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<b>13. ABSTRACT (Maximum 200 words)</b> To develop a label-free, field-deployable electronic interface to membrane proteins used as biosensing elements. We developed and demonstrated a new method for single-molecule electrophysiology, measuring changes in microwave-frequency transmission through a pore in a planar lipid bilayer simultaneously with conventional single-channel DC recordings. The results indicated that an increase in capacitance of order 0.5-1 fF was observed with blocking of the pore. The system consists of two sharpened coaxial probes aligned across a glass support with a lipid bilayer and a single pore-forming protein. Transmission through the pore was measured with a microwave spectrum analyzer functioning as a tuned receiver, whose output was recorded simultaneously with that of a patch-clamp amplifier. The protein staphylococcal $\alpha$ -hemolysin ( $\alpha$ HL) was used as the pore. This molecule is a heptameric pore which allows the passage of ions, small globular molecules (< 2000 D) or long polymer chains such as DNA. The $\alpha$ HL pore can be blocked by the molecular adapter $\beta$ -cyclodextrin, which lodges in the pore's lumen. These blocking events were observed simultaneously by decreases in transmembrane current and by increases in the microwave transmission at 900 MHz, due to increases in capacitance. The superior bandwidth of the spectrum analyzer (> 1 MHz) over that of the patch clamp amplifier (~ 10 kHz) ultimately promises higher temporal resolution, and the use of high frequencies moves the measurement away from the 1/f noise characteristics that dominate DC measurements. Finally, the use of near-field microwave transmission using low-impedance probes obviates the need for a giga-ohm seal in patch clamping.				
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## Single-Receptor interfaces for real-time kinetics

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### LONG-TERM GOALS and SUMMARY

To develop a label-free, field-deployable electronic interface to membrane proteins used as biosensing elements.

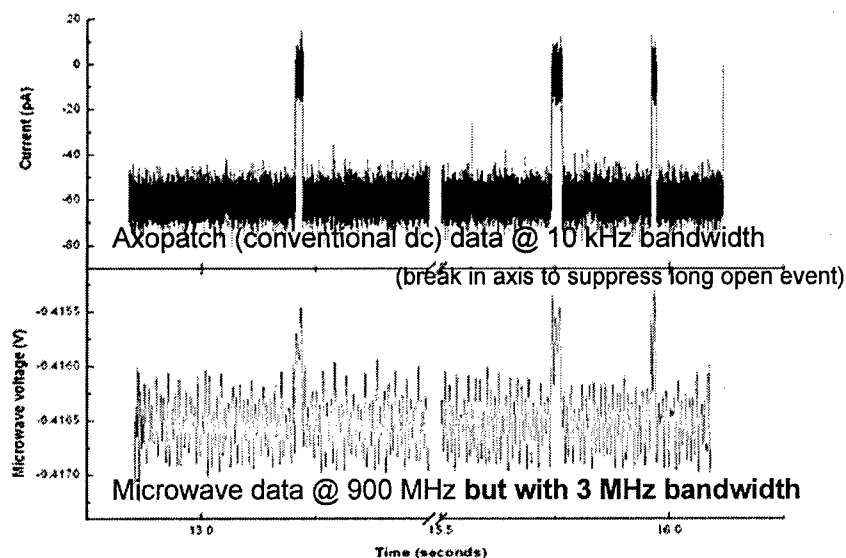
We developed and demonstrated a new method for single-molecule electrophysiology, measuring changes in microwave-frequency transmission through a pore in a planar lipid bilayer simultaneously with conventional single-channel DC recordings. The results indicated that an increase in capacitance of order 0.5-1 fF was observed with blocking of the pore. The system consists of two sharpened coaxial probes aligned across a glass support with a lipid bilayer and a single pore-forming protein. Transmission through the pore was measured with a microwave spectrum analyzer functioning as a tuned receiver, whose output was recorded simultaneously with that of a patch-clamp amplifier.

The protein staphylococcal  $\alpha$ -hemolysin ( $\alpha$ HL) was used as the pore. This molecule is a heptameric pore which allows the passage of ions, small globular molecules (< 2000 D) or long polymer chains such as DNA. The  $\alpha$ HL pore can be blocked by the molecular adapter  $\beta$ -cyclodextrin, which lodges in the pore's lumen. These blocking events were observed simultaneously by decreases in transmembrane current and by increases in the microwave transmission at 900 MHz, due to increases in capacitance. The superior bandwidth of the spectrum analyzer (> 1 MHz) over that of the patch clamp amplifier (~ 10 kHz) ultimately promises higher temporal resolution, and the use of high frequencies moves the measurement away from the 1/f noise characteristics that dominate DC measurements. Finally, the use of near-field microwave transmission using low-impedance probes obviates the need for a giga-ohm seal in patch clamping.

