Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supplementary information

For

Contribution of Convection and Diffusion to the *β*-Galactosidase/Glucose Oxidase Cascade Reaction confined in a Microchannel

Zeng-Qiang Wu‡, Zhong-Qiu Li‡, Jin-Yi Li, Jing Gu, Xing-Hua Xia*

State Key Laboratory of Analytical Chemistry for Life Science and Collaborative Innovation Center of Chemistry for Life Sciences, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing, 210093, China.

‡These authors contributed equally.

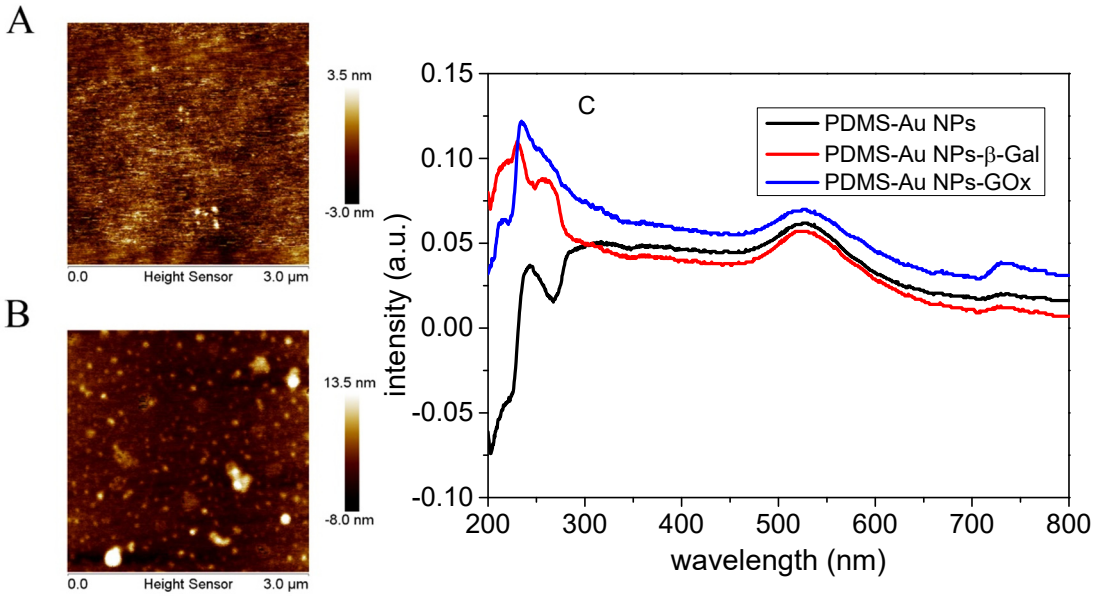

Fig. S1. AFM images of a bare PDMS surface (A) and Au-films PDMS surface (B). (C) UV-vis absorbance spectra of gold nanoparticles patterned PDMS (black curve) and after enzymes immobilization (red curve).

FEM model

In the established cascade enzyme reactor, the initial substrate lactose will be first hydrolyzed by *β*-Gal to form glucose and galactose (*eq.* 1). The intermediate glucose is further oxidized by dissolved oxygen to electrochemically active product hydrogen peroxide when GOx is present (*eq.* 2).

$$\beta - lactose + H_2O \xrightarrow{\beta-gal} \beta - D - glucose + D - galactose \quad (1)$$

$$\beta - D - glucose + O_2 + H_2O \xrightarrow{GOx} gluconicacid + H_2O_2 \quad (2)$$

In order to understand the influence of mass transport of substrate and intermediate on the kinetics of enzyme cascade reaction in a microchannel, we establish 2D numerical models based on Navier-Stokes (*eq*. 3) and convection-diffusion equations (*eqs.* 8-9). The whole models are carried out in the COMSOL 3.5a (COMSOL, Stockholm). In the case of *β*-Gal/GOx cascade reaction, effective transfer of intermediate (glucose) between two enzymes is essential to the cascade productivity[1]. Therefore, the enzyme catalytic reaction equations are introduced into the convection-diffusion equation.

$$\rho\left(\frac{\partial u}{\partial t} + u \bullet \nabla u\right) = -\nabla p + \mu\nabla^2 u + f \quad (3)$$

where, ρ is the fluid density, μ is the dynamics viscosity, p is the pressure, u is the flow velocity of fluid, and f is the external force.

