

Introduction

Photosynthetic reaction center (RC, Figure 1) is one of the most important proteins, because it is Nature's solar battery converting light energy into chemical potential in the photosynthetic membrane assuring conditions for carbon reduction in cells [1]. Although it is developed in nanometer scale, and is working in nanoscopic power, this is the protein that assures the energy input practically for the whole biosphere. The extremely large quantum yield of the primary charge separation (close to 100 %, [2]) offers a big challenge to use it in nano-devices. The key questions of researches these days are as follows: new applications, reproducibility of the measurements and stability – mainly that of the biological component. Results of optical and electro-chemical investigations on RC/ITO bio-nanocomposite materials will be presented here.

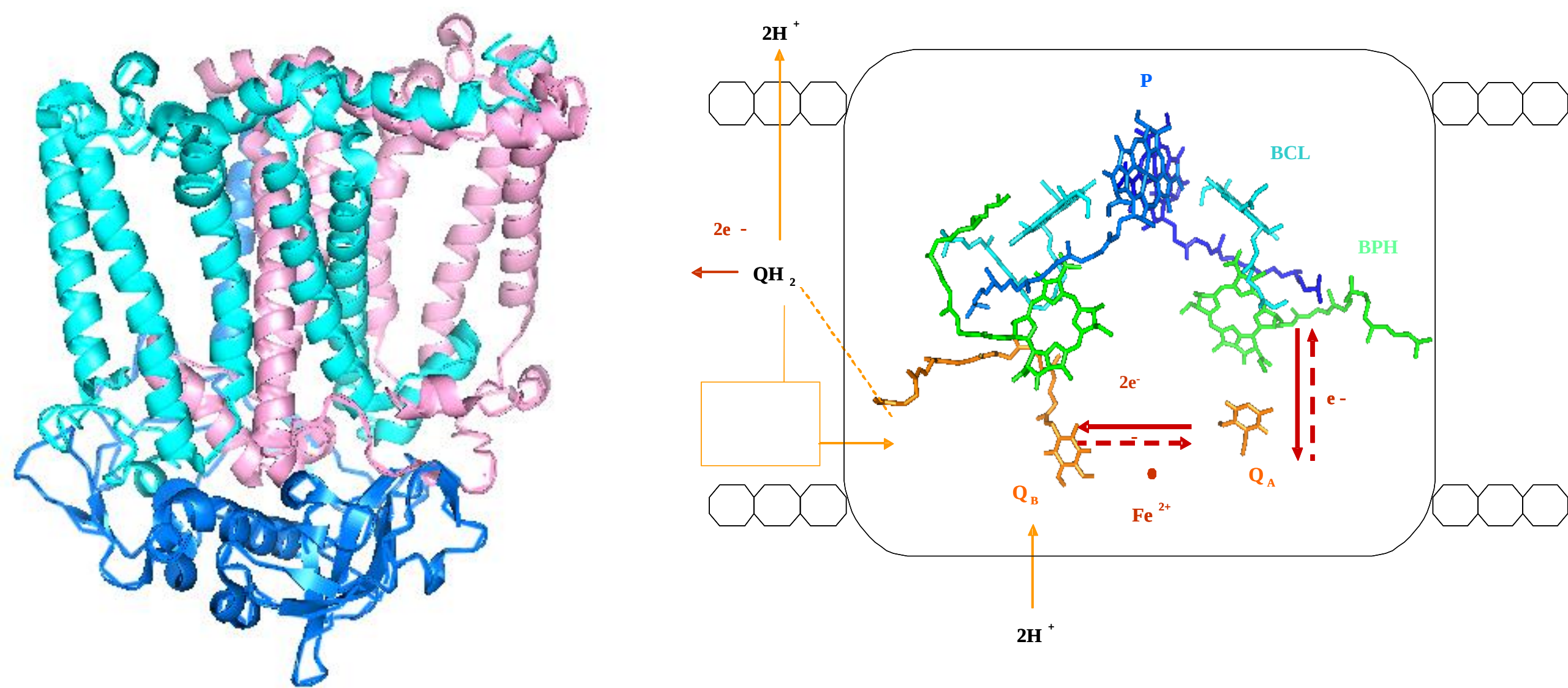


Figure 1. The protein subunits (left) and cofactors (right) of the photosynthetic reaction centers. The helix structures indicate the membrane spanning region of the protein. P: primary electron donor bacteriochlorophyll dimer; BCL: bacteriochlorophyll monomer; BPH: bacteriopheophytine; Q_A: primary quinone; Q_B: secondary quinone; QH: reduced quinone in the membrane pool.

Aims

It was shown recently [3] that RCs can be attached to CNTs and there is an electronic connection between the two types of materials.

