Supporting Information

The Use of Lipid Bilayers for the Construction of Reagentless Biosensors Based on NAD^+ Dependent Glutamate Dehydrogenase.

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EXPERIMENTAL SECTION

Materials. GLDH (E.C. 1.4.1.3) from bovine liver, as suspension in saturated ammonium was purchased from Biozyme (UK), nicotinamide adenine nucleotide (NAD^+), L-glutamic acid monosodium salt, dimethylformamid (DMF), *n*-$C_{18}H_{37}NH_2$, sodium dihydrogen phosphate, sodium hydroxide, *ortho*-phosphoric acid, sodium chloride, thionyl chloride, NH_4PF_6, 2,2'-bipyridine-4,4'-dicarboxylic acid, absolute 1,4-dioxane were obtained from Sigma-Aldrich (USA), K_2OsCl_6 was from Alfa (Spain), ethylene glycol and sodium dithionite were obtained from Pancreac (Madrid, Spain), 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine from Larodan Fine Chemicals (Sweden), 1,10-phenanthroline-5,6-dione was prepared by the published procedure.[27]

[Os(1,10-phenanthroline-5,6-dione)$_2$Cl$_2$] was synthesized according to the adapted method.[26] Generally the synthesis procedure involved refluxing of K_2OsCl_6 with 1,10-phenanthroline-5,6-dione in DMF under argon in the dark for 1 hr, followed by reduction with aqueous sodium dithionite solution.

Synthesis of 4,4′-[$CH_3(CH_2)_{17}NHCO]_2$bpy (I)

0.82 mmol of 2,2'-bipyridine-4,4'-dicarboxylic acid were refluxed during 24 h in 4 ml of thionyl chloride (Aldrich, USA), next thionyl chloride was evaporated under vacuum during 4 h. The reaction product was dissolved in 40 ml of absolute 1,4-dioxane. This solution was added dropwise to a solution 1.8 mmol of *n*-$C_{18}H_{37}NH_2$ in 200 ml of absolute 1,4-dioxane during 20 min, the reaction mixture was left to stay overnight. The next day the solid was filtered off, washed with 1,4-dioxane and air-dried to give compound I.

Synthesis of [Os(1,10-phenanthroline-5,6-dione)$_2$(4,4′-[CH$_3$(CH$_2$)$_{17}$NHCO]$_2$bpy)](PF$_6$)$_2$ (II) (Os-phendione-surfactant)

0.1468 mmol of [Os(1,10-phenanthroline-5,6-dione)$_2$Cl$_2$] and 0.1762 mmol of compound I were refluxed during 1 h in 5 ml of deaerated ethylene glycol under argon in the dark. Next the product was precipitated by mixing with a solution of 1 g of NH_4PF_6 in 100 ml of water. The solid was filtered off and washed with water, extracted with acetone and air dried to yield compound II. The synthesis route is presented in Figure S-1. ^{1}H NMR performed in $CDCl_3$ with a tetramethylsilane standard confirmed the presence of long alkane chains: 1.29 p. p. m. (m) 1.26 (m), 0.88 (t).

Preparation of bovine GLDH solution

In order to purify mesophilic bovine GLDH 4 ml of meso GLDH suspension in saturated ammonium sulphate solution were centrifuged during 10 min at 14000 RPM at 5°C. The precipitated enzyme was isolated from the ammonium sulfate solution and dissolved in 3 ml of 0.1 M sodium phosphate buffer (pH 7.4) containing 0.15 M sodium chloride, next the resulting enzyme solution was extensively dialyzed at 5°C against 200 ml of the same buffer during 24 h, the buffer solution being changed every two hours.

Preparation of graphite electrodes. Spectrographic graphite rods of 3 mm in diameter (Carbone of America, USA) were cut into pieces of 2 cm in longitude, introduced into heat-shrinkable PVC plastic tubes, shrunk by heating, wet polished on fine (grit 400 and 600) emery paper (Buehler, USA) and sonicated in water.

Preparation of reagentless glutamate biosensors based on immobilization between lipid bilayers. 1.5 mg of Os-phendione-surfactant and 10 mg of 1,2-dioleoyl-sn-glycero-3-phosphatiylcholine were dissolved in 0.5 mL of chloroform. Then this solution was evaporated under vacuum in a pear shaped flask to form the solid thin layer on the bottom. Next 1 mL of a solution containing 5.8 mg mL^{-1} (38 U mL^{-1} at 30°C) of bovine glutamate dehydrogenase and 40 mg of NAD^+ in 0.1 M phosphate buffer pH 7.6 were stirred during 2 h to form the lamellar phase. The suspension was washed 6 times with 1 mL of 0.1 M phosphate buffer (pH 7.6), using centrifugation at 4000 rpm during 10 min every time. Next the volume of this suspension was adjusted to 1 mL and 5 μL of it was deposited per graphite electrode and air-dried during 1 h.

