Supplementary Material for Chemical Communications

Supplementary Information

Building three-dimensional nanostructures with active enzymes by surface templated layer-by-layer assembly

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Experimental Section

Materials Biotin-disulphide N-hydroxysuccinimide ester (biotin-disulfide), avidin, sodium chloride, di-sodium hydrogen phosphate were purchased from Sigma-Aldrich Company (Dorset, UK) and used as received. Peroxidase from horseradish, biotin-xx conjugate (Biotin-HRP) and Amplex red dye were obtained from Invitrogen Ltd (Paisley, UK). Hydrogen peroxide (6%, 20 Volume) was purchased from Breckland Scientific Supplies (Thetford, UK). 11-mercaptoundecylhexa (ethylene glycol) alcohol ($(EG)_6OH$) was synthesized and purified as described previously.[1] Phosphate buffer (PBS, 10 mM phosphate, 150 mM NaCl, 2 mM NaN_3, pH 7.4) was prepared with ultra-pure MilliQ water (resistance > 18 MΩ.cm). The buffer solution was filtered through a Whatman syringe filter (0.2 μm pore size, Whatman Plc., USA). Avidin (0.1 mg/ml) and Biotin-HRP (0.2 mg/ml) were prepared with the PBS. 1 mM solution of biotin-disulphide was prepared in ethanol and filtered through Whatman syringe filter. Hydrogen peroxide solution (5 mM) was prepared by diluting 13.75 μl of 6 % stock solution to 6 ml with the PBS.

Layer-by-layer Assembly of Avidin/Biotin-HRP

A gold substrate was first coated with a self-assembled monolayer (SAM) of the biotin-disulfide by incubation it with a biotin-disulfide solution (1 mM in ethanol) for 1.5 hours. The substrate was then rinsed with ethanol and methanol to remove unbound biotin-disulfide, and finally rinsed with MilliQ water. After this, the substrate was incubated in 0.1 mg/ml avidin solution for 0.5 hour, rinsed with PBS, and then soaked in 0.2 mg/ml biotin-HRP solution for 0.5 hour, and then washed with PBS. This constitutes the 1st protein bilayer and abbreviated as $(avidin/biotin\text{-}HRP)_1$. To assemble more bilayers, the gold substrate was sequentially incubated in avidin (0.1 mg/ml) and biotin-HRP (0.2 mg/ml) solutions as described above. Repeating the assembly process led to the buildup of the multilayer protein films.

Ellipsometry Measurements

The thickness of the proteins films on evaporated gold surfaces (~200 nm, with ~ 10 nm Cr adhesion layer) were measured on an ELX-02C ellipsometer (DRE, Germany) at an incident angle of 70º using a He-Ne laser at 632.8 nm. A three phase model (gold/organic/air) was employed by using a fixed refractive index of 1.50 for the organic layer to evaluate the thickness.[2] The imaginary part of the refractive index was assigned to be zero because the protein films are transparent at 632.8 nm. After a specific number of protein bilayer was assembled on a particular substrate, it was washed with PBS, MilliQ water and then dried under a stream of nitrogen because measurement. At least five measurements were taken from different spots for each sample, and averaged values were used. The film thickness at each number of layers was obtained from independent samples directly assembled without drying in between each assembly step to minimize the possibility of protein denaturation.

Micro-contact Printing

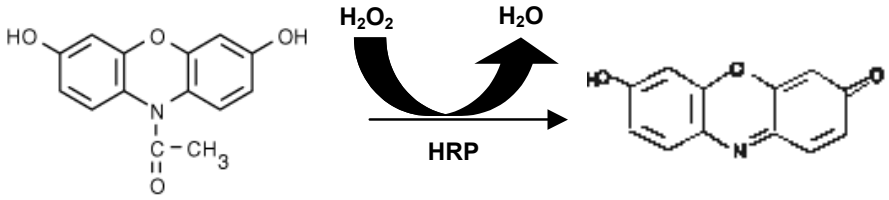
A PDMS stamp having 2 μm stripes separated by 2 μm gaps was used for the printing following a literature procedure.[1] The stamp was inked by using a cotton swab with 2 mM solution of the $(EG)_6OH$ in ethanol. Then the stamp was blown dry with N_2 and brought into conformal contact with flat semi-transparent thin-gold coated glass coverslips (~5 nm thick, with a titanium adhesion layer obtained from Ssens BV, Hengelo, the Netherlands) and allowed to contact for 20-30 seconds. After printing, the gold substrate was incubated with the biotin-disulfide solution for 1 hour to fill the unstamped bare gold region with a biotin terminated SAM. The substrate was thoroughly rinsed with ethanol, methanol and MilliQ respectively, to remove the unbound biotin-disulfide and then used to template the layer-by-layer assembly of avidin/biotin-HRP.

