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Measuring Rapid Enzymatic Kinetics by Electrochemical Method in Droplet-Based Microfluidic Devices with Pneumatic Valves

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This paper describes a droplet-based microfluidic chip with pneumatic valves for measuring millisecond enzyme kinetics using amperometric detection method. Aqueous streams containing reactants were injected to an oil flow to form droplets, and each droplet represented one microreactor. Pneumatic valves were used to control the moving distance and in turn the reaction time of the droplets. The reaction time was also fine-tuned by varying the flow rate of the droplets in microchannels. A complete Michaelis−Menten kinetics of catalase was successfully measured by amperometric method in a single-run experiment, and the total consumption of reagents was less than 50 μL. In the current experiment, the best time resolution was about 0.05 s, and the reaction time measured was from 0.05 to 25 s. This microfluidic system is applicable to many biochemical reactions, as long as one of the reactants or products is electrochemically active. With appropriate quenching method at the outlet, various detection methods can be integrated into the microfluidic system, further extending the application of the combination of pneumatic valves and droplets in microchannels.

Rapid kinetics measurement is essential to understanding many biological and chemical processes, such as protein and RNA folding, protein−protein interaction, and enzymatic mechanism. For rapid kinetics within microseconds to seconds, stopped-flow[1,2] and quenched-flow[3,4] are the two most often used measurement methods. In both methods, two or more reactant solutions are rapidly mixed in a mixing chamber. At desired reaction time, the flow is either stopped or quenched, and the reaction can be monitored using an appropriate detection method. Both of these techniques provide fast mixing and short dead time for studying rapid kinetics. However, the conventional instrumentation using these two methods consumes large volumes of samples, which are not compatible with bioanalysis as the reagents are usually expensive or in limited supply.

To solve the problem, various microfluidic platforms have been developed. Microfluidics has the advantage of low sample consumption, therefore is an attractive platform for bioanalysis. One of the major difficulties in utilizing microfluidics for bioanalysis, especially for kinetics measurement, is to mix nanoliter of solutions rapidly.[5] Several tricks have been played to enhance mixing in microfluidics.[6−8] Knight et al. developed a flow focusing microfluidic device capable of squeezing the central stream to submicrometer in thickness, thus reducing the diffusive mixing times to less than 10 μs. The flow focusing device facilitated the study of fast reaction kinetics such as RNA folding on time scales of hundreds of microseconds by monitoring the fluorescence signal in the central stream.[6,9,10] Song et al. demonstrated millisecond kinetics measurement on a droplet-based microfluidic chip by moving the droplets through winding microchannels and inducing chaotic flow inside the droplets. Kinetics of the cleavage of a fluorogenic substrate by RNase A was studied by analysis of the fluorescent image of the microfluidic device.[11] Droplet-based microfluidic system is a powerful method for kinetics measurement with advantages such as no dispersion, rapid mixing and on-chip dilution.[12,13] In both flow focusing and droplet-based microfluidic systems, fluorescence detection is the most widely used detection method, with excellent sensitivity and convenience to implement by using fluorescence microscope. Since most biomolecules have no or extremely weak fluorescence, these molecules need to be labeled with fluorescence tags. The potential issues of using fluorescence tags in kinetics measurement include photobleaching of the fluorescence tag and interference of the

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(1) Olson, S. T.; Bjork, I. *J. Biol. Chem.* **1991**, *266*, 6353–6364.
(2) Khorasanizadeh, S.; Peters, I. D.; Butt, T. R.; Roder, H. *Biochemistry* **1993**, *32*, 7054–7063.
(3) Eyles, S. J.; Radford, S. E.; Robinson, C. V.; Dobson, C. M. *Biochemistry* **1994**, *33*, 13038–13048.
(4) Kane, D. J.; Fendler, K.; Grell, E.; Bamberg, E.; Taniguchi, K.; Froehlich, J. P.; Clarke, R. J. *Biochemistry* **1997**, *36*, 13406–13420.
(5) Ottino, J. M.; Wiggins, S. *Philos. Trans. R. Soc., A* **2005**, *362*, 923–935.
(6) Knight, J. B.; Vishwanath, A.; Brody, J. P.; Austin, R. H. *Phys. Rev. Lett.* **1998**, *80*, 3863–3866.
(7) Song, H.; Tice, J. D.; Ismagilov, R. F. *Angew. Chem., Int. Ed.* **2003**, *42*, 768–772.
(8) Stroock, A. D.; Dertinger, S. K. W.; Ajdari, A.; Mezic, I.; Stone, H. A.; Whitesides, G. M. *Science* **2002**, *295*, 647–651.
(9) Pollack, L.; Tate, M. W.; Darnton, N. C.; Knight, J. B.; Gruner, S. M.; Eaton, W. A.; Austin, R. H. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 10115–10117.
(10) Pollack, L.; Tate, M. W.; Finnefrock, A. C.; Kalidas, C.; Trotter, S.; Darnton, N. C.; Lurio, L.; Austin, R. H.; Batt, C. A.; Gruner, S. M.; Mochrie, S. G. J. *Phys. Rev. Lett.* **2001**, *86*, 4962–4965.
(11) Song, H.; Ismagilov, R. F. *J. Am. Chem. Soc.* **2003**, *125*, 14613–14619.
(12) Huebner, A.; Sharma, S.; Srisa-Art, M.; Hollfelder, F.; Edel, J. B.; Demello, A. J. *Lab Chip* **2008**, *8*, 1244–1254.
(13) Song, H.; Chen, D. L.; Ismagilov, R. F. *Angew. Chem., Int. Ed.* **2006**, *45*, 7336–7356.

