Supporting Information

A Detailed Model of Electroenzymatic Glutamate Biosensors to Aid in Sensor Optimization and in Applications *in vivo*

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Table S1. Governing Equations and Boundary Conditions

<table>
<tr><td colspan="3">Enzyme Layer Governing Equation</td></tr>
<tr><td rowspan="3">$\varepsilon_E \frac{\partial C_i}{\partial t} = -\varepsilon_E^2 D_i \frac{\partial^2 C_i}{\partial x^2} + r_i$</td><td colspan="2">$r_{glut} = \frac{-k_{cat}[enz]C_{glut}C_{O2}}{\left(K_{m,O_2}C_{glut} + C_{O2}(C_{glut} + K_{m,glut})\right)}$</td></tr>
<tr><td colspan="2">$r_{O_2} = r_{glut}$</td></tr>
<tr><td colspan="2">$r_{H_2O_2} = -r_{glut}$</td></tr>
<tr><td colspan="3">Permselective Layer Governing Equation</td></tr>
<tr><td colspan="3">$\varepsilon_N \frac{\partial C_i}{\partial t} = -\alpha D_i \frac{\partial^2 C_i}{\partial x^2}$</td></tr>
<tr><td colspan="3">Initial Conditions (t = 0, all x)</td></tr>
<tr><td>$C_{glut} = 0$</td><td>C_{O2} = see text</td><td>$C_{H2O2} = 0$</td></tr>
<tr><td colspan="3">Electrode/Pemselective Layer Boundary Conditions[a]</td></tr>
<tr><td>$\alpha D_{glut} \frac{\partial C_{glut}}{\partial x} = 0$</td><td colspan="2" rowspan="3">$r_{EOx} = \varepsilon_{PPY}\left(\frac{k_2 N\, k_1\, C_{H_2O_2}}{1 + k_1 C_{H_2O_2} + k_4 C_{0_2}}\right)$
current $= 2FAr_{EOx}$</td></tr>
<tr><td>$\alpha D_{O_2} \frac{\partial C_{O_2}}{\partial x} = r_{EOx}$</td></tr>
<tr><td>$\alpha D_{H_2O_2} \frac{\partial C_{H_2O_2}}{\partial x} = -r_{EOx}$</td></tr>
<tr><td colspan="3">Permselective (Nafion) Layer/Enzyme Layer Boundary Conditions</td></tr>
<tr><td>$\varepsilon_E^2 D_i \frac{\partial C_{i,\text{Enzyme}}}{\partial x} = \alpha D_i \frac{\partial C_{i,\text{Nafion}}}{\partial x}$</td><td colspan="2">$\frac{C_{i,\text{Nafion}}}{C_{i,\text{Enzyme}}} = K_i$</td></tr>
<tr><td colspan="3">Enzyme Layer/Sample Solution Boundary Condition[b]</td></tr>
<tr><td>$\varepsilon_E^2 D_i \frac{\partial C_i}{\partial x} = m_i\left(C_{i,s} - C_i\right)$</td><td colspan="2">$m_i = \left(\frac{D_i}{D_{O_2}}\right)^{2/3} m_{O_2}$</td></tr>
</table>

[a] F is the Faraday constant, and A is the electrode area.

[b] $C_{i,s}$ is the concentration in the sample solution external to the sensor. An alternate boundary condition was used to simulate sensor response to pulsed Glut transients (see text).

Table S2. Parameters, parameter definitions, and parameter values.

ε_E	Enzyme layer porosity	0.5
ε_N	Nafion layer porosity	0.3
ε_{PPY}	Permselective polymer film porosity	0.5
α	Multiplier to give Nafion D_{eff}	0.052
A	Electrode surface area	4800 μm^2
$C_{i,S}$	Sample solution concentration	See text
D_{glut} D_{O2} D_{H2O2}	Molecular diffusivities in water	7.6×10^{-6} cm^2/s 1.92×10^{-5} cm^2/s 1.43×10^{-5} cm^2/s
K_{m,O_2} $K_{m,glut}$ k_{cat}	GlutOx intrinsic kinetic parameters	1.5 mM 1.3013 mM 400.16 s^{-1}
m_{o2} m_{H2O2} m_{glut}	Mass transfer coefficients	0.0200 cm/s 0.0164 cm/s 0. 0108 cm/s
K_{glut} K_{H2O2} K_{O2}	Partition coefficients in Nafion layer	0.001 1 1
k_2N k_1 k_4	Kinetic parameters for H_2O_2 electrooxidation	1.01 mmol/m^2/s 0.357 m^3/mol 0.149 m^3/mol

Variables. It is important to note that the dependent variables, C_i, represent the concentrations of Glut, O_2 and H_2O_2 in the *pore spaces* in the sensor layers,[1] thus a parameter representing the enzyme layer void volume fraction, ε_E, appears in the accumulation term of the material balance equation for dimensional consistency. The void volume fraction in the permselective layer, ε_N, is similarly included. The independent variables obviously are time, *t*,

and the distance from the electrode surface, x, where the electrode surface is set at $x = 0$ (Fig. 10, text).

Partition coefficients. The partition coefficient between the electronegative permselective film (Nafion) and the enzyme layer was assumed to be 1 for the charge neutral O_2 and H_2O_2 species and 10^{-3} for negatively charged Glut, thereby essentially excluding Glut from the permselective layer.

GlutOx concentration in the enzyme layer. The concentration of GlutOx in the enzyme layer ([enz], mmol/cm^3), which is considered to be a porous, hydrated mix of crosslinked GlutOx and BSA, was calculated using an enzyme density of 1.41 g/mL[2] and an enzyme molecular weight of 70,000 g/mol,[3] according to the following expression, $1410 \times f_{glutox} \times (1 - \varepsilon_E)/70{,}000$, where f_{glutox} is the fraction of protein (*i.e.*, GlutOx and BSA) in the enzyme layer that is GlutOx and ε_E is the porosity.

