Supplementary Information

A Multistage Volumetric Bar Chart Chip for Visualized Quantification of DNA

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1. Materials and methods

Glass slides (75×50×1 mm) were purchased from Corning Inc. (Corning, NY). SPR220-7 was purchased from MicroChem Corp. (Newton, MA). MF-CD26 was obtained from Rohm and Haas Electronic Materials (Marlborough, MA). Tridecafluoro-1,1,2,2-tetrahydrooctyl-1-trichlorosilane was obtained from Pfaltz and Bauer, Inc. (Waterbury, CT). FC-70 (a mixture of perfluoro-tri-*n*-butylamine and perfluoro-di-*n*-butylmethylamine) was purchased from Hampton Research (Aliso Viejo, CA). Fetal bovine serum (FBS) was obtained from Invitrogen (Grand Island, NY). PBS buffer (0.1 M, pH7.4) was obtained from Lonza, Inc. (Allendale, NJ). Catalase, (3-glycidoxypropyl) trimethoxysilane (3-GPS), hydrogen peroxide solution (35% wt in H_2O), NH_4F, HF, and HNO_3 were obtained from Sigma-Aldrich, Inc. (St. Louis, MO). Red ink was purchased from Fisher Scientific and was diluted 2× before use. Polyvinyl chloride sealing tape was obtained from 3M (St. Paul, MN). Amorphous diamond-coated drill bits (0.031 inch cutter diameter) were purchased from Harvey Tool (Rowley, MA). All devices were designed as computer graphics using AutoCAD software and then printed out as transparency photomasks by CAD/Art Services, Inc. (Bandon, OR) with resolution at 10 μm. All DNA oligomers were synthesized by Integrated DNA Technologies, Inc (Coralville, IA) and used as received:

Ebola virus (EV),

EV1, 5’-GGA GTA AAT GTT GGA GAA CAG TAT CAA CAA-3’

EV1’, 5’-GGA ATA AAT GTT GGA GAA CAG TAT CAA CAA-3’ (one-base mismatch)

EV1”, 5’-GGA ATA AAC GTT GGA GAA CAG TAT CAA CAA-3’ (two-base mismatch)

EV2, 3’-NH_2-A_{12}-CCT CAT TTA CAA CCT-5’

EV3, 5’NH_2-C_6-A_{12}-TTG TTG ATA CTG TTC-3’

Bacillus anthracis (BA),

BA1, 5’-GAG GGA TTA TTG TTA AAT ATT GTA AAG GAT-3’

BA2, 3’- NH_2-A_{12}-CTC CCT AAT AAC AAT-5’

BA3, 5’-NH_2-C_6-A_{12}-ATC CTT TAC AAT ATT-3’

Tubercle bacillus （TB)

TB1, 5’-CAT CGT GGA AGC GAC CCG CCA GCC CAG GAT-3’

TB2, 3’- NH_2-A_{12}-GTA GCA CCT TCG CTG -5’

TB3, 5’-NH_2-C_6-A_{12}-ATC CTG GGC TGG CGG-3’

2. Experiments

A. Fabrication and operation

A1.Photolithography

A standard photolithography process was used to fabricate the V-chip device, as shown in Figure S1. Glass slides (75×50×1 mm) were first spin-coated with a 10-μm layer of SPR220-7 photoresist. The slides were dried by baking at 75°C for 3 min and 115°C for 5 min to promote adhesion of the photoresist.[1] After cooling to room temperature, the glass slides with the photoresist coating were aligned with a photomask containing the designed wells and channels. The photomask and glass slides were exposed to ultraviolet light for 50 s and then the photomask was removed from the glass slide, which was developed by immersion in SPR developer solution (MF-CD26) for 3 min. In this step, only the photoresist in the exposed area was dissolved in the solution. The slides were thoroughly rinsed with Millipore water and dried with nitrogen gas.

A2. Glass etching and loading hole preparation

Before glass etching, the back of the glass slide was covered with polyvinyl chloride sealing tape to protect it from unnecessary etching. The taped glass slides were then carefully immersed in a plastic container with a glass etchant (1:0.5:0.75 mol/L $HF/NH_4F/HNO_3$) to etch the photolithography pattern.[1] A 35°C constant-temperature water bath was used to control the etching speed. At the etching time of 45 min, wells and channels were etched into the glass slides with a depth of approximately 50 μm. Afterwards, the tape was peeled off and the slides were thoroughly rinsed with acetone and isopropanol to remove the photoresist. Access holes were prepared with a diamond drill of 0.031-inch diameter.

A3. Deposition of platinum films

The standard lift-off method was used to prepare the platinum films in the bottom slides.[2, 3] The bottom glass slides were spin-coated with a 10-μm layer of SPR220-7 photoresist again and dried by baking at 75°C for 3 min and 115°C for 5 min to promote photoresist adhesion. After cooling to room temperature, the glass slides with the photoresist coating were aligned with a photomask in which only the circle wells are transparent. The photomask and glass slides were exposed to ultraviolet light for 50 s. The photomask was then removed and the pattern was developed by immersing the glass slides in SPR developer solution (MF-CD26) for 3 min. In this step, only the photoresist in the exposed circles was dissolved in the solution. The slides were thoroughly rinsed with Millipore water and dried with nitrogen gas. The bottom slides were sputter-coated with 2 nm chromium and then 10 nm platinum/ palladium (80:20) in the circle wells using a Cressington Sputter Coater 208 HR (Ted Pella, Inc.). Afterwards, the slides were thoroughly washed with acetone, Millipore water, and then dried with nitrogen gas.

A4. Surface hydrophobic modification

For surface modification, the glass slides were acid-cleaned in piranha solution (H_2SO_4:H_2O_2 = 7:3) for 1 h and rinsed with Millipore water. After being subjected to an oxygen plasma treatment, the glass slides were given a hydrophobic coating by silanizing with tridecafluoro-1,1,2,2-tetrahydrooctyl-1-trichlorosilane.[1] The glass plates were baked at 120°C for 30 min, rinsed with isopropanol and Millipore water, and then dried with nitrogen gas.

