Exploring Galvanic Replacement as a Method to Engineer Peroxidase-mimics Nanoparticles

Kuryn T. MaGloire
University of Central Florida

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Exploring Galvanic Replacement as a Method to Engineer Peroxidase-mimics Nanoparticles

By

Kuryn T. Ma Gloire

A thesis submitted in partial fulfillment of the requirements for the degree of Bachelor of Science
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ABSTRACT

Peroxidase enzymes are of critical importance within the scientific community for their applications in biosensing assays. In a living system, natural peroxidases function as catalysts in the oxidation of peroxide (e.g., H2O2) - a harmful byproduct of aerobic processes and convert them into harmless compounds. Such an ability allows peroxidases to serve as labels in biosensing assays, where they are conjugated to antibodies and accurately produce a detection signal by catalyzing substrates. However, due to intrinsic limitations, namely instability, peroxidase made of proteins substantially inhibit broader applications.

Alternatively, nanoparticles produced from noble metals have been found to exhibit peroxidase-like abilities and, therefore, can be used as synthetic enzymes with the potential to replace their natural counterparts. Given that the stability of most peroxidase mimics is already much better than their natural counterparts, in this field, the principal challenge has been creating substantial improvements to the catalytic efficiency of the mimics. This study sought to create a cage-like nanostructure (denoted as nanocages) consisting of two platinum group metals. This experiment uses Galvanic replacement as a mechanism to hollow all Nanocages formed. Galvanic replacement has been primarily demonstrated using coinage metals (Ex. Ag and Au). This experiment seeks to show that this process is viable for other Nobel metals, as well. Palladium cubes were used as scaffolds or sacrificed templates to induce the reaction with a precursor containing a secondary Nobel metal (Platinum, Rhodium, or Ruthenium). Once viable samples where produced (checked via TEM (Transmission Electron microscope)), the
peroxidase-like activity was compared to the activity of a non-hollowed nanostructure of the same material composition using TMB colorimetric assay.
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INTRODUCTION

Peroxidase

Traditionally, peroxidase is a protein-based enzyme found within most living systems capable of aerobic respiration (plants, animals, microbial organisms). During Aerobic respiration; the mitochondria facilitates the transfer of electrons called oxidative phosphorylation used to synthesis ATP. As a byproduct of this mechanism, reactive oxygen species can form, causing cytotoxic effects. This enzyme is of importance because of the role it plays in scavenging reactive oxygen species before cellular damage can occur. In the process of reducing oxygen to water, intermediaries such as peroxide (H$_2$O$_2$) form. Peroxidase can transfer electrons reducing peroxide into water and oxidizing a secondary substrate in the process. Peroxidase can transfer electrons using its heme group composed of an iron atom (Fe(III) state) coordinated within a porphyrin ring. This iron atom can perform heterolytic cleavage at the bond between each oxygen resulting in a water molecule and a Fe(IV) state iron. The additional electrons will transfer from the Iron group returning the enzyme to initial state.
Figure 1: This is a 3D model of a peroxidase enzyme showing clearly the secondary and tertiary structure the enzyme assumes. The arrow indicates the placement of peroxidase's heme group.²

$$\text{H}_2\text{O}_2 + \text{substrate}_{\text{reduced}} \xrightarrow{\text{Peroxidase}} \text{H}_2\text{O} + \text{substrate}_{\text{oxidized}}$$

Figure 2: This figure depicts the mechanism by which peroxidase reduces its primary substrate hydrogen peroxide while oxidizing a secondary substrate.²⁴
This ability to oxidize substrates in the presence of hydrogen peroxide has made peroxidase a popular enzyme for research purposes. The model enzyme used by scientists is Horseradish peroxidase (HRP). As the name implies, HRP is an extract of the root of horseradish plants, which are a naturally rich source of Peroxidase isoenzyme C (HRPC). HRP has become one of the principal enzymes for industrial applications such as health screening, treatment of wastewater, and quality assurance of food and beverages.