### OBJECTIVES

To use single membrane proteins as ultrasensitive chemical and biological weapon detectors, a label-free and field-deployable interface is required. Our objectives are to use coaxial microwave probe interfaces to these proteins. With these tools we will read out the state of the protein without the 1/f noise sources associated with conventional electrical recording techniques.

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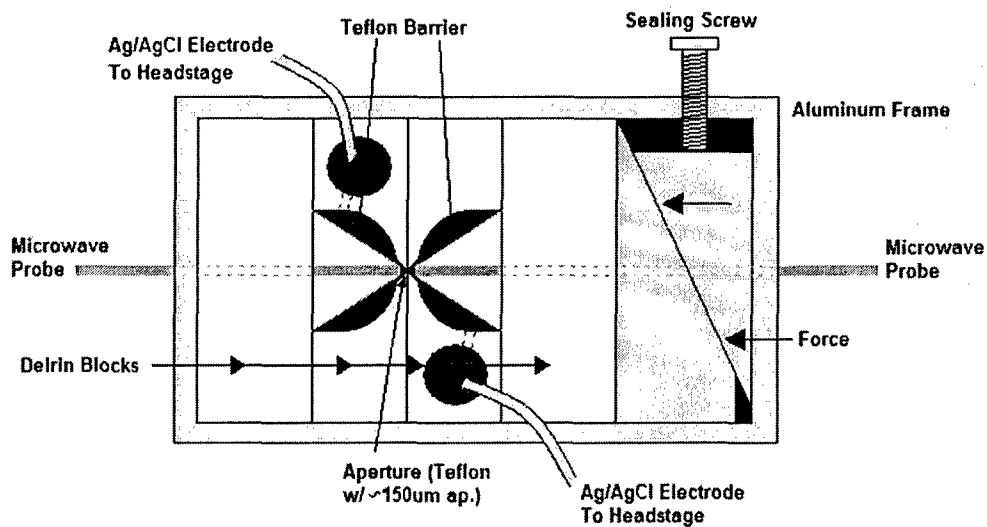


*1. Simultaneous recording of  $\alpha$ -hemolysin activity using conventional DC technique (above) and 900 MHz microwave transmission (below). Break in horizontal axis signifies a long unblocked (open) event.*