A5. Assembly and operation of MV-chip

Five microliters of Fluorinert liquid FC-70 were added to the top device plate with the patterns facing up and pressed with a blank glass slide to spread the oil. After the oil was completely spread, the blank glass slide was discarded and the device slide was spun at 2000 rpm for 20 s to remove the excess FC-70 and form an ultrathin layer of FC-70.[1] The top device plate was then assembled with the bottom plate; the ultrathin FC-70 layer bonded the two glass slides. The oil layer also served as a lubricant preventing air pockets during operation. The wells on the top plate were positioned to partially overlap with the wells on the bottom plate, forming a continuous "N" shaped fluidic path in the horizontal direction. To load a solution into the MV-chip, a 20-μL pipette tip containing the sample or reagent was inserted into the left inlet of the fluidic path in the assembled device. The solution was loaded into the wells by pushing the pipette manually. To obtain readout, the top plate was slid obliquely to change the flow direction from horizontal to vertical. Operation of the MV-Chip is simple: To start the reaction, an oblique slide of the chip causes the horizontal fluidic paths to separate and re-form into independent units arranged in parallel in the vertical direction; to stop the movement of the ink bar for a stable reading, the glass plates can be slid back to the original loading position.

B. Conjugation and capture DNA immobilization

B1.Procedures for the preparation of probe DNA-catalase complexes

Conjugation of probe DNA (EV3, BA3, or TB3) with catalase was performed according to previously reported methods. [4]Briefly, 30 μL sodium borate buffer (0.1 M, pH 9.2) was added to 60 μL of 1 mM probe DNA in ddH_2O water. Then, 1 mL of 20mg/mL PDITC in DMF was added and the solution was mixed and kept shaking for 2 hours at room temperature in the dark. Next, 6 mL of ddH_2O and 6 mL 1-butanol were mixed with the solution and the resulting solution was centrifuging for 20 min to remove the upper organic phase. The PDITC-activated DNA solution was obtained by extracting the resulting aqueous phase with 4 mL of 1-butanol three times and then purified using Amicon-10K with PBS buffer eight times. To conjugate catalase with probe DNA, 10 mg of catalase were added to the PDITC-activated DNA solution in PBS buffer to give a final concentration of 5 mg/mL; the solution was incubated with

shaking in the dark at room temperature up to 48 hours. Finally, the solution was purified using Amicon-100K with PBS buffer eight times to remove un-reacted PDITC-activated DNA. The characterization of the probe DNA-catalase conjugates is shown in figure S11.

B2. Fifteen-nucleotide capture DNA immobilization

The wells were cleaned by applying drops of piranha solution for 1 hour, then rinsed with Millipore water and dried with nitrogen gas. Next, 10% 3-GPS in toluene was added to each well using a pipette. The solution was kept in each well for 1 hour, and then rinsed with fresh toluene to remove extra 3-GPS molecules. The glass slides were then blown by dry nitrogen gas and baked at 120°C for 30 min. Epoxy groups were covalently modified onto the surface in each well.[5]

Fifteen-nucleotide capture DNA (EV2, BA2, or TB2) was covalently immobilized in the assay wells by reaction with the epoxy group on the glass surface. The amino-modified capture DNA was carefully added to each well and kept at 4°C overnight.[1] The wells were then washed with 5% bovine serum albumin (BSA) several times to avoid nonspecific binding.

C. MV-Chip assay

C1. Measurements of uniform and gradient catalase concentrations

For uniform catalase measurements, 20μL of 10μg/mL catalase was loaded into the right bottom hole and allowed to flow through the assay lane. For gradient catalase measurements, the assay lane was first loaded with PBS buffer, and then 2 μL of 30μg/mL catalase was loaded into the right bottom hole to generate the catalase gradient. We used food dye to represent catalase in the test performance and showed that the assay lane exhibited an excellent gradient.

C2.DNA detection

To detect 30-nucleotide DNA targets, 1μL of different concentrations of EV1 DNA (0, 20 pM, 200 pM, 2 nM, 20 nM, and 200 nM in PBS buffer, pH 7.4) solution were loaded in the assay wells and incubated at 4^{o}C in a sealed chamber for 1 h. The slide was then washed with washing solution (2% BSA and 0.05% Tween-20 in PBS buffer) three times. Afterwards, the probe DNA-catalase was added and incubated for another 1 h, and then washed four times with washing solution and once with PBS buffer.

The readout was obtained by sliding the MV-Chip to allow the catalase probe to make contact with hydrogen peroxide. For detection of DNA in serum, DNA targets was dissolved in 10 %, 30% and 50 % fetal bovine serum (FBS) in PBS buffer and add into each wells as the above procedures.

C3. Ink and H_2O_2 loading

Twenty microliters of red ink and 0.45 M H_2O_2 were loaded into their respective fluidic paths from the left inlet. The solution filled the fluidic path because of the hydrophilic interaction between the solution and the glass surface in the fluidic path.

D. Readout

After the completion of the DNA hybridization reaction and reagent loading, the MV-chip was carefully slid by moving the top slide at an oblique angle. The wells on the top and bottom slides then formed a "Z"-shaped fluidic path in the vertical direction. The red ink was propelled into the small channels and the inked bands moved into the channels due to the oxygen pressure generated (Video S1, Figure 1 and S3). The results were recorded with a Canon camera and the frontiers of ink advancement were marked for quantitation.

E. Characterization

Atomic force microscopy (AFM)

The platinum film was characterized using atomic force microscopy (AFM). AFM measurements were performed with a Veeco MultiMode Nanoscope IIIA (Bruker AXS, Madison, WI). The glass slides were cut into small pieces and washed with acetone and isopropanol, and then dried with nitrogen gas. Tapping mode was used to acquire the images under ambient conditions. Image analysis was carried out using Veeco NanoScope Analysis software version 1.20.

REFERENCES

1. Song, Y. J.; Zhang, Y. Q.; Bernard, P. E.; Reuben, J. M.; Ueno, N. T.; Arlinghaus, R. B.; Zu, Y. L.; Qin, L. D., *Nat. Commun.* 2012, 3, 1283.
2. Liao, X.; Brown, K. A.; Schmucker, A. L.; Liu, G.; He, S.; Shim, W.; Mirkin, C. A., *Nat. Commun.* 2013, 4, 2103.
3. Lam, B.; Das, J.; Holmes, R. D.; Live, L.; Sage, A.; Sargent, E. H.; Kelley, S. O., *Nat. Commun.* 2013, 4, 2001.
4. Xiang, Y.; Lu, Y., *Nat. Chem.* 2011, 3, 697.
5. Nam, Y.; Branch, D. W.; Wheeler, B. C., *Biosens. Bioelectron.*2006, 22, 589.

