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13. ABSTRACT (*Maximum 200 words*)

Several new and important results were enabled by this grant, which was to build a bioelectronic interface to single protein molecules. This interface consists of both environmental and mechanical supports for a membrane protein. While supported membranes are being studied in many laboratories, single channel probing has been performed almost exclusively with voltage-clamp techniques, by using fragile glass pipettes, or with cumbersome planar bilayer equipment unsuitable for field deployment. Single channel recording cannot yet be done on supported membranes. In this work, multifunctional scanned probe microscope tips were designed and fabricated to use in accessing the state of a membrane porin protein using microwave reflection and transmission, as well as fluorescent probing using photodiode probes.

Specific accomplishments were: development of a micromachined probe processand several probes to perform combined scanning force microscopy together with microwave probing, development of a companion process for combined optical and scanning force probing, imaging of calcium waves in brain slices, and preliminary results on single-protein probing with both direct-current and high-frequency probes.

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FINAL REPORT

GRANT #: N0014-00-1-G-0924

PRINCIPAL INVESTIGATOR: Daniel van der Weide

INSTITUTION: University of Wisconsin

GRANT TITLE: Nanoscale Field Localization for Manipulation and Probing of Computationally Interesting Biomolecules

AWARD PERIOD: 14 Aug 2000 - 30 Apr 2001

OBJECTIVE: In order to employ biomolecules in advanced computational structures, we need a deeper understanding of their structure and dynamics, as well as an electrical interface for communicating with them. To support this pursuit are applying newly developed micromachined silicon probes to probe protein function with minimal invasiveness.

APPROACH: We are designing, fabricating, and applying multifunctional scanning probe microscope (SPM) tips to probe single protein channels. We are using both photonic and electronic probes so we can examine local fluorescence as well as the electronic (i.e. conducting/non-conducting) state of ion channel proteins.

ACCOMPLISHMENTS: Several new and important results were enabled by this grant, which was to build a bioelectronic interface to single protein molecules. This interface consists of both environmental and mechanical supports for a membrane protein. While supported membranes are being studied and developed in many laboratories, single channel probing has been performed almost exclusively with fragile glass pipettes, or with cumbersome planar bilayer equipment, both of which are unsuitable for field deployment. Single channel recording cannot yet be accomplished with membranes on planar supports, although there are several startup companies attempting to achieve this goal. In this work, multifunctional scanned probe microscope tips were designed and fabricated to use in accessing the state of a membrane porin protein using microwave reflection and transmission, as well as fluorescent probing using photodiode probes.

Specific accomplishments were: development of a micromachined probe process and several probes to perform combined scanning force microscopy and microwave probing, development of a companion process for combined optical and scanning force probing, imaging of calcium waves in brain slices, and preliminary results on single-protein probing with both direct-current and high-frequency probes.

With new photodiode SPM tips, we can observe local optical phenomena such as fluorescence while simultaneously mapping the topography of a protein in a membrane. Although various groups have achieved each of these goals separately, no group has demonstrated the combined measurements needed to understand single channel responses more completely. Using new near-field microwave SPM tips, we are preparing to probe changes in both the conductance and capacitance of the channel, a significant advance in biochemical metrology. When the binding site is occupied, the channel changes its conformation and conductivity to a measurable extent, regardless of the charge of the analyte.

During the grant period, we built an inverted laser scanning confocal microscope with atomic force microscope (AFM) compatibility, then acquired a Park Scientific Bioprobe AFM to work with the confocal. We designed and constructed new planar horizontal bilayer fixtures to accommodate single proteins in black lipid membrane (BLM) for simultaneous optical and mechanical/electrical probing. Finally, and most importantly, we achieved preliminary results in probing single alpha-hemolysin porin proteins using both DC and radio-frequency techniques, in both reflection and in transmission. This was the primary goal of the proposal.

During this period we also collaborated with Prof. P.G. Haydon (University of Pennsylvania) to make single-channel calcium channel recordings in rat hippocampal neurons. Together we have also built a fluorescence microscope with a remote-controlled patch clamp manipulator for correlating conventional single-channel recordings with fluorescent probes and the microwave probes. This instrument can be controlled via an Internet interface, to enable biologists in other laboratory to eventually access our unique instrumentation.

CONCLUSIONS: A new type of electrical interface to single membrane proteins (porins) was developed with the support of this grant. Both microwave and optical scanning probe microscope tips were developed through a complex but high-yielding micromachining process, and near-field probing of single proteins was accomplished with probe tips of similar design. More opportunity to probe cells using force microscopy now exists, and should be the next step taken in this effort.

SIGNIFICANCE: Previous work on probing the states of individual membrane proteins used fragile probes and noise-sensitive techniques; ours paves the way for field-deployable sensing of protein activity at the single-channel level.

PATENT INFORMATION: A patent application on "Method and Apparatus for High Frequency Interfacing to Biochemical Membranes" was filed.

AWARD INFORMATION: ONR Young Investigator (1998), Alexander von Humboldt research fellowship (2000, postponed), promoted to Associate Professor (1998)

PUBLICATIONS AND ABSTRACTS (for total period of grant):

[1-11]

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