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13. ABSTRACT (Maximum 200 words) The focus of this program is to correlate structure and function in biological membranes using nanohybrids as artificial models and to develop new sensors based on nanohybrids. We have already demonstrated both objectives. Nanohybrid artificial membranes exhibit characteristics similar to biological membranes and they can be used as sensors. The nanohybrid membranes are synthesized by intercalating amphiphile molecules into the galleries of a layered host producing an alternating amphiphile/inorganic multilayer. We have established how the nanohybrid membranes respond to changes in temperature, pH, pressure and electric field. For example, permeation through the nanohybrids can be modulated by changing the pH or by switching on and off the electric field across the membrane. We have also shown that the nanohybrid membranes can be used as sensors for different analytes including saccharin and quinine. Different responses have been observed even for molecules that have similar features for example, saccharin and its sodium salt suggesting that the nanohybrid might be useful in developing an electronic nose. The dynamic range of the current sensor for saccharin is 6uM to 500uM. Recent work has enabled us to optimize the response time (from several minutes to seconds) as well as better understand the sensing mechanism.				
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Title: Nanobiohybrids: New Model Systems for Membranes and Sensors

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Abstract

The focus of this program is to correlate structure and function in biological membranes using nanohybrids as artificial models and to develop new sensors based on nanohybrids.

We have already demonstrated both objectives. Nanohybrid artificial membranes exhibit characteristics similar to biological membranes and they can be used as sensors. The nanohybrid membranes are synthesized by intercalating amphiphile molecules into the galleries of a layered host producing an alternating amphiphile/inorganic multilayer. We have established how the nanohybrid membranes respond to changes in temperature, pH, pressure and electric field. For example, permeation through the nanohybrids can be modulated by changing the pH or by switching on and off the electric field across the membrane.

We have also shown that the nanohybrid membranes can be used as sensors for different analytes including saccharin and quinine. Different responses have been observed even for molecules that have similar features for example, saccharin and its sodium salt suggesting that the nanohybrid might be useful in developing an electronic nose. The dynamic range of the current sensor for saccharin is $6\mu\text{M}$ to $500\mu\text{M}$. Recent work has enabled us to optimize the response time (from several minutes to seconds) as well as better understand the sensing mechanism. We have found that adsorption of saccharin renders the membrane more hydrophilic. The more hydrophilic membrane allows for increased adsorption of water molecules on both the surfaces and galleries of the membrane, which leads to changes in the electrostatic field and polarization of the membrane.

Recently we have also focused on demonstrating more complex, biological functionality by introducing, for example, specific proteins and enzymes into the nanohybrids. When membrane proteins are inserted into supported lipid bilayers they are generally rendered non-functional because of the unfavorable interactions of the protein with the underlying substrate. We have developed a new method to immobilize membrane proteins that overcomes these problems. The method involves micellizing the protein into a mixture of ionic and non-ionic surfactants followed by intercalation into the galleries of the inorganic core. The immobilized proteins are in an environment similar to their natural habitat, which prevents them from being deactivated. The nanohybrid approach represents an excellent method to immobilize different biomolecules to prevent denaturing and develop active biohybrids for applications ranging from novel therapeutics to sensors.

Nanohybrids containing gramicidin show good selectivity (8x enhancement) towards sodium ions compared to control membranes (without the gramicidin). We have also synthesized glucose oxidase nanohybrids and demonstrated their sensing capability towards glucose. The sensor is fairly robust and reproducible.

We are currently extending our work to immobilize heat sensitive proteins like TPRV1 in collaboration with *Dr. T. Pappas (UTMB)* to produce nanobiohybrids with *thermosensing and heat detecting* capabilities relevant to the mission of the Air Force.

Accomplishments –New Findings

Nanohybrid Membranes as Sensors

While simple artificial membranes lack specific proteins they can still act as sensors. The working hypothesis is that the analyte interacts directly with the amphiphilic molecules and depolarizes the membrane.