An example of frequent use for HRP is the ELISA or enzyme-linked immunosorbent assay. An ELISA can be used to quickly identify the amount of antigen within a sample using antibodies covalently linked to an enzyme and the enzyme’s substrate. Researchers often utilize HRP as the covalently bound enzyme in the presence of hydrogen peroxide and a chromogenic substrate such as TMB (3,3′, 5,5′-Tetramethylbenzidine). The oxidation of TMB creates a colorimetric signal that can be quantified using a spectrophotometer by measuring absorbance. TMB can also be used to gain a rough estimation of catalysis for enzymes or enzyme mimics capable of oxidizing the reagent.
Figure 3: This figure depicts a sandwiched ELISA using HRP to facilitate the oxidation of TMB, producing a bright blue color when the target antigen is bound.

While the use of peroxidase enzymes has some industrial advantages, the inherent restrictions faced by natural protein-based enzymes continue to limit further applications. HRP, like all protein-based enzymes, shows susceptibility to factors such as changes in temperature. The denaturation of HRP's tertiary structure begins at approximately 45°C, and it loses structural stability around at a pH of 4.5 and below.\textsuperscript{5,4} To maintain a protein's efficacy, a scientist must control the temperature and pH of its environment during transportation, storage, and application, which significantly limits the scope of use for HRP. These strict physiological requirements make the necessary process of extraction and purification expensive and time-consuming.\textsuperscript{26,4} Furthermore, the function of a protein-based enzyme is determined by its tertiary structure, meaning its protection is paramount.\textsuperscript{7} The fixed nature of the tertiary structure also means the catalytic capabilities of HRP are set as well, leaving little chance for optimization.
Peroxidase Mimics

Considering the inherent limitations of protein-based enzymes such as HRP, the formation of enzyme mimics to avoid such constraints was a logical step. Enzyme mimics are artificial enzymes often made from inorganic materials that retain the same functions as their protein counterparts. Various materials such as cyclodextrins, metal complexes, porphyrins, polymers, dendrimers, and biological molecules, have been found to mimic enzymatic activity and would make suitable mimic candidates. There are many advantages associated with using an enzyme mimic such as modifiable structures, increased catalytic efficiencies, increased resistance to environmental conditions, simplified production, as well as lowered production costs. For instance, Xia et al., in a study of Nobel metal peroxidase mimics, were able to show (using a TMB analysis) a significant improvement in catalytic efficiency by merely utilizing a Palladium (Pd) nanocubes in place of an HRP enzyme. The experiment took it one step further and compared the efficacy of the first two samples against a Pd cube with a secondary skin of Iridium (Ir). Once again, a significant increase in the catalytic efficiency showing how open to modification enzyme mimics are.
Figure 4: This figure provides a comparison of the catalytic capabilities of HRP to both a Pd and Pd-Ir nanocubes. Furthermore, this figure shows the capabilities Nobel metal nanoparticles possess for modification, and the effect said modification could have on their catalytic proficiency; by displaying the increase in Kcat value relative to one another.⁴

Of all known materials capable of simulation enzymatic activity Nobel metals (which include Palladium, Iridium, Platinum (Pt), Ruthenium (Ru), Rhodium (Rh), Silver (Ag), Osmium (Os), and Gold (Ag)) have shown a high aptitude for peroxidase mimicry when compared to other material of similar dimensions.⁴ The unreactive nature of Nobel metals negates the denaturing effect associated with any temperature and pH changes seen with traditional protein-based enzymes. They are also resistant to oxidation an important to trait to have to carry out peroxidase-like activity.⁴ Once formed the surface of an artificial enzyme provides a diversity of binding sites, vertexes, edges, terraces, and pseudo terraces allowing for a wider assortment of ligands to bind to the surface of the nanostructure.¹¹ Diversity concerning ligand binding is particularly helpful for immunoassays; for example, the previously mentioned sandwiched
ELISA pathway requires an antibody to be conjugated to an enzyme to catalyze a colorimetric reaction. The variety of binding sites offered on a peroxidase mimic could very easily accommodate the Fc portion of an antibody.