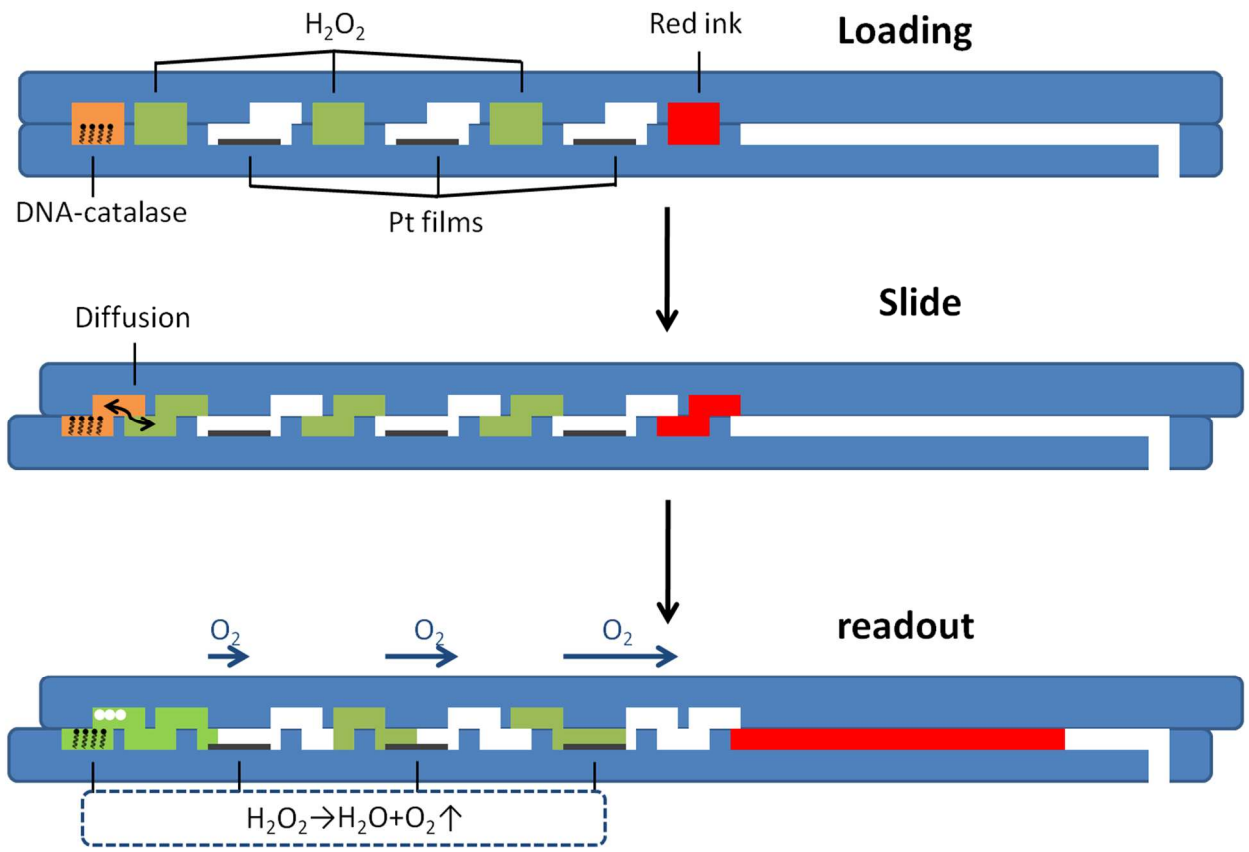

Figure S1. Schematic side view of the MV-Chip in the state of reagent/sample loading, oblique slide and readout.