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fluorescence tag with the kinetics or function of the target molecules.

Herein, we develop a complementary amperometric method based microfluidic system to monitor rapid kinetics in droplets. Many small molecules involved in biochemical reactions, such as metal ions, amino acids and ascorbic acid, can be directly studied by amperometric or other electrochemical methods. Electrochemical detection methods have great potential in microfluidics because of their high sensitivity, low cost, and compatibility with microfabrication technologies.[14] Previously electrochemistry system has been implemented in droplet-based microfluidic system for analysis of equilibrium state concentrations,[15] electroporation of cells,[16,17] and studying droplets flow behavior.[18] In the current work, we combine droplets and pneumatic valves[19] in microchannels to control the reaction time, and concentrations at different reaction times are measured by amperometric method. We validate the system by measuring the Michaelis−Menten kinetics of the decomposition of H_2O_2 by catalase.

EXPERIMENTAL SECTION

Fabrication of Electrodes. 5-mm-long Pt wire (diameter 30 μm, Sino-Platinum Metals) was soldered with a 2 cm-long Ag wire (diameter 150 μm, Sino-Platinum Metals) and was used as Pt working electrode. AgCl was deposited electrochemically on another piece of 3 cm-long Ag wire (diameter 50 μm, Sigma-Aldrich) in 0.15 M NaCl aqueous solution for 1 h with 0.5 mA current[20] and was used as quasi-reference electrode.

Fabrication of Multilayer Devices. The fabrication of multilayer devices was performed as described previously.[19,21] Master molds for multilayer devices were fabricated by photolithography. Fluid layer mold was made by spin-coating 50 μm-thick positive photoresist (AZ P4620, AZ electronic materials) on silicon wafer and patterning it with a high-resolution photomask. The mold with the patterned photoresist was baked at 110 °C for an hour to transform the cross section of the patterned photoresist to a half-moon geometry that allowed full valve closure.[19] Control layer mold was made by spin-coating and patterning a 25 μm-thick SU-8 photoresist (GM 1060, Gersteltec) on silicon wafer. A degassed 5:1 mixture of a polydimethylsiloxane (PDMS) precursor with the curing agent (Sylgard 184, Dow Corning) was cast as a fluid layer with thickness of 5 mm. A degassed 20:1 mixture of a PDMS precursor with the curing agent was spin-coated at 3000 rpm for 60 s to form a 25 μm-thick control layer. Both fluid and control layers were first cured for 30 min at 80 °C and then the fluid layer was aligned and sealed on the control layer to form the microchannels for the fluid layer. Bonding between these two layers was accomplished by curing the devices for another 60 min at 80 °C. The combined PDMS piece was then bound to a flat PDMS slab to form the microchannels for the control layer (Figure 1a). Pt and Ag/AgCl microelectrodes were inserted into the microchannel in the fluid layer and sealed with epoxy glue (inset of Figure 1b).

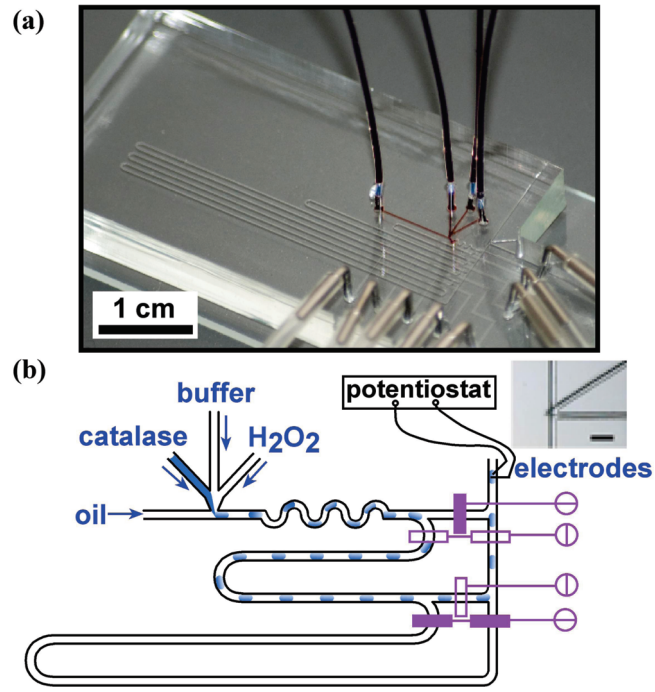

Figure 1. (a) Photograph of the multilayer PDMS device. (b) Scheme of multitime range access controlled by pneumatic valves (in purple) in a multilayer microfluidic device. Inset is the microphotograph of microelectrodes inserted near the outlet of the microchannel. Scale bar is 500 μm.

Sample Preparation and Operation. Catalase (from bovine liver, 2000−5000 units/mg, Sigma) was dissolved in 50 mM phosphate buffered saline (PBS, pH 7.4) at 10.0 μg/mL. Hydrogen peroxide (33%, Panreac) was diluted by 50 mM PBS buffer to 200 mM. Precision syringe pumps (PHD 2000, Harvard Apparatus) were used to drive the flow of catalase solution, the diluting buffer, and the carrier fluid. Teflon tubing (Stranco) was used to connect syringes with microfluidic channels. Silicon oil (viscosity 50 cP, Brookfield) was used as the carrier fluid, and droplets containing the mixture of PBS buffer, substrate solution and catalase solution were formed at the junction (Figure 1b).

