Journal Name

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COMMUNICATION

Photo-electrochemical communication between cyanobacteria (*Leptolyngbia* sp.) and osmium redox polymer modified electrodes

Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

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Electronic Supplementary Information (ESI):

Chemicals

Diuron [(1,1-dimethyl, 3-(3', 4'-dichlorophenyl) urea)], sodium phosphate dibasic, and sodium phosphate monobasic, magnesium chloride, sodium chloride and potassium hexacyanoferrate (III) were purchased from Sigma-Aldrich (Munich, Germany) and Merck (Darmstadt, Germany) and were of either research or analytical grade. All aqueous solutions were prepared by using water purified and deionized (18 MΩ) with a Milli-Q system (Millipore, Bedford, MA, USA).

Osmium redox polymer

[Os(4,4´-dimethoxy-2,2´-bipyridine)$_2$(poly-vinylimidazole)$_{10}$Cl]$^{2+/+}$, E°´ = -70 mV vs. Ag|AgCl (3 M KCl) Os-A[1,2], [Os(4,4´-dimethyl-2,2´-bipyridine)$_2$(poly-vinylimidazole)$_{10}$Cl]$^{2+/+}$, E°´ = 120 mV vs. Ag|AgCl (3 M KCl) Os-B[3], [Os(2,2´-bipyridine)$_2$(poly-vinylimidazole)$_{10}$Cl]$^{2+/+}$, E°´ = 220 mV vs. Ag|AgCl (3 M KCl) Os-C[4] and [Os(4,4'-dichloro-2,2'-bipyridine)$_2$(PVI)$_{10}$Cl]$^{2+/+}$, E°´ = 350 mV vs. Ag|AgCl (3 M KCl), Os-D[2,5] were synthesized and reported as described previously in the literature.

Measurements and instrumentation

All electrochemical experiments (cyclic voltammetry, CV and chronoamperometry, CA) were carried out using a PalmSens potentiostat (model Emstat^2, Palm Instruments BV,Utrecht, The Netherlands) equipped with PSTrace software with a conventional three electrode configuration; a Ag|AgCl (sat. KCl) (Sensortechnik, Meinsberg, Germany), a bare/polymer modified graphite (active surface area A = 0.0731 cm^2) and platinum foil served as the reference, working and counter electrodes, respectively. A Metrohm 827-pH lab meter (Metrohm AG, Herisau, Switzerland) was used for setting the pH values of the solutions. In order to perform photo-electrochemical experiments, a fibre optic illuminator FOI-150-220 (150 W and 220 V) with FOI-5 Light Guide (Titan Tool Supply Inc., Buffalo, NY, USA) was used to illuminate the electrode surface. The illuminator was adjusted using a light intensity meter (Techtum Lab AB, Umeå, Sweden). To excite the photosynthetic activity of CYN82, a light intensity of 44 mWcm^{-2} was used. Phosphate buffer (5 mM NaH_2PO_4, 5 mM Na_2HPO_4, 10 mM NaCl, 5 mM $MgCl_2$, at pH 7.0) was used as an electrolyte in all these studies. The electrolyte solutions were

degassed with pure argon for ≈10 min before measurements, which were performed at room temperature. All reported data were based on three independent experimental replicas. All potential mentioned in this manuscript is according to Ag|AgCl (sat. KCl) as a reference electrode.

Modification of working electrode

Graphite rods (Alfa Aesar GmbH & Co KG, Karlsruhe, Germany, AGKSP grade, ultra ″F″ purity, and 3.05 mm diameter) were used for making the working electrodes. The end of the graphite rod was polished on fine emery SiC paper (Turfbak Durite, P1200), carefully washed with Milli-Q water, and finally dried. Then an aliquot of 5 μL of an ORP solution (10 mg mL^{-1} in Milli-Q water) was spread onto the entire active surface of the electrode (0.0731 cm^2). Afterwards the electrode was dried at room temperature for 10 to 15 min and then 9.5 μg of CYN82 was spread onto the surface. Before use, a dialysis membrane (Spectrum Laboratories Inc., Rancho Dominguez, CA, USA, molecular mass cut-off: 6000–8000) was used to keep the ORP and CYN82 on the electrode surface. The dialysis membrane (pre-soaked in phosphate buffer at pH 7.0) was pressed onto the electrode and fixed tightly with a rubber O-ring and Para film. Note that the amount of ORP (5 μL) and CYN82 bacteria (9.5 μg) are the optimized standards.