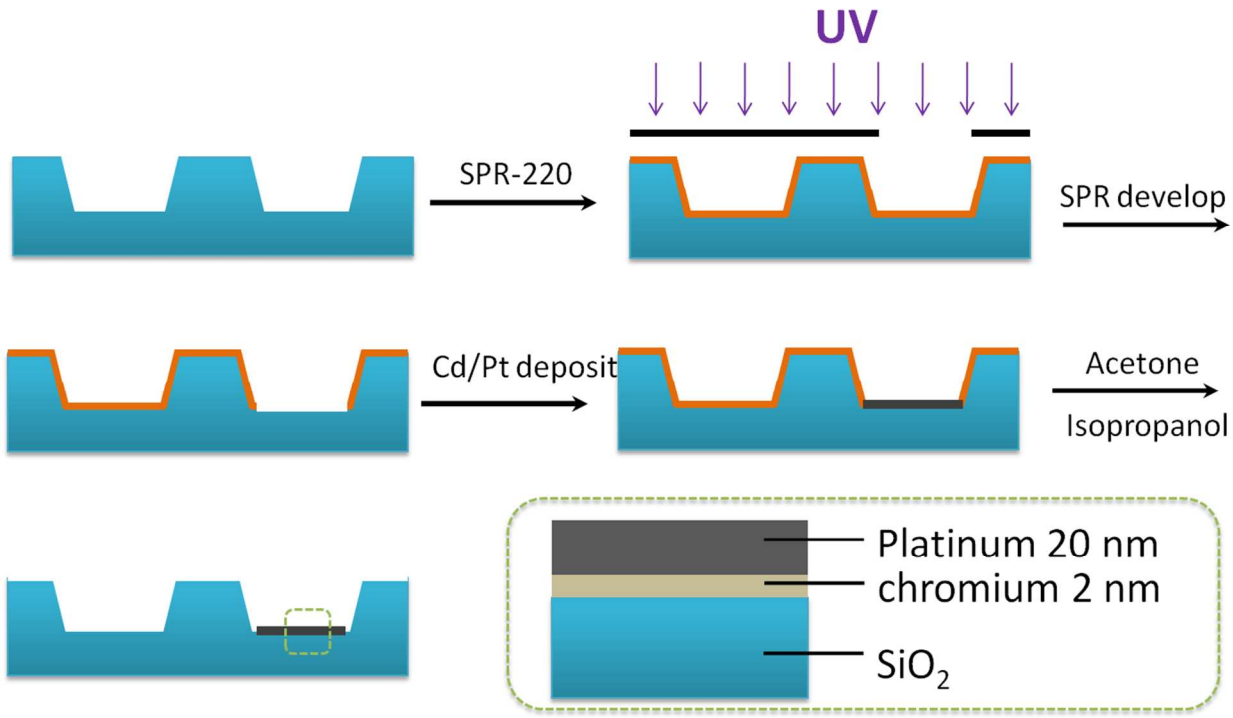

Figure S2. Schematic representation of the lift-off procedures for the deposition of platinum films in specific wells. The films and its thickness are showing in the green box.

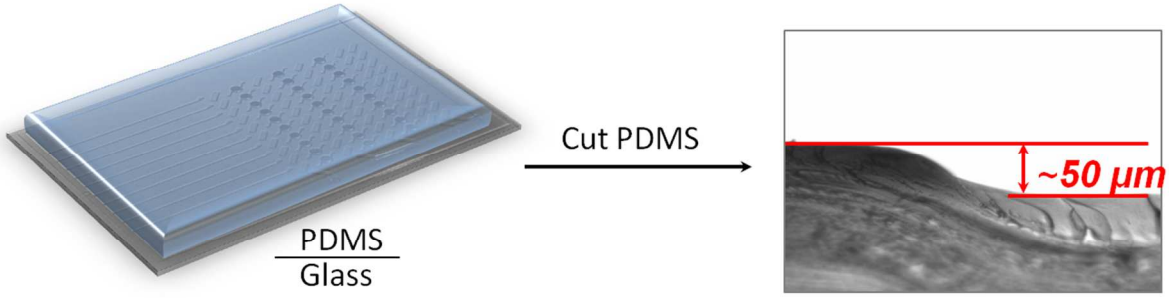

Figure S3. Channel height measurement. Polydimethylsiloxane (PDMS) elastomer was used to replicate the channels on the glass slide for the height measurement. The PDMS elastomer was cured and removed from the glass, cut into small pieces, and measured using a microscope. The microscope image on the right shows the channel height; 50 µm was used for all the MV-Chip devices in this paper.

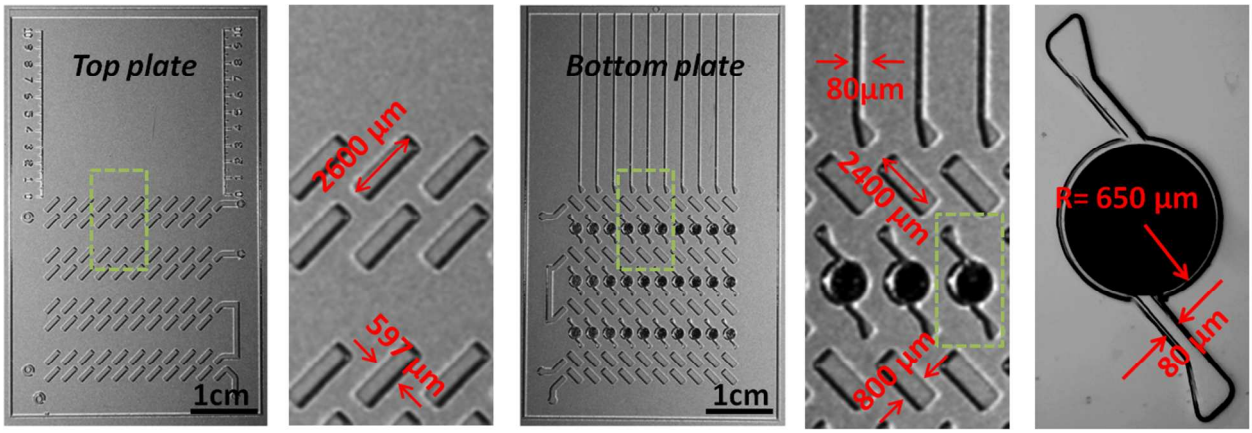

Figure S4. Top and bottom plates of a 10-plexed MV-Chip with dimensions marked in the images.

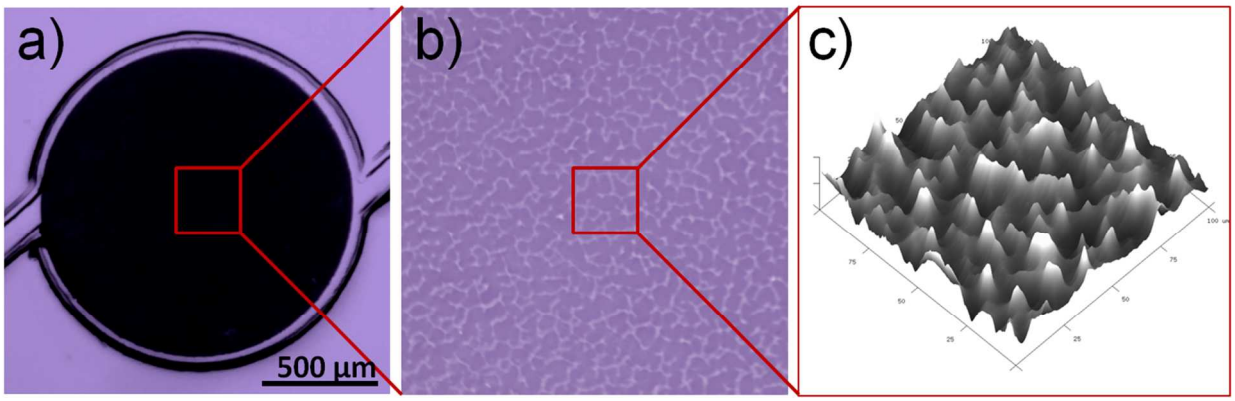

Figure S5. Characterization of the platinum film surface. (a) Microscope image shows that the platinum had completely covered the entire well without contaminating the boundary. (b) Microscope image of the rough platinum surface. (b) AFM image of the rough platinum surface shows nanosized protrusions. The roughness is at the scale of about 400 nm.

