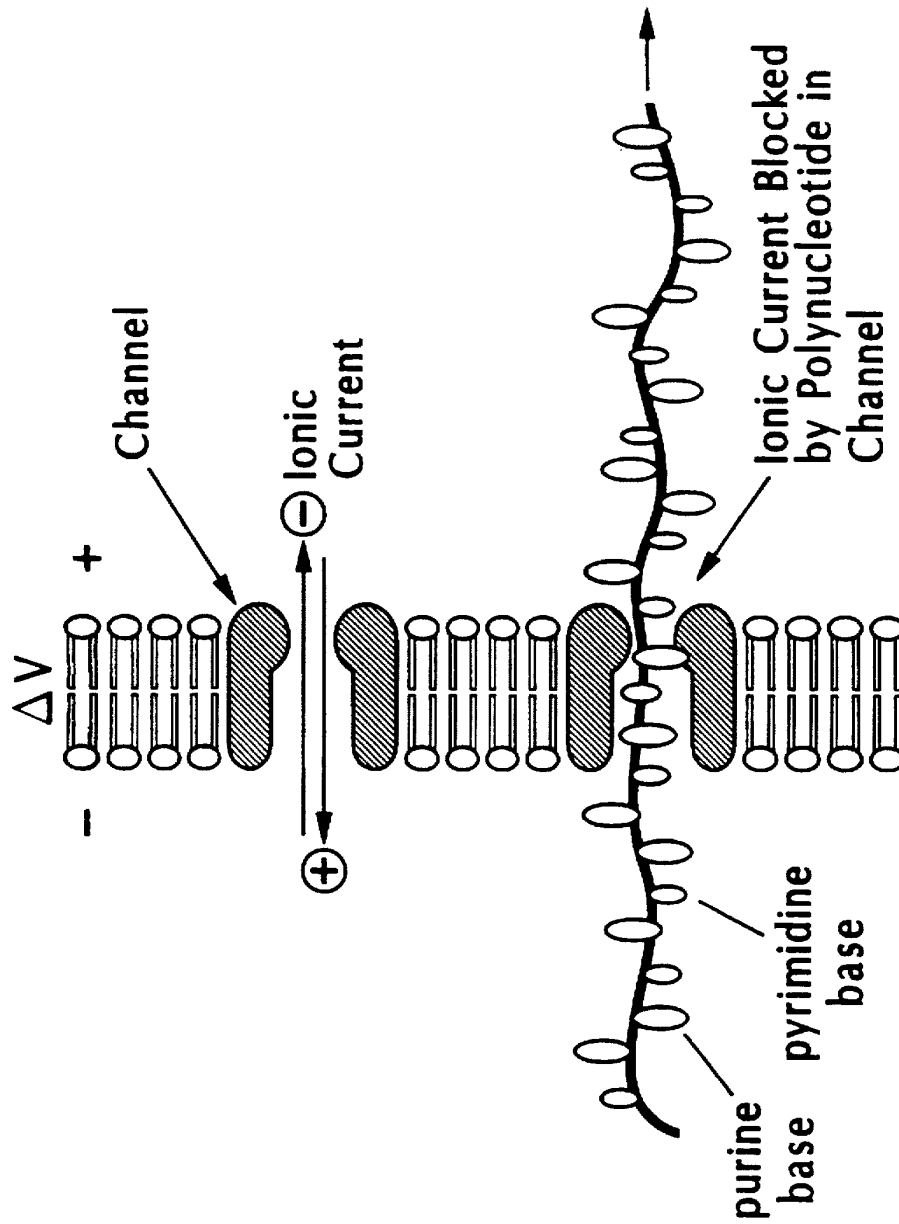


FIG. 1

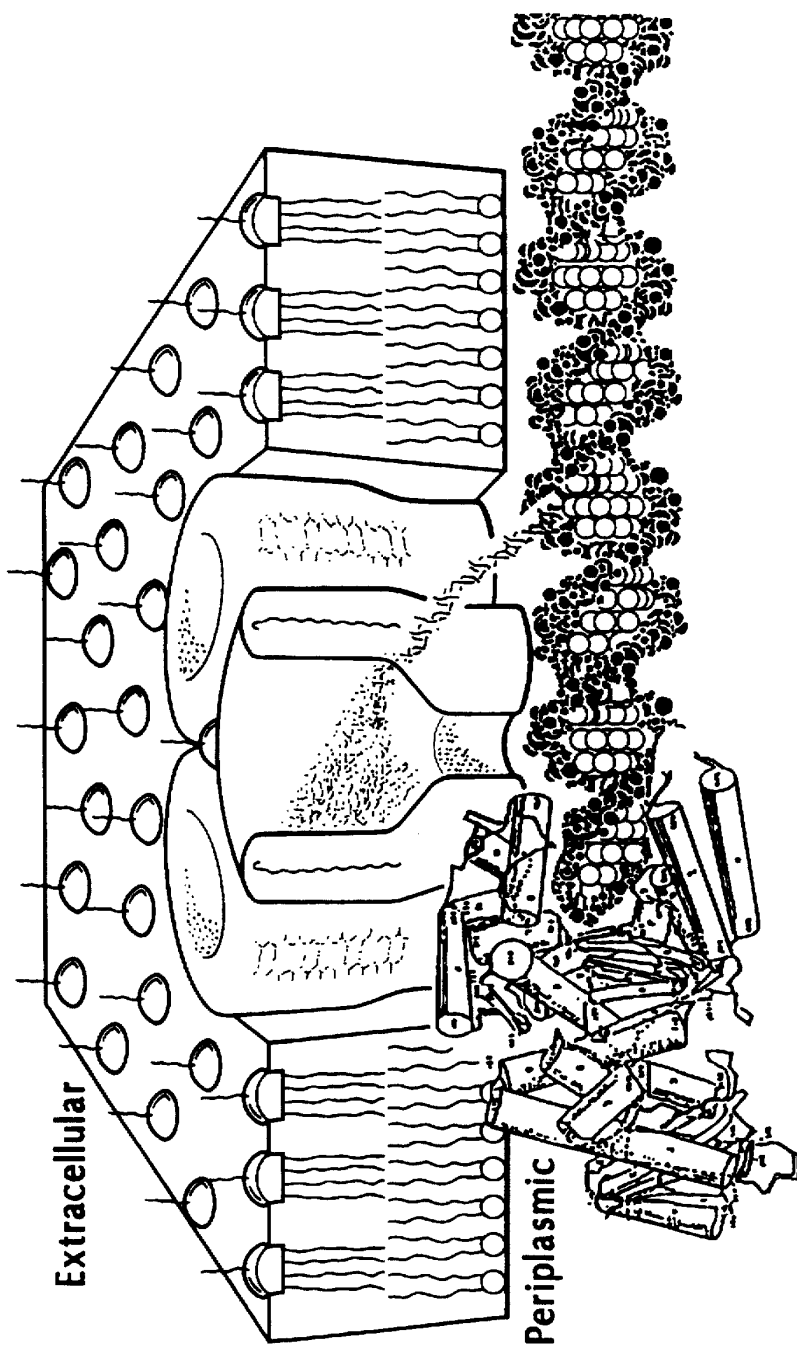


FIG. 2

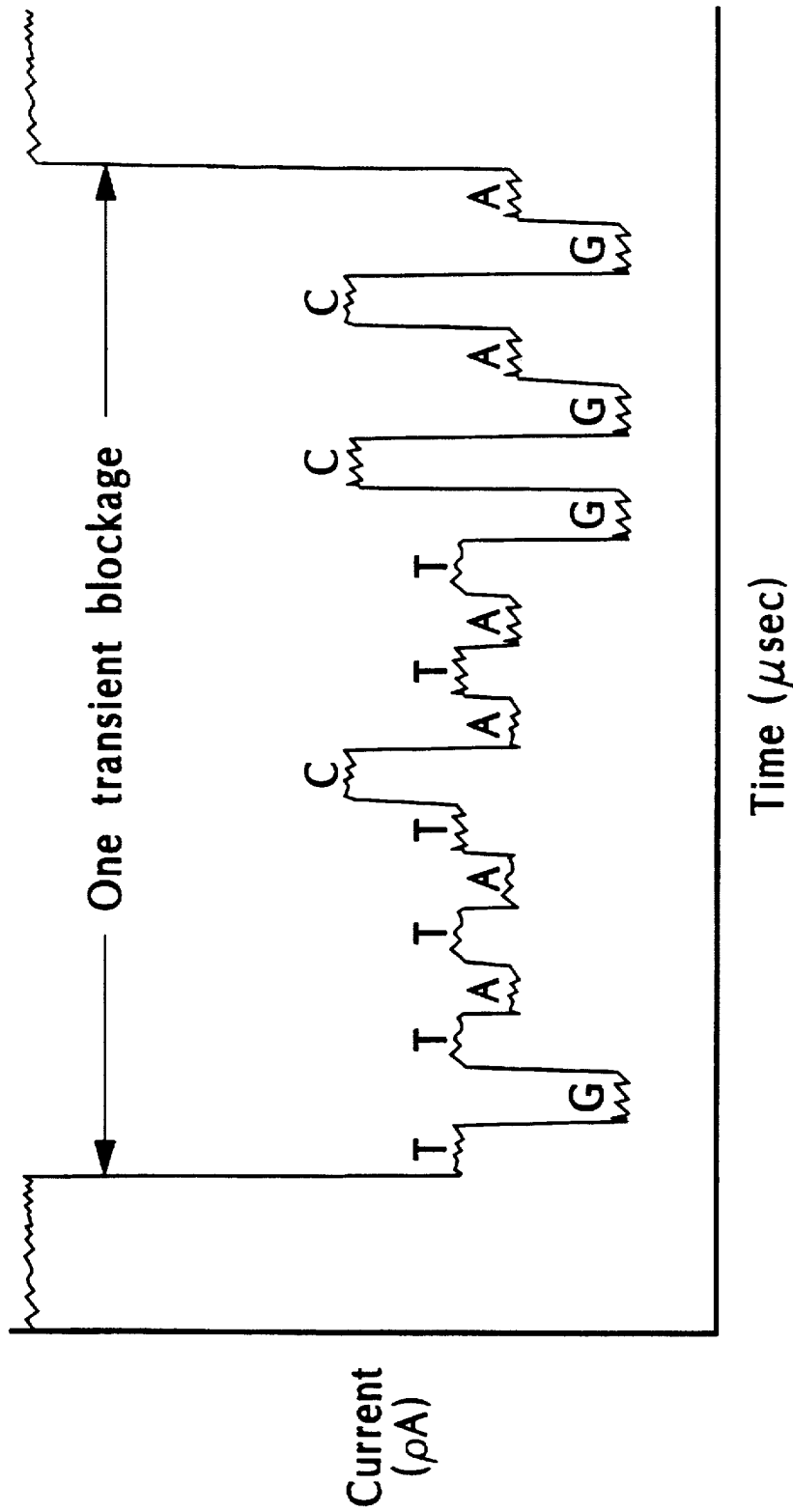


FIG. 3

# Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

## Real-Time Litigation Alerts



Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

## Advanced Docket Research



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

## Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

## API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

## LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

## FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

## E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.