REPORT DOCUMENTATION PAGE

AFRL-SR-AR-TR-08-0039

1. REPORT DATE *(07-25-2007)*	2. REPORT TYPE **final**	3. DATES COVERED *(03-01-2005 to 03-01-2008)*

4. TITLE AND SUBTITLE

Addressable Immobilized Ion Channels: Optimization of Ion Channels Tethered to Device Surfaces

5a. CONTRACT NUMBER

5b. GRANT NUMBER
F49620-03-1-0393

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release: Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

This work is performed by a team at Florida, Mainz, Texas, Agave Biosystems and Miami. We are attempting to develop a sensitive and selective biomolecular detection system based on arrays ion channel covalently tethered over microelectronic devices.

The technical approach involves inserting ion channels in non-native membrane bilayers, characterizing and optimizing the single channel stochastic response, and tethering these assemblies over device gate surfaces. Enhancing the stability of the assemblies is another important part of the program. The sensing will be based on this device architectures whereby the placement of individual analyte molecules at the throat of the ion channel will in turn change the stochastic signature associated with the ion translocation measured by the device. Optimizing and tethering the channels, enhancing stability, and demonstrating chem/bio agent signatures as well as interferants

There have been a number several recent accomplishments in this program. We have obtained gigaohm seals and formed these seals on gold micro electrodes with >80% pixel-to-pixel seal formation. We have measured single channel activity from more than ten different ion channel systems including three genetic variants of Maxi-K, Granacidin A, and a mechanosensitive channel. We obtained on-device pharmacological response from micromolar organophosphate and could wash it off the device. We assembled S-layer proteins at the lipid bilayer interface, enhancing stability. We studied bilayer formation by QCN-d.

15. SUBJECT TERMS
biosensor, ion channel, chemical agent detection, stochastic

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE		5	19b. TELEPHONE NUMBER *(include area code)*

Final Performance Report, July, 2006

Agreement number: F49620-03-1-0393

Program Title: *Addressable Immobilized Ion Channels: Optimization of Ion Channels Tethered to Device Surfaces*

Principle Investigator: *R. Duran, Univ. of Florida*

Co-Principle Investigators:

Status of effort: We have successfully incorporated a number of different ion channels in devices and measured single channel activity. Pharmacological and current levels studies match well literature values from conventional electrophysiology methods

Project Summary:

Detection of CB agents in water is of enormous importance for warfighters as well as domestic water supplies. Unfortunately, current methods for assessing water quality are, for the most part, neither rapid, nor reliable, are not man portable, and not easily deployed. This project will enhance the feasibility of using ion channels as sensitive, real-time detectors of aqueous CB agents, with a view to ultimately use ion channels in an on-chip, tethered configuration, as reliable and highly specific detectors of CB agents.

Ion channels are transmembrane proteins that regulate the movement of ions through the membrane of various cells, most notably nerve and muscle, where they control the electrical excitability of these cells. Ion channels open and close on a millisecond time scale, and do so stochastically, with the signature of a particular channel being defined by a series of factors such as mean open and closed times, probability of opening, etc. Moreover, the pattern of channel opening (i.e. the stochastic signature) is altered in the presence of pharmacological agents and other species that bind to the channel. Thus, the activity of ion channels that bear binding sites for CB agents will likely change in a predictable manner in the presence of those agents, providing a potentially powerful and sensitive means of detection.

Ion channels normally function in a biological environment that differs significantly from that at microelectronic device gate surfaces in a variety of respects. This project seeks to functionally incorporate the Maxi_K ion channel in tethered bilayers and to characterize the functionality and stability of the channel.

The technical approach involves inserting ion channels in non-native membrane bilayers, characterizing and optimizing the single channel stochastic response, and tethering these assemblies over device gate surfaces. Enhancing the stability of the assemblies is another important part of the program. The sensing will be based on this device architectures whereby the placement of individual analyte molecules at the throat of the ion channel will in turn change the stochastic signature associated with the ion translocation measured by the device. Optimizing and tethering the channels, enhancing stability, and demonstrating chem/bio agent signatures as well as interferants will set the stage for the development in Phase II of complete prototype systems, including electronics and fluidics.

