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By

Hugh O'Neill[1], Barbara R. Evans[2], Ida Lee[3], and Elias Greenbaum[4]

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[1]Department of Biochemistry and Cellular and Molecular Biology, University of Tennessee, Knoxville, TN 37996. Tel. (865) 574 5004. Fax. (865 574 1275). Email oneillhm@ornl.gov
[2]Chemical Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831. Tel. (865) 241 3185. Fax. (865) 574 1275. Email evansb@ornl.gov
[3]Department of Electrical and Computer Engineering, University of Tennessee, Knoxville, TN 37996. Tel. (865) 241 6695. Fax. (865) 574 1275. Email leei@ornl.gov
[4]Chemical Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831. Tel. (865) 574 6835. Fax. (865) 574 1275. Email greenbaum@ornl.gov

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Hugh O'Neill[1], Barbara R. Evans[2], Ida Lee[3], and Elias Greenbaum[2]

[1]Department of Biochemistry and Cellular and Molecular Biology, University of Tennessee, Knoxville, TN 37996. [2]Chemical Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831. [3]Department of Electrical and Computer Engineering, University of Tennessee, Knoxville, TN 37996.

Abstract

Photosystem I (PSI) is one of the photosynthetic reaction centers that converts light energy into electrical and chemical energy. We report on two different experimental techniques to measure the open and closed circuit photovoltaic properties of PSI reaction centers in an aqueous environment. The combined techniques of atomic force microscopy and scanning surface probe microscopy were used to measure the light induced electrostatic surface potentials at the gas-liquid interface. The orientation and magnitude of the light-induced photovoltages of PSI entrained in the pores of microchannel glass plates were recorded. A simple photocatalytic hydrogen-evolving system based on intermolecular electron transfer using isolated PSI reaction centers as the photoactive element is also reported. The experimental system comprised of sodium ascorbate as a sacrificial electron donor, plastocyanin (PC), PSI and sodium hexachloroplatinate. Photo-dependent platinization of PSI resulted in the formation of an efficient hydrogen evolving

catalyst. Hydrogen photoevolution by crosslinked platinized PSI-PC was increased 3-fold both in initial rate and total yield compared to the free enzymes. The native pathway for electron flow yielding enzymatic $NADP^+$ reduction activity was not observed in the presence of ferredoxin and ferredoxin-$NADP^+$ oxidoreductase. This indicated that the crosslinking procedure interfered with the interaction of PSI and ferredoxin.

Introduction

Nature has supplied us with a vast array of "nanomachines" each optimized to carry out a specific task as part of a larger organizational structure, usually a living cell. The ability to extract these molecules from living organisms, reconstitute them in a non-native environment and realize work from their specific functionalities is a central theme of nanoscale science and engineering.

Photosynthesis, the biological process that converts solar energy into chemical energy, is fundamental to the existence of life on earth. It is carried out by plants, algae and cyanobacteria. The molecular organization of the multisubunit transmembrane protein complexes Photosystems I and II in green plants is illustrated in Figure 1. These proteins each capture a photon and use it to create a high energy electron generating the necessary electrochemical potential to oxidize H_2O to O_2 and reduce CO_2 to biomass (Chitnis, 2001; Barber, 2002). Under certain metabolic conditions several types of unicellular microalgae divert reducing equivalents away from the Calvin-Benson cycle to the simultaneous production of O_2 and H_2 (Boichenko et al., 2001).

Photosystem I (PSI) is composed of a light harvesting complex and a reaction center with 12 core subunits (Ben-Shem et al., 2003). Its molecular mass is

approximately 525 kDa. PSI captures the reducing equivalents liberated from the oxidation of H_2O to O_2 by Photosystem II via plastocyanin. The light harvesting complex serves as an accessory antenna to harvest and funnel absorbed photons to a special chlorophyll dimer in PSI called P700 (Trissel and Wilhelm, 1993). After absorption of a photon, the photochemistry of P700 induces a charge separation ($P700^+A_0^-$) in the reaction center by rapid electron transfer to peripheral iron sulfur complexes (F_A and F_B) via the intermediates A_0, A_1 and F_x (Owens et al., 1987; Golbeck, 1987). This results in the generation of a weak oxidant (E'_o= +0.4 V) at the primary electron donor (P700) and a strong reductant (E'_o= -0.7 V) at the terminal electron acceptor (4Fe-4S center) (Barber and Andersson, 1994). P700 is rereduced by plastocyanin and the terminal Fe-S cluster is oxidized by another electron relay protein ferredoxin. This completes one electron transfer reaction for PSI. The photochemical reaction is completed within 10-30 ps (Jia et al.,1992) and generates approximately a 1 volt potential difference (Lee et al., 2000) over a distance of 6 nm (Ben-Shem et al., 2003). The quantum yield of this photochemical reaction is close to 100% (Zankel et al., 1968; Hiyama, 1985).