Electrochemical measurements. A three-electrode conventional thermostabilised cell (3 mL) equipped with an Ag/AgCl/KCl$_{sat}$ reference electrode, a platinum auxiliary electrode, and a graphite electrode modified according to one of the above mentioned methods as working electrode. The buffer, pH 7.4 (0.1 M sodium phosphate containing 0.15 M sodium chloride) served as supporting electrolyte. The response to successive additions of stock glutamate solution was registered as steady state current on a three-electrode potentiostat Autolab PGSTAT 10 (Eco chemie, Holland) controlled by a computer. The working potential was 150 mV *vs.* Ag/AgCl/KCl$_{sat}$ and the temperature of a thoroughly stirred solution was 30°C.

GLDH assay method. The activity of GLDH was tested spectrophotometrically by following the increase in absorbance at 340 nm, 30°C. The assay solution contained 5 mM NAD^+ and 10 mM in 0.1 M

sodium phosphate buffer (pH 7.4). The ΔA_{340} (Au/min) was obtained using the maximum linear rate for both the test and blank (without the enzyme) mixtures. The activity was calculated using ε_{NADH}=6.22 $mM^{-1}cm^{-1}$ extinction coefficient of β-NADH. One unit of GLDH oxidizes 1 μmol of L-glutamate per minute at pH 7.4 at 30°C. Protein concentration was determined spectrophotometrically at A_{280} using an absorbance coefficient of 0.973 $cm^2\,mg^{-1}$.

Figure 2S illustrates the kinetic scheme for the operation of reagentless biosensors based on the enzyme glutamate dehydrogenase (E) catalyzing the reaction of immobilized NAD^+ (A) with a L-glutamate (B) to give immobilized NADH (P), α–ketoglutarate (Q), and NH_4^+ (R). Then a mediator (M), which can stay in reduced (M_{red}) and oxidized (M_{ox}) forms, immobilized on the electrode surface reoxidizes NADH to NAD^+. A_{im} and P_{im} are the concentrations of immobilized A and P. B_o, Q_o, R_o are the concentrations of the substrate and the products in the lamellar phase B_∞, Q_∞ and R_∞ are the concentrations of the substrate and the products in the external medium. k'_B, k'_Q and k'_R are mass transfer coefficients of the substrate and the products (cm s^{-1}), respectively. L is the thickness of the film in which the enzymatic reaction occurs. The following expressions for the flux of B to the electrode surface (j_{el}), which determines the current density of a biosensor (j) through the equation $j = nFj_{el}$, where n is the number of exchanged electrons and F is the Faraday's constant, can be obtained.

$$j_{el} = k'_B(B_\infty - B_o) \quad \text{(S-1)}$$
$$j_{el} = L(k_1 A_{im}[E] - k_{-1}[E\cdot A]) \quad \text{(S-2)}$$
$$j_{el} = L(k_2 B_o[E\cdot A] - k_{-2}[E\cdot A\cdot B]) \quad \text{(S-3)}$$
$$j_{el} = L(k_3 [E\cdot A\cdot B] - k_{-3}[E\cdot P\cdot Q\cdot R]) \quad \text{(S-4)}$$
$$j_{el} = L(k_4 [E\cdot P\cdot Q\cdot R] - k_{-4}[E\cdot P\cdot Q]Q_o) \quad \text{(S-5)}$$
$$j_{el} = L(k_5 [E\cdot P\cdot Q] - k_{-5}[E\cdot P]Q_o) \quad \text{(S-6)}$$
$$j_{el} = L(k_6 [E\cdot P] - k_{-6}[E]P_{im}) \quad \text{(S-7)}$$
$$j_{el} = k'_Q Q_o \quad \text{(S-8)}$$
$$j_{el} = k'_R R_o \quad \text{(S-9)}$$
$$j_{el} = k_7 P_o \Gamma_{Mox} - k_{-7}\Gamma_{M\cdot P} \quad \text{(S-10)}$$
$$j_{el} = k_8 \Gamma_{M\cdot P} \quad \text{(S-11)}$$
$$j_{el} = k_S \Gamma_{Mred} \quad \text{(S-12)}$$
$$E_t = [E] + [E\cdot A] + [E\cdot A\cdot B] + [E\cdot P\cdot Q\cdot R] + [E\cdot P\cdot Q] + [E\cdot P] \quad \text{(S-13)}$$
$$\Gamma_M = \Gamma_{M\cdot P} + \Gamma_{Mox} + \Gamma_{Mred} \quad \text{(S-14)}$$
$$A_t = A_{im} + P_{im} \quad \text{(S-15)}$$

Where Γ_{Mox} and Γ_{Mred} are the surface coverages of the mediator in oxidized and reduced forms, respectively. Γ_M is the total mediator surface coverage. $\Gamma_{M\cdot P}$ is the surface coverage of the intermediate complex between the mediator and NADH. A_t and E_t are the total concentrations of NAD^+ and the enzyme immobilized in the lamellar phase, respectively. The equations (S-2 – S-7, S-13) yield the equation for the enzymatic kinetics:

