Supporting Information

**A Nernstian Biosupercapacitor**

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Section 1. Selection of Os-complex-modified redox hydrogel

There are some minor disadvantages of using poly(vinyl imidazole-co-allylamine)-[Os(bpy)2Cl] (Os-P) in the construction of a Nernstian biosupercapacitor (Nernstian BSC). For example, electrocatalytic reduction of oxygen (O2) by Os-P occurs, at least to a certain extent (cf. solid and dashed curves in Supporting Figure S1), which suppresses the anodic current output during operation of Os-P-based biodevices. Moreover, there can be kinetic limitations in electron transfer (ET) rates due to counter ion mobility or limitations in the self-exchange process between neighbouring redox centres in the polymer. Such concerns are suggested by the results of electrochemical studies of pure Os-P (without enzymes), immobilised on graphite electrodes, which revealed a midpoint potential (Em) equal to 0.44 V vs. NHE (here and below all potentials are given vs. NHE) with a peak separation equal to 0.05 V (Supporting Figure S1). In previous electrochemical investigations using differential pulse voltammetry it was shown that the coordination sphere of the Os in the Os-P is uniform for all Os complexes bound to the polymer backbone. However, Em value of Os-P in previous studies was measured to be 0.04 V lower, i.e. 0.40 V vs. NHE. Thus, in the present work, a possibility for the presence of Os complexes with slightly different coordination spheres cannot be completely disregarded (vide infra).

Supporting Figure S1. Cyclic voltammograms of graphite electrodes modified with the poly(vinyl imidazole-co-allylamine)-[Os(bpy)2Cl] in glucose free Ar-saturated PBS (dashed trace) and 20 mM glucose containing O2-saturated PBS (solid trace); potential scan rate: 0.2 mV s−1.

Os-P has useful characteristics that can outweigh the minor disadvantages. For example, the redox potential of Os-P is well-suited for combining with both enzymes used in this work, since there is sufficient driving force for anodic and cathodic bioelectrocatalytic processes (Figure 4 in the main text). Moreover, electrochemical studies of Os-P showed that the polymer is highly capacitive, with a total capacitance density as high as 151 mF cm−2, with pseudo and double-layer capacitances being 101 mF cm−2 and 50 mF cm−2, respectively, whereas capacitance densities below 2 mF cm−2 were measured in the case of bare graphite electrodes. To conclude, despite some shortcomings, Os-P was found to be well-suited for the design of a high performance Nernstian BSC.

Section 2. Electric setup

In order to simultaneously monitor the open-circuit voltage (OCV) of the biodevice as well as the open-circuit potentials (OCPs) of the bioanode and biocathode separately, during both self-charging and external discharging processes, an automated electric measurement setup was developed which allows amperometric and potentiometric testing of several working electrodes simultaneously in both continuous and pulse modes (Experimental Section and Supporting Scheme 1). The setup relies on computer-controlled relay banks that allow various loads – ranging from 1 kΩ to infinite impedance – to be applied across the BSC or between a given electrode and the reference electrode. Scheme S1 shows the setup for measuring the OCV of the biodevice. When the relays are all in their resting state, the external impedance across the biodevice is infinite, and self-charging is measured. By activating particular relays, a specific desired external impedance in the range 1 kΩ to 1 MΩ is connected across the biodevice, and discharging is measured. Using extra modules, multiple measurements can be carried out in parallel.
Section 3. Optimisation of bioelectrodes

To realise a highly capacitive and simultaneously lightweight Nernstian biodevice, the bioanode and biocathode should have identical total capacitance. Thus, the total capacitance of the electrodes was one of the main factors determining the optimisation process. Evidently, incorporation of redox enzymes into Os-P significantly decreases the total capacitance of the optimised electrodes (cf. dashed curves in Supporting Figures S1 and S2), i.e. from 151 mF cm$^{-2}$ for Os-P down to 117 mF cm$^{-2}$ and 134 mF cm$^{-2}$ for GDh-Os-P and BOx-Os-P conjugates, respectively.

