Gold Nanorod-mediated Photothermal Enhancement of Biocatalytic Activity of Polymer Encapsulated Enzyme

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Materials and Methods

Materials: Gold(III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$, ≥99.9%), ascorbic acid (AA, ≥99.0%), silver nitrate (>99%), sodium borohydride ($NaBH_4$, 98%), cetyltrimethylammonium bromide (CTAB, ≥99%), horseradish peroxidase(HRP) type VI-A, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS, 10mg/tablet), 3-mercaptopropyl)triethoxysilane (MPTES), (3-aminopropyl)trimethoxysilane (APTMS) and trimethoxy(propyl)silane (TMPS), sodium dodecyl sulfate (>99%) (SDS) were obtained from Sigma-Aldrich. The phosophate buffer saline (PBS) (10X) buffer was obtained from Thermofisher. Methoxy poly-(ethylene glycol) silane (*m*PEG-silane, Mw = 5000 g/mol) was obtained from JenKem Technology. All chemicals were used as received.

Synthesis of gold nanorods: Gold nanorods were synthesized using a seed-mediated growth approach. Seed solution was prepared by adding 0.6 mL of an ice-cold solution of sodium borohydride (10 mM) into 10 mL of vigorously stirred CTAB (0.1 M) and $HAuCl_4$ (2.5×10^{-4} M) aqueous solution at room temperature. The color of the seed solution changed from yellow to brown. Growth solution was prepared by mixing 1 ml of $HAuCl_4$ (10 mM), 20 ml of CTAB (0.1 M), 0.2 mL of silver nitrate (10 mM), 0.16 mL of ascorbic acid (0.1 M), 0.4 ml of HCl (1N) in the same order. The solution was homogenized by gentle stirring. To the resulting colorless solution, 48 µL of freshly prepared seed solution was added and set aside in dark for 14 h. The AuNRs solution with a LSPR peak position of 890 nm was centrifuged at 10,000 rpm for 10 min to remove excess CTAB and redispersed in nanopure water.

AuNR-HRP conjugation: 80 µl of HRP solution (2.5 mg/mL in phosphate buffer pH 5) was added to 1 ml of AuNR solution of extinction 2.0 and left overnight at 4°C. The conjugate solution was filtered in a 100 kDa centrifugal filter at 3,500 rpm for 10 min to remove the excess HRP.

Substrate Modification: Glass substrates were cleaned using piranha solution (3:1 concentrated sulfuric acid to 30% hydrogen peroxide solution) before use. *Caution: Piranha solution is extremely dangerous and proper care needs to be taken while handling and disposal.* The cleaned glass substrates were coated with MPTES by exposure to 1% (w/v) MPTES solution in ethanol for 1 hour followed by immersion in ethanol for 30 minutes. Subsequently, the substrates were thoroughly rinsed with water and dried under a stream of dry nitrogen. MPTES modified substrates were used to adsorb AuNR-HRP conjugates by drop casting filtered AuNR-HRP conjugates on the glass substrate followed by incubation for 1 hour at 4°C. The modified

substrates were washed with nanopure water and dried under a stream of dry nitrogen for subsequent experiments.

Polymer Encapsulation: Glass substrates with AuNR-HRP conjugates were immersed in 4 mL of PBS (pH 7.4) containing 10 µl of TMPS and 10 µl of APTMS for 9 minutes, followed by rinsing and drying under a stream of dry nitrogen. Subsequently, the substrates were exposed to 1 mg/ml *m*PEG-silane solution for 10 minutes, rinsed with water and dried. HRP was released from the polymer shell by shaking the substrates in 3 ml of 2% aqueous SDS solution. After release, the glass substrates were left at room temperature for 12 hrs. The substrates were then exposed to 500 µg/ml of HRP solution for 1 hour, followed by rinsing with water and drying with nitrogen. Finally, the substrates were immersed in 4 mL of PBS (pH 7.4) containing 0.5 µl of TMPS and 0.5 µl of APTMS for different time periods (1, 3 and 5 minutes) to form a capping layer.

Activity measurements: Enzyme activity was measured using an ABTS assay. The AuNR-HRP adsorbed substrates was exposed to 2.1 ml of potassium phosphate buffer at pH 5.0 and 0.3 ml of ABTS (9.1 mM) followed by the addition of 750 µl of H_2O_2 (0.3%). The absorbance at 420 nm was measured as a function of the time and the rate of the increase in absorbance with time (slope of absorbance vs. time plot) is used to calculate the enzyme activity. The activity was measured over a reaction time of 3 minutes unless otherwise stated. The 'activity %' values are calculated based on the ratio of the slopes of absorbance vs. time plots, before and after exposure to unfavorable conditions. The activity of lysozyme was measured using a standard *Micrococcus lysodeikticus* cell lysate assay. The decrease in absorbance at 450 nm is plotted against time to measure the enzyme activity of lysozyme.

