VSoE Research Innovation Fund Report
Single-molecule optical sensing of transport through membrane channel proteins

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1. Program summary

The long-range goal of this research program is to develop optical techniques based on resonant cavity microsensors and single-molecule microscopy that are capable of detecting single-molecule transport through channel proteins. By developing a general technique for observing transport through channel proteins, we will accomplish two goals. First, we will facilitate single-molecule biophysical studies of nonionic channel transport proteins. Second, we will create a new range of nanoscale biotechnology applications for such proteins. Consider, for example, the current work going towards using channel proteins for single-molecule sensing and DNA sequencing. An optical detection technique will expand the range of these technologies by allowing discrimination between transported species based on their effect on the evanescent optical field at the surface of optical microcavities.

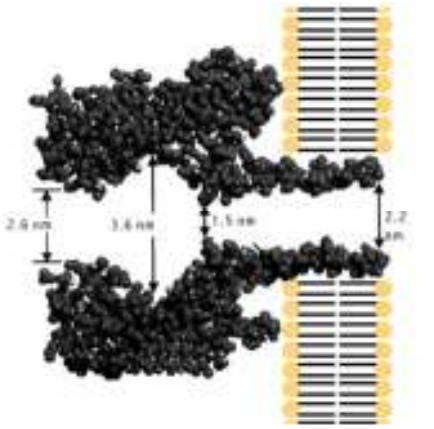

Figure 1: A schematic showing a cross-section of a single α-hemolysin heptamer inserted into a lipid bilayer. The tightest constriction through the pore restricts transport to molecules with diameters smaller than 1.5 nm. Source: Reference 3.

The early-stage research supported by the VSoE RIF in the first year of this project focused on labeling a model channel protein and optimizing its insertion into supported lipid bilayers. This model protein, α-hemolysin, is heptameric pore-forming molecule that spontaneously inserts into the membrane from the external aqueous phase, creating a channel, 1.5 nm in diameter at its narrowest point (see Figure 1). This protein channel allows for molecular transport across the bilayer. The VSoE funds supported the labor and supplies necessary to obtain and label this protein, insert it into model supported lipid bilayers, and characterize the resultsing protein-bilayer system.

2. Outcomes and impact

The major in outcome of the project in the first year was the development of a major new class of hybrid lipid bilayer system capable of supporting membrane protein insertion. This system, Covalently Anchored Bilayers On Nanotubes (CABONs) is shown schematically in Figure 2. A cross section of the system is shown at the center, indicating a multiwalled carbon nanotube (MWCNT, grey) with a lipid bilayer membrane covalently anchored to the surface (purple). The image of the vial in the upper left demonstrates a stable aqueous suspension of the CABONs; the covelent link between the nanotube and bilayer facilitates a remarkable stable dispersion of the otherwise difficult-to-disperse carbon nano-

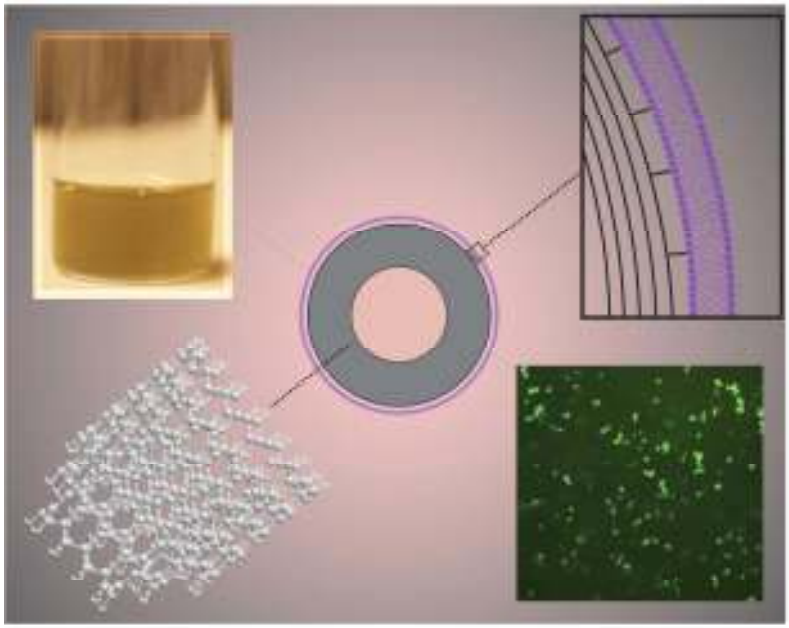

Figure 2: Diagram of a CABON showing the multiwalled nanotube core and a bilayer anchored to the surface.

material. The micrograph in the lower right shows CABONs labeled with fluorescent lipids in this aqueous suspension.

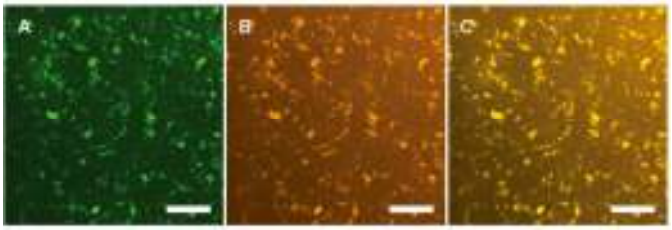

Figure 3: Insertion of α-hemolysin in CABONs showing (A) fluorescence from lipids in the bilayer (B) fluorescence from labeled α-hemolysin and (C) overlap image showing colocalization.

This work was recently published (Dayani and Malmstadt, *Langmuir* **28**:8174–8182, 2012); since the publication about a month ago, the paper has been continuously listed as one of the 20 "most read" articles on the journal's website. A major result presented in this paper is the capacity of CABONs to facilitate the insertion of α-hemolysin. As shown in Figure 3, the fluorescently labeled α-hemolysin developed using RIF support colocalizes specifically with CABONs; similar colocailization is not seen with bare carbon nanotubes or carbon nanotubes stabilized with noncovalent lipid monolayers. This biologically active conductive nanomaterial has a broad range of potential applications in sensing and bioelectronics.

Additional work accomplished with RIF funding involved the measurement of molecular transport across lipid bilayers using a fluorescence microscopy-based approach. Work performed in the past year focused on identifying relationships between the lipids composition of the membrane and the rate of transport across it. In one set of experiments, we have altered the concentration of oxidized lipid species in a membrane, synthetically simulating the damage that occurs with many aging-related physiological disorders. We found that at about 20% oxidation, the mode of transport across the membrane transitioned from a passive diffusion mechanism that could be explained with a single-exponential model to something more complex. We suspect that the change in transport mode is do to the formation of transient pores in membranes containing oxidized lipids, and are currently attempting to model this pore-facilitated transport.

3. Future directions

As the project continues, we will combine the labeled α-hemolysin we have used in the past year with the microresonator-supported bilayers described in our 2011 *Applied Physics Letters* paper. Initial work will focus on resolving the single-molecule insertion of α-hemolysin hetamers into the bilayer, or the insertion of monomers followed by assembly into heptamers. After these experiments are optimized, we will move towards detecting transport through membrane-supported protein channels.