

## Electronic Supplementary information:

### Printing Enzymatic Reactions

Junfei Tian and Wei Shen\*

*Department of Chemical Engineering, Monash University, Wellington Rd, Clayton, Vic. 3800, Australia. Tel: +61 3 99053447, Email: wei.shen@monash.edu*

#### Materials

Nitrocellulose film (Hybond-C Extra, GE Healthcare) was used to make the enzyme carrying relief and planographic printing plates. Relief printing plate was made by cutting circular nitrocellulose discs of 5 mm diameter and then sticking them onto an overhead transparency film.

Horse raddish peroxidase (dry powder), PBS concentrated solution (x10) and TMB liquid substrate were obtained from Sigma–Aldrich. PBS buffer solution was diluted with MilliQ water to the concentration of x1 for laboratory use. HRP stock solution was made by dissolving the dry HPR powder with the laboratory PBS solution (pH 7.4) to a concentration of 1.0 mg/mL.

#### Printing with the relief plate

The stock solution of HRP was further diluted to 1 µg/ml. Six micro litres of the diluted HRP solution was introduced drop by drop onto each nitrocellulose disc using a micro pipette to prepare the circular disc plates (Fig. S1). HRP solution spread gradually on the nitrocellulose discs and eventually absorbed by the discs. The discs were then incubated in the PBS solution for 2 minutes. After incubation the discs were rinsed with fresh PBS to remove non-immobilized enzyme; the discs were then allowed to dry under ambient condition.

Blotter paper (Dalton Paper, Melbourne, Australia, 255 gsm, 203 mm x 203 mm) was cut into a rectangular shape (4 cm x 1 cm) to make the paper sheet for printing. This blotter paper is made of cellulose and does not contain lignin; it is a standard grade used in papermaking laboratories to absorb water from wet paper sheets. The blotter paper was briefly dipped into the TMB liquid substrate solution and quickly retrieved out of the solution. It was transferred into a Petri dish and maintained moist.

Printing was made by placing the plate on top of the TMB treated paper for 30 seconds; a fixed weight placed on the back of the plate to provide the impression force. After one printing cycle the plate was rinsed with the PBS solution to remove any back transferred product of the reaction from the plate surface. The subsequent prints were then made following the same procedure. Supplementary Fig. 1 shows a fresh relief plate before printing. Fig. S2 shows the same plate after making 3 prints and after PBS rinsing to remove the blue product of TMB.



**Fig. S1** A relief plate before any print was made.



**Fig. S2** The same plate after making 3 prints and was rinsed using PBS solution after each print.

Since the catalytic activity of HRP reduces after each print, the colour density of each subsequent print decreases. This is expected; a plot of the print colour density vs the number of prints made shows that the decrease in colour density appears to be linearly correlated with the number of prints made.

### **Printing with planographic plate**

Ink jet printing of HRP solution was employed to fabricate the plate. A Cannon inkjet printer (PIXMA IP4500) and its ink cartridges were reconstructed to print the HRP ink solution (1 mg/ml) on a nitrocellulose film cut into a rectangular shape (5 cm x 4 cm) to form the printing plate. An electronic pattern of a cat's head was used as the printing pattern. The ink jet printed planographic plate was rinsed with PBS solution and was allowed to dry under ambient condition. Printing with planographic plate was carried out following the same procedure as with the relief plate. After each printing cycle, the plate was rinsed with PBS solution before subsequent printing was made.

### **Print density measurement**

Print density measurement was made for prints made using relief printing. The printed papers were kept into a dark box for 2 minutes. A PC interfaced digital scanner (EPSON PERFECTION 2450 PHOTO) was used to take a scan of the printed image. Finally, the color intensity (cyan) of the printed patterns on scanned images was measured by using Adobe<sup>®</sup> Photoshop CS2 software. The background colour density from the unprinted paper surface was measured and subtracted from the colour density data of the prints.