Supporting information

Enhanced Electroactivity and Substrate Affinity of Microperoxidase-11 Attached to Pyrene-linkers \( \pi-\pi \) Stacked on Carbon Nanostructure Electrodes

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Fig. S1. Raman spectra of MWNT and MWNT/Py films on HPG electrodes.

**FTIR Characterization.** The HPG electrode and MWNT showed the carbonyl vibration peaks at 1660 cm\(^{-1} \) (Figure S2). The FTIR spectra were taken after each modification step on HPG electrodes (Figure S2). MP-11 exhibited peaks between the regions 1500-1600 and 1600-1700 cm\(^{-1} \), which were assigned to the amide-I C=O and the amide-II N-H bending frequencies typical of a peptide backbone.\(^1\)\(^-\)\(^2\) The free amine groups of MP-11 vibration bands (Lys-13 and N-terminus Val-11) were assigned at 1627 cm\(^{-1} \). MWNT/Py modified HPG electrodes exhibited bands at 1665 cm\(^{-1} \) for carbonyl stretching and a broad band at 3480 cm\(^{-1} \) for the –OH stretching, both are attributed to the end –COOH groups of the Py-linker molecules.

The EDC-NHS treatment of MWNT/Py assemblies led to the disappearance of the –OH stretching band of –COOH (originally appeared at 3480 cm\(^{-1} \)) due to its conversion to N-succinimidyl groups and the appearance of new peaks centered at 1452 and 1769 cm\(^{-1} \) were attributed to C-N and C=O stretchings, respectively.\(^3\)\(^-\)\(^5\) The IR-spectrum obtained after the covalent bonding of MP-11 to MWNT/Py assemblies depicted characteristic bands for peptide amide-I and amide-II bonds in the region of 1500 to 1700 cm\(^{-1} \). Additionally the appearance of bands in the region of 3270 to 3400 cm\(^{-1} \) represent the amide A and B bonds of MP-11.\(^2\) Taken together, the FTIR characterization confirmed the covalent immobilization of MP-11 to MWNT/Py units on HPG electrodes (Figure S3).
**Fig. S2.** FTIR spectra of (a) only HPG and (b) only MWNT.

**Fig. S3.** FTIR spectra of (a) MWNT/Py; (b) EDC-NHS treated MWNT/Py; (c) MP11-amine$_{\text{cov}}$ films; and (d) only MP-11.

**Fig. S4.** TEM images for (A) MWNT/Py and (B) MP11-amine$_{\text{cov}}$ films (MP-11 features around the tubes are circled).
**Fig. S5.** EDS of the MP11-amine$_{\text{cov}}$ film. The presence of P, K, and Cl peaks is attributed to arise from PBS.

**Table S1.** Comparison of the electroactive MP-11 amounts on MWNT/Py electrodes with control films and the reported myoglobin-amine$_{\text{cov}}$ film.

<table>
<thead>
<tr>
<th>MP-11 Film</th>
<th>$\Gamma$ / pmol cm$^{-2}$</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MP11-amine$_{\text{cov}}$</td>
<td>$2385 \pm 103$</td>
<td></td>
</tr>
<tr>
<td>HPG/MWNT-MP11</td>
<td>$1250 \pm 66$</td>
<td>This study</td>
</tr>
<tr>
<td>HPG/MP11</td>
<td>$619 \pm 28$</td>
<td></td>
</tr>
<tr>
<td>HPG/Py-MP11</td>
<td>$746 \pm 30$</td>
<td></td>
</tr>
<tr>
<td>myoglobin-amine$_{\text{cov}}$</td>
<td>$300 \pm 60$</td>
<td>4</td>
</tr>
</tbody>
</table>

**Fig. S6.** Current density versus scan rate plot for the charging currents measured for (a) HPG/MWNT/Py electrode and (b) polished HPG electrode in the absence of immobilized MP-11.

**Calculation of the direct electron transfer rate constant of MP11-amine$_{\text{cov}}$ films.**

\[
E_{pc} = \left[ E^0 - \frac{RT}{nF} \ln \left( \frac{anF}{RTk_s} \right) \right] - \frac{RT}{anF} \ln \left( v \right)
\]

\[
E_{pa} = \left[ E^0 + \frac{RT}{(1 - \alpha)nF} \ln \left( \frac{(1 - \alpha)nF}{RTk_s} \right) \right] + \frac{RT}{(1 - \alpha)nF} \ln \left( v \right)
\]
\[
\Delta E_p = \frac{2.3RT}{a(1-a)nF} [a \log \alpha + (1-a) \log k_s - \log(\frac{RT}{nF})] + \frac{2.3RT}{a(1-a)nF} \log(\nu)
\]  

(3)

Where \(E^0\) is the formal potential, \(\alpha\) is the electron-transfer coefficient, \(k_s\) is the heterogeneous electron transfer rate constant for a surface confined redox process, \(\nu\) is the scan rate, and \(n\) is the number of electrons transferred (\(n = 1\) for MP11-heme). The average value of the transfer coefficient for the MP11-amine\(_{cov}\) film was calculated using the slopes of the plots of \(E_{pa}, E_{pc}\), versus \(\ln(\nu)\) according to Equations 1 and 2. Then Equation 3 was used to plot \(\Delta E_p\) values against \(\log(\nu)\) to obtain the \(k_s\) value (Figure S7).\(^5\)

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**Fig. S7.** Relationship between peak potentials \((E_p)\) and natural logarithm of scan rate for MP11-amine\(_{cov}\) films at pH 7.4, PBS. The linear fit lines at higher scan rates and the corresponding equations are shown.

**Fig. S8.** Reduction currents versus t-BuOOH concentration at 1000 rpm catalyzed by the MP11-amine\(_{cov}\) film in pH 7.4 PBS for a. 0.8, b. 1.6, c. 3.2, and d. 4.8 mM t-BuOOH.

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