While the formation of Nobel metal nanoparticles itself represents a notable advancement in artificial enzyme kinetics, namely, through expressing a significant increase in Kcat values, their potential for further improvement should not be overlooked. Like HRP, the enzymatic activity of a Noble metal nanoparticle is a function of its structure, specifically surface area, unlike HRP, the overall structure, composition, and size of a peroxidase mimic and be modified to optimize the efficiency of the particle. Size alone has a tremendous impact on the way nanoparticles function within a body. The "Nano" size of these particles allows for various communications with biomolecules on cell surfaces and within cells in a way that can be decoded and assigned to multiple biochemical and physicochemical properties of these cells.\textsuperscript{13,22} One of the most significant advantages of using an artificial enzyme is that it can be structurally altered to obtain the specific parameters need for the exact purpose the scientist intends.

An enzyme's efficiency is associated with the relationship $K_{\text{cat}}/K_{\text{m}}$. $K_{\text{cat}}$ or the catalytic constant refers to the number of substrates converted into a product by an enzyme over a specific unit of time once the enzyme reaches saturation with the available substrate.\textsuperscript{15} Whereas $K_{\text{m}}$, commonly known as the Michaelis-Menten constant, is the measure of an enzyme’s affinity for binding a particular substrate. A larger $K_{\text{cat}}/K_{\text{m}}$ ratio indicates a more efficient enzyme. $K_{\text{cat}}$ values are often solely compared when equating the catalytic proficiency of artificial enzymes to their natural counterparts because a higher $K_{\text{cat}}$ value directly corresponds to an enzyme's ability to convert substrates into product over a given period. Therefore, the enduring goal of scientists
pursuing synthetic enzyme mimics is to engineer a particle that shows a significant rise in its Kcat value.\(^4\)

\[
E + S \overset{k_1}{\underset{k^{-1}}{\rightleftharpoons}} ES \overset{k_2}{\underset{k^{-2}}{\rightleftharpoons}} E + P
\]

Figure 5: This figure describes the general pathway taken by an enzyme when in the presence of substrate, including the rate constants associated with each step of the reaction, both forward and reverse.\(^5\)

The primary focus of this experiment is to enhance artificial peroxidase enzymes by boosting Kcat values while keeping the costs of producing the particles relatively the same. If production costs are kept the same while catalytic efficiency is increasing, the enzyme will become more cost-effective. Once optimized, mimics accommodate a variety of scientific or medical applications such as catalysis, sensing, imaging, biomedicine.\(^6\) The addition of a more efficient enzyme into an enzyme-mediated immune assay should lead to an enhancement of diagnostic sensitivity. Improvement in sensitivity will allow medical professionals utilizing said assays to detect diseases much earlier than previously possible.\(^4\) Conditions such as cancer, diabetes, and atherosclerosis.\(^6\) Early detection increases the likelihood of diagnosing an illness at a treatable stage.

As all catalytic activity takes place on an enzyme’s surface, increasing the available surface area on which a reaction can take place is the top goal of most researchers in the nanoscience field. Despite nanostructures with longer and thinner dimensions characteristically having a larger
surface area to volume ratios, this experiment will focus on nanocubes. Nanocubes can be easily reproduced and synthesized in the most basic of lab environments with high quality and high yield. High quality and yield are of particular importance if the cubes produced will be used as frameworks for further synthesis; the final product would depend on the consistency of quality of the cubes used to produce it.

Hollow Structure

The hollowing of nanocubes is a simple yet effective way of increasing surface area. As stated previously, catalysis is a function of surface area; any increase to the surface area should confer a higher catalytic output. Hollowed cubes also exhibit a decrease in density that makes them easier to disperse within a solution and, therefore, more comfortable to use. The traditional way of augmenting the structure of Nanoparticles is through chemical etching. Chemical etching is a standard methodology by contact with a chemical agent selectively etches a nanoparticle. While this method can hollow out a nanostructure's core, it often leads to products that form too much aggregation. The excess aggregate, in turn, blocks portions of the outer surface area, negatively impacting catalysis and the overall Kcat value. However, some scientists have found success with this method, experiments conducted by Wang et all show the proficiency of chemical etching to hallow Pt-on-Pd bimetallic dendritic nanoparticles. It is important to note that chemical etching is a viable reaction mechanism when working with cubes, which contain a core comprised of only of palladium. If a nanostructure includes a palladium core, it is likely to be sufficiently hollowed with minimal aggregation. Nevertheless, alternative methods of
structural modifications are necessary to simplify the experimental procedure and ensure environmentally conscious synthetic enzymes are the way of the future.\textsuperscript{19}