As described above, reducing equivalents from the oxidation of H_2O can be diverted to H_2 by certain unicellular micro-algae (Boichenko et al., 2001). In this case, ferredoxin transfers electrons to hydrogenase resulting in the simultaneous production of hydrogen and oxygen. As an interesting variant to this pathway, ferredoxin and hydrogenase can be substituted by metallic platinum (Greenbaum, 1985). The metallic platinum is precipitated at the stromal side of isolated photosynthetic membranes at the site of electron emergence from the PSI complexes such that the platinized chloroplast thylakoid membranes are capable of sustained and simultaneous photoproduction of

hydrogen and oxygen. The platinization reaction takes place at ambient temperature and physiological pH according to the following reaction:

$$PtCl_6^{2-} + 4e^- + h\nu \rightarrow Pt\downarrow + 6Cl^-$$

The source of electrons for deposition of platinum can be exogenous hydrogen gas or as the above equation implies reducing equivalents from the light activated PSI reaction center itself (Greenbaum, 1988). Platinum can be substituted for other metals such as osmium or ruthenium in these reactions (Lee et al., 1998).

In a further development of this technique it has been shown that isolated PSI reaction centers can be modified with platinum also (Millsaps et al., 2001). In this case sodium ascorbate was used as an electron donor to plastocyanin. The platinization of PSI was achieved by photochemical deposition of metallic platinum from sodium hexachloroplatinate in solution. The electrons were photogenerated by PSI with sodium ascorbate as a sacrificial pool of reductant. The photochemistry of platinum deposition and catalyst formation precedes the onset of hydrogen evolution. The ability to direct the deposition of metallic Pt at the reducing side of PSI to produce a biomimetic photosynthetic structure may be viewed as an example of a part bioorganic part inorganic hybrid nanomachine.

The isolation of functional single biomolecules and their reconstitution on patterned surfaces or in defined geometries is another defining element of nanoscience and technology. A number of approaches have been used to immobilize PSI on surfaces to study its properties while maintaining its optoelectronic properties. These include the

incorporation of PSI into lipid films (Munge et al., 2003) and polymer matrices such as albumin-glutaraldehyde (Bonenfant and Carpentier, 1990) and acrylamide (Govorunova et al., 1995). Our research group demonstrated that PSI can be contacted with metallic platinum and anchored to a metal surface (Lee et al., 1996). This is akin to "molecular welding." In addition, we have carried out studies using atomic force microscopy and related technologies to investigate the properties of PSI molecules immobilized on surfaces. Lee et al. demonstrated that PSI reaction centers can be self-assembled and oriented on organosulfur modified gold electrodes and are stable nanometer scale diodes (Lee et al., 1995; Lee et al., 1997). The photovoltaic properties of the protein were stable during long-term storage (Lee et al., 2003). Scanning surface probe microscopy was used to measure the light-induced electrostatic potentials above single PSI reaction centers coordinated to mercaptoethanol functionalized atomically flat gold. The photodependent voltage found to be approximately 1 volt, in good agreement with previously reported data (Lee et al., 2000). The polarity and magnitude of the light-induced voltage are consistent with the known structural and energetic features of PSI. In addition, the discovery of a local central potential minimum, corresponding to energy interactions exceeding the Boltzmann energy *kT* at room temperature, suggests a docking and orientation mechanism for the transfer of electrons from theF_{AB}^{-} site to ferredoxin.

In summary, PSI is a molecular photovoltaic structure that is capable of generating a 1 V potential over a 6 nm distance after absorption of a photon. It can carry out efficient electron transfer directly from its active site to a metal surface in the absence of a mediator. Its protein architecture is such that two hydrophilic ends are separated by a hydrophobic middle region and therefore it has a natural disposition to orient itself. These

properties can be applied to the nanofabrication of self-assembled biomolecular electronic devices such as photovoltaics and photodetectors.