Each control channel for the pneumatic valves was connected to a homemade three-way switch through Tygon tubing (Cole-Parmer). Regulated compressed air (0.2 MPa) was applied to the three-way switch, and control channel can be pressured or vented to atmosphere by controlling the three-way switch.

The Pt and Ag/AgCl microelectrodes were separately connected to a potentiostat (263A, Princeton Applied Research) for electrochemical analysis of the droplets in the microchannel (Figure 1b).

Microphotographs were taken by using a stereoscope (MZ 16, Leica) equipped with a CCD camera (SPOT Insight, Diagnostic Instruments). A Machine Vision Strobe X-Strobe X1200 (Perkin-Elmer Optoelectronics) provided lighting. The flash duration of the strobe light was ~10 μs.

(14) Wang, J. *Talanta* **2002**, *56*, 223–231.
(15) Lindsay, S.; Vazquez, T.; Egatz-Gomez, A.; Loyprasert, S.; Garcia, A. A.; Wang, J. *Analyst* **2007**, *132*, 412–416.
(16) Luo, C. X.; Yang, X. J.; Fu, O.; Sun, M. H.; Ouyang, Q.; Chen, Y.; Ji, H. *Electrophoresis* **2006**, *27*, 1977–1983.
(17) Zhan, Y.; Wang, J.; Bao, N.; Lu, C. *Anal. Chem.* **2009**, *81*, 2027–2031.
(18) Liu, S.; Gu, Y.; Roux, R. B. L.; Matthews, S. M.; Bratton, D.; Yunus, K.; Fisher, A. C.; Huck, W. T. S. *Lab Chip* **2008**, *8*, 1937–1942.
(19) Unger, M. A.; Chou, H. P.; Thorsen, T.; Scherer, A.; Quake, S. R. *Science* **2000**, *288*, 113–116.
(20) Hassel, A. W.; Fushimi, K.; Seo, M. *Electrochem. Commun.* **1999**, *1*, 180–183.
(21) Duffy, D. C.; McDonald, J. C.; Schueller, O. J. A.; Whitesides, G. M. *Anal. Chem.* **1998**, *70*, 4974–4984.

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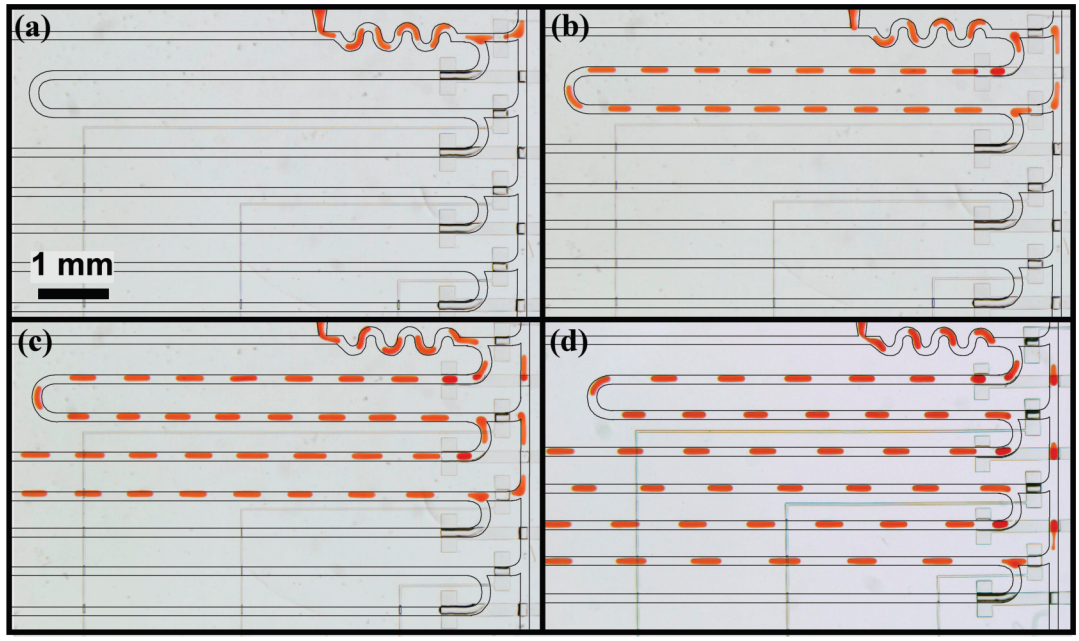

Figure 2. Microphotograph of the droplets moving along different routes controlled by pneumatic valves. (a) Droplets taking the shortest route of ~5 mm-long; (b) Droplets taking the second route of ~20 mm-long; (c) Droplets taking the third route of ~50 mm-long; (d) Droplets taking the fourth route of ~110 mm-long. The droplets contained $Fe(SCN)_x^{(3-x)+}$ for better observation. The length of droplets was shorter at the position where a valve was located, because the flexible PDMS membrane between the fluid channel and valve was forced to go down, increasing the height of the fluid channel.

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RESULTS AND DISCUSSION

Control of Reaction Time in Droplets. The reaction started once the droplets were generated by combining the aqueous streams and injecting them into the flow of silicone oil at the junction of the microchannel (Figure 1b). The reaction time in the droplets was the time that the droplets spent in the microchannel until they arrived at the microelectrodes. There were two ways to vary the reaction time in the droplets in our microfluidic system: (1) changing the flow rate to tune the reaction time; (2) using the pneumatic valves to change the flow route and in turn the travel time of the droplets (Figure 2).

