Membrane Proteins in Biomemeric Systems
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With the evident limitations in size miniaturization in conventional batteries, there is a need to develop an alternative power supply to drive more compact devices in future technology. As a potential nano-scale hybrid device which generates energy from biological sourse, the biocompatible fabric embedded with energy converting proteins were explored. There are two different types of energy converting proteins embedded in artificial membrane: bacteriorhodopsin (BR) and cytochrome oxidase (COX). Upon the presence of light, bacteriorhodopsin starts pumping protons from one side to another side of the membrane creating proton gradient. This electro-chemical gradient across the membrane forces cytochrome oxidase to function in reversed action. Reversed Cox mechanism generates intermediates from O_2 to H_2O along with electrons. The electrons are detected and attracted to the electrode placed in the vicinity of the protein in the membrane. The system converts optical energy to electrical energy, eventually allocating the derived energy to an external source.

We have successfully purified BR from purple membrane of *Halobacterium Salinarium* and Cox from the genetically engineered plasmid inserted in *Rhodobacter Sphaeroides*. The activities of the purified enzymes have shown in lipid vesicles as well as in polymer vesicles and planar membranes. Phosphatidylcholine derived lipid vesicles created the most nature like environment for the enzymes. Triblock copolymer membrane was the alternative choice for membrane protein reconstitution since polymers are more durable, ideal for industrial applications and support enzyme activities better. We also demonstrated the backward function of Cox *in vitro* by changing proton concentration in the surrounding medium. Langmuir-Blodgett method was used to reconstitute the enzymes into the planar lipid or polymer membranes. The enzyme activities of the enzymes in planar membrane system were tested by impedance spectroscopy. Orienting the enzymes is necessary to improve the efficiency of the system. Our Cox has been genetically engineered to have six histidine residues which are easily attracted to metals such as nickel. BR also has a cysteine residue exposed to the external side of the enzyme. The enzymes are oriented to gold surface evaporated on the substrates. The ratio of BR: Cox = 57:1 gives a steady state proton transfer rate of one proton by BR for every proton by Cox. One electron is transported per proton in Cox, yielding 37mA/m^2 in combined BR/Cox monolayer system.

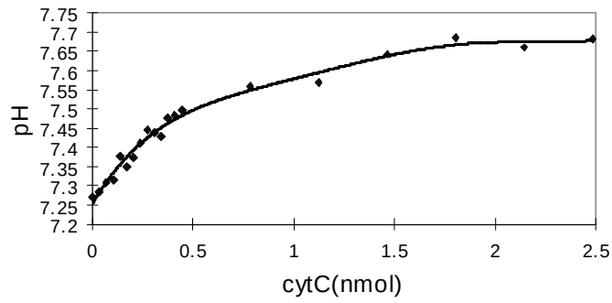

Figure 1. Forward Cox functionality in proteoliposomes. 0.034nmol cyt C added first 13 times, then 0.34nmol cyt C added every minute into 13.5μg COX reconstituted in proteoliposomes. Upon the addition of cytochrome c, cytochrome c oxidase started pumping protons out of the vesicles, increasing the pH of the internal side of the membrane. Notice that the first 14 data points made a linearly increasing slope while the later 6 data points had a smaller slope. The rate of formation of steady-state ΔpH was greater at the earlier stage of cytochrome c addition.

Figure 2. Reversal of COX monitored by spectrophotometer. **Bold: external pH**, *italic: internal pH*; a. **7.39**, *9.5*, b. **5.45**, *7.4*, c. **4.8**, *7.3*. Notice formation of the 330nm absorption peak of heme a. The spectral shift that we saw was clearly the consequence of energy-linked reversed electron transfer. Although O_2 generation from water has not been demonstrated despite numerous attempts, a partial reversal of the O_2 reaction remains possible.

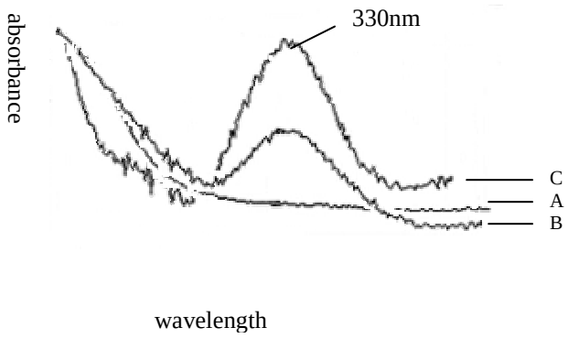

Figure 3. Impedance spectroscopy of polymer monolayer with BR inserted by LB method. The membrane and protein was deposited on quartz or silicon dioxide substrate evaporated with gold on top. The solid line: initial point (no light), open square: light illumination (1min), dark circle: light illumination (2min).