Electronic Supplementary Information (ESI)

# Biosurfactants Functionalized Single-Walled Carbon Nanotubes to Promote Laccase Bioelectrocatalysis

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**Experimental Section**

*Preparation of single-walled carbon nanotubes (SWCNTs)*

SWCNTs were synthesized on a gold (Au) wire surface by chemical vapor deposition, as described previously.[1–4] FE-SEM and TEM results indicated the pieces of SWCNTs were bundled with each other, resulting in tubes with a diameter of 5–20 nm (Fig. S2). A prominent feature in the Raman spectrum of the SWCNTs is the *G*-band at *ca*. 1,590 $cm^{-1}$ (Fig. S3).[5,6] The *G*-band is a doubly degenerate phonon Raman-active mode for $sp^2$-hybridized carbon networks. The other feature is the *D*-band at *ca.* 1,350 $cm^{-1}$. This is localized at imperfect lattice structure regions, particularly the edges and defects of $sp^2$-hybridized structures.[5,6] The *G*-band/*D*-band intensity ratio ($I_G/I_D$) was used to evaluate the crystallinity of the $sp^2$-hybridized structure. The $I_G/I_D$ ratio of the SWCNTs was *ca.* 20 when a 514.5-nm excitation source was used. The *D*-band was very weak, indicating that the SWCNTs were of high crystallinity and had few defects. The radial breathing mode of the SWCNTs provides information about the diameter distribution of the SWCNTs. The estimated diameter distribution of the SWCNTs is 0.9–1.6 nm according to:

$$d/\mathrm{nm} = 248/(v/\mathrm{cm}^{-1}),$$

where $d$ is the SWCNT diameter and $v$ is the Raman shift.[7,8]

*BET surface area of the bundled SWCNTs*

The Brunauer–Emmet–Teller (BET) specific surface area of the SWCNTs was measured using an adsorption analyzer (Quantachrome Instruments, NOVA2200e) and liquid $N_2$ (77 K). The mass of the synthesized SWCNTs on the Au surface was estimated to be 0.12 mg $cm^{-2}$, where the surface area was the apparent surface area of Au. The mass of 0.12 mg $cm^{-2}$ gives an estimated specific surface area of 2280 $cm^{-2}$ considering the BET surface area of 1900 $m^2$ $g^{-1}$ for closed-cap SWCNTs.[9] Here, we should consider that the BET surface area of the SWCNTs is decreased by the formation of bundles. The decrease ratio of the BET surface area of SWCNT bundles with a diameter of ca. 5–50 nm (in which ca. 5–50 pieces of SWCNTs form the bundle structure) can be evaluated to be 95%. Thus, we estimated that for the SWCNTs in this work, 1 $cm^2$ (apparent surface area) equals 110 $cm^2$ (BET surface area). This estimated value is in agreement with a previous report.[9]

*Surface concentration of Lac*

The concentration of Lac adsorbed onto modified SWCNTs was determined by using a color reagent as described in a previous report.[4] The obtained results are shown in Table S1.

*Surface concentration of modifiers*

The surface concentration of modifiers adsorbed onto SWCNTs was determined by the following method when Lac was not adsorbed onto the modified SWCNTs. To evaluate the quantity of modifier on the SWCNTs, the mass decrease was evaluated by thermogravimetric analysis. In this analysis, the temperature was raised up to 450 °C at a heating rate of 20 °C $min^{-1}$ because the combustion temperatures of BIGCHAP, deoxyBIGCHAP, CHAPS, CHAPSO, OGP, DMP, MEGA10, and SC are evaluated to be 208 and 397, 207 and 400, 296, 307, 249, 287, 290, and 358 °C, respectively. The combustion temperature of highly crystalline SWCNTs is at least ca. 500 °C.[10] From the determined quantity of modifiers and the evaluated BET surface area of the SWCNTs, the surface concentration of each modifier adsorbed onto the SWCNTs was determined (Table S2).

*Simulated analysis of the steady-state current density*

The steady-state current density ($j_s$) for the adsorption model is given by[11-13]

$$j_a = nFk_c\Gamma_a / [1 + (k_c/k_f) + (k_b/k_f)]$$

$$k_f = k^\circ \exp [-\alpha (nF/RT) (E - E^{\circ\prime})]$$

$$k_b = k^\circ \exp [(1 - \alpha) (nF/RT) (E - E^{\circ\prime})] \qquad (1)$$

where, $n$ and $F$ are the number of electrons ($n$ = 1 for the T1 Cu site of Lac) and the Faraday constant, respectively. $k_c$ is the catalytic constant ($s^{-1}$), and was assumed to be 2600 $s^{-1}$ because the theoretical rate of $O_2$ reduction by the T2/3 Cu site of bilirubin oxidase is expected to be as high as 2600 $s^{-1}$ under air-saturated conditions.[14,15] $k_f$ and $k_b$ are the surface electron transfer rate constants expressed by the Butler–Volmer-type equation. $E^{\circ\prime}$ is similar to the formal redox potential for the T1 Cu site of Lac. In this study, the $E^{\circ\prime}$ was estimated from the half-wave potential from the steady-