When the flow rate was varied to tune the reaction time, the maximum flow rate was limited by the highest pressure allowed at the inlets of the PDMS device. On the other hand, low flow rate led to long droplets, which required long mixing time as the droplets flowed through the winding microchannel.[22] In our experiment, the flow rate varied between 10 and 100 mm/s. Since most reactions are of positive reaction order, it is necessary to obtain more data points in the initial stage of the reactions in the kinetics measurement. We varied the flow rates when the droplets moved in the shortest route (Figure 2a), and the reaction time ranged from 0.05 to 0.5 s. When the pneumatic valves were used to vary the reaction time in the droplets, the flow rate of the droplets was maintained constant at 10 mm/s. As a result, the combined reaction time ranged from 0.05 s (in the shortest route) to 25 s (in the longest route).

Features of the Microfluidic Device. The time resolution of the current device is 0.05 s, which is limited by the maximum total flow rate v_{max} (100 mm/s) and the distance between the T-junction for droplets formation and the microelectrodes (5 mm). The segment of winding channel induced chaotic advection inside the droplets, which led to rapid mixing of the reagents in each droplet.[7] It has been shown that efficient mixing can be achieved by driving the droplets through only a short segment of such winding channel.[23] We used pyranine to characterize the mixing time in our droplet-based microfluidic system. Fluorescence intensity of pyranine increased dramatically when the solution pH changed from acidic to basic. By monitoring the fluorescence change of the droplets containing acidic pyranine solution (0.5 mM, pH ~ 1) and NaOH solution (pH ~ 14), we found that the mixing was completed within 0.02 s in our experiment (Supporting Information (SI) Figure S1), which was shorter than the time resolution of the device.

The Selwyn's test[11,24] was performed in the droplet system to demonstrate that there was no denaturation of the enzyme. In this test, the kinetics of the substrate consumption was monitored at different enzyme concentration. The resulting curves of the percentage of converted H_2O_2 versus the product of catalase concentration and time were found superimposable (SI Figure S2), suggesting that there was no apparent denaturation of catalase in our microfluidic system.

We observed that some droplets were split after the droplets passed by the Ag/AgCl electrode (Figure 3). Although the splitting did not interfere with the electrochemical measurement, it would be desirable to control the splitting in case more studies of the droplet content were required after the electrochemical measurement. We found that the splitting could be avoided by

(22) Bringer, M. R.; Gerdts, C. J.; Song, H.; Tice, J. D.; Ismagilov, R. F. *Philos. Trans. R. Soc. Lond. A* **2004**, *362*, 1087–1104.
(23) Song, H.; Bringer, M. R.; Tice, J. D.; Gerdts, C. J.; Ismagilov, R. F. *Appl. Phys. Lett.* **2003**, *83*, 4664–4666.
(24) Selwyn, M. J. *Biochim. Biophys. Acta* **1965**, *105*, 193–195.

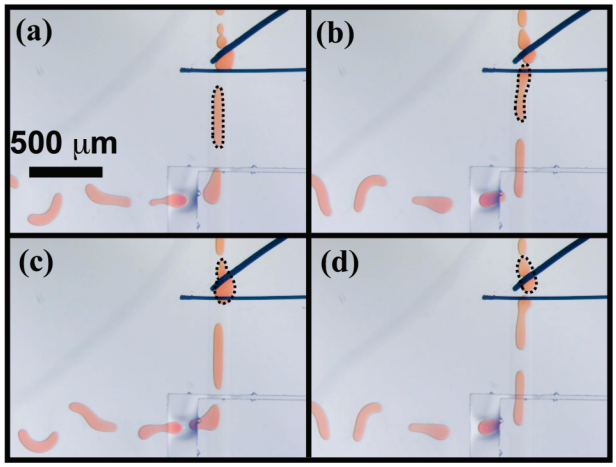

Figure 3. A series of microphotographs showing droplets passing through the two microelectrodes. The droplets contained $Fe(SCN)_x^{(3-x)+}$ for better observation. One droplet was highlighted by dashed line. (a) The highlighted droplet was approaching the microelectrodes; (b) The droplet just contacted the first microelectrode, Pt electrode; (c) The droplet moved further to connect both microelectrodes and a current signal was generated; (d) The droplet disconnected with the first microelectrode and current returned to zero.

using smaller-sized microelectrodes (SI Figure S3). In addition, reducing the length of the microelectrodes presented in the microchannel might also help prevent the droplets from splitting (SI Figure S4).

Five kinetic profiles were obtained at different concentrations of H_2O_2 in a single run. Each kinetic profile was measured within 1 min, consuming less than 10 μL of the combined reagent solutions. Therefore the total consumption of sample for a complete Michaelis−Menten kinetics with five different substrate concentrations was less than 50 μL. We envision that the reagent consumption can be significantly reduced by automating the flow rate control[25] and using smaller microchannels.

Electrochemistry of Hydrogen Peroxide. H_2O_2 is oxidized at the surface of metal electrodes such as platinum polarized to 600 mV:[26] $H_2O_2 \rightarrow 2H^+ + O_2 + 2e$.

