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A conventional symmetric biosupercapacitor based on rusticyanin modified gold electrodes

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Abstract: Here we report on an entirely new kind of bioelectronic device – a conventional biosupercapacitor, which is built from copper containing redox proteins. Prior to biodevice fabrication, detailed spectroelectrochemical studies of the protein, viz. *Acidithiobacillus ferrooxidans* rusticyanin, in solution and in adsorbed state, were performed, including estimation of the redox potential of the T1 site (0.62 V vs. NHE), protein midpoint potential when adsorbed on a self-assembled monolayer (0.34 V vs. NHE), as well as biocapacitance of rusticyanin modified gold electrodes (115 $\mu\text{F cm}^{-2}$). The symmetrical biosupercapacitor based on two identical gold electrodes modified with rusticyanin is able to capacitively store electricity and deliver electric power accumulated mostly in the form of biopseudocapacitance, when charged and discharged externally. When charged during just 5 sec, the biosupercapacitor with a total capacitance of about 73 $\mu\text{F cm}^{-2}$ provided a maximum of 4 $\mu\text{A cm}^{-2}$ peak current at 0.40 V. The biodevice, which can be charged and discharged at least 50 times without a significant loss of ability to store electric energy, had a low leakage current below 50 nA cm^{-2} .

Keywords: biosupercapacitor; biopseudocapacitance; direct electron transfer; double-layer capacitance; rusticyanin

1. Introduction

Recently an entire new class of bioelectronics devices, *viz.* self-charging biosupercapacitors, has been disclosed [1]. These hybrid biodevices are able to convert different types of energies into electric energy and simultaneously store the electric power in the same volume used for conversion. Nowadays chemical [2-7] and solar [8, 9] biodevices are known. In chemical biosupercapacitors redox active proteins with catalytic activities (*i.e.* redox enzymes), electronically wired to the electrode surface, are used to accelerate energy transformation, whereas their potential biosupercapacitive properties do not play an actual role in biodevice function. Even though biosupercapacitive properties of redox proteins, *i.e.* faradaic contribution to the total electrode capacitance due to their intrinsic redox activities [10] attributed to quasi-reversible or even reversible electrochemistry of surface confined biomolecules [11, 12], are well-known [1], a conventional biosupercapacitor, *i.e.* incapable of self-charging, in which the biopseudocapacitance is exploited (*i.e.* redox proteins serve as the pseudocapacitive component determining the total capacitance of the biodevice) has not been fabricated and characterized so far, as illustrated below chronologically.

The fundamental concept of a conventional biosupercapacitor was demonstrated as early as 2012 by Malvankar and co-workers, using a bioanode which was able to store electrical charges in the cytochrome *c* network of a bacterial biofilm [13]. Moreover, due to the special design of the electrode, which consisted of two gold anodes separated by a non-conducting gap, successful galvanostatic charge/discharge measurements of the biodevice were performed. However, a biofilm with catalytic activities and thus ability for self-charging was exploited to store electrical charges. Moreover, the term “biosupercapacitors” was actually not used in this study, which nevertheless should be highly valued and considered as the pioneering report in the field. The term “biosupercapacitor” was used one year later by Sattarahmady with co-workers to emphasize the biocompatibility of a double-layer supercapacitor based on electrodes coated with a redox inactive protein – albumin [14]. Nowadays, because of significant interest in implantable bioelectronics in general, and implantable biosupercapacitors in particular [15], this technological approach is widely used to design biocompatible supercapacitors using different proteins including redox active molecules, like myoglobin, hemoglobin, and cytochrome *c* [16, 17]. In addition to biocompatibility, biodevices based on protein modified electrodes often show superior properties in term of total capacitance [16, 17], unexpectedly, even when redox inactive proteins, like albumin and fibroin, are used [14, 18]. Of course, redox activity of proteins containing only amino acids is known for decades [19, 20]. Moreover, it has