Atomic Force Microscopy

All AFM experiments were carried out on a Digital Instrument (Veeco, Santa Babara, CA) Dimension 3100 AFM with a Nanoscope IV controller.[3] All the AFM experiments were carried out at 24 ± 1 ºC. Ultra-sharp MikroMasch silicon cantilevers (NSC15 series, 125 μm long, tip radius < 10 nm, spring constant ~ 40 N/m, resonant frequency ~300 KHz) were used. Images were collected at a scan rate of 0.5-1 Hz with 512 × 512 pixels per image, and analyzed with the Nanoscope image analyzing software (version 5.12) using first order flattening. Topographic images were taken under light tapping to minimize the effect of tip compression towards the bio-assembly features.

Determination of Horseradish Peroxidase Activity

HRP is a 44,000 Dalton protein which catalyzes the reduction of hydrogen peroxide (H_2O_2) to water (H_2O).[4] In the presence of specific substrates acting as hydrogen donors, HRP can convert colorless substrates into colored products. Amplex red is used as the substrate here. In the presence of HRP, Amplex red reacts with H_2O_2 in a 1:1 stoichiometry to produce highly coloured product, resorufin, (**Scheme S1**), which has an absorption maximum of 571 nm.



Amplex Red **Resorufin**

Scheme S1. Schematic showing the conversion of Amplex red into Resorufin during the horse radish peroxidase (HRP) catalyzed reduction of hydrogen peroxide into water.

Evaporated gold surfaces (~ 200 nm) containing different numbers of protein bilayers were used to measure the immobilized biotin-HRP activity. The reaction was carried out in PBS with 5 mM H_2O_2 and 25 μM Amplex red in a quartz cuvette (1 cm path length) with total volume of 3 mL under magnetic stirring. The enzyme activity was investigated by monitoring the peak absorption of resorufin at 571 nm (Amplex red is transparent at this wavelength) at different time intervals on a Cary 300 Bio UV-Vis spectrophotometer at 24±1 ºC. The enzyme activity was estimated by using the absorbance at first 5 minute, where the absorbance-time course was roughly linear for the immobilized enzymes.

The amount of the enzymes immobilized in the first bilayer on a given surface is estimated from the film thickness (~1.0 nm for the HRP layer) measured by ellipsommetry (assuming a homogenous film of a density of 1 g/cm^3). This gives an enzyme surface coverage of ~100 ng/cm^2. The relative activity of the immobilized enzymes is then estimated by comparing the relative catalytic rate of the immobilized enzyme (1 cm^2 surface, ~100 ng of enzyme) with that of the same amount (100 ng) of free solution enzymes, where the later enzyme activity is taken as 100%. The relative enzyme activity of the immobilized enzymes is found to be ~ 14% that of the same amount of soluble enzymes.

Reference:

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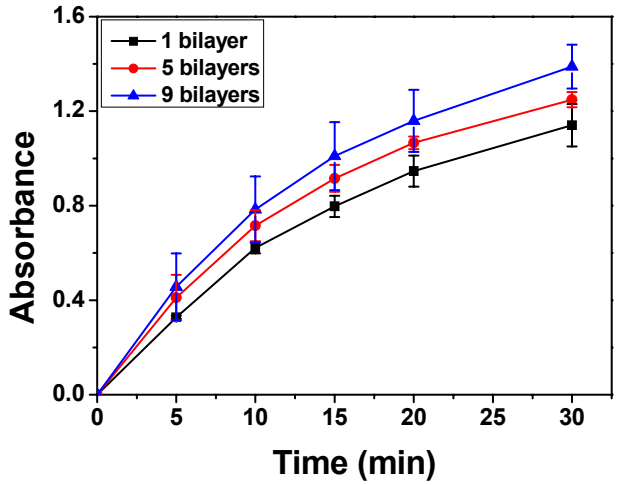

Figure S1. Plot of the absorbance at 571 nm versus the reaction time for different layered avidin/biotin-HRP films immobilized on gold surfaces. The reaction was carried out in PBS (10 mM phosphate, 150 mM NaCl, 2 mM NaN_3, pH 7.4) with 5 mM H_2O_2 and 25 μM Amplex red.