# Supplementary

- **Using the extracted parameters from the experiment we can simulate a continuous glucose addition experiment.**
- **Coupled reaction diffusion equations to fit the transient simulation to the experimental amperograms.**

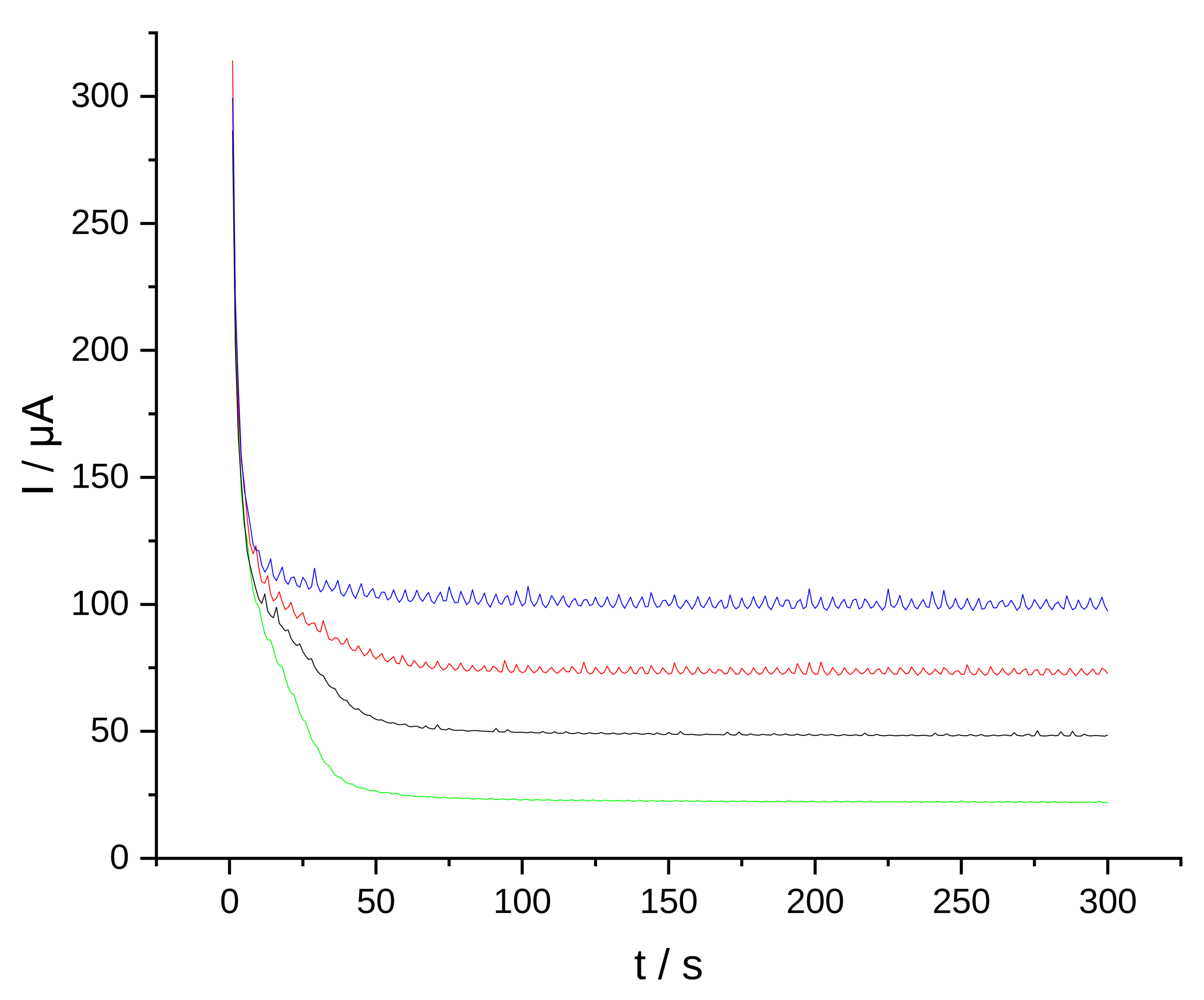


**Figure 1s: Experimental chronoamperograms recorded at 350 mV vs. Ag/AgCl at 10, 20, 50 and 100 mM glucose concentration. Flowrate of 0.01 mL/s without in plain PBS buffer of pH 7.4 buffer. Green: 10mM glucose, black: 20 mM glucose, red: 50 mM glucose, blue: 100mM glucose.**