recently been shown that redox active proteins are surprisingly good electric conductors, resembling solid-state semiconductors [21, 22]. Nevertheless, it is commonly accepted that proteins, when adsorbed on electrode surfaces, significantly decrease total capacitance due to an insulating layer formed, when redox inactive proteins are used or when direct electron transfer reactions between redox active proteins and electrodes are not established [1, 23]. Thus, despite the fact that several highly capacitive protein modified electrodes have already been disclosed, conventional biosupercapacitors (*i.e.* based on the biosupercapacitive properties of redox proteins) have not yet been realized. Here we demonstrate a complete functional conventional biosupercapacitor based on gold electrodes modified with a copper containing redox protein, rusticyanin (Rc). Whereas there are many papers regarding the electrochemistry of cupredoxins, *e.g.* azurin [24, 25] and plastocyanin [26], as well as pseudoazurin, umecyanin, stellacyanin, and plantacyanin [27], only two electrochemical studies of Rc are reported in the literature, *i.e.* Refs. [28, 29]. While Alcaraz with co-workers carried out electrochemical investigations of the protein in solution [28], the only paper regarding electrochemistry of immobilized Rc, *viz.* Ref. [29], contains several contentious issues, which raise some doubts regarding the direct electric wiring of the protein to the electrode surface (*vide infra*). Thus, we begin our paper with detailed spectroelectrochemical studies of free and immobilized Rc, as the fundamental basis for its practical applications in bioelectronics.

2. Experimental

2.1. Chemicals and materials

$\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, KH_2PO_4 , NaCl , H_2SO_4 , $(\text{NH}_4)_2\text{SO}_4$, Whatman® cellulose DE52 and CM52, glutaraldehyde (grade II), mercaptopropionic acid (MPA), HAuCl_4 , $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$, and indium tin oxide (ITO) conductive glasses ($15\text{--}25\ \Omega\ \text{sq}^{-1}$) were purchased from Sigma Aldrich (Steinheim, Germany). 99.5% ethanol was acquired from CCS Healthcare AB (Malmö, Sweden). Argon (Ar) and oxygen (O_2) were supplied by AGA Gas AB (Sundbyberg, Sweden). All chemicals were of analytical grade and used without further purification. All solutions were prepared using water (H_2O) purified with the PURELAB UHQ II system from ELGA Labwater (High Wycombe, United Kingdom). Polycrystalline gold (Au) disk electrodes with a geometric area of $0.02\ \text{cm}^2$ were from Bioanalytical Systems (Indiana, USA).

2.2. Redox protein

Acidithiobacillus ferrooxidans Rc was isolated and purified according to a previously described protocol [30], modified by replacing the cation exchange resin cellulose CM52 with CM32. The highly purified preparation stored at -23 °C contained > 95% of Rc with a molecular mass of 17 kDa with a few minor protein impurities, as determined by SDS/PAGE (Supporting Figure S1). The protein concentration equal to 16 mg mL⁻¹ was determined using two independent methods, *viz.* in accordance with methodology presented in Ref. [31], as well as using extinction coefficients provided in Ref. [30].

2.3. Electrode preparation

Gold disk electrodes (Au electrodes) were used as working electrodes in fundamental studies. Prior to surface modification, the electrodes were polished with 0.3 µm agglomerated alpha alumina suspension from Struers (Copenhagen, Denmark), thoroughly cleaned with ethanol and ultrapure H₂O, followed by sonication using the XB2 ultrasonic cleaner. After polishing, the Au electrodes were cleaned electrochemically by performing cyclic voltammetry (CV) for 25 cycles from -0.2 V to 1.7 V *vs.* Ag|AgCl|3M NaCl in 0.5 M H₂SO₄.

For surface modification, MPA was used to form a self-assembled monolayer (SAM). The cleaned Au electrodes were immersed overnight in 10 mM MPA ethanol solution. The Au electrodes were then rinsed with ethanol and ultrapure H₂O. 1 µL of glutaraldehyde solution (25% in H₂O) was drop casted onto the chemically modified electrode surface followed by 2 µL of Rc solution. The Au electrodes were covered to prevent evaporation, left for 2 h at room temperature, and finally gently rinsed with ultrapure H₂O.

Au nanoparticles (AuNPs) modified ITO based electrodes were used to demonstrate the functionality of biosupercapacitors. To fabricate nanostructured electrodes, ITO conductive glasses were cut into chips of 10 mm × 25 mm × 1.1 mm. The following cleaning procedure involved sonication in ethanol and subsequently in methylene chloride, 15 min each, and additionally storing in ultrapure water for 20 min. 20 nm AuNPs were synthesized, concentrated, and characterized as previously described [32]. The dispersion of AuNPs was immersed in an ultrasonic bath for 20 min for an efficient homogenization. Afterwards, 5 µL of the dispersion were dropped on the ITO chips and dried in air. Rc was immobilized on the nanostructured Au surface as mentioned just above. The geometric area of AuNPs modified ITO electrode (ITO-AuNPs) with immobilized protein (ITO-AuNPs-SEM-Rc) was measured to be *ca.* 0.07 cm².