There have been a number several significant accomplishments in this program. We have obtained gigaohm seals on both gold and silicon oxide layers and maintained the gigaseal for a month on gold. We also formed these seals on gold micro electrodes with >80% pixel-to-pixel seal formation. We have measured single channel activity from nine different ion channel systems including two genetic variants of Maxi-K. We obtained on-device pharmacological response from micromolar TEA and organophosphate and demonstrated that the organophosphate could be easily washed off the device, maintaining the seal in such a way that it could be used again. We incorporated a variety of very different channels: Gramacidin a, the F1Fo enzyme, Maxi-K channel, the mechanosensitive ion channel - MSCL, and the M2 peptide from the acetylcholine receptor. The ion currents for these channels on the device ranged from fractions of picoamps to 100 picoamps. We verified that the pharmacology observed for TEA and Maxi-K matches that seen in the literature by conventional electrophysiology methods. The currents observed for Gramacidin on the device match those from tip-dip measurements. We assembled S-layer proteins at the lipid bilayer interface, enhancing stability and allowing a 90% channel incorporation rate for one M2 channels. The S-layer stabilization also allowed us to achieve sustained single channel response for 20 hours. We have produced a number of genetically engineered mutants of Maxi-K and we detected a response to 10 nanomolar Ricin B subunits via whole cell currents. Furthermore, the Ricin response could be easily be differentiated from a closely related lectin. We demonstrated externally applied electrical potentials gate both channel systems studies. We have collaborated closely with the group at Texas to optimize a bipolar junction transistor and demonstrated that its current sensitivity was easily sufficient to measure single channel events. We also measured reproducible gigaseals and initial channel fluctuations on this amplified device configuration. We are also collaborating with two groups on signal analysis and denoising. We organized a symposium at the national ACS meeting in Mar 2005. Some recent results are shown below.

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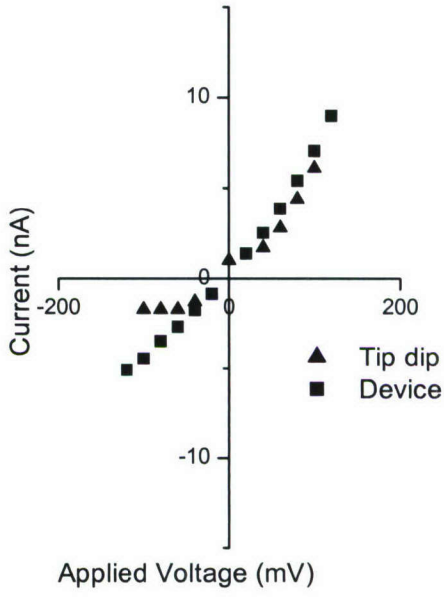
Funding Profile:

FY01	FY02	FY03	FY04	FY05
FY06	FY07	FY08		
0K	0K	0K	0K	250K
0K	0K	0K		

Personnel Supported: Graduate students:

References, Transitions, and Presentations at major Meetings:



1) Martin Andersson, Ashwin Madgavkar, Yanrong Wu, Weihong Tan and Randolph S. Duran Using optical tweezers for measuring the interaction forces between human bone cells and implant surfaces: System design and force calibration," in press *Rev Sci Inst* **78** (7) 2007

2) Functional Ion Channels in Tethered Bilayer Membrane Arrays; Implications for Biosensors
Henk M. Keizer, Brian R. Dorvel, Daniel Fine, Rebecca B. Price, Joanna R.Long, Ananth Dodabalapur, Ingo Köper,Wolfgang Knoll, Peter A. V. Anderson, and Randolph S. Duran* *ChemBioChem* **8** 1246 (2007)