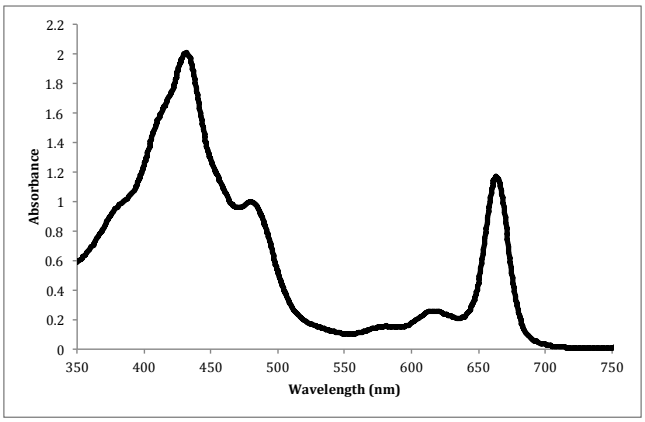
CYN82 growth condition and inoculum preparation

The cyanobacterium investigated in this work is *Leptolyngbia* sp. CYN826 and was collected from the Cawthron Institute Collection Culture of Microalgae (CICCM), New Zealand. The growth and culture condition of CYN82 reported by Luimstra et al[6]. In brief a low ionic strength MLA medium was used as growth medium and a light source of 40 μmol photons m^{-2} s^{-1} from a white cool fluorescent lamp was arranged over the culture with a regime of 12:12 light and dark. The culture growth was maintained at room temperature (≈ 21° C). To harvest the CYN82, the cells were centrifuged at 4000 rpm for 10 min at 20° C and later on washed with electrolyte and centrifuged again at the same condition. Finally, the CYN82 cells were re-suspended in the same electrolyte to adjust the concentration at 1 g/ml and used immediately for electrochemical measurements. MLA is a complex growth medium that is comprised of $NaNO_3$ (2.00 mM), $NaHCO_3$ (2.019 mM), $MgSO_4.7H_2O$ (200.43 μM), $CaCl_2$ $2H_2O$ (200 μM), K_2HPO_4 (199.77 μM), NaEDTA (11.7 μM), H_2SeO_3 (10.00 μM), H_3BO_3 (39.95 μM), $MnCl_2$ $4H_2O$ (18.19 μM), $FeCl_3$ $6H_2O$ (5.85 μM), $CuSO_4$ $5H_2O$ (40.1 pM), $ZnSO_4$ $7H_2O$ (76.5 pM), $CoCl_2$ $6H_2O$ (79.86 pM), Na_2MoO_4 $2H_2O$ (24.8 pM), biotin (0.05 μg/L), vitamin B_{12} (0.05 μg/L), and thiamine HCl (100 μg/L)7.

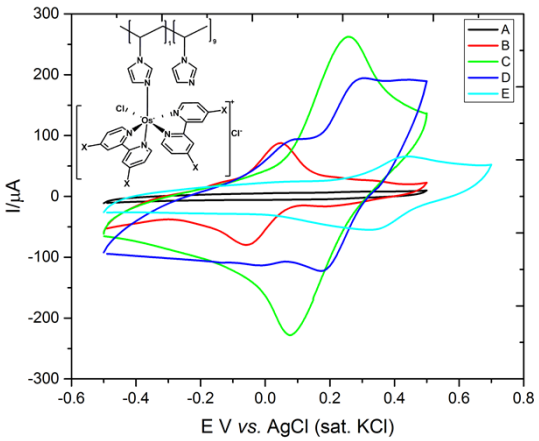
Photosynthetic dye extraction

For chlorophyll extraction the cells were spun down for 5 min at 4000 rpm and 5 mL of a 7:2 acetone/methanol solution was added to the pellet. The mixture was then incubated over night under slow shaking. The green supernatant was then either added into an extractor or the spectrum was immediately determined with a spectrophotometer.

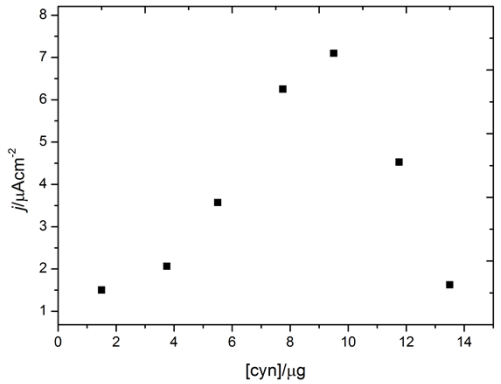
Supplementary figure



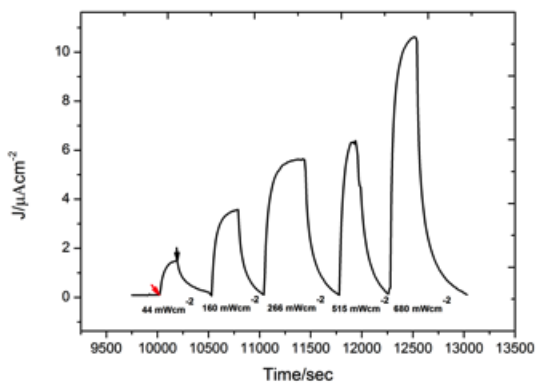
Supplementary Figure 1. Absorbance spectrum of CYN82. Photosynthetic pigment extracted from *Lyptolyngbia* sp. (CYN82). The distinguished peak of chlorophyll a appears at 665 nm wavelengths and chlorophyll b, carotenoid at 400 nm.