$$j_{el} = \frac{L(V_1 A_{im} B_o - (V_1 P_{im} Q_o R_o)/K_{eq})}{\begin{aligned} &K_{AB} + K_B A_{im} + K_A B_o + A_{im} B_o + K_{QR} K_{iA} K_B P_{im}/(K_{iP} K_{iQ} K_R) + K_{PQ} K_{iA} K_B R_o/(K_{iP} K_{iQ} K_R) + \\ &K_P K_{iA} K_B Q_o R_o/(K_{iP} K_{iQ} K_R) + K_R K_{iA} K_B P_{im} Q_o/(K_{iP} K_{iQ} K_R) + K_{iA} K_B P_{im} Q_o R_o/(K_{iP} K_{iQ} K_R) + \\ &K_P K_{iA} K_B R_o P_{im}/(K_{iP} K_{iQ} K_R) + K_A B_o P_{im}/K_{iP} + K_A B_o P_{im} Q_o/(K_{iQ} K_{iP}) + \\ &K_{iR} K_P K_B A_{im} B_o Q_o/(K_{iB} K_{iP} K_{iQ} K_R) + K_P K_B A_{im} R_o Q_o/(K_{iP} K_{iQ} K_R) + K_{PQ} K_B A_{im} R_o/(K_{iP} K_{iQ} K_R) + \\ &K_P K_B A_{im} B_o Q_o R_o/(K_{iB} K_{iP} K_{iQ} K_R) + K_A B_o R_o P_{im} Q_o/(K_{iR} K_{iP} K_{iQ}) + K_{PQ} K_B A_{im} B_o R_o/(K_{iB} K_{iP} K_{iQ} K_R) \end{aligned}} \quad \text{(S-16)}$$

Where the constant of equilibrium $K_{eq}= k_1k_2k_3k_4k_5k_6/(k_{-1}k_{-2}k_{-3}k_{-4}k_{-5}k_{-6})$. $V_1 = E_t(k_1k_2k_3k_4k_5k_6)/(k_1k_2k_5k_6k_{-3} + k_1k_2k_4k_5k_6 + k_1k_2k_3k_5k_6 + k_1k_2k_3k_4k_6 + k_1k_2k_3k_4k_5)$ and $V_2 = E_t(k_{-1}k_{-2}k_{-3}k_{-4}k_{-5}k_{-6})/(k_{-2}k_{-3}k_{-4}k_{-5}k_{-6} + k_{-1}k_{-3}k_{-4}k_{-5}k_{-6} + k_{-1}k_{-2}k_{-4}k_{-5}k_{-6} + k_3k_{-1}k_{-4}k_{-5}k_{-6})$ are the maximum velocities in forward and reverse directions, the Michaelis constants for A, B, P, Q, R are $K_A = (k_2k_3k_4k_5k_6)/(k_1k_2k_5k_6k_{-3} + k_1k_2k_4k_5k_6 + k_1k_2k_3k_5k_6 + k_1k_2k_3k_4k_6 + k_1k_2k_3k_4k_5)$, $K_B = (k_1k_5k_6k_{-2}k_{-3} + k_1k_4k_5k_6k_{-2} + k_1k_3k_4k_5k_6)/(k_1k_2k_5k_6k_{-3} + k_1k_2k_4k_5k_6 + k_1k_2k_3k_5k_6 + k_1k_2k_3k_4k_6 + k_1k_2k_3k_4k_5)$, $K_{AB} = (k_5k_6k_{-1}k_{-2}k_{-3} + k_4k_5k_6k_{-1}k_{-2} + k_{-1}k_3\ k_4k_5\ k_6)/(k_1k_2k_5k_6k_{-3} + k_1k_2k_4k_5k_6 + k_1k_2k_3k_5k_6 + k_1k_2k_3k_4k_6 + k_1k_2k_3k_4k_5)$, $K_P = k_{-1}k_{-2}k_{-3}k_{-4}k_{-5}/(k_{-2}k_{-3}k_{-4}k_{-5}k_{-6} + k_{-1}k_{-3}k_{-4}k_{-5}k_{-6} + k_{-1}k_{-2}k_{-4}k_{-5}k_{-6} + k_3k_{-1}k_{-4}k_{-5}k_{-6})$, $K_Q = k_{-1}k_{-2}k_{-3}k_{-4}k_{-6}/(k_{-2}k_{-3}k_{-4}k_{-5}k_{-6} + k_{-1}k_{-3}k_{-4}k_{-5}k_{-6} + k_{-1}k_{-2}k_{-4}k_{-5}k_{-6} + k_3k_{-1}k_{-4}k_{-5}k_{-6})$, $K_R = (k_{-1}k_{-2}k_{-3}k_{-5}k_{-6} + k_4k_{-1}k_{-2}k_{-5}k_{-6} + k_3k_4k_{-1}k_{-5}k_{-6})/(k_{-2}k_{-3}k_{-4}k_{-5}k_{-6} + k_{-1}k_{-3}k_{-4}k_{-5}k_{-6} + k_{-1}k_{-2}k_{-4}k_{-5}k_{-6} + k_3k_{-1}k_{-4}k_{-5}k_{-6})$, $K_{QR} = (k_5k_{-1}k_{-2}k_{-3}k_{-6} + k_4k_5k_{-1}k_{-2}k_{-6} + k_3k_4k_5k_{-1}k_{-6})/(k_{-2}k_{-3}k_{-4}k_{-5}k_{-6} + k_{-1}k_{-3}k_{-4}k_{-5}k_{-6} + k_{-1}k_{-2}k_{-4}k_{-5}k_{-6} + k_3k_{-1}k_{-4}k_{-5}k_{-6})$, $K_{PQ}= k_6k_{-1}k_{-2}k_{-3}k_{-4}/(k_{-2}k_{-3}k_{-4}k_{-5}k_{-6} + k_{-1}k_{-3}k_{-4}k_{-5}k_{-6} + k_{-1}k_{-2}k_{-4}k_{-5}k_{-6} + k_3k_{-1}k_{-4}k_{-5}k_{-6})$, $K_{PQR}=(k_5k_6k_{-1}k_{-2}k_{-3} + k_4k_5k_6k_{-1}k_{-2} + k_{-1}k_3\ k_4k_5\ k_6)/(k_{-2}k_{-3}k_{-4}k_{-5}k_{-6} + k_{-1}k_{-3}k_{-4}k_{-5}k_{-6} + k_{-1}k_{-2}k_{-4}k_{-5}k_{-6} + k_3k_{-1}k_{-4}k_{-5}k_{-6})$. The inhibition constants for A, B, P, Q, R are $K_{iA} = k_{-1}/k_1$, $K_{iB} = (k_{-2}k_{-3})/(k_2(k_{-3} + k_3))$, $K_{iP} = k_6/k_{-6}$, $K_{iQ} = k_5/k_{-5}$, $K_{iR} = (k_3k_4)/(k_{-4}(k_3 + k_{-3}))$.

