Synthesis of Gold-Platinum Core-Shell Nanoparticles Assembled on a Silica Template and Their Peroxidase Nanozyme Properties

Xuan-Hung Pham 1, Van-Khue Tran 2, Eunil Hahm 1, Yoon-Hee Kim 1, Jaehi Kim 1, Wooyeon Kim 1 and Bong-Hyun Jun 1,*

1 Department of Bioscience and Biotechnology, Konkuk University, Seoul 05029, Korea; phamricky@gmail.com (X.-H.P.); greenice@konkuk.ac.kr (E.H.); hilite2201@naver.com (Y.-H.K.); susia45@gmail.com (J.K.); buzinga5842@konkuk.ac.kr (W.K.)
2 VNUK Institute for Research and Executive Education, The University of Danang, Danang 550 000, Vietnam; khue.tran@vnu.edu.vn
* Correspondence: bjun@konkuk.ac.kr; Tel.: +82-2-450-0521

Abstract: Bimetallic nanoparticles are important materials for synthesizing multifunctional nanozymes. A technique for preparing gold-platinum nanoparticles (NPs) on a silica core template (SiO2@Au@Pt) using seed-mediated growth is reported in this study. The SiO2@Au@Pt exhibits peroxidase-like nanozyme activity has several advantages over gold assembled silica core templates (SiO2@Au@Au), such as stability and catalytic performance. The maximum reaction velocity (Vmax) and the Michaelis–Menten constants (Km) were and 2.1 × 10^-10 M^-1·s^-1 and 417 µM, respectively. Factors affecting the peroxidase activity, including the quantity of NPs, solution pH, reaction time, and concentration of tetramethyl benzidine, are also investigated in this study. The optimization of SiO2@Au@Pt NPs for H2O2 detection obtained in 0.5 mM TMB; using 5 µg SiO2@Au@Pt, at pH 4.0 for 15 min incubation. H2O2 can be detected in the dynamic liner range of 1.0 to 100 mM with the detection limit of 1.0 mM. This study presents a novel method for controlling the properties of bimetallic NPs assembled on a silica template and increases the understanding of the activity and potential applications of highly efficient multifunctional NP-based nanozymes.

Keywords: gold-platinum bimetallic nanoparticles; nanoparticle; gold-platinum-assembled silica nanostructures; nanozyme; peroxidase-like activity

1. Introduction

Nanozymes, a new functional nanomaterial with enzyme-like catalytic activity, have several advantages when compared with natural enzymes, including high stability in harsh environments, low production costs, large specific surface areas, and customizable catalytic activities based on size, morphology, and composition [1–11]. A series of nanomaterials made from metals, metal oxides, and other materials including Pt [12–14], Au [15–18], Ag [19], Cu [20], Fe3O4 [21], CeO2 [22], MnO2 [23], Mn3O4 [24,25], conducting polymers [26], metal–organic frameworks [27], carbon nanomaterials [28], and single-atom catalysts [29] have been prepared for use as nanozymes. These nanozymes have been used as bio(chemical) sensors, in immunoassays, for drug delivery, and as antibacterial agents [3,12,13,30–34]. While the physical and chemical properties of nanoparticles (NPs) can be adjusted by changing their size, shape, or structure, monometallic NPs are limited by other physical or chemical properties such as size-dependent optical properties, electronic properties, and thermal and catalytic effects that control the chemical compositions of NPs [35].

Several studies have been conducted on bimetallic NPs owing to the increased awareness of the options to adjust their physical and chemical properties. Compared with
monometallic NPs, bimetallic NPs allow a greater adjustment of their magnetic, optical, and catalytic properties via adjustments of their composition and chemical configuration [36]. Bimetallic NPs have unique structure- and composition-dependent properties that allow the optimization of enzyme-like activities more than those of single-metal NPs [37,38]. The Pt family of bimetallic NPs has been developed using various morphologies including core–shell structures, solid solution alloys, intermetallic alloys, and phase-segregated structures. Owing to the synergistic effect caused by the presence of Pt NPs, a super catalyst for electrochemical reactions, which are designed to maximize Pt utilization, tune the energetics, and assemble the geometry of exposed Pt atoms for high catalytic reactivity and selectivity, has been created [39,40]. Bimetallic NPs exhibit various enzyme-like activities including peroxidase, catalase, polyphenol oxidase, ferrooxidase, and superoxide dismutase activities [40–44]. Au@Pt bimetallic NPs have intrinsic peroxidase-like activities [45–47], have been studied extensively for electrochemical formic acid and alcohol oxidation, and Pt-rich compositions exhibit enhanced electrocatalytic activity [48,49]. Au@Pt NPs have extensive catalytic properties as they can effectively scavenge superoxide free radicals, H$_2$O$_2$, or formic acid, enabling their use in fuel cells, hydrogenation, air purification, anti-aging therapy, and cancer therapy [48,50–52]. However, Pt tends to aggregate in catalytic reactions, resulting in reduced catalytic activity [53].

Au@Ag or Au NPs immobilized on SiO$_2$ NPs have been developed recently [54–58]. In these nanostructures, the SiO$_2$ NPs serve as a template and the metal NPs are introduced to the surface using the seed-mediated growth method. Because of the SiO$_2$ core, the metal NPs assembled on SiO$_2$ nanostructures are cost-effective, more stable during surface modification, easily separable from the reaction solution, aggregate less than fine metal NPs, and redisperse into solution with enhanced separation compared with single metal NPs. Furthermore, the effects of various synthesis factors on metal NPs assembled on SiO$_2$ have been studied using near-infrared surface enhanced Raman scattering nanoprobes for bioimaging [55,56]. However, research regarding the high peroxidase-like activity of the metal NPs assembled on SiO$_2$ is limited. Therefore, the development of an improved method for preparing metal NPs assembled on SiO$_2$ templates is needed to enhance peroxidase activity. This study improves the understanding of the activity and applications of highly efficient multifunctional nanozymes.