In our microfluidic system, the aqueous droplets containing H_2O_2 and the carrier fluid alternatively passed by the microelectrodes, and the electrochemical circuit was connected and disconnected, resulting in a periodic current rise and fall (SI Figure S5). The peak value was stable and reproducible, with a CV of 5%. The occurrence of each current peak signal can be divided into three stages (Figure 3): (1) the aqueous droplet approached the two microelectrodes, and became in contact with only the first microelectrode (Figure 3b); (2) the droplet moved further along the channel, and became in contact with both microelectrodes, and a current peak was generated (Figure 3c); (3) the droplet was disconnected from the first microelectrode, the current signal returned to zero (Figure 3d). To make a complete electric circuit, the droplet must be longer than the gap between the two microelectrodes. In the microfluidic device, the two microelectrodes were placed with a gap smaller than the cross-sectional dimension of the microchannel. In our experiments, the capillary number ($Ca = \mu v/\gamma$, where μ is viscosity, v is the flow velocity of the carrier fluid, and γ is interfacial tension) was less than 0.005, and the width of the main channel was comparable to the width of the aqueous inlet (Figure 2). Under these experimental conditions, the droplets formed in the microchannel were in the form of "plugs",[27,28] with the length longer than the cross-sectional dimension of the microchannel and the microelectrodes gap, ensuring that the electrochemical circuit was always completed when a droplet passed by the microelectrodes.

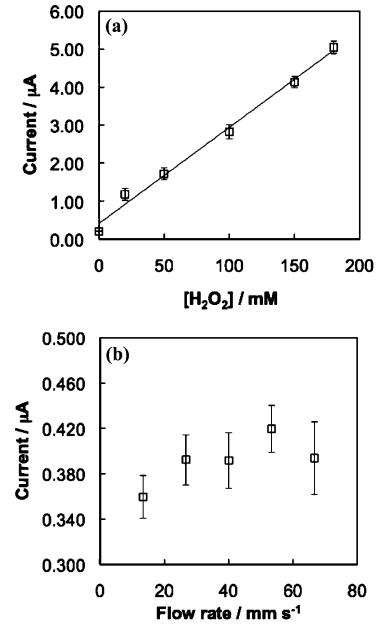

Figure 4. (a) Calibration curve for H_2O_2 concentration ranging from 10 to 200 mM with the linear fitting of the experimental data. (b) Relationship between current and flow rate of the droplets. The concentration of H_2O_2 was 50 mM and the flow rates were from ~10 to ~70 mm/s.

In our electrochemical detection setup, we found that the current peak was independent of the flow rate of the droplets (Figure 4b), indicating that diffusion was not the limiting step in the electrochemical oxidation of H_2O_2. Our observation was consistent with previous studies on the electrode reaction of H_2O_2,[29−32] which found the reaction to be irreversible and sensitive to the surface roughness of the electrode. The quantitative treatments of the flow and of the response of the hydrodynamic microelectrode in the microchannel are beyond the scope of the current work, and the complete understanding of the mechanism of the electrochemical response in our two-phase system requires further study.

Analysis of the Enzymatic Kinetics. The current amplitude in the electrochemical system depends on two factors: (1) the bulk concentration of the analyte; (2) the flow rate of the droplets when diffusion is a limiting factor in the electrochemical reaction.

(25) Zheng, B.; Roach, L. S.; Ismagilov, R. F. *J. Am. Chem. Soc.* **2003**, *125*, 11170–11171.
(26) Lingane, J. J.; Lingane, P. J. *J. Electroanal. Chem.* **1963**, *5*, 411–419.
(27) Garstecki, P.; Fuerstman, M. J.; Stone, H. A.; Whitesides, G. M. *Lab Chip* **2006**, *6*, 437–446.
(28) Tice, J. D.; Song, H.; Lyon, A. D.; Ismagilov, R. F. *Langmuir* **2003**, *19*, 9127–9133.
(29) Evans, S. A. G.; Elliott, J. M.; Andrews, L. M.; Bartlett, P. N.; Doyle, P. J.; Denuault, G. *Anal. Chem.* **2002**, *74*, 1322–1326.
(30) Hall, S. B.; Khudaish, E. A.; Hart, A. L. *Electrochim. Acta* **1997**, *43*, 579–588.
(31) Nakanishi, S.; Mukouyama, Y.; Karasumi, K.; Imanishi, A.; Furuya, N.; Nakato, Y. *J. Phys. Chem. B* **2000**, *104*, 4181–4188.
(32) Zhang, Y.; Wilson, G. S. *J. Electroanal. Chem.* **1993**, *345*, 253–271.

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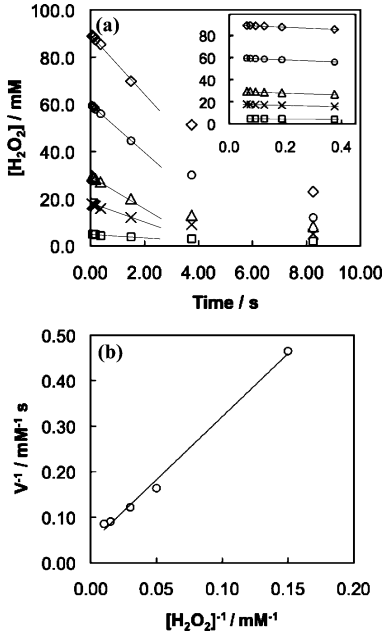

Figure 5. Plots for Michaelis–Menten kinetics analysis. (a) Plot of $[H_2O_2]$ vs the reaction time. The starting H_2O_2 concentrations are 100 mM ("◇"), 66.7 mM ("○"), 33.3 mM ("Δ"), 20.0 mM ("×"), and 6.67 mM ("□"), respectively. Inset is the blow-up of the plot in the time range from 0 to 0.5 s. (b) Plot of V^{-1} vs $[H_2O_2]^{-1}$ for Michaelis–Menten kinetics analysis.