The equations (S-10 – S-12, S-14) give the equation (S-17) governing the kinetics of NADH oxidation at the electrode surface modified with the Os-phendione-surfactant mediator

$$j_{el} = \frac{\Gamma_M k_7k_8k_S P_o}{k_8k_S + k_{-7}k_S + (k_7k_8 + k_7k_S)P_{im}} = \frac{\Gamma_M P_{im} k_7k_8k_S/(k_7k_8 + k_7k_S)}{(k_8k_S + k_{-7}k_S)/(k_7k_8 + k_7k_S) + P_{im}} = \frac{k_{cat}\Gamma_M P_{im}}{k_M + P_{im}} \quad \text{(S-17)}$$

The Michaelis constant for the mediator M is $k_M = (k_8k_S + k_{-7}k_S)/(k_7k_8 + k_7k_S)$ and $k_{cat} = k_8k_S/(k_8 + k_S)$. On the basis of the equations (S-15) and (S-17) the concentrations of NAD^+ and NADH (A_{im} and P_{im}) as well as B_o, Q_o and R_o from (S-1), (S-8) and (S-9) can be found: $P_{im} = j_{el}K_M/(k_{cat}\Gamma_M - j_{el})$, $A_{im} = A_t - P_{im} = A_t - j_{el}K_M/(k_{cat}\Gamma_M - j_{el})$, $B_o = B_\infty - j_{el}/k'_B$, $Q_o = j_{el}/k'_Q$, $R_o = j_{el}/k'_R$. Substitution of A_{im}, B_o, P_{im}, Q_o, R_o into the equation (S-16) yields the equation of the fourth order:

$$LV_1\Gamma_Mk_{cat}E_tA_tB_\infty = j_{el}[K_{AB}\Gamma_M k_{cat} + K_B\Gamma_Mk_{cat}A_t + K_A\Gamma_Mk_{cat}B_\infty + A_t\Gamma_Mk_{cat}B_\infty + LV_1(A_t + K_M)B_\infty + (LV_1A_t\Gamma_Mk_{cat})/k'_B] + j^2_{el}[(K_{PQ}K_BA_t\Gamma_Mk_{cat})/(K_{iP}K_{iQ}K_Rk'_R) - LV_1(A_t + K_M)/k'_B - K_{AB} - K_B(A_t + K_M) - K_AB_\infty + K_MK_{QR}K_{iA}K_B/(K_{iP}K_{iQ}K_R) - (A_t + K_M)B_\infty - A_t\Gamma_Mk_{cat}/k'_B + \Gamma_Mk_{cat}K_{PQ}K_{iA}K_BR_o/(K_{iP}K_{iQ}K_Rk'_R) + K_AK_MB_\infty/K_{iP} + K_{iR}K_PK_BA_tB_\infty\Gamma_Mk_{cat}/(K_{iB}K_{iP}K_{iQ}K_Rk'_Q) + K_{PQ}K_BA_tB_\infty\Gamma_Mk_{cat}/(K_{iB}K_{iP}K_{iQ}k'_R)] + J^3_{el}[LV_1K_M/(K_{eq}k'_Qk'_R) + K_A/k'_B + (A_t + K_M)/k'_B - K_{PQ}K_{iA}K_B/(K_{iP}K_{iQ}K_Rk'_R) + \Gamma_Mk_{cat}K_PK_{iA}K_B/(K_{iP}K_{iQ}K_R\,k'_Qk'_R) + K_MK_RK_{iA}K_B/(K_{iP}K_{iQ}K_Rk'_Q) + K_MK_PK_{iA}K_B/(K_{iP}K_{iQ}K_Rk'_R) - K_AK_M/(K_{iP}k'_B) + K_AK_MB_\infty/(K_{iQ}K_{iP}k'_Q) - K_{iR}K_PK_B(A_t + K_M)B_\infty/(K_{iB}K_{iP}K_{iQ}K_Rk'_Q) - K_{iR}K_PK_BA_t\Gamma_Mk_{cat}/(K_{iB}K_{iP}K_{iQ}K_R\,k'_Bk'_Q) + K_PK_BA_t\Gamma_Mk_{cat}/(K_{iP}K_{iQ}K_Rk'_Qk'_R) - K_{PQ}K_B(A_t + K_M)/(K_{iP}K_{iQ}K_Rk'_R) + K_PK_BA_tB_\infty\Gamma_Mk_{cat}/(K_{iB}K_{iP}K_{iQ}K_Rk'_Qk'_R) - K_{PQ}K_BB_\infty(A_t + K_M)/(K_{iB}K_{iP}K_{iQ}K_Rk'_R) - A_t\Gamma_Mk_{cat}K_{PQ}K_B/(K_{iB}K_{iP}K_{iQ}K_Rk'_Bk'_R)] + j^4_{el}[K_{iA}K_BK_M/(K_{iP}K_{iQ}K_Rk'_Qk'_R) - K_PK_{iA}K_B/(K_{iP}K_{iQ}K_Rk'_Qk'_R) - K_AK_M/(K_{iQ}K_{iP}k'_Bk'_Q) + K_{iR}K_PK_B(A_t + K_M)/(K_{iB}K_{iP}K_{iQ}K_Rk'_Bk'_Q) - K_PK_B(A_t + K_M)/(K_{iP}K_{iQ}K_Rk'_Qk'_R) - K_PK_BB_\infty/(K_{iB}K_{iP}K_{iQ}K_Rk'_Qk'_R) - K_PK_BA_t\Gamma_Mk_{cat}/(K_{iB}K_{iP}K_{iQ}K_Rk'_Bk'_Qk'_R) + K_AB_\infty K_M/(K_{iR}K_{iP}K_{iQ}k'_Qk'_R) + K_{PQ}K_B(A_t + K_M)/(K_{iB}K_{iP}K_{iQ}K_Rk'_Bk'_R)] + j^5_{el}[K_PK_B(A_t + K_M)/(K_{iB}K_{iP}K_{iQ}K_Rk'_Bk'_Qk'_R) - K_AK_M/(K_{iR}K_{iP}K_{iQ}k'_Bk'_Qk'_R)] \quad \text{(S-18)}$$

Use of the inhibition constants allows to redefine: $K_{PQ}=K_{iP}K_Q$ and $K_{QR}=K_{iQ}K_R$.

The model takes into consideration the general flux of glutamate to the biosensor from a bulk solution, the general flux of enzymatic oxidation of glutamate by NAD^+ to give NADH, the general flux of NADH reoxidation by Os-phendione-surfactant, and the general flux of charges from reduced Os-phendione-

surfactant to the electrode surface. In steady state all these fluxes are equal to each other and to hence to j_{el}. The possible polarization of glutamate and the products of its oxidation due to their slow transport through the lipid bilayers can make individual fluxes of enzymatic NAD^+ reduction by glutamate, individual fluxes of NADH reoxidation by the mediator be dependent of the distance from the graphite electrode. This polarization is taken into consideration by using general apparent mass transfer coefficients k'_B, k'_Q and k'_R. Another kind of polarization can be caused by the slow transport of charges from reduced Os-phendione-surfactant in lipid bilayers to the electrode surface, but this fact is reflected in the values of the general apparent constants k_M and k_{cat} which are defined through the kinetic constants k_7, k_{-7}, k_8 and k_S, where k_S is the apparent constant of the heterogeneous charge transfer between the electrode surface and Os-phendione-surfactant molecules distributed throughout the film. So, the model does not describe the distribution of individual fluxes and concentrations throughout the lipid bilayers, but it describes the general current generated by glutamate oxidation in the film using apparent general kinetic constants which actually consider lipid bilayers' geometry.

Figures and Figure Captions.