We investigated the physico chemical properties (photochemical activity, electric conductivity) of the bio-nanocomposite materials prepared by depositing RCs onto ITO surface.

Our aim is to show the direct electronic connection between RCs and ITO on dried samples and mediator helped connection in electrochemical cell.

Experimental

Sample preparation

RCs were prepared by detergent solubilization and purified by ammonium sulfate precipitation, followed by anion-exchange chromatography. Calculated amount of RCs were dried on the surface of ITO.

Measurements

Flash-induced absorption change was measured by a single-beam kinetic spectrophotometer of local design. The redox changes of the primary bacteriochlorophyll dimer, the electrochromic response of the absorption of bacteriopheophytins were detected at 860 or 771 nm, respectively [3,4].

Light induced change in the conductivity of RC film deposited on ITO surface was measured by a Keithley 2400s multimeter (7 digits, 4 points connection). 10mA current was set and the voltage was measured. Data are represented in ohms. Measurement was regulated through the USB port by using the home developed software (<http://www.noise.physx.u-szeged.hu/>).

Special electrochemical cell with three electrodes was designed in our laboratory. The two parts of the cell was separated by a membrane with a 10 kDa cut-off. The working, the counter and the reference electrode was ITO, platinum and Ag\AgCl, respectively. The RC concentration was 6 μ M and horse heart cytochrome c and UQ-0 was used as mediators.

