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Characterization of Proton Transport across a Waveguide-Supported Lipid Bilayer

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Cellular energy transduction processes are often driven by transmembrane ion gradients, and a number of artificial membrane systems have been developed that allow for chemically or light-induced transport of ions across lipid bilayers.[1−4] Light-induced proton pumping across lipid bilayer vesicles, for example, can be used to drive ATP synthesis.[1] These liposomal architectures, however, are not readily interfaced to a solid-state transducer. Formation of ion gradients across planar-supported membranes, "wired" to substrate electrodes, may ultimately allow utilization of the potential energy to drive other electrochemical processes. We describe here a significant step toward this goal, using a quinone proton shuttle embedded in a planar supported lipid bilayer (PSLB), coupled to a planar optical waveguide electrode through a pH-sensitive, self-assembled conductive polymer film. This construct provides for highly sensitive characterization of transmembrane proton transport.

A redox-driven proton shuttle, 1,4-diphenyl-2,5-benzoquinone (BQ), was inserted into a PSLB, coupled to an optically transparent electrode (indium tin oxide, ITO) through a self-assembled poly(aniline) (PANI)/poly(acrylic acid) (PAA) multilayer film (Figure 1a).[6,7] The hydrophilic, water-swollen (PANI/PAA)$_2$ film (ca. 25 Å thick)[7] planarizes the ITO surface, is physically and chemically compatible with deposition of a continuous PSLB (whereas ITO is not), and serves as a ionic reservoir for charge transported across the PSLB.

At near-neutral pH, three major forms of PANI exist in equilibrium in the (PANI/PAA)$_2$ film (Figure 1b; also see structures in Supporting Information (SI)).[8,9] Two are partially oxidized forms, the protonated emeraldine salt (ES) and the deprotonated emeraldine base (EB). The third is fully reduced leucoemeraldine (LE). LE is transparent at visible wavelengths, whereas the absorbance of EB and ES in the 400−900 nm range is pH-dependent (see SI). Although PANI films are typically doped into their conductive ES forms at low pH, PAA co-deposition renders PANI electroactive and pH-responsive at near-neutral pH,[5,6,9−11a] such that ITO/(PANI/PAA)$_2$ films can function both as potentiometric sensors and as absorbance pH indicators.

Proton transport across the PSLB is initiated by a redox reaction between ascorbic acid (AA) in the superstrate solution and BQ in the membrane (Figure 1a). The reduced product, BQH$_2$, can diffuse across the membrane, where it can be reoxidized by either the ES or EB forms of PANI. At open-circuit, this reaction shifts the ITO/PANI interfacial potential and the LE/EB equilibrium according to eq 1(see SI for derivation):

$$\Delta E_{\mathrm{cell}} = E^\circ_{\mathrm{ES/LE}} - 0.0296 \log\{[\Delta \mathrm{LE}]K_{\mathrm{a}}[\Delta \mathrm{EB}]^{-1}\} - 0.0592(\Delta \mathrm{pH}) \quad (1)$$