Supplementary Figure 2. The cyclic voltammograms (CVs) of graphite electrode modified with different osmium redox polymers (ORP); (A) bare graphite electrode; (B) Os-A; (C) Os-B; (D) Os-C; (E) Os-D. Inset shows the general chemical structure of osmium redox polymer, where Os-A (X = OCH_3), Os-B (X = CH_3), Os-C (X = H) and Os-D (X = Cl).

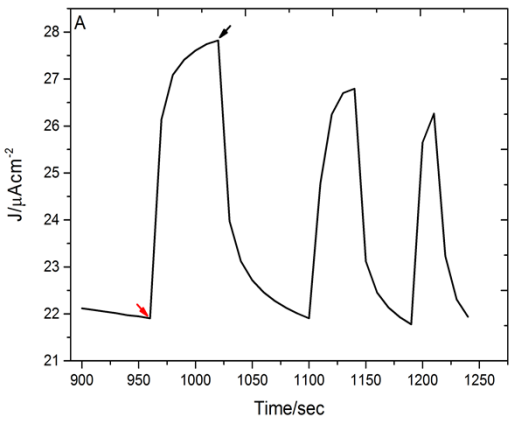


Supplementary Figure 3. The optimization of CYN82 concentration over Os-C polymer modified electrode, CYN82 concentration varied from 1.50, 3.75, 5.50, 7.75, 9.50, 11.75 and 13.50 µg and optimized concentration fixed at 9.50 µg, applied potential: 350 mV vs Ag|AgCl (sat. KCl), electrolyte: 10 mM phosphate buffer at pH 7.0, 10 mM NaCl and 5 mM $MgCl_2$, light intensity: 44 $mWcm^{-2}$.

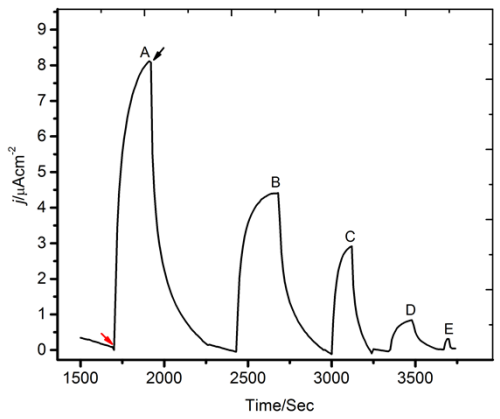


Supplementary Figure 4. The effect of light intensity on photocurrent generation. The figure shows background corrected (light off conditions) current density. The light intensity raises from 44, 160, 266, 515 and 680 $mWcm^{-2}$ that results photocurrent increases in 2.32, 3.87, 5.46, 6.00 and 9.21 μAcm^{-2}. CYN82 (1.50 µg) immobilized on Os-C modified eletrode, applied potential: 350 mV vs Ag|AgCl (sat. KCl), electrolyte: 10 mM phosphate buffer at pH 7.0, 10 mM NaCl and 5 mM $MgCl_2$, light intensity: 44 $mWcm^{-2}$, black and

red arrow stand for light off and on phenomena and valid to all curves.



Supplementary Figure 5. Photocurrent generation from ferricyanide (1 mM) mediated electron transfer. CYN82 (9.50 μg) immobilized on bare graphite electrode, applied potential: 350 mV vs Ag|AgCl (sat. KCl), electrolyte: 10 mM phosphate buffer at pH 7.0, 10 mM NaCl and 5 mM $MgCl_2$, light intensity: 44 $mWcm^{-2}$, black and red arrow stand for light off and on phenomena and valid to all curves.



Supplementary Figure 6. The inhibition of photocurrent by diurion, a specific inhibitor for photosystem II. The figure shows background corrected (light off conditions) current density. The diuron concentration increases from 0.2, 0.3, 0.4 and 0.5 mM and consequences of photocurrent down to 4.78, 3.27, 1.20 and 0.65 μAcm^{-2}, while non-inhibited photocurrent was 8.52 μAcm^{-2}. CYN82 (9.50 μg) immobilized on Os-C modified electrode, applied potential: 350 mV vs. Ag|AgCl (sat. KCl), electrolyte: 10 mM phosphate buffer at pH 7.0, 10 mM NaCl and 5 mM $MgCl_2$, light intensity: 44 $mWcm^{-2}$, black and red arrow stand for light off and on phenomena and valid to all curves.

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