state sigmoidal wave obtained at each modified SWCNT electrode. $k^{\circ}$ and $\alpha$ are the heterogeneous electron transfer rate constant ($s^{-1}$) at $E^{\circ\prime}$ between the adsorbed Lac and SWCNT electrode, and the transfer coefficient, respectively. In this study, $k^{\circ}$, $\Gamma_a$, and $\alpha$ were adjustable parameters. We assumed that the dependence of the catalytic reduction current on the mass transport of oxygen could be ignored.

**References**


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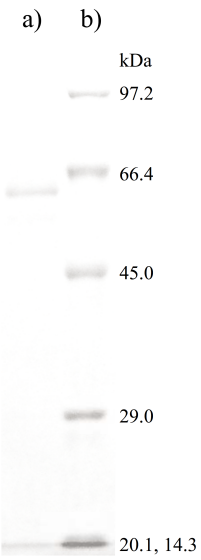


**Figure S1.** SDS-PAGE of a) purified Lac and b) molecular weight marker sample.

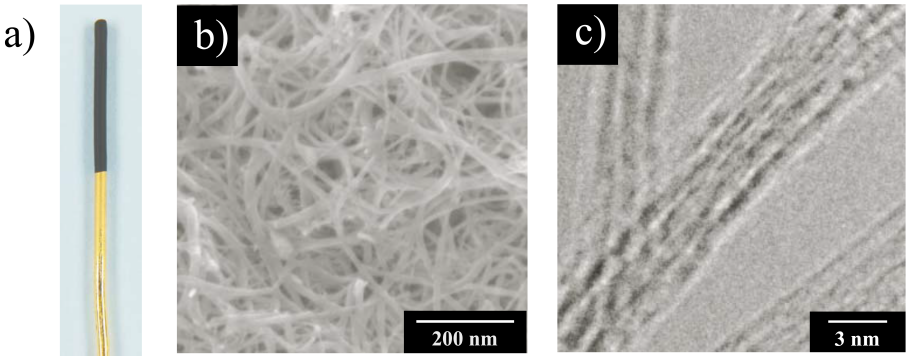


**Figure S2.** a) Photograph of SWCNT-coated Au electrode. b) FE-SEM and TEM images of SWCNTs.

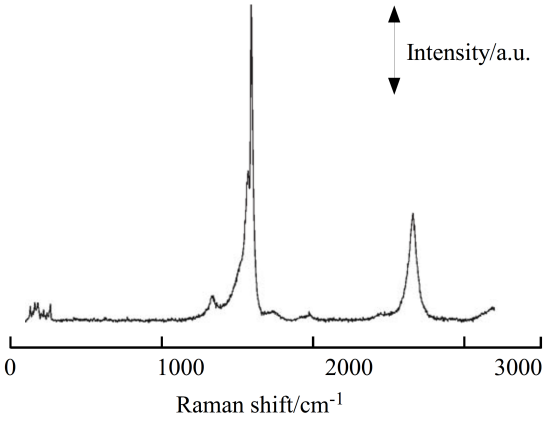


**Figure S3.** Raman spectrum of SWCNTs. Excitation laser wavelength: 514.5 nm (2.41 eV).

**Table S1.** Estimated surface concentrations of Lac.

| Surfactant | Δ Abs (at 577 nm) | Adsorbed Lac [a] $\Gamma$/pmol cm$^{-2}$ | DET active Lac $\Gamma_a$/pmol cm$^{-2}$ | $\Gamma_a/\Gamma$ |
|---|---|---|---|---|
| BIGCHAP | 0.0593 | 7.6 | 0.36 | 0.05 |
| | 0.0585 | 7.5 | | 0.05 |
| | 0.0441 | 5.7 | | 0.06 |
| | 0.0372 | 4.8 | | 0.08 |
| | 0.0369 | 4.7 | | 0.08 |
| | 0.0343 | 4.4 | | 0.08 |
| | 0.0339 | 4.4 | | 0.08 |
| | Average | 5.6 (±2.0) | | 0.07 (±0.02) |
| deoxy BIGCHAP | 0.0507 | 6.5 | 0.11 | 0.02 |
| | 0.0357 | 4.6 | | 0.02 |
| | 0.0331 | 4.3 | | 0.03 |
| | 0.0331 | 4.3 | | 0.03 |
| | 0.0280 | 3.6 | | 0.03 |
| | 0.0267 | 3.4 | | 0.03 |
| | 0.0221 | 2.8 | | 0.04 |
| | Average | 4.2 (±2.3) | | 0.03 (±0.01) |
| CHAPS | 0.0953 | 12.2 | 2.1 | 0.17 |
| | 0.0925 | 11.9 | | 0.18 |
| | 0.0697 | 9.0 | | 0.23 |
| | 0.0691 | 8.9 | | 0.24 |
| | Average | 10.5 (±1.7) | | 0.20 (±0.04) |
| CHAPSO | 0.1077 | 13.8 | 1.4 | 0.10 |
| | 0.0857 | 11.0 | | 0.13 |
| | 0.0812 | 10.4 | | 0.13 |