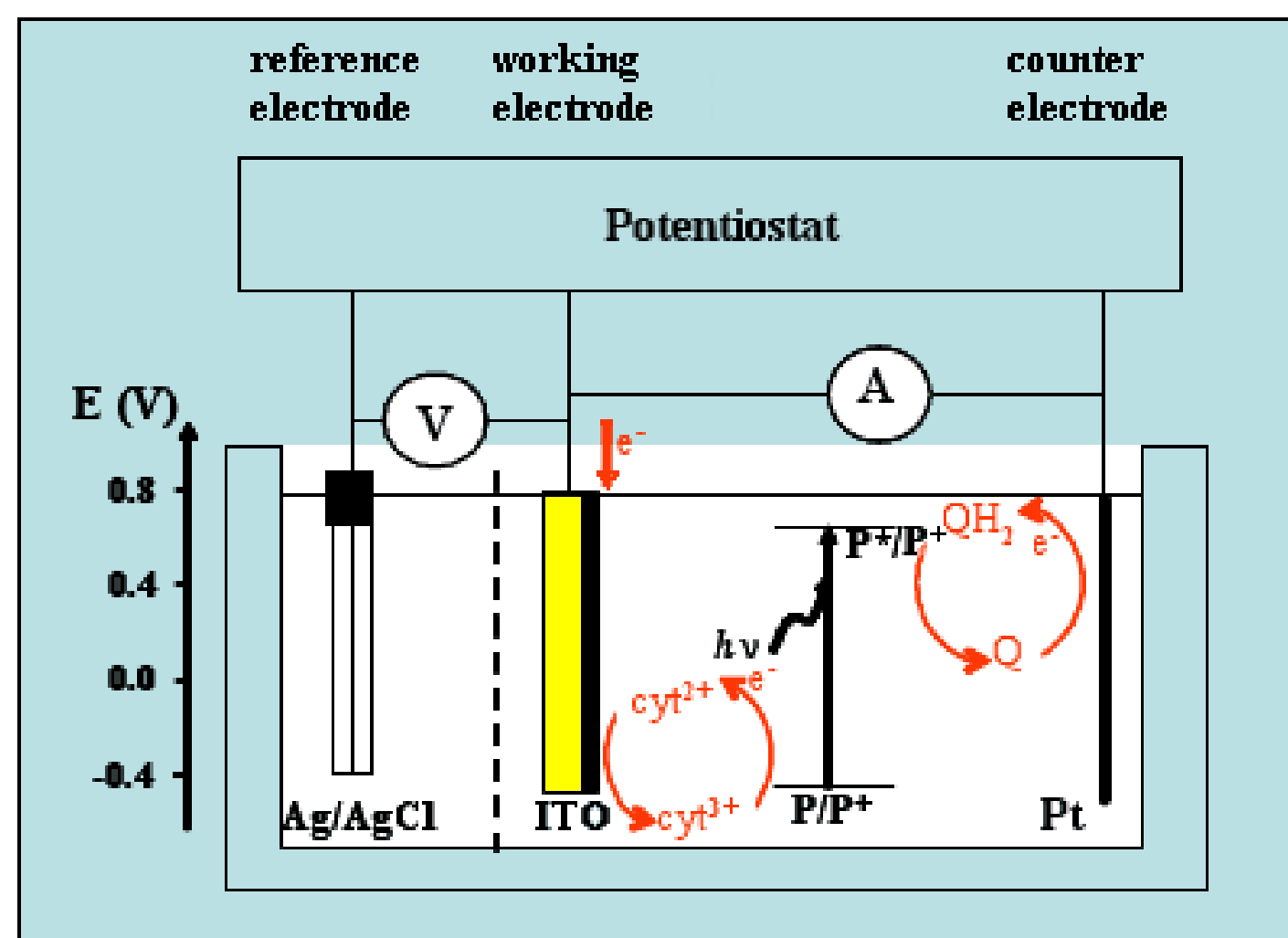


Figure 2. The scheme for the electrochemical cell

Bibliography

- [1] Nagy et al. (2010) Notulae Scientia Biologica, 2, 07-13.
- [2] Wraight, C.A. and Clayton, R. (1974) Biochim. Biophys. Acta, 333, 246.
- [3] Dorogi et al. (2006) J. Phys. Chem. B., 110, 21473-21479.
- [4] Nagy et al. (2004) Biochemistry, 43, 12913-12923.

Results and discussions

Interaction between the RCs and conductive glass – ITO

Flash-induced absorption change

The submillisecond phase of the absorption change at 771 nm reports the electronic structure of the RC protein around the BPheo monomer. After charge separation there is a structural rearrangement of the hydrogen bonding network around the acceptor site. Figure 3 shows that the amplitude of the pheophytine shift is about two times larger when the RC is deposited on the ITO as compared to the glass surface.