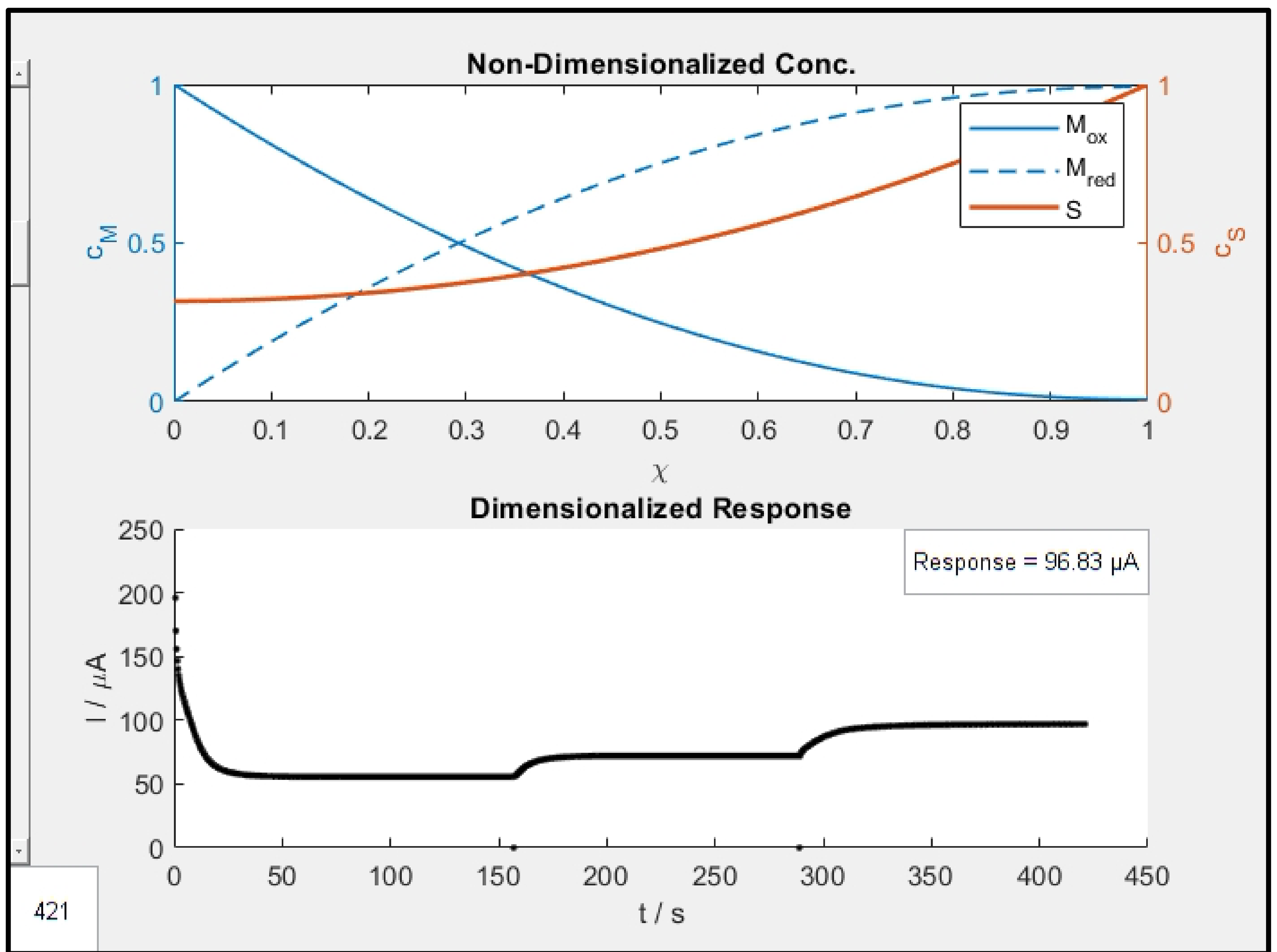


**Figure 2s: Simulated glucose step experiments for addition of 20, 50 and 100 mM glucose in the electrolyte solution (QR code given below).**