In this study, gold-platinum NPs are prepared on a silica core template (SiO$_2$@Au@Pt) using seed-mediated growth combined with the dropping method and utilize it as a nano-material to detect hydrogen peroxide (H$_2$O$_2$) as a model because H$_2$O$_2$ plays an important role in cellular metabolism and in various industry such as food industry, gas sensors, pharmaceuticals, catalysis, environment, and solar energy [59–61]. Therefore, the need to develop a low cost, simple, fast, and sensitive method for monitoring the concentration of H$_2$O$_2$ is of practical significance in both industry and academia. The preparation of SiO$_2$@Au@Pt consists of two steps: tiny Au seeds are embedded on the SiO$_2$ surface, and the addition of a Pt$^{2+}$ precursor in the presence of ascorbic acid (AA) reductant allows the deposition and growth of the Pt layer. First, a suspension of Au seeds (approximately 2.5 nm) was prepared using tetrakis(hydroxymethyl)phosphonium chloride (THPC) and HAuCl$_4$. Then, the Au seeds were mixed with aminated SiO$_2$ NPs (approximately 160 nm) overnight to obtain SiO$_2$@Au seeds, as previously reported [54,55,57,58,62–64]. On the SiO$_2$@Au seeds, the reduction of Pt$^{2+}$ to Pt(0) was directly induced using AA, a mild reducing agent, in the presence of polyvinylpyrrolidone (PVP). These mildly reducing conditions allow a greater control over the growth of the Pt layer as the reaction proceeds much slower than that in strongly reducing conditions [65]. A low concentration of the Pt$^{2+}$ precursor and AA were added onto the SiO$_2$@Au seeds in 5 min intervals to enable the precise control of the size of the Pt NPs. After the optimization of synthesis, SiO$_2$@Au@Pt was used as a peroxidase-like nanomaterial to detect H$_2$O$_2$ efficiently.
2. Results and Discussion

2.1. Preparation of Au@Pt NPs-Assembled Silica Nanostructures

The seed-mediated growth from SiO$_2$@Au seeds was used to prepare the gold-platinum-embedded silica nanospheres (SiO$_2$@Au@Pt). First, the surface SiO$_2$ template was assembled using small Au NPs (2.6 ± 0.52 nm) (Figure 1a). Then, 10 mM H$_2$PtCl$_4$ and AA solutions were added dropwise into the dispersion of SiO$_2$@Au seeds to obtain a final Pt$^{2+}$ concentration of 200 µM. The Au@Pt NPs on the surface of the SiO$_2$ core were bigger (3.6 ± 0.56 nm) than SiO$_2$@Au seeds. The presence of Pt on the surface of SiO$_2$@Au was confirmed by the line energy dispersive X-ray (EDS) mapping of SiO$_2$@Au@Pt (Figures 1b and S1). In Figure 1b, the signal of both Pt and Au elements could obtain in the image. The quantitative EDS analysis in Table S1 of SiO$_2$@Au@Pt synthesized at 200 µM Pt$^{2+}$ consists of 74.6% Pt and 23.6% Au as mentioned in Table S1.

Figure 1. (a) Transmission electronic microscopy images of SiO$_2$@Au seed and gold-platinum-embedded silica nanospheres (SiO$_2$@Au@Pt) at different magnifications. (b) Line energy dispersive X-ray mapping of SiO$_2$@Au@Pt for Pt and Au elements. (c) UV-Vis absorbance spectroscopy of SiO$_2$@Au seeds and SiO$_2$@Au@Pt NPs. (d) UV-Vis absorbance spectroscopy of SiO$_2$@Au@Pt in various solutions. The inset shows the colors of the solutions.

The UV-Vis spectroscopy of SiO$_2$@Au@Pt was conducted when the Pt$^{2+}$ solution was added into the SiO$_2$@Au seed suspension in the presence of AA. The absorbance intensity of SiO$_2$@Au showed a slight increase at ~500 nm. This peak was suppressed in the absorbance spectrum of SiO$_2$@Au@Pt but the spectrum of SiO$_2$@Au@Pt increased in the UV region (Figure 1c) owing to the Pt layer, consistent with previous results of pure Pt hydrosol \[66,67\]. The results of TEM, EDS mapping, UV-Vis spectrum of SiO$_2$@Au@Pt indicated that Pt was deposited to the SiO$_2$@Au.

2.2. Peroxidase-like Activity of SiO$_2$@Au@Pt NPs

The peroxidase-like activity of SiO$_2$@Au@Pt NPs was evaluated using the oxidation reaction of 3,3',5,5'-tetramethylbenzidine (TMB) substrate prepared in a buffer (pH = 4)
containing TMB or a TMB-H2O2 mixture. TMB oxidation involves the exchange of two electrons. When TMB transfers one electron to form TMB\(^{+}\), the solution changes from colorless to blue. However, TMB\(^{+}\) is unstable in acidic conditions and must oxidize to TMB\(^{2+}\), forming a yellow solution [68]. In this study, the TMB, H2O2, and TMB+H2O2 solutions without SiO2@Au@Pt NPs were colorless and no absorbance peaks were observed at 453 nm (Figure 1d), indicating that there was no peroxidase-like catalytic activity in the absence of SiO2@Au@Pt. A small absorbance band was observed for the SiO2@Au@Pt + TMB solution, and a strong absorbance band with peaks at 370 and 652 nm was observed for the SiO2@Au@Pt + TMB-H2O2 solution. The SiO2@Au@Pt + TMB-H2O2 solution changed from blue to yellow and an absorbance band with a peak at 453 nm was observed (Figure S2a). These results indicate that the conversion of TMB to TMB\(^{2+}\) was catalyzed by the SiO2@Au@Pt NPs with H2O2, suggesting that SiO2@Au@Pt NPs has a peroxidase-like activity. To confirm the synergic qualities of SiO2@Au@Pt, an Au layer was deposited on the surface of the SiO2@Au seeds using a 200 µM Au\(^{3+}\) solution. The peroxidase-like catalytic activity of SiO2@Au@Pt (5 µg) and SiO2@Au@Au (5 µg) in TMB and TMB-H2O2 solutions was then verified (Figures 2a and S3b).

Figure 2. (a) UV-Vis absorbance spectroscopy of SiO2@Au@Pt and SiO2@Au@Au (5 µg) in TMB and in TMB-H2O2 solutions. The inset shows the colors of the solutions. (b) The absorbance of recycled SiO2@Au@Au and SiO2@Au@Pt is shown in a TMB-H2O2 solution. (c) UV-Vis absorbance spectroscopy and (d) Lineweaver-Burk plot at 652 nm for 5 µg SiO2@Au@Pt in a TMB-H2O2 solution (0–600 µM TMB).
The blue color of the SiO$_2$@Au@Pt in TMB-H$_2$O$_2$ is darker and the absorbance intensity at 370 and 652 nm is stronger than those of the SiO$_2$@Au@Au suspension. The absorbance intensities of the SiO$_2$@Au@Pt suspension were 5.7- and 7.7-fold of those of SiO$_2$@Au@Au at 370 and 652 nm, respectively. Similarly, the absorbance intensity of the SiO$_2$@Au@Pt suspension at 453 nm was stronger than that of SiO$_2$@Au@Au (Figure S3b). In addition, the recycling of both SiO$_2$@Au@Au and SiO$_2$@Au@Pt in the TMB-H$_2$O$_2$ solution at 453 nm was compared when the NPs were reused five times. The absorbance intensity of SiO$_2$@Au@Au in a TMB-H$_2$O$_2$ solution at 453 nm decreased to approximately 40% while that of SiO$_2$@Au@Pt in a TMB-H$_2$O$_2$ solution at 453 nm decreased to 80%. These results indicate that the catalytic ability of SiO$_2$@Au@Pt is better than that of SiO$_2$@Au@Au, and that SiO$_2$@Au@Pt NPs are reusable and separatable from the reaction mixture.