Accordingly two calibration curves would be required for using amperometric method to analyze the droplet content in the current microfluidic system: (1) the current signal dependence on the concentration of the electrochemically active molecule; (2) the current signal dependence on the flow rate of the droplets. Both calibration curves could be obtained in a single experimental run by manipulating the individual flow rates of H_2O_2 stream, the diluting buffer stream, and the carrier fluid. The first calibration curve (Figure 4a) was established at varying concentration of H_2O_2 in the droplets by changing the ratio of the flow rates of the buffer and H_2O_2 streams while maintaining the combined flow rate constant. In the concentration range in our experiment for enzyme activity measurement, the peak heights of the current signal were linearly proportional to the concentration of H_2O_2 in the droplets. The second calibration curve was established by gradually increasing the flow rates of both H_2O_2 solution and the buffer, while the ratio of the two aqueous streams was maintained constant (Figure 4b). As we mentioned in the previous section, the current signal of H_2O_2 oxidation was independent of the flow rate, therefore the second calibration curve was essentially a flat line in the case of electrochemical oxidation of H_2O_2. As a result, the analysis of the current signal of H_2O_2 oxidation was simplified by referring to only the calibration curve of the current versus the bulk concentration of H_2O_2.

The calibration of the current signal versus H_2O_2 concentration was performed both before and after the Selwyn's test. The two superimposable calibration curves suggested that there was no obvious adsorption of protein or other contaminants on the surface of the microelectrodes during the kinetics measurement (SI Figure S6).

The reaction kinetics was analyzed following the typical Michaelis–Menten kinetics. The Michaelis–Menten kinetics does not always apply to the decomposition of H_2O_2 by catalase because of the two-step nature of the catalytic reaction.[33] However, in our experiment with H_2O_2 concentration below 200 mM, catalase does exhibit a Michaelis–Menten kinetics.[34] In a single-run experiment, we obtained multiple reaction kinetics at different starting H_2O_2 concentration and constant catalase concentration (Figure 5a). Each curve in Figure 5a was composed of two segments. The first segment was from 0.05 to 0.5 s and was obtained by changing the flow rate of the droplets in the shortest route (inset of Figure 5a). The second segment was from 1.5 to 8.0 s and was obtained by using the pneumatic valves to change the flow routes of the droplets. The initial velocities (V) of the enzymatic reaction were calculated at different concentration of H_2O_2 from the linear portion of the kinetics curves in the initial stage of the reaction. By plotting initial velocities versus different concentrations of H_2O_2 at the same catalase concentration, a Michaelis–Menten kinetics curve was obtained (SI Figure S7). The Michaelis constant K_m and the maximum velocity V_{max} were calculated as 62 mM and 22 mM/s by plotting V^{-1} against $[H_2O_2]^{-1}$ (Figure 5b). These values were consistent with previous measurements of bovine catalase under similar experimental conditions.[34]

CONCLUSION

We integrated the electrochemical detection method into a droplet-based microfluidic system for measuring rapid kinetics. Pneumatic valves were implemented to control the reaction time. In this work, the best time resolution was about 0.05 s. A complete Michaelis–Menten kinetics measurement with five different H_2O_2 concentrations was achieved in a single experimental run, and the total consumption of samples was less than 50 μL.

This microfluidic system is complementary to the fluorescence-based microfluidic system for rapid kinetics measurement. The system has high sensitivity of the electrochemical detection without the need of labeling. Only one pair of microelectrodes was employed in the microfluidic system, making the PDMS chip simple and inexpensive to fabricate, and the chip can be disposable. It is possible to further simplify the fabrication of the chip by separating the microelectrodes from the microfluidic channels.[35] The current microfluidic system is applicable to many biochemical reactions, as long as one of the reactants or products is electrochemically active. With appropriate quenching method at the outlet, the reaction in the droplets can be "frozen" at different reaction times, allowing the droplets to be analyzed offline, for example by mass spectrometry.[36] In this way various detection methods can be integrated into the microfluidic system, further extending the application of the combination of pneumatic valves and droplets in microchannels.

ACKNOWLEDGMENT

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(33) Nicholls, P.; Fita, I.; Loewen, P. C. Enzymology and structure of catalases. In *Advances in Inorganic Chemistry*; Academic Press Inc: San Diego, 2001; Vol. 51, pp 51–106.
(34) Switala, J.; Loewen, P. C. *Arch. Biochem. Biophys.* **2002**, *401*, 145–154.
(35) Wang, J.; Tian, B.; Sahlin, E. *Anal. Chem.* **1999**, *71*, 5436–5440.
(36) Lin, Y.; Gerfen, G. J.; Rousseau, D. L.; Yeh, S. R. *Anal. Chem.* **2003**, *75*, 5381–5386.

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SUPPORTING INFORMATION AVAILABLE

Figure S1 shows the fluorescence intensity of pyranine solution during mixing the acidic pyranine solution with NaOH solution. Figure S2 shows the result of the Selwyn's test. Figures S3 and S4 show the microphotographs of droplets passing by two microelectrodes of 20 μm in diameter without splitting. Figure S5 shows the current profile measured at 0.6 V (vs Ag/AgCl quasi-reference electrode) for different H_2O_2 concentration. Figure S6 shows the calibration curves of current versus H_2O_2 concentration before and after the Selwyn's test. Figure S7 shows the plot of initial velocity (V) vs different substrate concentrations ($[H_2O_2]$) at the same catalase concentration. This material is available free of charge via the Internet at http://pubs.acs.org..

