Electronic Supplementary Information

Nanoparticle Embedded Enzymes for Improved Lateral Flow Sensors

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Experimental

Synthesis of nanoparticles embedded HRP

Horse radish peroxidase embedded polyacrylamide nanoparticles coated with amino groups were synthesized by microemulsion polymerization according to Daubresse *et. al.*[1] as reported previously.[2, 3] Briefly, 38.8%T solution of acrylamide monomer solution (1.35 g acrylamide, 0.4 g N,N´-methylenebisacrylamide, 0.35 g N-(3-aminopropyl) methacryl amide) dissolved in 4.5 ml of 10 mM sodium phosphate buffer, pH 7.2), 200 µl of 250 µM HRP and 20 µl of 50 mg/ml Texas Red Dextran was mixed together. 2.0 ml of this monomer solution was added dropwise into a hexane solution (3.08 g of dioctyl sulfosuccinate (AOT) and 1.08 g of Brij 30 disolved in 43 ml of hexane and degassed with sonication). Polymerization was started by adding 50 µl of 10 % ammonium persulfate and 10 µl TEMED. After 3 hours of reaction, hexane was removed under vacuum and nanoparticles were precipitated by adding 100 ml of ethanol. The suspension was filtered through a 100 kDa filter (Millipore corp., Bedford, USA) and washed with ethanol. The nanoparticles were dried under vacuum to obtain about 450 mg of dry particles. Vacuum dried particles were stored in -20 °C until use. Resoloubilized particles were used in the experiments within one hour. The control nanoparticles were prepared with the same procedure, but without adding HRP.

The amino functionalized nanoparticles were conjugated to avidin molecules through glutaraldehyde linker (GA).[4] The nanoparticles were transferred into 1 % GA solution. The reaction were carried out at 4 °C for 12 hours with continuous stirring. Then, the activated nanoparticles were washed by centrifugation. The particles were solubilized in PBS buffer with 10 µl of 100 µM avidin.

The size of nanoparticles were determined by Dynamic Light Scattering (DLS) as 46 nm. About 1 mg nanoparticles were dissolved in 1 ml of PBS buffer (0.01 M phosphate buffered saline-0.138 M NaCl, pH=7.4) and analysed by a Zetasizer Nano-S (Malvern instruments, Worcestershire, UK).

Leaching experiments on particles were performed by following a procedure reported by Nielsen *et. al.*[2] A 1.0 ml of a 50 mg/ml solution of sensor particles was placed in the dialysis casettes of MWC_20000 (Thermo Fisher Scientific Inc., Rockford, USA) according to manufacturer's procedures and left in 200 mL of PBS buffer. The fluorescence of the surrounding buffer was monitored for 15 min. No significant leaching of Texas red was detected in 15 minutes after solubilisation of sensor particles in buffer.

Immobilization methods

In order to compare the effect of immobilization on catalytic activity, we performed DAB-Peroxidase assay for three different immobilization strategies. For direct adsorption, HRP solution was directly adsorbed on nitrocellulose membrane surface. Similarly, nanoparticle embedded HRP sensor was applied on the nitrocellulose membrane for protein adsorption. For the last method, avidin was absorbed on nitrocellulose membrane. After treating the avidin immobilized membranes with blocking solution (1% BSA), biotinylated HRP was applied for biotin-avidin conjugation. For all immobilization, equal amount of catalytically active HRP (0.12 units) was prepared in equal volume (5 µl). The specific activity of the enzyme was described as formation of 1 mg purpurogallin from pyrogallol in 20 sec at pH=6.0 at 20 °C according to manufacturer (Sigma-Aldrich inc).

Lateral flow assays

5 µl of nanoparticle solution (either sensor particles or control particles) was manually deposited on the specific zones of nitrocellulose membrane (HF240MC100, Millipore) of lateral flow strips. Sample pad (CFSPOO 1700, Millipore) and absorbent pad (GFCOOO800, Millipore) were assembled on an overlapping sequence on the adhesive tape at both ends of nitrocellulose membrane. During assays, 200 µl of sample was directly applied on sample pad. The samples were prepared in either PBS buffer or Dulbecco´s Modified Eagle (DMEM) cell culture medium containing 10 % Feotal Calf Serum (FCS).

DAB-Peroxidase activity assay

Diamino benzidine peroxidase assays were performed with a metal enhanced DAB substrate (Piercenet) according to manufacturer´s recommendations. This sensitive peroxidase buffer was reported to give an intense dark-brown precipitate in the presence of HRP as low as 0.16 femto mole. After lateral flow strips with immobilized HRP or nanoparticle embedded

HRP were prepared, 250 µl of the working solution of DAB/Metal concentrate (containing hydrogen peroxide) was applied directly on the sample pad region and incubated for 5 min. at room temperature.

Spectrofluorometry and fluorescence scanning

Fluorescence of sensor particles were measured in a Nanodrop 3300 (Thermo Scientific, Willmington, USA). Texas red was excited at 535 nm (Blue excitor) and emission was recorded at 600 nm. The LFA strips were scanned on a slide with a GenePix 4100A (Molecular Devices, California, USA). The intensities of fluorescent or color bands on the strips were determined by ImageJ 1.46r.[5]

References

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