The catalytic performance of SiO$_2$@Au@Pt NPs at TMB concentrations of 0–600 μM was also investigated as the absorbance intensity at 652 nm in TMB-H$_2$O$_2$ every 3 min (Figure 2b). The absorbance intensity of the oxidation of TMB increased as the TMB concentration increased, following Michaelis–Menten behavior. The signal of SiO$_2$@Au@Pt NPs increased as the TMB concentration increased from 100 to 500 μM and reached saturation at 600 μM. The relationship of the TMB concentration and absorbance intensity at 652 nm after incubation for 180 s was plotted according to the Lineweaver–Burk equation to calculate the maximum reaction velocity (V$_{\text{max}}$) and the Michaelis–Menten constants (K$_{\text{m}}$) (Figure 2d). The kinetic activity of SiO$_2$@Au@Pt at various TMB concentrations (100–500 μM) revealed a linear relationship. The K$_{\text{m}}$ was 417 μM and the V$_{\text{max}}$ was $2.1 \times 10^{-10}$ M$^{-1}$·s$^{-1}$. The K$_{\text{m}}$ of SiO$_2$@Au@Pt indicates a higher affinity of SiO$_2$@Au@Pt for TMB compared with that for horseradish peroxidase enzyme (K$_{\text{m}}$ = 438 μM). The K$_{\text{m}}$ of SiO$_2$@Au@Pt was higher than those of Au NPs (K$_{\text{m}}$ = 123 μM), SiO$_2$@Au@Au NPs (K$_{\text{m}}$ = 60 μM), glucose oxidase-conjugated Au NPs (K$_{\text{m}}$ = 208 μM), MnO$_2$ NPs (K$_{\text{m}}$ = 83 μM), and latex-conjugated MnO$_2$ NPs (K$_{\text{m}}$ = 99 μM). The K$_{\text{m}}$ of SiO$_2$@Au@Pt was lower than those of Au NPs-decorated porous silica microspheres (K$_{\text{m}}$ = 523 μM) and Prussian-blue-decorated latex NPs (K$_{\text{m}}$ = 2.19 mM) [55]. The V$_{\text{max}}$ of SiO$_2$@Au@Pt was higher than that of SiO$_2$@Au@Au (V$_{\text{max}}$ = $2.1 \times 10^{-10}$ M$^{-1}$·s$^{-1}$), indicating that SiO$_2$@Au@Pt oxidizes TMB at a faster rate than SiO$_2$@Au@Au. SiO$_2$@Au@Pt had a comparatively more stable catalytic activity that remained at 80% after five reuses (Figure 2b).

2.3. Effects of Synthesis and Experimental Conditions on the Catalytic Activity of SiO$_2$@Au@Pt NPs

Various concentrations of the Pt$^{2+}$ precursor (100–400 μM) were added to the SiO$_2$@Au seeds. The size of Pt increased as the Pt$^{2+}$ concentration increased (Figures 3a and S2). In particularly, the size of Au@Pt synthesized at 100, 200, 300, 400 μM Pt$^{2+}$ were 3.1 ± 0.61; 3.6 ± 0.56; 3.9 ± 0.59; 4.6 ± 0.62 nm, respectively. At a high concentration of Pt$^{2+}$ (>200 μM), the Au@Pt on the SiO$_2$ surface partly merged. The quantitative EDX analysis of Pt and Au elements on the surface of SiO$_2$ synthesized at various Pt$^{2+}$ concentration was carried out to investigate the composition of the Au@Pt on the SiO$_2$ surface and the results were shown in Table S1. All Au@Pt NPs contained both Pt and Au elements, but their Au and Pt components were different. The atomic Pt component increased from 71.59% to 87.07% while the atomic Au component decreased from 28.41 to 12.93% when Pt$^{2+}$ increased from 100 μM to 400 μM. Therefore, the reciprocal of Pt and Au increased from 2.5 (at 100 μM Pt$^{2+}$) to 6.7 (at 400 μM Pt$^{2+}$). The results matched to the TEM images, and it indicated that Pt was gradually deposited on the surface of SiO$_2$@Au.
Although the Au@Pt NPs grew as the concentration of the Pt $^{2+}$ precursor increased, the size of the Au@Pt NPs increased from 3.1 to 4.6 nm, which increased the surface area for reactions between NPs and reactants. Therefore, the catalytic activity of SiO$_2$@Au@Pt NPs increased from 3.1 to 4.6 nm, which increased the surface area for the catalytic reaction between the Au NPs and the TMB-H$_2$O$_2$ mixture [55]. The UV-Vis absorption spectra of all SiO$_2$@Au@Pt NPs showed an absorbance peak at 453 nm (Figure 3c), indicating that all of the SiO$_2$@Au@Pt NPs had peroxidase-like activity. As the concentration of Pt $^{2+}$ increased, the size of the Au@Pt NPs increased (Figure S2). The growth of Au@Pt NPs on SiO$_2$ could be controlled well (Figure 3).

The correlation between the peroxidase-like activity of SiO$_2$@Au@Pt NPs and the concentration of Pt$^{2+}$ was investigated (Figure 3c). The peroxidase-like activity of SiO$_2$@Au@Pt NPs synthesized with 100–400 µM Pt$^{2+}$ was estimated using a TMB assay. The SiO$_2$@Au seeds showed very weak peroxidase-like activity because of the lack of spaces on the SiO$_2$@Au NPs and the small Au NPs on the SiO$_2$ template, resulting in an insufficient surface area for the catalytic reaction between the Au NPs and the TMB-H$_2$O$_2$ mixture [55]. The UV-Vis absorption spectra of all SiO$_2$@Au@Pt NPs showed an absorbance peak at 453 nm (Figure 3c–e), indicating that all of the SiO$_2$@Au@Pt NPs had peroxidase-like activity that was dependent on the initial Pt$^{2+}$ concentration.

In contrast, SiO$_2$@Au@Pt NPs treated with a Pt$^{3+}$ precursor concentration >100 µM had high peroxidase-like activity. As the concentration of Pt$^{3+}$ increased, the size of the Au@Pt NPs on the SiO$_2$@Au@Pt NPs increased from 3.1 to 4.6 nm, which increased the surface area for reactions between NPs and reactants. Therefore, the catalytic activity of SiO$_2$@Au@Pt NPs increased as the concentration of the Pt$^{2+}$ precursor increased from 100 to 200 µM because of the formation of sublayers or a monolayer of Pt on the surface of SiO$_2$@Pt [45,67]. This result consistent with the previous report where the increase of Pt component of Au-Pt led an better catalytic activity because the alloying of Pt with Au can change the electronic structure of Pt, leading the catalytic performance of Au@Pt changes [11,37,69].