| | 0.0810 | 10.4 | | 0.13 |
| | 0.0739 | 9.5 | | 0.15 |
| | Average | 11.0 (±2.8) | | 0.13 (±0.03) |
| OGP | 0.0776 | 10.0 | 2.8 | 0.28 |
| | 0.0656 | 8.4 | | 0.33 |
| | 0.0596 | 7.7 | | 0.37 |
| | 0.0586 | 7.5 | | 0.37 |
| | 0.0564 | 7.2 | | 0.39 |
| | 0.0481 | 6.2 | | 0.45 |
| | Average | 7.8 (±2.2) | | 0.37 (±0.09) |
| MEGA10 | 0.0602 | 7.7 | 3.0 | 0.39 |
| | 0.0483 | 6.2 | | 0.48 |
| | 0.0395 | 5.1 | | 0.59 |
| | 0.0360 | 4.6 | | 0.65 |
| | Average | 5.9 (±1.8) | | 0.53 (±0.14) |
| DMP | 0.0988 | 12.7 | 1.9 | 0.15 |
| | 0.0962 | 12.4 | | 0.15 |
| | 0.0930 | 12.0 | | 0.16 |
| | 0.0788 | 10.1 | | 0.19 |
| | 0.0649 | 8.3 | | 0.23 |
| | 0.0633 | 8.1 | | 0.23 |
| | Average | 10.6 (±2.5) | | 0.19 (±0.04) |
| None | 0.0604 | 7.8 | 3.4 | 0.44 |
| | 0.0533 | 6.8 | | 0.50 |
| | 0.0508 | 6.5 | | 0.52 |
| | 0.0502 | 6.4 | | 0.53 |
| | Average | 6.9 (±0.9) | | 0.50 (±0.06) |

[a]BET surface area.

**Table S2.** Estimated surface concentrations of biosurfactants.

| Surfactant | Mass [a] /μg cm$^{-2}$ | Surface concentration[b] /nmol cm$^{-2}$ | Coverage [b] |
|---|---|---|---|
| BIGCHAP | 12 | 0.12 | 2.7 |
| | 12 | 0.12 | 2.7 |
| | 8 | 0.08 | 1.8 |
| | 8 | 0.08 | 1.8 |
| | Average | 0.10 (±0.02) | 2.2 (±0.5) |
| deoxy BIGCHAP | 36 | 0.38 | 8.2 |
| | 36 | 0.38 | 8.2 |
| | 36 | 0.38 | 8.2 |
| | Average | 0.38 (±0.00) | 8.2 (±0.0) |
| CHAPS | 20 | 0.30 | 3.4 |
| | 12 | 0.18 | 2.0 |
| | 12 | 0.18 | 2.0 |
| | Average | 0.22 (±0.08) | 2.5 (±0.9) |
| CHAPSO | 12 | 0.17 | 2.3 |
| | 8 | 0.12 | 1.5 |
| | 8 | 0.12 | 1.5 |
| | Average | 0.13 (±0.04) | 1.8 (±0.5) |
| OGP | 32 | 1.00 | 6.0 |
| | 32 | 1.00 | 6.0 |
| | 20 | 0.62 | 3.7 |
| | Average | 0.87 (±0.25) | 5.2 (±1.5) |
| MEGA10 | 44 | 1.14 | 9.2 |
| | 32 | 0.83 | 6.7 |
| | Average | 0.99 (±0.16) | 7.9 (±1.3) |
| DMP | 296 | 5.58 | 61.1 |
| | 164 | 3.09 | 33.9 |
| | 108 | 2.03 | 22.3 |
| | Average | 3.57 (±2.01) | 39.1 (±22.0) |

[a]Geometric surface area.

[b]BET surface area.