On the one hand, high loading of electrodes with biocatalysts is needed to obtain high bioelectrocatalytic currents. On the other hand, a complex interplay between self-charging ability, electrode capacitance, and biodevice performance was found. For instance, an increased amount of Os-P on the electrode surface resulted in deterioration of bioelectrocatalytic performance of both electrodes (Supporting Figure S3). Specifically, bioelectrocatalytic currents from cathodes were found to be 116, 82, 65 µA cm$^{-2}$ at 0.35 V applied potential for electrodes consisting of a bioelectrocatalytic layer, charge-storing layer/bioelectrocatalytic layer, and charge-storing layer/charge-storing layer/bioelectrocatalytic layer, respectively, whereas these values from bioanodes at 0.53 V applied potential were 56, 54, 31 µA cm$^{-2}$, respectively. Thus, based on the data regarding both bioelectrocatalytic current outputs and total capacitances of bioelectrodes, as well as taking into account certain peculiarities of the performance of Nernstian BSC compared to conventional capacitors and biological fuel cells (BFCs), as described directly below, electrodes consisting of the charge-storing layer/bioelectrocatalytic layer (red curves in Supporting Figure S2) were chosen as the optimised ones for stability investigations (vide infra; see also Fig. 3 and its description in the main text), as well as for proof-of-principle demonstration (Supporting Video S1).

One can also notice a quite pronounced cathodic current output at 0.55 V in Supporting Figure S2a. Taking into account a negligible cathodic current from Os-P modified electrodes at this potential under aerobic conditions (Supporting Figure S1), this current is attributed to bioelectrocatalytic reduction of oxygen by Box based on direct electron transfer. This suggestion is supported by the fact that the high potential current (at 0.55 V) disappears, when two charge-storing layers are applied to the electrode surface prior to immobilisation of BOx-Os-P composite (solid blue curve in Supporting Figure S2a). The hypothesis can be further corroborated by the following calculations. In previous investigations the diffusion coefficient of laccase (structurally very similar enzyme to Box) in Os complex containing redox polymers was estimated to be 1.5 $10^{-9}$ ± 0.5 $10^{-9}$ cm$^2$s$^{-1}$.[2] Taking into account that Myrothecium verrucaria BOx[3] is a ca. 20 % smaller enzyme as compared to Trametes versicolor laccase[4] used in Ref.[2], one can safely assume the diffusion coefficient of Box to be about 2 $10^{-9}$ cm$^2$s$^{-1}$. Thus, despite knowing that Os-P is a non-Newtonian fluid and the simplified Stokes-Einstein formula becomes imprecise, one can roughly calculate the distance of BOx diffusion during electrode modification (ca. 30 min) to be about 27 µm. A thickness of several µm can be assumed for one Os-P layer. Hence, if only one bioelectrocatalytic layer is applied on the graphite electrode, some BOx molecules may diffuse and adsorb on the bare graphite surface enabling direct ET based bioelectrocatalysis, a well-known phenomenon in BOx electrochemistry[5]. In contrast, when two charging layers are applied and dried prior to the immobilisation of the bioelectrocatalytic layer, the enzyme cannot reach the graphite surface and, thus, direct ET based bioelectrocatalysis is impossible (cf. solid black and blue curves in Supporting Figure S2a).

