Supplementary Information

Mediatorless high-power glucose biofuel cells based on compressed carbon nanotube-enzyme electrodes

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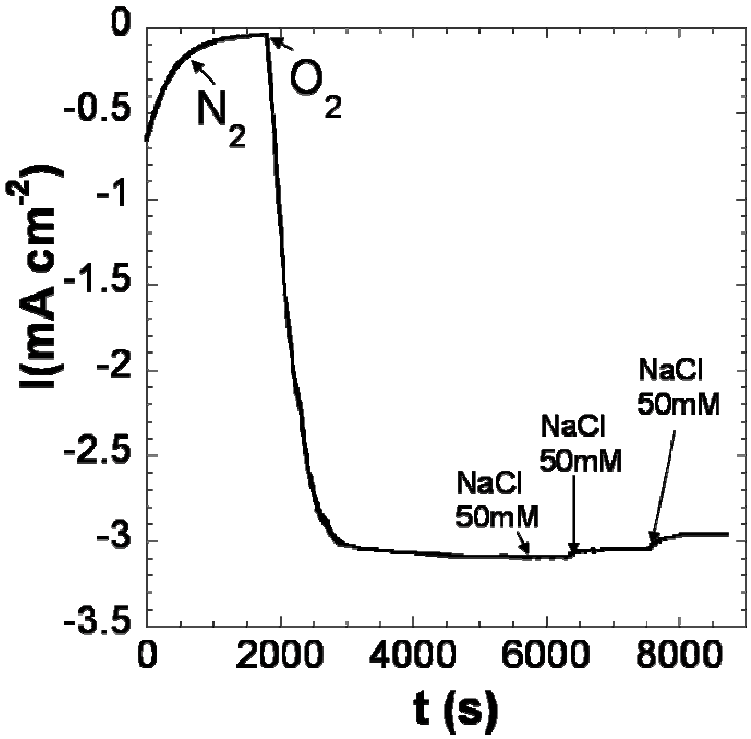
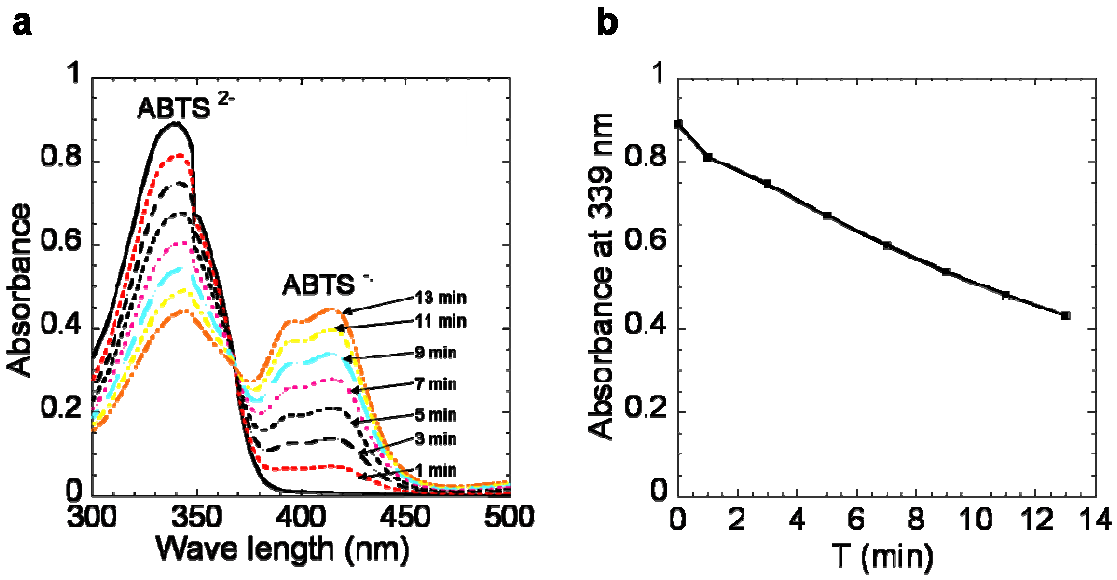


Supplementary Figure S1. Inhibition of laccase biocathode by chloride. Inhibitive effect of chloride on the reduction current response of the biocathode containig laccase (20 %) at 0.2 V vs. SCE, after saturation with dioxygen of a phosphate buffer (pH 7) previously saturated with N_2.