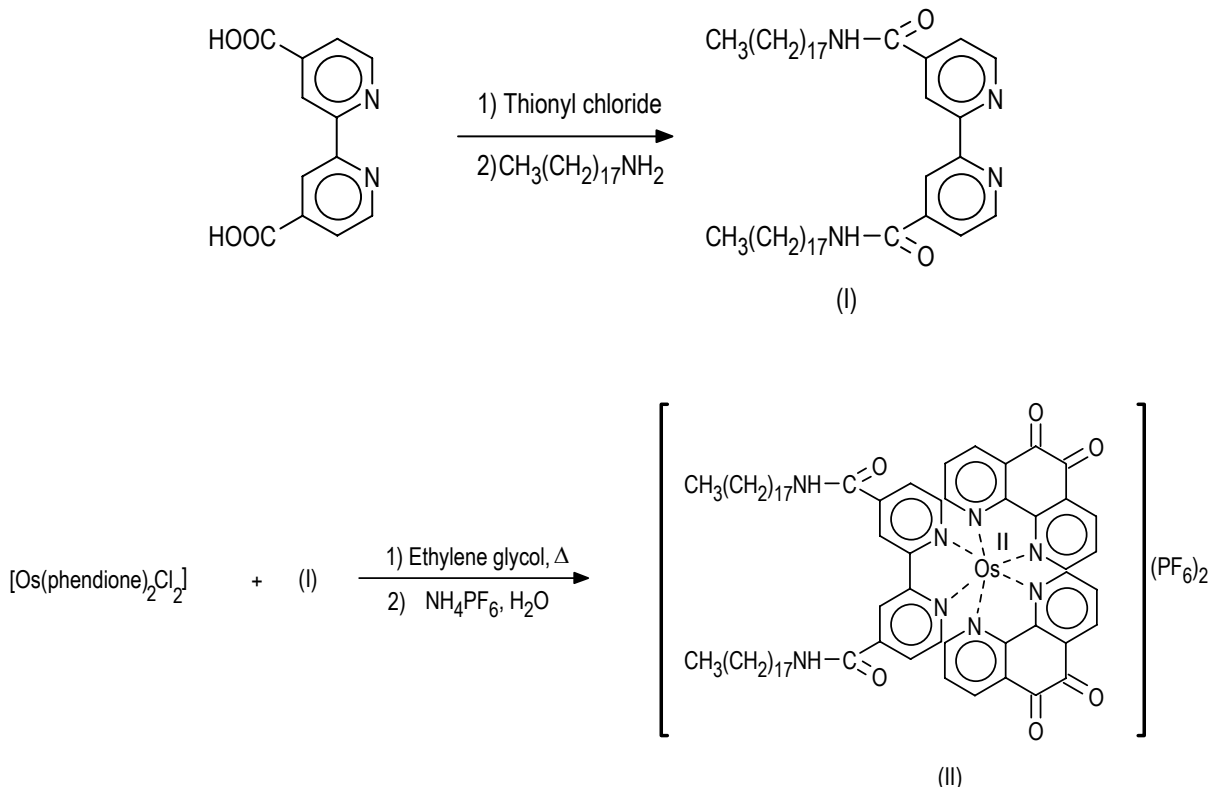

Figure S-1. Synthetic route to the NADH oxidizing tensoactive mediator Os-phendione-surfactant.

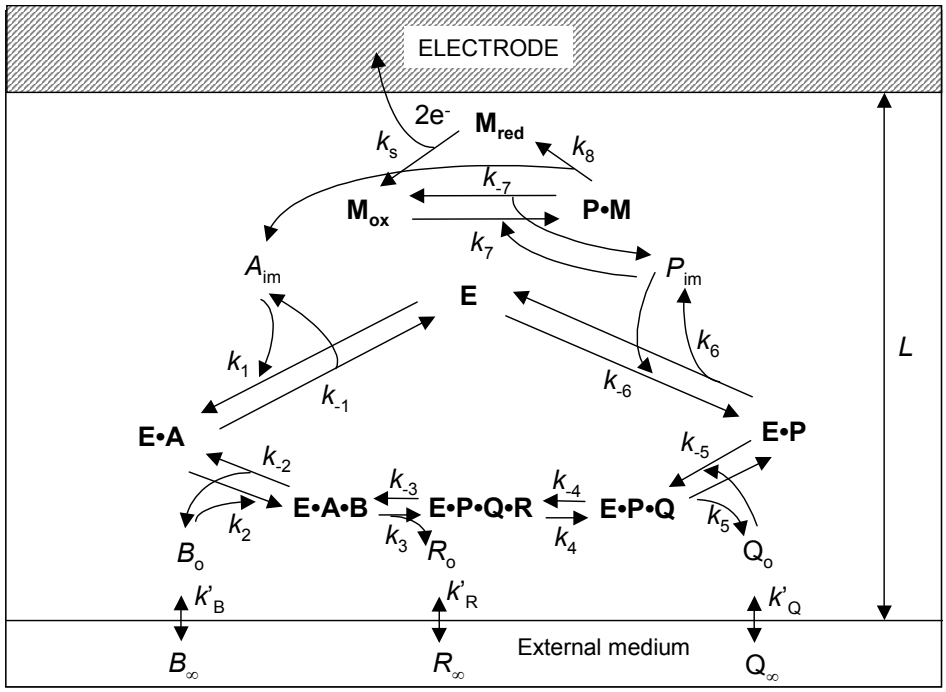

Figure S-2. Reaction scheme for a reagentless glutamate biosensor.