In this paper we present two techniques to measure the photovoltaic properties of PSI reaction centers in solution. We have used the photochemical platinization reaction to carry out a kinetic study of intermolecular and intramolecular electron transfer by PSI. This allowed us to measure the closed circuit potential of photoinduced electron transfer. We have also investigated the use of atomic force microscopy (AFM) and scanning surface probe microscopy (SSPM) to study the open circuit photovoltaic properties PSI reaction centers in solution.

Results

Closed Circuit Photovoltage Measurements

The experimental system was comprised of sodium ascorbate as a sacrificial electron donor, plastocyanin (PC), PSI and sodium hexachloroplatinate in a buffered phosphate solution at pH 7.0. PSI and PC were both isolated from spinach chloroplasts. The isolation protein procedures and PSI-PC crosslinking procedure using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) are described elsewhere (Evans et al., 2004). The apparatus for measuring photodependent H_2 evolution was also described previously (Millsaps et al., 2001). Briefly, it is a closed continuous flow system that uses helium or nitrogen as a carrier gas. The production of H_2 was monitored with a Figaro tin oxide semiconductor gas sensor. Absolute calibration of the sensor was

achieved using electrolysis and application of Faraday's laws of electrochemical equivalence.

Initial experimental data indicated a marked difference in rate of photodependent electron transfer between the crosslinked and native proteins. At 2 mM Na_2PtCl_6 a copious precipitation of metallic Pt was observed during the first light cycle with the crosslinked protein preparation and no H_2 evolution was evident. In contrast, no precipitation was observed with the native enzymes and photodependent H_2 evolution proceeded as expected. These data gave good qualitative insights into the reaction mechanism. During the first light cycle Pt catalyst formation at the surface of the reducing end of PSI is followed by H_2 production. The catalyst must first grow to a size that is capable of catalyzing the reduction of H^+ to H_2 (Greenbaum, 1988). This in turn prevents further increase in the catalyst size as electrons are diverted from the $4e^-$ reduction of Pt^{4+} to Pt to a $2e^-$ molecular H_2 forming reaction. However, with the crosslinked system the $4e^-$ transfer to Pt is favored over the $2e^-$ reduction of H^+ ions resulting in the formation of a large metallic surface not suitable for H_2 evolution. On reduction of the starting concentration of Na_2PtCl_6 to 0.5 mM the rate of diffusion of the Pt^{4+} to the surface of PSI was such that H_2 synthesis was favored over Pt formation. All further experiments were carried out at this concentration of Na_2PtCl_6.

The light-catalyzed evolution of hydrogen during 2h steady-state illumination periods was increased 3-fold for the cross-linked proteins (Figure 2). The initial rates of H_2 evolution were also determined to increase about 3-fold by cross-linking PC to PSI. The free system had an initial rate of about 30 nmol $(mg\ chl)^{-1}\ h^{-1}$, whereas that for the cross-linked system was approximately 90 $(mg\ chl)^{-1}\ h^{-1}$.

It was also determined that the ability of the PC-PSI crosslinked complex to catalyze photodependent production of NADPH via ferredoxin and ferredoxin $NADP^+$ reductase was abolished. This is thought to be an artifact of the crosslinking procedure that results in internal crosslinks in PSI subnit PsaD, the binding site for ferredoxin. The rate of NADPH formation was 355 nmol $(mg\ chl)^{-1}\ min^{-1}$ with the native enzymes. This is approximately 10 times faster than the rate of H_2 formation by the metallized PSI complex.

The data reported demonstrates that a system composed of Na ascorbate + PC + platinized PSI is a biomimetic photosynthetic system. It is among the class of biomimetic structures that used extracted functional biomolecules to perform simplified and alternative versions of photosynthesis. The simplification is predicated on fewer components accompanied by an alternative conversion of light energy into chemical energy. Chemically cross-linking PC and PSI can increase the photocatalytic production of hydrogen compared to a system in which these two biomolecules are not crosslinked.

Open-circuit Photovoltage Measurements

In this study an experimental approach was developed that directly measured the light induced electrostatic potential at the gas liquid interface (Lee et al., 2003). The combined techniques of atomic force microcopy and scanning surface probe microscopy were used to measure the open-circuit photodependent voltages of PSI in solution. Previous studies carried out by our research group on the photovoltaic properties of PSI were carried out with single PSI molecules adhered to atomically flat gold. In these

studies the PSI molecules were dried on the gold surface before measurements were made (Lee et al., 1995; 1997; 2000).