The nanohybrid membranes are synthesized by intercalating amphiphilic molecules into the galleries of a layered host producing an alternating amphiphile/inorganic multilayer. To evaluate the sensing capability of our bioinspired membranes, films were formed on interdigitated electrodes by solvent casting a suspension of the nanohybrid in toluene. After drying, membranes between 7-8 μm were obtained as measured by SEM. The membrane was immersed into a buffer containing beaker and known amounts of analyte were added. The electrical response was monitored by impedance spectroscopy. Measurements were made using a two-electrode array by applying a 10mV amplitude AC voltage and measuring the current and the phase angle through the electrodes.

Figure 3 shows the response of the nanohybrid membrane as rate of conductivity in the presence of saccharin. All conductance measurements were in the range of 200 to 400 μS falling into the linear log-log response of the electrodes. Using the rate of conductivity (i.e. the slope of conductivity over time) rather than conductivity per se we were able to decrease the response time of the sensor to a few seconds.

The dynamic range of the current sensor is 6 μM to 500 μM . We expect further optimization of the size and the geometry of the electrodes to increase sensitivity. To evaluate the selectivity of the sensor, the response of our sensor to glucose and sucrose was investigated. No significant interference was found in the response of the sensor.

In an attempt to better understand the sensing mechanism and response of the nanohybrid membranes several experiments were designed to shed more light on how the analyte interacts with the membrane. Absorption of the analyte can cause a change in the hydrophilicity/hydrophobicity of the membrane. For example, if the membrane becomes more hydrophobic, more water molecules can be incorporated into the membrane. An increase in hydrophilicity, apart from increasing permeation of ions, can also change the "effective area" of the head group of the amphiphile and the orientation/association of water, which leads to changes in the electrostatic field at the surfaces of the membrane.

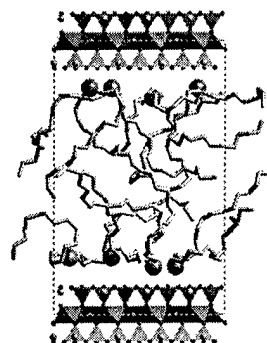


Figure 1. Computer simulations of amphiphile/inorganic bilayer in the nanohybrid. Note that only one of the bilayers is shown.

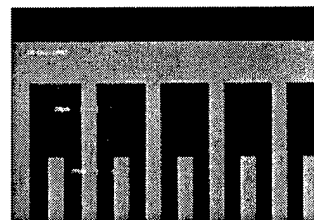


Figure 2. SEM picture of microfabricated interdigitated electrodes. Width of electrodes 20 microns.

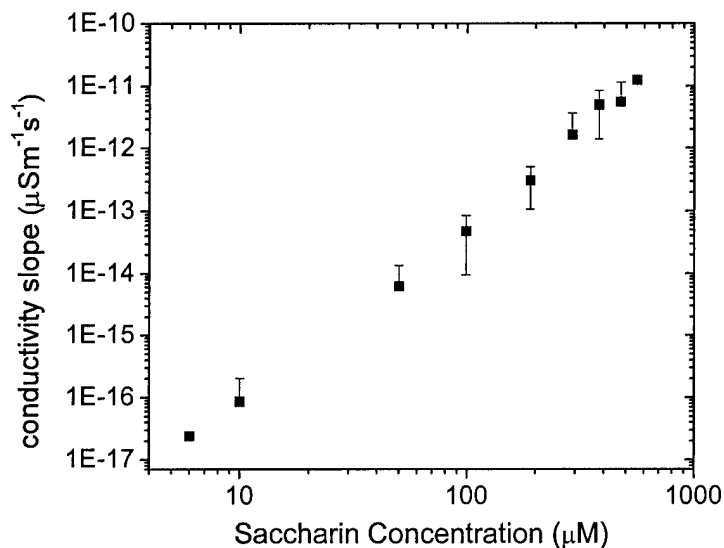


Figure 3. Response of nanohybrid sensor to saccharin

Contact angle measurements show a systematic decrease in contact angle with increasing saccharin content. The decrease in contact angle supports the suggestion that the membrane becomes more hydrophilic upon exposure to saccharin. X-ray diffraction profiles of the nanohybrid membrane show also a progressive increase in gallery height by 0.6 nm upon exposure to saccharin (Figure 4). The expansion is proportional to the concentration of the analyte solution for a given time and is due to the incorporation of water in the galleries of the nanohybrids.