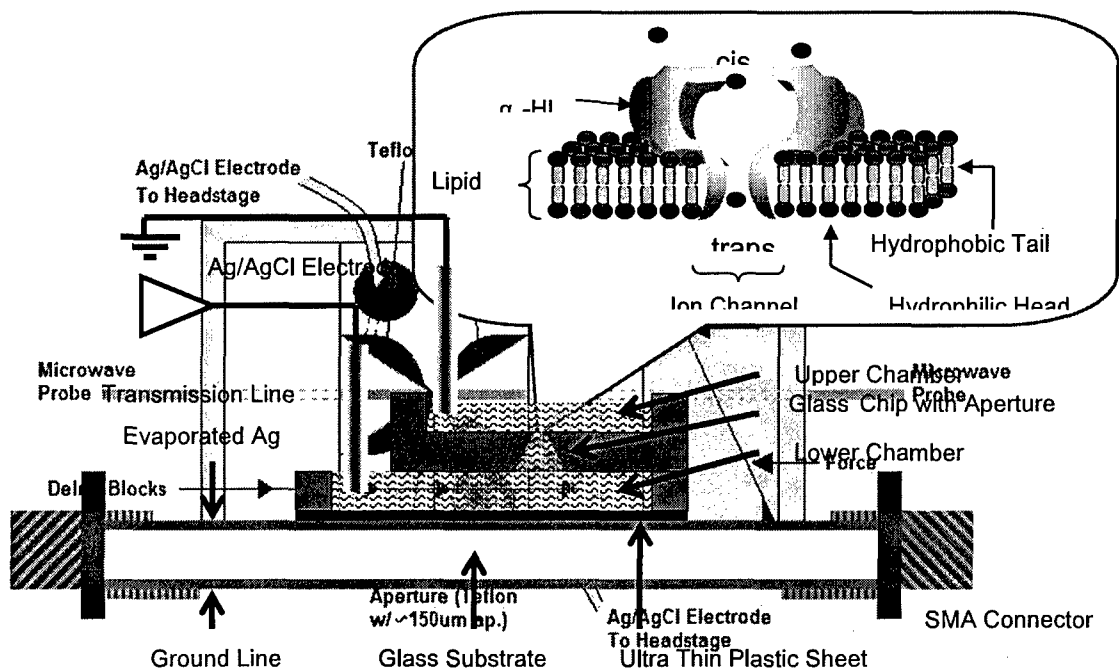
## APPROACH

We used coaxial transmission lines whose tips are  $\sim 10 \mu\text{m}$  in diameter to probe the local changes in capacitance and hence microwave transmission associated with membrane protein activity (Figure 1), specifically that of an exotoxin,  $\alpha$ -hemolysin (from Hagan Bayley). Incorporating these probes into an advanced bilayer recording setup (Figure 2 a) enabled us to simultaneously measure single-protein activity using accepted ionic current recording (patch clamp amplifiers) and our new high-frequency approach. The setup consisted of a micromachined glass chip that supported a lipid bilayer, along with passivated coaxial probes on either side of the bilayer, aligned along a central axis containing the pore.

In addition we fabricated a setup combining conventional microstrip measurements with on-chip patch clamp measurements (Figure 2 b). This setup enabled us to determine insertion of pores of ion channels while we simultaneously determined the global microwave transmission change. This is of great importance for the overall goal of fast broadband readout. Again we used  $\alpha$ -HL and recorded blocking events. Similar studies were undertaken with alamethicin (ALA), which is a simple rod-like peptide forming pores when more than three are combined. The particular advantage of ALAs is the ability to control their membrane insertion via an applied DC voltage. Hence, the setup enabled us to combine microscopic events with global microwave response.



2 a. Four separate machined Delrin blocks with an aluminum frame. Thin (25 µm) Teflon barriers isolate the electrolyte and provide an aperture for the lipid membrane.



2 b. On-chip patch clamping unit mounted on a microstrip transmission line. Patch clamp amplifier is connected to the upper chamber where the bilayer is painted on the glass chip aperture.

## WORK COMPLETED

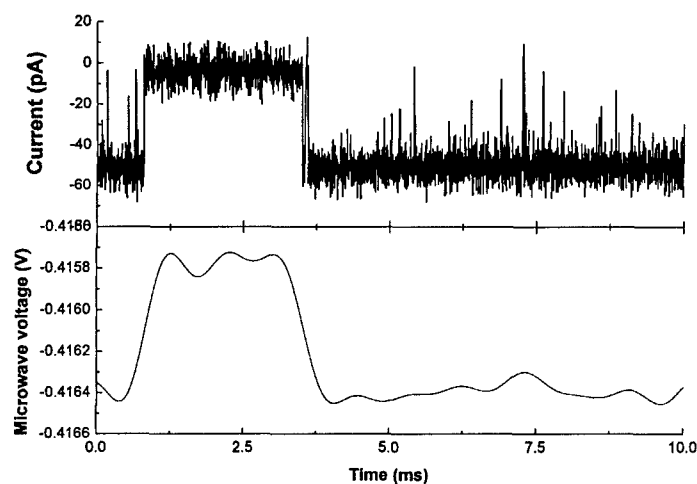
We observed correlations between conventional DC recordings of  $\alpha$ -hemolysin activity with simultaneous recordings of localized microwave transmission, using both wild-type and mutant M113N protein. We found a predictable decrease in microwave transmission "spiking" frequency when hepta-S<sub>7</sub>  $\beta$ CD was introduced to the *trans* side of the pore, and we further observed a linear relationship between transmembrane potential and spiking frequency that helped us develop an intuitive model of the protein's microwave response.

The microwave transmission is observed in spikes whose spacing in time shows a direct relation to the amount of current across the pore. Furthermore, as the number of  $\alpha$ -hemolysin pores formed in the lipid bilayer increases, there is a corresponding decrease in the spacing. Current block caused by  $\beta$ -cyclodextrin results in the disappearance of the spikes during the dwell time of the molecule in the pore.

We found that with the concentration of  $\beta$ CD at 40  $\mu$ M, the  $k_{on}$  was  $2.58 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$  and the  $k_{off}$  was  $0.0806 \text{ s}^{-1}$ , for a dissociation constant  $K_d$  of 3.13  $\mu$ M. These numbers differ from the published values of Gu *et al.*, which are  $k_{on} = 2.7 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ ,  $k_{off} = 0.038 \text{ s}^{-1}$ , and  $K_d = 0.13 \mu\text{M}$ , possibly due to differences in pH or temperature.