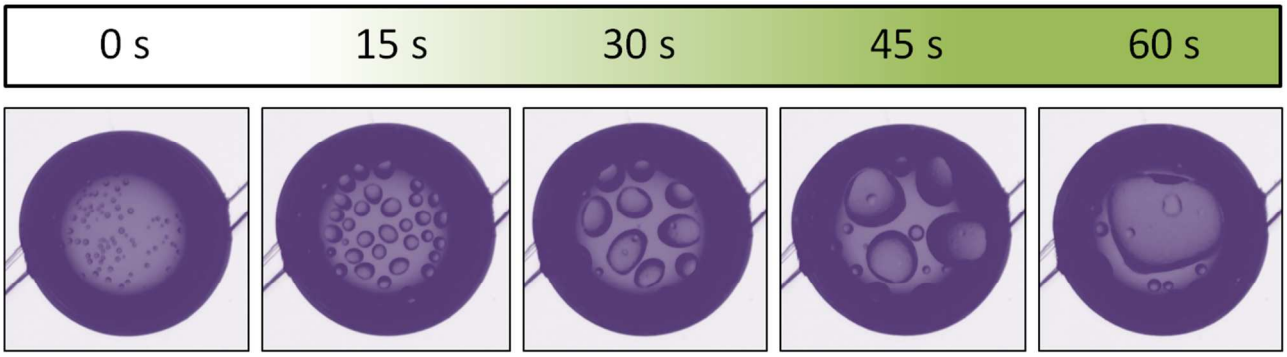

Figure S6. The platinum films in the circle wells will generate oxygen bubbles in the presence of H_2O_2 in less than 1 minute.

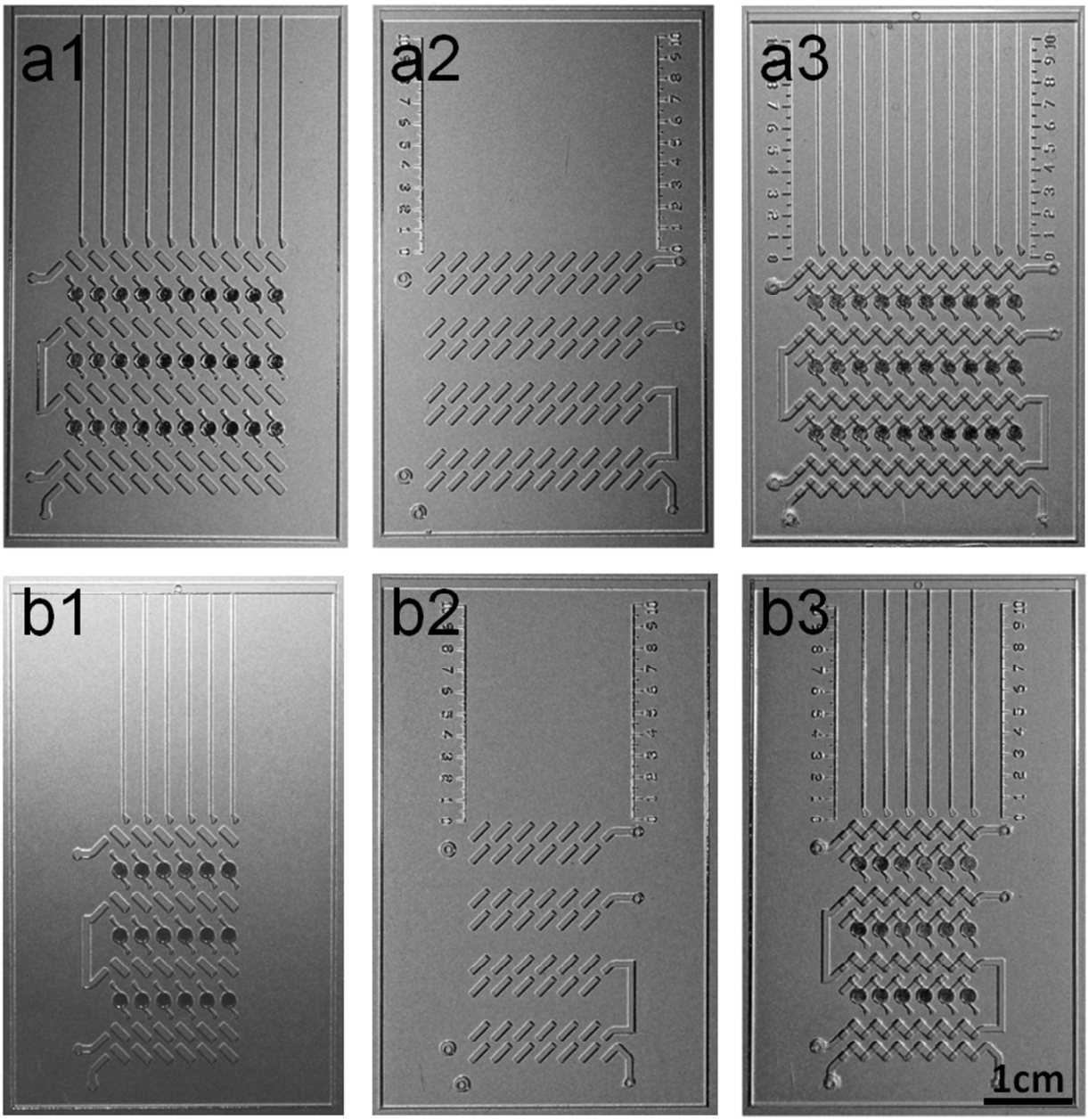

Figure S7. Photographs of 10- and 6-plexed MV-chip devices. Pictures from left to right are the top plates, the bottom plates, and the assembled MV-chips, respectively. The scale bar is 1 cm for all pictures.