Microchannel glass was used as the sample holder and delivery system for microcantilever sensing of molecules at the gas-water interface. It has hollow channels arranged in a 2D hexagonal close packed array. The key advantage of this material is that it minimizes liquid movement so the SSPM technique can be used. The microchannel glass substrate was ~400μm thick and the channels had a diameter of 5-6μm. The fabrication of the channeled glass (Tonucci et al., 1992) and the procedure for the isolation of spinach Photosystem I from thylakoid membranes were previously described (Owens et al., 1987). A modified Nanoscope IIIa (Digital Instruments) was used to measure the surface potentials. Two sources of light illuminated the PSI preparation. One was an external diode laser (670 nm, 3.98 mW) that could be turned on or off. The second laser light source was an integral part of the optical technique that was used for cantilever measurement. Therefore, it was not possible to obtain a true dark value for the surface potentials of PSI sample.

The SSPM image of the microchannels with nanopure water indicated that the electrostatic potential was slightly negative. This suggested that the H_2O molecules were oriented with their O atoms towards the air interface and the OH groups towards the bulk liquid. In the case of the PSI solution, the sample was illuminated with a diode laser during the measurement. The size of the electrostatic potential above the PSI solution was opposite to that measured over the pure water surface. The surface potential of pure water and different PSI preparations was measured as a function of time (Figure 3). For data acquisition, the zero vertical position of the probe was first established on the glass

surface. The probe was then moved over the central region of the microchannel well and the surface potential was measured versus time. The surface water potential of pure water was acquired over a 30 sec period and was -30 mV (Figure 3A). A PSI sample stored for 6 months at 4^{o}C exhibited a +58 mV relative to the substrate (Figure 3B). However, a 1 month old sample had a surface potential of +150 mV (Figure 3C). This demonstrates that the photovoltaic properties of PSI changed over time. In the absence of the external diode laser the surfaces of the PSI solution were 20 mV less negative suggesting that the electron-transport vector of the reaction centers point towards the bulk water phase.

This work demonstrates that the pores of microchannel glass can be used to immobilize columns of liquid to study electrostatic potentials of interfacial photoactive biomolecules. It provides a new analytical method to characterize electrostatic features of single molecules in solution and the dynamic forces that act at the air-liquid interface. Quantitative light-induced electrostatic measurements of PSI were performed using an AFM and SSPM. By use of the combined AFM/SSPM technique, absolute orientation of the photon-activated electron transport vector could be deduced from the sign of the photovoltage. The presence of the illuminated PSI reaction centers at the gas-water interface has a dramatic effect on the sign of the interfacial surface potentials.

Discussion

Two methods have been presented to investigate of the photovoltaic properties of PSI reactions centers in a solution environment. The first method demonstrates how a biomimetic photosynthetic system that is comprised of PSI, PC, Na ascorbate and Na_2PtCl_6 can be used to measure the closed circuit photovoltaic properties of PSI

molecules in solution. In this system hydrogen evolution is used as an effective ammeter and voltmeter to measure the rate of electron transfer and energetics of the reaction. The reduction of H^+ to H_2 requires a potential more negative than -0.4 V (versus the standard H_2 electrode). The terminal e- acceptor on PSI has a potential of approximately -0.6 V (vs. SHE) at the reducing side of PSI. The data show that crosslinking PSI and PC increases the rate of electron transfer by removal of the diffusion-limited intermolecular electron transfer step. The observed rates appear to be supported by kinetic analysis of free and cross-linked PC electron transfer reported in the literature (Drepper et al., 1996). In addition, the effect of the initial concentration of Na_2PtCl_6 on the pathway of electron flow shows that H^+ and Na_2PtCl_6 are in competition to sequester the high energy electrons generated at the reducing end of PSI molecules. This is a very powerful and simple method to investigate the photoelectronic properties of PSI molecules in their nonnative environment.

The second method presented demonstrated how scanning probe techniques can be used to measure the open circuit photovoltages of PSI molecules in solution. In this work a combination of atomic force microscopy and scanning surface probe microscopy was used to compare the surface potentials of water and PSI preparations at different intervals after purification. It was carried out using microchannel glass to create a gas-liquid interface that was stable enough to allow the SSPM technique to be used. Pure water exhibited a slightly negative potential similar to previously described work. In contrast the PSI sample exhibited a positive surface potential. In addition, the magnitude of the potential decreased as the PSI sample aged over 6 months. One limitation of the study that was carried out was that it was not possible to carry out a light versus dark

measurement of the photovoltages of PSI. This was due to the configuration of the instrument. A laser light source is required to as part of the optical technique used for cantilever measurement. This is intrinsic to the measuring techniques and caused scattering and multiple reflections within the microchannel plate. This prevented a true dark value being obtained for the surface potentials in the presence of PSI. Black microchannel glass was not available for these experiments. This would decrease scattering phenomena and reflections and may lessen the contribution of this light source on the measurement of the photovoltaic properties of PSI.