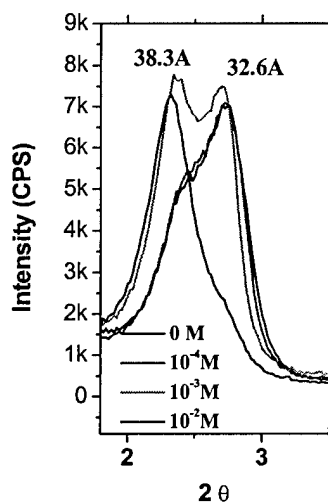


Figure 4. X-Ray diffraction patterns of nanohybrids exposed to progressively increasing concentrations of saccharin showing an increase in the gallery height from the original spacing attributed to intercalation of water molecules.

Gramicidin Nanohybrids

Integration of gramicidin was accomplished by ion exchanging gramicidin containing cationic micelles into the host nanoparticles shown schematically in Figure 5. The success of immobilization was investigated using X-ray diffraction, XRD, and FTIR. The nanohybrids exhibit the signature absorption peaks characteristic of gramicidin (amide peaks). In addition, an increase in the repeat unit of the organic/inorganic multilayer is consistent with the incorporation of gramicidin in the nanohybrid.

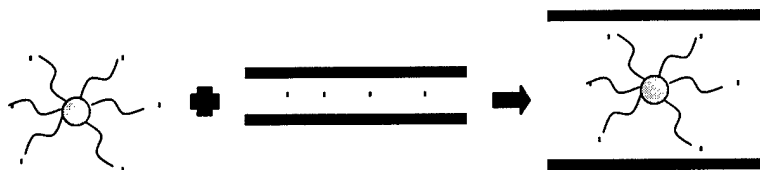


Figure 5. Schematic of the synthetic approach to introduce biomolecules like membrane proteins into the nanohybrids using micelles

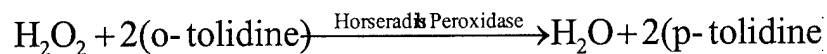
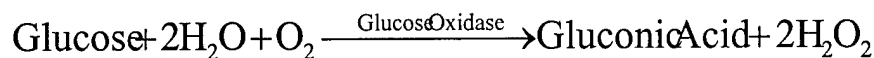
Preliminary experiments show an increase in selectivity of the membrane towards sodium ions. Further experiments are in progress to evaluate the function of the nanohybrid membrane as an ion channel.

Glucose Nanohybrids-Sensors

Similar to gramicidin (i.e. by first encapsulating the biomolecule into a cationic micelle following by ion exchange) we have succeeded in incorporating Glucose oxidase in the nanohybrid membranes. XRD and FTIR suggest that the glucose oxidase has indeed been incorporated into the nanohybrids. Nanohybrid films were solution cast onto glass slides and their activity towards glucose was evaluated spectrophotometrically by monitoring by UV-Vis the color change accompanying exposure to glucose due to the production of H_2O_2 as shown below.

The slide with the enzyme containing nanohybrid was exposed to a solution of glucose for a given time (i.e. 3min). The hydrogen peroxide produced is assayed using o-tolidine as a substrate in the presence of horseradish peroxidase. The reaction from o-tolidine to p-tolidine turns the solution blue and the extent of reaction is measured spectrophotometrically.

The reactions can be seen below.