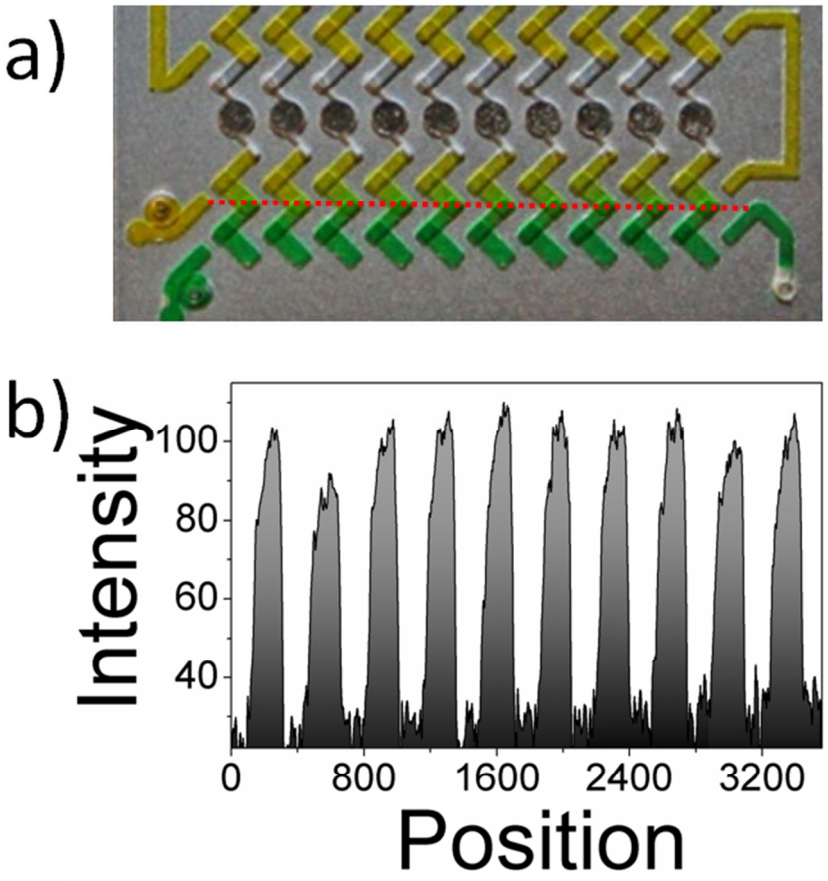

Figure S8. (a) An oblique slide forms ten isolated 'Z'-shaped paths in the vertical direction and uniform gradual changes could be observed at the bottom of each detection unit because of the food dye diffusion. (b) The intensity of the green color at the position of red line in (a).

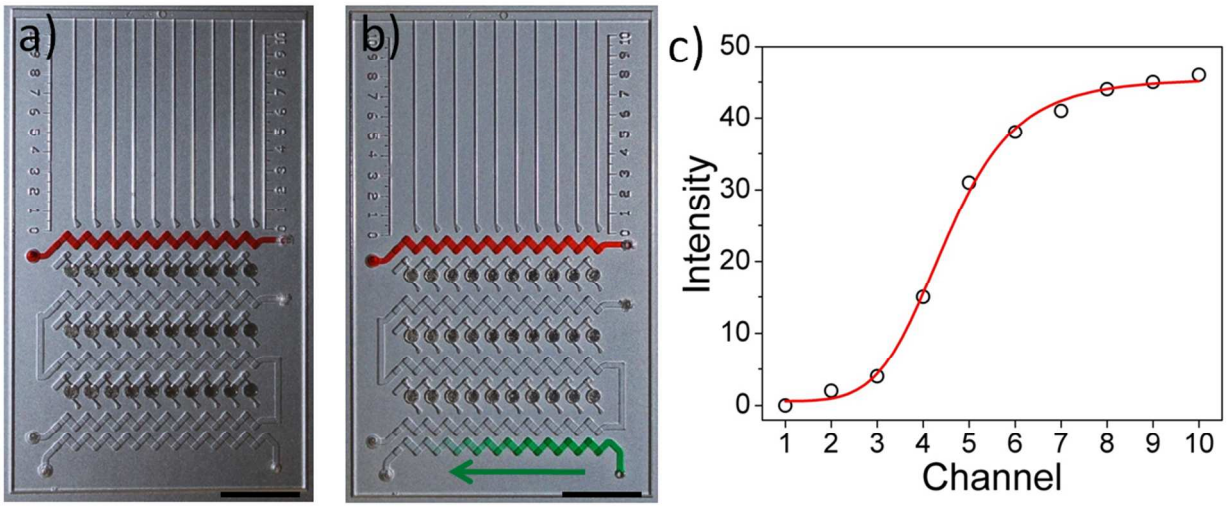

Figure S9. a) MV-Chip loading with reagents and samples. b) A concentration gradient was generated by loading 2 μL green dye from the right bottom hole. c) The intensity in arbitrary units of the diffused green food dye at the center of each bottom wells. Scale bar is 1 cm for a) and b).The data was obtained by measuring the center of the bottom wells, which exhibits a sigmoidal curve.