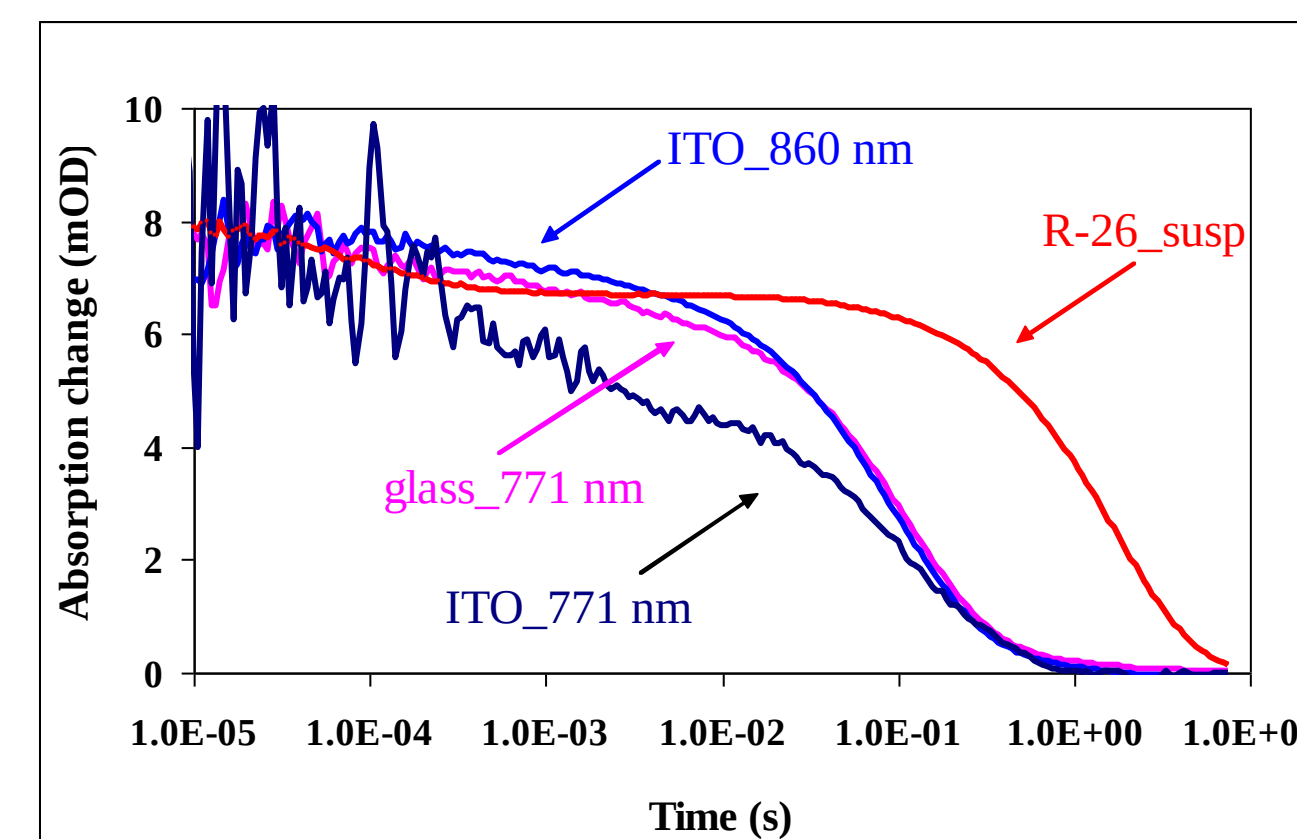


Figure 3. The absorption change of RCs after single saturating laser pulse in different surfaces as indicated.

Resistivity measurement on RCs dried on the surface of ITO

Figures 4-5 show that there is an electric interaction between the RCs and the conductive ITO layer. The electric resistance decreases if the pure ITO is illuminated. The decrease is smaller if RCs are deposited on the surface and reaches a saturation level by increasing the RC concentration. After reaching the saturation, i.e. multiple layers are formed, the resistance increases mainly due to heat dissipation. If the photochemically inactive BChl was deposited the resistance increases linearly with the concentration indicating only the temperature effect.