The electrochemically active (real) area of AuNP/Au was calculated from the experimentally measured charge (q_{real}) associated with the gold oxide reduction process performed by running CV in H₂SO₄. The theoretical charge density (σ_t) associated with the reduction of the gold oxide is $390 \pm 10 \mu\text{C cm}^{-2}$ [33]. The electrochemically active (real) area of ITO-AuNPs was calculated to be 2.47 cm^2 (Supporting Figure 2)

2.4. Redox titration

The redox potential of the T1 site of Rc was determined by mediated spectroelectrochemical redox titration following the procedure reported previously [34, 35]. The home-built spectroelectrochemical cell consisted of a 1 cm long capillary Au electrode with an inner diameter of 350 μm , serving both as the working electrode and as a cuvette with a total volume of just 1 μL . The input and output optical fibers (FCB-UV 400/050-2 and FCUV 200, respectively) were purchased from Ocean Optics (Dunedin, Florida, USA) and attached to the ends of the capillary. The system comprised a DH-2000 light source and an HR4000CG-UV-NIR spectrometer from Ocean Optics. The spectra were recorded with the Ocean Optics Spectra Suite software. The potential of the Au capillary electrode was controlled using a three-electrode potentiostat (Zata Electronics, Lund, Sweden). A platinum wire served as a counter electrode, and a Ag|AgCl|3M NaCl electrode, separated from the enzyme solution by two ceramic frits and a buffer solution salt bridge, was used as a reference electrode. The working capillary Au electrode was cleaned for *ca.* 24 h in freshly prepared 3:1 (v:v) 96% sulfuric acid: 30% hydrogen peroxide solution (piranha). For enhancement of the electrochemical communication between the electrode and the enzyme molecules, the reduced forms of three redox mediators, K₄[Fe(CN)₆], K₄[W(CN)₈] and K₄[Mo(CN)₈], were used. The final concentration of each compound in the cell was 2 mM, and their midpoint redox potentials were 0.43, 0.52 and 0.78 V vs. NHE [36].

2.5. Electrochemical measurements and characterization

Electrochemical impedance spectroscopy (EIS), CV, and chronoamperometric charge/discharge procedures were performed using a μ Autolab Type III/FRA2 potentiostat/galvanostat from Metrohm Autolab B. V. (Utrecht, The Netherlands). The reference and counter electrodes were a Ag|AgCl|3M NaCl and a Pt wire, respectively. All

electrochemical measurements were performed in 50 mM phosphate buffer (PB) pH 7.4, purged with Ar in order to remove O₂. EIS spectra of bare Au electrodes (Au), MPA modified Au electrodes (Au-SAM), and Rc modified Au electrodes with immobilized MPA (Au-SAM-Rc) were recorded in 50 mM PB, 0.1 M KCl, pH 7.4, in the presence and absence of 5 mM [Fe(CN)₆]^{3-/4-} (1:1) redox couple from 0.1 Hz to 70 kHz with 10 mV amplitude at 0.43 V (the formal potential of [Fe(CN)₆]^{3-/4-} redox couple) and 0.15 V (the redox potential of Rc), using ZView software from Scribner Associates Inc. (North Carolina, USA) to fit the data. Potentiostatic charge-discharge cycles of Au-SAM-Rc based biosupercapacitors were performed using amperometry and two symmetric electrodes, one as the working electrode and the other as a combined reference/counter electrode. The potential steps used were 0 V and 0.4 V. Different times between pulses were applied, *i.e.* 5 and 40 sec, respectively.

A single potential amperometry was employed to charge a biosupercapacitor based on two ITO-AuNPs-SAM-Rc electrodes at a constant potential of 0.4 V during 60 s. Chronopotentiometry was used to monitor the OCV of the biosupercapacitor at zero current after charging and while discharging, when connecting a resistor from Velleman Inc. (Forth Worth, TX, USA) or a low voltage liquid crystal display from RISE Acreo (Kista, Sweden) to the circuit.