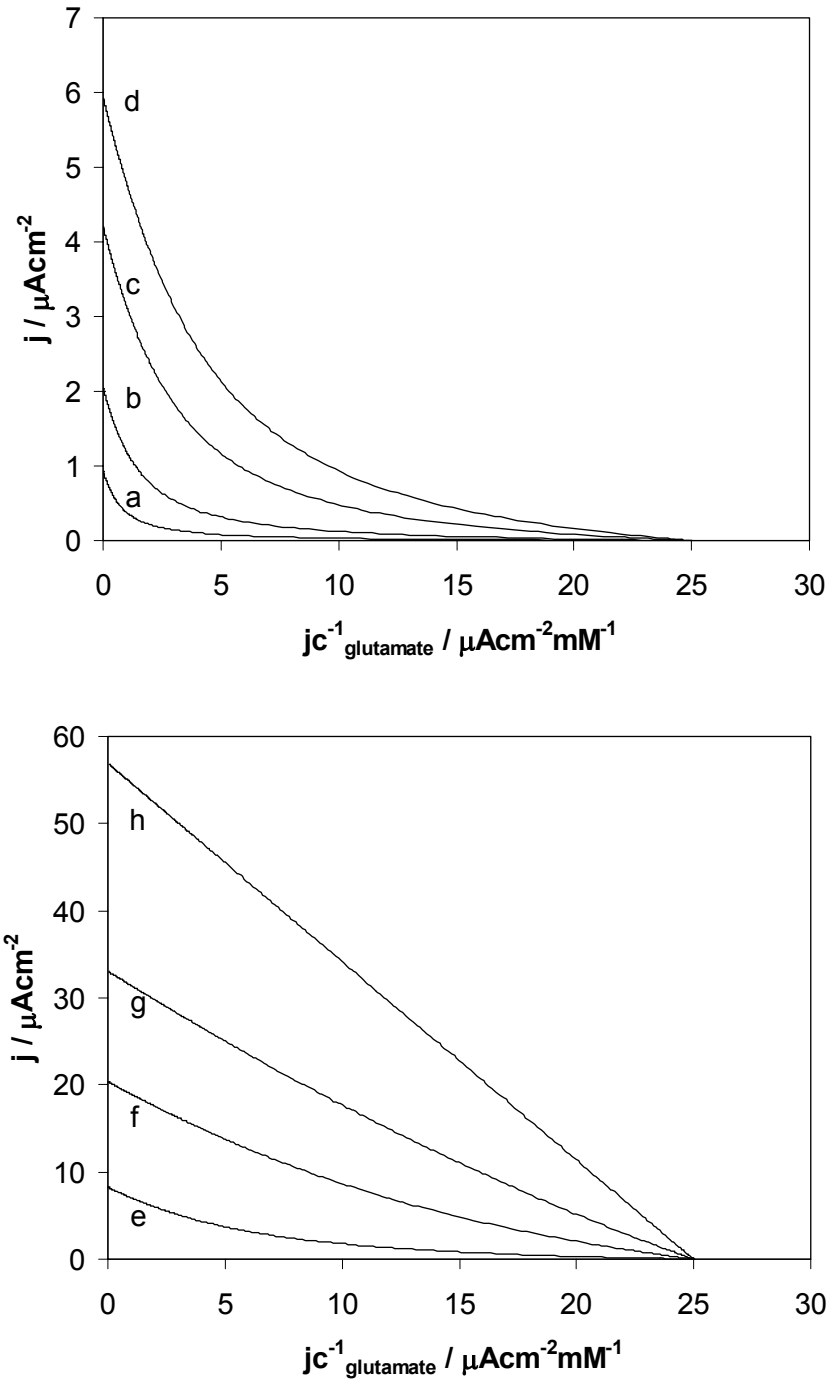

Figure S-3. Simulation of the Eadie-Hofstee plots for the reagentless glutamate biosensors with varying maximum fluxes of the electrochemical NADH oxidation (Γk_{cat}): a) 2.6×10^{-11} mol s^{-1} cm^{-2}; b) 1.04×10^{-10} mol s^{-1} cm^{-2}; c) 4.15×10^{-10} mol s^{-1} cm^{-2}; d) 8.3×10^{-10} mol s^{-1} cm^{-2}; e) 1.7×10^{-9} mol s^{-1} cm^{-2}; f) 1.3×10^{-8} mol s^{-1} cm^{-2}; g) 5.178×10^{-8} mol s^{-1} cm^{-2}; h) ∞. The values of other parameters are: A_t=1 mM; $k'_B \rightarrow \infty$; $k'_Q \rightarrow \infty$; $k'_R \rightarrow \infty$; LV_l=3.63×10^{-10} mol s^{-1} cm^{-2}; K_M = 0.8 mM; K_A = 0.23 mM; K_B = 2.5 mM; K_{AB} = 0.3 mM^2; K_{iA} = 10 mM; K_{iB} = 11 mM; K_R = 20 mM; K_Q = 0.25 mM; K_P = 0.04 mM; K_{iR} = 9 mM; K_{iQ} = 1.6 mM; K_{iP} = 0.03 mM.