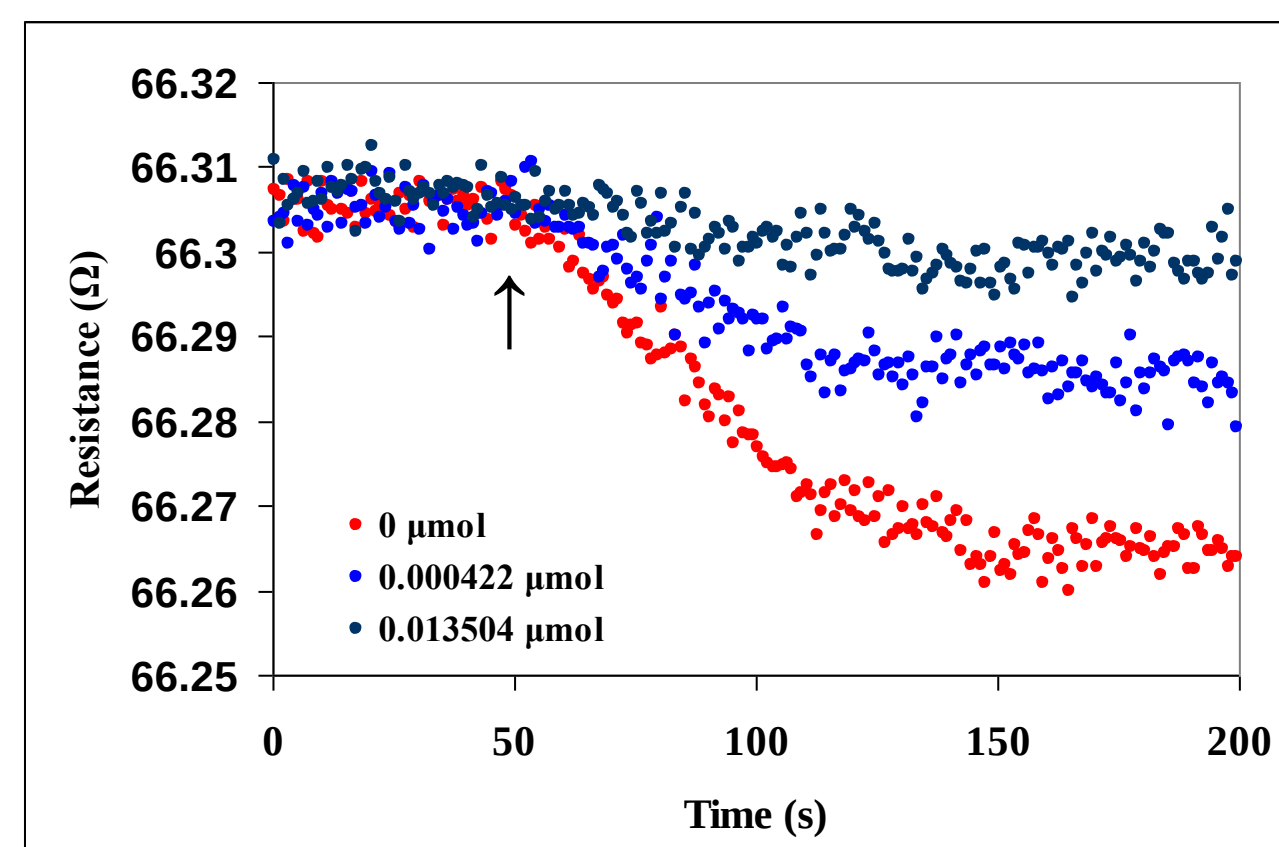


Figure 4. Resistance of ITO with or without RCs as a function of time under light excitation. The concentration of RCs and the time when the light was switched on are indicated.