Although the Au@Pt NPs grew as the concentration of the Pt$^{2+}$ precursor increased, the peroxidase reaction of SiO$_2$@Au@Pt treated with >200 µM of the Pt$^{2+}$ precursor did not increase because TMB and H$_2$O$_2$ cannot gain access to inner part of the thick Pt layer [66]. It means that the catalytic activities of SiO$_2$@Au@Pt reached the highest value at the Pt/Au ratio of 3.2 and decreased when Pt$^{2+}$ concentration increased further. Therefore, 200 µM
of Pt\(^{2+}\) precursor-treated SiO\(_2\)@Au@Pt NPs, which exhibited high peroxidase-like activity, were used in subsequent experiments.

Reaction conditions affect the catalytic activity of nanozymes similarly to the effects of reaction conditions on enzymes [21,70–74]. Therefore, the peroxidase-like activity of different amounts of SiO\(_2\)@Au@Pt NPs were investigated at different incubation times, pH values of the buffer solution, and TMB concentrations (Figure 4).

![Figure 4](image.png)

**Figure 4.** The effects of different conditions on the peroxidase-like catalytic activity of SiO\(_2\)@Au@Pt NPs in a mixture of TMB and H\(_2\)O\(_2\). (a) The amount of SiO\(_2\)@Au@Pt, (b) incubation time, (c) pH of the solution, and (d) TMB concentration were varied.

The amount of SiO\(_2\)@Au@Pt varied from 0.02 to 20 µg (Figures 4a and S3). The absorbance intensity of SiO\(_2\)@Au@Pt in a TMB-H\(_2\)O\(_2\) solution at 453 nm increased as the amount of SiO\(_2\)@Au@Pt increased from 0.02 to 5 µg. When the amount of SiO\(_2\)@Au@Pt increased to >10 µg, the poor solubility of TMB in the aqueous solution resulted in significant aggregation, inducing precipitation with oxidation [55].

The absorbance intensity increased as the incubation time increased from 5 to 15 min (Figures 4b and S4). The absorbance intensity reached saturation after 15 min of incubation.

The highest peroxidase catalytic activity was obtained at a pH of 4.0, at which TMB dissolved maximally and H\(_2\)O\(_2\) was the most stable (Figures 4c and S5), which is consistent with the results of previous studies [17,21,75–78].

The absorbance of TMB\(^{2+}\) at 453 nm increased as the TMB concentration increased and reached the saturation at 600 µM TMB (Figures 4d and S6).

2.4. Effects of H\(_2\)O\(_2\) Concentration on Peroxidase-like Activity of SiO\(_2\)@Au@Pt NPs

After optimizing the detection conditions, the absorbance intensity was measured at 0–400 nm for the SiO\(_2\)@Au@Pt in 500 µM TMB with various concentrations of H\(_2\)O\(_2\) (Figure 5). The yellow color of the 5 µg SiO\(_2\)@Au@Pt suspension became darker as the H\(_2\)O\(_2\) concentration increased to 200 mM, indicating that more TMB\(^{2+}\) was produced as the H\(_2\)O\(_2\) concentration increased. The catalytic activity of SiO\(_2\)@Au@Pt increased as the
H$_2$O$_2$ concentration increased to 200 mM and reached saturation at 300 mM because of aggregation (Figure S7).

A linear curve-fitting procedure was used to calibrate the reaction (Figure 5c). A significant relationship was found between the absorbance intensity at 453 nm and the H$_2$O$_2$ concentration from 1.0 to 100 mM (calibration curve: $y = 0.0185 \times + 0.63285$, where $x$ is the H$_2$O$_2$ concentration, $y$ is the absorbance intensity at 453 nm, and $R^2 = 0.99$). The theoretical LOD was 1.0 mM, estimated using the 3sblank criterion. This LOD is higher than the LOD of silver NPs modified cellulose nanowhiskers [79], polyoxometalate [80], Ce$_2$(WO$_4$)$_3$, papain [81], Ag-nanoparticle-decorated silica microspheres [82] and magnetic mesoporous silica nanoparticles [83]. These results suggest that this material can be used to detect H$_2$O$_2$.

Figure 5. (a) Colors of the solutions. (b) An absorbance signal plot at 453 nm and (c) the dynamic linear range of SiO$_2$@Au@Pt@Pt at various H$_2$O$_2$ concentrations in the presence of 0.5 mM TMB. The optimized conditions were 5 µg SiO$_2$@Au@Pt@Pt, 0.5 mM TMB, a 15 min incubation period, and a pH of 4.0.

3. Materials and Methods

3.1. Chemicals and Reagents

Tetraethylorthosilicate (TEOS), chloroauric acid (HAuCl$_4$), THPC, chloroplatinic acid (H$_2$PtCl$_6$), 3-aminopropyltriethoxysilane (APTS), AA, TMB, and PVP (MW 40,000) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ammonium hydroxide (NH$_4$OH, 27%), sodium hydroxide (NaOH), ethyl alcohol (EtOH, 99.9%), and sulfuric acid (H$_2$SO$_4$) were obtained from Samchun (Seoul, Korea). H$_2$O$_2$ was purchased from Daejung (Siheung, Gyeonggi-do, Korea). Phosphate buffer saline containing 0.1% Tween 20 (PBST, pH 7.4) was obtained from Dynebio (Seongnam, Gyeonggi-do, Korea).

3.2. Characterization

The TEM images of the samples were obtained using a JEM-F200 electron microscope (JEOL, Akishima, Tokyo, Japan) at an accelerated voltage of 200 kV. The UV-Vis spectra of the samples were recorded using an Optizen POP UV/Vis spectrometer (Mecasys, Seoul,
Korea). The samples were centrifuged using a 1730R microcentrifuge (LaboGene, Lyngen, Denmark).

3.3. Synthesis of Gold-Platinum Nanoparticles Assembled on a SiO\textsubscript{2} Nanostructure (SiO\textsubscript{2}@Au@Pt NPs)

The SiO\textsubscript{2}@Au seed NPs were synthesized as previously reported [54]. Briefly, colloidal Au NPs were prepared from HAuCl\textsubscript{4} and THPC. Silica templates (approximately 160 nm) were prepared using the modified Stöber method [84]. The surfaces of 50 mg SiO\textsubscript{2} NPs were modified using amino groups via incubation with 62 \(\mu\)L APTS overnight at 25\(^{\circ}\)C. Animated SiO\textsubscript{2} NPs (2 mg) were incubated with 10 mL colloidal Au (approximately 2.5 nm) for 12 h at 25\(^{\circ}\)C. After the suspension was centrifuged for 10 min at 8500 rpm and washed with EtOH, 2 mg of SiO\textsubscript{2}@Au seed NPs were dispersed in 2 mL of 1 mg/mL PVP solution. Subsequently, 200 \(\mu\)L of SiO\textsubscript{2}@Au seed (1 mg/mL) suspension was mixed with 9.8 mL of PVP solution. Under stirring, 20 \(\mu\)L of 10 mM HPtCl\textsubscript{6} solution (in water, Pt\textsuperscript{2+} precursor) and 40 \(\mu\)L of AA reducing agent (10 mM AA in water) were added to the mixture. The mixture was reacted for 5 min under stirring to convert Pt\textsuperscript{2+} to Pt(0). The same volumes of Pt\textsuperscript{2+} precursor and AA were added every 5 min to obtain the desired concentration of Pt\textsuperscript{2+}. The SiO\textsubscript{2}@Au@Pt NPs were then carefully washed with EtOH several times using centrifugation at 8500 rpm for 10 min. The washed SiO\textsubscript{2}@Au@Pt NPs were redispersed in 0.1% PBST solution (1 mL) to obtain a 0.2 mg/mL SiO\textsubscript{2}@Au@Pt NP suspension.