Laser Illumination: The organosilica encapsulated HRP immobilized on AuNRs was illuminated with 808 nm laser at 400 mW/cm^2 with a laser spot size of 1 cm X 1 cm for 240 seconds time followed by the measurement of enzyme activity for 10 seconds, for four cycles (total time ~ 16 minutes). The substrate with encapsulated HRP is introduced in a solution of ABTS and H_2O_2 to measure the enzyme activity. The substrate is exposed to 808 nm laser for a specific period of time followed by the measurement of enzyme activity. The relative activity in the case of laser illumination is calculated based on the increase in the activity with respect to the original activity.

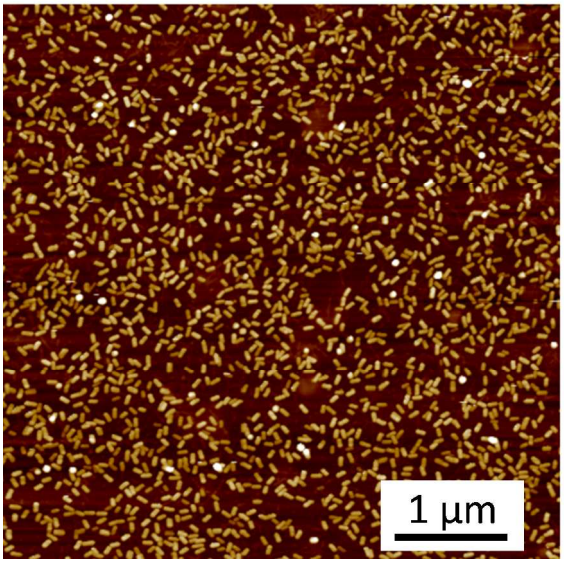

Figure S1: AFM image of the AuNR-HRP bionanoconjugates uniformly adsorbed on a glass substrate.

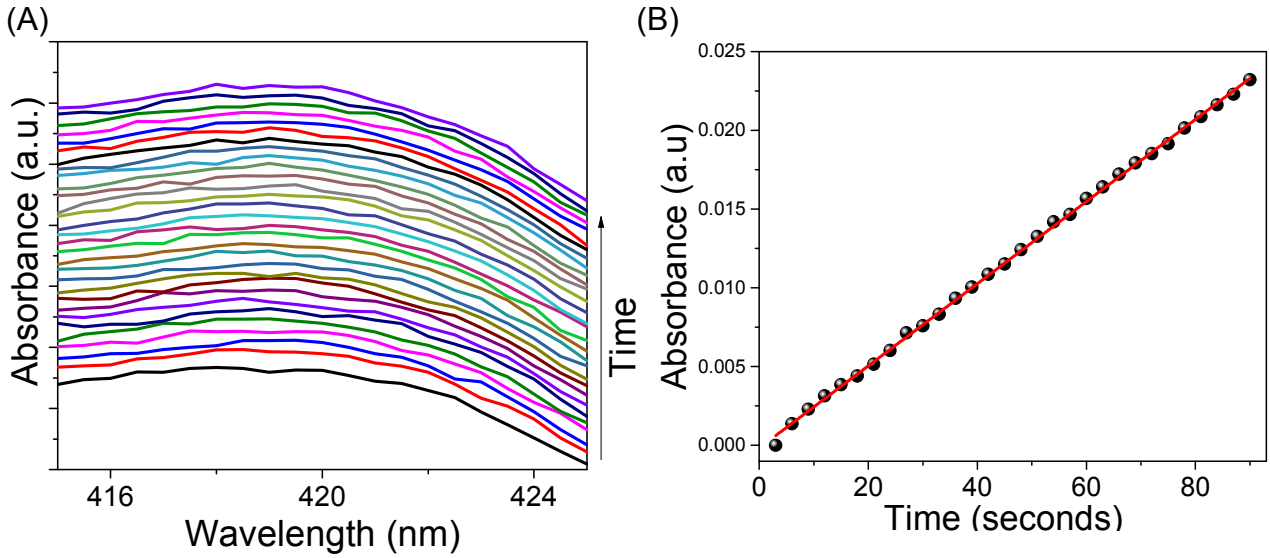

Figure S2: (A) Representative absorption spectra showing the time dependent increase in the absorbance corresponding to the product formation during the enzymatic reaction (B) Absorbance of $[ABTS]^{+}$ measured at 420 nm as a function of time. The slope of this plot is an estimate of the relative enzyme activity.