The continuity equation can be described by

$$\frac{\partial \rho}{\partial t} + \frac{\partial(\rho u)}{\partial x} + \frac{\partial(\rho u)}{\partial y} = 0 \quad (4)$$

when the fluid is incompressible, ρ maintains a constant in any fluid parcel, and $d\rho/dt = 0$. The *eq.*4 can be reduced to

$$\nabla u = 0 \quad (5)$$

Since substrates are transported by electrokinetic flow in the experiments, the electrostatic force in the double layer as a source term (*f*) is introduced into the Navier–Stokes equation.

$$f = \rho_E E \quad (6)$$

where, ρ_E is the charge density, E is the electrical field strength. The source term (*f*) can then be solved by coupling the numerical solutions of the conductive media DC equation (*eq.* 7) in COMOSOL 3.5a.

$$-\sigma\nabla^2 V + \nabla J^e = Q_j \quad (7)$$

where, σ is the conductivity of fluid, V is the applied potential to microchannel, J^e is the current density in microchannel under the applied potential, Q_j is the current source.

For calculating the models, we divide he whole computer domain into 3 subdomains in the FEM simulations (Figure S2). In the microchannel domain, the convection-diffusion equation (*eq.* 8) is incorporated into the numerical models. For the subdomains of enzymes (*β*-Gal and GOx), the terms (*R*) for enzyme catalytic reactions are introduced to the convection and diffusion equation (*eq.* 9).

$$\frac{\partial c}{\partial t} = D\nabla^2 c - u \bullet \nabla c \quad (8)$$

$$\frac{\partial c}{\partial t} = D\nabla^2 c - u \bullet \nabla c + R \quad (9)$$

where, D is the diffusion coefficient of species, c is the concentration of species, R is the term for enzyme catalytic reactions.

In the regions of immobilized enzymes (Figure S2), the locally initial reaction rate follows the enzyme catalytic reaction equations (*eqs*. 10 and 11).

$$R_1 = -\frac{dc_{\text{lactose}}}{dt} = \frac{dc_{\text{glucose}}}{dt} = \frac{V_{\text{max,β-Gal}} c_{\text{lactose}}}{K_{\text{m,β-Gal}} + c_{\text{lactose}}} \quad (10)$$

$$R_2 = -\frac{dc_{\text{glucose}}}{dt} = \frac{dc_{H_2O_2}}{dt} = \frac{V_{\text{max,GOx}} c_{\text{glucose}} c_{O_2}}{K_{\text{m,glucose}} c_{O_2} + K_{\text{m},O_2} c_{\text{glucose}} + c_{O_2} c_{\text{glucose}}} \quad (11)$$

where, K_m is the Michaelis–Menten constant of enzyme reaction, V_{max} is the maximum velocity of enzyme catalytic reaction, c_i is the concentration of species.

Herein, *eqs*. 3, 5 and 6-11 are used to simulate the *β*-Gal/GOx cascade reaction in microchannel. The detailed information about boundary conditions of numerical models is described in supporting information. Scale of the microchannel (4 cm in length, 50 μm in width and 18 μm in depth) and parameters used in experiments are chosen as the representative conditions of models. The representative values of rate constants were measured experimentally and the diffusion coefficients are selected from *ref.* 2. For each series of numerical calculations, all parameters are fixed in the models except for systematically varied parameters.

The kinetic parameters of the immobilized GOx in microchannel were electrochemically determined. The kinetic parameters of *β*-Gal reaction are varied until the best sets which reproduce the experimental data are obtained.

Boundary conditions for FEM model

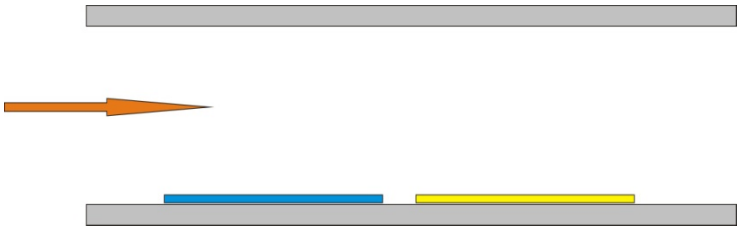

Fig S2. Computation domain in the FEM model. The blue and yellow regions denote the immobilized enzymes in the microchannel. The gray region represents the channel wall of microchip. The arrow with orange color represents flow direction of fluid.