3. Results and discussion

Electrochemical capacitors, also known as supercapacitors, are used for rapid power delivery and recharging (*i.e.*, high power density) [37]. Following this definition, as well as taking into account the presence of self-charging biosupercapacitors (*vide supra*), the “conventional biosupercapacitors” term should be used for biodevices with high biocapacitance and low double-layer capacitance, and which can be rapidly charged and discharged externally. Below a conventional symmetric biosupercapacitor based on Rc modified gold electrodes is presented.

Rc is a rather small (*ca.* 16 kDa) blue copper (Cu) containing protein. Thus, by immobilizing the protein at the electrode surface, *e.g.* modified Au, the Cu ion should be directly accessed electronically (Fig. 1). In this case, Rc can be utilized as the biocapacitive component of the conventional biosupercapacitor, since the charge will be primarily stored at the T1 Cu site (faradaic term, additional details can be found in Ref. [10]), with protonation/deprotonation of accessible residues (non-faradaic term [37]) contributing to a lesser extent to the overall capacitance. However, as mentioned above, there is only one report in the literature regarding electrochemistry of immobilized Rc [29]. In that report, quantum dots

modified electrodes were used to obtain a direct quasi-reversible electrochemical reaction of surface confined protein. Below a direct quasi-reversible electrochemistry of Rc on planar (*i.e.* without additional nanostructuration) Au electrodes is described.

The Au-SAM-Rc electrodes were first characterized individually using CV and EIS, and the results were compared with the data using MPA modified electrodes. Fig. 2 shows typical cyclic voltammograms of Au, Au-SAM, and Au-SAM-Rc electrodes. As expected, upon surface modification with MPA the capacitance is reduced from $50 \mu\text{F cm}^{-2}$ down to $14 \mu\text{F cm}^{-2}$. Contrary to previous studies of the immobilized protein, in which cathodic peaks of bare electrodes (Au and ZnS quantum dots modified Au electrodes without Rc) were observed with peak potentials and currents close to the cathodic peak of the immobilized protein (Fig. 4a in [29]), no pronounced electrochemical signals on non-biomodified electrodes were registered by us (Fig. 2). However, after modification with Rc, well-pronounced redox peaks from the T1 Cu site of the protein are observed. Also, in contrast to the previous study of the immobilized protein [29], in which huge peak-to peak separation (*ca.* 0.35 V) was observed along with completely different shapes and incommensurable areas of the cathodic and anodic peaks, a quasi-reversible direct electron transfer based electrochemistry of Rc (peak-to peak separation of about 0.08 V only) were registered in the present work (Fig. 2).

A surface coverage of 0.73 nmol/cm^2 could be estimated from integration of the area of the reduction peak, corresponding to a complete densely packed multi-layer of electronically addressable Rc on the electrode surface. Modification with Rc dramatically increased the total capacitance of MPA modified Au electrodes, from $14 \mu\text{F cm}^{-2}$ to $146 \mu\text{F cm}^{-2}$, where a majority of the capacitance can be attributed to the faradaic process related to the redox transformation of Rc ($115 \mu\text{F cm}^{-2}$, Fig. 2b). Thus, modification with the redox protein increased the capacitance of the MPA-Au system more than ten-fold.

In previous studies, the midpoint potential of the immobilized protein was found to be 0.88 V *vs.* NHE at pH 6.86 [29], *i.e.* 0.20-0.26 V higher compared to the redox potential of Rc in solution (0.62-0.68 V) [28, 38]. Such a high value is unexpected, considering especially the equilibrium redox potential of $\text{O}_2/\text{H}_2\text{O}$ couple, 0.82 V, under these conditions. Thus, the anodic peak reported in Ref. [29] could be attributed to H_2O electrooxidation on biomodified electrode surfaces rather than to the electrooxidation of the T1 site of the protein. This suggestion regarding the different origin of the cathodic and anodic processes could be also used to explain the different temperature dependency of peak potentials observed by the authors: while the potential of the cathodic peak was highly dependent on temperature, the anodic peak remained unchanged when the temperature was increased from 18 °C to 43 °C [29]. Contrary to previous

studies, in present work the potential of the T1 Cu site of surface confined Rc has been estimated to be 0.34 V vs. NHE, which is significantly lower than values previously reported for Rc in solution.