3.4. Peroxidase-like Activity of SiO\textsubscript{2}@Au@Pt NPs

To verify the peroxidase-like catalytic activity of SiO\textsubscript{2}@Au@Pt NPs, TMB solution (6 mM in EtOH, 100 \(\mu\)L), the SiO\textsubscript{2}@Au@Pt NPs suspension (100 \(\mu\)L) syntheses with 100, 200, 300, and 400 \(\mu\)M Pt\textsuperscript{2+}, and freshly prepared H\textsubscript{2}O\textsubscript{2} solution (2 M in pH 4 buffer, 100 \(\mu\)L) were added to each mixture. The mixture was incubated for 5 min under stirring to convert Pt\textsuperscript{2+} to Pt(0). Then, the mixture was reacted for 5 min under stirring to obtain the desired concentration of Pt\textsuperscript{2+}. The SiO\textsubscript{2}@Au@Pt NPs were then carefully washed with EtOH several times using centrifugation at 8500 rpm for 10 min. The washed SiO\textsubscript{2}@Au@Pt NPs were dispersed in 0.1% PBST solution (1 mL) to obtain a 0.2 mg/mL SiO\textsubscript{2}@Au@Pt NP suspension.

3.5. Peroxidase-like Activity of SiO\textsubscript{2}@Au@Pt in Various Reaction Conditions

3.5.1. Amount of SiO\textsubscript{2}@Au@Pt NPs

A mixture of 6 mM TMB solution in EtOH (100 \(\mu\)L), 2 M H\textsubscript{2}O\textsubscript{2} (100 \(\mu\)L), and 700 \(\mu\)L buffer (pH = 4) was added to 100 \(\mu\)L PBST containing 0, 0.2, 2, 5, 10, or 20 \(\mu\)g of SiO\textsubscript{2}@Au@Pt. The resulting solution was incubated at 25\(^{\circ}\)C for 15 min. To terminate the reaction, 1 M H\textsubscript{2}SO\textsubscript{4} solution (500 \(\mu\)L) was added to each mixture and the resulting mixture was incubated at 25\(^{\circ}\)C for 10 min. The absorbances of the suspensions were measured at 300–1,000 nm using a UV-Vis spectrometer.

3.5.2. Reaction Time

A mixture containing 6 mM TMB (100 \(\mu\)L), a SiO\textsubscript{2}@Au@Pt NP suspension (0.05 mg/mL, 100 \(\mu\)L), and 700 \(\mu\)L buffer (pH = 4) was added to 100 \(\mu\)L of buffer (pH = 4.0) then incubated at 25\(^{\circ}\)C for various incubation times. The mixture was terminated using 1 M H\textsubscript{2}SO\textsubscript{4} (500 \(\mu\)L). The absorbances were measured at 453 nm using a UV-Vis spectrometer.

3.5.3. PH Value of the Buffer

A mixture containing 6 mM TMB (100 \(\mu\)L), a SiO\textsubscript{2}@Au@Pt NP suspension (0.05 mg/mL, 100 \(\mu\)L), and 2 M H\textsubscript{2}O\textsubscript{2} (100 \(\mu\)L) was added to 700 \(\mu\)L of buffer at a pH range from 3.0 to 11.0. The mixture was incubated for 15 min and terminated using 1 M H\textsubscript{2}SO\textsubscript{4} (500 \(\mu\)L). The absorbances of the mixtures were measured at 453 nm using a UV-Vis spectrometer.

3.5.4. TMB Concentration

A mixture containing 700 \(\mu\)M buffer (pH 4.0), 2 M H\textsubscript{2}O\textsubscript{2} (100 \(\mu\)L), and 0.05 mg/mL SiO\textsubscript{2}@Au@Pt suspension (100 \(\mu\)L) was added to various concentrations of TMB (1, 2, 3, 4, 5, and 6 mM). The final TMB concentration in the reaction mixture was 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 mM. After incubating the mixture for 15 min, the reaction was terminated using
1 M H₂SO₄ (500 µL). The absorbances of the mixtures were measured at 453 nm using a UV-Vis spectrometer.

4. Conclusions

In summary, SiO₂@Au@Pt NPs were successfully synthesized using the seed-mediated growth method under mild conditions. Compared with SiO₂@Au@Au, the SiO₂@Au@Pt NPs exhibited more synergic and stable catalytic abilities that remained at 80% after five uses of 5 µg SiO₂@Au@Pt NPs. In addition, the peroxidase-like activity of SiO₂@Au@Pt NPs under various conditions such as the amount of SiO₂@Au@Pt NPs, pH of the buffer solution, incubation time, and TMB concentration were also investigated, revealing optimized conditions of 5 µg SiO₂@Au@Pt at pH 4.0, with 15 min of incubation in the presence of 500 µM TMB. SiO₂@Au@Pt was used to detect H₂O₂. The dynamic linear range was obtained from 1 to 100 mM, with an LOD of 1.0 mM. Therefore, this study suggests novel uses of bimetallic metal-assembled silica nanostructures in various fields and provides a suitable method for the development of nanoparticle-based multi-functional nanozymes.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms23126424/s1.

Author Contributions: Conceptualization, X.-H.P.; V.-K.T., and B.-H.J.; methodology, V.-K.T. and X.-H.P.; investigation, W.K. and Y.-H.K.; formal analysis, X.-H.P., E.H., and J.K.; writing—original draft preparation, V.-K.T., X.-H.P.; writing—review and editing, J.K. and B.-H.J.; supervisor, B.-H.J. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the KU Research Professor Program of Konkuk University and funded by the Ministry of Science and ICT (NRF-2022R1A2C2012883).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is available in the manuscript and supporting information.