Navier-Stokes equation (*eqs* 3 and 5):

Inlet and outlet: normal flow/pressure ($t \cdot u=0$, $p=p_{atm}$)

Wall of microchannel: electroosmotic velocity ($u = -\varepsilon_r \varepsilon_0 \frac{\zeta}{\eta} E$)

Immobilized enzyme regions: no slip (u=0)

continuity (internal boundary)

Conduct media DC (*eq*.7):

Inlet: electric potential ($V=V_{applied}$)

Outlet: electric potential (V=0)

Wall: electric insulation ($\boldsymbol{n \cdot J}$=0)

Immobilized enzyme regions: electric insulation ($\boldsymbol{n \cdot J}$=0)

Convection Diffusion equation (*eqs*. 8 and 9):

eq.8 (microchannel) Inlet: concentration

Outlet: concentration

Wall: insulation/symmetry

eq.9 (Immobilized enzyme regions)

For β-Gal region: $c_1 = c_1^* \exp\left(-V_{\max,gal} t / k_{m,gal}\right)$, $c_2 = c_1^* \exp\left(-V_{\max,gal} t / k_{m,gal}\right)$, c_3=0

For GOD region: $c_1 = c_1(t)$, $c_{2,GOD} = c_2(t) - V_{\max,GOD} t + k_{m,GOD} \ln\left(\frac{c_2(t)}{c_{2,GOD}}\right)$

$$c_3 = -V_{\max,GOD} t + \ln\left(\frac{c_2(t)}{c_{2,GOD}}\right)$$

Simulated current with different lactose concentrations at 50 μm distance

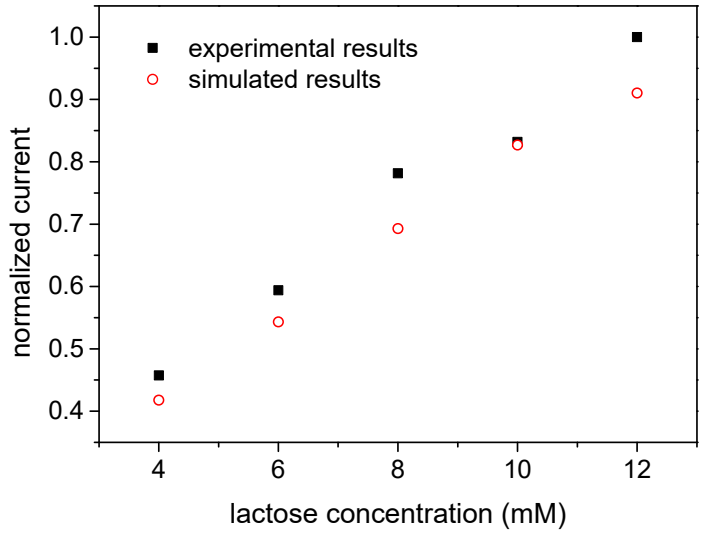

Fig. S3 Relationship between lactose concentration and the electrochemical response current in microchannel. (■, square) experimental results; (○, circle) simulated results. Experimental and Simulated currents of H_2O_2 were normalized by using the current for 12 mM lactose at a 50 μm gap distance.

Flow rate in microchannel with and without immobilized enzymes

For understanding the influence of fluidic velocity on the β-Gal/GOx cascade reaction, we firstly analyzed the velocity of microchannel without enzymes immobilized. In experiments, H_2O_2 was used as the velocity tracer to investigate velocities of the microchannel under different separation voltages (Figure S3). The fluidic velocities in microchannel under different separation voltages were calculated using the eluted times of H_2O_2 (Figure S4), since a relation of velocities versus separation voltages (without enzymes immobilized in microchannel) exists, $u = 1.1954 \times 10^{-4}\mathrm{V}$, where u is the fluid velocity in microchannel without enzymes immobilized; V is the separation voltage. When a microchannel was modified with enzymes, the electroosmotic flow (EOF) velocity in microchannel correspondingly changes due to the variation of surface properties. The retention times of cascade reaction product (H_2O_2) at different separation voltages were measured (Figure S5), and a relationship between retention time and separation voltages— $t = 31416.539/\mathrm{V}$ can be obtained. To acquire the ratio of fluidic velocity in microchannels without and with enzymes immobilized, we simply divided the