Electrically active photosynthetic protein complexes such as PSI will form the basis for a new generation of solid-state devices such as photodetectors and photovoltaic cells. It is very important to understand the molecular interactions at the interface of hard materials and soft materials to orient and spatially arrange the protein molecules such that they create a functional nanostructured device. This is a 'bottom-up' approach in that it deals with building nano-structured devices from the molecular to the system level. The techniques described here to measure the open and closed circuit photovoltages of PSI molecules in solution have a very important role in attaining these goals. It allows functional changes to the molecules to be assessed in their native environment before progressing to the solid state.

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Figure list

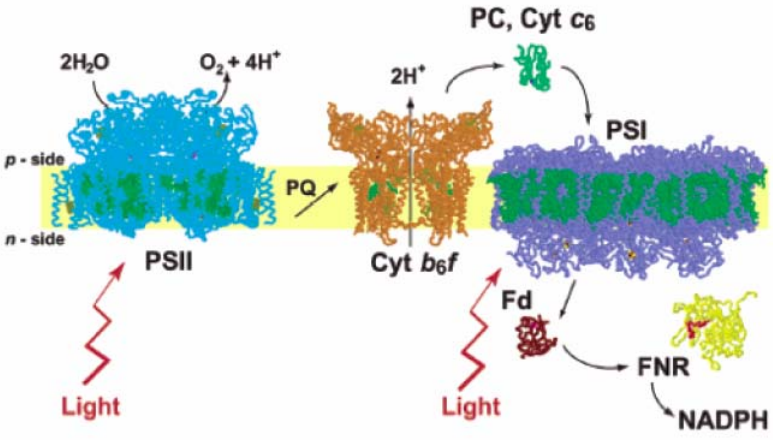

Figure 1. Schematic illustration of the molecular architecture of the photosynthetic membrane. The transmembrane protein complexes, PSI and PSII, operate in series to oxidize water to O_2 and reduce $NADP^+$ to NADPH. PC is the water-soluble electron relay that transports electrons from cytochrome b_6f to PSI. [Adapted from Kurisu et al., 2003. Copyright 2003 American Association for the Advancement of Science. Used by permission.]

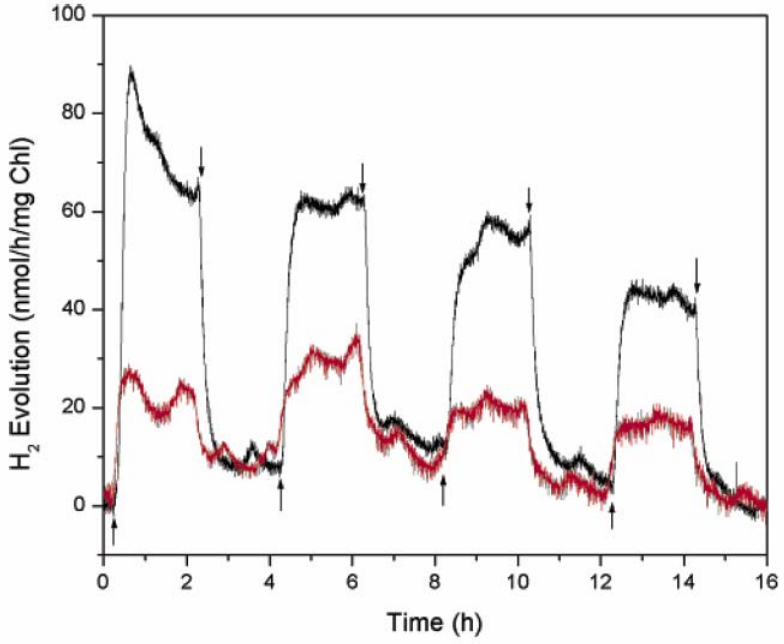

Figure 2. Photoevolution of hydrogen during alternating periods of illumination and darkness. Upper curves: cross-linked PC-PSI; lower curves: free PC and PSI. The reactions contained PSI (143 μg chl), 16.3 nmol PC, 8 mM Na ascorbate, 0.5 mM Na_2PtCl_6, and 50 mM sodium phosphate buffer pH 7.1 in a 15 ml reaction. Red light (531 uE/m^2/s) was used to illuminate the sample with a 2-h-on/2-h-off cycle. The vessel was thermostatted at 25^{o}C. [Adapted from Evans et al., 2004. Copyright 2004 American Association for the Advancement of Science. Used by permission]