Acknowledgments: The authors are grateful for the financial support from the NRF of Korea. Further, the authors give thanks for the financial support from Konkuk University.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ragg, R.; Tahir, M.N.; Tremel, W. Solids Go Bio: Inorganic Nanoparticles as Enzyme Mimics. Eur. J. Inorg. Chem. 2016, 2016, 1906–1915. [CrossRef]
2. Zhou, Y.; Liu, B.; Yang, R.; Liu, J. Filling in the Gaps between Nanozymes and Enzymes: Challenges and Opportunities. Bioconjug. Chem. 2017, 28, 2903–2909. [CrossRef] [PubMed]
3. Wang, X.; Hu, Y.; Wei, H. Nanozymes in bionanotechnology: From sensing to therapeutics and beyond. Inorg. Chem. Front. 2016, 3, 41–60. [CrossRef]
4. Wu, J.; Li, S.; Wei, H. Multifunctional nanozymes: Enzyme-like catalytic activity combined with magnetism and surface plasmon resonance. Nanoscale Horiz. 2018, 3, 367–382. [CrossRef] [PubMed]
5. Wu, J.; Li, S.; Wei, H. Integrated nanozymes: Facile preparation and biomedical applications. Chem. Commun. 2018, 54, 6520–6530. [CrossRef] [PubMed]
6. Jiang, D.; Ni, D.; Rosenkrans, Z.T.; Huang, P.; Yan, X.; Cai, W. Nanozyme: New horizons for responsive biomedical applications. Chem. Soc. Rev. 2019, 48, 3683–3704. [CrossRef]
7. Wei, H.; Wang, E. Nanomaterials with enzyme-like characteristics (nanozymes): Next-generation artificial enzymes. Chem. Soc. Rev. 2013, 42, 6060–6093. [CrossRef]
8. Gao, L.; Fan, K.; Yan, X. Iron Oxide Nanozyme: A Multifunctional Enzyme Mimetic for Biomedical Applications. Theranostics 2017, 7, 3207–3227. [CrossRef]
9. Wu, J.; Wang, X.; Wang, Q.; Lou, Z.; Li, S.; Zhu, Y.; Qin, L.; Wei, H. Nanomaterials with enzyme-like characteristics (nanozymes): Next-generation artificial enzymes (II). Chem. Soc. Rev. 2019, 48, 1004–1076. [CrossRef]
10. Wang, X.; Gao, X.J.; Qin, L.; Wang, C.; Song, L.; Zhou, Y.-N.; Zhu, G.; Cao, W.; Lin, S.; Zhou, L.; et al. eg occupancy as an effective descriptor for the catalytic activity of perovskite oxide-based peroxidase mimics. Nat. Commun. 2019, 10, 704. [CrossRef]
11. Zhang, K.; Hu, X.; Liu, J.; Yin, J.; Hou, S.; Wen, T.; He, W.; Ji, Y.; Guo, Y.; Wang, Q.; et al. Formation of PdPt Alloy Nanodots on Gold Nanorods: Tuning Oxidase-like Activities via Composition. Langmuir 2011, 27, 2796–2803. [CrossRef] [PubMed]
12. Zhang, Y.; Wang, F.; Liu, C.; Wang, Z.; Kang, L.; Huang, Y.; Dong, K.; Ren, J.; Qu, X. Nanozyme Decorated Metal–Organic Frameworks for Enhanced Photodynamic Therapy. *ACS Nano* **2018**, *12*, 651–661. [CrossRef] [PubMed]

13. Wang, X.; Qin, L.; Zhou, M.; Lou, Z.; Wei, H. Nanozyme Sensor Arrays for Detecting Versatile Analytes from Small Molecules to Proteins and Cells. *Anal. Chem.* **2018**, *90*, 11696–11702. [CrossRef]

14. Han, L.; Zhang, H.; Li, F. Bioinspired Nanozymes with pH-Independent and Metal Ions-Controllable Activity: Field-Programmable Logic Conversion of Soluble Logic Gate System. *Part. Part. Syst. Charact.* **2018**, *35*, 1800207. [CrossRef]

15. Zhang, Z.; Wang, J.; Nie, X.; Wen, T.; Ji, Y.; Wu, X.; Zhao, Y.; Chen, C. Near infrared laser-induced targeted cancer therapy using thermoresponse polymer encapsulated gold nanorods. *J. Am. Chem. Soc.* **2014**, *136*, 7317–7326. [CrossRef] [PubMed]

16. Wang, F.; Ju, E.; Guan, Y.; Ren, J.; Qu, X. Light-Mediated Reversible Modulation of ROS Level in Living Cells by Using an Activity-Controllable Nanozyme. *Small* **2017**, *13*, 1603051. [CrossRef]

17. He, W.; Zhou, Y.-T.; Wamer, W.G.; Hu, X.; Wu, X.; Zheng, Z.; Boudreau, M.D.; Yin, J.-J. Intrinsic catalytic activity of Au nanoparticles with respect to hydrogen peroxide decomposition and superoxide scavenging. *Biomaterials* **2013**, *34*, 765–773. [CrossRef]

18. Das, R.; Dhimant, A.; Kapil, A.; Bansal, V.; Sharma, T.K. Aptamer-mediated colorimetric and electrochemical detection of Pseudomonas aeruginosa utilizing peroxidase-mimic activity of gold NanoZyme. *Anal. Bioanal. Chem.* **2019**, *411*, 1229–1238. [CrossRef]

19. Pham, X.-H.; Seong, B.; Bock, S.; Hahn, E.; Huynh, K.-H.; Kim, Y.-H.; Kim, W.; Kim, J.; Kim, D.-E.; Jun, B.-H. Nonenzymatic Hydrogen Peroxide Detection Using Surface-Enhanced Raman Scattering of Gold–Silver Core–Shell-Assembled Silk Nanostructures. *Nanomaterials* **2021**, *11*, 2748. [CrossRef]

20. Wurth, R.; Winther, A.K.; Fraugergard, A.S.; van den Akker, W.; Sersen, L.; Nielsen, S.M.; Jarlsted Olesen, M.T.; Dai, Y.; Jeppesen, H.S.; Lamagni, P.; et al. Identification and Directed Development of Classical and Apparent Pan-Enzymatic Mimicry into Nanozymes for Efficient Prodrug Conversion. *Angew. Chem. Int. Ed.* **2019**, *58*, 278–282. [CrossRef]

21. Gao, L.; Zhuang, J.; Nie, L.; Zhang, J.; Zhang, Y.; Gu, N.; Wang, T.; Feng, J.; Yang, D.; Perrett, S.; et al. Intrinsic peroxidase-like activity of ferromagnetic nanoparticles. *Nat. Nanotechnol.* **2007**, *2*, 577–583. [CrossRef] [PubMed]

22. Bhagat, S.; Srikant Vallabani, N.V.; Shutthandanan, V.; Bowden, M.; Karakoti, A.S.; Singh, S. Gold core/ceria shell-based redox active nanozyme mimicking the biological multienzyme complex phenomenon. *J. Colloid Interface Sci.* **2018**, *513*, 831–842. [CrossRef] [PubMed]

23. Huang, Y.; Liu, Z.; Liu, C.; Ju, E.; Zhang, Y.; Ren, J.; Qu, X. Self-Assembly of Multi-nanozymes to Mimic an Intracellular Antioxidant Defense System. *Angew. Chem. Int. Ed.* **2016**, *55*, 6646–6650. [CrossRef] [PubMed]

