Supporting Information to

Biomimetic oxygen reduction reaction catalyzed by microperoxidase-11 at liquid/liquid interfaces

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S1. Adsorption of MP-11 at the water/DCE interface

Figure 1 in the manuscript shows an apparent capacitive current in particular at positive potentials. Moreover, this capacitive current increased with increasing the potential scan rate. As shown in **Figure S1**, plotting the dependence of the current at a specific positive potential, e.g., 0.16 V, on the potential scan rate yielded a linear correspondence, suggesting that MP-11 is not involved in any heterogeneous charge transfer reactions at the water/DCE interface and that the capacitive current most probably arises from a surface process related to the adsorption of positively charged MP-11 molecules.



Figure S1. The dependence of the current at 0.16 V on the potential scan rate. The data was extracted from CVs shown in **Figure 1b**.

Figure 2c shows that the surface excess charge density due to adsorbed MP-11 apparently increases with the concentration of MP-11 in the aqueous solution. The solid lines correspond to the fittings in terms of the Langmuir adsorption

isotherm by eq. 1, from which the Langmuir adsorption constant, namely β , at different Galvani potential differences can be attained. β is defined by,

$$\beta = \exp\left[-\frac{\Delta G}{RT}\right] \tag{S-1}$$

where *R* and *T* are the gas constant and the absolute temperature, respectively. ΔG is the adsorption Gibbs energy, which includes the potential independent and dependent contributions by,

$$\Delta G = \Delta G_a^{\circ} - zFb^{\mathsf{w}} \Delta_o^{\mathsf{w}} \phi \tag{S-2}$$

where ΔG_a^* is the standard Gibbs energy of adsorption that is potential independent. The second term on the equation right side represents the potential dependent contribution. b^* denotes the fraction of the Galvani potential difference in the aqueous phase side that operates on the adsorption of MP-11 from the aqueous phase to the interface. Thus, combining eqs. S-1 and S-2 yielded eq. 2 in the manuscript

From a linear fitting (see **Figure S2**, supporting information), a value of $\Delta G_a^{\bullet} = -16.5 \text{ kJ mol}^{-1}$ was obtained, indicating the adsorption is thermodynamically favorable and the spontaneous nature of the adsorption process. Such a system can be akin to the biological environment where the enzyme is embedded in the bi-lipid layer.



Figure S2. The linear fitting of the dependence of β values on the Galvani potential difference. β values were obtained from the Langmurian fit by eq. 1.

S2. CVs of O₂ reduction with DFc catalyzed by MP-11

Figure S3 compares the cyclic voltammograms of 5 mM DFc at the water/DCE interface in the absence and presence of MP-11. Apparently, a PCET current was observed in the positive potential regime, which is accompanied by an ion transfer current wave at –0.05 V.



Figure S3. (a) CVs of DFc at the polarized water/DCE interface in the absence (x = 0, z = 5, black curve) and presence (x = 5, z = 5, red curve) of MP-11 using the electrochemical cell shown in **Scheme 3**: y = 2, scan rate 50 mV s⁻¹. The potential was swept from negative to positive and was called the forward scan. (b) Illustration of the oxygen reduction reaction catalyzed by MP-11, i.e., a proton coupled electron transfer (PCET), and the ion transfer (IT) of DFc⁺ at the water/DCE interface.

S3. CVs of O₂ reduction catalyzed by MP-11 under argon

In order to prove the involvement of O_2 in the PECT process, cyclic voltammetric measurements were also performed with the solutions prepurged by argon stream for ca. 20 min. As shown in Figure S4, less amounts of TTF^{•+}/DFc⁺ were produced under the nitrogen condition and the PCET current waves at positive potentials also turned smaller, indicating the participation of O_2 in the reaction.



Figure S4. 20 cycles of CVs in the presence of both 5 μ M MP-11 and 5 mM TTF (a) or DFc (b) (x = 5, z = 5) under aerobic (red line) and anaerobic (black line) using the cell shown in **Scheme 3**: y = 2, scan rate 50 mV s⁻¹.

S4. Calculation of the Galvani potential difference

As shown in the paper, two-phase reactions were performed, where the Galvani potential difference across the liquid/liquid interface was controlled by the distribution of all of ions between two phases. In order to calculate this potential, we first considered the Nernst equation for the different ionic species *i* present in the system,

$$\Delta_{o}^{w}\phi = \Delta_{o}^{w}\phi_{tr,i}^{0,w\to o} - \frac{RT}{z_{i}F}\ln\frac{c_{i}^{o}}{c_{i}^{w}}$$
(S-3)