3) Dorvel BR, Keizer HM, Fine D , Vuorinen J , Dodabalapur A, Duran RS Formation of tethered bilayer lipid membranes on gold surfaces: QCM-Z and AFM study *LANGMUIR* **23** 7344-7355 (2007)

4) Andersson M (Andersson, Martin), Keizer HM (Keizer, Henk M.), Zhu CY (Zhu, Chenyu), Fine D (Fine, Daniel), Dodabalapur A (Dodabalapur, Ananth), Duran RS Detection of single ion channel activity on a chip using tethered bilayer membranes *LANGMUIR* **23** 2924-2927 (2007)

5) Atanasov V., Knorr N., Duran R.S., Ingebrandt S., Knoll W., Köper I "Membrane on a Chip. A functional tethered lipid bilayer membrane on Silicon oxide surfaces.", *Biophys. J.* **89** (3): 1780-1788 (2005)

6) Francis, R., Louche, G., Duran. R.S. "Effect of close packing of octadecyltriethoxysilane molecules on monolayer morphology at the A/W interface: an AFM study", in press *Thin Solid Films*

7) B.S. Kang, G. Louche, R.S. Duran, Y. Gnanou, S.J. Pearton, F. Ren "Gateless AIGaN/GaN HEMT Response to Block Co-Polymers", *Solid-State Electron* **48**, 851 (2004)

Meeting presentations: (25 total and one symposium)

MEETINGS:

ACS National Meeting, Atlanta, GA, Mar. 26-30, 2006

1) Electrophysiological studies of M2δ ion channel using a patch clamp - Danyell Wilson, Henk M. Keizer, Chenyu Zhu, Joanna R. Long, and Randy Duran

2) Single channel activity from ion channels in engineered tethered bilayer membrane arrays - Henk M. Keizer, Brian Dorvel, Dan Fine, Joanna R. Long, Ananth Dodabalapur, Ingo Köper, Wolfgang Knoll, Peter AV. Anderson, and Randolph S Duran

3) Characterization of the M2δ oligopeptide ion channel mutant in an artificial lipid membrane Chenyu Zhu, Henk M. Keizer, Danyell Wilson, Joanna R. Long, and Randolph S Duran

4) Biosensors using Maxi-K potassium channels in engineered tethered bilayer membrane arrays - Henk M. Keizer, Brian Dorvel, Rebecca B. Price, Peter AV. Anderson, Dan Fine, Ananth Dodabalapur, and Randolph S Duran

5) Purification and reconstitution of Maxi-K ion channels into artificial membranes - George O. Okeyo, Brian Dorvel, Henk M. Keizer, Christopher R. Williams, Rebecca B. Price, Peter AV. Anderson, and Randolph S Duran

6) Single Channel activity from Gramicidin A ion channels in engineered tethered bilayer membrane arrays - Martin PJ. Andersson, Henk M. Keizer, Daniel Fine, Joanna R. Long, Ananth Dodabalapur, Ingo Köper, Wolfgang Knoll, Peter AV. Anderson, and Randolph S Duran

7) Ion channels reconstituted into tethered black lipid membranes: Engineering functional biomimetic systems - Brian R. Dorvel, Henk M. Keizer, Christopher R. Williams, Peter AV. Anderson, and Randolph S Duran

8) Ion channels reconstituted into tethered bilayer lipid membranes: Engineering functional biomimetic systems - Brian R. Dorvel, Henk M. Keizer, Christopher R. Williams, Peter AV. Anderson, and Randolph S Duran

SESAPS 2005 72nd Annual Meeting of the Southeastern Section of the APS, Nov. 10–12, 2005, Gainesville, FL

9) Kinetics of the Formation of Tethered Bilayer Lipid Membranes on Ultraflat Gold Supports: A QCM-Z and AFM Study , Brian Dorvel, Henk Keizer, Randy Duran

10) Single Channel Activity from Ion Channels in Engineered Tethered Bilayer Membrane Arrays, Henk Keizer, Brian Dorvel, Joanna Long, University of Florida, Daniel Fine, Ananth Dodabalapur, University of Texas at Austin, Ingo Koper, Wolfgang Knoll, Max Planck Institute, Peter Anderson, Randolph Duran