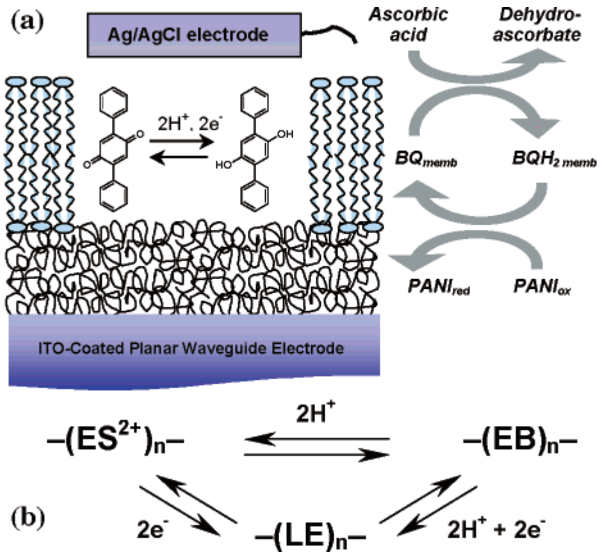

Figure 1. (a) Schematic of the ITO/(PANI/PAA)$_2$/PSLB structure. AA in the aqueous superstrate reduces membrane-bound BQ. BQH$_2$ diffuses across the lipid bilayer and is oxidized by PANI, donating two protons and two electrons to the (PANI/PAA)$_2$ film. (b) Coupled equilibria between the three dominant forms of PANI near neutral pH. When the (ES+EB)/LE ratio is held constant by potential control, BQH$_2$ oxidation shifts the ES/EB equilibrium. Under open-circuit (potentiometric) conditions, the reaction shifts both the LE/EB and LE/ES equilibria.

where $E^\circ_{\mathrm{ES/LE}}$ is the standard reduction potential for the ES/LE half-cell, and K_{a} is the ES diprotic dissociation constant.

Prior to PSLB deposition, the potential of the ITO/(PANI/PAA)$_2$ electrode was first held at +0.14 V vs Ag/AgCl for 15−30 min to create a mixture of LE, EB, and ES forms.[12] The PSLB was then deposited by vesicle fusion.[13] For potentiometric (open-circuit) experiments, the cell was then disconnected from the potentiostat and immediately connected to a high-input impedance voltmeter. When 50 μM AA in pH 7 buffer was injected into the cell without a PSLB deposited on the ITO/(PANI/PAA)$_2$/PSLB electrode, there was a rapid negative shift in interfacial potential (Figure 2, curve 1), corresponding to a shift in the LE/EB ratio due to direct reduction of the partially oxidized PANI film by AA. When AA was injected over the ITO/(PANI/PAA)$_2$/PSLB electrode, with no embedded BQ in the PSLB, the shift in E_{cell} was less than ca. 5 mV over 60 min (Figure 2, curve 3), showing that the (PANI/PAA)$_2$ film composition was stable and that the PSLB blocked AA diffusion to the film. When AA was injected over a PSLB containing 2% BQ,[13] the interfacial potential shifted negatively due to a shift in the LE/EB ratio due to reduction by BQH$_2$ (Figure 2, curve 2) but at a slower rate than curve 1, which is expected if quinone diffusion in the PSLB is rate-limiting.[4,14] The rate of potential decay was dependent upon BQ concentration (range of 1−3%) in the PSLB.

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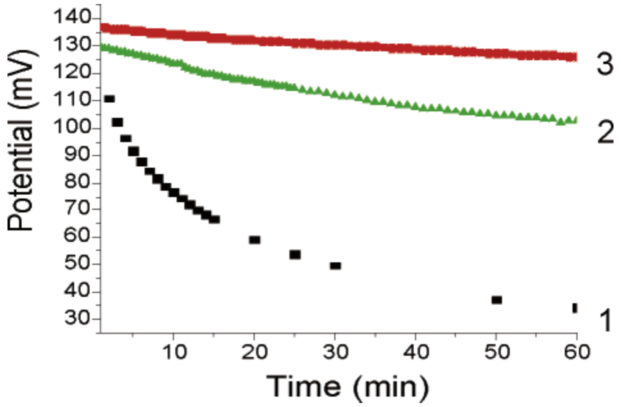

Figure 2. Change in potential during reaction of 50 μM AA with ITO/(PANI/PAA)$_2$ films: 1, direct reaction in the absence of a PSLB; 2, injection of AA over a PSLB containing BQ; 3, injection of AA over a PSLB lacking BQ.