**Galvanic Replacement**

Galvanic replacement is a practical and versatile approach to altering the internal structure of a nanoparticle.\textsuperscript{20} This method differs from chemical etching, which relies on the capabilities of temperature controlled etching agents to produce the desired shape, size, composition, and structure.\textsuperscript{20} Galvanic follows the same trend as a single replacement reaction. The process works through a coupling of a reduction and oxidation reaction. First, one metal (denoted the scaffold or sacrifice) is oxidized by the ions of another metal in solution. The ions introduced to the solution must have a higher reduction potential to ensure the oxidation of the scaffold. A precursor solution adds the secondary metal.\textsuperscript{20} Another ion often a halogen is used to help oxidize atoms off the surface of the framework allowing the secondary metal to reduce onto the surface. For every three particles removed, only two can deposit back onto the surface. This ratio of oxidation and reduction leads to the eventual hollowing of the scaffold. The two steps associated with galvanic replacement occur concurrently in solution as a one-step mechanism. Conversely, chemical etching is a two-step process requiring both the growth of a secondary metal and the etching of the core to take place separately.
Figure 6: This figure depicts the mechanism by which Galvanic replacement occurs within the solution. First, 18 nm Pd seeds (scaffolds) are added to a solution and introduced to the ions of secondary metal. The redox reaction that ensues results in a hollowed cube made of Pd-X metal alloy with a porous surface.

1) $3\text{Pd} + 2\text{Ir}^{3+} + 12\text{I}^- \rightarrow 3\text{PdI}_4^{2-} + 2\text{Ir}$

   Galvanic replacement

2) $\text{PdI}_4^{2-} \rightarrow \text{Pd}$

   Reduce Pd precursor into Pd atoms
Originally it was shown that Galvanic replacement is capable of hollowing bimetallic cubes comprised of only silver and gold.\textsuperscript{20} However, work has been done to expand the understanding of galvanic replacement. Research has shown Pd-Pt cubes can also form cages via galvanic replacement using the difference in reduction potential generated by the addition of KBr.\textsuperscript{21} While this study shows great promise, further examination is warranted to explore the extent to which this approach can be employed — especially using materials such as Iridium, Ruthenium, and Rubidium. This experiment seeks to explore the production of bimetallic hollowed Nano cages (Pd-Ir, Pd-Rh, Pd-Ru, Pd-Pt) as well as altering and improving on the known experimental procedure of Pd-Pt cages as needed.
METHODS

Seeds

For this experiment, Pd cubes were used as scaffolding agents. To synthesis, these seeds the methodology proposed by Xia et al. was used. The particles were synthesized using a one-pot technique, an 8.0 ml aqueous solution which contained 105 mg of Polyvinyl pyrimidine (PVP), 60 mg of Ascorbic Acid (AA), and 600 mg of KBr were held in a flask and preheated at 80 °C in oil bath under consistent magnetic stirring for 10 min. Subsequently, 3.0 mL of a Na2PdCl4 aqueous solution (19 mg/mL) was added with a micropipette. After the flask had been sealed, the reaction could continue at 80 °C for 3 hrs. After being washed with deionized (DI) water three times via centrifugation using an Eppendorf 5430/R rotor at rpm for minutes The Pd cubic seeds were stored in a 20 mL conical tube of Ethelyn Glycol (EG) for future use (∼1 mg/mL). Results were viewed using a Transmission Electron Microscope (TEM) for quality assurance.