The presence of direct ET based bioelectrocatalysis might imply a mixed potential of the biocathode, in which the Nernstian behaviour of the Os complex containing polymer is only one potential determining redox couple, the other being the direct ET bioelectrocatalytic reduction of oxygen. The mixed potential could be responsible for unexpectedly high X values obtained (Figure 1b). On the
on hand, the overpotential of direct ET based bioelectroreduction of oxygen on graphite electrode modified with BOx was measured to be 0.14 V at pH 8\(^6\), and namely this value was obtained in our studies (OCP equal to 0.65 V at pH 7.4). On the other hand, direct ET bioelectrocatalytic reduction of oxygen on BOx modified cathodes based on graphite electrodes can occur with an overpotential of 0.08 V only\(^7\), but such high OCP values (around 0.71 V) were never achieved despite stabilisation of the potentials during measurements (Figures 2 and 3, Supporting Fig. S3). Moreover, as can be seen from Figure 2, the OCP of the bioanode is shifted from \(E_m\) of the polymer by 0.30 V, which implies X value as high as 5.08 (Figure 1b), whereas the OCP of the biocathode is shifted by 0.22 V only (X = 3.73), despite the fact that the bioelectroreduction current is at least 1.5 times higher as compared to the bioelectrooxidation current. Thus, the possibility for a mixed potential of biocathode can be safely disregarded.

Changes in operating voltages and OCVs of the assembled biodevice involved bioelectrodes with equal capacitances, along with changes in steady-state potentials and OCPs of separate bioelectrodes under charging/discharging processes, are presented in Supporting Figure S3a. When a constant load of 10 kΩ was applied to the fully charged Nernstian BSC (full charge was evident from the stabilisation of OCV- and OCP-values during the self-charging process in the presence of enzyme substrates), a rapid initial voltage/potential change was observed (Supporting Figure S3), corresponding to the transformation of Os\(^{3+}\) to Os\(^{2+}\) and vice versa in the case of bioanode and biocathode, respectively (Figure 1). At the initial stage, the potential of the biocathode promptly (in less than in 1 min) dropped from 0.65 V down to 0.55 V, however, accompanied by a subsequent slight increase from 0.55 V to 0.57 V in about 7 min of the discharging process (blue trace in Supporting Figure S3a). In contrast, the potential of the anode continuously increased from an OCP of 0.22 V, reaching a value of 0.45 V after 8 min of discharge, i.e. 0.01 V higher than the midpoint redox potential of Os-P (0.44 V). Such behaviour of bioelectrodes can be explained by their complex nature - they combine properties of supercapacitor and conventional BFC electrodes, as described below.
At the beginning of the discharging process, the Nernstian BSC behaves as a conventional electrochemical capacitor, thus, its power output and operating voltage decrease with time (Supporting Figure S3). However, at the end of the discharging event, the Nernstian BSC ideally should behave as a BFC, i.e., its power and current output, as well as operating voltage, should be non-zero and constant. This was actually the case in our studies, as demonstrated in Supporting Figure S3, when an equilibrium, or, in other words, a BFC regime, was reached, and an almost constant steady-state voltage of the biodevice was registered. However, since bioelectrocatalytic current output from the biocathode is significantly higher compared to the bioanode (82 µA cm⁻² and 54 µA cm⁻², respectively, cf. red curves in Supporting Figure S2), and both bioelectrodes are highly and equally capacitive, the biocathode is slightly self-charging during discharging of the Nernstian BSC, depolarising (discharging even further) the bioanode (Supporting Figure S3a).

For a Nernstian BSC constructed from a biocathode with twice the capacitance of the bioanode and, consequently, a lower bioelectrocatalytic current output from the O₂-reducing bioelectrode compared to the glucose-oxidising bioelectrode (82 µA cm⁻² and 56 µA cm⁻², vide supra), discharge led to steady-state potentials on both bioelectrodes (Supporting Figure S3b). However, for a Nernstian BSC built from a bioanode with twice the capacitance of the biocathode, a fast growth of the anodic potential, up to 0.56 V after 8 min of discharge (Supporting Figure S3c), i.e., 0.12 V higher than the redox potential of Os-P, was observed. This was