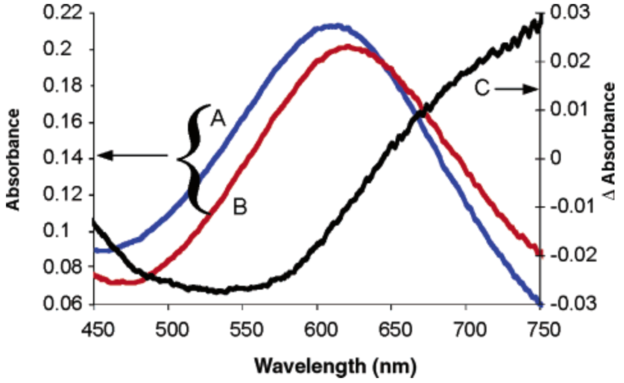

Figure 3. Absorbance spectra acquired before (A) and 10 min after (B) injection of AA over a PSLB containing BQ, and the difference spectrum (C = B − A). The decreased absorbance in the 450−650 nm region and the increased absorbance at >650 nm result from BQ-mediated proton transport across the PSLB.

Equation 1 shows that PANI reduction and EB protonation generate *equal and opposite shifts* in interfacial potential. However, PAA and surface sites on ITO can also be protonated,[15] whereas PANI is the only electron acceptor in the ionic reservoir (see SI); thus the overall shift of curve 2 is negative. Clearly, the reaction can be monitored potentiometrically, but the pH change in the ionic reservoir *cannot be determined*.

To monitor this process spectroscopically, the ITO-coated glass substrate was used as a broadband electroactive planar optical waveguide to perform attenuated total reflectance (ATR) spectroscopy.[11] Multiple total internal reflections along the length of the substrate provide a large optical path length, allowing small absorbance changes in the (PANI/PAA)$_2$ film to be measured over a bandwidth of ca. 400 nm. After (PANI/PAA)$_2$ deposition, the electrode potential was set to −0.40 V to convert the PANI film entirely to the LE form, providing a spectral "blank" for the subsequent measurements. The potential was then stepped to +0.14 V and held there for the duration of the experiment, so that the ([EB]+[ES])/[LE] ratio *was constant*. Under these conditions, transport of protons across the PSLB converts EB to ES, causing a striking red shift in the ATR spectrum (see Figure 3 and SI), and co-transport of electrons, due to oxidation of BQH$_2$, produces a current in the circuit.

After PSLB deposition,[13] no spectral shifts were observed when buffers of varying pH were injected into the ATR cell (see SI), verifying the blocking nature of the PSLB. After returning the pH in the ATR cell to 7.0, 50 μM AA was injected. Spectra acquired before and 10 min after AA injection are shown in Figure 3. Increases in ES, caused by BQ-mediated proton transport across the PSLB, produced a spectral shift of 12 nm. Control experiments performed without BQ in the PSLB did not produce spectral changes. The pH change in the ionic reservoir was estimated from a calibration plot of peak absorbance wavelength versus pH in the absence of a PSLB (see SI). The 12 nm red shift in Figure 3 corresponds to an effective pH decrease of 1.1. Measuring the pK_a for ITO/(PANI/PAA)$_2$ and calculating the number of proton acceptor sites in the ionic reservoir leads to an estimate of 740 pmol for the effective number of protons transported across the PSLB in 10 min. The calculated transport rate, 0.07 s^{-1} (see SI), agrees well with previous estimates of transmembrane charge transport by benzoquinone and its structurally related analogues in lipid bilayers.[4,14]

In conclusion, we have constructed a novel conductive polymer/planar waveguide transducer that provides for highly sensitive detection of proton transport across a PSLB. The general and flexible nature of this architecture makes it adaptable to many different types of transmembrane transport chemistries, particularly light-activated systems.[1]

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Supporting Information Available: ATR spectra and pH calibration curve for ITO/(PANI/PAA)$_2$, with/without the PSLB, PANI structures, and eq 1 derivation and calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) The number of protonatable sites on the buffer molecules in the ionic reservoir is negligible compared to the number of sites in the (PANI/PAA)$_2$ film (see SI).

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