Salt Precursor

In a glass vial, 10 mg of metal salt (IrCl3, RhCl3, RuCl3, PtCl4) and 10 ml of Ethylene glycol were mixed. The mixture was sonicated to ensure a homogenous solution and stored away from light (wrapped in foil or placed in the refrigerator). This protocol creates a stock of 1 mg/ml that was diluted as needed throughout the experiment.
Hollowed Cages (Pd-Rh, Pd-Ru, Pd-Pt)

The hollow Pd-X nanostructures produced in this experiment were synthesized based partially off a method proposed by Ye et al. In short, 15 mg of PVP, 20 mg of AA, and 100 mg KI were added to a vial containing 7.5 mL of EG and 0.5 ml of 18 nm Pd cubes. The flask was vacuum sealed with a rubber stopper and then placed in an oil bath preheated to 200°C, where it incubated in the presence of N2 gas, distillation, and magnetic stirring for 2 hrs. After the initial incubation period, a hole was pierced through the rubber stopper. A capillary tube attached to a syringe by a thin plastic tube was inserted into the stopper. The syringe contained 20ml (0.1 mg/ml) of the secondary metal precursor. Its release into solution was automated via a syringe pump at a rate of 5ml/hr. Once complete, the nanostructures were washed first in acetone at 7,800 rpm for 15 minutes in an Eppendorf 5430/R then in DI water two times at 14,000 rpm for 10 minutes each.

Nanocubes (Pd-Ir)

Pd-Ir nanocubes were synthesized via chemical etching to provide a basis of comparison to Nanocages prepared via Galvani Replacement. The method was based in part from a paper by Zhang et al. First, 50 mg of PVP and 100 mg of Ascorbic acid were added to a vial containing 7.0 mL of EG and 1.0 ml of 18 nm Pd cubes. The flask was vacuum sealed with a rubber stopper and then placed in an oil bath preheated to 200°C, where it incubated in the presence of N2 gas, distillation, and magnetic stirring for 2 hrs. Afterward, a hole was pierced through the rubber stopper, where a capillary tube was inserted, this capillary tube is attached to a thin plastic tube.
which was attached to a syringe containing the Iridium metal precursor. A syringe pump was used to slowly inject a solution of 8 ml of a 0.1 mg/ml concertation of Ir precursor into the solution at a rate of 0.4 ml/hr. Once complete, the Pd-Ir nanocubes were washed first in acetone at 7800 rpm for 15 minutes using an Eppendorf 5430/R rotor then in DI water twice at an rpm of 14,000 for 10 mins each time. The resulting sample was stored for future use.

**Hollowed cages (Pd-Ir)**

Hollow Pd-Ir nanostructures were also synthesized by Galvanic replacement using a method based partially off Ye et al.\textsuperscript{30}. First, 50 mg of PVP and 200 mg KI were added to a vial containing 7.0 mL of EG and 1.0 ml of 18 nm Pd cubes. The flask was vacuum sealed with a rubber stopper and then placed in an oil bath preheated to 200°C, where it incubated in the presence of N2 gas, distillation, and magnetic stirring for 2 hrs. Afterward, a hole was pierced through the rubber stopper, where a capillary tube is inserted into the rubber stopper. This capillary tube is attached to a thin plastic tube that is inserted into a syringe containing the metal precursor. A syringe pump was used to inject 8 ml of a 0.1 mg/ml Ir precursor solution at a rate of 1ml/hr. Once complete, the Pd-Ir nanostructures were washed first with acetone via centrifugation using an Eppendorf 5430/R rotor at an rpm of7,800 rpm for 15 minutes then in DI water four times at 14,000 rpm for 10 minutes each. The resulting sample was stored for future use.
TMB Analysis

To prep first, a TMB stock solution was created by adding 0.0375 g of TMB to 3 ml of DMSO this mixture was vortexed to ensure homogeny, wrapped in foil and stored in the fridge until time of use. Next, in a 50 ml conical tube, a buffer is prepared consisting of 2.34 ml of acetic acid, and 0.74 g of sodium acetate were mixed the solution is brought up to a total of 50 ml with deionized water. Each cuvette was filled with 200 ml of buffer, 15.4 ml of TMB solution, 584 ml of deionized water, and 1ml of the sample. Pipette up and down rapidly to mix, load the cuvette into a spectrophotometer where absorbances were checked every minute for 10 minutes.
RESULTS

Pd Cubes

Figure 7: TEM image of 18nm Pd cubes used as a scaffold for all subsequent experiments
Pd-Pt/Rh/Ru cages

Figure 8: TEM images of Pd-X cages A. palladium platinum (Pd-Pt), B. Palladium Rhodium (Pd-Rh), C. Palladium Ruthenium (Pd-Ru).
Pd-Ir Cubes

Figure 9: TEM images of Pd-Ir cubes.

