NDnano UNIVERSITY OF NOTRE DAME
CENTER FOR NANO SCIENCE AND TECHNOLOGY

ND*nano* Undergraduate Research Fellowship (NURF)
2013 Project Summary

1) Student name: **Christopher Walker**
2) Faculty mentor name: Professor Hsueh-Chia Chang
3) Project title: *Home-Use Nucleic Acid-Based Nanosensors for Screening Chronic Diseases*

4) Briefly describe any new skills you acquired during your summer research:

Through my research this summer, I have been introduced to the intriguing world of biosensing, microfluidics, and membrane electrokinetics. In addition to gaining valuable general laboratory skills, I have learned how to create a low-cost nucleic acid sensing device from raw materials, and how to test the nanomembrane sensor devices using a potentiostat. I learned the chemistry involved in functionalizing target-specific oligoprobe on the nanomembrane surface, and the fundamental principle of biosensing and the device's operation. Furthermore, I got to experience firsthand the development process for a prototype device.

5) Please briefly share a practical application/end use of your research:

The nanomembrane sensor that I helped to develop has potentially great implications in the developing world where many chronic infectious diseases go undetected and untreated due to the lack of equipment, funding, or trained technicians. A vision of Professor Chang's Lab is to develop a portable, integrated platform for biosensing which can be mass-produced at a low cost to be used in the field or at home. The device would include units for pretreatment of a biological fluid sample, pre-concentration of nucleic acids from the lysed sample, and my focus, the actual sensing. Since different viruses and cancer cells contain distinct biomarkers in their nucleic acid sequences, it is possible to apply this sensing technique to a plethora of diseases.

Project summary:

The goal of my project was to develop the sensing component of the integrated chip mentioned above, optimizing the sensitivity and selectivity to the DNA or RNA targets of interest. Issues to be dealt with were first creating a reliable standalone sensor chip and setup to test it, which in itself was a challenge; determining an optimal method of functionalizing an anion-exchange membrane - that is, covalently attaching probes composed of a sequence of ssDNA complementary to the biomarker target of interest to the membrane surface; and finding a way to remove non-target nucleic acids, which may be highly similar in sequence to the target, from the membrane.

Working with Assistant Research Professor Satyajyoti Senapati and Postdoctoral Research Associate Zdenek Slouka, I was able to fabricate the sensor chips to test at a relatively low cost: first, a cutout of nanoporous anion-exchange membrane was embedded in a polyurethane well cast from monomer resins in a custom 3D-printed mold. A chip with two

reservoirs, one on each side of the membrane, was created by adhering the molded polyurethane to a glass slide using UV-curable glue. The experimental setup for the biosensing involved four electrodes - a platinum working electrode and silver reference electrode at each reservoir; both were filled with PBS buffer solution. In this way it is possible to obtain a nonlinear current-voltage curve distinct to the system by running a voltage sweep via a potentiostat. The membrane between the two reservoirs, being positively charged, allows only small negative ions to pass through. At a certain voltage, fixed positively charged anion exchange membrane shows nonlinear current−voltage characteristics (CVC) with three distinguishable regions. The first region, referred to as the under-limiting region, behaves similarly to an ohmic resistor which occurs at low voltages where the electrical current is directly proportional to the voltage applied across the membrane. The second region known as the limiting region (LR) occurs when the electrical current starts to saturate at a limiting current beyond a critical cross-membrane voltage drop as a result of the ion-transport limitation introduced by ion depletion on one side of the membrane. The third region, known as the over-limiting region, develops when an inflection point appears on the CVC curve at a critical voltage, corresponding to the end of the LR, and the electrical current rises abruptly, giving rise to a second linear region. The nucleic acid, when bound to the depleted side of the membrane, being negatively charged, is cation-selective and thus develops a bipolar characteristic (positive charge comes from the membrane and negative charge comes from DNA molecules). This leads to suppression of electroconvection vortices and generation of water-splitting reactions as the concentration of DNA increases at the membrane surface. At high DNA concentration, the water-splitting reaction predominates. As a result, a significant shift is observed in the overlimiting regions of the current-voltage curve.

The membrane surface was functionalized by UV-crosslinking with benzophenone-tetracarboxylic acid, followed by the addition of the ssDNA probe complementary to the target facilitated by the carboxyl-activating agent EDC. The presence or lack of probe attached to the membrane could be confirmed by a shift in the current-voltage characteristics of the system in the overlimiting region. The primary probe that I tested this summer was for RNA from *Brucella* cells, the pathogen that causes Brucellosis, but the sensing device could be readily adapted to a very wide range of nucleic acid sequences. Experiments I ran included concentration studies of the functionalized sensors, testing the current-voltage curve response for both synthetic DNA with the complementary *Brucella* sequence as well as real RNA from lysed *Brucella* cells. The sensors developed have shown to be sensitive to concentrations as low as 10pM of target DNA and 10pg/uL of target RNA. The implications of this are that even lower concentrations may be detected by integrating a pre-concentration unit close to the sensor. Optimizations which were explored included the composition of the anion-exchange membrane, the pH of the carboxylic acid and amount of UV-exposure needed to functionalize the membrane, and the sensing area exposed to the reservoirs. An issue that remains to be solved is fully removing unwanted non-target nucleic acid from the membrane surface without disrupting the bound target, which may be dehybridized by a wash with strong base; a wash with concentrated PBS buffer has been found to be insufficient in cases where the non-target and target sequences are similar.