Electronic Supplementary Material (ESI) for Lab on a Chip

Supporting information

Insights into "Free State" Enzyme Reaction Kinetics in Nanoconfinement

Chen Wang[1,2], De-Kai Ye[1], Yun-Yi Wang[1], Tao Lu[2]*, and Xing-Hua Xia[1]*

1. State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, China

Fax: +86-25-83686106; Tel: +86-25-83597436; E-mail: xhxia@nju.edu.cn

2. Department of Physical Chemistry, School of Basic Science, China Pharmaceutical University, Nanjing 211198, China

Fax: +86-25-86185179; Tel: +86-25-86185180; E-mail: lutao@cpu.edu.cn

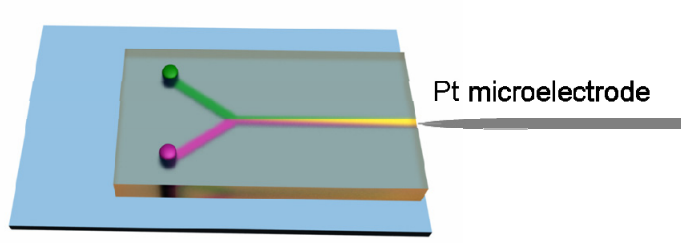

Figure S1. Schematic layout of the nanofluidics chip. (*Green colors*) Glucose oxidase; (*Pink colors*) Substrate glucose; (*Yellow colors*) Product H_2O_2. The working electrode is aligned at the end of nanochannel with a 20 μm distance.

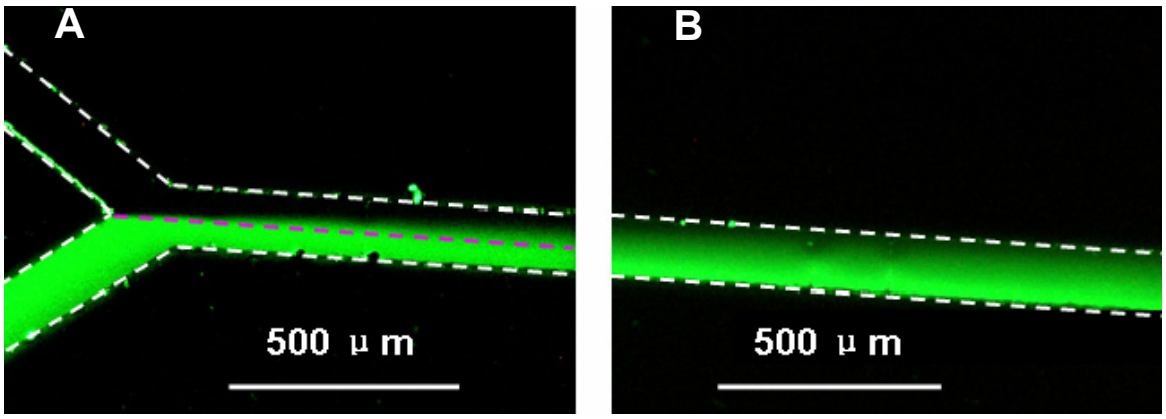

Figure S2. The photo images of streams flow in nanochannel after driving 10 μg ml^{-1} fluorescein isothiocyanate (FITC) in 10 mM PBS buffer (pH 7.0) and 10 mM PBS Buffer (pH 7.0) through nanofluidics chip. Images were taken after applying a voltage of 600 V with FITC (e) and buffer reservoir (s) anode, waster reservoir (r) cathode as indicated in Figure 2D. The liquid flow rate is controlled as 200 μm/s. White dotted line indicates outlines of the nanofluidics chip, and pink dotted line shows midsplit of the nanochannel. Image (A) shows the junction part of the nanofluidics chip, where laminar flow forms, and transverse diffusion between the two fluids starts; (B) the photo image at downstream, where the two fluids gradually mix thoroughly within short distance.