microchannels into regions without enzymes (S1) and with enzymes (S2). The flow time of sample plug (t) can be formulated by:

$$t = \frac{S1}{u} + \frac{S2}{u_e} \tag{12}$$

where, u is the sample velocity in microchannel without enzymes and can be described by $u = 1.1954 \times 10^{-4}\mathrm{V}$; u_e is the sample velocity in microchannel with enzymes and can be described by $u_\mathrm{e} = 1.1954 \times 10^{-4}\lambda\mathrm{V}$, where, λ is the coefficient of ratio. So, *eq*.12 can be further simplified into:

$$t = \frac{1}{1.1954 \times 10^{-4}V}\left(S1 + \frac{S2}{\lambda}\right) \tag{13}$$

As shown in Figure S5, the flow time of product is inversely proportional to the separation voltage. Based on the above fitted equation—$t = 31416.539/V$, the constant term in eq. 13 can be displaced into *eq*.14.

$$31416.539 = \frac{1}{1.1954 \times 10^{-4}}\left(S1 + \frac{S2}{\lambda}\right) \tag{14}$$

Since S2 and S3 are constants in microchannel (S1=1.1 cm, S2=2 cm), λ can be solved as $\lambda = 0.7532$. The value of λ denotes that the velocity of sample plug became slower in the microchannel with enzymes immobilized. This difference in velocities is considered in the FEM model.

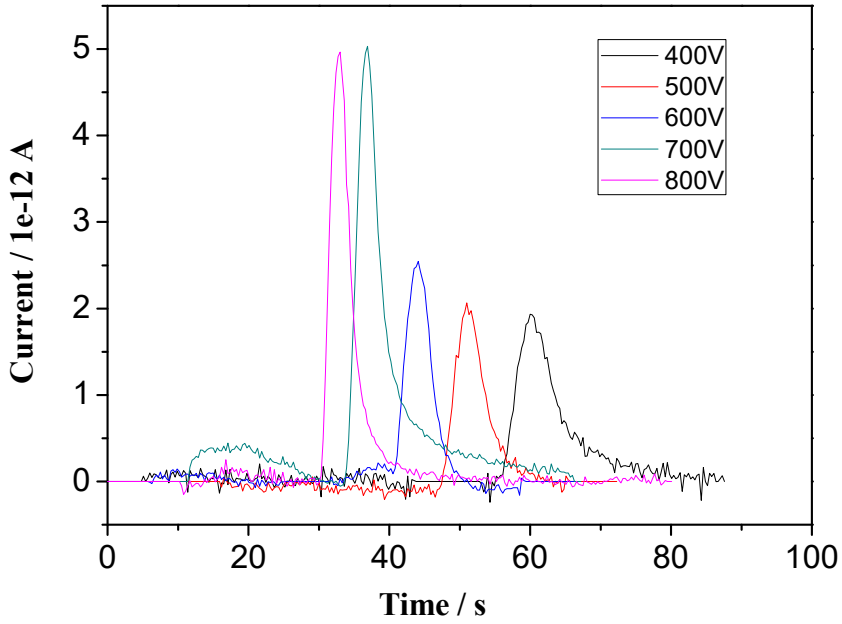

Fig S4. The eluted curves for H_2O_2 in microchannel without enzymes under different separation voltages.

