Computational Optimization of Enzyme-Based Biosensors with Cyclic Signal Amplification

Romas Baronas, **Linas Litvinas** and **Karolis Petrauskas**

Vilnius University, Institute of Computer Science,
Mathematical and Computer Modeling Research Group, Vilnius, Lithuania

Introduction

Biosensors are analytical devices that convert biological recognition events into measurable signals. In catalytic electrochemical biosensors, enzyme-catalyzed reactions generate electroactive species, producing signals proportional to analyte concentration. Amperometric enzyme biosensors are widely used due to their simplicity, cost-effectiveness, and reliable performance in clinical diagnostics, environmental monitoring and industrial bioprocesses [1]. Their sensitivity can be enhanced through cyclic reactions that amplify electrochemical signals via repeated regeneration and conversion of reaction products, known as the triggering mechanism [2,3].

Computational modeling enables the prediction and optimization of biosensor responses under steady-state and transient conditions. By accounting for sensor geometry, enzyme-catalyzed reaction kinetics and mass transport, numerical simulations provide valuable tools for evaluating biosensor performance, optimizing design and operating conditions and developing novel biosensing systems [2].

This study investigates and optimizes amperometric bienzyme biosensors based on catalysis-catalysis-electrochemistry (CCE) triggering mechanisms, considering internal and external diffusion, interfacial partitioning and Michaelis-Menten kinetics [3,4].

Mathematical model

The time-dependent concentrations of substrate S and products P_1 and P_2 **in the enzyme layer** are governed by the following reaction-diffusion equations ($t > 0$),

$$\frac{\partial S_e}{\partial t} = D_{S_e}\frac{\partial^2 S_e}{\partial x^2} - R_1(S_e)\,,$$

$$\frac{\partial P_{1e}}{\partial t} = D_{P_{1e}}\frac{\partial^2 P_{1e}}{\partial x^2} + R_1(S_e) - R_2(P_{1e})\,,$$

$$\frac{\partial P_{2e}}{\partial t} = D_{P_{2e}}\frac{\partial^2 P_{2e}}{\partial x^2} + R_2(P_{1e}),\ x \in (0,\, a_1),$$

where R_1 and R_2 are the rates of biochemical reactions

$$R_1(S_e) = \frac{V_1\, S_e}{K_1 + S_e},\ R_2(P_{1e}) = \frac{V_2\, P_{1e}}{K_2 + P_{1e}}$$

where V_i is the maximal enzymatic rate and K_i is the Michaelis constant (i = 1, 2).

Only the mass transport by diffusion of all three compounds takes place **in the outer layer** ($t > 0$),

$$\frac{\partial S_d}{\partial t} = D_{S_d}\frac{\partial^2 S_d}{\partial x^2}\,,$$

$$\frac{\partial P_{id}}{\partial t} = D_{P_{id}}\frac{\partial^2 P_{id}}{\partial x^2}\,,\ i = 1, 2, \quad x \in (a_1,\, a_2),$$

The governing equations, together with the appropriate initial and boundary conditions, define an initial-boundary value problem that constitutes the mathematical model of the biosensor. In particular, the interfacial boundary conditions relate concentrations through formal partition coefficients.

Computational simulation

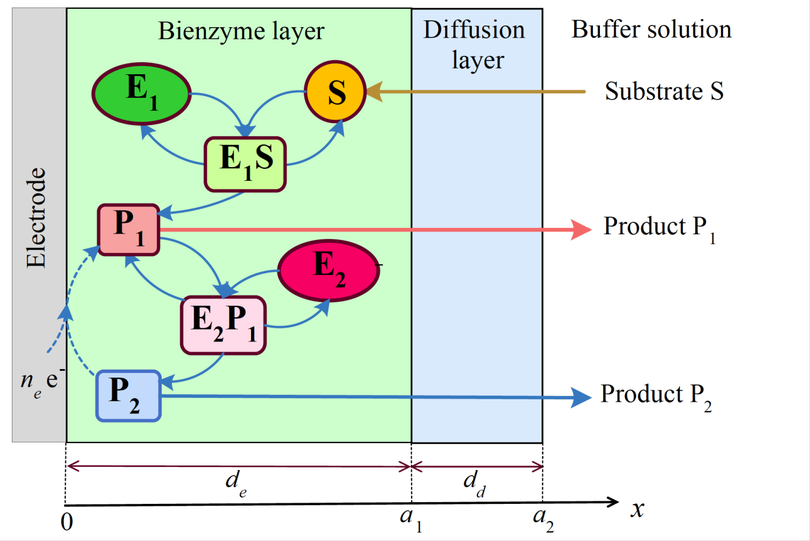
Transient system behavior was simulated using a semi-implicit finite difference scheme, with the resulting algebraic equations solved by the Thomas algorithm [3].