International Symposium on the Role of Adsorbed Films and Particulate Systems in Nano and Biotechnologies Gainesville, FL August 24-26, 2005

11) Single ion channel current fluctuations via films tethered to microelectronic devices: towards a direct biology-to-digital biosensor? – RS Duran

12) The Physical Characterization and Stabilization of Archaebacteria Phospholipids Using Alkoxysilanes – B Dorvel, H. Keizer, K. Fung and R. Duran

Pittcon Orlando, FL, Mar. 13-17, 2005
13) Membrane on a Chip: Applications of Tethered Bilayers, I Koeper, RS Duran, W. Knoll

Biophysical society, Long Beach, Feb 12-16, 2005
14) "Functional analysis of a re-engineered K+ channel (maxi-K) for development of an ion channel-based hybrid biosensor device" CR Williams, B Price, J Zhang, J Long, PAV Anderson, RS Duran.

MRS Spring Meeting, San Francisco, Ca., Mar 28-Apr1, 2005

15) Design Considerations of Solid State Devices for Integration with Immobilized Ion Channels
Daniel Fine, Debarshi Basu, Liang Wang , Wolfgang Knoll, Ingo Koepper , Joanna Long, Peter Anderson , Randolph Duran, Ananth Dodabalapur

ACS National Meeting, San Diego, CA, Mar. 13-17, 2005
a) ++ organizer of syposium "Transmembrane Biopolymers at Engineered Surfaces" with W. Knoll and H. DeLong

16) Interfacial behavior between synthetic phospholipids and polymerizable alkoxysilanes
Brian R. Dorvel, Henk M. Keizer, Kun Fang, and R.S. Duran

17) Stabilizing ion channel containing membranes with bacterial S-layer proteins, J. S. Tabb, W. P. Laratta, C. J. Chase, H. M. Keizer, R. S. Duran

18) Membrane on a chip; tethered bilayer membranes as a biosensor platform. I. Koeper, N. Knorr, V. Atanasov, J. R. Long, P. A. V. Anderson, R. Duran, W. Knoll

19) High resolution structural studies of membrane active peptides interacting with lipid phases of varying composition. J. R. Long, F. Mills, H. Keiser, K. Fang, R. Duran, I. Koper, W. Knoll

20) Molecular Engineering of the Maxi-K channel. P. A. V. Anderson, C. R. Williams, J. Zhang, R. B. Price, R. Duran

MRS Fall Meeting, Nov 29-Dec 3, 2004, Boston, MA

21) "Ion Channels Embedded in Synthetic Bilayers: Sensing their Stochastic Ion Translocation", R. S. Duran, P. Anderson, J. Long, J. Zhang, I. Koeper, W. Knoll, C. Williams, F. Raucci, K. Fang and H.M. Keizer
22) "Towards Tethering Ion Channels Embedded in Synthetic Bilayers Over Microelectronic Devices", R. S. Duran, P. Anderson, J. Zhang, I. Koeper, W. Knoll, A. Dodabalapur, D. Fine, F. Ren, S. Pearton, A. Offenhauser and S. Ingebrandt
23) "Membrane on a Chip- Applications of Tethered Lipid Bilayers", I. Koper, R.S. Duran, W. Knoll, P. Anderson, A. Offenhauser, S. Ingebrandt, N. Knorr, V. Atanasov, J. Li and R. Naumann

AIChE Annual Meeting, Nov 7-12, 2004, Austin, Texas

24) "Tethering Ion Channels Over Microelectronic Devices for Stochastic Sensing",(featured, lengthened lead-off presentation) R. Duran, J. Zhang, K. Fang, I. Koeper, W. Knoll, P. Anderson, H. Keizer, J. Li, N. Knorr