Pd-Ir Cages

Figure 10: TEM images of Pd-Ir cages.
Figure 11: Absorbance peaks for Pd-Ir cubes diluted.

Figure 12: Absorbance values for Pd-Ir nanocages undiluted.
DISCUSSION

The purpose of this experiment was to investigate Galvanic Replacement's practicality as a method to Hollow a broader range of Nobel metal nanostructures. Previous research has already shown that Galvanic replacement is a viable mechanism for hollowing coinage metals (Ag, Au), a subset of Noble metals. Therefore, it is not a stretch to believe the method would also work across the scope of noble metals, especially once the mechanism by which Galvanic replacement hollows Nanostructures is considered. Galvanic replacement will occur if there exists a sufficient difference in electrochemical potential between the scaffold and secondary metal used for seeding. The secondary metal should have a higher reduction potential than the scaffold to ensure oxidation of the framework's core. Palladium acted as the scaffold for each reaction, as a scaffold; it is an extremely stable metal, making it a suitable base for most other noble metals to replace. Through the course of experimentation, the results of this experiment were able to prove definitively Galvanic replacements capabilities to hollow a broader range of Nobel Metals nanoparticles.

The quality of synthesized Nanocages was determined visually, using the images captured using a Transmission Electron Microscope (TEM) pictured above. The overarching desire in the field of nanoscience is to increase the catalytic capabilities of synthesized particles. As the catalytic output is a function of available surface area, the amount of unobstructed surface area produced can be used to determine cube quality. An unsuccessful synthesis can lead to particles whose surface area is obstructed by the aggregation of particulates (likely secondary metal-based), creating a rough outer surface. Individual particles can also aggregate together, blocking each other’s surface.
Aggregation of particles has been shown to occur less with Galvanic replacement than standard chemical etching. However, aggregation can still happen. A careful balance must exist between components of the reaction to create an environment where there is enough energy to drive the reaction forward without driving the reaction past its optimum state and producing particles with rugged exteriors. Maintaining an even, slightly concaved surface is vital in providing high indexed facets (100, 101), which are responsible for the high catalytic capabilities the particles exhibit. To achieve a well-formed exterior, the ratio between deposition on the edges and corners, as well as diffusion of ions across the entire surface, must be well controlled. Too much of either deposition or diffusion resulted in low indexed facets and decreased catalytic output. There must be a balance between oxidation and reduction. This ensures both the hollowing of the cube while producing an even outer shell. The secondary metal ions must reduce onto the surface without over reducing to form surface aggregation. Each component of the solution plays an essential role in maintaining said balance. A successful equilibrium of reagents was struck, and hollowed bi-metallic cages were successfully synthesized for all four Noble metals attempted.