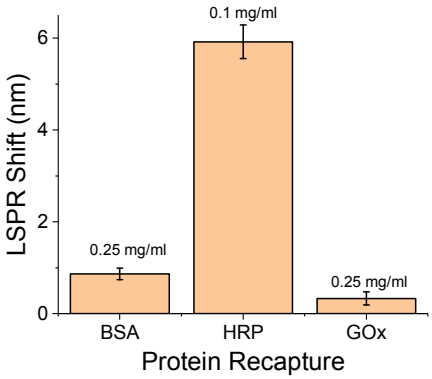

Figure S3: LSPR wavelength of AuNRs with HRP biomolecular imprints after exposure to HRP, BSA and GOx.

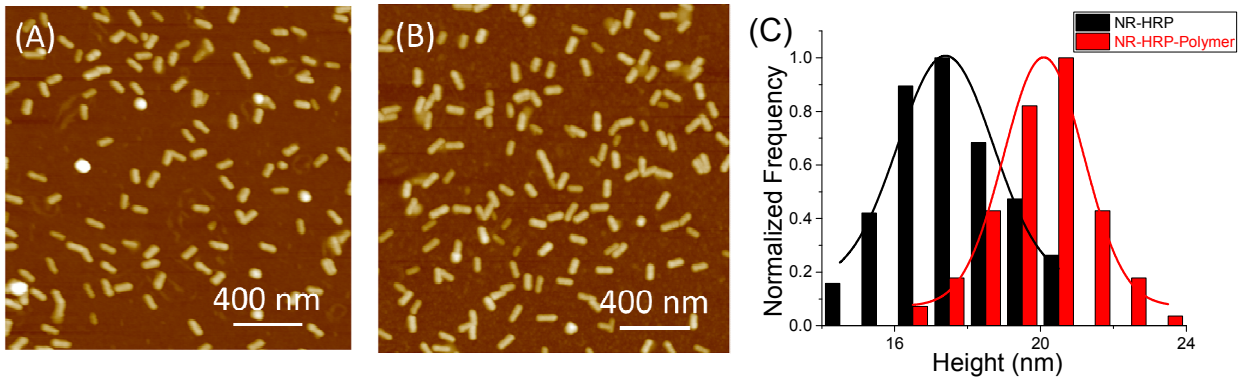

Figure S4: AFM images of (A) AuNR-HRP bionanoconjugates uniformly adsorbed on a glass substrate. (B) AuNR-HRP bionanoconjugates with polymer encapsulation. (C) Height distribution of the AuNR-HRP bionanoconjugates with and without polymer encapsulation.