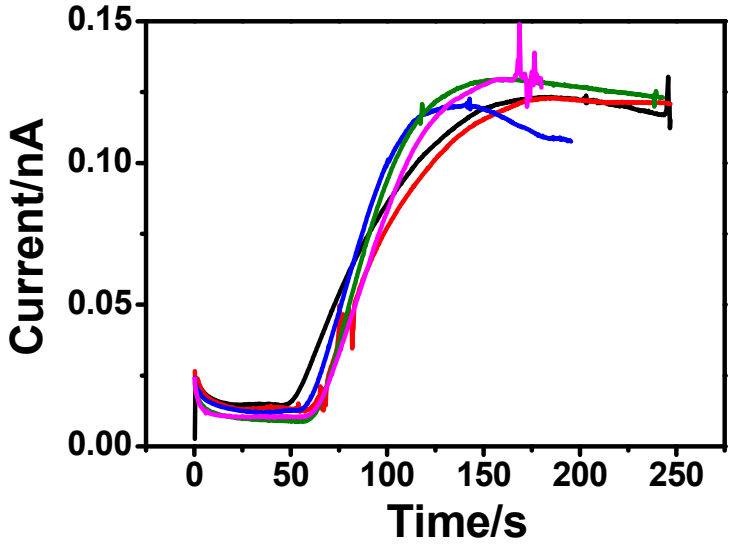

Figure S3. Five replications of current responses as a function of time for introduction of a 0.5 mM glucose solution and 6.5 μM GOx in the Y-shaped nanofluidics chip (RSD=4.53%). The current responses are detected on a Pt ultramicroelectrode (10 μm in diameter) at 0.8 V.

Control of liquid flow values in nanochannel. To control the liquid flow rate in nanofluidics chip, the electroosmotic flow (μ_{EOF}) and the electroosmotic velocity (V_{EOF}) were determined with the elution time of a neutral marker hydrogen peroxide solute. The results are listed in Table S1. The neutral marker will be carried through the channel under the action of only the electroosmotic flow and the μ_{EOF} can thus be evaluated by equation (S1), where L is the length of separation channel, V is the applied separation voltage, and t is the migration time of the neutral marker. The electroosmotic velocity (V_{EOF}) can also be calculated by equation (S2). The liquid flow rate in the channel can be estimated according to V_{EOF}.

$$\mu_{EOF} = \frac{L^2}{Vt} \qquad \text{(S1)}$$

$$V_{EOF} = \frac{L}{t} \qquad \text{(S2)}$$

Table S1. The effect of channel size on the values of μ_{EOF} and V_{EOF}.

Migration time/*s*	The applied separation voltage/*v*	μ_{EOF} / $cm^2\ v^{-1}\ s^{-1}$	V_{EOF} / $cm\ s^{-1}$
120	300	4.0×10^{-5}	0.010
60	600		0.020
36	1000		0.033
30	1200		0.040

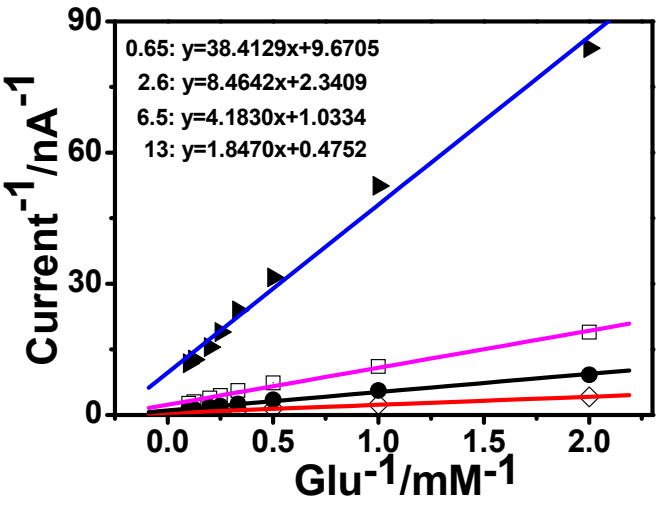

Figure S4. The Lineweaver–Burke plots for substrate concentrations ranging from 0.05 to 5 mM corresponding to Figure 4B at different enzyme concentrations (the linear correlation coefficients are respectively 0.9951, 0.9966, 0.9961, 0.9978). Enzyme concentration: 0.65 (solid triangles), 2.6 (empty squares), 6.5 (solid cycles), and 13 (empty diamonds) μM.