**QR code for Figure 2s**

**Table 1s: Normalized Variables**

| | |
|---|---|
| $\alpha$ | $\frac{D_m}{D_s}$ |
| $\kappa$ | $l\sqrt{\frac{k_m c_{e_{layer}}}{D_m}}$ |
| $\eta$ | $\frac{D_s k_m K_M}{D_m k_{cat}}$ |
| $\gamma$ | $\frac{k_m P_m c_{m_{layer}} K_M}{k_{cat} P_s c_{s_{bulk}}}$ |
| $\mu$ | $\frac{P_s c_{s_{bulk}}}{K_M}$ |
| $\tau_m$ | $\frac{D_m t}{l^2}$ |
| $\tau_s$ | $\frac{D_s t}{l^2}$ |

**Table 2s: Parameters and their definitions**

| Parameter Name | Symbol |
|---|---|
| Thickness of the enzyme immobilized mediator layer (cm) | $l$ |
| Diffusivity coefficient for substrate in the layer ($cm^2s^{-1}$) | $D_s$ |
| Diffusivity coefficient for mediator in the layer ($cm^2s^{-1}$) | $D_m$ |
| Enzyme tunrover number ($s^{-1}$) | $k_{cat}$ |
| Michalis constant ($mol/cm^3$) | $K_M$ |
| Initial substrate concentration in the bulk ($mol/cm^3$) | $c_{s_{bulk}}$ |
| Initial enzyme concentration in the layer ($mol/cm^3$) | $c_{e_{layer}}$ |
| Initial mediator concentration in the layer ($mol/cm^3$) | $c_{m_{layer}}$ |
| Partition Coefficient for glucose | $P_s$ |
| Partition Coefficient for mediator | $P_m$ |
| Mediator rate constant ($(molcm^{-3})^{-1}s^{-1}$) | $k_m$ |
| Time span for the experiment (s) | $t$ |
| Diffusivity coefficient for enzyme in the layer is ignored ($cm^2s^{-1}$) | $D_e$ |

**Table 3s: Analytical expression for cases**

| | |
|---|---|
| **I** | $\kappa^2 a_\epsilon$ |
| **II** | $\kappa a_\epsilon$ |
| **III** | $a_\epsilon + \frac{\eta}{\gamma}$ |
| **IV** | $\frac{\sqrt{\eta}\kappa}{\gamma\sqrt{(1+0.5\mu)}}$ |
| **V** | $\frac{\kappa^2}{\gamma(1+\mu)}$ |
| **VI** | $\sqrt{\frac{2a_\epsilon\kappa^2\eta}{\gamma}}$ |
| **VII** | $\sqrt{\frac{2a_\epsilon\kappa^2}{\gamma(1+\mu)}}$ |

**Equations: Reaction diffusion equations that define our system.**

$$\alpha\frac{\partial c_S(\chi,\tau_M)}{\partial\tau_M} = \frac{\partial^2 c_S(\chi,\tau_M)}{\partial\chi^2} - \frac{\gamma\eta^{-1}\kappa^2 c_S(\chi,\tau_M)c_{M_{ox}}(\chi,\tau_M)}{\gamma c_{M_{ox}}(\chi,\tau_M)\big(1+\mu c_S(\chi,\tau_M)\big)+c_S(\chi,\tau_M)} \quad 1s$$

$$\frac{\partial c_{M_{ox}}(\chi,\tau_M)}{\partial\tau_M} = \frac{\partial^2 c_{M_{ox}}(\chi,\tau_M)}{\partial\chi^2} - \frac{\kappa^2 c_S(\chi,\tau_M)c_{M_{ox}}(\chi,\tau_M)}{\gamma c_{M_{ox}}(\chi,\tau_M)\big(1+\mu c_S(\chi,\tau_M)\big)+c_S(\chi,\tau_M)} \quad 2s$$