Supplementary Figure S2. **Activity of laccase**. (**a**) Spectra of a phosphate buffer (pH 7) containing 10^{-5} mole L^{-1} $ABTS^{2+}$, before and after addition of laccase (3 µg). (**b**) Evolution of $ABTS^{2+}$ absorbance with time at 339 nm after addition of laccase.

Supplementary Note 1: Inhibition studies of laccase by chloride

We investigated the inhibitory effect of chloride ions on the activity of laccase under physiological conditions. The chronoamperometric response of the biocathode was recorded at 0.2 V vs SCE with and without dioxygen (figure S1). After the repetitive addition of NaCl, the catalytic reduction current of O_2 decreases slowly for the concentrations of Cl^- higher than 0.05 mole L^{-1}. This decrease reaches about 5% of the initial value of the catalytic current for 0.15 mol L^{-1} Cl^- which corresponds to the physiological conditions. Indeed, the concentrations of chloride and sodium ions in human serum are normally between 95 and 105 mM for chloride and between 135 and 145 mM for Na^+.

Supplementary Note 2: The activity of free laccase at pH 7

The specific activity of laccase free in buffer (pH 7) was determined by using ABTS as substrate for the enzymatic reduction of dioxygen into water. The enzymatic oxidation of $ABTS^{-2}$ into $ABTS^{\cdot-}$ was monitored by UV-spectroscopy. The absorbance change of a phosphate buffer (pH 7, dioxygen saturated) containing 10^{-5} M of $ABTS^{-2}$ (ε_{max} = 3.45x10^4 M^{-1} cm^{-1}) was recorded as a function of time after addition of 3 µg of laccase (figure S2.a). The slope of the absorbance intensity at 339 nm versus time (S2.b), indicates the velocity of the enzymatic consumption of $ABTS^{-2}$ and hence reflects the activity of laccase. Taking into account that one unit of laccase activity is defined as the amount oxidizing 1 µmol substrate per min, the activity was calculated using the equations:

$d[ABTS]/dt = (d[Abs]/dt)/(\varepsilon.l) = X$ mol L^{-1} min^{-1}

Activity = $X.v.10^6/m = Y$ µmol min^{-1} $mg^{-1} = Y$ unit mg^{-1}

ε is the extinction coefficient of $ABTS^{2-}$, *l* the path length, v the volume of the solution and m the amount of laccase in solution. The resulting activity of 1 U/mg at pH 7 is 20 times smaller than the activity of laccase at pH 4.

Supplementary Note 3: Activity of the entrapped and wired enzymes

The surface activity and the specific activity of the electrically wired enzymes immobilized in the CNT disks were calculated from the current densities measured at each bioelectrode using the following equation

Surface activity (U cm^{-2}) = J_{max} x 60 / (F x n)
Specific activity (U mg^{-1}) = J_{max} x 60 x A / (F x n x m)
U is the enzyme activity in µmol min^{-1}; n is the number of electrons involved in electrode reaction (n = 2 for glucose oxidation by GOx, n= 1 for $ABTS^{2-}$ oxidation by laccase); A is the electrode surface in cm^2; F is the Faraday constant, m is the mass of enzyme in mg. J_{max} is the current density in A cm^{-2}.
For the biocathode, the laccase activity was conventionally investigated via the one-electron oxidation of the co-substrate $ABTS^{2-}$ participating to the reduction of dioxygen. The surface activity and specific activity of the electrically connected laccase were thus estimated at 3.84 U cm^{-2} and 0.1 U mg^{-1}, respectively. If we consider the activity of laccase towards the dioxygen reduction (n= 4 for dioxygen reduction by laccase), the surface activity and specific activity were 0.96 U cm^{-2} and 25 mU mg^{-1}, respectively.
For the bioanode, the surface activity and specific activity of the electrically wired GOx were estimated at 2.48 U cm^{-2} and 65 mU mg^{-1}, respectively.