At the same time, the mass balance for the different species was considered,

$$\frac{V_{\rm o}}{V_{\rm w}}c_{i,\text{ initial}}^{\rm o} + c_{i,\text{ initial}}^{\rm w} = \frac{V_{\rm o}}{V_{\rm w}}c_{i}^{\rm o} + c_{i}^{\rm w}$$
(S-4)

where $c_{i,\text{initial}}^{\text{o}}$ and $c_{i,\text{initial}}^{\text{w}}$ stand for the concentrations of *i* initially added in the organic and aqueous phases, respectively. The concentrations at the equilibrium are denoted c_i° and c_i^{w} . In our particular case, the volume ratio between the organic and the aqueous phase V_{o}/V_{w} was always unitary, ergo it will be no further considered. On the other hand, the calculations are always performed assuming equilibrium conditions; therefore the mass balance equilibrium concentrations correspond to those involved in the Nernst equation for the ion transfer process. Additionally, the electroneutrality condition must be fulfilled and reads,

$$\sum_{i}^{j} z_i c_i^{\mathsf{w}} = 0 \tag{S-5}$$

which after being combined with eqs. S-3 and S-4 finally yields,

$$\sum_{i}^{J} z_{i} \frac{c_{i,\text{total}}}{1 + \exp\left[\frac{F}{RT}\left(\Delta_{o}^{W}\phi - \Delta_{o}^{W}\phi_{\text{tr},i}^{\Theta,W \to o}\right)\right]} = 0$$
(S-6)

From above derivation, we can:

- By solving eq. S-6, the potential drop across the interface can be obtained. Nonetheless, prior knowledge of the standard ion transfer potential is required for all the ionic species.
- 2) From the potential value thus obtained, the concentrations for all the ions in both phases can be calculated after applying eqs. S-3 and S-4.

Solving eq. S-6 was carried out by using Mathematica 6.0. Considering the solution composition shown by **Scheme 4**, the Galvani potential difference across the interface calculated was +0.58 V, respectively. And the calculated equilibrim concentrations after putting two phases in contact are shown in **Tables S1**.

Table S1. Calculated Equilibrim Concentrations after Contacting 5 mM DFc/1 mM TTF in DCE with 10 mM HCl and 5 mM LiTB in Water

_	$c_{\rm H}^+/{ m mM}$	$c_{\rm Li}^+/{ m mM}$	c_{TB}/mM	$c_{\rm Cl}/{\rm mM}$
Water	5.75	4.33	0.07	10.00
DCE	4.25	0.67	4.93	7.33×10^{-18}

The calculated Galvani potential difference is approximately +0.58 V.

S5. Biphasic reaction using DFc as the electron donor

Figure S5 shows the biphasic reaction results using DFc as the electron donor. After 2 h of the biphasic reaction, the generation of DFc⁺ was witnessed by the green color of DFc⁺ (see **Figure S5a**) and its absorption band at 652 nm (see **Figure S5b**). The generation of H_2O_2 was proved by titration with NaI and two absorption bands at 287 and 352 nm due to thus formed I_3^- (see **Figure S5c**). Apparently, in the presence of MP-11, the amount of DFc⁺ and H2O2 is significantly higher, proving its catalytic role in the O2 reduction reaction.



Figure S5. (a) Photographs of biphasic reaction before (left) and after stirring 2 h (right) using the cell shown in **Scheme 4**: x = 0 (flask 1) and x = 5 (flask 2). The initial Galvani potential difference was +0.58 V. (b) UV-visible spectra of DCE solutions separated from the flask 1 (blue curve) and flask 2 (red curve) after biphasic reaction. A freshly prepared DCE solution containing 5 mM DFc is also compared. (c) UV-visible spectra of aqueous solutions separated from the flask 2 (red curve) after biphasic reaction, treated by excess NaI. A freshly prepared aqueous solution containing 5 μ M MP-11 is also compared.

Time profiles of the formation of DFc⁺ and H_2O_2 are compared in **Figure S6**. In the presence of MP-11, the generation of DFc⁺ and H_2O_2 is clearly much faster, proving the catalytic role of MP-11. However, remarkable decrease of H_2O_2 amount was observed after 45 min due to further reduction of H_2O_2 to H_2O_2 .



Figure S6. Time profile of the formation of DFc⁺ (a) and H₂O₂ (b) in the absence (black) and presence (red) of 5 μ M MP-11 in a biphasic reaction with the solution composition illustrated by the cell in **Scheme 4**. The Galvani potential difference across the interface was +0.58 V. The quantities of DFc⁺ (a) and H₂O₂ (b, diluted by 4 times) were represented by the absorbance of DFc⁺ at 652 nm and of I₃⁻ generated by titration with excess NaI at 352 nm, respectively.

S6. Dependence of the PCET current on the MP-11 concentration

As shown in **Figure S7**, the PCET current and the DFc⁺ transfer current increased with increasing the MP-11 concentration, indicating more DFc⁺ produced and the catalytic activity of MP-11 in the O₂ reduction reaction by DFc (see **Figure S7a**). In addition, the PCET current tended to reach a steady value at a higher MP-11 concentration (see **Figure S7b**).



Figure S7. (a) CVs of 5 mM DFc in DCE (z = 5) and different concentration of MP-11 in water: x = 0.5, 1, 3 and 5 (from inner to outer), using the electrochemical cell shown in **Scheme 3**: y = 2, scan rate 50 mV s⁻¹. (b) The dependence of electrical current on the MP-11 concentration at three different Galvani potential differences.