Supplemental Information

Unveiling Pseudocapacitance: A Kinetic Treatment of the Pseudocapacitive Biosensor

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Materials and Methods:

Electrode Preparation: FcMe2-LPEI (14 uL, 10 mg/mL in water), EGDGE (0.75 uL , 10 v/v % in water), BOD (3 uL, 12 mg/mL in water), FAD-GDH (3uL, 0.375 mg/mL in water) were combined and 4 uL was drop-cast onto 0.25 cm^2 carbon paper electrodes and dried overnight. Electrodes prepared for OCP-monitoring during polymer swelling were prepared and dried under Ar atmosphere, while those prepared for glucose monitoring were prepared under atmospheric air and dried under vacuum.

OCP Monitoring During Swelling: Experiments were conducted in 5 ml of sodium phosphate buffer (100 mM, pH 7.4, Ar atmosphere) with a Pt-mesh counter-electrode, and a saturated calomel reference electrode. OCP monitoring was initiated prior to the electrode being immersed in electrolyte. Electrolyte was stirred at 400 rpm with 10x3 mm stir bar. For the atmospheric air test, the same electrode was removed from the glovebox and the experiment was repeated under atmospheric air.

OCP Monitoring with glucose addition: Electrodes were prepared, and electrochemical experiments were conducted as previously described[1]. After 24 hours of cross-linking, the dual-enzyme bioelectrode was immersed in 7 mL of 100 mM phosphate buffer solution (pH 7.4), serving as the working electrode. Before glucose addition, the solution was pre-saturated with O_2 for 20 minutes at a flow rate of 0.5 mL/min, ensuring the stabilization of the open-circuit potential (OCP). Glucose was then added at varying concentrations. Upon introducing glucose, a drop in the OCP was observed, followed by stabilization at a steady-state potential, indicating the sensor's recovery and the system's response to the specific glucose concentration. Subsequent glucose additions led to a further drop in the OCP, corresponding to the increased glucose levels.

Kinetic simulation: We conduct a kinetic simulation by employing approximate and literature derived values for these kinetic parameters to calculate changes in V_{BOD} and V_{GDH} (per main text eqs. 3 and 4) over time in response to an input glucose concentration. V_{GDH} responds to glucose immediately, while V_{BOD} increases slowly in response to M accumulation until it approaches 0.1% below the value of V_{GDH}, when glucose concentration is increased again. From these varying enzyme velocities, one may calculate the concentrations of M^+ and M, and at the time of velocity convergence, the OCP of the system according to main text eqn. 5. The code for this simulation and the associated optimization is available on our GitHub: github.com/MinteerLab

Response Time vs. Enzyme Loading

One valuable insight suggested by this model is that the sensor's response time is proportional to total enzyme concentration (while maintaining a constant X_{BOD}:X_{GDH} ratio). This follows from response time corresponding to the time required for BOD activity to increase to $2V_{BOD} = V_{GDH}$. The rate of change in M with respect to time is expressed as:

$$\frac{dM}{dt} = 2V_{GDH} - 4V_{BOD} \tag{S1}$$

Consider for simplicity the conditions just after BOD pseudocapacitive charging of the polymer, such that M^+>>M. if we further (though not necessarily) assume from the overpotential differences between BOD, M, and GDH that $K_M >> K_{M+}$, it follows that V_{BOD} is dominated by its K_M/M term, while V_{GDH} is dominated by its K_G/G term, such that:

$$V_{BOD} = \frac{X_{BOD}}{\frac{K_{O_2}}{O_2} + \frac{K_M}{M} + 1} \approx \frac{X_{BOD} M}{K_M} \tag{S2}$$

$$V_{GDH} = \frac{X_{GDH}}{\frac{K_G}{G} + \frac{K_{M^+}}{M^+} + 1} \approx \frac{X_{GDH} G}{K_G} \quad \text{(S3)}$$

Combining eqs. 3, S2, and S3 the acceleration of BOD activity (dV_{BOD}/dt) in response to an addition of glucose is expressed as:

$$\frac{dV_{BOD}}{dt} = \frac{X_{BOD}}{K_M}\left(\frac{2X_{GDH}G}{K_G} - \frac{4X_{BOD}M}{K_M}\right) \quad or \quad \frac{4MX_{BOD}{}^2}{K_M{}^2}\left(\frac{X_{GDH}GK_M}{K_G 2X_{BOD}M} - 1\right) \quad \text{(S4)}$$

Conveniently, the left parenthetical term in the final form of eqn. S4 is equivalent to V_{GDH} / $2V_{BOD}$. Thus, as the two velocities equilibrate the entire parenthetical approaches a value of 0, thus BOD acceleration nullifies. Furthermore, BOD acceleration having a square dependence on total BOD concentration explains the modeled response time decreasing proportionally to an increase in total enzyme concentration. For example, the kinetic simulation in main text Figure 3 was conducted with both X_{BOD} and X_{GDH} decreased to $4.1*10^{-5}$ times the predicted values of 14 and 1 respectively, to more closely match the 34 second experimental response time in the 2.8 to 4.1 mM glucose transition.

If we execute the optimized kinetic simulation with X_{GDH} decreased to 1/2 (now $1/4^{th}$ of the initial prediction), the model suggests a change in response time in the 2.8 mM to 4.2 mM glucose transition from 34 seconds, to 34.1 seconds, a 0.34% increase. Our experimental data instead demonstrates a response time shift of 34.5 seconds to 35 seconds, a 1.4% increase, at the same transition of glucose concentration. This deviation from the prediction may be attributed to a crosslinker-mediated shift in the enzyme kinetic constants, or a nonlinear shift in GDH denaturation in response to differing crosslinker:GDH ratios, or simply experimental signal noise.