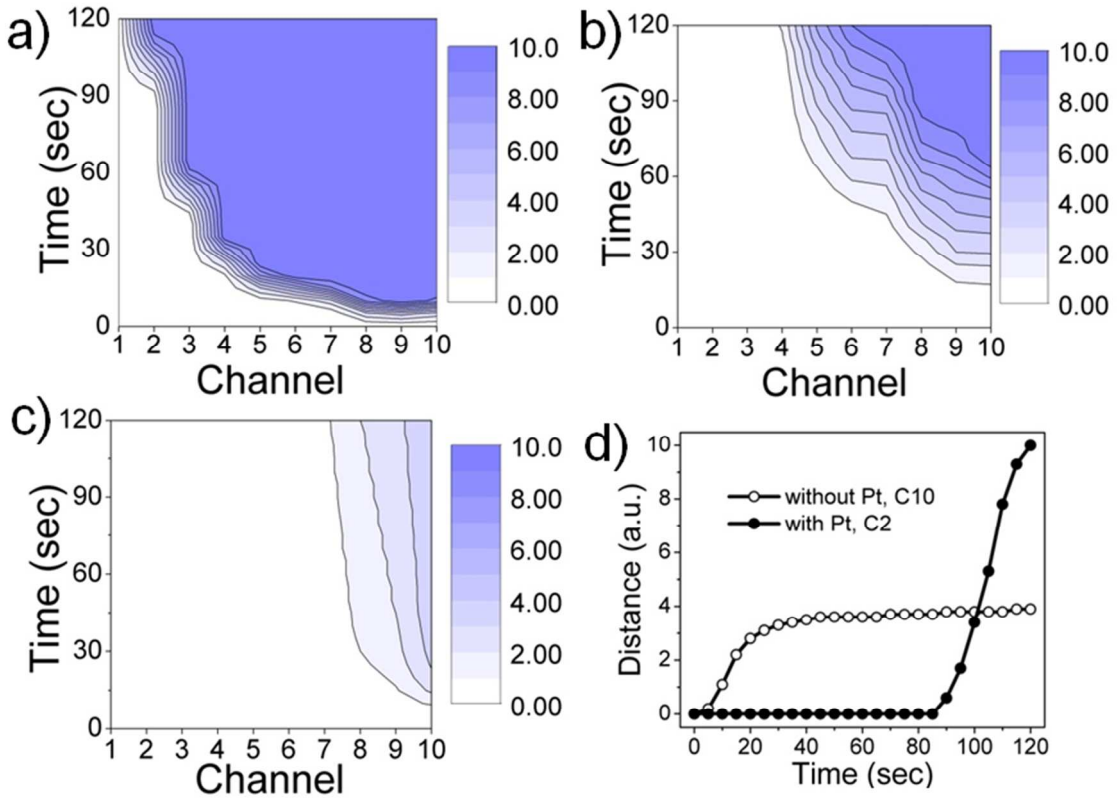

Figure S10. (a,b,c) Heat map images of the distance changes of each channel with 2 min diffusion of 2 µl catalase from the right loading holes. (a) 0.45 M H_2O_2 with three stage amplification. (b) 0.25 M H_2O_2 with three stage amplification. (c) 0.45 M H_2O_2 without amplification. (d) Changes in bar chart advancement of the second channel (closed circles, with Pt, C2) in the MV-Chip and with the tenth channel (open circles, without Pt, C10) in the V-Chip in 2 min.

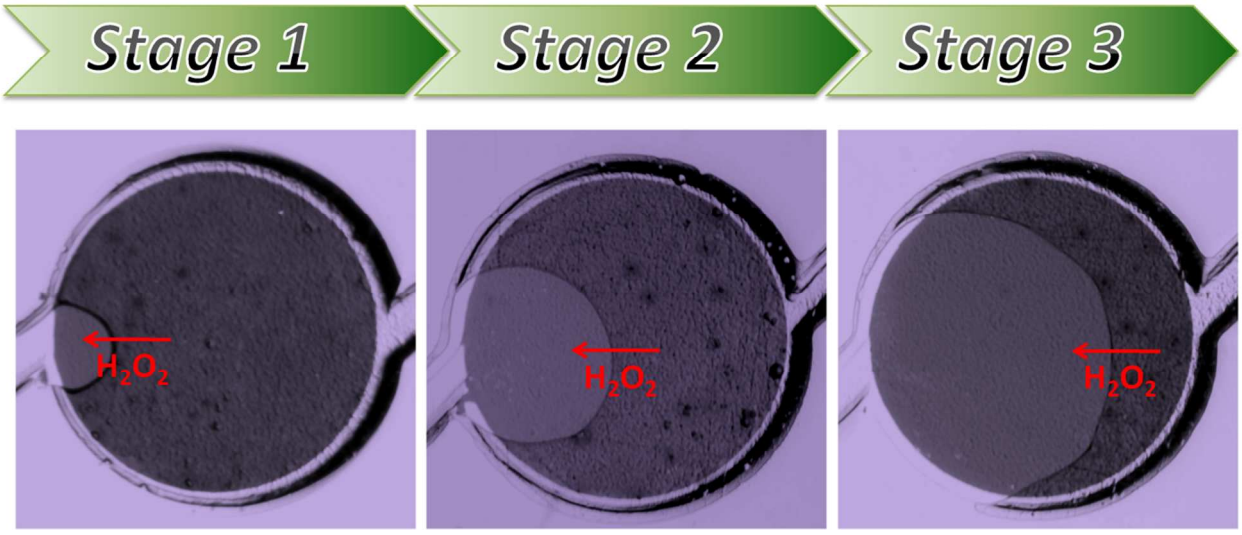

Figure S11. Demonstration of hydrogen peroxide entering the platinum wells. The amplification effect could be observed as more hydrogen peroxide has been generated in stage 2 and 3 than in stage 1 and 2.