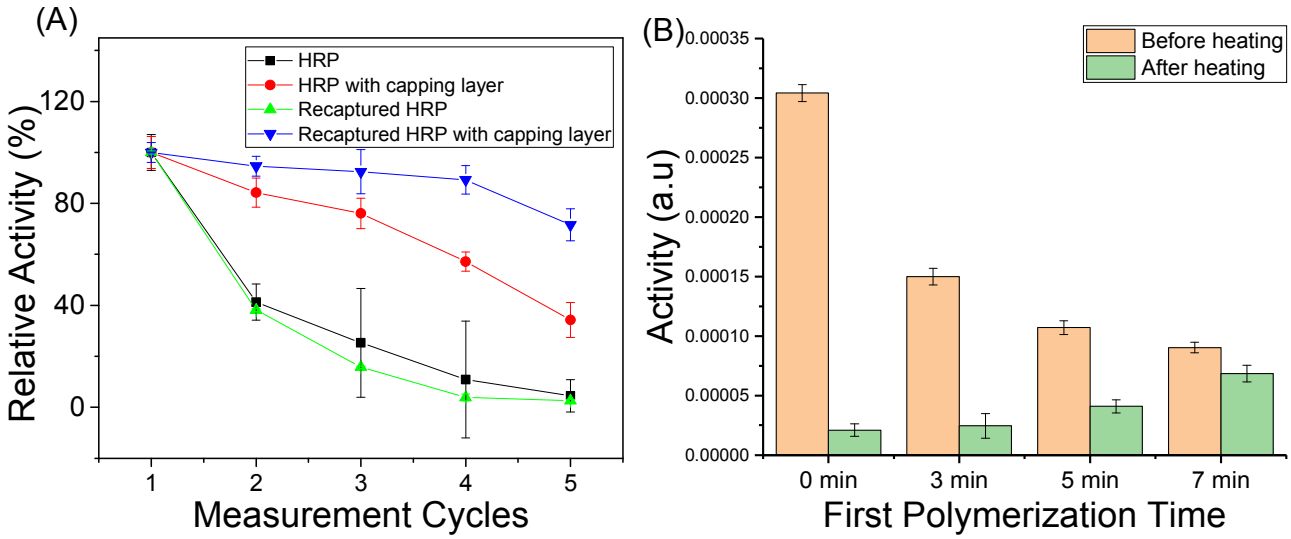

Figure S5: (A) Recycling stability of HRP nanoconjugates in different configurations indicated in the plot. Error bars indicate the standard deviation from three independent samples. (B) Activity of HRP immobilized on AuNRs after first polymerization for different polymerization times before and after heating at 55°C for 1 hour. Error bars indicate the standard deviation from three independent measurements.

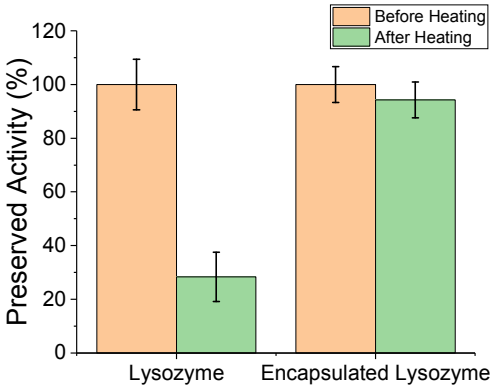

Figure S6: Activity of lysozyme and encapsulated lysozyme before and after heat treatment at 55°C for 1 hour. Error bars indicate the standard deviation from three independent samples.

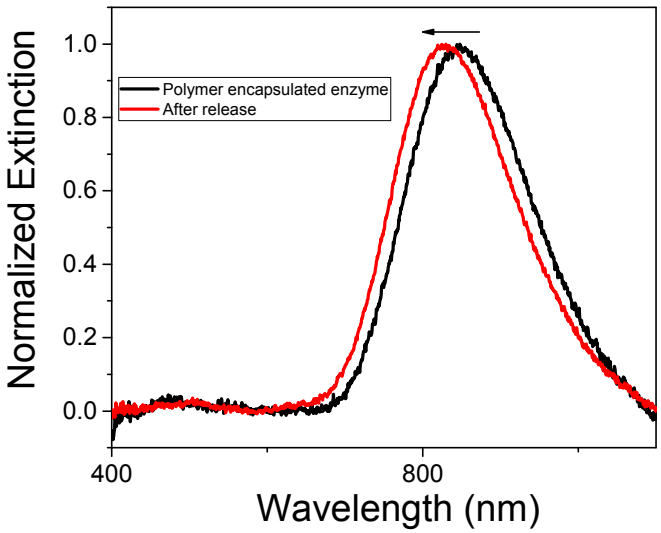

Figure S7: Extinction spectra of AuNR before and after release of enzymes from the polymer encapsulation corresponding to the complete removal of enzymes.