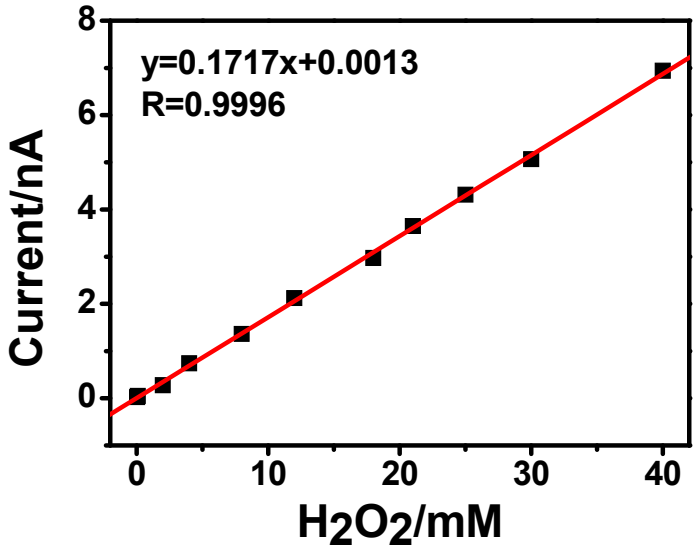

Figure S5. H_2O_2-current calibration in nanofluidics. A series of different concentrations of H_2O_2 solutions are electrokinetically driven through the nanofluidic chip for electrochemical measurement. The linear rang is 0.05-40 mM of H_2O_2. Based on H_2O_2-current calibration, the concentration of reaction product H_2O_2 ($[H_2O_2]$) can be achieved as 0.59, 2.47, 5.61, 12.21 mM responding to 0.65, 2.6, 6.5, 13 μM GOx according to the values of i_{max}. Given the reaction time (t) 40 s for 200 μm/s liquid flow rate in nanochannel, the maximum reaction rate are calculated as 14.82, 61.83, 140.30, 305.32 μM/s, according to the equation $v_{\max} = [H_2O_2]/t$.

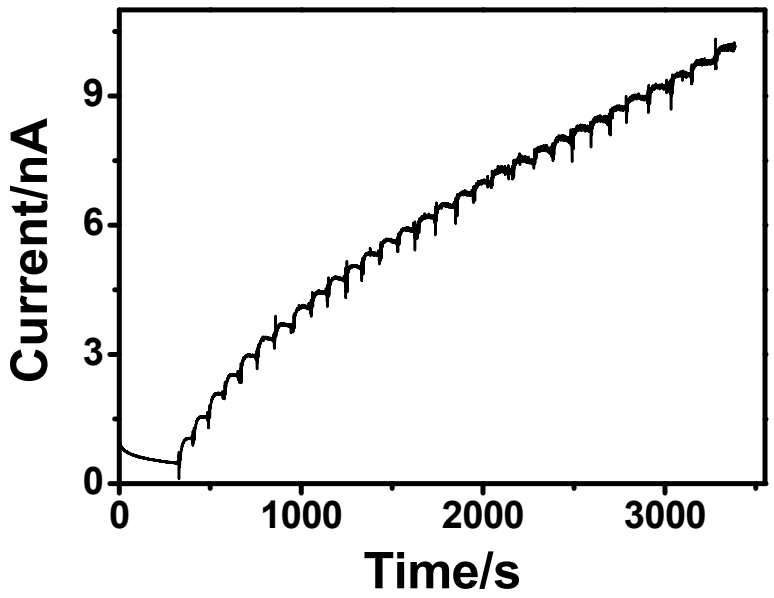

Figure S6. Steady-state current versus time (i–t) curve for the detection of the enzyme reaction product hydrogen peroxide on a Pt ultramicroelectrode at 0.8 V in bulk solutions with an enzyme concentration of 13 μM. 0.50 mM glucose was successively injected into the stirred 10 mM PBS (pH 7.0). From this figure, it is clear that the time for reaching steady-state current is about 50 s.

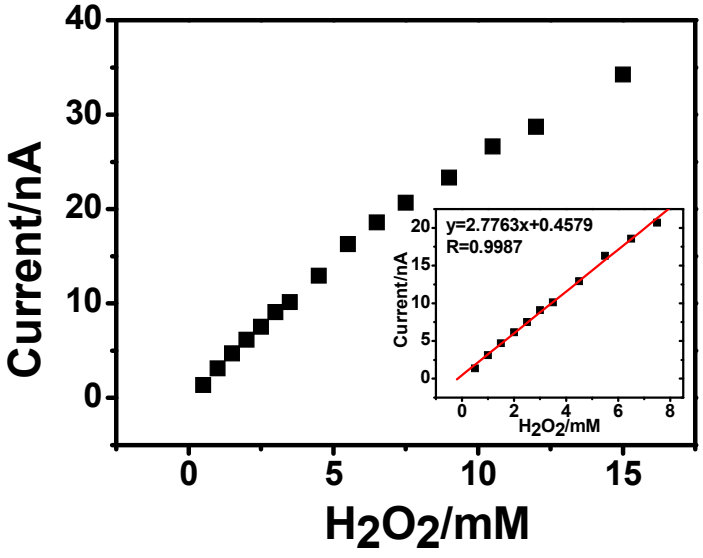

Figure S7. H_2O_2-current calibration in bath solution. A series H_2O_2 solutions are injected into bath system in PBS buffer (pH=7.0) for electrochemical measurement. The linear rang is 0.05-8 mM of H_2O_2, as indicated in the inserted figure. The linear equation is y=2.7763x+0.4579. The maximum current i_{max} in bath solution is 17.61 nA (Table 1). Consequently, the corresponding concentration of reaction product H_2O_2 ($[H_2O_2]$) is calculated as 6.18 mM based on H_2O_2-current calibration (17.61=2.7763× 6.18+0.4579). Since the reaction time is about 50 s, the resulted v_{max} is 123.53 μM/s according to $v_{\max} = [H_2O_2]/t$, where t is the reaction time (50 s).