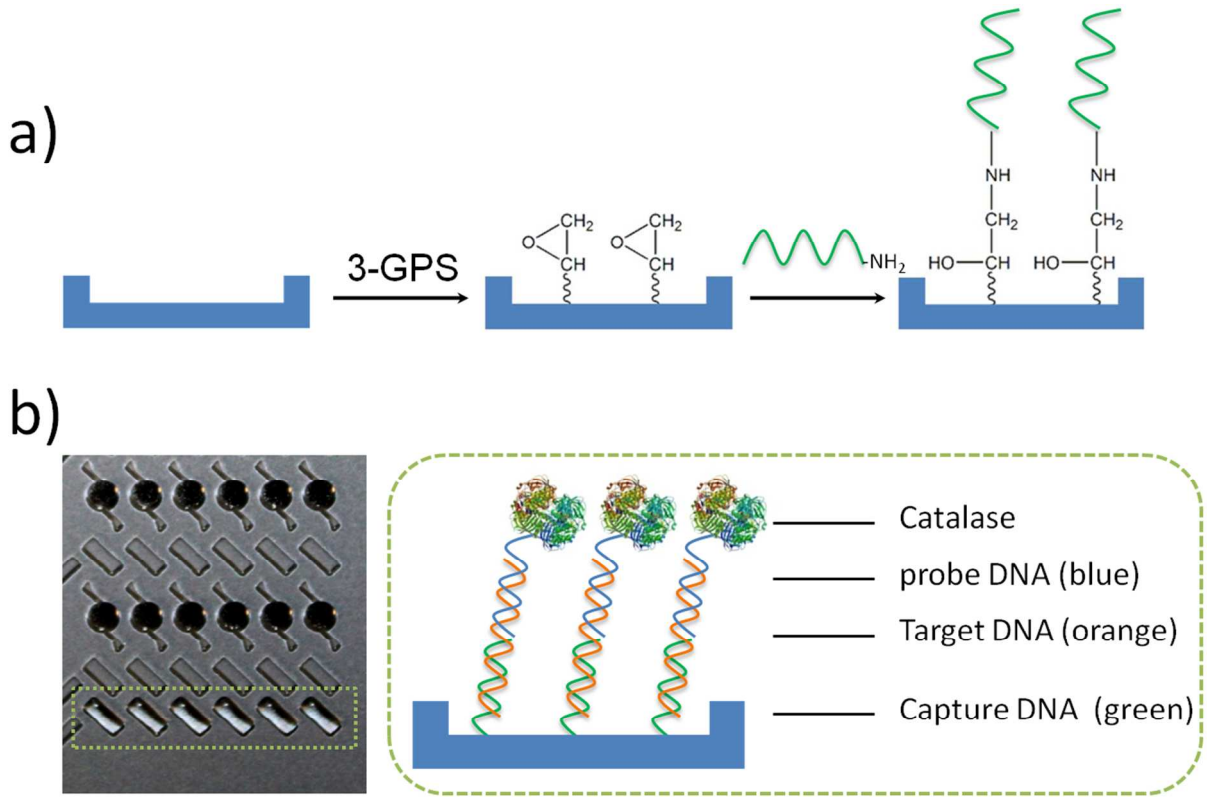

Figure S12. Capture DNA loading and catalase probe binding. (a) The assay wells are modified with epoxy groups and then reacted with capture DNA. The capture DNA is covalently immobilized on the glass surface by the reaction between amino groups and the epoxy groups. (b) The capture DNA solution (1 μL) was loaded into each sample well. Because the assay wells were treated to be hydrophilic and the boundaries are hydrophobic, the solution stayed inside and covered the entire well. The final DNA hybridization structures in each well are shown in the green box.

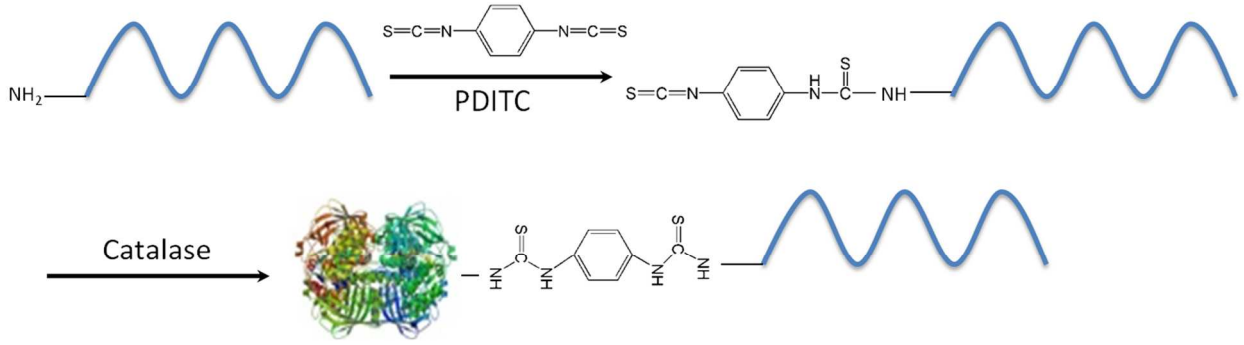

Figure S13. Procedures for conjugating amino probe DNA with catalase.

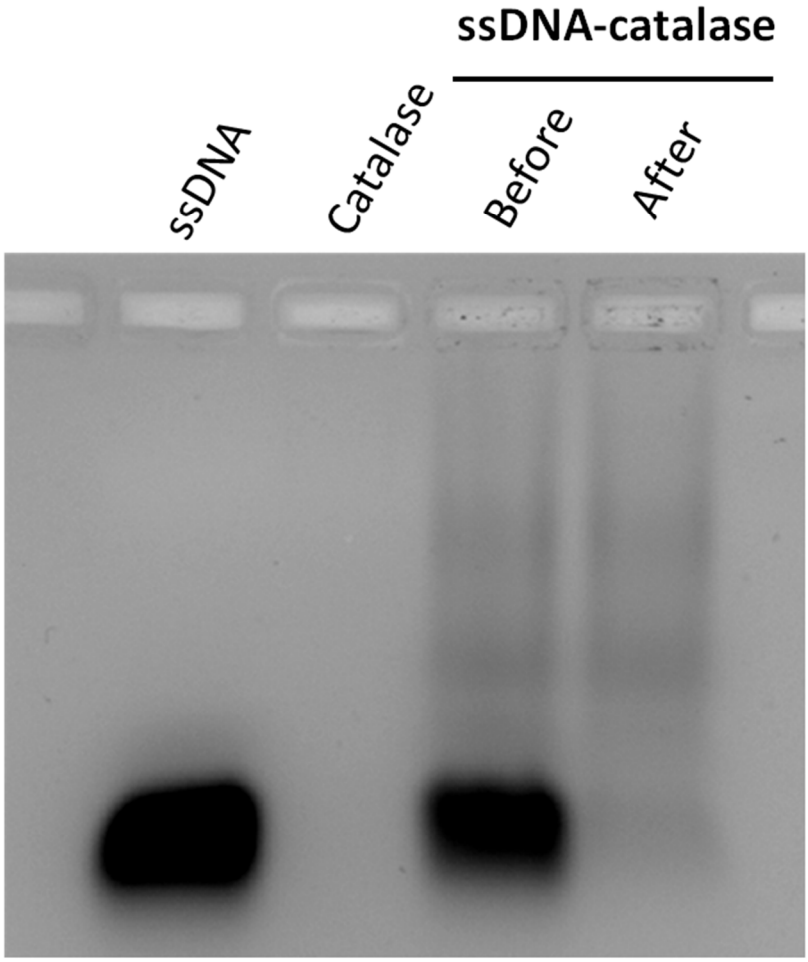

Figure S14. Agarose gel (1.0 %) image for EV_3, catalase, and EV_3-catalase conjugates before and after purification.