Supporting Figure S3. Discharge/charge traces of Nernstian BSC with different relative capacitances of bioanode and biocathode in O₂-saturated PBS containing 20 mM glucose. a) Equal total capacitance of both bioelectrodes, b) capacitance of the bioanode is twice higher than the capacitance of the biocathode, c) capacitance of the bioanode is twice lower than the capacitance of the biocathode. 1: discharging of fully charged BSC at a constant load of 10 kΩ, 2: disengaging the load. Red curve: OCP of bioanode, blue trace: OCP of biocathode, black trace: OCV of BSC.
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because of a tremendous difference in bioelectrocatalytic current outputs of biocathode and bioanode, viz. 82 µA cm\(^{-2}\) vs. 31 µA cm\(^{-2}\), respectively. Significantly longer charging time of this non-optimised Nernstian BSC should also be noted. To conclude, for complete optimisation of BSCs, not only capacitances but also bioelectrocatalytic current outputs should be equal on the anodic and cathodic sides.

Section 4. Stability of the Nernstian BSC

An operational stability test of a Nernstian BSC in long-term pulse mode showed that the assembled biodevice was quite stable over 48 h of operation (Figure 3).

Supporting Figure S4. Operational stability test in long-term pulse mode in O\(_2\)-saturated PBS containing 20 mM glucose: red trace: bioanode, blue trace: biocathode. The Nernstian BSC was discharged by applying a constant load of 22 kΩ for 20 min.

OCP-values of biocathode and bioanode, when the Nernstian BSC was fully charged, were 0.66 V and 0.21 V, respectively, when fully charged, changing to 0.61 V (0.05 V difference) and 0.22 V (only 0.01 V difference) after 48 h of biodevice operation. During 20 min discharging pulses at the beginning of the test, the potential changed by -0.06 V and +0.26 V for biocathode and bioanode, respectively, while at the end of the test these potentials changed by -0.08 V and +0.20 V. Since the total capacitances of both bioelectrodes were identical and the discharging time was quite long, the observed difference (-0.06+0.08 V vs. 0.26-0.20 V) can be attributed to the different bioelectrocatalytic activities of the bioelectrodes (82 and 54 µA cm\(^{-2}\), respectively, vide supra).

These changes are consistent with a gradual deactivation of the O\(_2\)-reducing bioelectrode, whereas the glucose-oxidising bioelectrode seemed to be stabilised and even slightly “activated” after 15 h of self-charging/discharging cycles. Similar behaviour was also observed when charge-storing stabilities of separate bioelectrodes were evaluated, viz. looking at the perturbation of the bioanode after 15 h of the experiments (Supporting Figure S4), which resulted in a slight decrease of the potential of the electrode, even in glucose- (enzyme substrate) free electrolyte, when just long-term OCP measurements were performed (cf. red curves in Figure 3b and Supporting Figure S4). Specifically, while the OCP of the biocathode gradually decreased with time from 0.65 V down to 0.56 V, the OCP of the bioanode first increased (from 0.2 V to 0.26 V), but after 15 h decreased again to 0.24 V (Figure 3b). Thus, in all likelihood, the interesting feature of bioanode “self-activation” is not an experimental error/artefact because this effect was seen in totally different measurements performed in our studies. However, to give a reasonable explanation for this effect, additional measurements are needed, which is beyond the scope of the current work.