The Java-based biosensor model was integrated with the C++ implementation of the HybAlg optimization algorithm, combining Bayesian global search with Hooke–Jeeves local refinement [4,5].

Constrained multi-objective optimization sought to maximize sensitivity and signal gain while minimizing enzyme consumption.

Schematic representation of the treated system

- The treated system consists of two relatively thin layers:
  - bienzyme-loaded membrane applied to the surface of an electrode,
  - an outer diffusion layer.
- In the bienzyme membrane, two enzyme-catalyzed biochemical reactions occur.
- An electrochemical conversion takes place at the electrode surface.
- The mechanism follows a catalysis-catalysis-electrochemistry (CCE) sequence.



Biosensor characteristics

Biosensor output current: $I(t) = n_e F D_{P_{2e}} \left.\frac{\partial P_{2e}}{\partial x}\right|_{x=0}$

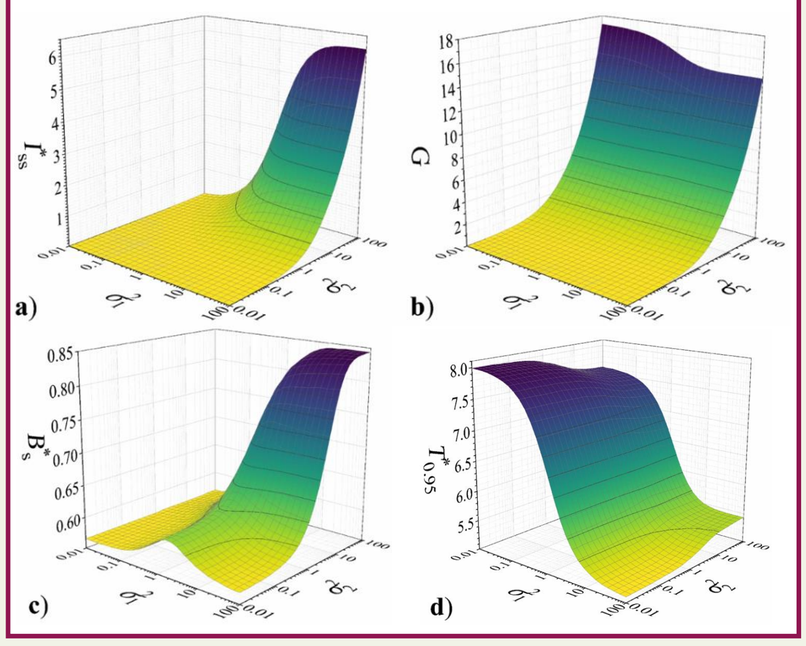
Steady-state current: $I_{\mathrm{SS}} = \lim_{t\to\infty} I(t),$

Response time: $T_\eta = \inf\{t > 0 : I(t) = \eta I_{\mathrm{SS}}\},$

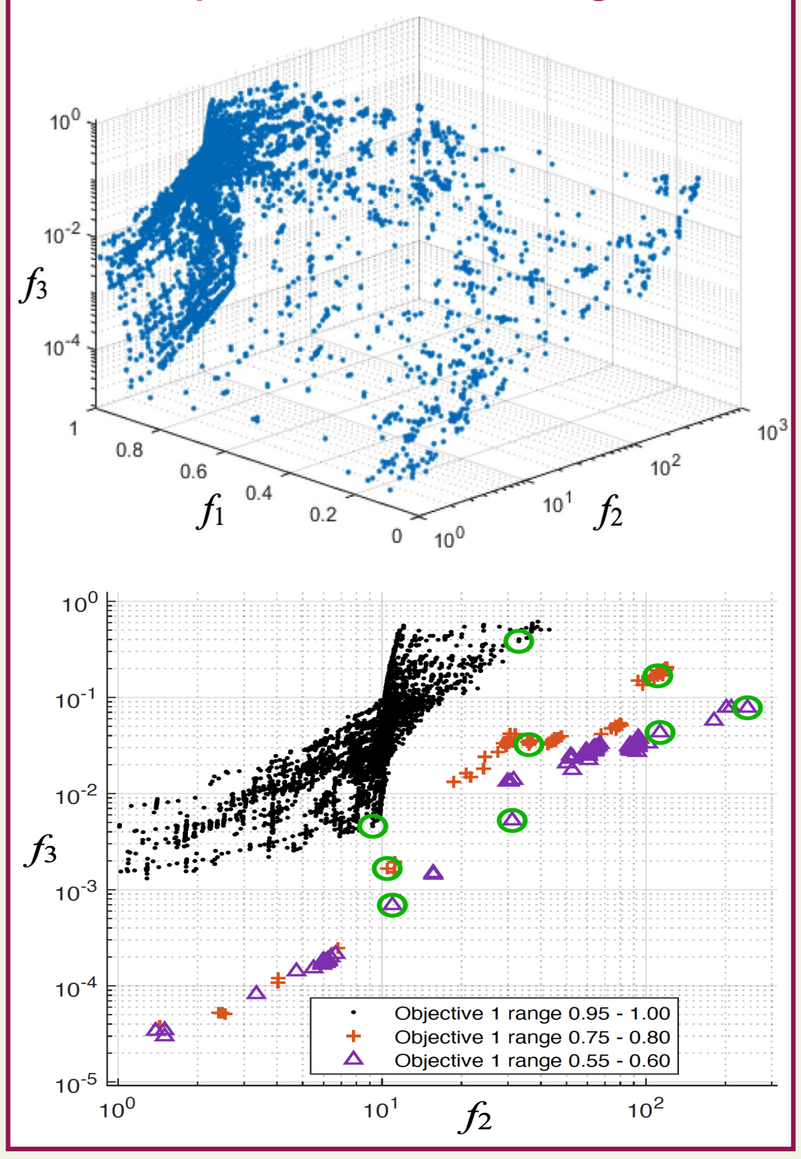
Biosensor sensitivity: $B_{\mathrm{s}}(S_0) = \frac{\partial I_{\mathrm{SS}}(S_0)}{\partial S_0},$