The reaction mixture contained Potassium Iodide (KI), a potent chemical etching agent; it acts as a catalyst lowering the activation energy required to begin the oxidation of the Pd seed. Initially, in earlier attempts at producing nanocages, Ascorbic acid (AA) was incorporated into solution in small amounts. Ascorbic acid served to aid in the reduction of palladium ions. This was necessary for synthesis using Platinum, Rhodium, Ruthenium as secondary metals. However, it was found not to be necessary for palladium iridium cages; in fact, excess reduction lead to an aggregation of particles on the surface of each cage, obstructing the formation of
facets. Polyvinylpyrrolidone (PVP) is a surfactant added to the reaction mixture to act as a capping agent. PVP stops the excess diffusion of secondary metal ions, thereby stabilizing the overall structure of each nanocage. Lastly, Ethylene Glycol is the solvent chosen to dissolve all other reagents; Ethylene glycol was the solvent of choice as it has a Boiling point of 200°C. The overall ΔG of this reaction mechanism is positive and therefore requires heat energy to be inputted into the system for it to occur. Ethylene Glycol’s boiling point is sufficient for the heat required to drive this reaction forward. Each piece plays its part in sustaining the balance; however, Temperature and Injection rate play the most crucial role in regulating the deposition and diffusion of ions during Galvanic replacement. As stated previously, the input of heat energy is required to drive the reaction mechanism forward due to unfavorable ΔGs. Therefore, the temperature of the experiment must be adequately high; however, if temperatures are too high excess diffusion occurs, which leads to clumping on the surface of the nanocage as well as over-etching of corners and edges blocking the formation of facets needed for catalysis. The boiling point of Ethylene Glycol sets an upper limit for temperature manipulation. Through the course of experimentation, 200°C to be the optimum temperature, as anything below could not adequately hollow cubes. The only exception to this trend was the Pd-Pt cages. When occurring in the presence of platinum, the reaction mechanism could take place at 180°C, reducing the amount of ion diffusion across the surface of the particles, creating the optimal shape. Picture A from figure 8 showed the smooth convex surface of each Pd-Pt cage along with a well-defined outer layer and hollowed interior. It would appear the combination of a palladium scaffold with a secondary metal of platinum lends itself well to galvanic replacement. However, this does not mean that Pd-Rh or Pd-Ru cubes were unsuccessful.
When comparing the Pd-Pt cages to Pd-Ru, Pd-Rh, and Pd-Ir, all three have rounded edges where excessive oxidation occurred. Likely, this is the result of the higher temperature needed to hollow Pd-Rh Pd-Ru and Pd-Ir cages but not Pd-Pt, the heat could cause excess diffusion and remove the ions deposited on the corners of each particle, giving them a more rounded appearance. There could also be properties associated with Rhodium, Ruthenium, and Iridium when in the presence of palladium — leading to an increased reactivity along their corners and edges, causing the observed result.

The injection rate also played a crucial role in maintaining the balance between deposition and dispersion. The injection rate refers to the speed at which the precursor containing the secondary metal (Pt, Rh, Ru, or Ir) injects into the solution. The slower the injection of precursor, the more time ions are given to deposit and disperse along the surface of the particle leading to a smoother product. From past trials, if the reaction happened too quickly, there would be an aggregation of secondary metal deposits on corners and edges of the particle. Time is necessary to allow the proper diffusion of particulates along the surface. During this experiment, a multitude of different injection amounts and rates were tested to discern the optimal parameters for a well-formed particle. Ultimately Pd-Pt, Pd- Rh, Pd-Ru, required a total of 20ml of precursor injected at a rate of 5ml/hr. This a large amount of precursor for a relatively short amount of time when compared to Pd-Ir synthesis. However, successful cubes were synthesized.

Pd-Ir cages required 8ml of the precursor at a rate of 1ml/hr. The synthesis needed much less precursor and a slower injection rate to achieve roughly the same amount of surface smoothing.
Visually the Pd-Ir particles resemble Pd-Rh, Pd-Ru cages, rounded edges. Despite the adjustment in injection amount and rate, Pd-Ir cages were not able to maintain the cube-like shape of their scaffold. As stated earlier, the cause of said shape could stem from the same issue faced in Pd-Rh/Ru synthesis, high temperatures, or due to specific properties of the metal. It was observed with this specific combination; the process of hollowing was significantly more difficult. Earlier trails produced particles without hollowed cores or completely obscured by aggregation. The difficulty could be due to an unfavorable difference in electrochemical potential stunting the redox reaction, which might also explain the need for a decreased injection rate. Nevertheless, a fruitful balance was struck, and the formation of Pd-Ir nanocages was successful.