24. Singh, N.; Savanur, M.A.; Srivastava, S.; D’Silva, P.; Mugesh, G. A Redox Modulatory Mn3O4 Nanozyme with Multi-Enzyme Activity Provides Efficient Cytoprotection to Human Cells in a Parkinson’s Disease Model. *Angew. Chem. Int. Ed.* **2017**, *56*, 14267–14271. [CrossRef] [PubMed]

25. Singh, N.; Geethika, M.; Eswarappa, S.M.; Mugesh, G. Manganese-Based Nanozymes: Multienzyme Redox Activity and Effect on the Nitric Oxide Produced by Endothelial Nitric Oxide Synthase. *Chem. A Eur. J.* **2018**, *24*, 8393–8403. [CrossRef]

26. Yang, Z.; Wang, C.; Lu, X. Conducting polymer-based peroxidase mimics: Synthesis, synergistic enhanced properties and applications. *Sci. China Mater.* **2018**, *61*, 653–670. [CrossRef]

27. Zheng, H.-Q.; Liu, C.-Y.; Zeng, X.-Y.; Chen, J.; Lu, J.; Lin, R.-G.; Cao, R.; Lin, Z.-J.; Su, J.-W. MOF-808: A Metal–Organic Framework with Intrinsic Peroxidase-Like Catalytic Activity at Neutral pH for Colorimetric Biosensing. *Inorg. Chem.* **2018**, *57*, 9096–9104. [CrossRef]

28. Garg, B.; Bisht, T. Carbon Nanodots as Peroxidase Nanozymes for Biosensing. *Molecules* **2016**, *21*, 1653. [CrossRef]

29. Zhang, X.; Li, G.; Chen, G.; Wu, D.; Zhou, X.; Wu, Y. Single-atom nanozymes: A rising star for biosensing and biomedicine. *Coordin. Chem. Rev.* **2020**, *418*, 213376. [CrossRef]

30. Huang, Y.; Ren, J.; Qu, X. Nanozymes: Classification, Catalytic Mechanisms, Activity Regulation, and Applications. *Chem. Rev.* **2019**, *119*, 4357–4412. [CrossRef]

31. Sindhu, R.K.; Najda, A.; Kaur, P.; Shah, M.; Singh, H.; Kaur, P.; Cavallu, S.; Jaroszuk-Sierocińska, M.; Rahman, M.H. Potentiality of Nanoenzymes for Cancer Treatment and Other Diseases: Current Status and Future Challenges. *Materials* **2021**, *14*, 5965. [CrossRef] [PubMed]

32. Wang, P.; Pang, T.; Hong, J.; Yan, X.; Liang, M. Nanozymes: A New Disease Imaging Strategy. *Front. Bioeng. Biotechnol.* **2020**, *8*, 15. [CrossRef] [PubMed]

33. Wang, Q.; Wei, H.; Zhang, Z.; Wang, E.; Dong, S. Nanozyme: An emerging alternative to natural enzyme for biosensing and immunoassay. *Trends Anal. Chem. TrAC* **2018**, *105*, 218–224. [CrossRef]

34. Das, B.; Franco, J.L.; Logan, N.; Balasubramaniam, P.; Kim, M.I.; Cao, C. Nanozymes in Point-of-Care Diagnosis: An Emerging Futuristic Approach for Biosensing. *Nano-Micro Lett.* **2021**, *13*, 193. [CrossRef] [PubMed]

35. Sharma, G.; Kumar, A.; Sharma, S.; Naushad, M.; Prakash Dwivedi, R.; Alothman, Z.A.; Mola, G.T. Novel development of nanoparticles to bimetallic nanoparticles and their composites: A review. *J. King Saud Univ. Sci.* **2019**, *31*, 257–269. [CrossRef]

36. Langlois, C.; Li, Z.L.; Yuan, J.; Alleyeaud, D.; Nelayah, J.; Bochicchio, D.; Ferrando, R.; Richelleau, C. Transition from core–shell to Janus chemical configuration for bimetallic nanoparticles. *Nanoscale* **2012**, *4*, 3381–3388. [CrossRef]

37. Hu, X.; Saran, A.; Hou, S.; Wen, T.; Ji, Y.; Liu, W.; Zhang, H.; He, W.; Yin, J.-J.; Wu, X. Au@PtAg core/shell nanorods: Tailoring enzyme-like activities via alloying. *RSC Adv.* **2013**, *3*, 6095–6105. [CrossRef]
38. Cai, S.; Qi, C.; Li, Y.; Han, Q.; Yang, R.; Wang, C. PtCo bimetallic nanoparticles with high oxidase-like catalytic activity and their applications for magnetic-enhanced colorimetric biosensing. J. Mater. Chem. B 2016, 4, 1869–1877. [CrossRef]

39. Lapp, A.S.; Duan, Z.; Marcella, N.; Luo, L.; Genc, A.; Ringnalda, J.; Frenkel, A.I.; Henkelman, G.; Crooks, R.M. Experimental and Theoretical Structural Investigation of AuPt Nanoparticles Synthesized Using a Direct Electrochemical Method. J. Am. Chem. Soc. 2018, 140, 6429–6429. [CrossRef]

40. Gawande, M.B.; Goswami, A.; Asefa, T.; Guo, H.; Biradar, A.V.; Peng, D.-L.; Zboril, R.; Varma, R.S. Core–shell nanoparticles: Synthesis and applications in catalysis and electrocatalysis. Chem. Soc. Rev. 2015, 44, 7540–7590. [CrossRef]

41. He, W.; Wamer, W.; Xia, Q.; Yin, J.-j.; Fu, P.P. Enzyme-Like Activity of Nanomaterials. J. Environ. Sci. Health Part C 2014, 32, 186–211. [CrossRef] [PubMed]

42. Liu, J.; Jiang, X.; Wang, L.; Hu, Z.; Wen, T.; Liu, W.; Yin, J.; Chen, C.; Wu, X. Ferroxidase-like activity of Au nanorod/Pt nanodot structures and implications for cellular oxidative stress. Nano Res. 2015, 8, 4024–4037. [CrossRef]

43. Liu, Y.; Wu, H.; Chong, Y.; Wamer, W.G.; Xia, Q.; Cai, L.; Nie, Z.; Fu, P.P.; Yin, J.-j. Platinum Nanoparticles: Efficient and Stable Catechol Oxidase Mimetics. ACS Appl. Mater. Interfaces 2015, 7, 19709–19717. [CrossRef] [PubMed]

44. Zhou, Y.-T.; He, W.; Wamer, W.G.; Hu, X.; Wu, X.; Lo, Y.M.; Yin, J.-j. Enzyme-mimetic effects of gold@platinum nanorods on the antioxidant activity of ascorbic acid. Nanoscale 2013, 5, 1583–1591. [CrossRef]

45. Wu, Q.; Li, Y.; Xian, H.; Xu, C.; Wang, L.; Chen, Z. Ultralow Pt-loading bimetallic nanoflowers: Fabrication and sensing applications. Nanotechnology 2012, 24, 25091. [CrossRef]