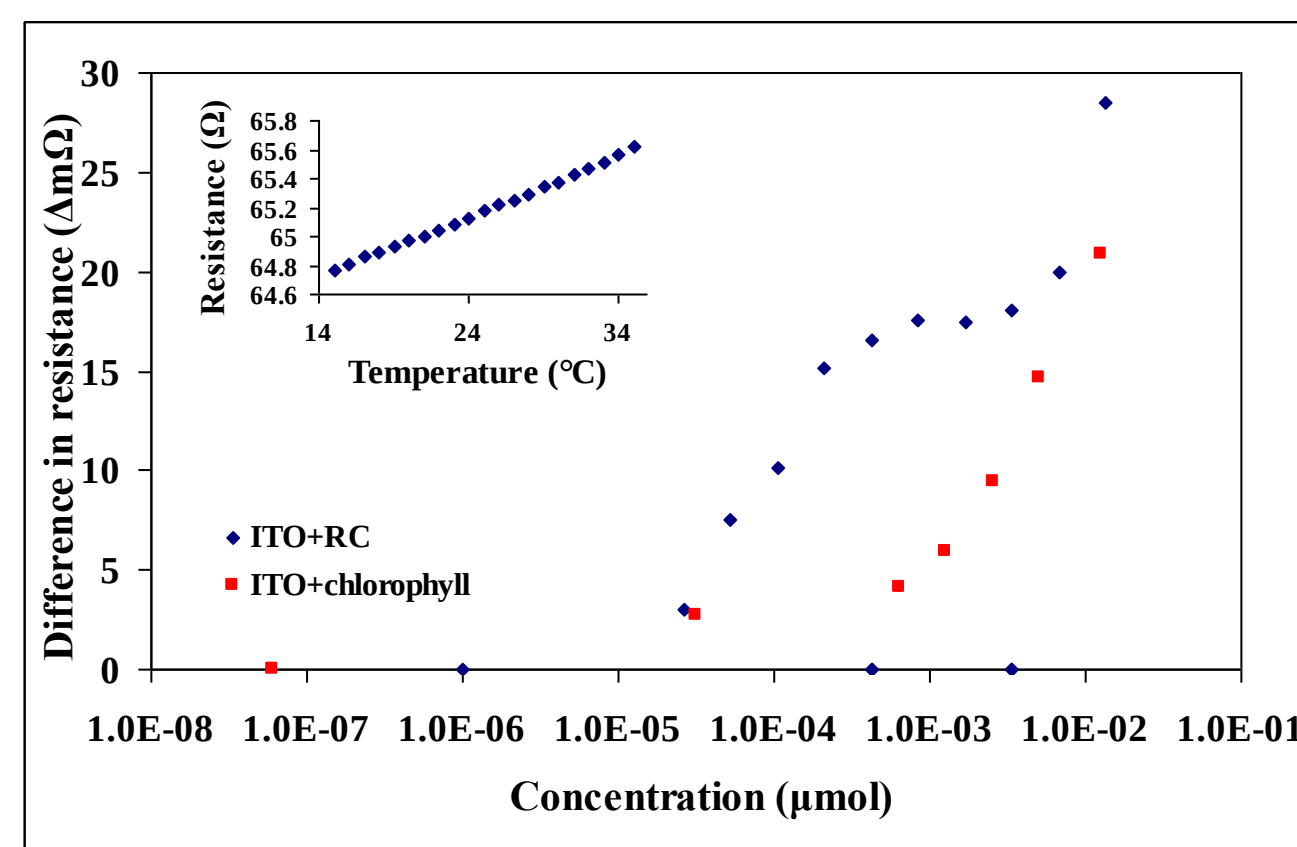


Figure 5. Change of the resistance of ITO as a function of the RC (blue) and bacteriochlorophyll (red) concentration. Insert shows the resistance of ITO as a function of the temperature.

Photocurrent of electrochemical cell containing RC

All the measurements were done at the dark equilibrium open circuit potential. After the system has reached its equilibrium, it was illuminated for 400 s with red light ($\lambda > 700$ nm). Figure 6 shows that starting the illumination the magnitude and the direction of the current depends on the type of the mediator used. In the presence of excess mediator the integral of the curve is the function of the RC concentration. After the RC turnover is saturated there is an undefined phase that needs further investigation for better understanding. If both the donor and the acceptor sides are reconstituted (i.e. cytochrome c and quinone are present in the same time, Figure 7) the current direction is determined by the mediator which is present in limiting concentration.