Once synthesis of an adequate sample occurred, a preliminary TMB analysis was run via spectrophotometer. This test was administered solely on Pd-Ir cubes and cages as there is a stronger basis for comparison in previously published work for Pd-Ir nanostructures. As stated in the introduction, Xia et al. proved when modifying palladium cubes, a significant increase in catalytic capability is possible if a thin skin of iridium is introduced to the surface of a palladium cube when compared to just a palladium cube (see figure 4 for a comparison). The hope is that hollowing a Pd-Ir cube will increase the catalytic output even further. However, once conducted, the results showed that the nanocages exhibited a much lower catalytic output than their solid cube counterparts. When considering data, both samples show a peak around 650 nm, the expected absorbance for these particles. However, the difference in the size of the absorbance peak is the opposite of expectations. This experiment hypothesized that hollowing Nanocubes would provide double the surface area from which enzymatic activity could take place. If the quality of the samples were to be validated, TMB results would have to show significantly higher
absorption peaks than their cube counterparts; however, this did not occur. As seen in the data, cubes showed an absorbance peak of nearly 1.5 while cages also showed a peak of approximately 1.5. This issue is further exacerbated by the fact that the cube sample ran was diluted (1ml of the sample in 1 ml of Deionized water) while the cage samples were used undiluted. The results suggest a notable disparity in the catalytic capabilities of the cages produced is significantly less than those of their cage counterparts. This result defies the central assumption this experiment is based on; hollowing nanocubes provides double the surface area for enzymatic activity and therefore doubling the rate of product turnover. However, when the issues faced while cleaning samples are considered the TMB results take on a different perspective. Throughout the process of Galvanic Replacement, the Pd-Ir cage sample produced far more extraneous particles, likely a byproduct of the removed core than any other sample. These particulates can be observed in figure 10. It should also be noted and observed in figure 8 that no other bimetallic cage experienced this issue to the degree Pd-Ir cages exhibited. Standard centrifugation techniques used for all other samples were not adequate for cleaning Pd-Ir cages. These samples required at minimum two additional rounds of centrifugation (in water at the same rpm for the same amount of time). It is likely that the additional washings led to particle loss and lowered the overall concentration of the Pd-Ir cage sample. This loss could potentially account for the TMB results gathered. Concentrations were not calculated for any samples collected, and therefore, it is not guaranteed that the issues stemmed from concentration. If concentrations were known, then a more accurate TMB analysis could be administered, and a more precise and accurate picture of their enzymatic activity relative to one another could form.
CONCLUSION

In review, this experiment sought to exploit the versatility of Noble metal nanostructures to form a hollowed caged shape. Traditional methods of structure modification often result in excessively aggregated products negating the benefits associated with an increased surface area. Galvanic replacement has already been shown to be apt at hollowing nanostructures comprised of coinage metals. The purpose of this experiment is to show that Galvanic replacement is a practical method for the hollowing of a broader range of bimetallic Nanocubes. A hollowed cube is desired as it is a simple and yet effective way of increasing surface area, which should lead to an increase in the overall catalytic capabilities of the enzyme mimic. Once a particle with a sufficiently opsonized enzymatic activity is synthesized, it can be substituted into enzyme-mediated immunological assays such as the ELISA and facilitate an increase in diagnostic sensitivity. Through the course of this experiment, four different bimetallic Nobel metal Nano cages were successfully formed via Galvanic replacement, and the procedures used were reported on in this paper. However, unexpected TMB results show there is further work to be done. In the future, if this experiment is attempted again. All samples should have their concentrations calculated prior to analysis. If this does not solve this issue, more work will need to be done to refine the process by which the cages are washed. A gentler procedure such as dialysis or perhaps centrifugation in a PVP buffer could reduce particle loss. Also, more work should be done testing the catalytic capabilities of the Pd-Pt, Pd-Ru, and Pd-Rh samples as these samples showed a more superior aptitude for galvanic replacement, which could lead to the increased catalytic output sought after.
REFERENCES


4. Xia, X., Ye, H., Xi, Z. and Magloire, K. Noble-metal Nanostructures as Highly Efficient

5. Chattopadhyay, K., and Mazumdar, S., Structural and Conformational Stability of
   Horseradish Peroxidase: Effect of Temperature and pH. Biochemistry, 2000, Volume 39,
   (1), 263-270

6. Wei, H., Wang, E., Nanomaterials with enzyme-like characteristics (nanozymes): next-

7. Fujinaga, M., Cherney, M. M., Oyama, H., Oda, K., and James, M. N. The molecular
   structure and catalytic mechanism of a novel carboxyl peptidase from Scytalidium
   lignicolum. Proceedings of the National Academy of Sciences of the United States of


   Nanomaterials with enzyme-like characteristics (nanozymes): next-generation artificial