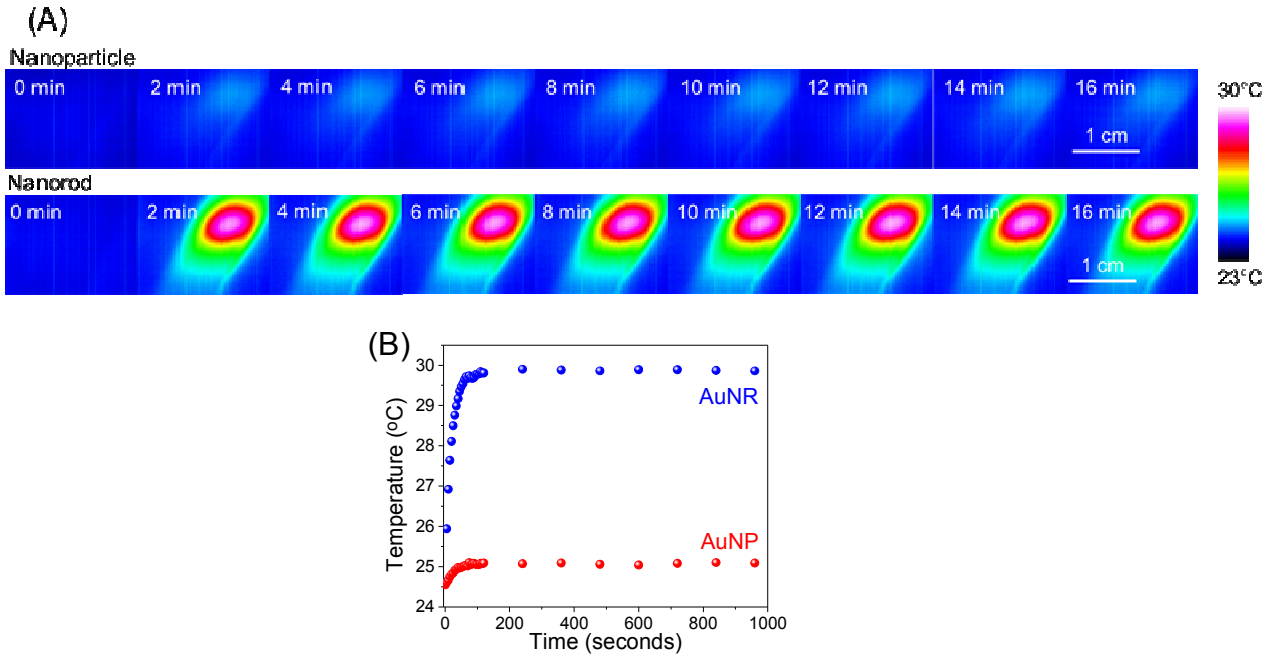

Figure S8: (A) IR images depicting the temperature rise of glass substrates adsorbed with (top) AuNR-HRP (on-resonance) and (bottom) AuNP-HRP (off-resonance) exposed to 808 nm laser. (B) Temperature profile in AuNR-HRP and AuNP-HRP as a function of laser irradiation time.

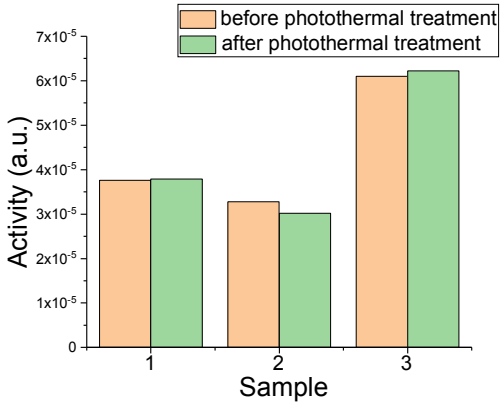

Figure S9: Biocatalytic activity measurements of encapsulated enzymes before and after photothermal treatment. Measurements from three independent samples.

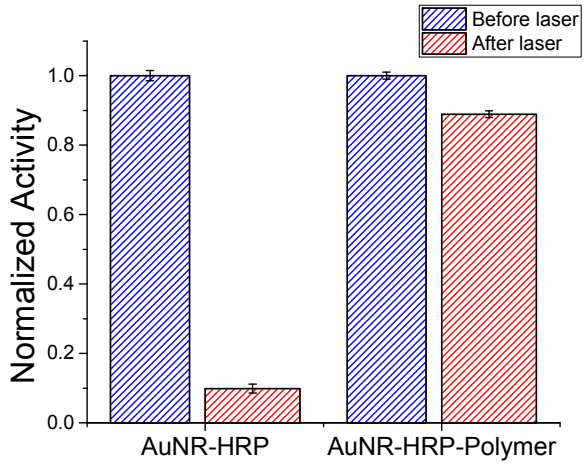

Figure S10: Biocatalytic activity measurement at room temperature after laser exposure (800 mW/cm^2) for 30 minutes. Error bars indicate the standard deviation from three independent measurements.

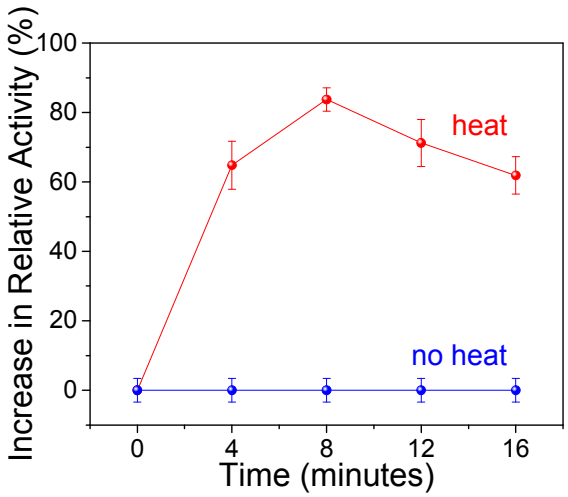

Figure S11: Activity measurement in the presence of external heating at 30°C on the polymer encapsulated enzyme. Error bars indicate the standard deviation from three independent samples.