One could speculate that the immobilization of the protein destabilizes the coordination to the Cu ion. Indeed, for unfolded Rc one of the histidine ligands to the copper center has been observed to detach, lowering the redox potential by 0.1 V [28]. Thus, the protein's coordination to the carboxylated SAM along with covalent linkage of the protein on the electrode surface may result in a lowering of the redox potential. To confirm the fact that a significant shift of redox potential of Rc is solely attributed to the protein immobilization, spectroelectrochemical studies of Rc in solution were performed, as described below.

The redox potential of the T1 Cu site of Rc was accurately measured by mediated redox titration following a previously developed procedure [34]. Least-squares linear regression analysis of the 597-nm Nernst plots provided a midpoint potential (E_m) value of 0.63 V vs. NHE and a slope of 0.064 V (Fig. 3A, insert), which implies a number of electrons (n) equal to 0.92. The linear correlation coefficient was high, 0.998. In addition to the least-squares regression analysis, the titration curves were also analyzed by direct data fitting. In this case, even better results were obtained, as presented in Fig. 3. Thus, the measured potential of Rc in solution coincide with previously reported values; indeed, *ca.* 0.3 V shift is attributed to a conformational change of the protein due to its immobilization on the electrode surface.

The Au-SAM-Rc electrodes were further characterized by EIS, an effective method to monitor the changes in double layer capacitance (C_{dl}) and charge transfer resistance (R_{ct}) induced by the electrode modification. Fig. 4b shows the impedance responses of the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox couple in 50 mM PB, 0.1 M KCl pH 7.4 on bare electrode (Au), MPA modified electrode (Au-SAM), and MPA modified electrode with immobilized Rc (Au-SAM-Rc). The impedance spectra were fitted in the equivalent circuit shown in Fig. 4a. Supporting Table S1a shows the numeric values obtained from the fitting of each spectrum. As evident from the data, modification of Au electrodes with MPA did not affect solution resistance (R_s), whereas this parameter was slightly affected by Rc immobilization. The formation of the SAM decreased R_{ct} from 288 down to 172 Ω , which can be attributed to the presence of -OH groups on the bare Au surface that repelled the negatively charged redox couple. After Rc immobilization, R_{ct} increased up to 8500 Ω , since Rc forms an insulating layer on the electrode surface. A slightly higher C_{dl} value was obtained for bare Au electrode since the SAM seems to insulate the electrode surface as well, which is in good agreement with the results obtained by CV (Fig. 2a). For the electrode with immobilized Rc, the highest C_{dl} value was obtained.

The Warburg impedance (Z_w) increased significantly, *viz.* from 8295 for Au to 10105 for Au-SAM and to 37751 $\Omega \text{ s}^{1/2}$ for Au-SAM-Rc, as the resistance for the diffusion of the redox species from the bulk electrolyte to the electrode surface increased.

The Au-SAM-Rc electrodes were additionally characterized by EIS in the absence of the redox mediator (Supporting Figure S3). R_{ct} , which is inversely proportional to the rate of electron transfer, notably differ for Au-SAM and Au-SAM-Rc (Supporting Table S1b), *viz.* the value obtained for Au-SAM is more than 10 times higher than for Au-SAM-Rc electrode. Since EIS measurements were performed at the redox potential of Rc, the lower R_{ct} value reflects a higher rate of electron transfer, as expected. As for C_{dl} , a higher value is obtained for the electrode modified with Rc in accordance with the capacitive feature of the Au-SAM-Rc electrodes due to the contribution of the T1 copper center of Rc to the faradaic charge storage.

After detailed (spectro)electrochemical characterizations, two Au-SAM-Rc electrodes were combined to assemble a symmetric conventional biosupercapacitor. The biodevice was investigated using chronoamperometric charge/discharge procedure (Fig. 5). The biosupercapacitor was charged at 0.4 V to allow the complete oxidation/reduction of the accessible Cu ions, and displayed a rapid charge/discharge profile with good reproducibility with a low leakage current below 50 nA cm^{-2} . Initial currents of 5 $\mu\text{A cm}^{-2}$ and even higher were obtained, when the conventional biosupercapacitor was tested every 40 sec (Fig. 5a). The biodevice also showed quite good operational stability with no substantial decrease in the performance after 50 charge/discharge cycles (Fig. 5b). A total capacitance equal to 61 $\mu\text{F cm}^{-2}$ was derived from the discharge curves, in good agreement with the CV measurements, since the total capacitance of an assembled capacitor is determined by the series connection of the two electrode and theoretically should be half of 146 $\mu\text{F cm}^{-2}$, *i.e.* 73 $\mu\text{F cm}^{-2}$. No conductive additives were used and the biodevice was almost solely based on the capacitive properties of the immobilized Rc, enabling a ten-fold increase in capacitance compared with an electrochemical capacitor without redox protein. On the one hand, examination of basic supercapacitor/rechargeable battery features of the designed electric power device [37] revealed that the biodevice has some typical characteristics of a “rechargeable battery”, *e.g.* a peak-shaped CV profile (Fig. 2). On the other hand, a setup with two identical electrodes (Fig. 1), the fast charging time and a well-pronounced current decrease during potentiostatic discharging (Fig. 5) substantiate the “supercapacitor” moniker for the biodevice.