Colorless

Blue

The response of the glucose oxidase nano hybrid is shown in Figure 6. The nano hybrids were

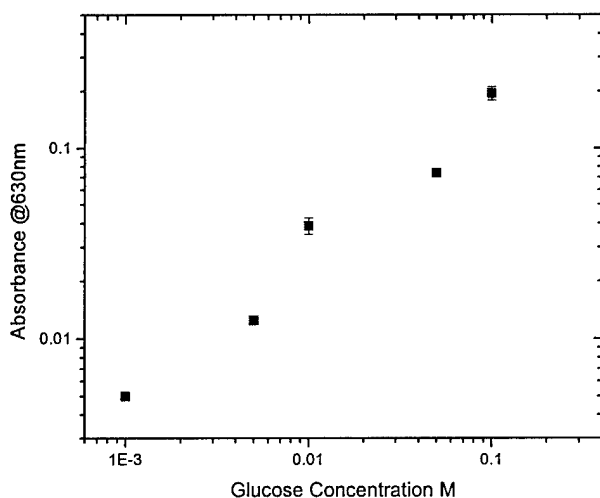


Figure 6. Response of Glucose Oxidase Nano hybrid sensor

active with a reproducible response up to 5 days. Further studies will focus on establishing a better understanding of the sensing mechanism as well as sensor optimization. Future work is also directed towards incorporation of other enzymes including catalase, urease and others.

In addition to the sensor work described above, we are attempting to extend this work to immobilize heat sensitive proteins like TPRV1 in collaboration with *Dr. T. Pappas (UTMB)* to produce nanobiohybrids with thermosensing and heat detecting capabilities.

Personnel Supported.

Nikolaos Chalkias (graduate student)

Evangelos Tsagarakis (visiting scientist)

Publications

1. M.M.M. Muthu, E. Hackett and E.P. Giannelis, "From Nanocomposite to Nanogel Polymer Electrolytes", Journal of Materials Chemistry, **13**, 1, **2003**.
2. D. Anglos, A. Stassinopoulos, R.N. Das, G. Zacharakis, M. Psyllaki, R. Jakubiak, R. A. Vaia, E. P. Giannelis, S. H. Anastasiadis "Random Laser Action in Organic/Inorganic Nanocomposites", J. Opt. Soc. Amer. B., **21**, 208, **2004**.
3. S. Ramesh, B.A. Sutzberg, C. Huang, J. Gao and E.P. Giannelis, R. Sivarajah, "Dielectric Nanocomposites for Integral Thin Film Capacitors", IEEE Transactions of Advanced Packaging, **26**, 17, **2003**.
4. S. Cypes, W.M. Saltzman and E.P. Giannelis, "Organosilicate-Polymer Drug Delivery Systems: Controlled Release and Enhanced Mechanical Properties", Journal of Controlled Release, **90**, 163, **2003**.
5. V. Vohra, D.F. Schmidt, C.K. Ober and E.P. Giannelis, "Deintercalation of a Chemically Switchable Polymer from a Layered Silicate Nanocomposite", Journal of Polymer Science, B: Polymer Physics, **41**, 3151, **2003**.
6. H. Chen, D.F. Schmidt, M. Pitsikalis, N. Hadjichristidis, Y. Zhang, U. Wiesner and E.P. Giannelis, "Poly(Styrene-*block*-Isoprene) Nanocomposites: Kinetics of Intercalation and Effects of Copolymer Architecture on Intercalation Behavior", Journal of Polymer Science, B: Polymer Physics, **41**, 3264, **2003**.
7. S. Abbrent, S.H. Chung, S.G. Greenbaum, J. Muthu and E.P. Giannelis, "Nuclear Magnetic Resonance Studies of Nanocomposite Gel Electrolytes", Electrochimica Acta, **2113**, **2003**.
8. J-H. Chang, Y. U. An, S.C. Ryu and E.P. Giannelis, "Synthesis of Poly(butylenes terephthalate) Nanocomposites by In-situ Interlayer Polymerization and Characterization of its Fiber", Polymer Bulletin, **51**, 69, **2003**.
9. J-H. Chang, Y. U. An, D. Cho, and E.P. Giannelis, "Poly(lactic acid) nanocomposites: comparison of their properties with montmorillonite and synthetic mica", Polymer, **44**, 3715, **2003**.
10. K.M. Tyner, S. Schiffman and E.P. Giannelis, "Nanobiohybrids as Delivery Vehicles for Camptothecin", Journal of Controlled Release, **95**, 501, **2004**.
11. K.M. Tyner, M.S. Roberson, K.A. Berghorn, L. Li, R.F. Gilmour Jr., and E.P. Giannelis, "Intercalation, Delivery, and Expression of the Gene Encoding Green Fluorescence Protein Utilizing Nanobiohybrids", Journal of Controlled Release, **xx**, 00, **2004**.
12. D. Shah, P. Maiti, E. Gunn, D.F. Schmidt, D.D. Jiang, C.A. Batt and E.P.