Section 5. “Nernstian” enzymatic fuel cell

On the one hand, based on theoretical considerations (Figure 4), the fabricated biodevice described herein may not have been expected to display favourable operational characteristics, when operating as a conventional BFC. This somewhat negative expectation is supported by theoretical polarisation and power output curves (Supporting Figure S5), which give the impression that an ideal Nernstian system consisting of surface-confined species would generate only minor amount of power due to negligible
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current outputs (limited by the heterogeneous ET; solid voltammograms in Figure 4) from both bioelectrodes along with a low operating voltage of the biodevice equal to 0.0906 V (25 °C)[9] when exploiting just one redox mediator (Figure 4, dotted red curve in Supporting Figure S5). On the other hand, in the real setup compared to an ideal theoretical system, sufficient power density at much higher operating voltages than predicted was still experimentally realised, when the Nernstian BSC was continuously operated (solid curves in Supporting Figure S5). Since these measurements were done under equilibrium, higher operating voltage equal to 0.1218 V instead of 0.0906 V (cf. red curves in Supporting Figure S5) could not reflect a certain degree of irreversibility of the system consisting of Os-P modified electrodes (vide supra). Moreover, as can be seen from the shape of the polarisation curves (cf. solid and dashed blue curves in Supporting Figure S5), neither Ohmic nor actually kinetic limitations were registered for the Nernstian BSC operating in BFC mode. In previous studies peak broadening has been related to repulsive interactions that result in a distribution of activity coefficients dependent on the degree of film oxidation[8]. In later reports, however, it was shown that at high ionic strength (above 0.1 M), peak widths are quite close to the ideal value of 90.6 mV for a one-electron reaction[9]. In our studies, an electrolyte with high ionic strength (above 0.1 M) was used. Moreover, in previous theoretical and experimental investigations, thin polymer layers (thickness ~ few nm) were used, whereas in our studies, thick polymer layers (thickness ~ few μm) were formed to ensure high total capacitance of electrodes. Thus, the presence of Os complexes with slightly different coordination spheres, rather than limitations in ET or a distribution of activity coefficients, is the most reasonable explanation for the superior properties of the real Nernstian BSC compared to the ideal biodevice. Actually, a Gaussian distribution of formal redox potentials due to heterogeneities in the layer was shown previously[10].

Quite pronounced mass transfer limitations were observed in the region close to the biodevice short circuit. Despite the relatively low maximal current density (below 40 μA cm⁻²), mass transfer limitations are not surprising taking into account the quite thick layer of enzyme-Os-P conjugate, where enzyme substrate depletion can easily occur. Despite these mass transfer drawbacks, the Nernstian BSC displays strong performance characteristics. This suggests that, ultimately, neither conventional BFCs nor continuously operating BSCs will be able to compete with capacitive biodevices operating in pulse mode under otherwise identical circumstances, e.g., usage of the same biocatalysts, redox mediators, electrode materials, etc.

Supporting Figure S5. Polarisation (blue traces, left ordinate) and power output (red traces, right ordinate) curves of a Nernstian BSC. Experimental data (solid traces) were obtained in 20 mM glucose in O₂-saturated PBS. Theoretical curves (dashed traces) were plotted based on the maximal bioelectrocatalytic current of the limiting electrode in the experimental setup (anode) using potentials for an ideal Nernstian system consisting of surface-confined redox species.

Section 6. Experimental Section

Chemicals

All chemicals were of analytical grade and were used without further purification. KCl, NaOH, and CaCl₂ were obtained from J.T. Baker; Na₂HPO₄·2H₂O and KH₂PO₄ were from VWR; NaCl was from Fisher Scientific. Pyrrolequinoline quinone (PQQ) was purchased from Fluka. Poly(ethylene glycol) diglycidyl ether and Triton X-100 were obtained from Sigma-Aldrich. 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and D-(+)-glucose monohydrate were purchased from Applichem. Argon (Ar) and O₂ were obtained from Air Liquide. All solutions were prepared using deionised water purified with a Milli-Q system from Millipore. All experiments were carried out at room temperature, (25 ± 3) °C.