ACS fall National Meeting, Philadelphia, PA, Aug 22-26, 2004

25) Gateless AlGaN/GaN HEMT Response to Block Copolymers Sophie Bernard, Claire Mathieu Byoung Sam Kang, Guillaume Louche, Yves Gnanou, Fan Ren, and R. S. Duran

Interactions:

1) DARPA MOLDICE Phase 2 Kick-off Meeting, "Addressable Immobilized Ion Channels", San Diego, CA July 28, 2005

2) Pontifical Catholic University of Peru, "Tethered Ion Channels on Microelectronic Devices: an Example of Biology and Nanoscience", Lima, Peru Nov 12, 2004
3) Miami University of Ohio, "Probing Electronic Signatures Associated with Ion Translocation Through Synthetic Bilayers", Oxford OH, Oct 7, 2004
4) Claflin University, "Core-Shell Nanoparticles", Orangeburg SC, Sept 23, 2004
5) Washington University St. Louis, "Probing Electronic Signatures Associated with Ion Translocation Through Synthetic Bilayers", St. Louis, MO, Sept 16, 2004
6) SAIC Inc, "Addressable Immobilized Ion channels", McLean VA, Feb 1, 2005
7) University of Hawaii, "Interfacing Ion Channels to Microelectronics: Comparing Single Channel Activities from Three Systems", Honolulu HI Dec 9, 2005
8) Morehouse College, "Ion Channels interfaced to Microelectronic devices", Gainesville FL Sept 7, 2005.
9) Univ. California San Diego, "Stochastic Sensors using M2", La Jolla, CA Aug 1, 2005.
10) Harvey Mudd College, "Towards a Direct Biology-to-Microelectronics Interface: Probing Electronic Signatures Associated with Ion Translocation Through Synthetic Bilayers", Claremont CA, July 26, 2005.
11) MMI Molecular Machines & Industries AG, "Stochastic Sensors using Ion Channels", Glattbrugg, Switzerland July 4, 2005
12) Cell Robotics Inc., "Stochastic Sensors using Ion Channels", Albuquerque, NM, June 24, 2005
13) Centre des Etudes atomique (CEA), " Interfacing Ion Channels with Microelectronics – Towards direct biology-to-digital information conversion ", Grenoble, France June 15, 2005
14) PALM Microlaser Technologies AG, "Stochastic Sensors using Ion Channels", Bernried, Germany, May 11, 2005
15) University Paul Sabatier – CNRS LCC, "Interfacing Ion Channels with Microelectronics – Towards direct biology-to-digital information conversion", Toulouse, France, May 9, 2005
16) Pasteur Institute, "Interfacing Ion Channels with Microelectronics – Towards direct biology-to-digital information conversion", Paris, France, May 4, 2005
17) Chalmers University, "Interfacing Ion Channels with Microelectronics – Towards direct biology-to-digital information conversion", Gothenburg, Sweden, May 2, 2005
18) Universite de Bourgogne, "Interfacing Ion Channels with Microelectronics – Towards direct biology-to-digital information conversion", Dijon, France, Apr 29, 2005
19) AFOSR Program review "Interfacing Ion Channels to Microelectronics: Comparing Single Channel Activities from Three Systems", Marathon Fl 3 Jan, 2006

20) Maui NanoBioTech "Interfacing Ion Channels to Microelectronics: Comparing Single Channel Activities from Three Systems", Maui 12/13/2005

Transitions:

The UF/MPI team established an agreement with Diverchem, a company in France to commercially synthesize one of the phytanyl-based tethered lipids we developed

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100, rue Louis Blanc
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The UF/MPI team signed NDA agreements with Electronic Bio Sciences Inc. and is in discussion with them regarding technology transitions for a potential Phase 2 DARPA proposal.

Contact: Any Hibbs, CEO
Telephone: (858) 0232 Fax: (858) 288-1017

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Suite 108
San Diego, CA 92121

A large HHMI award entitled "Science for Life" was received in June 2006 and would not have been possible without results from this research

Proposals are in review at NIH (SBIR) with Agave Biosystems, ARO, and NIH on research that is based on results from this work.