GlutOx kinetics. The GlutOx enzyme kinetics were modeled according to the common ping-pong reaction mechanism of oxidase enzymes (Table S1),[4] although the reaction mechanism for GlutOx has not been established. Apparent values for k_{cat} and for the glutamate K_m of 53.2 s^{-1} and 173 µM, respectively, have been reported for the recombinant GlutOx from *Streptomyces* sp. X-119-6 that commonly is used in biosensors.[5] However, these apparent parameters generally are determined at a roughly constant O_2 concentration corresponding to near saturation from air at ~230 µM,[6] which is well below the actual, intrinsic $K_{m,O2}$ for most oxidases. Thus according to the ping-pong mechanism, the apparent k_{cat} and glutamate K_m should be multiplied by $(1 + K_{m,O2}/C_{O2})$ to give an estimate for the intrinsic k_{cat} and $K_{m,glut}$. Unfortunately, the $K_{m,O2}$ for this GlutOx has not been measured. A $K_{m,O2}$ of 1.5 mM was estimated based on the range of known values for L-amino acid oxidases, 1.2 to 1.86 mM. [7-18] These kinetic parameters, applicable to dilute enzyme solutions, were used under the assumption mentioned in the text that the immobilized enzyme exhibits the same kinetics as in dilute solutions.

References

[1] Bird, R. B., Stewart, W. E., and Lightfoot, E. N. (2002) *Transport phenomena*, 2nd, Wiley international ed., J. Wiley, New York.

[2] Fischer, H., Polikarpov, I., and Craievich, A. F. (2004) Average protein density is a molecular-weight-dependent function, *Protein Sci 13*, 2825-2828.

[3] Arima, J., Sasaki, C., Sakaguchi, C., Mizuno, H., Tamura, T., Kashima, A., Kusakabe, H., Sugio, S., and Inagaki, K. (2009) Structural characterization of l-glutamate oxidase from Streptomyces sp X-119-6, *Febs J 276*, 4318-4327.

[4] Parker, J. W., and Schwartz, C. S. (1987) Modeling the Kinetics of Immobilized Glucose-Oxidase, *Biotechnol Bioeng 30*, 724-735.

[5] Utsumi, T., Arima, J., Sakaguchi, C., Tamura, T., Sasaki, C., Kusakabe, H., Sugio, S., and Inagaki, K. (2012) Arg305 of Streptomyces L-glutamate oxidase plays a crucial role for substrate recognition, *Biochem Biophys Res Commun 417*, 951-955.

[6] Kusakabe, H., Midorikawa, Y., Fujishima, T., Kuninaka, A., and Yoshino, H. (1983) Purification and Properties of a New Enzyme, L-Glutamate Oxidase, from Streptomyces Sp X-119-6 Grown on Wheat Bran, *Agr Biol Chem Tokyo 47*, 1323-1328.

[7] Negri, A., Massey, V., Williams Jr., C. H., and Shopfer, L. M. (1988) The Kinetic Mechanism of Beef Kidney D-Aspartate Oxidase, *Journal of Biological Chemistry 263*, 13557-13563.

[8] Sehanobish, E., Shin, S., Sanchez-Amat, A., and Davidson, V. L. (2014) Steady-state kinetic mechanism of LodA, a novel cysteine tryptophylquinone-dependent oxidase, *FEBS Lett 588*, 752-756.

[9] Radler, F., and Torokfalvy, E. (1973) Affinity for oxygen of polyphenol oxidase in grapes, *Zeitschrift guer Lebensmittel Untersuchung und Forschung 152*, 38-41.

[10] Bordeleau, L. M., and Bartha, R. (1972) Biochemical transformations of herbicide-derived anilines. Purification and characterization of causative enzymes, *Canadian Journal of Microbiology 18*, 1865-1871.

[11] Niekus, H. G. D., and Stouthamer, A. H. (1981) Formate oxidase in glutaraldehyde-treated Campylobacter sputorum subspecies bubulus, *FEMS Microbiology Letters 11*, 83-87.

[12] Shigehara, T. (1984) Quantitative analysis by the oxygen electrode method of superoxide and hydrogen peroxide generated by xanthine oxidase reaction, *Okayama Igakkai Zasshi*, 77-85.

[13] Humphreys, K. J. (2009) Galactose Oxidase as a Model for Reactivity at a Copper Superoxide Center, *Journal of the American Chemical Society*.

[14] Gibson, Q. H., Swoboda, B. E. P., and Massey, V. (1964) Kinetics and Mechanism of Action of Glucose Oxidase, *J. Biol. Chem. 239*, 3927-3934.

[15] Gadda, G. (2003) Kinetic mechanism of choline oxidase from Arthrobacter globiformis, *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics 1646*, 112-118.

[16] Ghadem, M., Fan, F., Francis, K., and Gadda, G. (2003) Spectroscopic and Kinetic Properties of Recombinant Choline Oxidase from Arthrobacter globiformis, *Biochemistry 42*, 15179-15188.

[17] Nordkvist, M., Nielsen, P. M., and Villadsen, J. (2007) Oxidation of lactose to lactobionic acid by a Microdochium nivale carbohydrate oxidase: kinetics and operational stability, *Biotechnol Bioeng 97*, 694-707.

[18] Koide, Y., Koide, N., Ross, S., Saaf, J., and Wetterberg, L. (1981) Monoamine-Oxidase in Human-Platelets - Kinetics and Methodological Aspects, *Biochem Pharmacol 30*, 2893-2900.