Giannelis, "Dramatic Enhancements in Toughness of Polyvinylidene Fluoride Nanocomposites via Nanoclay-Directed Crystal Structure and Morphology", Advanced Materials, **16**, 1173, **2004**.

13. D. Shah, P. Maiti, D.D. Jiang, C.A. Batt and E.P. Giannelis, "Effect of Nanoparticle Mobility on Toughness of Polymer Nanocomposites", Advanced Materials, in press.
14. A. B. Bourlinos, R. Herrera, N. Chalkias, D. D. Jiang, Q. Zhang, L. A. Archer, and E. P. Giannelis, "Solvent-Free Functionalized Nanoparticles with Liquid-like Behavior", Advanced Materials, in press.
15. A. B. Bourlinos, S.R. Chowdhury, D. D. Jiang, Y-U. An, Q. Zhang, L. A. Archer, and E. P. Giannelis, "Layered Organosilicate Nanoparticles with Liquid-like Behavior", Small, in press.

Invited Talks to Conferences, Seminars

175. ACS, Colloid & Surface Chemistry, New York, NY (September 2003)
176. ACS, Polymer Chemistry & Nanotechnology, New York, NY (September 2003)
177. IEEC Symp. on Emerging Technologies in Packaging, Binghamton, NY (Sept. 2003)
178. ACS Rubber Division, Cleveland, OH (October 2003)
179. Functional Fillers –Workshop on Nanocomposites, Atlanta, GA (October 2003)
180. Workshop in Drug Delivery, Ithaca, NY (October 2003)
181. 1st World Congress on Nanocomposites, San Francisco, CA (November 2003)
182. Symposium on Nanostructured Polymeric Materials, Strasbourg, France (March 2004)
183. IPC, Opportunities in Disruptive Technologies, Baltimore (May 2004)
184. 11th European Conference on Composite Materials, Rhodes, Greece (May 2004)
185. PPS-20, Akron, OH (June 2004)

Invited Seminars to Industry

123. Rhodia, Paris, France, (September 2003)
124. Rohm and Haas, Springfield, PA (October 2003)
125. Bayer, Pittsburg, PA (November 2003)
126. Atofina, King of Prussia, PA (December 2003)
127. OilDri Corporation, Lincolnshire, IL (March 2004)
128. Saint-Gobain Innovation Day on Nanomaterials, Northboro, MA (May 2004)
129. Pactiv, Canandaigua, NY (June 2004)
130. UOP, Des Plaines, IL (June 2004)

Interactions/Transitions

We are collaborating with Bristol-Myers-Squibb to evaluate the use of nanohybrids as a means to increase the solubility and bioavailability of water insoluble drugs.

New discoveries, inventions, or patent disclosures.

1. J. Zhu, B. Park and E.P. Giannelis, "Composite of High Melting Polymer and Nanoclay with Enhanced Properties"

2. A.B. Bourlinos and E.P. Giannelis, "Functionalized Nanostructures with Liquid-like Behavior".

Honors/Awards

EPG, Member of Editorial Board, *Small*, Wiley-VCH