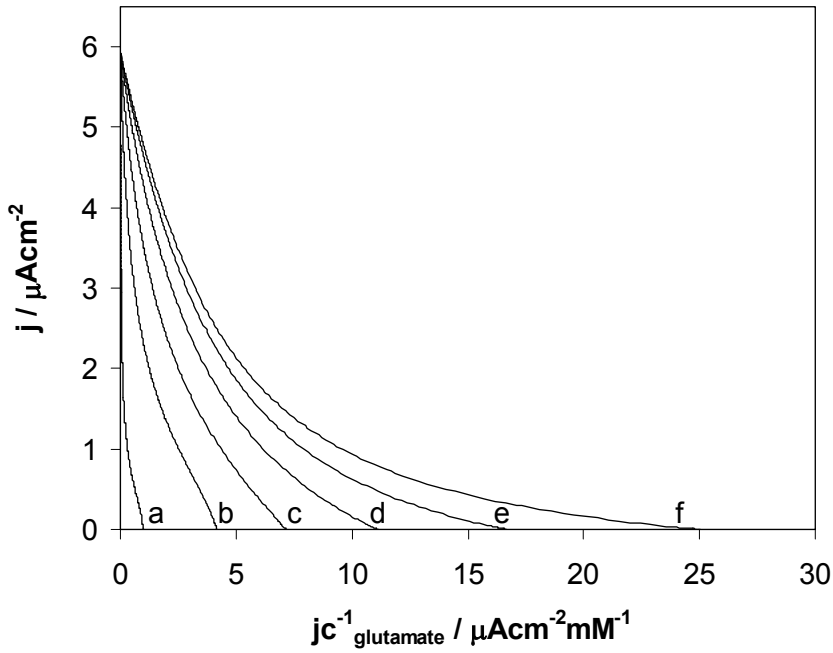

Figure S-4. Simulation of the Eadie-Hofstee plots for the reagentless glutamate biosensors with varying mass transfer coefficients (k'_B, k'_Q, k'_R): a) 5.18×10^{-12} cm s^{-1}; b) 2.6×10^{-11} cm s^{-1}; c) 5.18×10^{-11} cm s^{-1}; d) 1.03×10^{-10} cm s^{-1}; e) 2.6×10^{-10} cm s^{-1}; f) ∞. The values of other parameters are: A_t=1 mM; $LV_1 = 3.63\times10^{-10}$ mol s^{-1} cm^{-2}; $\Gamma k_{cat} = 8.3\times10^{-10}$ mol s^{-1} cm^{-2}; K_M = 0.8 mM; K_A = 0.23 mM; K_B = 2.5 mM; K_{AB} = 0.3 mM^2; K_{iA} = 10 mM; K_{iB} = 11 mM; K_R = 20 mM; K_Q = 0.25 mM; K_P = 0.04 mM; K_{iR} = 9 mM; K_{iQ} = 1.6 mM; K_{iP} = 0.03 mM.

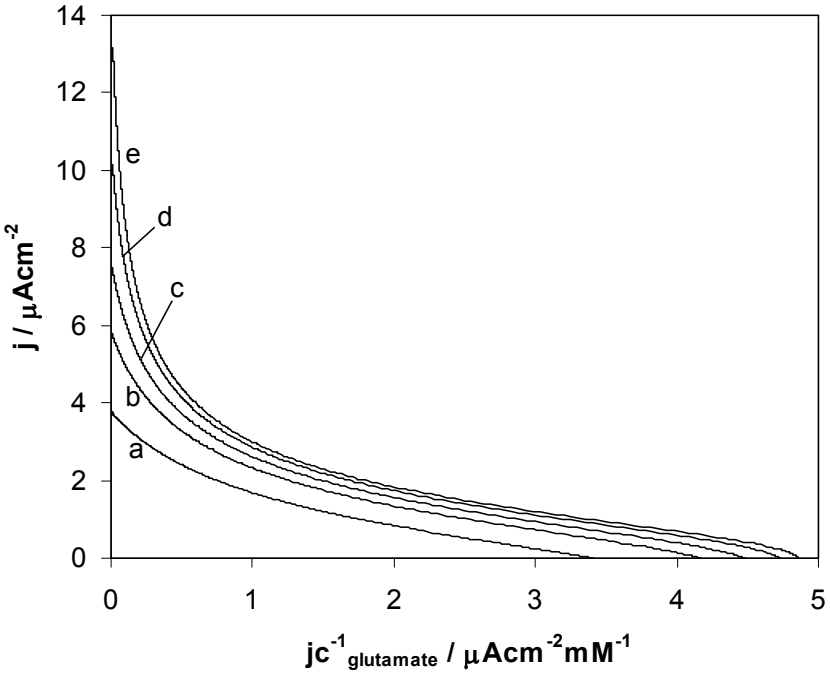

Figure S-5. Simulation of the Eadie-Hofstee plots for the reagentless glutamate biosensors with varying maximum flux of enzymatic reduction of NAD^+ (LV_1): a) 1.55×10^{-10} mol s^{-1} cm^{-2}; b) 3.63×10^{-10} mol s^{-1} cm^{-2}; c) 6.22×10^{-10} mol s^{-1} cm^{-2}; d) 1.24×10^{-9} mol s^{-1} cm^{-2}; e) 2.49×10^{-9} mol s^{-1} cm^{-2}. The values of other parameters are: k'_B =2.6×10^{-11} cm s^{-1}; k'_Q =2.6×10^{-11} cm s^{-1}; k'_R =2.6×10^{-11} cm s^{-1}; A_t=1 mM; Γk_{cat} = 8.3×10^{-10} mol s^{-1} cm^{-2}; K_M = 0.8 mM; K_A = 0.23 mM; K_B = 2.5 mM; K_{AB} = 0.3 mM^2; K_{iA} = 10 mM; K_{iB} = 11 mM; K_R = 20 mM; K_Q = 0.25 mM; K_P = 0.04 mM; K_{iR} = 9 mM; K_{iQ} = 1.6 mM; K_{iP} = 0.03 mM.