Signal gain: $G(S_0, V_1, V_2) = \frac{I_{\mathrm{SS}}(S_0, V_1, V_2)}{I_{\mathrm{CE}}(S_0, V_1)},$

Effect of internal diffusion limitations



Pareto-optimal biosensor design



Multi-objective optimization problem

$$\mathbf{F_P} = \min_{\mathbf{x}\in A} F(\mathbf{x}),\ F(\mathbf{x}) = (-f_1(\mathbf{x}), -f_2(\mathbf{x}), f_3(\mathbf{x}))^T$$

$$f_1(\mathbf{x}) = B^*_{\mathrm{s}}(S_0),$$
$$f_2(\mathbf{x}) = G(S_0, V_1, V_2),$$
$$f_3(\mathbf{x}) = E^*_{\mathrm{T}}(E_{1,\mathrm{T}}, E_{2,\mathrm{T}}, d_e),$$

Variable		Description	Min	Max
x_1	d_e	Enzyme layer thickness (μm)	2	100
x_2	σ_1	Thiele modulus for enzyme E_1	0.1	10
x_3	σ_2	Thiele modulus for enzyme E_2	0.1	10
x_4	δ	Diffusivity ratio D_d/D_e	1	10
x_5	β	Biot number	0.1	30
x_6	θ	Partition coefficient	0.1	10
x_7	κ	Ratio of Michaelis constants	0.1	10

Constraints: $\frac{\sigma_1}{\sigma_{1,\max}\sqrt{\delta}} \le \frac{d_e}{d_{e,\max}},\ \frac{\sigma_2}{\sigma_{2,\max}}\sqrt{\frac{\kappa}{\kappa_{\max}\delta}} \le \frac{d_e}{d_{e,\max}}$

HybAlg optimization algorithm parameters [5]:
$(N_{\max}, I_{\max}, N, q, p, h_0, h_n)^T = (\infty, 1, 1000, 10, 0.1, 4, 6)^T$

Conclusions

Computational modelling provides a framework for analysis of the bienzyme kinetics, diffusion limitations and partitioning on biosensor performance and optimization of design parameters for industrial applications.

The design of a CCE-triggered amperometric biosensor can be formulated as a constrained multi-objective optimization problem involving conflicting objectives.

Using primarily dimensionless parameters reduces redundancy, while retaining dimensional enzyme-layer thickness preserves the link to physical geometry.

Physical and performance constraints restrict the search to feasible and practically relevant designs.

The parallel HybAlg algorithm enables efficient exploration of the computationally demanding seven-dimensional design space and identification of Pareto-optimal biosensor configurations.

References

1. C.M. Pandey, B.D. Malhotra. *Biosensors: Fundamentals and Applications*, De Gruyter, 2nd ed., 2019.
2. R. Baronas, F. Ivanauskas, J. Kulys. Mathematical Modeling of Biosensors, 2nd ed. Springer, 2021.
3. R. Baronas, F. Ivanauskas, J. Kulys. Mathematical model of the biosensors acting in a trigger mode. *Sensors*, 4(4):20–36, 2004.
4. R. Baronas, K. Petrauskas. Effects of diffusion limitations and partitioning on signal amplification and sensitivity in bienzyme electrochemical biosensors employing cyclic product conversion. *Applied Sciences*, 16(3):1171, 2026.
5. L. Litvinas. An open-source parallel algorithm of Bayesian-based global search with Hooke-Jeeves local refinement for multi-objective optimization problems. *Nonlinear Analysis: Modelling and Control*, 31(2):463-476, 2026.