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Supporting Information

Measuring Rapid Enzymatic Kinetics by Electrochemical Method in Droplet-Based Microfluidic Devices with Pneumatic Valves

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Supporting Information

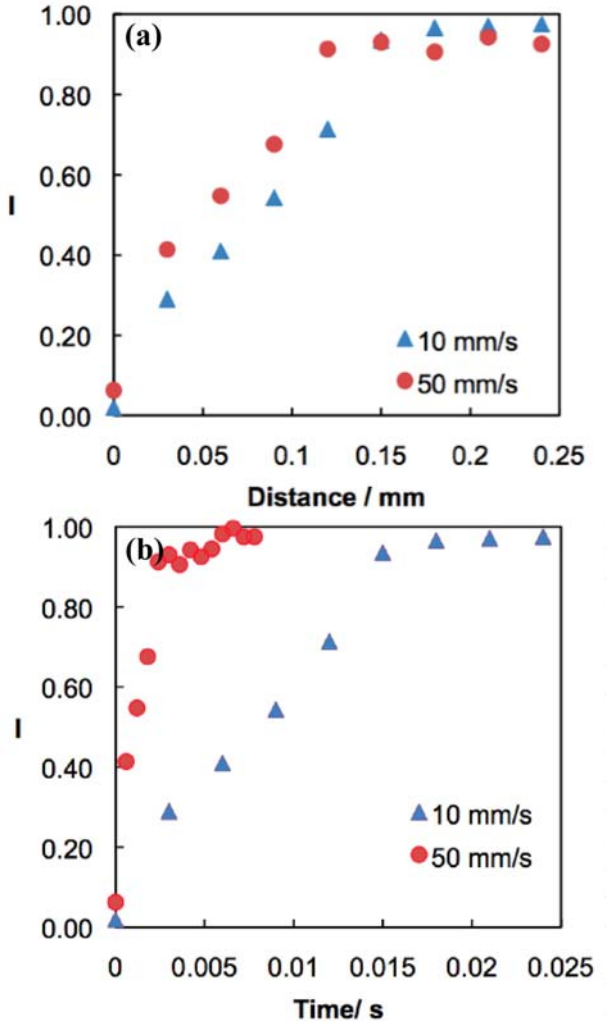

Figure S1. Normalized fluorescence intensity (*I*) obtained from microphotograph (2 s exposure) as a function of the travel distance (a) and as a function of travel time (b) of the droplets at two different flow rates. Fluorescence intensity increased when acidic pyranine solution (0.5 mM, pH ~ 1) mixed with NaOH solution (pH ~ 14) inside of droplets. The average intensity across the width of the microchannel was measured.

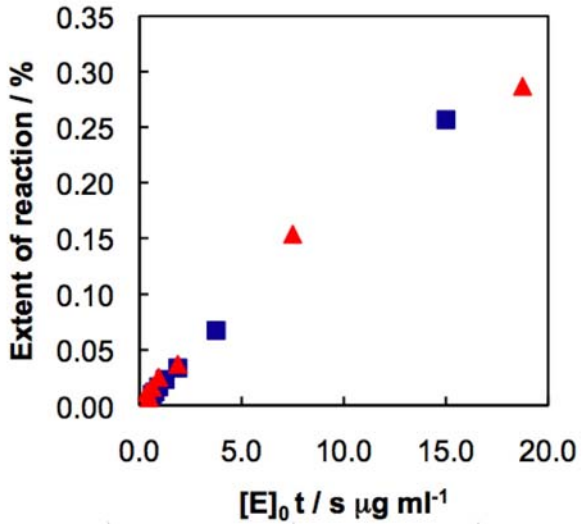

Figure S2. The Selwyn plot for catalase. H_2O_2 concentration was 66.7 mM. Two catalase concentrations, 5 μg/ml (red triangles) and 10 μg/ml (blue squares), were tested.

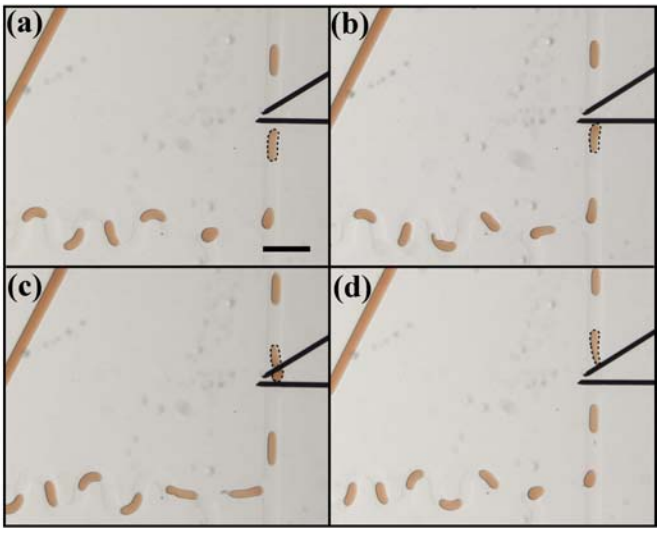

Figure S3. A series of microphotographs showing droplets passing by the two stainless steel wires (diameter ~ 20 μm). The two steel wires were extended laterally across the microchannel. The droplets contained $Fe(SCN)_x^{(3-x)+}$ for better observation. One droplet was highlighted by dashed line. (a) The highlighted droplet was approaching the metal wires; (b) The droplet just contacted the first metal wire; (c) The droplet moved further to connect both metal wires; (d) The droplet disconnected with the first metal wire. Scale bar is 500 μm.