46. Tseng, C.-W.; Chang, H.-Y.; Huang, C.-C. Detection of mercury ions based on mercury-induced switching of enzyme-like activity of gold/platinum nanoparticles. Nanoscale 2012, 4, 6823–6830. [CrossRef]

47. Li, X.-R.; Xu, M.-C.; Chen, H.-Y.; Xu, J.-J. Bimetallic Au@Pt@Au core–shell nanoparticles on graphene oxide nanosheets for high-performance H2O2 bi-directional sensing. J. Mater. Chem. B 2015, 3, 4355–4362. [CrossRef]

48. Li, D.; Meng, F.; Wang, H.; Jiang, X.; Zhu, Y. Nanoporous AuPt alloy with low Pt content: A remarkable electrocatalyst with enhanced activity towards formic acid electro-oxidation. Electrochem. Acta 2016, 190, 852–861. [CrossRef]

49. Suntivich, J.; Xu, Z.; Carlton, C.E.; Kim, J.; Han, B.; Lee, S.W.; Bonnet, N.; Marzari, N.; Allard, L.F.; Gasteiger, H.A.; et al. Surface Composition Tuning of Au–Pt Bimetallic Nanoparticles for Enhanced Carbon Monoxide and Methanol Electro-oxidation. J. Am. Chem. Soc. 2013, 135, 7985–7991. [CrossRef]

50. Kataj, M.; Hikosaka, K.; Itsuka, M.; Kanayama, A.; Toshima, N.; Miyamoto, Y. Platinum nanoparticle is a useful scavenger of superoxide anion and hydrogen peroxide. Free Radic. Res. 2007, 41, 615–626. [CrossRef]

51. Kim, J.; Takahashi, M.; Shimizu, T.; Shirasawa, T.; Kataj, M.; Kanayama, A.; Miyamoto, Y. Effects of a potent antioxidant, platinum nanoparticles, on the lifespan of Caenorhabditis elegans. Mech. Ageing Dev. 2008, 129, 322–331. [CrossRef] [PubMed]

52. Hamasaki, T.; Kashiwagi, T.; Imada, T.; Nakamichi, N.; Aramaki, S.; Toh, K.; Morisawa, S.; Shimakoshi, H.; Hisaeda, Y.; Shirahata, S. Kinetic analysis of superoxide anion radical-scavenging and hydroxyl radical-scavenging activities of platinum nanoparticles. Langmuir 2008, 24, 7354–7364. [CrossRef] [PubMed]

53. Li, J.; Xu, K.; Chen, Y.; Zhao, J.; Du, P.; Zhang, L.; Zhang, Z.; Lu, X. Pt Nanoparticles Anchored on NH2-MIL-101 with Efficient Peroxidase-Like Activity for Colorimetric Detection of Dopamine. Chemosensors 2021, 9, 140. [CrossRef] [PubMed]

54. Seong, B.; Bock, S.; Hahn, E.; Huyhn, K.-H.; Kim, J.; Lee, S.H.; Pham, X.-H.; Jun, B.-H. Synthesis of Densely Immobilized Gold-Assembled Silica Nanostructures. J. Mater. Sci. 2021, 22, 2543. [CrossRef] [PubMed]

55. Seong, B.; Kim, J.; Kim, W.; Lee, S.H.; Pham, X.-H.; Jun, B.-H. Synthesis of Finely Controllable Sizes of Au Nanoparticles on a Silica Template and Their Nanoparticle Properties. J. Mater. Sci. 2021, 52, 10382. [CrossRef]

56. Bock, S.; Choi, Y.-S.; Kim, M.; Yun, Y.; Pham, X.-H.; Kim, J.; Seong, B.; Kim, W.; Jo, A.; Ham, K.-M.; et al. Highly sensitive near-infrared SERS nanoprobes for in vivo imaging using gold-assembled silica nanoprobes with controllable nanogaps. J. Nanobiotechnol. 2022, 20, 130. [CrossRef]

57. Pham, X.-H.; Hahn, E.; Kang, E.; Ha, Y.N.; Lee, S.H.; Rho, W.-Y.; Lee, Y.-S.; Jeong, D.H.; Jun, B.-H. Gold-silver bimetallic nanoparticles with a Raman labeling chemical assembled on silica nanoparticles as an internal-standard-containing nanoprobe. J. Alloys Compd. 2019, 779, 360–366. [CrossRef]

58. Pham, X.-H.; Hahn, E.; Huyhn, K.-H.; Son, B.S.; Kim, H.-M.; Jeong, D.H.; Jun, B.-H. 4-Mercaptobenzoic Acid Labeled Gold-Silver-Alloy-Embedded Silica Nanoparticles for an Internal Standard Containing Nanostructures for Sensitive Quantitative Thiram Detection. Int. J. Mol. Sci. 2019, 20, 4841. [CrossRef]

59. Yang, H.; Wang, J.; Li, X.; Zhang, L.; Yu, H.; Zhang, L.; Ge, S.; Yu, J.; Zhang, Y. Self-Circulation Oxygen–Hydrogen Peroxide–Oxygen System for Ultrasensitive Cathode Photoelectrochemical Bioassay Using a Stacked Sealed Paper Device. ACS Appl. Mater. Interfaces 2021, 13, 19793–19802. [CrossRef] [PubMed]

60. Liu, H.; Weng, L.; Yang, C. A review on nanomaterial-based electrochemical sensors for H2O2, H2S and NO inside cells or released by cells. Microchem. Acta 2017, 184, 1267–1283. [CrossRef]

61. Elias, H.; Vayssié, S. Reactive Peroxo Compounds Generated in Situ from Hydrogen Peroxide: Kinetics and Catalytic Application in Oxidation Processes. In Peroxide Chemistry; Wiley: Hoboken, NJ, USA, 2000; pp. 128–138. [CrossRef]

62. Shim, S.; Pham, X.-H.; Cha, M.G.; Lee, Y.-S.; Jeong, D.H.; Jun, B.-H. Size effect of gold on Ag-coated Au nanoparticle-embedded silica nanospheres. RSC Adv. 2016, 6, 48644–48650. [CrossRef]
82. Zhang, Z.; Liu, Q.; Liu, Y.; Qi, R.; Zhou, L.; Li, Z.; Yun, J.; Liu, R.; Hu, Y. Colorimetric H2O2 Detection Using Ag-Nanoparticle-Decorated Silica Nanoparticles. *Int. J. Mol. Sci.* 2019, 20, 1258. [CrossRef] [PubMed]

83. Wang, Y.; Zhou, B.; Wu, S.; Wang, K.; He, X. Colorimetric detection of hydrogen peroxide and glucose using the magnetic mesoporous silica nanoparticles. *Talanta* 2015, 134, 712–717. [CrossRef] [PubMed]

84. Deutsch, J.C. Ascorbic Acid Oxidation by Hydrogen Peroxide. *Anal. Biochem.* 1998, 255, 1–7. [CrossRef] [PubMed]