Deviation from Linearity at High Glucose vs. GDH Loading

It is critical to recognize that GDH activity maintains a stable elevated value in response to glucose addition only while it remains catalytically limited by glucose concentration. If GDH continues to consume M^+ without compensation in M^+ concentration by growing BOD activity, the activity of the former will become M-limited and begin to decrease. This is demonstrated when X_{GDH} : X_{BOD} is too large, especially at high glucose concentrations (Figure S1 B). at high X_{GDH} : X_{BOD}, BOD activity fails to accelerate sufficiently in response to M^+ accumulation. In this regime, the final value for V_{GDH} at stable OCP falls below its initial value achieved in response to the change in glucose concentration. At low-glucose concentrations in contrast, V_{GDH} is too small to impact the concentration of M^+, so V_{GDH} remains stable for the entire OCP stabilization time even at relatively lower loadings of BOD. In other words, at low glucose concentrations, e.g. ln(glucose) = -7, equivalent curves to Figure S1B all overlap perfectly, independent of modifications to X_{GDH}.

It is curious to note that at lower GDH loadings, where V_{GDH} remains relatively stable across the OCP stabilization time, (Figure S1B, blue), the system fails to maintain linearity at high glucose concentrations (Figure S1A, blue). This is to say the capacity of the experimental data to maintain linearity at these high glucose concentrations (Figure 1) seems to arise precisely from a failure of GDH to maintain high activity and decrease as a result of its own consumption of M^+. However, because this simulation does not account for glucose consumption or diffusional effects, we might expect experimental active-layer glucose concentrations to respond more significantly to V_{GDH} activity, causing the activity to decrease in a similar manner to its response to M^+ losses seen in these simulations. Put simply, this simulation likely overestimates the deviation from linear OCP-ln(glucose) response at lower X_{GDH} values (blue), because the combined effect glucose and M^+ consumption by GDH activity would cause the blue curves to approach the shape of the purple curve.

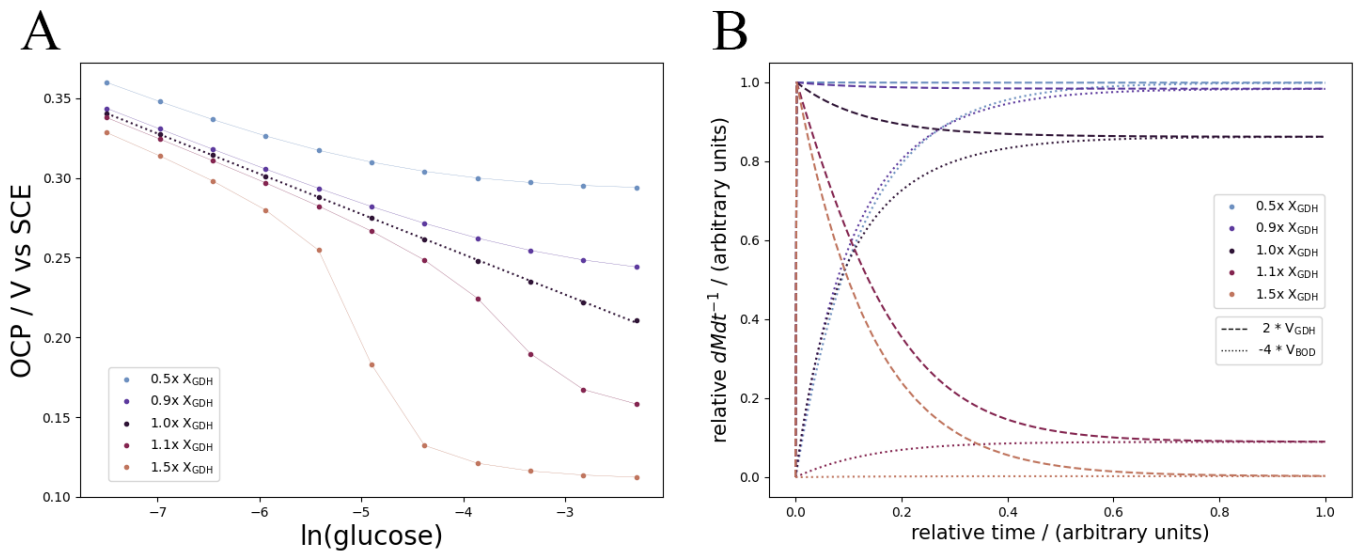

Fig S1. (A) Simulated variation in sensor behavior across glucose concentrations relative to different loadings of GDH. 1.0x X_{GDH} is equivalent to the optimized parameters fit in figure 3 of the main manuscript. (B) Zoomed-in view of relative dM dt^{-1} from V_{BOD} (dashed lines) and V_{GDH} (dotted lines) in the high-glucose 59.6 to 100 mM transition. Axis have been normalized to account for different response times and magnitudes of initial V_{GDH} response to glucose addition. Notice that at high X_{GDH} (beige), BOD fails to recover M^+ losses to GDH, causing GDH activity to slow in response to its own depletion of M^+, versus a steady, glucose-limited V_{GDH} magnitude at lower loadings of GDH (blue).

References:

1 W. El Housseini, *et al.*, *ACS Sensors*, 2024, **9**, 3357–3366.