

Bio/Nano Sensors

Charles R. Martin, Sang Bok Lee, Butler J. Raines, David T. Mitchell, Erich D. Steinle, Marc S. Wirtz, Rahela Gasparac, Elizabeth H. Medeiros, Lacramioara Trofin, Department of Chemistry
Center for Chemical Research at the Bio/Nano Interface
University of Florida
Gainesville, Florida 32611

Our research group has been exploring the transport properties of novel synthetic membranes that contain a parallel collection of gold nanotubules that span the complete thickness (~10 μm) of the membrane (1-4). These nanotubule membranes are prepared via the template method, a general and versatile approach for preparing nanomaterials (5,6). The Au nanotubules are prepared by electroless deposition of Au within the pores of a commercially available polycarbonate template membrane. We have shown that the inside diameters of the Au nanotubules can be controlled at will, down to molecular dimensions (< 1nm) by controlling the Au deposition time (1-3). Because the nanotubules have inside diameters of molecular dimensions, these membranes can be used to cleanly separate small molecules on the basis of molecular size (2).

In related work, we showed that these Au nanotubule membranes can be used in a new and highly sensitive method for electrochemical analysis (7,8). In this method, a salt solution is placed on either side of the membrane and an electrode in each solution is used to apply a constant potential across the membrane. The resulting transmembrane current, associated with migration of ions through the nanotubules, is measured. When an analyte molecule of dimensions comparable to the inside diameter of the nanotubules is added to one of the half-cells, these molecules enter the nanotubules and partially occlude the pathway for ion conduction across the membrane. As a result, the transmembrane current drops. We have shown that the magnitude in the drop in the transmembrane current is proportional to the concentration of the analyte (7,8). Detection limits as low as 10^{-11} M were obtained (7).

While this new nanotubule-membrane-based method of electrochemical analysis shows size-based selectivity, chemical selectivity is not observed. That is, if two analytes are of the same size, to a first approximation, the Au nanotubule membrane would not be able to distinguish one analyte from the other. This led us to explore ways of introducing chemical selectivity into these Au (and other) nanotubules membranes (1,3). For example, we have shown that chemical transport selectivity can be introduced by chemisorbing thiols to the inside Au tubule walls (1,3).

Another approach for introducing chemical selectivity entails incorporating a biochemical molecular-recognition agent (e.g., an enzyme, channel protein, antibody, oligonucleotide) into the nanotubules. From an electroanalytical point of view, the question then becomes how can the molecular-recognition event between the analyte and the nanotubule-bound biochemical molecular-recognition agent be transduced as a measurable change in the ion-current across the membrane.

Our inspiration for this work comes from Mother Nature. In essence we are attempting to make bio/nano devices similar to an acetylcholine-gated ion channel (9). In analogy to the concept presented above, in this case, a molecular-recognition event (the binding of acetylcholine to the protein channel) causes the channel to switch from a closed to an open state, and in the open state, an ion current flows across the channel.

In one illustration of this concept, we have incorporated the enzyme urease within the pores of a nanoporous membrane. Urease catalyzes the hydrolysis of urea and in so doing converts this parent neutral molecule into a collection of daughter ions. As a result of this release of ions into the nanopores, the ionic conductivity of the membrane increases. Hence, in analogy to the acetylcholine channel, we have transduced a molecular-recognition event into a measurable change in the ion current across the membrane. An AC-impedance and a potential-step method were used to investigate the change in conductivity of the nanopore membrane as a function of urea (analyte) concentration. A number of examples of this general concept will be presented in this talk including the use of other proteins and oligonucleotides as the molecular-recognition agents.

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1. K.B. Jirage, J.C. Hulteen, C.R. Martin, *Anal. Chem.*, **71**, 4913 (1999).
2. K.B. Jirage, J.C. Hulteen, C.R. Martin, *Science*, **278**, 655 (1997).
3. J.C. Hulteen, C.R. Martin, *J. Amer. Chem. Soc.*, **120**, 6603 (1998).
4. M. Nishizawa, V.P. Menon, C.R. Martin, *Science*, **268**, 700 (1995).
5. J.C. Hulteen, C.R. Martin, *J. Mater. Chem.*, **7**, 1075 (1997).
6. C.R. Martin, *Science*, **266**, 1961 (1994).
7. Y. Kobayashi, C.R. Martin, *Anal. Chem.*, **71**, 3665 (1999).
8. Y. Kobayashi, C.R. Martin, *J. Electroanal. Chem.*, **431**, 29 (1997).
9. D. Voet, J.G. Voet, *Biochemistry*, 2nd Ed. Wiley, NY, pp. 1297,1298 (1995).