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12-20-2016

# Organosilica Imprints for Preserving and Enhancing Biocatalytic Activity of Immobilized Enzyme

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#### **Recommended Citation**

Yim, Jieun and Singamaneni, Srikanth, "Organosilica Imprints for Preserving and Enhancing Biocatalytic Activity of Immobilized Enzyme" (2016). *Mechanical Engineering and Materials Science Independent Study.* 25.

https://openscholarship.wustl.edu/mems500/25

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## Objective

Enzymes are naturally occuring sustainable catalysts that offer competitive catalysis to synthetic routes. There exist growing demands for environmentally-friendly and sustainable catalysis, which have led to a surge of research in biocatalysis.<sup>1-3</sup> However, the major bottleneck for the industrial applications of enzymes is the long term operational stability and difficultly in recovery and reusability of enzymes.<sup>4-6</sup> The goal of this study was to develop biocatalytic surface with organo-silica enzyme imprints that can recover, stabilize and reuse enzymes. The complete encapsulation was expected to enhance thermal and chemical stability, and the plasmonic nanostructures were expected to provide the ability to enhance the biocatalytic activity when light was used as an external trigger. The target enzyme was horseradish peroxidase (HRP).

# **Procedure and Result**

### 1) The Preparation of Organosilica Imprints for immobilized Enzyme

Figure 1 shows the various steps involved in the organo silica-based preservation of biocatalytic activity of bionanoconjugates.



*Figure 1: Schematic representing the polymer encapsulation strategy for enzyme stabilization.* 

First, gold nanorods (AuNRs) were used as optically-active inorganic supports for the immobilization of enzymes (Figure 2).



*Figure 2: The representative TEM image of AuNRs employed in the study.* 

As shown in Figure 3, the adsorption of HRP on the gold nanorods resulted in a redshift in the localized surface plasmon resonance (LSPR) wavelength corresponding to the increase in the refractive index of the medium surrounding AuNR.



*Figure 3: Extinction spectrum of AuNRs showing a red shift in the LSPR peak position after conjugation to HRP.* 

The filtered bionanoconjugates were adsorbed on a glass substrate uniformly as shown in the AFM image (Figure 4).



Figure 4: AFM image of the NR+HRP conjugates adsorbed on a glass substrate.

Following the immobilization of the bionanoconjugates on a glass substrate, an organosilica encapsulation layer was formed through copolymerization of trimethoxypropyl silane (TMPS) and (3-aminopropyl)trimethoxysilane (APTMS) around the AuNR-HRP surface. After the organo-silica template was formed around the enzyme, the template enzyme which was bound to the surface of the AuNR through electrostatic interactions was removed by exposure to sodium dodecyl sulfate (SDS). Then, the organo-silica template was exposed to the enzyme for recapture. After the recapture of the enzyme, a thin organo-silica capping layer was formed on the surface to provide thermal stability to the enzyme.

The LSPR wavelength of the AuNR exhibited a red shift corresponding to the formation of an organo-silica layer around the NR-HRP and a blue shift corresponding to the release of the enzyme. The rebinding of the enzyme and the formation of a capping layer exhibited a red shift due to the increase in the refractive index. The LSPR shift for each step is shown in Figure 5.



*Figure 5 LSPR wavelength of each step in the polymer encapsulation process of the enzyme showing the release and recapture of the enzyme.* 

The thickness of the capping layer was controlled by the polymerization time. The corresponding LSPR shift after the capping layer scaled with the polymerization time (Figure 6).



*Figure 6 LSPR shift after second polymerization on the recaptured enzyme for different times.* 

### 2) Tests on Biocatalytic Activity of Immobilized Enzyme

The adsorbed AuNR-HRP substrates are catalytically active when tested using 2,2'azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay.

The enzyme activity dropped as the thickness of the polymer capping layer increased (Figure 7).



*Figure 7 Relative activity of the polymer encapsulated HRP subjected to different polymerization times* 

Despite the drop in the relative enzyme activity after the polymer capping layer due to the slower diffusion of the substrate through the polymer shell, a significant improvement in the reusability was observed that justified the presence of the capping layer (Figure 8).



*Figure 8 Activity measurement after multiple cycles of catalysis in polymer encapsulated HRP with and without polymerization* 

We probed the ability of the organo-silica imprints to release and recover the enzymes. We exposed the polymer encapsulated enzymes to multiple cycles of release and recapture by alternating exposure to SDS and HRP. The relative activity of the polymer encapsulated enzyme followed a saw tooth pattern corresponding to the release and recapture of the enzyme (Figure 9).



*Figure 9 Relative activity of polymer encapsulated enzyme through multiple cycles of release and recapture.* 

In order to gain insight into the thermal stability of the polymer encapsulated enzyme, we exposed the encapsulated enzymes with different organo-silica capping layers to 55°C for 1 hour. The preserved activity increased with the increase in thickness of the capping layer (Figure 10)



Figure 10 Retained activity of the polymer encapsulated enzyme after subjecting it to a heat treatment of 55°C for 1 hour

We also probed the chemical stability of the organo-silica stabilized enzyme by exposure to protease digestion. The activity of NR-HRP without organo-silica shell was reduced to ~5% while the activity of organo-silica stabilized enzyme retained ~90% of the activity (Figure 11).



*Figure 11 Activity of polymer encapsulated HRP subjected to proteolytic treatment as opposed to bare NR+HRP conjugates.* 

In order to compare the bio-activity with an external trigger and without, we employed two different plasmonic nanostructures, namely, gold nanoparticles (AuNP) with an LSPR peak position at 520 nm and gold nanorods (AuNR) with an LSPR peak position at 800 nm. We compared the photothermal ability of these polymer encapsulated enzymes after exposure to 808 nm laser at a power of 400 mW/cm<sup>2</sup>. We observed a progressive increase in temperature with time in the case of AuNR while the temperature in the case of AuNP did not exhibit a significant change over the entire duration of laser exposure (Figure 12 A, B).



Figure 12 (A) IR camera images of HRP substrates exposed to 808 nm laser conjugated with on-resonance AuNR and off-resonance AuNP (bottom). (B) Temperature profile in AuNR-HRP and AuNP-HRP as a function of time.

We studied the effect of laser exposure on the biocatalytic activity of organosilica encapsulated enzymes immobilized on plasmonic nanostructures. We found that the activity of enzymes immobilized on AuNR increased by ~110% after 4 min laser exposure and retained the same activity for prolonged exposure (Figure 13 A). However, in the case of AuNP-HRP, the activity only increased by 10% after 4 min laser exposure followed by a drop in activity for prolonged laser exposures (Figure 13 B).



Figure 13 Relative activity after different laser exposure times in (A) on-resonance AuNR and (B) off-resonance AuNP.

# Conclusion

This study demonstrated organo-silica imprints that can realize multiple catalytic cycles, efficiently recover and reuse enzymes; provide enahanced thermal and chemical stability; and facilitate externally triggered biocatalytic activity.

The synthetic organosilica imprints on the plasmonic nanostructure presented complimentary shape and chemical interactions with the enzyme, thus enhancing the capture efficiency of the enzyme. The thin capping polymer layer provided enhanced resusability, and thermal and chemical stability of the enzyme to enable its use in harsh settings.

The plasmonic nanostructures coverted the light into the heat. The increase in the biocatalytic activity of polymer encapsulated enzyme in the plasmonic nanostructures is attributed to (i) enhanced diffusion of the substrate through the pores of the organo-silica polymer and (ii) possible increase in the pore size of the organo-silica due to the increase in the temperature.

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