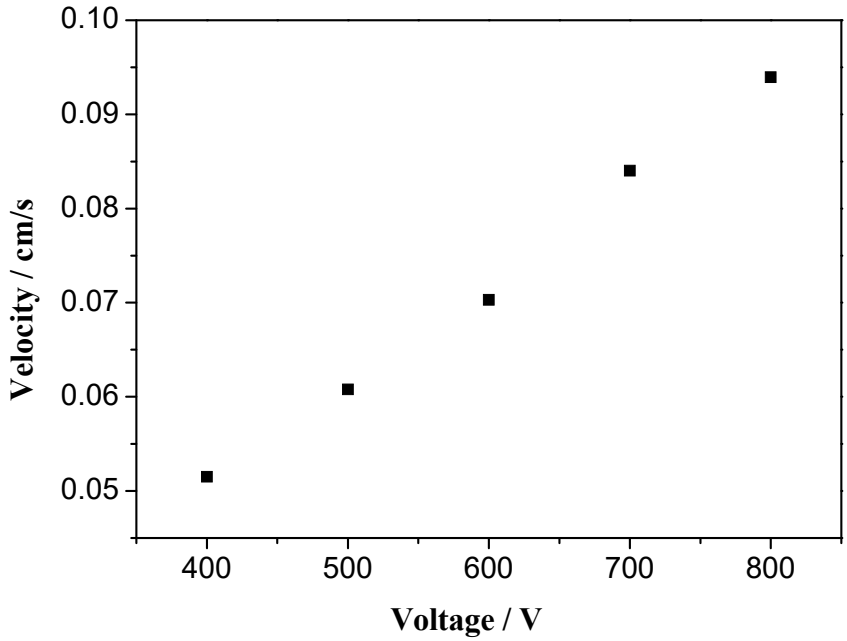

Fig S5. The velocity versus separation voltage in microchannel without enzymes.

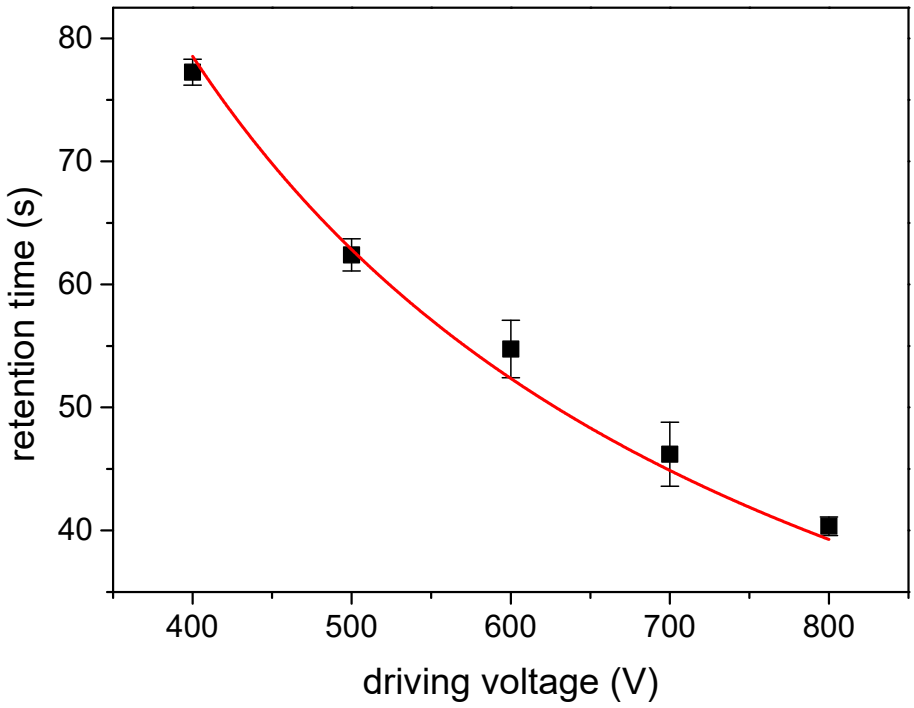

Fig S6. The retention time of cascade reaction product (H_2O_2) as a function of separation voltage. Substrate concentration: 12 mM lactose.

References:

1. Fu, J.; Liu, M.; Liu, Y.; Woodbury, N. W.; Yan, H., Interenzyme Substrate Diffusion for an Enzyme Cascade Organized on Spatially Addressable DNA Nanostructures. *J. Am. Chem. Soc.* **2012**, *134*, 5516-5519.
2. Wohlfahrt, G.; Trivic, S.; Zeremski, J.; Pericin, D.; Leskovac, V., The chemical mechanism of action of glucose oxidase from Aspergillus niger. *Mol. Cell. Biochem.* **2004**, *260*, 69-83.