Finally, to demonstrate the functionality of biosupercapacitors, high surface area nanostructured electrodes based on AuNPs were exploited (Supporting Figure 2). The biodevice was built from two ITO-AuNPs-SAM-Rc. The total charge (Q) equal to 39.2 μC , accumulated

in the biosupercapacitors, was calculated by integration of the area contained in the discharge curve (Supporting Figure S4). Fig. 6 shows the amperometric charge curve, demonstrating a fast charging process of the biosupercapacitors, and a discharge process, when connecting 500 k Ω resistor to the circuit. Another fast charging curve is also shown, followed by the second discharge curve, when connecting the liquid crystal display instead of the resistor (Fig. 6). Supporting Figure S5 demonstrates the ability of the designed conventional biosupercapacitor to power real electronic device.

4. Conclusions

In summary, detailed spectroelectrochemical studies of MPA modified Au electrodes with immobilized Rc were performed. Quasi-reversible direct electron transfer based electrochemistry of immobilized protein was shown with the midpoint potential of the redox center of the protein significantly shifted from the potential of the T1 Cu site of Rc in solution. A pseudobiocapacitance/double layer capacitance ratio equal to 3.7 for Rc modified electrodes was achieved. Based on fundamental studies a conventional symmetric biosupercapacitors was realized. The biodevice, which could be externally charged and discharged at least 50 times with initial current densities as high as 5 $\mu\text{A cm}^{-2}$ without significant loss of electrochemical activity, had a low leakage current below 50 nA cm^{-2} . Employment of redox active proteins, *i.e. renewable and biodegradable molecules*, which can be potentially produced at *low costs*, to design biomaterials with superior properties, like “on-off” characteristics, high capacitance and *good biocompatibility*, allows creation of new bioelectronic devices, *e.g.* protein based memory devices [39] or conventional biosupercapacitors, as the one reported herein.

Conflict of interest

There is no conflict of interest

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Figure legends

Fig. 1. Schematic representation of conventional symmetric biosupercapacitor based on Rc modified gold electrodes. The structure of Rc is visualized using known crystal structure of the proteins (PDB file 1RCY).

Fig. 2. Cyclic voltammograms of Au electrodes submerged in 50 mM PB pH 7.4 recorded with a scan rate of 20 mV s^{-1} , showing the contribution of pseudo (blue) and double-layer (grey) capacitances into the total capacitance of biomodified electrodes.

Fig. 3. Mediated redox titration of Rc. a) Potentiometric titration curve. Insert: Nernst plot of the dependence of the applied potential on the absorbance at 597 nm and parameters calculated from the titration. b) Spectra from the titration, corresponding to oxidized Rc (0.90 V), partly oxidized protein (0.65 V and 0.60 V), and fully reduced Rc (0.45 V).

Fig. 4. EIS studies of Au electrodes in 50 mM PB containing 0.1 M KCl, pH 7.4, in the presence of 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ couple. a) Equivalent circuit model used to perform theoretical fits for the EIS data. b) Nyquist plot of different electrodes at the midpoint potential of the redox couple. The inset shows the expansion of the high frequency range.

Fig. 5. Chronoamperometric studies of the conventional symmetric biosupercapacitor based on two identical Au-SAM-Rc electrodes. Potential pulses at 0.4 V and 0, every 40 sec (a) and every 5 sec (b).

Fig. 6. Charge (black) and discharge (blue) curves for the biosupercapacitor based on two ITO-AuNPs-SAM-Rc. The first discharge curve was obtained using 500 k Ω external resistor and the second curve by connecting the biodevice to the flexible low voltage display.

Graphics Abstract

Figure 1

Figure 2

Figure 3

b)

Figure 4

Figure 5

Figure 6