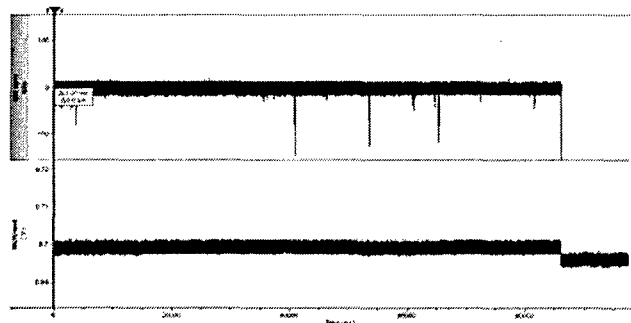
Furthermore, the combination of conventional microstrip line recordings with the on-chip patch clamp technique allowed us to link the microscopic response to the overall impedance of the circuit. We were able to observe insertion of single pores into bilipid membranes and simultaneously record the microwave response over a frequency range of 100 MHz to 14 GHz. We were able to show the bias as well as concentration dependence of the microwave response and a clear variation when channels were inserted. The glass chips for patch clamp measurements we also applied for studying stochastic resonance with ALA pores, as shown below. This topic is of relevance since we found that inherent noise in membrane patch measurements appears to stem from membrane fluctuations and not from the pores themselves.

## RESULTS

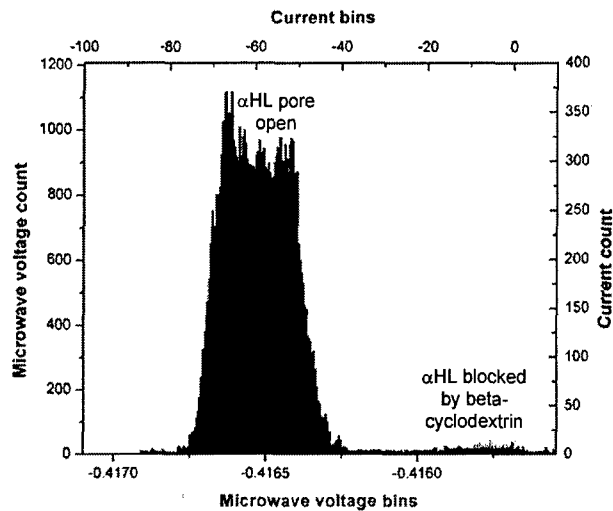


**3. Transient blockage of two  $\alpha$ -hemolysin pores due to  $\beta$ -cyclodextrin in conventional recording (above; increase in current is downward, scale is 10 pA/div) and filtered 900 MHz transmission (below; increase in power transmitted is upward and represented by detector voltage.)**

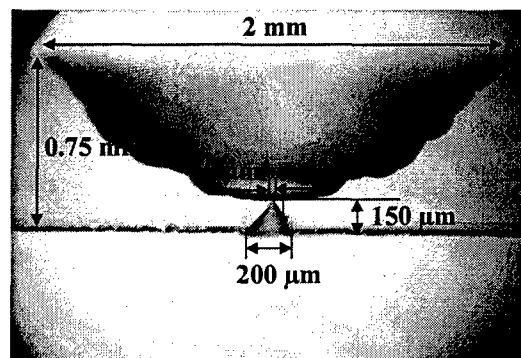
We obtained simultaneous single-porin recordings using both conventional patch clamp amplifiers and our high-frequency approach (in transmission mode) on native  $\alpha$ -hemolysin with  $\beta$ -cyclodextrin flowing through the pore (Fig. 3). The high-frequency recordings are consistent with a series-capacitor equivalent circuit model of the pore/membrane/buffer interface, with the change in capacitance due to the binding of  $\beta$ -cyclodextrin,  $\Delta C \sim 0.5$  fF, which is readily measured with high-frequency instrumentation. In Figure 4, we display the correlation between DC and microwave recordings for membrane rupture, which indicate a decrease in microwave transmission with the rupture event, consistent with the proposed mechanism for increase in microwave transmission with blockage, i.e. an increase in capacitance through exclusion of the conductive buffer upon blockage or with the membrane intact.