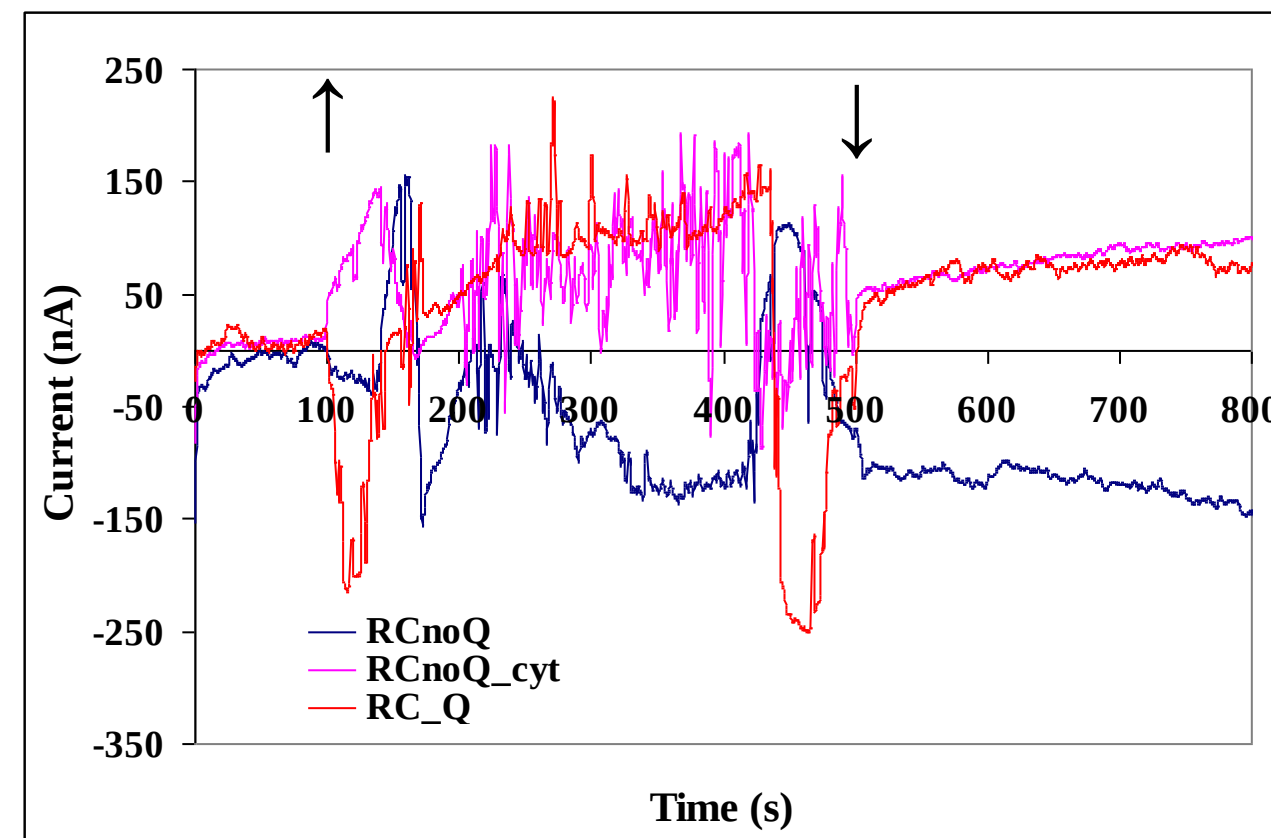


Figure 6. Photocurrent of the electrochemical cell containing quinone depleted RC (blue), quinone depleted RC with cytochrome c (pink), RC with quinone (red) as a function of time at open circuit potential. The concentrations of cytochrome c and quinone were 80-80 μ M, respectively. The time when the light was switched on(↑) and off(↓) are indicated.