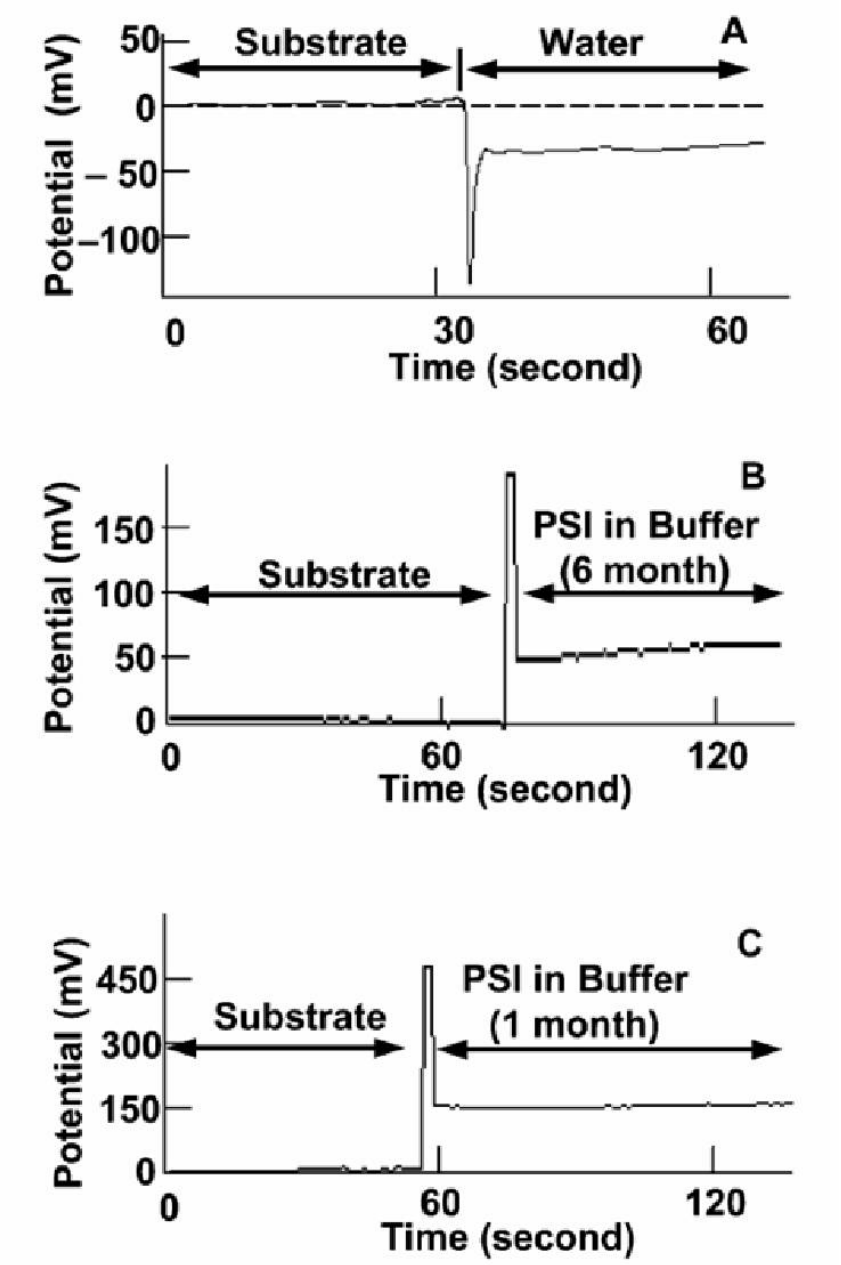

Figure 3. (A) The surface potential of a centrally located fixed point above the glass substrate and water in a microchannel as a function of time; similar measurements for a (B) six- and (C) one month old PSI solution stored at 4^{o}C. The sample was laterally positioned for measurement above the glass or an aqueous microchannel. [From Lee et al., 2003. Copyright 2003. American Chemical Society. Used by permission]