Enzymes

Apo-GDh and Myrothecium verrucaria BOx were kindly gifts provided by Roche Diagnostics and Novozymes, respectively. A highly purified preparation of BOx with a protein concentration of 3.61 mg mL⁻¹ and specific activity equal to 370 Units mg⁻¹ (determined as described in Ref.[11]) was stored in 20 mM Tris buffer (pH 8.0) with 100 mM NaClO₄. The BOx solution was used for electrode modification without any additional treatment. The PQQ-GDh holo-enzyme was reconstituted by dissolving 7.2 mg of apo-GDh in 100 μL of a 500 μM PQQ solution in 10 mM HEPES buffer (pH 7.0, adjusted using NaOH) with 150 mM CaCl₂ and 0.1% (v/v) Triton X-100. The mixture was incubated for at least 30 min at 4 °C prior to electrode preparation. The specific activity of the reconstituted enzyme was measured to be 3800 Units mg⁻¹ (determined as described in Ref.[12]).
Graphite electrodes (c.4.7 mm from SGL Carbon) were polished using emery paper with decreasing particle size (P2000, P1000, and P400), rinsed with water, and dried in air. The redox polymer, poly(vinyl imidazole-co-allylamine)-[Os(bpy)2Cl], was synthesised according to [1] to create a charge-storing layer in the bioelectrodes. 10 μL of the polymer solution (23 mg mL⁻¹) was mixed with 3 μL of the cross-linker (poly(ethylene glycol) diglycidyl ether), 1:50 v:v with PBS) and dried in air. To fabricate a bioelectrocatalytic layer, 3 μL of polymer solution, 3 μL of cross-linker, and 10 μL of Box solution or 2 μL of PQQ-sGDH solution, were mixed on the top of a charge-storing layer of the biocathode and bioanode, respectively. Prior to combination into Nernstian BSCs, the electrodes were polarised to the redox potential of the polymer for 15 min in Ar-saturated phosphate buffer consisting of 10 mM Na₂HPO₄, 2 mM KH₂PO₄, 2.7 mM KCl and 137 mM NaCl, pH 7.4 (PBS) to obtain an anodic/anodic ratio of 1.

**Electrochemical measurements**

Electrochemical characterisation of bioelectrodes was carried out using a multi-channel potentiostat/galvanostat Model 1030 from CH Instruments in a three-electrode configuration, where Ag/AgCl 3 M KCl (210 mV vs. normal hydrogen electrode (NHE)) and a platinum wire were used as reference and counter electrodes, respectively. All potentials in the are given vs. NHE.

Investigations of the performance of fabricated Nernstian BSCs, including stability tests in long-term mode, were performed using a four-electrode setup with the biocathode and biocathode serving as working electrodes, and Ag/AgCl and a platinum wire serving as reference and counter electrodes, respectively. Discharge of the biodevice was carried out by connecting the system to a resistor included in a relay box (Scheme S1). Charge/discharge measurements were controlled with an in-house-written software programmed using Visual Basic 6.0 and a D-8927-1 output board from Decision-Computer International including single-pole double-throw 12 V M4-12H relays from Meisei Electric to drive digital output lines. Each relay channel was used to control the ON/OFF state of the resistor/display selected and for closing the circuit between biocathode and biocathode. The individual potentials of the bioelectrodes were monitored using a PGU-BI 100 bi-potentiostat from IPS-Jaisse Elektronik, while the open-circuit voltage (OCV) of the biodevices was measured by connecting the terminals of a PPT 85 potentiometer from Bank Elektronik to the working electrodes. A module with a 16-bit CIO-DAS 1602/16 AD/DA board from Plug-In Electronic was used for data acquisition recording the potentials from the potentiostat and the potentiometer.

Operational stability tests were carried out using a two-electrode configuration in which the biocathode and bioanode were connected as working and combined reference/counter electrodes, respectively. Discharging in long-term pulse mode was performed by applying a constant load of 22 kΩ for 20 min followed by self-charging for 20 min, with discharge/charge done in repeated cycles. Discharging in short-term pulse mode was performed by applying a constant current of 5 μA for 1 s, followed by self-charging for 100 s, with discharge/charge done in repeated cycles. OCV during the charging process was continuously monitored using the PGU-BI 100 bi-potentiostat. Storage stability tests were carried out using a two-electrode configuration in which OCV values were continuously monitored using the PGU-BI 100 bi-potentiostat and the PPT 85 potentiometer.

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