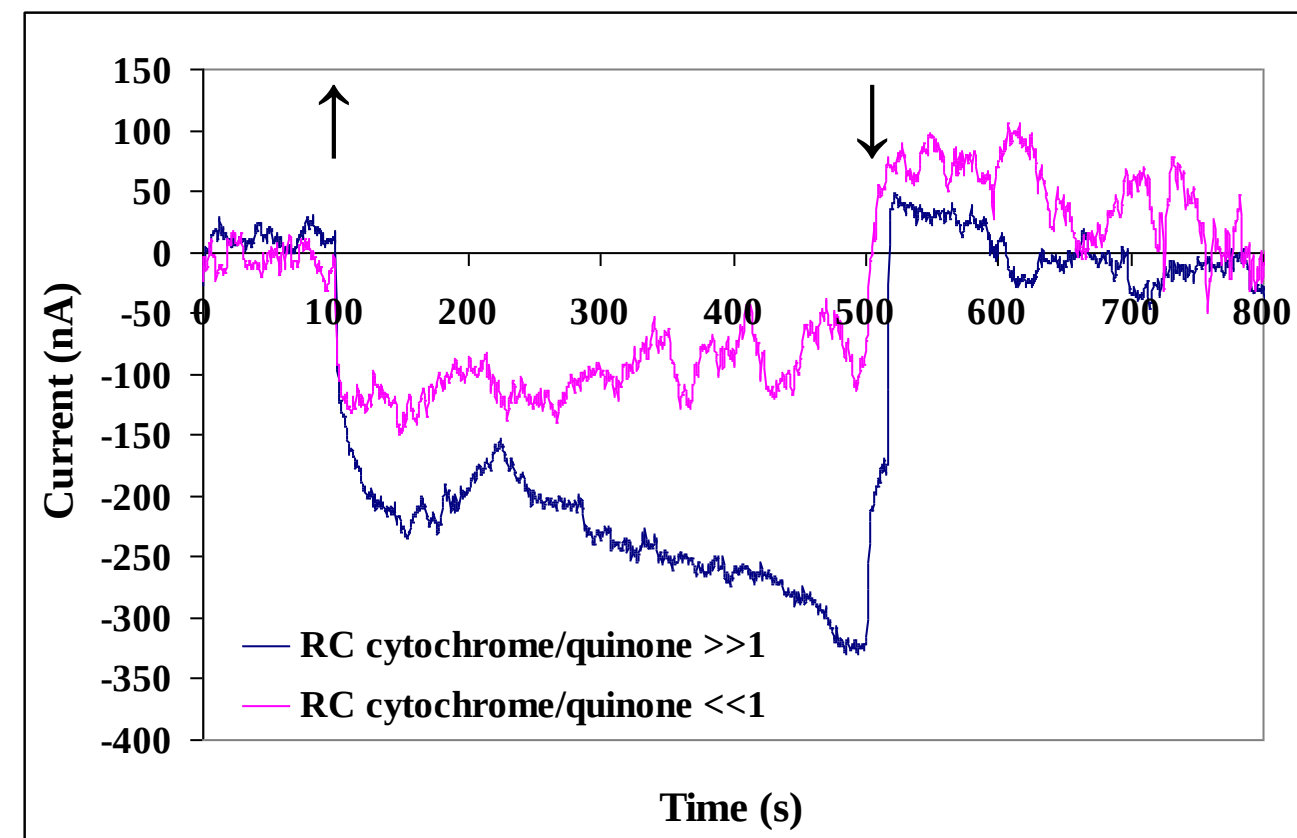


Figure 7. Current of RC with quinone and cytochrome c containing electrochemical cell as a function of time at open circuit potential in case of cytochrome c (blue) and quinone (pink) predominance. The concentrations of cytochrome c and quinone were sequentially 80 μ M and 10 μ M, and in the second case 10 μ M and 80 μ M. The time when the light was switched on(↑) and off(↓) are indicated.

Summary

Our results indicate that

- Resistivity measurements on dried samples indicate that there is a direct electric connection between the RCs and the ITO, which was further confirmed by optical investigations.
- The magnitude of the electrochromic shift in the absorption accompanying the Q_A⁻ to Q_B indicates that the local electrostatics is more pronounced on the ITO compared to the glass surface.
- A direct and mediator assisted photocurrent was measured in electrochemical cell containing RCs. The direction of the photocurrent depends on the donor or the acceptor side reconstitution.