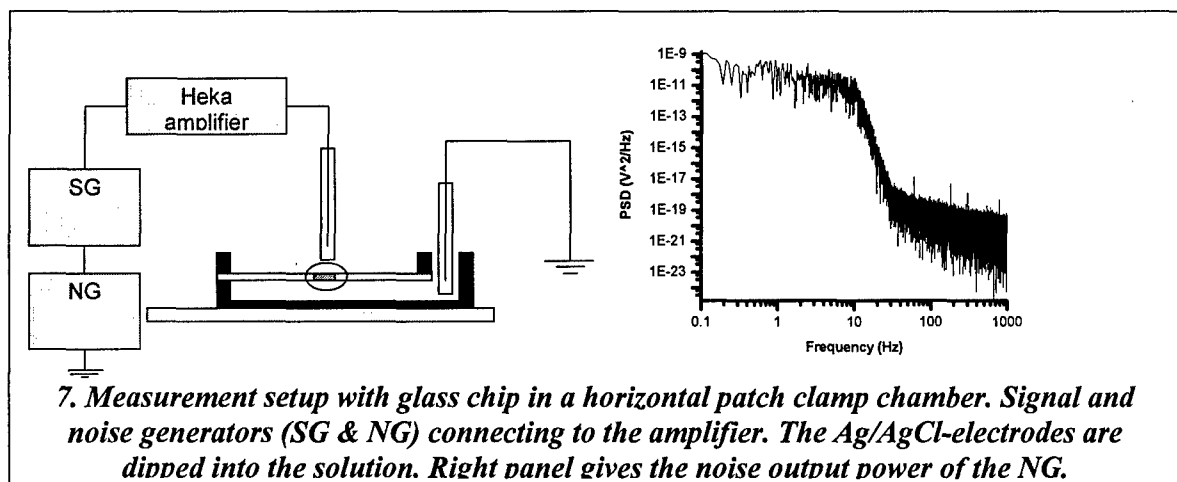
**4. Correlation of membrane rupture (DC recording, top; microwave recording, bottom). Presence of membrane increases microwave transmission via an increase in capacitance from one coaxial cable to the opposite one; this is consistent with the increase in transmission observed with pore blockage.**

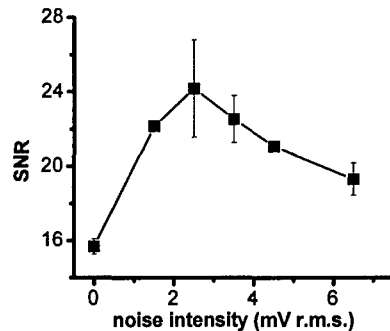


5. Statistics from full recordings (Figure 1); microwave signal (shown in red) appears to contain more detailed open-state information than contemporaneous DC data (black). The wider bandwidth of the microwave recording is responsible for this.



6. Cut-away view of the glass chip showing detail of the 20  $\mu\text{m}$  aperture which supports the membrane and pore.





**8. Stochastic resonance signal-to-noise ratio (SNR) vs. noise intensity for a membrane patch of 200  $\mu\text{m}$  diameter.**

### IMPACT/APPLICATION

These results represent the first high-frequency single-channel recordings ever made, and they provide proof-of-principle justification for integrating high-frequency protein readout circuitry and probes onto chips for rugged, field-deployable interfaces to single proteins used as chemical sensors.

These results give justification for developing arrays of noise-immune, high-speed protein probes as well as for developing a "scanning electrode microscope"[1-3] that can image the distribution and dynamics of single ion-channel proteins in cell membrane. Ultimately such devices can be employed in high-throughput screening of pharmaceutical candidate compounds and in clinical diagnostics.

### TRANSITIONS

The van der Weide laboratory is currently (as of 5/1/05) funded through an NIH Phase II STTR (with Prairie Technologies, LLC) to develop the scanning membrane probe aspects of this technology.

### RELATED PROJECTS

Studies on bilayers using a vertical chamber and specially configured microscope have shown the value of combining electrophysiological and optical techniques on the same sample. Hanyu, et. al. [4] have studied spectroscopic and physiological signals in a vertical planar bilayer system. In this work a special microscope was constructed to look at voltage dependant movement of a membrane incorporated peptide. Korchev, et. al. [1] have demonstrated localization of single ion channels on the surface of living cells, with a scanning ion conductance microscope (SICM), using a micropipette as both the scanning and conducting probe for a SPM. Amemiya and Bard [3] have used micropipette electrodes for probing ion transfer at bilayer lipid membranes. There is also a growing volume of literature using scanning force microscopy (SFM) to image lipid bilayers[5]. Building on these techniques and adding microwave-based probes to the correlative techniques used to presently study lipid bilayer structure, function and interaction will reveal distribution as well as dynamics of membrane proteins.

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