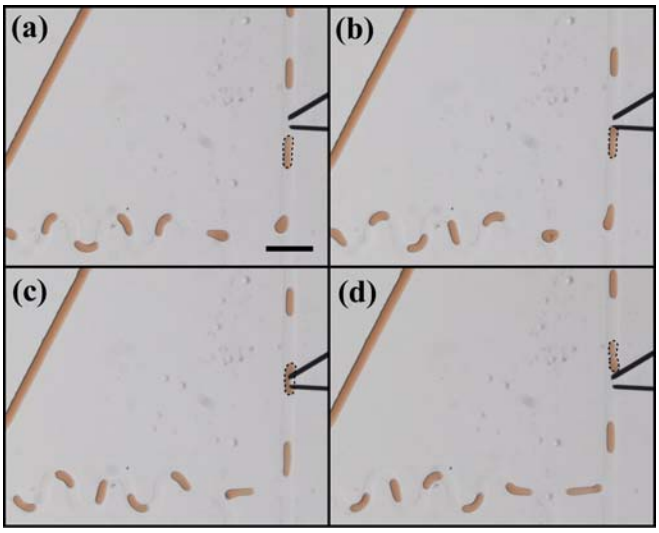

Figure S4. A series of microphotographs showing droplets passing by the two stainless steel wires (diameter ~ 20 μm). The two steel wires were extended laterally to only the middle of the microchannel. The droplets contained $Fe(SCN)_x^{(3-x)+}$ for better observation. One droplet was highlighted by dashed line. (a) The highlighted droplet was approaching the metal wires; (b) The droplet just contacted the first metal wire; (c) The droplet moved further to connect both metal wires; (d) The droplet disconnected with the first metal wire. Scale bar is 500 μm.

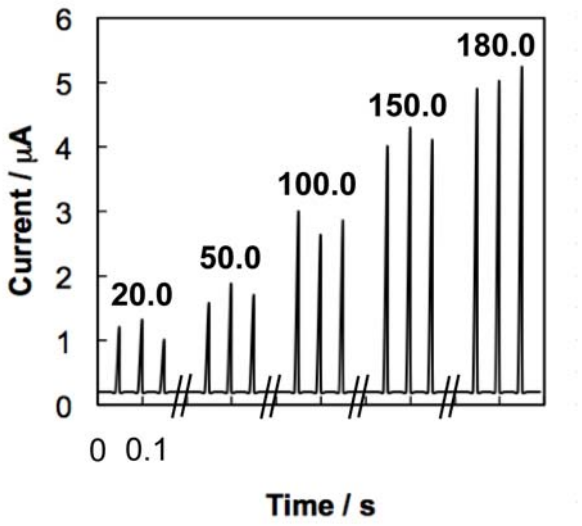

Figure S5. Current profile measured at 0.6 V (vs. Ag/AgCl quasi-reference electrode) for H_2O_2 concentration ranging from 10 mM to 200 mM. Numbers on top of each group of peaks indicate the concentrations of H_2O_2 in mM.

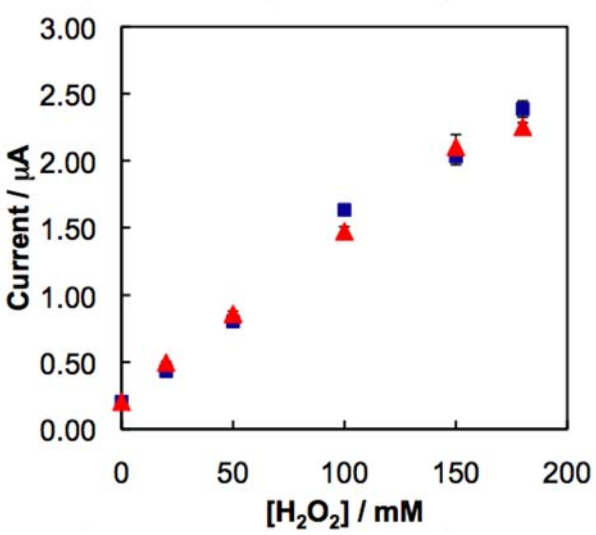

Figure S6. Calibration curves of the current versus H_2O_2 concentration before (blue squares) and after Selwyn's test (red triangles).

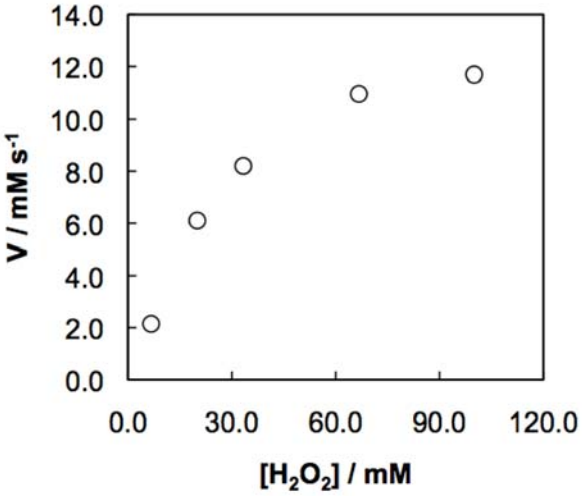

Figure S7. Plot of initial velocity (*V*) vs. different substrate concentrations ($[H_2O_2]$) at the same catalase concentration (10 μg/ml).